A. CANTANI

Pediatric Allergy, Asthma and Immunology

Springer

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With 649 Figures in 728 Partfigures, 341 in Color and 741 Tables

Springer

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ISBN-10 3-540-20768-6
Springer Berlin Heidelberg New York
ISBN-13 978-3-540-20768-9
Springer Berlin Heidelberg New York

Library of Congress Control Number: 2004117336

Title of the original Italian edition:
Allergologia e immunologia pediatrica –
dall'infanzia all'adolescenza
© 2000 Verduci Editore – Roma, ISBN 88-7620-545-4

Editor: Dr. Ute Heilmann, Heidelberg, Germany
Desk Editor: Dörthe Mennecke-Bühler, Heidelberg, Germany
Cover design: Frido Steinen-Broo, Pau, Spain
Production: Martha Berg, Heidelberg, Germany
Reproduction and typesetting:
AM-productions GmbH, Wiesloch, Germany
Printing and binding: Stürtz GmbH, Würzburg, Germany

21/3151bg – 5 4 3 2 1 0
Printed on acid-free paper

To my beloved wife
for so much help she has given me
and for so much time I robbed her of.

Preface

Science cannot be restricted
by the narrow frame of a book:
it is generally intolerant of frames

Pediatric Allergy, Asthma and Immunology is a new discipline that finds its foundation in this book, whose roots linke me to Elena and Luisa Businco, with whom I founded the first Italian Pediatric Allergy Division some 30 years ago, now called the Pediatric Allergy and Immunology Division. There I discovered this world, where I had the chance to revive the significance of the three Ss: science, safety and sympathy. Children and their parents consult us in the hope of finding scientific and medical knowledge as well as assurance and understanding sympathy, all necessary prerequisites for the successful outcome of our everyday tasks. Above all, one should appreciate how much the pediatrician–allergist is, more than any other doctor, dedicated to the care of his or her patients, since he or she must either deal with cases of extreme severity, such as anaphylactic shock, or perform ordinary jobs, such as giving suggestions on the diets or the furnishings of the home. The pediatrician–allergist should always find out how to protect the infant, the child and the adolescent against discrimination because of their allergy. With proper prescriptions and appropriate recommendations, such an objective is always within reach, and both the child and his or her parents will profit from a better quality of life.

The earliest roots of this book developed from my everyday work in the Pediatric Allergy and Immunology Division and have grown while preparing lessons and courses to be delivered to medical students and postgraduates in pediatrics. Of course, this ongoing work has found its expression in a host of papers that have inspired several chapters within this book. However, my primary aim was not one of doing something necessary; I have hoped only to do something that is useful to someone. With this book, I hope to have offered convincing proofs and foundations to colleagues committed to pediatric allergy and immunology. Often its main goal is one of *prevention*, in all senses and using all resources, as Arnaldo Cantani Sr. wrote in 1877 in the preface to the first edition of his *Textbook of Clinical Pharmacology*: " … only corresponding with a meticulous study and the greatest exactness to the precise indications of the case, the drugs may be use–ful to the patient … in the belief that air, water, and alimentation are the first and most powerful means to be well."

Pediatric allergy and immunology is a multidisciplinary field of research today, and familiarity with current concepts is important for medical students, for clinicians in every pediatric specialization and for researchers in this attractive area. However, the issue is not benefited by an easy approach, because pediatric allergy and immunology has characteristic features both different and larger in scope than adult allergy. Nor can we disregard significant events such as the *atopic-march*, the inexorably accelerating prevalence of atopic diseases, which develop in 80%–90% of cases within the very first months and years of life, while the intense efforts of research scientists and the greater awareness of pediatricians and of dedicated parents have widened the positive results of prevention and treatment. The avalanche of immunological progress shows no sign of abating in this new millennium. I have therefore begun with the fundamental concepts of basic immunology, whose inferences are relevant to the later chapters. For example, I have attempted to offer an exhaustive discussion in Chap. 1 to the interested reader trying to understand the significance of adhesion molecules from the pathogenic point of view. Therefore, after the chapters on fetal-neonatal immunology and the mucosal immune system, the neonate at risk of atopy, the genetic and environmental predisposing factors and the epidemiology and natural history of atopic diseases, a whole chapter encompasses the diagnosis of allergy, from the clinical history to the provocation tests. The book progresses chapter by chapter to elucidate the spectrum of several diseases, including atopic dermatitis, food allergy, asthma, rhinoconjunctivitis, and to discuss specific immunotherapy (SIT) for these diseases. It also places great emphasis on specialist disorders such as sinusitis and otitis media with effusion, which are frequently associated with allergic diseases. Many pages are devoted to autoimmune diseases, primary immunodeficiencies and to pediatric HIV infection. The last two chapters are comprehensively built on the earlier ones, introducing two emerging important advances, malnutrition and the immune system and another of capital importance, atopy prevention, which sums up the wealth of new data.

Until recently, the expansion of immunology was undervalued. In this breakthrough my major thrust was

to attest to the ferments of activity that have revolutionized, so to say, the exciting new area of research, such as the therapeutic strategies exemplified by the switch from Th2 to Th1 lymphocytes in the immune system manipulations through SIT and anti-IgE therapy, gene therapy of primary immunodeficiencies and the maternofetal treatment of HIV infection. A growing body of literature is shaping our knowledge of the fetal immune system. We are now aware that the fetus can be immunocompetent from the 18th to 20th week of intrauterine life, and that from the 22nd week it can react to food and inhalant allergens of maternal origin, suggesting that heredity and maternal intake of foods or drugs or allergen inhalation may anticipate the foundations of pediatric allergy and immunology in intrauterine life, thus requiring an advancement of preventive measures. In this context, immunology is the new milestone when one refers to the so-called collagen diseases, revisited as a deviation from the normal mechanisms of self-recognition, to the viruses that deceive the immune system, modulating apoptosis at will, and to the immunological components of breast milk, rich in prebiotics and TLRs and protecting infants even from diabetes. From this viewpoint we cannot underestimate the impact of transgenic foods and pesticides, which are revolutionizing foods, and of polluted air breathed by newborns. Among the food offenders, the first level refers to hidden allergens, or those regularly absent from the labels, and the growing number of cross-reactions, with the remarkable latex–fruit syndrome and the mite–mollusc correlations. The role of infectious agents could likely be the opposite of current theories, namely that of protecting infants from the onset of allergic disease, whose higher frequency could be favored by the improvement in the standard of living. The hygiene hypothesis is intriguing, but milk may kill by inhalation, casein may remain active for 2,500 years and egg for 500 years. We move forward in pediatric allergy and immunology: fascinating findings focus on the increasing number of wheezing infants and on the success of desensitization shared by food-allergic and asthmatic children, thus leaving these children without disease. Immunodeficiencies are radically cured by bone marrow transplantation, autoimmune diseases are starting to be cured with stem cell transplantation, diabetes seems to be cured by mother–daughter transplantation of pancreatic cells and immunodeficiencies by bone marrow transplantation. HIV infection can be "cured" by prevention.

In the presentation of the diverse conditions, I have preferred a complete description in a traditional sequence, beginning with an introduction, the definitions and the epidemiology, then continuing with the immunological characteristics, pathogenesis, symptoms, diagnosis and treatment. Further, being compelled to deal with aspects sometimes so distant or different has certainly implied possible errors in measure and a certain degree of overlap. A very hard task was that of selecting, among the relevant literature in an unending stream of data on pathogenic and therapeutic aspects, the most significant ones, especially in the field of pediatrics. It is not always easy and productive to interweave basic and clinical material. I have tried to inform the reader more comprehensively following a logical progression, synthetically reviewing the most recent state of this rapidly advancing specialization, leaving in the background the data pertaining to the basic knowledge of pediatricians and allergist–immunologists.

My purpose was also that of lightening the text with the aid of approximately 1,400 high-quality figures covering basic aspects and tables abounding with practical information facilitating day-to-day diagnosis and management. My approach has been that of utilizing the figures and tables as both a commentary and an extension of the text. The appendices complete the volume, while the abbreviations and acronyms are listed separately. In addition, I have adopted the Système International des Unités (SI) where appropriate. At the end of each chapter a list of references includes leading articles and subspecialty reviews, so that readers are referred to numerous points of departure from which to explore further the subjects closer to their interests.

I have attempted, therefore, to offer to dedicated pediatricians and family practitioners a comprehensive, clear and timely distillation of current information making it possible to keep abreast of recent advances and to acquire the basic principles necessary in their practice. The careful reader will find practical advice on which to base actions that will block the atopic and immunological march by preventing, managing and treating allergic–immunological diseases, and by appropriately informing parents, without neglecting to raise public awareness of the threat posed by the march and to provide the means to stop it. Managing childhood atopic and immune disorders requires a new strategy. Millions of children and their parents expect disease prevention and cure, and allergists or immunologists are challenged to provide interventions that achieve optimal health from childhood to adulthood. I hope that students and postgraduate doctors willing to find a detailed reference for this fascinating and demanding area of pediatrics and willing to develop an allergic–immunological viewpoint will succeed in identifying the diverse pathologies and will be motivated to become more actively involved in the daily health needs of atopic infants, children and adolescents.

I am deeply grateful to my wife, María Susana Campostrini, who assisted me in this challenging enterprise and helped me to add expressive illustrations to the book. I wish to acknowledge the assistance of several colleagues for their helpful discussions and contributions, including Doctors Daniele Ceccoli, Franco Frati, Oreste Marciano and my referees Professors Emanuele Errigo and Massimo Fiorilli. The consultation of numerous journals was of particular help, especially in the libraries of the Pediatric Department of Rome Uni-

versity "La Sapienza" and Rome University "Tor Vergata," the Pediatric Department of Sassari University, the National Council for Scientific Research, the Italian Institute of Public Health and several university libraries of the Hospital Policlinico Umberto I where I work, especially the Department of Experimental Medicine. I extend my gratitude to many colleagues and publishers who have kindly provided many figures including the late Professor Luisa Businco and the UCB that kindly supplied many figures related to the SCORAD and ETAC studies. In particular, I am deeply indebted to Professors Molkhou, Revillard and Wüthrich and their publishers. My thanks to Professors Mogi, Ring and Wüthrich, who presented me with their books and Professors Bernstein, Brandtzaeg, Buckley, Gerrard, Patriarca, Roos and Sullivan for sending me reprints not easily found otherwise. I owe particular gratitude to Springer-Verlag and especially to Ms Ute Bujard for her meticulous editing skills that allowed the publication of this book. I would also like to thank Martha Berg whose excellent assistance helped me very much.

To offer a wide panorama of results, several data have been presented throughout the book and reported in the tables and in the figures, independently of how the children were identified as affected with allergic-immunologic disease. Of course I do not expect that my opinions or my suggestions "to live better with allergy" meet the unconditioned favor of all readers: I would be grateful if they would point out "the errors and the omissions" so that I can correct them in a future edition.

Rome, September 2007
Arnaldo Cantani

Foreword

For those who believe that I may not be the best suited person to present *Pediatric Allergy, Asthma and Immunology* by Arnaldo Cantani, I would like to explain the reasons that encouraged me – a specialist of adult disease of intellectual development, with professional experiences substantially different from those of the author – to agree to his request with great pleasure. These reasons are either of a personal nature or of a more general and ideological nature.

Professor Cantani's knowledge has its deep roots in his extensive work at the Department of Pediatrics of the University of Rome "La Sapienza" and especially at the chair of the late Professor E. Rezza. Only recently, however, when I had more frequent opportunities of collaborating with him, was I struck by the profound "team spirit" that Arnaldo Cantani feels for pediatric allergy and immunology as well as by his exactness and precision in dealing with the commitment necessary to report his own experience.

As Past President of the European Academy of Allergology and Clinical Immunology, I am fully aware of the problems, both general and specific, that pertain to the discipline of "pediatric allergy and immunology." The opportunity to acknowledge this discipline as an autonomous specialization is of primary necessity, especially in north European centers. This orientation is opposed by some pediatric specialists or allergy specialists. However, it should be appreciated that pediatric allergy and immunology differs widely from that of adults, as evidenced by this textbook. This difference is particularly important not only in the newborn period but above all in the diseases typical and specific to the pediatric age as well as in those also common to adult patients, of wholly peculiar physiopathologic, diagnostic and therapeutic characteristics. These current points of view are confirmed by the recent proposal to consider both internal medicine and pediatrics as common branches of the allergology and clinical immunology specialization, as well as the decision of the European Academy of Allergology and Clinical Immunology to create a Section of Pediatric Allergology, thus satisfying the need to gather under a single roof specialists of the "general" discipline, thereby recognizing and warranting the importance and autonomy of the pediatric allergologist.

Professor Cantani's book is a concrete and cogent contribution to the foundation of pediatric allergy–immunology. This book is an impressive and comprehensive documentation of the progress in the understanding and management of allergic–immunologic disorders of infants, children and adolescents, and is divided into 24 chapters illustrated by more than 1,400 tables and figures that are helpful in clarifying complex points. I have greatly appreciated the author's approach of discussing, in addition to the ontogeny of the immune system, mucosal immunology and the typical pathology of infantile immediate hypersensitivity with its very early onset age, the mechanisms underlying specific disease states such as the developing neonatal immune response, autoimmune disease, immune deficiencies and pediatric AIDS, which are increasingly recognized as complex diseases. Such an approach entails, in fact, that one's eyes be kept open to the complexity of clinical allergology and immunology, and widened beyond the limited field of atopic disease and the "atopic march" to the genetic relation to atopy and bone marrow transplantation.

A critical analysis of how this complex and detailed information is condensed into a readable textbook suggests the author's far-sighted attitude: on the one hand the painstaking precision of the scientist (see the list of abbreviations that opens the book), and on the other the more typical Latin inclination to prefer clinical reasoning, which, even in its subjectivity, always represents the essential distinguishing feature between the clinical professor and accurately programmed reasoning.

The findings of several schools and disciplines different from those of Professor Cantani are critically evaluated, and the virtues of single authorship, compared to multi-authored textbooks that often lack sufficient coordination and revision by the editor, are evident throughout the book.

All chapters have reference lists with citations that will be stimulating for those interested in more in-depth study. Moreover, the suggestions at the end of each chapter and the numerous discussions, also in the form of tables and figures, promote an expert starting point for diverse specialists interested in evidence-based medicine, be they allergists, immunologists, pediatricians or practitioners. As a result, the reader has at hand a doubly useful book: one to be studied and consulted, the other to be read with pleasure, a book to be approached critically.

Although he had the excellent collaboration and editorial assistance of Springer-Verlag throughout the preparation of this textbook, Arnaldo Cantani has undertaken alone the fascinating burden of putting together *Pediatric Allergy, Asthma and Immunology*. I therefore compliment the author on assembling an outstanding opus in the interest of pediatric allergy and immunology and in the training of all those concerned with the "march." Anyone who cares for allergic children or wants to learn about immune diseases will surely benefit from frequent consultations of this textbook. I hope that the book will provide pleasure and insight to all prospective readers.

Rome, May 2007
Sergio Bonini

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Abbreviations

A	Alimentum
AA	Amino acid
AA	Arachidonic acid
AA	Aspartic acid
α_2M	α_2 Macroglobulin
α_2M-R	α_2 Macroglobulin receptor
AAAAI	American Academy of Allergy, Asthma and Immunology
Aab	Autoantibody
Aag	Autoantigen
AAF	Amino acid formula
AAP	American Association of Pediatrics
AAPSNAD	American Association of Pediatrics Subcommittee of Nutrition and Allergic Disease
ABA	Allergic bronchopulmonary aspergillosis
ABC	Abacavir
AC	Allergic conjunctivitis
ACAAI	American College of Allergy, Asthma and Immunology
ACAT	Automated computerized axial tomography
ACC	1-Aminocyclopropane-1-carboxylic acid
ACD	Allergic contact dermatitis
ACE	Angiotensin-converting enzyme
ACh	Acetylcholine
ACT	Immune-activating cytokine
ACT-2	Immune-activating cytokine-2
AD	Atopic dermatitis
AD	Autosomal dominant
ADA	Adenosine deaminase
ADAM	α Disintegrin and α metalloproteinase
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADD	Average daily dose
ADGL	Dihomo-γ-linolenic acid
ADHD	Attention-deficit hyperactivity disorder
ADH	Antidiuretic hormone
ADNI	Selective antibody deficiency with normal Ig isotypes
ADR	Adrenergic receptor
ADR	Adverse drug reaction
ADRB2	α_2-Adrenergic receptors
AEA	Antiendomysial antibodies
AEA	Antierythrocyte autoantibody
AECA	Antiendothelial cell antibodies
AF	Anchoring filaments
AF	*Aspergillus fumigatus*
AFP	α-Fetoprotein
AGA	Antigliadin antibodies
Ah	Aromatic hydrocarbons
AHS	Anticonvulsant hypersensitivity syndrome
AIC	Amb a 1 immunostimulatory oligodeoxynucleotide conjugate
AICDA	Activation-induced cytidine deaminase
AID	Autoimmune disease
AID	Activation-induced cytidine deaminase
AIDS	Acquired immunodeficiency syndrome
AIHA	Autoimmune hemolytic anemia
AIM	Activation inducer molecule
AIN	Autoimmune neutropenia
AKC	Atopic keratoconjunctivitis
ALA	α -Lactalbumin
ALCAM	Activated leukocyte cell adhesion molecule (CD166)
Alfaré	Alimentation facilement résorbable
Allergen	Allergy generator
ALPS	Autoimmune lymphoproliferative syndrome
AML	Acute myeloblastic leukemia
AMLR	Autologous mixed lymphocyte reaction
ANA	Antinuclear antibody
ANCA	Antineutrophil circulating antibodies
ANF	Antinuclear factor
ANP	Atrial natriuretic peptide
ANS	Autonomic nervous system
AOM	Acute otitis media
AP-1	Activator protein-1

The English medical abbreviations have been cross-referenced using Davis NM Medical Abbreviations, 8th edn, NM Davis Associates, Huntington Valley, 1997. To offer a wide panorama of results, several data have been presented throughout the book and reported in the tables independently of how the children were identified as affected with atopic disease. Drug availability has been assessed. Regarding drug usage, several tables specify the chemical names, types of packaging, administration routes and, where possible, the pediatric doses and schedules of treatment. I have taken care to make sure that the information is correct at the time of publication; however, the ultimate responsibility rests with the prescribing physician.

AP-1	Apolipoprotein 1
Apaf 1	Apoptotic protease activating factor 1
APC	Antigen-presenting cells
APO-1	Apoptosine-1 (CD95)
APP	Acute-phase proteins
APR	Acute-phase response
APT	Aptamil HA
APT	Atopy patch test
APV	Amprenavir
AR	Alfaré
AR	Allergic rhinitis
AR	Autosomal-recessive
AR3	Apoptose receptor 3
ARAM	Antigen recognition activation motif
ARC	Allergic rhinoconjunctivitis
ART	Antiretroviral therapy
ASA	Acetylsalicylic acid
ASAT	Aspartate aminotransaminase
ASCT	Autologous stem cell transplantation
ASO	Allele-specific oligonucleotide
AST	Antistreptolysin titer
ATA	Ataxia-telangiectasia
ATAC	Activation-induced, T cell-derived, and chemokine-related
ATG	Antithymocyte globulin
ATM	Ataxia-telangiectasia mutated
ATP	Deoxyadenosine triphosphate
AU	Allergy unit
AUR	Allergy unit by RAST
AXT	Deoxyadenosine nucleotides
AZT	Azidodeoxythymidine
BaDF	Basophil activating factor
BALF	Bronchoalveolar lavage fluid
BALT	Bronchus-associated lymphoid tissue
BAU	Bioequivalent allergy unit
BCF	Basophil chemotactic factor
BCG	Bacillus Calmette-Guérin
Bcl-2	B cell lymphoma-2
BcR	B cell receptor
BDP	Beclomethasone dipropionate
BE	Base excess
Bf	B factor
BGP-1	Biliary glycoprotein-1
BH1 to BH4	Bcl-2 homology domains
BHA	Butylated hydroxyanisole
β-HCB	β-Hexachlorobenzene
β-HCH	β-Hexachlorocyclohexane
BHR	Bronchial hyperreactivity
BHT	Butylated hydroxytoluene
bid	bis in die, twice a day
b.i.d.	*Bis in die*, twice a day
BIV	Bovine immunodeficiency virus
BK	Bradykinin
βLG	β-Lactoglobulin
β_2-m	β_2-Microglobulin
BM	Basement membrane
BM	Bone marrow
BM	Breast milk
BMA	Breast milk allergy
BMI	Body mass index
BMT	Bone marrow transplant
BP	Blood pressure
bp	base pair
BPI	Bacterial permeability increasing protein
BPO	Benzyl-penicilloyl
BPT	Bronchial provocation test
BSA	B-superantigens
BSA	Bovine serum albumin
BSE	Bovine spongiform encephalopathy
β-TG	β-Thromboglobulin
Btk	*Bacillus thuringiensis* subspp. *kurstaki*
Btk	Bruton's tyrosine kinase
BTS	Benzothiazole bisulfide
Btt	*Bacillus thuringiensis* subspp. *tenebrionis*
BU	Biologic units
BUD	Budesonide
bw	Body weight
C	Constant
c-ANCA	Cytoplasmic antineutrophil circulating antibodies
C/EDPα	CCAAT/enhancer binding protein α
C/EDP	CCAAT/enhancer binding protein η
C1-INH	C1-inhibitor
C4 bp	C4-binding protein
C8 bp	C8-binding protein
CA	Capsid
CAF	CD8 T-cell antiviral factor
CALC	Calcitonin
cALL	Common acute lymphoblastic leukemia
CALLA	Common acute lymphoblastic leukemia antigen
CALT	Conjunctiva-associated lymphoid tissue
cAMP	Cyclic adenosine monophosphate
CAP	Chemiluminescent assay
CAP	Chloramphenicol
CARD	Caspase activation and recruitment domain
CATCH 22	Cardiac abnormalities, Abnormal facies, Thymic hypoplasia, Cleft palate, Hypocalcemia, chromosome 22
CB	Cord blood
CBC	Complete blood count
CBIgE	Cord blood IgE
CBMC	Cord blood mononuclear cells
CCP	Complement control protein
CCR	CC chemokine receptor
CD	Celiac disease
CD	Cluster of differentiation
CD	Crohn's disease
Cd	Cadmium
CD11a/CD18	LFA-1
CD11b/CD18	CR3 Mac-1

CD11c/CD18	CR4 p150,95
CDC	Centers for Disease Control (and Prevention)
CDR	Complementarity-determining regions
CEA	Carcinoembryonic antigen
ced	Cell-death defective
CF	Cystic fibrosis
CFC	Chlorofluorocarbon
CFS	Chronic fatigue syndrome
CFU	Colony-forming unit
CFU-GM	Colony-forming unit, granulocytes and monocytes
CFU-S	Colony-forming unit, spleen
CFU-T	Colony-forming unit, thymus
CGD	Chronic granulomatous disease
CGM1	CEA gene member 1
CGM6	CEA gene member 6
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene-related peptide
CH_{50}	Hemolytic complement 50%
CHARGE	Coloboma, Heart anomalies, Atresia of choanae, Retardation, Genital hypoplasia, Ear anomalies
CHF	Casein hydrolyzed formula
CI	Confidence intervals
CIC	Circulating immune complexes
CID	Combined immunodeficiency
CIE	Crossed immunoelectrophoresis
CIEV	Caprine infectious encephalitis virus
CIITA	Class II transactivator
CJD	Creutzfeldt-Jakob disease
CKR-SF	Cytokine receptor superfamily
Cl	Chlorine
CLA	Conjugated linoleic acid
CLA	Cutaneous lymphocyte-associated antigen
CLA	System-chemiluminescent immunoassay
CLC	Charcot-Leyden crystals
CLE-0	Consensus lymphokine element-0
CLE-1	Consensus lymphokine element-1
CLE-2	Consensus lymphokine element-2
CLIP	Class II associated invariant chain peptide
CM	Cow's milk
CMA	Cow's milk allergy
CMI	Cell-mediated immunity
CMV	Cytomegalovirus
CN	Calcineurin
CNO	Chronic nasal obstruction
CNS	Central nervous system
CO_2	Carbon dioxide
Con-A	Concanavalin A
COV	Mean coefficient of variation
CpG	Deoxycytidyl-deoxyguanosine dinucleotide
CPK	Creatinine phosphokinase
CPS	Capsaicin
CPT	Conjunctival provocation test
CR	Complement receptor
CR	Crossed reactions
Cr	Chromium
CR3	Complement receptor type 3
CREST	Calcinosis-Raynaud-Esophageal (motility disorders)-Sclerodactyly-Telangiectasia
CRH	Corticotropin-releasing hormone
CRIE	Crossed radioimmunoelectrophoresis
CRP	C reactive protein
CS	Corticosteroids
CsA	Cyclosporin A
CSF	Cerebrospinal fluid
CSF	Colony stimulating factor
CSM	Costimulatory molecule
CT	Computerized tomography
CTAP-III	Connective tissue-activating protein-III
CTL	Cytotoxic T lymphocytes
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4 (CD152)
Cu	Copper
CVID	Common variable immune deficiency
CXCR	CX chemokine receptor
D	Dalton ($1.6605655 \times 10^{-24}$ g)
D	Diversity
d4T	Stavudine
DAF	Decay accelerating factor (CD55)
DAG	Diacylglycerol
DALIA	Distribution-analyzing latex immunoassay
DARC	Duffy antigen receptor complex
DBPC	Double-blind, placebo-controlled
DBPCCT	Double-blind, placebo-controlled challenge test
DBPCFC	Double-blind, placebo-controlled food challenge
DC	Dendritic cells
DC-CK1	Dendritic cell chemokine-1
DCC	Double-blind, controlled
ddC	Zalcitabine
DDE	Dichlorophenyl-dichloroethene
ddI	Dideoxyinosine
ddI	Didanosine
DDT	Dichlorodiphenyltrichloroethane
DEP	Diesel exhaust particles
Der f	*Dermatophagoides farinae*
Der p	*Dermatophagoides pteronyssinus*
DES	Diethylstilbestrol
DGS	DiGeorge syndrome
DGSC	DiGeorge syndrome, complete
DGSP	DiGeorge syndrome, partial
DGST	DiGeorge syndrome, transient
DHA	Docosahexaenoic acid
DHST	Delayed hypersensitivity skin test
DIC	Disseminated intravascular coagulation
DM	Diabetes mellitus
DMARDs	Disease-modifying antirheumatic drugs

DMN	Dimethylnitrosamine
DMV	Daily mean variations
DN	Double negative
DNA	Deoxyribonucleic acid
DNCB	Dinitrochlorobenzene
DP	Double positive
DPG	Diphenylguanidine
DPI	Dry powder inhaler
DPU	Delayed pressure urticaria
DR	D-related
DSCG	Disodium chromoglycate
DTH	Delayed-type hypersensitivity
DYM	Dynorphin
DZ	Dizygotic (twins)
E-L-R	Glutamic acid-leucine-arginine
e-NANC	Excitatory NANC
EA	Erythrocytes, antierythrocyte (antibody)
EAA	Extrinsic allergic alveolitis
EAACI	European Academy of Allergy and Clinical Immunology
EAC	Erythrocytes, antierythrocyte (antibody), complement
EACA	Epsilon aminocaproic acid
EAE	Experimental autoimmune encephalitis
EAEC	Eosinophil adhesion to endothelial cells
EAF	Eosinophil-activating factor
EBI3	Epstein-Barr virus-induced gene 3
EBV	Epstein-Barr virus
ECA	Eosinophil chemotactic activity
ECEF	Eosinophil cytotoxicity enhancing factor
ECF	Eosinophil chemotactic factor
ECHF	Extensively casein hydrolysate formula
ECHO	Enteric cytopathic human orphan (virus)
ECM	Extracellular matrix
ECP	Eosinophil cationic protein
ECU	Extracellular unique
ED	Emergency department
EDIF	Epithelium-derived inhibitory factor
EDN	Eosinophil-derived neurotoxin
EDRF	Endothelium-derived relaxing factor
EDTA	Ethylenediaminetetraacetic acid
EFA	Enhancing factor of allergy
EFA	Essential fatty acids
EFAD	Essential fatty acid dysfunction
EFV	Efavirenz
EGF	Epidermal growth factor
EIA	Enzyme immunoassay
EIA	Exercise-induced anaphylaxis
EIAV	Equine infectious anemia virus
ELA2	Elastase 2
ELAM	Endothelial-leukocyte adhesion molecule (CD62E, LECAM-1)
ELC	EBI1 ligand chemokine
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
EM	Electron microscope
EM	Erythema multiforme
EMA	Endomysium autoantibodies
ENA-78	Epithelial cell-derived neutrophil-activating protein-78
EMK	Enkephalin
ENR	Eosinophilic nonallergic rhinitis
env	Envelope (of HIV)
EPA	Eicosapentaenoic acid
EPD	Enzyme potentiated desensitization
EPO	Eosinophil peroxidase
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
ER	Endoplasmic reticulum
ERV	Expiratory reserve volume
ESL-1	E-selectin ligand 1
ESPACI	European Society of Pediatric Allergy and Immunology
ESPGAN	European Society of Pediatric Gastroenterology and Nutrition
et al.	*et alii*, and others
ET	Eustachian tube
ET-1	Endothelin-1
ET-2	Endothelin-2
ET-3	Endothelin-3
ET-4	Endothelin-4
ETAC	Early treatment of the atopic child
ETAC	European Task Force on Atopic Dermatitis
ETD	Eustachian tube dysfunction
ETO	Eustachian tube obstruction
ETS	Environmental tobacco smoke
EU	European Union
EWHF	Extensively whey hydrolyzed formula
FA	Food allergy
Fab	Fragment antigen binding
FADD	Fas-associated death domain
FAE	Follicle-associated epithelium
Fas	APO-1
FAS	Family atopy score
FAST	Fluoroallergosorbent test
FC	Flux cytometry
Fc	Fragment crystallizable
FcR	Fc-Receptor
FCT	Food challenge test
FDA	Federal Drug Administration
FDC	Follicular dendritic cells
Fe	Iron
FEF	Forced expiratory flow
FEF_{50}	Forced expiratory flow at 50%
FEIA	Fluoroenzymeimmunoassay
FEIA	Food-associated EIA
FEV_1	Forced expiratory volume in 1 s
FFA	Free fatty acids
FGF	Fibroblast growth factor
FH	Family history
FHA	Family history of atopy

FIC	Fibroblast-induced chemokine
FIS	Fetal immune system
FISH	Fluorescence in situ hybridization
FIV	Feline immunodeficiency virus
FKBP	FK-506 binding proteins
FMLP	Formylmethionyl leucylphenylalanin
FN	Fibronectin
FP	Fluticasone propionate
FR	Framework region
FR	Free radicals
FRC	Functional residual capacity
FSA	Family score of atopy
FVC	Flow-volume curves
FVC	Forced vital capacity
G-CSF	Granulocyte-colony stimulating factor
G6PD	Glucose-6-phosphate dehydrogenase
GA	Gestational age
GABA	γ-Aminobutyric acid
GAD	Glutamic acid decarboxylase
gag	Group-specific antigen
GAG	Glycosaminoglycan
GAL	Galanin
Gal-1	Galectin-1
Gal-3	Galectin-3
GALT	Gut-associated lymphoid tissue
GAPs	GTPase-activating proteins
GC	Germinal center
GCK	Glucokinase
GCP-2	Granulocyte chemotactic protein 2
GDP	Guanosine diphosphate
GE	Gas exhaust
GEF	Glycosylation enhancing factor
GER	Gastroesophageal reflux
GHD	Growth hormone deficiency
GIF	Glycosylation inhibiting factor
GINA	Global initiative for asthma
GLA	γ-Linolenic acid
GLUT-2	Glucose transporter-2 (protein)
GlyCAM-1	Glycosylation-dependent cell adhesion molecule 1
GM	Geometric mean
GM-CSF	Granulocyte macrophage-colony stimulating factor
GM-CSFR	Granulocyte macrophage-colony stimulating factor receptor
GMF	Genetically modified food
GMO	Genetically modified organism
GMP	Granule-associated membrane protein
GNA	*Galanthus nivalis* agglutinin
GPC	Giant papillary conjunctivitis
gps	Glycoproteins
GPM	Genetic polymorphism
GRO	Growth-related gene
GRP	Gastrin-releasing peptide
GS	Good Start
GTP	Guanosine triphosphate
GvHD	Graft-versus-host disease
H	Heavy
H	Humana HA
H/P	Hypolac/Profylac
HA	Hemoagglutination
HA	Hypoallergenic
HAART	Highly active antiretroviral therapy
HAV	Hepatitis A virus
HBV	Hepatitis B virus
H-CAM	Hematopoietic cell adhesion molecule
HC	Head circumference
HCC-1	Hemofiltrate CC chemokine-1
HCV	Hepatitis C virus
HDE	House dust endotoxin
HDM	House dust mite
HE	HIV-exposed
HEM	Heat escape method
HEP	Histamine equivalent potency
HEPA	High-efficiency particulate (or particle arresting) air (filter)
HET	Heterozygote, heterozygous
15-HETE	Hydroxyeicosatetraenoic acid
HEV	High endothelial venules
HF	Hydrolysate formula
HFI	Hydrofluorocarbon inhaler
HHM	Hypogammaglobulinemia with hyper-IgM
HHV	Human herpesvirus
5-HIAA	5-Hydroxyindoleacetic acid
HIES	Hyper IgE syndrome
HIgES	Hyper-IgE syndrome
HIgMS	Hyper-IgM syndrome
HIS	Hyper IgE syndrome
HIV	Human immunodeficiency virus
HIV-1 gp120	HIV-1 glycoprotein 120
HLA	Histocompatibility leukocyte antigens
HLA	Human leukocyte antigens
HML	Human mucosal lymphocytes
HMMBF	Home-made meat-based formula
HNF	Hepatocyte nuclear factor 1α, 4α
HFC	Hydrofluorocarbons
HPA	Hypothalamus-hypophysis-adrenal
HPLC	High-performance liquid chromatography
HR	Hazard risk
HR	(At) high risk (of atopy)
HR	Heart rate
HRF	Histamine release factor
HRF	Homologous restriction factor
HRF-P	Histamine release factor platelets
HRIF	Histamine release inhibition factor
HRQL	Health-related quality of life
HRP	Horseradish peroxidase
HSP	Heat shock proteins
HSCT	Hematopoietic stem cell transplantation
HSV	Herpes simplex virus
5-HT	5-Hydroxytryptamine
HTLV-I	Human T-cell leukemia virus
HVR	Hypervariable region
HZ	Homozygote, homozygous

I-309	I-309 protein
i-NANC	Inhibitory-NANC
I-TAC	Interferon inducible T-cell alpha chemoattractant
IA	Idiopathic anaphylaxis
Ia	I region-associated antigen
IAA	Insulin autoantibodies
IAC	Immunologically active casein levels
IAP	Inhibitors of apoptosis proteins
IAP	Integrin associated protein (CD47)
IAR	Immediate asthmatic reactions (see LAR)
IAW	Immunologically active whey protein levels
IB	Ipratropium bromide
IBD	Inflammatory bowel disease
IBE	Immunoreactive bacterial extracts
IBS	Irritable bowel syndrome
IC	Intracytoplasmatic
ICA	Islet-cell antibodies
ICAM-1 (CD54)	Intracellular adhesion molecule 1
ICAM-2 (CD102)	Intracellular adhesion molecule 2
ICAM-3 (CD50)	Intracellular adhesion molecule 3
ICAM-4 (CD242)	Intracellular adhesion molecule 4
ICD	International classification of diseases
ICD	Irritant contact dermatitis
ICDRG	International contact dermatitis research group
ICE	Interleukin $IL_1\beta$ converting enzyme
ICMA	Intracellular *Mycobacterium avium*
ICRM	Identifiable as casein raw material
ICS	Inhaled corticosteroids
ICT	Ice cube test
ICU	Intensive care unit
ID	Immune deficiency
ID	Intradermally
IDC	Interdigitating dendritic cells
IDDM	Insulin-dependent diabetes mellitus
IDV	Indinavir
IEF	Isoelectrofocalization
IEL	Intraepithelial lymphocytes
IF	Immunofluorescence
IFN	Interferon
IFR	Inspiratory flow rate
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgDs	Surface immunoglobulin D
IgE	Immunoglobulin E
IgE-BF	IgE binding factors
IgE-PF	IgE potentiating factor(s)
IgE-SF	IgE suppressive factor(s)
IgG	Immunoglobulin G
IgG-STS	IgG short time sensitization
Igs	Immunoglobulins
IgSC	Ig-secreting cells
IgSF	Immunoglobulin superfamily
IκB	Inhibitor of NF-κB
IκB-α	Inhibitor of NF-κB, type α
IKK	Inhibitor of B kinase
IL	Interleukin
IL_1RA	IL_1 receptor antagonist
IM	Intramuscular
IMN	Infectious mononucleosis
iNOS	Inducible NO synthase
IP-10	Inflammatory protein-10
IP-10	Interferon-inducible protein-10
IPD-1	Insulin promoter factor 1
IP3	Inositol-trisphosphate
IPPB	Intermittent positive pressure breathing
Ir	Immune response
IRAK	IL_1R-activating kinase
IRR	Incidence rate ratio
IRFI	Interferon regulatory factor-1
IRV	Inspiratory reserve volume
ISAAC	International Study of Asthma and Allergy in Children
ISP	Immature single positive
ISS	Immunostimulatory sequences
ITAM	Immunoreceptor tyrosine-based activation motif
IU	International Unit
IUIS	International Union of Immunological Societies
IV	Intravenous
IVAP	In vitro antibody production
IVIg	Intravenous immunoglobulins
J	Junction
JAK	Janus-family kinase
JCA	Juvenile chronic arthritis
JRA	Juvenile rheumatoid arthritis
JSC	Juvenile scleroderma
kb	Kilobase
kD	Kilodalton
KS	Kaposi's sarcoma
L	Light
LA	Linolenic acid
LAD	Leukocyte adhesion deficiency
LAD I	Leukocyte adhesion deficiency, type I
LAD II	Leukocyte adhesion deficiency, type II
LAD III	Leukocyte adhesion deficiency, type III
LAD IV	Leukocyte adhesion deficiency, type IV
LAD V	Leukocyte adhesion deficiency, type V
LAG-3	Lymphocyte activation gene-3
LAK	Lymphokine activated killer
LAM	Leukocyte adhesion molecule (CD62L)
LAMP	Lysosome-associated membrane protein
LAR	Late asthmatic reactions (see IAR)

LARC	Liver and activation-regulated chemokine
LBP	Lipopolysaccharide-binding protein
LBW	Low birth weight
LC	Langerhans cells
LC-SFA	Long-chain saturated fatty acids
LCA	Leukocyte common antigen (CD45)
LCAM	Liver cell adhesion molecule
LCP	Long-chain polyunsaturated fatty acids
LCP	Long-chain PUFA
LD	Lymphocyte-defined
LDH	Lactate-dehydrogenase
LDL	Low-density lipoprotein
LESN	Lupus erythematosus systemic, neonatal
LFA-1	Lymphocyte function-associated antigen-1 (CD11a/CD18)
LFA-2	Lymphocyte function-associated antigen-2 (CD2)
LFA-3	Lymphocyte function-associated antigen-3 (CD58)
LGL	Large granular lymphocytes
li	Invariant chain
LIF	Leukocyte-inhibiting factor
LIP	Lymphocyte (lymphoid) interstitial pneumonitis
LMI	Leukocyte migration inhibition
LMP	Low-molecular-weight polypeptide
LMPT	Lactulose mannitol permeability test
LMW	Low molecular weight
Lod	Logarithm of the odds
LPAM-1	Lymphocyte Peyer's patch HEV adhesion molecule 1
LPAM-2	Lymphocyte Peyer's patch HEV adhesion molecule 2
LPR	Late-phase reaction
LPS	Lipopolysaccharide
LR	(At) low risk (of atopy)
LRTI	Lower respiratory tract infection
LST	Long synthetic overlapping peptide
LST	Lymphocyte stimulation test
LT	Leukotriene
LTB_4	Leukotriene B_4
LTC_4	Leukotriene C_4
LTP	Lipid transfer protein
LTR	Long terminal repeats
LTT	Lymphocyte transformation test
LYST	Lysosomal trafficking
M	Microfold
mAb	Monoclonal antibodies
M-CSF	Monocyte/macrophage-colony stimulating factor
M-CSFR	Myeloid colony stimulating factor receptor
MAC	Membrane attack complex
MAC	Mid-arm circumference
Mac-1, -2	Macrophage-1 (-2) glycoprotein (CD11b/CD18)
MACIF	Membrane attack complex inhibitory factor (CD59)
MAD-2	Monocyte adhesion dependent protein-2
MAdCAM-1	Mucosal addressin cell adhesion molecule-1
MAG	Myelin associated glycoprotein
MALT	Mucosa-associated lymphoid tissue
MAMC	Mid-arm muscle circumference
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MAS	Macrophage activation syndrome
MASP	MBL-associated serine protease
MAST	Multiplied allergosorbent test
MBL	Mannose-binding lectin
MBP	Major basic protein
MBP	Mannose-binding protein
MBP	Myeline basic protein
MBT	Mercaptobenzothiazole
MCAF	Monocyte chemotactic and activating factor (MCP-1)
MCC	Mast cell chymase
MCD	Mad cow disease
MCP	Mast cell protease
MCP	Membrane cofactor protein (CD46)
MCP-1	Monocyte chemotactic protein-1
MCP-2	Monocyte chemotactic protein-2
MCP-3	Monocyte chemotactic protein-3
MCP-4	Monocyte chemotactic protein-4
MCP-5	Monocyte chemotactic protein-5
MCR	Monocyte complement receptor
MCS	Multiple chemical sensitivities
MCT	Medium-chain triglycerides
MDA-7	Melanoma differentiation-associated factor 7
MDC	Macrophage-derived chemokine
MDI	Metered-dose inhaler
MDV	Mean diurnal variation
ME	Middle ear
MEEs	Middle ear effusions
MEF	Mid-expiratory flow
MEF_{25-75}	Maximal expiratory flow at 25 %–75 % VC
MGF	Mast cell growth factor (SCF)
MGSA	Melanocyte growth stimulating activity
MHC	Major histocompatibility complex
MIF	(Monocyte) migration inhibiting factor
mig	Monokine inducible by IFN-γ
mIgD	Membrane IgD
mIgM	Membrane IgM
MIIC	MHC class II-loading compartment
MIP-1α	Macrophage inflammatory protein-1α
MIP-1β	Macrophage inflammatory protein-1β
MIP-2	Macrophage inflammatory protein-2
MIP-3α	Macrophage inflammatory protein-3α
MIP-3β	Macrophage inflammatory protein-3β
MIPF-1	Myeloid inhibitory factor-1
MIPF-2	Myeloid inhibitory factor-2
MLC	Mixed lymphocyte culture

MLR	Mixed lymphocyte reaction
MMEF	Maximal mid-expiratory flow
MMP	Matrix metalloproteinase
MMR	Measles, mumps and rubella (vaccine)
MMWR	Morbidity and Mortality Weekly Report
Mo	Molybdenum
MODY	Maturity-onset diabetes of the young
MP	Monopositive
MPO	Myeloperoxidase
MPS	Mononuclear phagocyte system
MR	Magnetic resonance
MR	Mannose receptor
mRAST	Modified RAST
MS	Multiple sclerosis
MSP-R	Macrophage-stimulating protein receptor
MT	Mantoux test
MUD	Matched unrelated donor
MVM	Microvillus membrane
MXT	Methotrexate
MyD88	Myeloid differentiation protein gene 88
MZ	Monozygous
N	Neutral
N	Nutramigen
N-CAM	Neural cell adhesion molecule
NA	Neutrophil antigen
NACDG	North American Contact Dermatitis Group
NADP	Nicotinamide-adenine dinucleotide phosphate
NADPH	Nicotinamide-adenine dinucleotide phosphate (reduced form)
NALT	Nasal-associated lymphoid tissue
NANC	Nonadrenergic noncholinergic
NAP-1	Neutrophil-activating factor-1
NAP-2	Neutrophil-activating factor-2
NARES	Nonallergic rhinitis, eosinophilic subgroup
NAT	Nucleic acid amplification technology
NBT	Nitroblue tetrazolium (test)
NC	Nucleocapsid
NCA	Neutrophil chemotactic activity
NCA	Non-cross-reactive antigen
NCAM	Neural adhesion molecule
NCF	Neutrophil chemotactic factor
NCF-A	Neutrophil chemotactic factor of anaphylaxis
NE	Norepinephrine
nef	Negative factor
NEMO	NF-κB essential modifier
NEP	Neutral endopeptidase
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor κB
NFV	Nelfinavir
NGF	Nerve growth factor
NGFR-SF	Nerve growth factor receptor superfamily
NHR	Nasal hyperreactivity
NI	Nidina HA
NIDDM	Non-insulin-dependent diabetes mellitus
NeuroD-1	Neurogenic differentiation factor 1
NK	Natural killer cells
NKA	Neurokinin A
NKAR	Natural killer-activating receptor
NKAT	Natural killer-associated transcripts
NKB	Neurokinin B
NKIR	Natural killer inhibitory receptor
NKR	Natural killer receptor
NKRP-1	Natural killer receptor P-1 (CD161)
NKSF	Natural killer cell stimulatory factor
NN	Neonatal neutrophils
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NO	Nitric monoxide
NO_2	Nitric dioxide
NOD	Nonobese diabetics
NOS	Nitric oxide synthase
NP	Nutrilon Pepti
NPP	Nutrilon Pepti Plus
NPT	Nasal provocation test
NPV	Negative predictive value
NPY	Neuropeptide tyrosine (Y)
NRL	Natural rubber latex
NRTI	Nucleoside reverse transcriptase inhibitor
NSAIDs	Nonsteroidal anti-inflammatory drugs
NT	Neurotensin
NVP	Nevirapine
NZB	New Zealand black
NZW	New Zealand white
$O_2^{\bullet-}$	Superoxide anion
O_3	ozone
OAS	Oral allergy syndrome
OCT	Oral challenge test
ODN	Oligodeoxynucleotides
OFC	Open food challenge
OME	Otitis media with effusion
q.i.d.	*Quarter in die*, four times a day
ORL	Otorhinolaryngologist
OVA	Ovalbumin
P	Pregestimil
P	Properdin
P+P	Prick by prick
p-ANCA	Perinuclear anti-neutrophil circulating antibodies
p150,95	CD11c/CD18
PA	Pseudoallergic, pseudoallergy
PAA	Proteins with anti-infective activity
PABA	*P*-aminobenzoic acid
PAC	Perennial allergic conjunctivitis
PACGT	Pediatric AIDS Clinical Trials Group

$PaCO_2$	Partial pressure of CO_2 in arterial blood
PAF	Platelet-activating factor
PALS	Periarteriolar lymphocyte sheath
PAN	Periarteritis nodosa
PaO_2	Partial pressure of O_2 in arterial blood
PAR	Perennial allergic rhinitis
PARC	Pulmonary and activation-regulated chemokine
PAS	*Para*-aminosalicylic (acid)
PBB	Polychlorinated biphenyl compounds
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells
PBP	Platelet basic protein
PC	Particle counter
PC	*Pneumocystis carinii*
PC_{20}	Methacholine/histamine provocative concentration causing a fall in FEV_1 of 20%
PCB	Polychlorinated biphenyls
PCD	Programmed cell death
PCD	Protein contact dermatitis
pCi	PicoCurie
PCIIINP	Amino terminal propeptide of type III procollagen
PCIP	Carboxy terminal propeptide of type I procollagen
PCP	Personalized care project
PCP	*Pneumocystis carinii* pneumonia
PCR	Polymerase chain reaction
PD_{20}	Provocation dose 20
PDE	Phosphodiesterase
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor (CD140)
PE	Progressive encephalopathy
PECAM 1	Platelet endothelial cell adhesion molecule (CD31)
PEF	Peak expiratory flow
PEFR	Peak expiratory flow rate
PEFV	Partial expiratory flow volume (curve)
PEG	Polyethylene glycol
PEM	Protein-energy malnutrition
PENTA	Pediatric European Network for Treatment of AIDS
PF	Perch fish
PF4	Platelet factor 4
PFC	Plaque-forming cells
PFM	Peak flow meter
PFT	Pulmonary function testing
PG	Polygalacturonase
PG	Prostaglandin
PGD	Prostaglandin D
PGE	Prostaglandin E
PGF	Prostaglandin F
PGL	Persistent generalized lymphadenopathy
PGP9.5	Neuron-specific protein 9.5
PH	Prophylac/Hypolac
PHA	Phytohemagglutinin
PHI	Peptide histidine-isoleucine
PHM	Peptide histidine-methionine
phox	Phagocyte oxidase
PHV	Peptide histidine-valine
PI 3K	Phosphatidylinositol-3-kinase
PID	Primary immune deficiency
pIgA	Polymeric IgA
pIgR	Polymeric Ig receptor
PIP2	Phosphatidylinositol-bisphosphate
PJ	PeptiJunior
PKA	Protein kinase A
PKC	Protein kinase C
PL	Phospholipase
PLA	Phospholipase A
PLC	Phospholipase C
PLCγ1	Phospholipase Cγ1
PLCγ2	Phospholipase Cγ2
PLD	Phospholipase D
PLH	Pulmonary lymphoid hyperplasia
PLP	Proteolipid protein
PM_{10}	Particulate matters <10 µm
PMA	Phorbol myristate acetate
pMDI	Pressurized metered dose inhaler
PMN	Polymorphonuclear (leukocytes)
PNM	Polynucleated neutrophils
PNP	Purine nucleoside phosphorylase
PNU	Protein nitrogen units
pol	Polymerase
poly	Polyarticular
POP	Persistent organic pollutants
PP	Peyer's patches
ppb	Parts per billion
PPD	Purified protein derivative
PPDA	Paraphenylenediamine
ppm	Parts per million
PPV	Positive predictive value
PR	Pregomin
PR	Protease
PR	Protein related to pathogenesis
PR3	Proteinase 3
prn	*pro re nata*, if required
PRIST	Paper radioimmunosorbent test
PrP	Prion protein
PRU	Phadebas RAST unit
PS	Polysaccharides
PSA	Polysaccharide antigen
PSGL-1	P-selectin glycoprotein ligand 1 (CD162)
PT	Patch test(ing)
PT	Provocation testing
PTF	Patch test with foods
PTK	Protein tyrosine kinase
PTP	Protein tyrosine phosphatase
PUFA	Polyunsaturated fatty acids
PUVA	Psoralen ultraviolet A
PV	Pulmonary volume
PVR	Polio virus receptor (CD155)
PWHF	Partial whey hydrolysate formula
PWM	Pokeweed mitogen

RA	Recombinant allergens
RA	Rheumatoid arthritis
RAG	Rice allergen
RAG-1 and -2	Recombination-activating gene-1 and -2
RANTES	Regulated on activation normal T expressed and secreted
RAP	Rapamycin
RAP	Rice allergenic protein
RAST	Radio allergosorbent test
Raw	Airway resistance
RBA	Radio-binding assay
RBC	Red blood cell
RCA	Regulator of complement activation
RDA	Recommended daily allowance
RES	Reticuloendothelial system
REV	Rev responsive element
rev	Regulator of viral expression
RF	Rheumatoid factor
RFLP	Restriction fragment length polymorphism
RFX5	Regulatory factor X5
RGS	Regulators of G protein-signaling
RH	Relative hazard
rHuG-CSF	Recombinant human G-CSF
RIA	Radioimmunological assay
Rint	Interrupter resistance
RLS	Restless legs syndrome
ROC	Receptor-operated channels
RP	Ratio of proportion
RR	Relative risk
RR	Respiratory rate
RRI	Recurrent respiratory infections
RSR	Respiratory system resistance
RSS	Conserved recombination signal sequences
RSV	Respiratory syncytial virus
RSV-IVIg	(Anti)respiratory syncytial virus-intravenous immunoglobulins
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase/polymerase chain reaction
RTC	Rapid thoracoabdominal compression
RTV	Ritonavir
Rx	Radiographic
SA	Superantigen
SAA	Serum amyloid A protein
SAC	Seasonal allergic conjunctivitis
SAFT	Skin application food test
SAH	*S*-adenosylhomocysteine, *S*-adenosylhomocysteine
SALT	Skin-associated lymphoid tissue
SaO_2	Oxygen saturation
SAP	Serum amyloid P component
SAR	Seasonal allergic rhinitis
SARAH	Skin activity reference allergen/ histamine
SARS	Severe acute respiratory syndrome
SBHR	Spontaneous basophil histamine release
SC	Secretory component
SC	Subcutaneous
SC-SFA	Short-chain saturated fatty acids
SCF	Stem cell factor
SCID	Severe combined immunodeficiency
SCN	Severe congenital neutropenia
SCORAD	Scoring of atopic dermatitis
SCT	Stem-cell transplantation
SCY	Small secreted cytokine
SD	Serologically defined (antigens)
SD	Standard deviation
SDF	Stromal cell derived factor
SDS	Standard deviation score
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SE	Staphylococcal enterotoxin
Se	Selenium
SEA	Staphylococcal enterotoxin A
SEB	Staphylococcal enterotoxin B
SEC	Staphylococcal enterotoxin C
SED	Staphylococcal enterotoxin D
SEE	Staphylococcal enterotoxin E
SEM	Standard error of the mean
SFA	Suppressive factor of allergy
SGOT	Serum glutamic-oxaloacetic transaminase
SH2, 2, 3	Src homology 2, 3
SH2DIA	SH2 domain containing gene 1A
SHS	Schönlein-Henoch syndrome
SI	Système International des Unités
SIAIC	Società Italiana di Allergologia e Immunologia Clinica
SIDS	Sudden infant death syndrome
sIgA	Secretory IgA
SIgAD	Selective IgA defect
sIgE	Specific IgE (food-specific)
sIgG	Specific IgG
sIgM	Secretory IgM
SIS	Skin immune system
SIT	Specific immunotherapy
SIV	Simian immunodeficiency virus
SJS	Stevens-Johnson syndrome
SL	Synovial liquid
SLAM	Signaling lymphocyte activation molecule
SLC	Secondary lymphoid tissue chemokine
SLE	Systemic lupus erythematosus
sLe^x	Sialyl-Lewis x (CD15s)
SLIT	Sublingual immunotherapy
SMOC	Second messenger-operated channels
SO_2	S dioxide
SOD	Superoxide dismutase
SOM	Somatostatin
SP	Single positive
SP	Substance P
SP-A	Surfactant protein A
SP-D	Surfactant protein D

SPA	*Staphylococcus aureus* protein A
SPB	Solid-phase binding
SPEA	Staphylococcal pyrogenic exotoxin A
SPEB	Staphylococcal pyrogenic exotoxin B
SPF	Soy protein formula
SPT	Skin prick test
SQ	Standardized quality unit
SQV	Saquinavir
sRaw	Specific airway resistance
SRBC	Sheep red blood cell
SRCR-SF	Scavenger receptor cysteine-rich superfamily
SSSS	Staphylococcal scalded skin syndrome
Stat	Signal transducers and activators of transcriptions
STCP-1	Stimulated T cell chemotactic protein-1
STS-IgG	Short-term sensitizing IgG
SV	Simian vacuolating (virus)
syk	Spleen tyrosine kinase
T	Tryptase-containing mast cells
T-bet	Transcription factors T-box expressed in T cells
TACTILE	T cell activation increased late expression
TAI	Transient autoimmunity
TAME	Tosylarginine methylester
TAP-1	Transporter associated with antigen presentation 1
TAP-2	Transporter associated with antigen presentation 2
TAPA-1	Target of an antiproliferative antibody 1
Tapr	T cell and airway phenotype regulator
TAR	Transactivation responsive sequence
TARC	Thymus and activation-regulated chemokine
tat	*Trans*-activant
TB	Tubercular
TC	Tryptase and chymase-containing mast cells
TCA	Trichloroacetic acid
TCA3	T cell activation gene 3
TCC	T cell clones
TCDD	Tetrachlorodibenzo-*p*-dioxin
TCL	T cell line
TcR	T cell receptor
TdT	Terminal-deoxynucleotidyl-transferase
TECK	Thymus-expressed chemokine
TEN	Toxic epidermal necrolysis
TF	Transcription factor
TGF	Transforming growth factor
TGFR	Transforming growth factor receptor
TGV	Thoracic gas volume
Th	T helper
ThP	Th precursors
THI	Transient hypogammaglobulinemia of infancy
TIF	(IL_{10}-related) T-cell derived inducible factor
TIM	T-cell immunoglobulin and mucin (domain)
TLC	Total lung capacity
TLR	Toll-like receptor
TM	Transmembrane
TM4-SF	Transmembrane 4 superfamily
Tme/Te	Percentage of expiratory time to reach peak tidal flow
TMJ	Temporomandibular joint
TN	Triple negative
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNFRSF	Tumor necrosis factor superfamily receptor
TNFRSF6	Tumor necrosis factor receptor superfamily receptor 6
Torr	Torricelli (1 Torr = 1 mmHg)
TPN	Total parenteral nutrition
Tr1	T-regulatory 1
TRADD	TNFR-1-associated death domain
TRUE	Thin-layer, rapid use epicutaneous (test)
Ts	T suppressor
TSP-1	Thrombospondin-1
TSP	Total suspended matter
TSST-1	Toxic shock syndrome toxin-1
tTG	Tissue transglutaminase
TV	Tidal volume
TVP	Tensor veli palatini
TVP	Textured vegetable proteins
TX	Thromboxane
TXA_2	Thromboxane A_2
UC	Ulcerative colitis
UCBT	Umbilical cord blood transplantation
UHT	Ultra-high temperature
UPA-R	Urokinase plasminogen activator receptor
URTI	Upper respiratory tract infection
UV	Ultraviolet
V	Variable
V	Vivena HA
VAP-1	Vascular adhesion protein
VC	Vital capacity
VCAM-1	Vascular cell adhesion molecule (CD106)
vCJD	Variant Creutzfeldt-Jakob Disease
VI	(HIV) virus-infected
vif	Viral infective factor
VIP	Vasoactive intestinal peptide
VKC	Vernal keratoconjunctivitis
VLA	Very late antigens
VNR	Vitronectin receptor
VOC	Volatile organic compounds
VOC	Voltage-operated channels
vpr	Viral protein R

vpu	Viral protein U
vpx	Viral protein X
VRI	Viral respiratory infection
VSV	Vesicular stomatitis virus
vWF	Von Willebrand factor
VZV	Varicella zoster virus
WARI	Wheezing associated respiratory infections
WAS	Wiskott-Aldrich syndrome
WASp	Wiskott-Aldrich syndrome protein
WB	Western blot
WCC	White-cell count
WGAA	Wheat germ agglutinin antibodies β
WHF	Whey hydrolyzed formula
WHO	Word Health Organization
WHO/IUIS	Word Health Organization/International Union of Immunological Societies
XL	X linked
XHIMS	X-recessive hyper-IgM syndrome
XLA	X-linked agammaglobulinemia
XLP	X-linked lymphoproliferative syndrome
XLT	X-linked thrombocytopenia
ZAP-70	Zeta-associated protein 70
ZDV	Zidovudine
Zn	Zinc

Immunology

Historical Milestones

The concept of forbidden foods that should not be eaten goes back to the Garden of Eden and apart from its religious meanings it may also have foreshadowed the concept of foods that can provoke adverse reactions. Thus we could say that allergic diseases have plagued mankind since the beginning of life on earth. The prophet Job was affected by a condition that following the rare symptoms described by the Holy Bible might be identified as a severe form of atopic dermatitis (AD). The earliest record of an apparently allergic reaction is 2621 B.C., when death from stinging insects was first described by hieroglyphics carved into the walls of the tomb of Pharaoh Menes depicting his death following the sting of a wasp. In 79 A.D., the death of the Roman admiral Pliny the Elder was ascribed to the SO_2-rich gases emanating from the eruption of Mount Vesuvius. Hippocrates (460–377 B.C.) was probably the first to describe how cow's milk (CM) could cause gastric upset and hives, proposing dietetic measures including both treatment and prevention for CM allergy. He also coined the term "ασθμα," meaning breathlessness. Subsequently, Thucydides described that during the plague afflicting Athens from 430 to 429 B.C., "those people who recovered from the disease rarely developed the illness a second time and never mortally," an observation that was verified by Panum in 1847 [407]. The Roman poet Lucretius (98–55 B.C.) stated that "what is food to one, is a bitter poison to others." Galen (129–202) was the first to describe allergy to goat's milk and also evidenced the fifth cardinal sign of inflammation, that is the loss of function or *functio laesa* not previously described by Celsus (first century B.C.). Perhaps an example of tolerance could be that of Mithridates (132–63 B.C.), who reportedly acquired immunity against poisons by assuming progressively increasing doses of each poison, a precursor of the oral desensitization by taking small incremental amounts of noxious foods. The concept of immunity, deriving from the Latin *immunitas* (meaning being exempt from), a century ago was linked by early immunologists to the resistance of an individual to infections; therefore immunology probably began its march as acquired immunity [33]. The concepts of immunology have a long history and began primarily as a branch of microbiology. Fracastoro wrote in *De contagione et contagiosis morbis*, published in 1610, that "an infection is the same in both the carrier and newly infected" and postulated the existence of "imperceptible" germs. Since then, several important discoveries have launched a renaissance of research into the field of immunology. Experimental immunology began in 1798 with Jenner [237], Pasteur developed killed and attenuated vaccines, and Miescher later discovered DNA (deoxyribonucleic acid) [33]. In 1875 Cantani Sr stated that the cause of diabetes mellitus was to be sought in a missing *ferment* which in the healthy metabolized glucose. He also demonstrated that dehydration should be cured by *fluid rehydration*. In 1890, Ehrlich expressed the concept of autoimmunity as horror *autotoxicus* [137], amply skimming the etiopathogenetic mechanisms. At the turn of the century, von Pirquet coined the term "allergy" from "αλλος" and "εργος" (meaning altered reaction) [611] and therefore included the development of protective immunity [33], coupled by Coca and Cooke in 1923 with the term "atopy," from "ατοπος" (meaning out of place and thus abnormal) [88]; thus many episodes based on the mechanisms of cause and effect were brilliantly documented [33]. In 1921, Prausnitz and Küstner demonstrated the presence of "reagins" in the serum of allergic patients [436]; in the same year a "Textbook of Immunology" was first published in Italy [75]. In 1966, the Ishizakas attributed a scientific meaning to reagins by identifying IgE as the carrier of reaginic activity in the sera of hay fever sufferers [228]: the first case of immunodeficiency (ID) was reported in 1952 (Chap. 22).

The Immune System

Definitions. The *immune system* was presumably evolved by animals during evolution as a means of self-preservation in a world teeming with microorganisms. It is not selective and immune responses ensue against foreign substances regardless of whether they are bacterial products or not. Therefore the immune system has become exquisitely specialized and highly complex, to protect the host from potentially noxious environmental agents (antigens). The introduction of foreign substances into the host may have an adverse effect on a variety of cells; hence this system synthesizes highly specialized molecules (antibodies), also generating selected cells,

called cytokines, adhesion molecules, chemokines, and the like. Related cells and cell products are consequently a defense system designed to interact with foreign agents to protect the host from any external injury [34].

- *Immunity* is the complex of cellular and/or humoral events following the entry of foreign substances (non-self) into the host. These events are overall free of adverse effects for the majority of subjects, therefore defined as normal.
- *Immunology* is the study of the ways used by the host to maintain homeostasis in the internal environment when confronted with non-self.
- *Allergy* currently implies all forms of hypersensitivity with detrimental consequences for the host and type I, IgE-mediated or cell-mediated reactions.
- *Atopy* is a term underlining the personal or familial hereditary aspects of allergic reactions and is associated usually in childhood or adolescence with increased production of IgE antibodies and/or altered specific reactivity in response to ongoing exposures to allergens, usually proteins.

A thoroughly functioning immune system serves three main functions: (a) defense against invasion of microorganisms and foreign substances, ingested, inhaled or achieved by mucosal contact, or by parenteral injection; (b) homeostasis fulfilling universal requirements of multicellular organisms to preserve uniformity of a given cell type, as well removing worn-out "self" components; and (c) surveillance devoted to perception and destruction of mutant cells [33]. Therefore, inability to relate to *unum* is intrinsic to atopic diseases that depend on hyperproduction of IgE antibodies being IgE-mediated. This is a genetic characteristic, although the mode of transmission is still a matter of debate, and the fact of being atopic does not result automatically in the development of clinical manifestations. Atopy is also polymorphous, manifesting in various forms, starting out as AD or food allergy (FA), to further develop into allergic rhinitis (AR) and/or asthma.

According to the classic theory of clonal selection (Chap. 2), the crucial function of the immune system from the first days of life is to distinguish self from non-self, for example, between what belongs to or is closely correlated to the organism (tolerance to self antigens) and what is foreign, with the goal of eliminating the latter, independent of its potential pathogenicity. This is the quintessential dichotomy of immunology: the *self versus non-self discrimination*. We shall see in Chap. 2 that theories regarding self/non-self are still valid (as long as they are not interpreted literally), with the exception of some recent studies that have clarified how the so-called *neonatal window* period represents an ontogenetic window only as far as tolerance induction is concerned [60]. Immunity is acquired at the first contact with non-self (*antigen recognition*), it is *specific* for a given foreign substance and *acquired,* since it is able to respond to molecules not encountered before. Following an initial antigen stimulus, finally familiar with self and non-self, a series of reactions results, sometimes as if it were reacting to invaders. In virtue of such events, the integrity of the organism, which becomes protected from successive entries of potentially pathogenic substances, is maintained. This is put into practice by the immune memory enabling the immune system to memorize previous exposures to a particular antigen, in a way analogous to natural memory that recalls past experiences. Amongst all defense mechanisms put into action by the host against any external aggressor, we find a network of organs, tissues, cells and molecules responsible for immunity: the *integrated immune system* [36]. The interconnected and coordinated responses following the entry of foreign substances are globally known as *immune reactions.* Relatively small modifications in the delicate and complex molecular and cellular structures of the immune system may cause a functional disequilibrium that is responsible for a cascade of organic perturbations that may become evident alterations, such as atopic diseases varying in nature and severity and primary immune deficiency (PID) [33].

Ideally, the immune reactions determine schematically ordered responses articulated on six levels: processing, presentation and recognition of non-self, cellular activation, elaboration of biologically active chemical substances, mediators, and cellular cooperation. The final outcome of the encounter between host and a foreign invader is now recognized as dependent upon an integrated network of multidirectional communication pathways and signaling, where the mechanisms of memory, effector responses and consequent regulation of secretory proteins and soluble molecules such as the interleukins (ILs) are clarified [34]. Antigen contact starts to form those elements necessary for its recognition, including the cells emitting signals, ILs transducing such signals and target cells receiving them by binding to appropriate receptors; following transduction and amplification of signaling, a detectable physiological response occurs [34]. Thus, immune responses usually culminate in the elimination of provoking agents. The specificity of antigen recognition, as defined in molecular terms, is entrusted to three structures: the variable (V) region of Igs (immunoglobulins), MHC (major histocompatibility complex) V regions and TcR (T-cell receptor) chains. A fourth component of molecular recognition, the cluster of differentiation (CD) markers, differentiates subsets of T from B lymphocytes (from *lympha*, water) and other cell populations [34].

Systems of Immunity

Two general systems of immunity with specialized roles in defending against infection have been selected during evolution [114]:

- *Innate immunity,* an ancient form of host defense, also called natural or congenital immunity, is an attribute of

Table 1.1. Comparison of the innate and acquired immune systems

Properties	Innate immune system	Acquired immune system
Cellular components	Macrophages, PMN, eosinophils, NK cells, DCs, IFN-producing cells, γδ T cells, $CD8^+$ T cells	T and B lymphocytes, macrophages, DCs, $CD4^+$ T cells, $CD8^+$ T cells
Soluble components	Enzymes (lysozyme, complement, etc.), acute-phase proteins, interferon, collectins, defensins, chemokines	Antibodies, interleukins
Memory	No	Yes
Physical barriers	Skin and mucous membranes	None
Recognition	++	+
Self–non-self discrimination	Yes	Yes
Specificity	No	Yes
Speed	tast	slow

Modified from references [115, 184, 537].

every living organism, present at birth, that is, before exposure to foreign agents and consisting of several non-specific factors. Innate responses occur to the same extent as the infectious agent is encountered. Not significantly modified after an encounter with non-self substances, it is void of both a fine-tuned discrimination of such substances and an increased activity following repeated encounters, thereby demonstrating that it does not possess memory. The phagocyte cells (macrophages, neutrophils and monocytes) and alternative complement pathway, although void of specificity, are essential as primary elements of defense against a large number of infectious agents.

- *Acquired immunity,* also called adaptive immunity, becomes involved when the first level of defense fails to fully prevent infection, exemplifying a recent evolutive process, distinct by a particular specificity for offending antigens and by memory. Unlike innate immunity, it is elicited or stimulated by exposures to intruders that escaped early elimination by the innate immune system, insofar as it is armed with a versatile discriminating capacity and potentiated by a successive encounter with such agents.

Table 1.1 [114, 184] shows the major differences between these two types of immunity: the effector mechanisms of innate immunity are activated immediately after infection and rapidly control the replication of infecting pathogens, so the infection is restrained until lymphocytes can accomplish their action. It takes 3–5 days for a sufficient number of clones to be produced and differentiated into effector cells, which allows time for pathogens to damage the host [353]. The greatest difference is that acquired immunity, to compete with genetic variability of microorganisms, has lost the cardinal characteristic seen in innate immunity, that is, the ability to distinguish between potential pathogens and harmless substances. However, innate immunity may have an additional role in determining which antigens the acquired immune system responds to and the nature of that response [148].

Acquired Immunity

As Table 1.1 shows, acquired immunity involves T and B lymphocytes, antibodies and ILs, distinguished schematically into humoral and cell-mediated immunity (CMI), each equipped with various functions, partly different and partly overlapping. Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Specialized cells, the antigen-presenting cells (APCs), display the antigen to lymphocytes and collaborate with them in the response to the antigen [537].

Humoral-mediated immunity, above all responsible for primary defense against bacterial infections, is passively transferable by serum or plasma, being mediated by antibodies with a specific aptitude for reacting with the configurations responsible for its production, which is typical of B lymphocytes, which in humans differentiate in mammalian bursal equivalent tissue and acquire features of B cells from plasma cells with an endoplasmic reticulum (ER) characterized by an abundant RNA (ribonucleic acid). Specific antibodies are responsible for the reactions of immediate hypersensitivity, cytotoxicity, Arthus reaction and, by means of Fc receptors, phagocytosis.

CMI, as well as being active in the defense against viral infections, is implicated in some cases of autoimmune disease and is characteristically associated with effector–target cell interactions involved in antimicrobial immunity, rejection of allografts, immune surveillance and rejection of tumor cells. This specific immunity is transferable by lymphoid cells and not by serum, where T lymphocytes play a fundamental role [481].

Delayed-type hypersensitivity (DTH) is a typical CMI reaction [33], first discovered by Jenner [237].

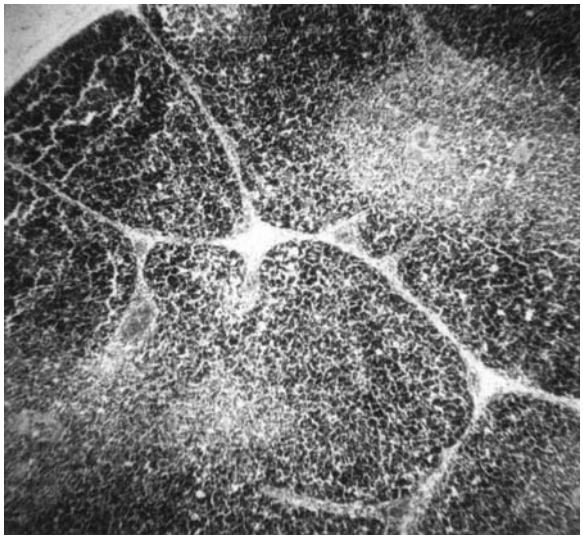
Organs and Cells of the Immune System

Several organs and tissues participate in the host defense and are classified into [33, 36, 47]:

- *Primary lymphoid organs* (forming the central lymphoid organs) where both T and B lymphocytes mature into antigen-recognizing cells and developing lymphocytes acquire antigen specific receptors. These cells develop from pluripotent stem cells (SC) *in bone marrow and only during fetal life in the liver* and then circulate throughout the extracellular fluid. B cells reach maturity within the bone marrow, but T cells must travel to the thymus to complete their development. An important and key role in homing-related responses and in the regulation of cell trafficking of stem/progenitor cells (SPC) is played by SDF-1 (stromal cell derived factor-1) bound to G-protein-coupled CXCR4 (chemokine receptor4). Supernatants of leukapheresis products activate SDF-dependent actin polymerization and significantly enhance the homing of human cord blood (CB) and bone marrow-derived CD34 cells in a NOD (nucleotide-binding oligomerization domain)/SCID mouse (severe combined immunodeficiency (ID). CXCR4 plays a critical role in the trafficing of other tissue/organ specific SPCs expressing CXCR4 on their surface, such as during embryo/organogenesis and tissue/organ regeneration [277, 654]. They are represented in mammals by the thymus, bone marrow and only during fetal life by the liver.
- *Secondary lymphoid organs* (forming the peripheral lymphoid organs) including lymph nodes, spleen, tonsils and linings of the digestive, respiratory and genitourinary tracts, skin, conjunctiva and salivary glands. These organs of different size, disseminated throughout the organism, build structures where antigen-driven proliferation and differentiation occur and lymphocytes circulate and recirculate. There T cells interact with foreign configurations by cytotoxicity or releasing nonspecific mediators, with the non-complementary help of ILs, and store, amplify, and disseminate information about macromolecules encountered in various parts of the body [33].

The primary lymphoid organs include the thymus and the bone marrow.

The *thymus* is a lymphoepithelial organ located in the anterosuperior region of mediastinum deriving from the endoderm of the third and fourth pharyngeal pouches. In the 6th week of fetal life, primitive mesenchymal and neural crest cells seed epithelial structures. Remarkably, parathyroids develop about the same time as the same pouches. During fetal development, the thymus size increases, then reaches its greatest relative weight shortly after birth, and its greatest absolute weight at puberty when contrary to other lymphoid organs, it progressively involutes and is replaced by adipose tissue. However, even if lymphocyte numbers progressively decrease, they do not disappear altogether. Apparently, a few fragments are adequate to ensure a compatible T cellularity in peripheral lymphoid tissues. By 8 weeks of gestation, $CD7^+$ hematopoietic SCs migrate from the yolk sac and fetal liver and later from the bone marrow via the bloodstream and enter the thymus through the epithelial cell linings of the cortex. The hematopoietic SC activity is regulated by C/EBPα (CCAAT-enhancer binding protein α) [679]. It has not yet been clarified whether these traveling stem cells are already committed to differentiate along the T-cell (T = thymus-derived) lineage or become committed only after entering the thymus and what excites them to such a gland. It has been hypothesized that these precursors may express certain as yet unidentified surface markers that selectively bind to their corresponding ligands on the thymic vascular endothelial cells. For this reason, the thymus is the central organ where the precursors, bone marrow-derived prothymocytes, undergo intense proliferation and differentiation, as well as do macrophages, epithelial and dendritic cells (DCs) [454]. The gland consists of two lobes surrounded by a thin capsule of connective tissue extending into the lobe, thus forming septa, partially dividing parenchyma into lobules (Fig. 1.1, a) [454]. The peripheral zone of each lobule forms the cortex with immature proliferating cells. The cortex is further subdivided into a superficial and a deeper zone, with a more central area forming the medulla containing more mature cells and Hassall's corpuscles, an aggregate of epithelial cells. While the thymus has a cortex and a medulla, there is no germinal center (GC), or plasma cells, as in normal situations [33]. The thymus, independent of antigen stimulation, may be viewed as a lymphoid organ controlling all peripheral lymphoid organs, by means of expanding immunocompetence of the lymphocytes associated with CMI. The thymus exercises its function in time by secreting several soluble hormones (thymosin, thymopoietin, etc.): in fact, epithelial thymic cells secrete a series of polypeptides (thymic hormones and ILs), contributing to the maturation of T lymphocytes, with which they can interact directly, influencing their differentiation [33]. During fetal cell development, the requirement for STAT5 (signal transducers and activators of transcriptions) in thymopoiesis is developmental stage specific. STAT5 is required for IL_7R-regulated TCR gene transcription, but it is not necessarily essential for TCR gene rearrangement, denoting that factors other than STAT5 activated by the IL_7R-JAK pathway control TCR locus accessibility to VDJ recombinase [246]. One of the major roles of STAT3 in the G-CSF signaling pathway is to augment the function of C/EBPα (CCAAT enhancer binding protein-α), which is essential for myeloid differentiation. Moreover, co-operation of C/EBPα with other STAT3-activated proteins is required for the induction of some G-CSF responsive genes [386]. The close connection between epithelial cells and lymphocytes is confirmed by the presence of large epithelial cells (*nurse cells*) in the superficial cortex, each containing 20–30 blasts in



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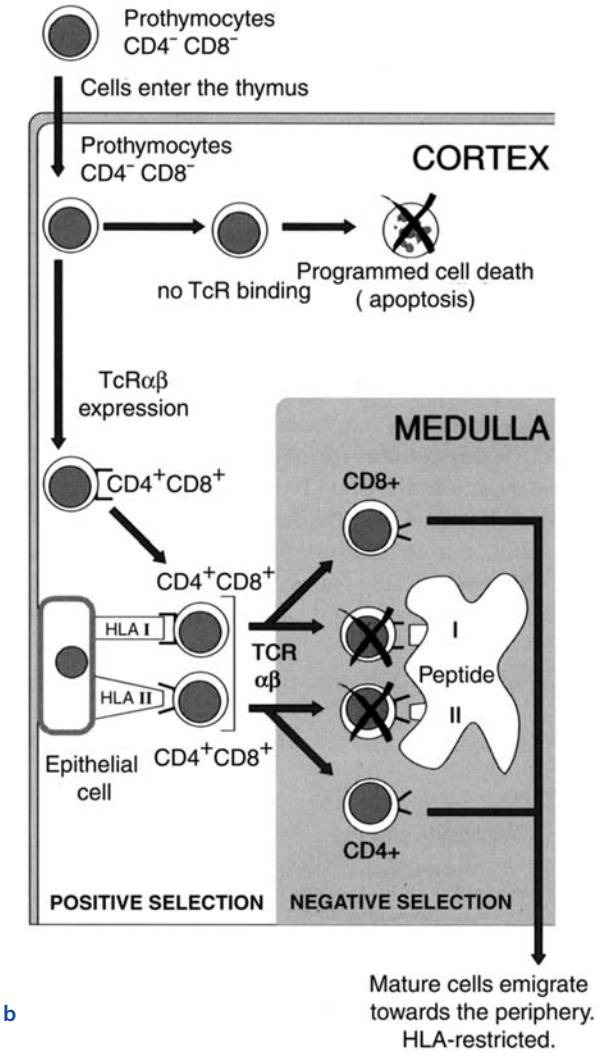


b

Fig.1.1. **a** The thymus of a normal newborn. Connective tissue septa, traversed by large blood vessels, divide the thymus into lobules, the very dense peripheral cortex is clearly distinguished from the more central, less dense medulla. **b** Positive and negative selection in the thymus

division. Prothymocytes (pro-T) express CD2 and CD7, immature T cells of the cortex, smaller (pre-T), such as CD1 and once migrated into the medulla, CD3, CD4, CD8, CD38 and CD71 (transferrin receptor) present not only on T cells, but also on cells of other lineages undergoing proliferation. A low number of $CD4^+8^+$ (DP, double positive) T cells located in the medulla learn how to discriminate self from non-self in a process known as education; they interact with stem cells expressing MHC molecules, then $CD4^-8^-$ (DN, double negative) T cells acquire a $CD4^+8^-$ or $CD4^-8^+$ (MP, mono-positive) phenotype. Targeted mutations of CD80 and CD86 substantially reduce the proliferation and survival of DN T cells in the thymus, since the DN development in the thymus is subject to modulation by the CD80–CD28 costimulatory pathway [682]. The earliest immune maturation of any lymphocyte compartment in humans is represented by αβ and γδ TcR and most likely reflects the importance of these cells in controlling pathology due to common environmental challenges. A significant difference between the γδ and αβ T cell lineages is the much earlier activation and conversion to memory of the γδ T cells, which illustrates the central role that γδ T cells have in adressing Ag challenge from birth onward. γδ T cells are distinguished by expression of Vδ1 vs Vδ2 δ-chains. The majority of Vδ2 cells display signs of early activation in neonates. Even in infants <1 year most of Vδ2 nonnaive cells stain for perforin and produce IFN-γ after short-term stimulation, yet nearly all naive Vδ2 cells disappear from blood by 1 year of life. Vδ1 cells predominate during fetal and early life, but represent the minority of γδ cells in healthy adolescents [111]. As regards TcR, only cells with an αβTcR-CD4/CD8 coreceptor combination that is able to bind the same MHC class I or class II molecules will fully mature. Cells with a mismatched combination will die [263]. Positive selection is conditioned by activation of the MAPK (mitogen-activated protein kinase) cascade, although it does not involve $CD4^-8^-$ (DN) T cells [10]. Most lymphocytes not surviving the thymic selection process die by apoptosis (from "απωπτοσις," cellular suicide): they are engulfed and digested by macrophages of the corticomedullary junction within 3–4 days of their last cell division [192] (Fig. 1.1 b). In the deep cortex, T cells interact with macrophages, FCDs and epithelial cells, which during a differentiation process modify expression of cytokeratins such as skin keratinocytes; medullary T cells are found in Hassall's corpuscles [225]. Several immune deficiencies are characterized by selective deficiency of T cells [33].

The *bone marrow* is a structure present in man and in higher mammals considered to be a primary lymphoid organ functionally equivalent to avian bursa. The location of pluripotent hemopoietic stem cells eventually differentiating into stromal cells guides hemopoiesis both by direct cellular contacts with developing lymphocytes (precursors) and by other blood cells, also under the control of growth factors. Many ILs are involved

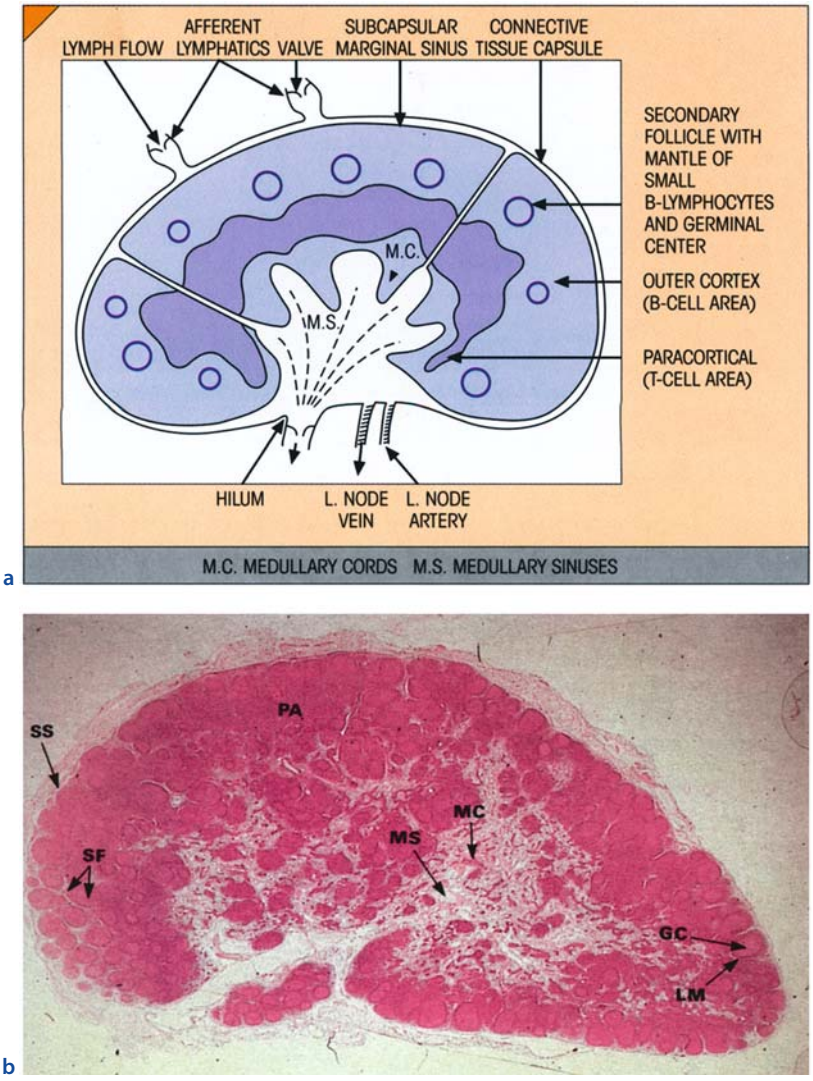
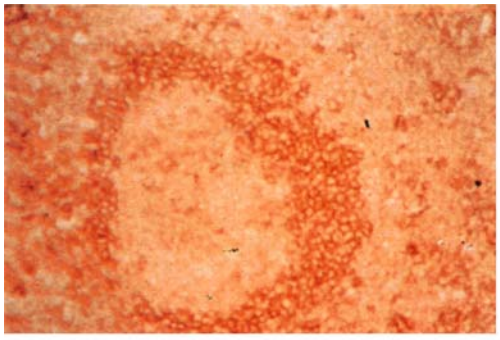


Fig.1.2. **a** Schematic structure of human lymph node. **b** Human lymph node (low magnification). **c** Secondary lymphoid follicle showing GC surrounded by a mantle of B lymphocytes. In the center there are few IgD-positive cells; both areas contain IgM-positive B cells. Lymphocyte mantle stained with anti-human IgD antibody. **d** Section of lymph node medulla stained in methyl green (DNA)/pyronin (RNA) to show the basophilic (*pink*) cytoplasm of plasma cells with abundant ribosomes. **e** Section of lymph node medulla showing macrophages lining the medullary sinus following the uptake of red dye. **f** Lymph node of a mouse immunized with pneumococcal polysaccharide antigen, thymus-independent, revealing a prominent stimulation of SFs with GC. **g** Green stain (methyl/pyronin) of a lymph node draining site of skin stained with the contact sensitizer oxazolone, with the generalized expansion and activation of the paracortical T-cell area, which is clearly noted, since T blasts are strongly basophilic. **h** Lymph node section from a congenitally athymic (nude) mouse showing paracortical depletion with failure of T-cell development. *LM* lymphocyte mantle with SF, *MC* medullary cords, *MS* medullary sinus, *PA* paracortical area, *PC* plasma cells, *PN* primary node, *SF* secondary follicle, *SM* sinus macrophage, *SS* subcapsular sinus

in different pathways; among them IL_4 produced by T cells regulates proliferation of B lymphocytes (B=bone marrow-derived or bursal equivalent). In mammals, B cell maturation occurs in bone marrow: precursor cells (pro- and pre-B; see Chap. 2) multiply when in contact with primitive reticular cells, which generate ILs necessary for cellular multiplication and maturation. As for T cells in the thymus, a great number of B lymphocytes die through apoptosis, while mature B cells leave the bone marrow traversing the walls of venous sinuses [469]. Then they are transported by the circulation to secondary lymphoid organs, where they encounter and respond to invading antigens.

Secondary lymphoid organs include lymph nodes, spleen, tonsils and MALT.

Lymph nodes are lymph filters placed at the junctions of sympathic vessels. They are aggregates of lymphoid tissue strategically positioned to form a complete network throughout the body and carry out the basic functions of filtering foreign material, favoring antigen-dependent differentiation of lymphocytes, and localizing and preventing the spread of infectious processes. Apart from collagenous capsules surrounding lymph nodes, two cortical zones are recognizable within ganglia parenchyma, a more external (cortex or B-cell area) and a deeper one (paracortex or T-cell area) and a central medulla, where parenchyma is organized into medullary cords interdigitating with medullary sinuses. T and B cells, plasma cells and several macrophages are found there (Fig. 1.2) [470]. B cells populate three principal areas, the GCs, with dark and light zones, the follicular mantle zone, including $CD5^+$ and $CD5^-$ with features of virgin cells, and the marginal zone with several subsets also present in the spleen, subepithelial areas of the tonsils and the dome region of Peyer's patches (PP) [63]. B memory cells coming from tonsils colonize subepithelial areas and directly present antigen to T cells by rapid up-regulation of CD80 and CD86 [304]. T lymphocytes reside in T-cell-dependent areas, the deep cortex or paracortical zone together with interdigitating DCs (IDC) and high endothelial venules (HEV) present in lymphoid tissues and possessing cubic epithelial cells



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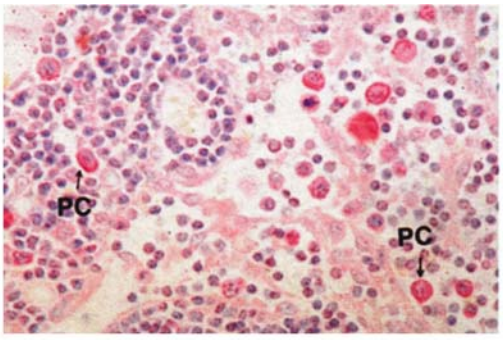

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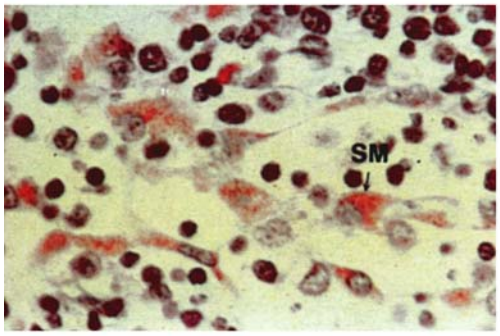

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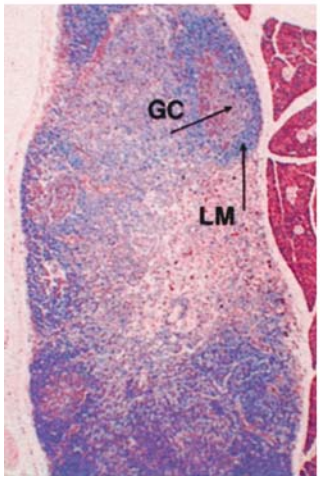
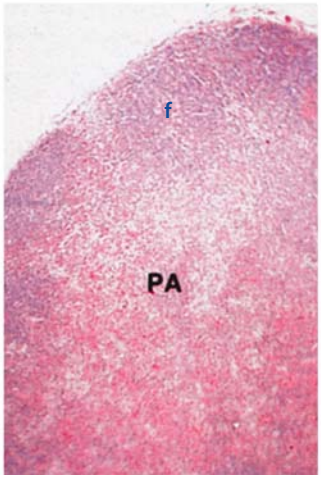
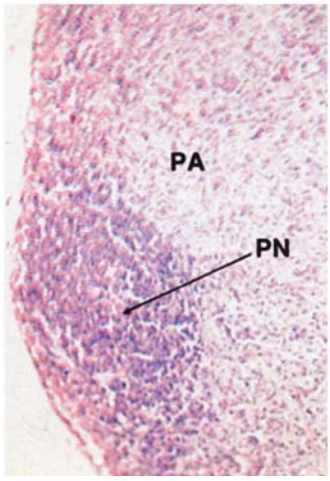




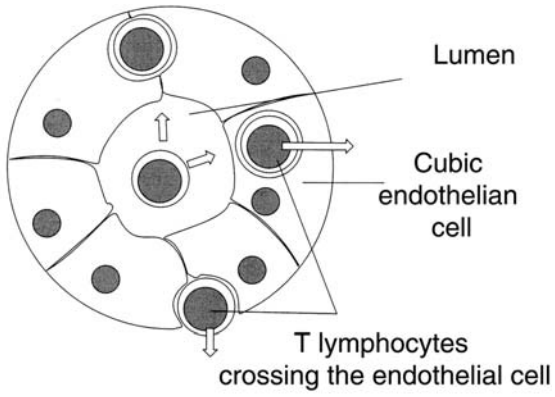


f–h

Fig.1.2. (Continued)

(Fig. 1.3). By crossing HEVs, non-antigen stimulated, virgin lymphocytes reach lymph nodes [454], where the paracortical area following antigen stimulation is hypertrophied and contains T lymphocytes and ICDs that are APCs. A specific subset of DCs (originating from circulating CD11c + DCs), which strongly stimulate T cells but not B cells, was recently identified in GCs [191]. Finally, the medullary layer, which is made up of cords separating lymphatic sinuses, contains essentially macrophages and plasma cells.

The *spleen*, in addition to being a filter eliminating senescent or worn-out cells, foreign particles and macromolecules from the circulation, is the only lymphatic tissue specialized in filtering blood as well as in filtering and processing antigens transported into the bloodstream (Fig. 1.4). Similar to lymph nodes, it is divided both from a functional and structural point of view into B and T zones. There are no lymphatic vessels, since blood enters the splenic parenchyma at the hilum via the splenic artery and follows along trabeculae until smaller arterial branches become surrounded by sheaths of lymphocytes, the white pulp. This zone contains lymphoid cells aggregating in lymphoid follicles or lymph nodules, and in *periarteriolar lymphoid sheaths* (PALS), which are similar to the cortex of lymph nodes. The pulp surrounding periarteriolar sheaths is called the marginal zone. In the peripheral area, cortical lymphocytes aggregate into follicles (as can be found in all peripheral lymphoid organs), distinguished into two types, primary and secondary follicles, also designated as GCs (see also "B Lymphocytes"). GCs also contain B cells and macrophages; B cells can also be found in red pulp, which is formed by venous sinuses, reticular fibers (Billroth's cords) and vessels [454]. The PALS mainly

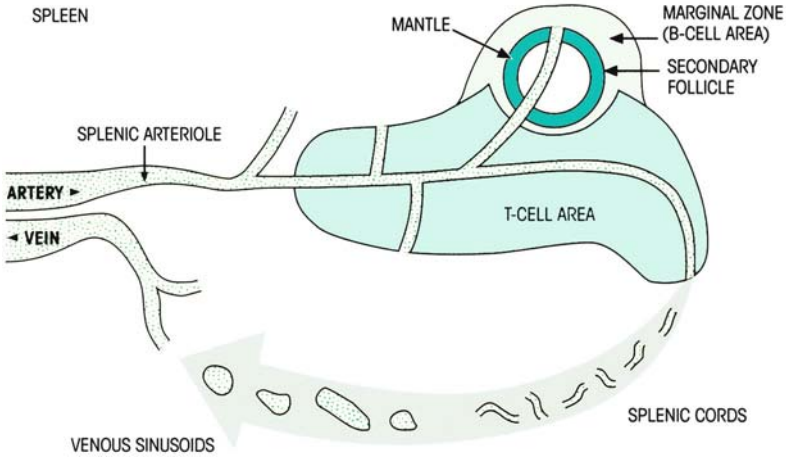


T lymphocytes		Endothelial cell
CD44	⟷	Hyaluronate
CD62L	⟷	Sialic acid
CD29/49	⟷	CD 106
CD11a	⟷	CD54, 58, 102

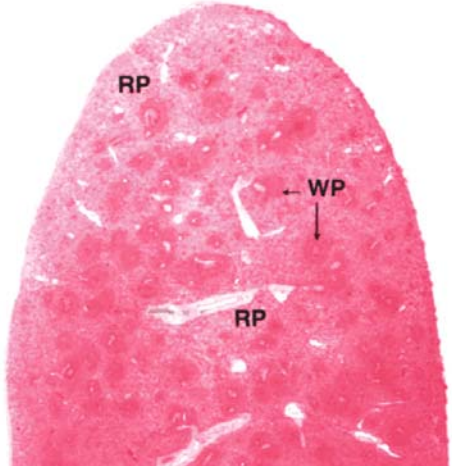
Fig.1.3. High endothelial venules

contain mature T lymphocytes with a CD4 phenotype in 65% of cases and CD8 in the remaining 35%. GCs and cells in active proliferation are present; the larger and medium-sized cells predominate in the central part of the GC, while the smaller cells localize at the periphery and form the mantle [454]. The surrounding follicles and marginal zone are composed mostly of B cells in addition to monocytes, plasma cells, red cells, platelets, DCs and several macrophages active in phagocytosis. The main immune function of the spleen is to initiate immune responses against polysaccharide antigens (PSA) traveling in the blood and fixed on the surface of follicular DCs (FDCs), differently from lymph nodes where immune responses to antigens take place if antigens enter via afferent lymphatics [33, 36]. The marginal zone is a natural reserve of memory B cells [48].

The *tonsils* (lingual, palatine and adenoidal) are important immune organs, containing many primary and secondary follicles and GCs, with morphology and cellular composition identical to those in lymph nodes. They are strategically placed for initiating immune responses, as they are continually in contact with inhaled or ingested antigens (Chap. 15).



a



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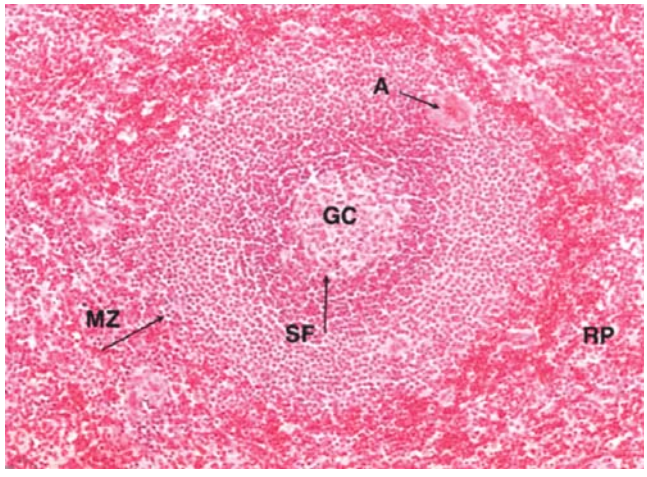


c

Fig.1.4. a Spleen: diagrammatic representation. **b** Low-power view showing lymphoid white pulp (*WP*) and red pulp (*RP*). **c** High-power view of a secondary follicle (*SF*) with germinal center (*GC*) and lymphocyte mantle (*M*) surrounded by marginal zone (*MZ*) and the red pulp (*RP*). Adjacent to the follicle is an arteriole (*A*) surrounded by periarteriolar lymphoid sheath (*PALS*, T cells)

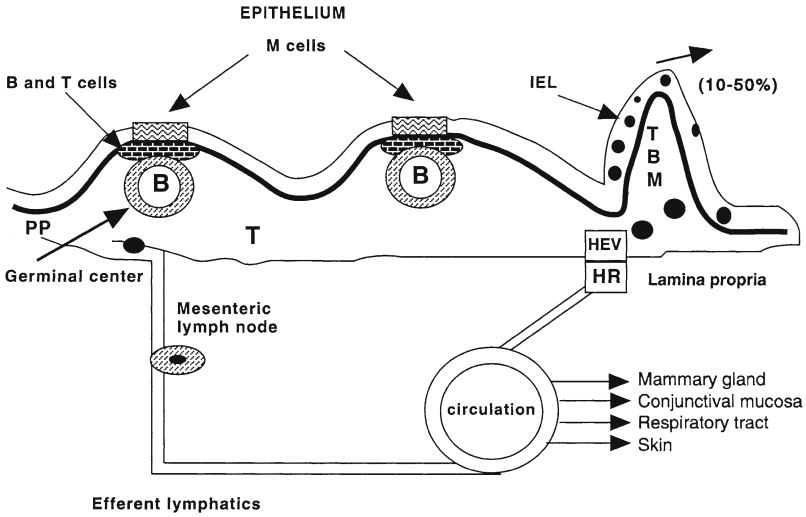


Fig.1.5. Schematic representation of recirculation of lymphocytes showing the relationships with MALT sites. After antigen presentation, T lymphocytes of the organized MALT leave the Peyer's plaques (*PP*) via efferent lymphatics and reach the circulation via mesenteric lymph nodes. Cells derived from the gut preferentially home back to the gut lamina propria via specialized homing receptors (*HR*) and the *HEV*. Cells originating from the gut can also reach other MALT sites: GALT, BALT, SIS, NALT, CALT (see text). *T, B* T and B lymphocytes, *M* macrophages, *IEL* intraepithelial lymphocytes, *HEV* high endothelial venules, *HR* homing receptors. (Modified from [546])

Mucosa-associated lymphoid tissue (MALT) is made of lymphocytes aggregated to form follicles in the lamina propria underlying basement membranes of mucosal epithelia of the respiratory, gastroenteric, cutaneous and urogenital systems. The most prominent of such tissues are termed GALT and BALT (gut- and bronchus-associated lymphoid tissue) (Fig. 1.5) [548]. Such distant anatomical sites not only share common immune effectors such as secretory IgA antibodies (sIgA), but they are also interrelated by a traffic of lymphoid cells.

GALT is represented by PPs in the mucosa of the outer wall of the terminal ileus (up to 200 PPs are present in mammals) and by the appendix, with a similar division into B- and T-dependent areas. Lymphoid tissue is also diffusely distributed in the lamina propria of intestinal villi and crypts and among epithelial cells of gut mucosa. GALT may intervene in differentiating stem cells into B lymphocytes that are mainly committed to IgA synthesis throughout the MALT. Primed B lymphocytes within PPs travel via mesenteric lymph nodes (where they differentiate into mature IgA B cells) and thoracic duct lymph, and subsequently to the systemic circulation, and finally they return mostly to reside at intraepithelial sites in the mucosa and to some extent to the spleen or to more distant sites such as BALT. Studies have shown that T lymphocytes may leave intravascular space via homing receptors on specialized HEVs where vascular adhesion molecules may direct their traffic [548].

BALT is structurally and perhaps functionally similar to GALT, associated with both upper (nasal mucosa) and lower (lungs) respiratory tracts. Lymphocytes are organized into lymphoid aggregates and follicles. They are commonly placed along main bronchi in all lobes that are found especially at bifurcations of bronchi and bronchioli; the so-called M (*microfold*) cells are found in both GALT, over the PP dome, and BALT overlying follicles (see Chaps. 9 and 11).

SIS (skin immune system). The term highlights the skin's immune characteristics, where intraepidermal lymphocytes, DCs, Langerhans' cells (LCs), keratinocytes, etc. are found.

Subsequently, additional lymphoid structures, including *NALT (nasal-associated lymphoid tissue),* corresponding to Waldeyer ring and *CALT (conjunctiva-associated lymphoid tissue)* have been characterized.

Cells of the Immune System

Two Families of Lymphocytes

Both T and B cells can be defined as two lines of immunocompetent cells, morphologically indistinguishable but functionally distinct, having a different origin. The immune response has indeed different functional aspects according to the reciprocal involvement of T or B cells; therefore, this response can be regarded as having two distinct components, the T system of lymphocytes producing immune or sensitized lymphocytes, and the B system of lymphocytes producing Igs. For their identification there is an internationally defined nomenclature, the group of CD antigens (CD followed by a number) (CD1–CD342) (Table 1.2) [4, 6, 16, 23, 34, 49, 70, 82, 85, 102, 103, 109, 112, 120, 132, 147, 158, 175, 242, 262, 343, 373, 407, 428, 437, 442, 484, 501, 515, 559, 571, 584, 608, 604], denoting that a cluster of antibodies will react with a particular antigen (see "Afferent Phase of Immune Response").

These effector cells of specific immunity share two cardinal features:

- They can be induced in that they have the ability to be activated by antigens evoking their formation and can be stimulated to proliferate and differentiate, so as to generate effector and memory cells.

Table 1.2. Leukocyte cell surface proteins: cluster of differentiation (*CD*) and their immune expression

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD1a (R4, Leu 6, VIT6, gp49)	Myeloid progenitors, DCs, LCs, activated T cells	49	Presentation of lipid and glycolipid antigens, antigen presentation to T γ/δ
CD1b (R1, NUT2, 4A76, gp45)	Thymocytes, DCs, LCs, T subpopulation	45	CD1a to C1b, noncovalently bound to β_2-microglobulin, include forms of non-HLA class I-like molecules, with a potential role alternative to known APCs
CD1c (R7, PHM3, M241, gp43)	Thymocytes, DCs, LCs, T subpopulation	43	Presentation of lipid and glycolipid antigens, αβ TcR
CD1d (R3)	Leukocytes, epithelium		Presentation of hydrophobic nonpeptide antigens, αβ TcR
CD1e (R2)			Presentation of lipid and glycolipid antigens, αβ TcR
CD2 (9.6, 35.1, T11, LFA-2)	Murine B, mature T and NK cells, thymocytes	47–58	Associated with CD3ε and ζ, promotes intercellular adhesion via the CD58 ligand, of CTLs to target cells, of T cells to endothelial cells and APCs, signal transduction
CD2R (T11.3, VIT13, D66)	Activated T and NK cells	50	Epitope of restricted CD2, activates thymic, T and NK cells
CD3-α (UCHT1, Leu 4, T3)	Thymocytes, T cells	25–28, 21,	Invariant part of TcR (5 chains), signal transduction for T cell activation
		20, 16, 22	Lineage-specific marker, Ig-SF
CD4 (91.D6, Leu 3, T4)	Thymocytes, T subpopulation, monocytes	59	Th marker, CD3 co-receptor, stabilizes HLA class II–TcR complexes, HIV receptor, signal transduction in association with $p56^{lck}$, Ig-SF
CD5 (T1, UCHT2, T101, gp67)	Mature (murine) B cells and T cells, thymocytes	67	Costimulation of T cells, binds CD 32, CD38, CD45RA, CD45RO, CD72, increases the pool of 2nd messengers, SRCR- SF
CD6 (T12, T411, gp100)	B and T subsets, thymocytes	100–130	Interacts in the TcR-mediated activation of T cells, binds CD166; SRCR-SF
CD7 (3A1, 4A, CL1.3)	Human fetal liver, thymocytes, T and NK cells	40	Signaling, early T-lineage marker, activates T and NK cells, FcR for IgM, Ig-SF
CD8 (α chain: T8, Leu 2a, T811; β chain: T8; gp32)	Most thymocytes, CTLs, intraepithelial lymphocytes, some DCs	34	Stabilizes HLA class II–TcR complexes, coreceptor for HLA class I CTL-restricted, signal transduction, Ig-SF
CD9 (PHN200, FMC56, p24)	Pre-B cells, monocytes, basophils, platelets, activated B and T cells	22–27	Adhesion of pre-B cells, possible role in signal transduction mediated by interaction with GTP-binding proteins, activation and aggregation of platelets, binds CD41/CD61, TM4-SF
CD10 (J5, NEP, BA-3, gp100, CALLA)	B precursors, TN thymocytes, PMNs, fibroblasts	100	Zn-dependent neutral endopeptidase, putative role in B-cell development

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD11a (MHM24, LFA-1, CRIS3, CR1)	Myeloid precursors, monocytes, macrophages, neutrophils, eosinophils, B and T cells	180	β2 Integrin (αL chain), receptor for CD50, CD54 and 102, mediates cell–cell leukocyte adhesion to endothelium (CD54/102), of CTL to target cells, heterotropic among B, T and monocytes and homotropic of neutrophils via CD50
CD11b (Mo1, MAC-1, CR3, gp155/95)	Monocyte-macrophages, neutrophils, NK cells	165	β2 Integrin (αM chain), receptor for CD54, iC3b and fibrinogen, modulates adhesion of neutrophils/monocytes and extravasation via CD54
CD11c (L29, B-LY6, BL-4H4, CR4, gp150/95)	Monocyte-macrophages, neutrophils, NK cells, subsets of activated B and T cells	150	β2 Integrin (αX chain), receptor for CD54, iC3b and fibrinogen, neutrophil/monocyte adhesion to endothelium, receptor for phagocytes
CDw12 (M67)	Eosinophils, monocytes, PMNs, basophils	90–120	Requires further analysis
CD13 (MY7, MCS-2, TÜK1, gp150)	Myeloid progenitors, monocytes, granulocytes	150–170	Aminopeptidase N: inactivates active peptides, receptor for coronaviruses
CD14 (Mo2, VIM 13, MoP15, gp55)	Eosinophils, monocytes, PMNs, basophils	55	Inhibits IgE synthesis on monocytes, receptor for LPS, suggested role in Gram– pathogen clearance, polymorphism in the flanking region, receptor for endotoxin, LPS/LPS binding complex
CD15 (My1, VIM-D5)	Eosinophils, monocytes, PMNs, basophils, LCs	–	Oligosaccharide; binds CD62E; modulates PMN adhesion and phagocytosis
CD15 s (sLe^x)	Granulocytes, NK, T and B cells, monocytes	–	Sialyl-Lewis, binds CD6E, CD62P and CD62L
CD15u			Sulfated CD15, carbohydrate structures
CD16a (BW209/2, HUNK2,3 GB)	Immature fetal T and B cells, monocytes, NK cells, PMN subsets, macrophages	50–65	FcγRIIIa, associated with TcR-ζ and FcεRI-ɣ, promotes signal transduction for NK cells (ADCC) and macrophages (phagocytosis), IgSF
CD16b	Granulocytes	48–60	FcγRIIIb, IgSF
CDw17 (GO35, Huly-m13)	Granulocytes, PMNs, monocytes, eosinophils	–	Lactosylceramide, possible role in phagocytosis, signaling
CD18 (MHM23, M232, 11H6)	All leukocytes	95	Integrin, β2 chain of CD11a, b, c, binds CD54, participates in cell adhesion
CD19 (B4, HD37)	Pan B-cell and pre-B precursors, FDCs	95	Membrane protein: B-cell activation and proliferation, part of signal transduction complex including CD21, CD81 and Leu-13, IgSF
CD20 (B1, IF5, p37/32)	Pan B-cell except pre-B and plasma cells	33, 35, 37	Modulates ionic channels, B-cell activation and proliferation, associated with CD53, CD81, CD82 and HLA

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD21 (B2, HB5, CR2, p140)	Mature B cells, FDCs, normal thymocytes	145	See CD19; binds C3d, EBV and CD23, which is implicated in IgE synthesis; intracellular domain contains potential PKC and PTK sites; RCA
CD22 (HD39, S-HCL1, To15, gp135)	B cells mature, pro- to pre-B cells	130/140	Homologous to MAG, the α-form mediates adhesion to monocytes and RBCs, the β-form to T cells via CD45RO and to B cells via CD75, the cytoplasmic domain has potential PKC sites and 6 tyrosine residues; IgSF
CD23 (Blast-2, MHM6, gp45/50, FcεRII)	Low-affinity IgE receptor (see text)	45	C-type lectin, ligand of CD21, inducible by IL_4 and IL_{13}, modulates cytotoxicity of eosinophils and macrophages, may prevent apoptosis of GC B cells
CD24 (VIBE-3, Ba-1, HSA, gp41/38)	Immature T, B/T cells, PMNs, eosinophils, FDCs	38–70	T-cell costimulation, adhesion and signaling, supports CD80 for T-cell growth
CD25 (7G7/B6, 2A3, Tac p55)	Activated B cells, T, NK, monocyte-macrophages	55	Integrin, IL_2R α chain, associating with β (CD122) and γ chain induces proliferation and activation of T, B and NK cells, and macrophages; CCP-like
CD26 (134–2C2, TS145, gp120)	Memory CD45RO, T and NK cells, macrophages	110	Dipeptidyl-peptidase IV (substrate is the V3 loop of HIV-1 and HIV-2); triggers T cells, to which anchors adenosine deaminase (ADA); binds collagen, fibronectin
CD27 (VIT14, S152, T18A, gp55)	T cells, B cells, subsets of thymocytes, NK cells	55	CD70 ligand, costimulatory signaling activating T cells; TNFR-like protein
CD28 (9.3, KOLT2, B7, gp44)	T-cell subsets, resting T cells, plasma cells	44	Binds CD80 (CD86); costimulatory signal for T cells distinct from TcR signal; IgSF
CD29 (K20, A-1A5)	T, B and NK cells, all leukocytes, monocytes, platelets, endothelial cells	110,130	VLA-β, β1 chain integrin, binds collagen, laminin and fibronectin, or heterodimer of CD49 modulates cell–cell and adhesion to cellular matrix
CD30 (Ki-1, Ber-H2, HSR4)	Activated T, B and NK cells, Reed-Sternberg cells	105	TNFR-like protein for MCP, enhances HIV replication in CD4 T cells, delivering a death signal, expresses Th2-like ILs, binds CD153; TNFRSF, and NGFRSF
CD31 (SG134, TM3, HEC-75, gp140, PECAM-1)	Platelets, monocytes, macrophages, neutrophils, B, naive T and NK cells, Lateral endothelium	140	Platelet antigen GPIIa, $\alpha_V\beta_3$ integrin, binds CD51/CD61, regulates adhesion to endothelium and transmigration of monocytes, PMNs, T and NK cells; IgSF
CD32 (CIKM5, 41H16, 2E1, ex CDw32)	Monocytes, B cells, all leukocytes, platelets	40	FcγRII, implicated in the phagocytosis of neutrophils and monocytes; IgSF
CD33 (My9, H153, L4F3, gp67)	Monocytes, all leukocytes, myeloid precursors	67	Sialoadhesin, binds sialylate glycoproteins

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD34 (My10, Bi-3C5, ICH-3, gp105–120)	Myeloid precursors, vascular endothelium, immature hematopoietic cells	105–120	Sialomucin, lymph node HEVs, CD62L-independent leukocyte adhesion to HEVs, receptor for CD62L, early marker of hematopoietic stem/progenitors cells
CD35 (TO5, CB04, J3D3, CR1)	Monocytes, some B and NK cells, erythrocytes, macrophages, neutrophils, eosinophils, FDCs	250	Receptor for C3b and C4b, RCA, reversible complement inactivation; supports phagocytosis of monocytes and neutrophils, APC putative role on DCs
CD36 (5F1, CIMeg1, ESIVC7, gp90)	Monocyte-macrophages, DCs, platelets, B cells	88	Platelet antigen GPIV (IIIb), binds thrombospondin, collagen, LDL receptor for macrophages (phagocytosis of apoptotic cells)
CD37 (HD28, HH1, G28–1, gp52–40)	Mature B, T and myeloid cells, PMNs, monocytes	40–52	Role in signaling, intercellular traffic with CD53, CD81, CD82, HLA-class II with function of ion channel; modulates B cell activation and proliferation (?), TM4-SF
CD38 (HB7, T10, p45)	Early and activated T, B cells, myeloid progenitors	45	Signal transduction, and cell adhesion
CD39 (AC2, G28–2, gp 80, gp70–100)	T, B and NK cells, monocytes, vascular endothelium	78	Directs NK cell activation, may deliver activating signals from B cells to T cells
CD40 (G28–5, gp50)	Normal and neoplastic B cells, FDCs, macrophages, epithelial and endothelial cells, keratinocytes	50	Ligand for CD40L, important in B cell differentiation and activation, GC formation and isotype switching, prevents apoptosis, TNFRSF
CD40L (see CD154)			
CD41 (PBM6.4, PL 273)	Platelets, megakaryocytes	125/22, 105	Platelet antigens GPIIb/IIIa, mediates platelet aggregation; β3 integrin; associates with CD61; binds fibrinogen, fibronectin, vitronectin, vWF and thrombospondin
CD42a (FMC25, BL-H6, GR-P, gp23)	Platelets, megakaryocytes	22	Platelet antigen GPIX
CD42b (PHN89, AN51, GN287)	Platelets, megakaryocytes	135, 25	Platelet antigen GPIbα
CD42c	Platelets, megakaryocytes	22	Platelet antigen GPIbβ
CD42d	Platelets, megakaryocytes	85	Platelet antigen GPV The a+b+c+d complex promotes platelet adhesion, binds vWF and thrombin
CD43 (OTH71C5, G19–1, gp95)	Hematopoietic precursors, CD4, pre-B, leukocytes	115	Sialomucin, leukosialin; T cell proliferation, costimulation, and adhesion
CD44 (H-CAM, Pgp-1, gp80–95)	Fetal and immature adult thymocytes, memory T	80–95	Binds hyaluronic acid, primes T cell activation, adhesion, HEV homing, apoptosis

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD44R (CD44-restricted or variant)	Expressed in different epithelia	110–250	Possible role in DC migration, binding and presentation of chemokines
CD45 (T29/33, BMAC 1, LCA, T200)	Expressed on all leukocytes	180–220	Tyrosine phosphatase, ligand for CD22; signal transduction for T and B cells
CD45RA (G1–15, F8–11–13, gp220)	Naive T cells, T CD8 subset, B and NK cells	205, 220	T200 with expression restricted to T cells, isoform with A exon
CD45RB (PTD/26/16)	Naive T cells, T, B and NK cells	205, 220	T200 with expression restricted to naive T cells, isoform with B exon
CD45RC			CD45 restricted epitope
CD45RO (UCHL-1, gp180)	Granulocytes, monocytes, T cells	180	T200 with expression restricted to memory T cells, isoform without A/C exons
CD46 (HULYM5, J4B, MCP, gp66/56)	Myeloid precursors, hematopoietic cells and non-myeloid precursors, T, B cells, all leukocytes	56/66	Membrane cofactor protein, prevents C3 breakdown
CD47 (BRIC 125, BRIC 126, gp47–52)	Myeloid precursors, T, B cells, all leukocytes	47–52	β1 integrin associated protein, signaling molecule facilitating adhesion, antigen associated with Rh group
CD47R ex CDw149 (MEM-133)	B and T cells, neutrophils, eosinophils, monocytes	120	Unknown
CD48 (WM68, BCM1, OX-45, Blast-1)	distributed to hematopoietic cells and nonhematopoietic T, NK, capillary, endothelial cells, fibroblasts	45	Binds CD2, involved in T cell adhesion to APCs and their costimulation, may be required by γδ cells for antigen recognition; IgSF
CD49a	T, NK, capillary, endothelial cells, fibroblasts	200–210	α1 Chain associated with CD29 to form VLA-1, binds laminin and collagen I, IV
CD49b (Gi14, CLB/thromb4)	Platelets, B and T cells, endothelia, fibroblasts	155–165	α2 Chain associated with CD29 to form VLA-2, receptors for GPIa, laminin and collagen I-IV, regulates the expression of metallo-proteinase-1
CD49c	B cells, fibroblasts, keratinocytes, epithelia	145–150	α3 Chain associated with CD29 to form VLA-3, binds laminin, collagen and fibronectin
CD49d (B5G10, HP2/1, HP1/3)	Myeloid precursors, thymocytes, NK cells DCs, B- and T-cell lineages	150, 80, 70	α4 Chain associated with CD29 to form VLA-4, binds fibronectin, CD106, MAdCAM-1, role in T cell adhesion/migration to lymph nodes and homing to HEVs
CD49e	Platelets, epithelia, endothelia, thymocytes, monocytes, myocytes, T and B cells	160, 135/25	α5 Chain associated with CD29 to form VLA-5, binds fibronectin and GPIc, T cells, involved in cell adhesion and migration mediates proliferation and differentiation

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD49f (GoH3)	Widespread: T cells, thymocytes, monocytes, platelets, epithelial cells, eosinophils	150, 130	α6 Chain associated with CD29 to form VLA-6, binds laminin; the different α6 isoforms have a distinct developmentally regulated distribution in various tissues
CD50 (101–1D2, 140–11, ICAM-3)	T, B and NK cells, monocytes, PMNs, LCs	116–140	Binds CD11a/CD18, promotes signal transduction and costimulation on T cells; signaling via CD50 stimulates β1 and β2 integrin function on T cells; IgSF
CD51 (13C2, 23C6, NKI-M7)	Platelets, endothelia, monocytes, macrophages	125–24	Complex with CD61, binds fibronectin, vitronectin (α chain) and vWF; role in platelet aggregation, cell-cell adhesion (via CD31) and γδ costimulation
CD52 (O97, YTH66.9, Campath-1)	B and T lymphocytes, monocytes, macrophages	25–29	Target of complement-mediated lysis, may contribute to T lymphocyte depletion and GvHD prevention
CD53 (MEM-53, HI29, HI36, gp32–40)	Granulocytes, B and T lymphocytes	32–40	Bound to phosphoinositol
CD54 (RR7/7F7, ICAM-1)	B (and T) cells, endothelia, epithelia, DCs, fibroblasts	90–115	Protein implicated in B-cell activation, ligand of CD11a/CD18, CD43 and monocytes, Rhinovirus, mediates leukocyte adhesion to epithelium in inflammation sites and in T-cell interactions with APCs and target cells; IgSF
CD55 (BRIC110, BRIC128, DAF)	All leukocytes, activated T cells, endothelium	70	Bound to phosphatidylinositol, RCA, binds CD97 and C3b/C3bBb and C4b/C4b2a convertase, thus accelerating the decay of the C3 convertase and C5
CD56 (Leu19, NKH1, L185, gp220/135)	NK, T and Schwann cells, neurones, astrocytes	200–220	Isoform of NCAM, NK-cell marker, putative role in tissue architecture (during embryogenesis) and in non-restricted cytotoxicity; IgSF
CD57 (Leu 7, L183, L186, gp110)	NK and T cells (CD8), B subpopulation	110	Binds CD62-L, -P, NNK-1, role in non-HLA-restricted cytotoxicity after activation
CD58 (BRIC5, G26, TS2/9, LFA-3)	Myeloid precursors, erythrocytes, all leukocytes	55–70	Role in APCs and T-cell interactions via CD2, bound to phosphatidylinositol; IgSF
CD59 (MEM-43, YTH53.1, p18, gP18)	Many hematopoietic cells and nonhematopoietic cells, T cells	18–20	MACIF, 2nd ligand of CD2, binds C8, C9, phosphatidylinositol, signaling
CD60 (M-T32, M-T21, M-T41)	T-cell and NK-cell subsets, platelets		Carbohydrate with NeuAc-NeuAc-Gal sequence modulates T-cell activation
CD60a			GD3, carbohydrate structures
CD60b			9-*O*-acetyl-GD3, carbohydrate structures

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD60c			7-*O*-acetyl-GD3, carbohydrate structures
CD61 (CLB thromb/1, BL-E6)	Platelets, megakaryocytes, monocytes	105	β3 Chain integrin, receptor for vitronectin (β chain), fibronectin, fibrinogen; platelet antigens GPIIb/IIIa, platelet aggregation, heterodimer with CD41 CD51
CD62E (ELAM-1, LECAM-1, E selectin)	Vascular endothelium	110	C-type lectin, binds ESL-1, promotes tethering and rolling of monocytes, some memory T cells, and for neutrophils also the adhesion to endothelia with CD15s
CD62L (LAM-1, LECAM-2, L selectin)	All circulating leukocytes, except some memory cells	70–90	C-type lectin, migration of lymphocytes to lymph nodes and leukocytes to sites of T cells inflammation, mediates T-cell tethering and rolling and adhesion to HEVs, binds CD34, ESL-1, GlyCAM-1, and MAdCAM
CD62P (gp140, PADGEM, P selectin)	Activated platelets and endothelial cells	140	C-type lectin binds CD162, adhesion of platelets to neutrophils and monocytes, as well as tethering and rolling of leukocytes on activated endothelium
CD63 (CLB gran/12, gp53)	Activated platelets, PMNs, monocyte-macrophages	53	Platelet-activating antigen, associated with con CD9, CD81, VLA 3–6; TM4-SF
CD64 (Mab22, Mab32.2, gp75)	Monocyte-macrophages, DCs, neutrophils	75	FcγRI; high-affinity receptor for IgG Fc, promotes ADCC; IgSF
CD65 (VIM2, VIM8, HE10)	Granulocytes, monocytes, myeloid leukemia cells		Ceramide-dodecasaccharide 4c
CD65 s	Sialylated form of CD65		
CD66a (BGP-1, CEA)	Granulocytes, epithelial cells	140–180	Binds CD62E, adhesion molecule for neutrophils, signaling role; IgSF
CD66b (ex CD 67, CGM6, p100)	Granulocytes	95–100	Adhesion molecule, can activate neutrophils, signaling; IgSF
CD66c (NCA, CEA)	Granulocytes, epithelial cells	90	Binds CD62E, adhesion molecule, capable of activating neutrophils
CD66d (CGM1, CEA)	Granulocytes	30	Adhesion molecule, can activate neutrophils
CD66e (CEA)	Colon epithelium, colon carcinoma	180–200	Adhesion molecule
CD66f	Myeloid lineage cells		
CD67 now CD66b			
CD68 (EBM11, Ki-M7, Ki-M6, gp110)	DCs, PMNs, monocyte-macrophages, basophils	110	Marker of macrophages, lysosomal protein implicated in endocytosis

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD69 (MLR3, VEA, gp 34/28, AIM)	All activated leukocytes, neutrophils, platelets	85	C-type lectin involved in signal transduction for lymphocytes, apoptosis of eosinophils and in early events of T γδ cell cytotoxicity
CD70 (Ki-24, HNE 51, HNC 142)	Activated B and T cells, Reed-Sternberg cells	75, 95, 170	CD27 ligand, implicated in T-cell activation, TNRSF
CD71 (138–18, 120–2A3, T9)	Proliferating, activated T and B cells, macrophages	95	Receptor for transferrin and proteins binding Fe, activated on proliferating cells
CD72 (J3–109, BU-40, BU-41)	pan-B including progenitors, macrophages	43–43	C-type lectin, CD5 ligand, elicits B-cell activation/proliferation, inducible by IL_4
CD73 (AD2, 1E9.28.1, gp69)	B- and T-cell subsets, endothelial/epithelial cells	69	Ecto-5′-nucleotidase, may induce T-cell activation, B-cell interactions with FDCs
CD74 (LN2, BU-43, BU-45)	HLA class II, mature B cells, T cells, monocytes	41–35–33	Invariant chain associated with HLA class II prevents binding of foreign peptides
CD75 (LN1, HH2, EBU-141)	GC and mature B cells, T-cell subpopulation	53	GC B cells, putative ligand for CD22 supports B-B cells interactions
CD75s			α-2,6-Sialylated lactosamines, carbohydrate structures
CD76 (HD66, CRIS-4, ex CD76)	Mature B cells, T-cell subpopulation	53–87	Directs mantle zone and extrafollicular B cells in tonsil areas
CD77 (424/4A11, 424/3D9)	Activated GC and centrofollicular B cells, FDCs	–	Globotriaosylceramide (Gb3), may control transmembrane signals → apoptosis
CDw78 (Anti Ba, Leu21, 1588)	Resting and activated B cells, macrophages	–	Can inhibit or enhance B-cell activation
CD79a (mb-1, Igα)	Specific for B-cell ontogeny, BcR complex	33	BcR component, important for signal transduction comprising ITAM; IgSF
CD79b (B29, Igβ)	Specific for B-cell ontogeny, BcR complex	39	BcR component, important for signal transduction, comprising ITAM; IgSF
CD80 (B7–1, BB1)	Activated B and T cells, DCs, macrophages	60	Early activation marker, binding to CD28 and CD152 (CTLA-4) regulates IL_2 gene expression and activates T lymphocytes
CD81 (TAPA-1)	All B, T, NK cells, FDCs, thymocytes, eosinophils	26	Part of complex including CD19 and CD21: cross-linking CD81 is thought to have a role in signal transduction; TM4-SF
CD82 (R2, IA4, 4F9)	B, T, NK cells, macrophages, monocytes, platelets	60	Participates in T-cell activation, probably in signal transduction; TM4-SF
CD83 (HB15)	GC B cells, B cells, circulating DCs, LCs	43	Mature DC specific marker, may function in antigen presentation; IgSF
CDw84 (GR6, BPC6)	Mature B and T cells, platelets, macrophages	68–80	May be a signaling molecule

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD85 (VMP-55, GH1/75)	Mature circulating and neoplastic B cells	120, 83	ILT/LIR family, dendritic cells (DC)
CD86 (FUN-1, BU63, B7–2)	Activated, mature circulating B cells, DCs, monocytes	80	Rapid expression on activated B cells; interacts with CD28 and CD152 (CTLA-4) to regulate IL_2 gene expression and prevent T-cell anergy; IgSF
CD87 (UPA-R)	Activated PMNs, monocytes, NK and T cells	35–39	Binds vitronectin, receptor for urokinase, role in leukocyte extravasation
CD88 (C5aR)	Monocytes, granulocytes, NK cells, DCs, microglia	43	G-protein-coupled, C5a receptor on phagocytes
CD89 (Fcα-R)	Neutrophils, monocyte-macrophages, eosinophils	45–70	Fc receptor for serum/secretory IgA, signal transduction for phagocytes; IgSF
CD90 (Thy-1)	CD34 subset of bone marrow, CB and fetal liver hematopoietic stem cells, HEV endothelium	25–35	Associated with CD45, participates in T-cell recirculation, adhesion, activation, in cell–cell modulation and cell signaling; IgSF
CD91 (α_2M-R)	Monocyte-macrophages, non hematopoietic cells	600	Binds LDL, receptor for α2M-R mediator of endocytosis
CD92 (CTLL1, GR 9)	Monocytes, granulocytes, B, T, epithelial cells	70	Unknown
CDw93 (GR 11)	Monocytes, granulocytes, endothelial cells	110	Unknown
CD94 (kp43)	γ/δ T subsets, human NK cells and αβ CD8	70–43	Implicated in signal transduction for NK and T cells; the CD94 receptor (43kD) can inhibit HLA class I molecules; CD94 with a new associated protein (94AP) forms a NK receptor involved in the recognition of HLA-A, HLA-B, HLA-C molecules
CD95 (Fas/Apo-1)	Thymocytes, activated B and T cells	36–45	Transduces apoptosis signal, role in T-cell clonal deletion, TNFRSF
CD96 (TACTILE)	Activated T and NK cells	160	Promotes T and NK cells activation; IgSF
CD97 (GR1, BL-KDD/F12)	Monocytes, granulocytes, NK cells	74, 80, 89	Receptor for CD55 (?) and TNF; TNFR
CD98 (4F2, 2F3)	Monocytes, B and T cells, several cell lines	85, 40	Actin-associated, modulates intracellular Ca^{++} levels and cell proliferation (?)
CD99 (E2, MIC2)	All hematopoietic cells, thymocytes, T and B cells	32	T-cell rosette formation with erythrocytes, DP thymocytes adhesion to T cells
CD99R (CD99-mAb restricted)	B and T cells	32	Restricted CD99 hematopoietic cells
CD100 (BB18, A8, GR3, ST-003, -005)	Most hematopoietic cells, activated B, T, NK cells	150	Semaphorin, associated with CD45, PBMC proliferation, role in T-cell adhesion
CD101 (BB27, BA27, GR14, ST-004)	Monocytes, granulocytes, mucosal T cells	140	Inhibition of T-cell proliferation, possible role in T-cell signaling, IgSF

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD102 (ICAM-2)	Lymphocytes, monocytes, vascular endothelia, NK cells, HEVs, platelets	55–65	Binds CD11a/CD18 on endothelial cells, mediates T-cell recirculation, trafficking and activation, and stimulates leukocyte activity in inflammation; IgSF
CD103 (HML-1, M290)	Intraepithelial T cells, activated CD8 T cells	175–105	Integrin, $\alpha_E\beta_7$ subunit, binds E-cadherin, role in mucosal lymphocyte adhesion
CD104 (β1)	Epithelia, monocytes, T cells, keratinocytes	150–125	β4 Chain integrin, binds laminin
CD105 (GR7, CELL-CAM)	Endothelia, PMNs, activated monocyte-macrophages	90	Endoglin, receptor for TGF-β1 and TGF- β3; TGF-βR type III and ectoATPase
CD106 (VCAM-1, INCAM-110)	IL-activated vascular endothelia, bone marrow stromal cells, embryonic tissues, APCs	90–110	Binds CD49d/CD29; lymphocyte migration, recruitment, activation/stimulation by APCs, PBMC and eosinophil adhesion stimulated by endothelial ILs; IgSF
CD107a (LAMP-1)	Activated platelets, T cells, neutrophils	110	Lysosome-associated protein
CD107b (LAMP-2)	Activated platelets, T cells, neutrophils	120	Lysosome-associated protein
CD108 (SEMA7A)	Splenic T lymphocytes, some stromal cells	80	Human blood group antigen
CD109 (8A3, 7D1, PAF)	Endothelial cells, T cells, activated platelets	170/150	Possible role in activation, proliferation and signal transduction
CD110 (MPL, TPO-R)	Platelets		
CD111 (PRR1/Nectin1)	Myeloid cells		
CD112 (HVER, PRR2)			
CD113 (PRR2)	Myeloid cells		
CD114 (CSF3R, G-CSFR)	Monocytes, granulocytes, endothelial cells, platelets	130	G-CSF receptor; CSF3R
CD115 (CSF-1R, M-CSFR)	Monocyte-macrophages and progenitors, placenta	150	M-CSF receptor (CSF-1) promotes phagocyte proliferation/ differentiation
CD116 (GM-CSF)	Macrophages, eosinophils, PMNs, DCs, fibroblasts	75–85	Binds GM-CSF, with higher affinity if coexpressed with CD131, supports proliferation and differentiation; class CSFR
CD117 (SCF-R, c-kit)	TN thymocytes, hematopoietic progenitors	145–150	SCF receptor, signal transduction, differentiation and adhesion; IgSF
CD118 (IFN-α/βR)	Broad cell expression	–	Receptor for IFN-α and IFN-β; CSFR
CD119 (IFN-γR)	Macrophages, B, T, NK and epithelial cells	90	IFN-γα chain receptor induces macrophage activation, B-cell differentiation; A CSFR

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD120a (TNFR.I, p55)	Granulocytes, epithelial cells, FDCs, monocytes	55	Type 1 TNFR, up-regulates leukocyte adhesion molecules and inflammation; TNFRSF1A
CD120b (TNFR.II, p75)	Granulocytes, myeloid cells, monocyte-macrophages	75	Type 2 TNFR, signaling triggers biological effects TNFRS1B
CD121a (IL_1R 1)	Thymocytes, fibroblasts, T cells, endothelial cells	75–85	Type 1 IL_1R, promotes cell growth in synergy with IL_2 and IL_4, protects against intracellular pathogens, inhibits the IL_{12} protective effects; IgSF
CD121b (IL_1R 2)	Neutrophils, monocytes, bone marrow and B cells	68	Type 2 IL_1R; IgSF
CD122 ($IL_2R\beta$)	Activated T cells (α, β, γc), $\beta\gamma$ chain on NK cells, CD8 T cells	70–64	β Chain of IL_2R, responsible for all the effects induced by IL_2, γc chain could prevent induction of anergy, the complex IL_2 + CD122 + CD25 forms a high-affinity receptor for the activation of thymocytes, B, T and NK cells and macrophages, incrementing the NK cytolysis and Ig synthesis; CKR-SF
CD123 ($IL_3R\alpha$)	Bone marrow stem cells, megakaryocytes, granulocytes		$IL_3R\alpha$ CKR-SF (cytokine receptor-superfamily)
CD124 (IL_4R)	Mature B and T cells, hematopoietic precursors, fibroblasts, epithelial and endothelial cells, pre-B, B and T lymphocytes	130–150	Activates B cells increasing CD23 and IgM expression; is a switch factor implicated in IgE regulation. Associates with $IL_{13}R\alpha$ to form $IL_{13}R$ complex; CKR-SF
CDw125 ($IL_5R\alpha$)	B cells, eosinophils, basophils	80	Combines with CDw131 to form the high-affinity receptor for IL_5; CKR-SF
CD126 ($IL_6R\alpha$)	T and B cells, monocytes, fibroblasts, hepatocytes	60	α Subunit, forms with CD130 the high-affinity receptor for IL_6; CKR-SF
CD127 (IL_7R, $IL_7R\alpha$)	Thymocytes, T/B cell progenitors, mature T cells, monocytes, lymphoid and myeloid cell lines	68–64	The two chains associate to form the high-affinity receptor for IL_7, triggers proliferation of pro- and pre-B cells and immature T-cell growth; CKR-SF
CDw128 (IL_8R)	NK cells, neutrophils, monocytes	58–67	Receptor for IL_8 and α chemokines
CD129 (IL_9R)	T and B cells, macrophages, megakaryoblasts	64	Receptor for IL_9, 40% homology with $IL_2R\beta$, inhibits apoptosis; CKR-SF
CD130 (gp130, SIG, IL_6R)	All hematopoietic cells, many other cell lines	130–140	β Subunit; signal transduction chain for IL_6 receptors; CKR-SF
CD131	Widespread on myeloid cell types	120–140	Common β subunit associated with α subunit of IL_3R (CD123), IL_5R (CDw125) and GM-CSFR (CD116) ; class BCSFR (see Table 1.5: it links to IL_6R)

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD132	B, T, NK cells, monocytes, PMNs, macrophages	65–70	IL_2R γc (common) chain, subunit of IL_2R, IL_4R, IL_7R, IL_9R, $IL_{15}R$, $IL_{21}R$ and similar signaling components of receptors; associated with JAK3; CKR-SF
CD133 (AC133)	Stem/progenitor cells		Prominin1 (PROM1)
CD134 (OX40)	Expressed on activated T cells and macrophages	50	Adhesion of activated T cells to vascular endothelial cells via gp 34, TNFRSF4
CD135 (Flt3, Flk2)	All hematopoietic stem cells, B-cell progenitors	130	Tyrosine kinase receptor, growth factor for hematopoietic progenitors, IgSF
CDw136 (MSP-R)	Tissue macrophages, some epithelial cell lines	180	Receptor for macrophage-stimulating protein (MSP-R)
CD137 (4–1BBL)	B and T cells, macrophages		T-cell activation and differentiation, distinguishes between IgE- and non-IgE-mediated atopic dermatitis and asthma, TNFRSF9
CD138	B cells		Glycosaminoglycan, binds fibronectin, collagen, thrombospondin
CD139	B cells, granulocytes, monocyte-macrophages		
CD140a (PDGF-Rα)	Endothelial cell lines		Receptor for PDGF α chain; CKR-SF
CD140b (PDGF-Rβ)	Monocytes, PMNs, fibroblasts, smooth muscle cells	160	Receptor for PDGF β chain, tyrosine kinase directing signal transduction
CD141	PMNs, monocyte-macrophages, endothelia, platelets, smooth muscle cells, keratinocytes, megakaryocytes	75	C-type lectin, thrombomodulin
CD142	Monocytes, vascular endothelial cells, fibroblasts, keratinocytes, stromal cells	45–47	Serine protease cofactor, binds factors VIIa and Xa coagulation factor III
CD143 (ACE)	Endothelial cell lines		Peptidylpeptidase, binds angiotensin
CD144	Vascular endothelial cell lines		Cadherin-5 or VE-cadherin, binds β-cadherin
CDw145	Endothelial cell lines (?)		
CD146 (MUCI8, 5-endo)	All leukocytes, endothelial cells, platelets	50–60	Likely adhesion molecule; IgSF
CD147	Widespread cell diffusion		Neurotelin, basigin; IgSF
CD148 (HPTP-η)	Granulocytes, monocytes, DCs, Kupffer cells	240–260	Proteintyrosinphosphatase (PTP)
CD150 (SLAM, IPO-3)	B and T cells		SLAMF1, IgSF
CD151 (PETA-3)	Macrophages, platelets		TM4-SF

SLAM signaling lymphocytic activation molecule.

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD152 (CTLA-4)	Activated cytotoxic T cells molecule, modulates lymphocyte homeostasis		CTLA-4; 70% similarity with CD28, binds CD80 and CD86, costimulatory; killer cell Ig-like receptors IgSF
CD153 (CD30L)	Activated T cells, B cells, neutrophils, macrophages		CD30L; cross-linking supports T-cell proliferation and IL production; TNFSF5
CD154 (CD40L, T-BAM, gp39)	T and NK cells, basophils, mast cells, eosinophils production; CD154 deletion results in the ID with hyper-IgM	33	Ligand for CD40, activates B cells, costimulates T-cell proliferation and IL production, CD154 deletion results in the hyper-IgM syndrome, TNFSF5, belongs to the family of TNF-α and -β, NGF, and of Fas, CD27, CD30 ligands
CD155 (PVR)	Monocytes	80–90	Poliovirus receptor; IgSF
CD156 (ADAM-8, MS2)	Neutrophils, monocytes	69	Metalloprotease, binds peptidase, possibly involved in leukocyte extravasation
CD156b (TACE/ADAM17)	Adhesion structures		Snake venom-like protease
CD157 (BST-1, MO-5)	B and T lines, monocyte-macrophages, granulocytes	50	Unclear function in myeloid cells
CD158e, i, k (KIR family)	NK cells		NK receptors specific for class I HLA; IgSF
CD159a (NKG2A)	NK cells		Killer cell lectin-like receptor subfamiliy C
CD160 (BY55, NK1, NK25)	NK cells		
CD161 (NKR-P1)	NK cells		Killer cell lectin-like receptor subfamiliy B, binds NK cells
CD162 (PSGL-1)	Myeloid cells, granulocytes, monocytes	120	Sialomucin, binds CD62P
CD162R (PEN5)	NK cells		
CD163 (M130)	Tissue macrophages, LPS-stimulated monocytes	110	Unknown function; SRCR-SF
CD164 (MGC-24)	Epithelium, monocytes, bone marrow stromal cells	80	Potential adhesion molecule
CD165 (GP37/AD2)	T cells		Potential adhesion molecule
CD166 (ALCAM)	Activated T cells and monocytes, epithelium, fibroblasts	100–105	Adhesion molecule binding CD6; IgSF
CD167 (DDR1)	Adhesion structures		Discoidin
CD168 (RHAMM)	Adhesion structures		
CD169	Adhesion structures		Sialoadhesin
CD170 (Siglec-5)	Adhesion structures		
CD171 (L1)	Adhesion structures		
CD172a (SIRP α)	Adhesion structures		

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD173	Carbohydrate structures		
CD174 (Lewis y)	Carbohydrate structures		
CD175 (Tn)	Carbohydrate structures		Tn antigen (T antigen novelle)
CD175s	Carbohydrate structures		Sialyl-Tn
CD176 (TF)	Carbohydrate structures		
CD177 (NB1)	Myeloid cells		
CD178	Cytokine/chemokine receptors		Fas ligand (CD95L), TNFSF6
CD179a	B cells		Vpre-B
CD179b (×5)	B cells		
CD180 (RP105)	B cells		
CD183	Cytokine/chemokine receptors		CXCR3
CD184	Cytokine/chemokine receptors		CXCR4
CD195	Cytokine/chemokine receptors		CCR5
CDw197	Cytokine/chemokine receptors		CCR7
CD200 (OX2)	Thymocytes, B cells, T cells		
CD200R	Monocyte/macrophage, DCs		
CD201 (EPC R)	Endothelial cells		
CD202b	Endothelial cells		Tie2 (Tek)
CD203c (NPP3/PDNP3)	Myeloid cells		Expressed on blood basophils but not on other blood leukocytes
CD204	Myeloid cells		Macrophage scavenger R
CD205 (DEC205)	Dendritic cells		
CD206	Dendritic cells		Macrophage mannose R
CD207	Dendritic cells		Langerin
CD208 (DC-LAMP)	Dendritic cells		
CD209 (DC-SIGN)	Dendritic cells		
CDw210 (IL_{10} R)	Cytokine/chemokine receptor		$IL_{10}RA$, $IL_{10}RB$
CD212 (IL_{12} R)	Cytokine/chemokine receptor		$IL_{12}R\beta$

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD213a1	Cytokine/chemokine receptor		$IL_{13}R\alpha1$
CD213a2	Cytokine/chemokine receptor		$IL_{13}R\alpha2$
CDw217	Cytokine/chemokine receptor		$IL_{17}R$
CD220	Non-lineage molecules		Insulin R
CD221 (IGF1R)	Non-lineage molecules		
CD222	Non-lineage molecules		Mannose-6-phosphate/RIGF2 R
CD223 (LAG-3)	Non-lineage molecules		Lymphocyte activation gene 3
CD224	Non-lineage molecules		γ-Glutamyl transferase
CD225 (Leu13)	Non-lineage molecules		
CD226 (DNAM-1 PTA1)	T cells		
CD227 (MUC.1)	Non-lineage molecules		
CD228	Non-lineage molecules		Melanotransferrin
CD229 (Ly9)	Non-lineage molecules		
CD230	Non-lineage molecules		Prion protein
CD231 (TALLA-1/A15)	Non-lineage molecules		
CD232 (VESPR)	Non-lineage molecules		
CD233 (Band 3)	Erythroid cells		
CD234	Erythroid cells		fy-Glycoprotein (DARC)
CD235a	Erythroid cells		Glycophorin A
CD235b	Erythroid cells		Glycophorin B
CD235ab	Erythroid cells		Glycophorin A/B cross-reactive mabs
CD236	Erythroid cells		Glycophorin C/D
CD236R	Erythroid cells		Glycophorin C
CD238 (Kell)	Erythroid cells		
CD239 (B-CAM)	Erythroid cells		
CD240CE (Rh30CE)	Erythroid cells		Cross-reactive mabs
CD240D (Rh30D)	Erythroid cells		

Table 1.2. (Continued)

CD (synonyms)	Main cellular distribution	MW in kD	Main functions and properties/ligands/receptors/superfamily
CD240DCE (Rh30D/CE)	Erythroid cells		
CD241 (RhAg)	Erythroid cells		
CD242 (ICAM-4)	Erythroid cells		
CD243 (MDR-1)	stem/progenitor cells		
CD244 (2B4)	NK cells eosinophils		Elicites ERK, activates NK cells and causes eosinophils to release EPO, IL_4 and IFN-γ
CD245 (p220/240)	T cells		
CD246	T cells		Anaplastic lymphoma kinase
CD247	T cells		ζ Chain of TcR

CDw128 has been divided into CDw128a (CXCR1, IL_8RA) and CDw128b (CXCR2, IL_8RB). The main cells are underlined following the Immune Receptor Supplements [4, 6, 262] and International Workshops on Human Leukocyte Differentiation Antigens [501]. Data from the 5th International Workshops on Human Leukocyte Differentiation Antigens [453], Immune Receptor Supplement, 1st [4] and 2nd ed [5], 1997, CD antigens 1996 [257], CD Designations, 7th HLDA Workshop [331] and new CD designations (2002) [691] and from [4, 6, 16, 23, 34, 47, 70, 82, 85, 102, 103, 109, 112, 120, 132, 147, 158, 175, 242, 262, 343, 407, 428, 437, 442, 484, 501, 515, 559, 571, 584, 608, 688, 691]. The 8th HLDA Workshop has extended the CD nomenclature up to CD339 (S. Zola pers. com., March 5, 2005). Abbreviations are in the list.

- They are committed to specific, initial antigen recognition by interacting with a restricted part of the macromolecule called epitope by means of specific membrane receptors present on both B and T cells [326].

There are two major sets of lymphocytes. The antibodies produced by B lymphocytes play a crucial role in interacting with extracellular antigens, when found outside the cell (for example, when viruses are encountered in the blood), whereas these antibodies are ineffective against endocellular antigens, because overall they are unable to penetrate the cells. Accordingly, T lymphocytes are probably originally derived to ensure a high specificity in the critical phase of immune responses to pathogens of various sources. *Antigen recognition takes place in a different way for each phenotype,* since T cells respond to antigens only when encountered inside or on the target cell surface, thereby showing that T-cell antigen recognition is fundamentally different from B-cell antigen recognition. Such properties are gradually acquired during ontogenesis when lymphocytes undergo a series of differentiation events, each characterized by sequential expansion or regression of the genes coding for expression of membrane proteins and glycoproteins (gps) [35, 481, 647].

Structure and Molecular Framework

Igs or antibodies are molecules of specific immunity, present in soluble form in biological fluids or as membrane receptors at BcR (B-cell receptor) and TcR surface. The immune system consists on the whole of $\approx 10^{12}$ T cells and of $\approx 10^{20}$ B cells: in the bloodstream ≈80% of lymphocytes are T, 10%–15% are B cells and 10%–15% are *null cells* known as LGLs (*large granular lymphocytes*), non-T non-B since they lack markers of B and T cells. The Ig N-terminal part has a binding site or paratope providing specific recognition. The C-terminal transmits biological signals following epitope-paratope binding. TcR, BcR, and Ig polypeptide chains are composed of subunits, each made up of ≈100 amino acids, bound to disulfide (-S-S) bonds, termed domains or regions. Such striking similarities are also found in molecules belonging to the *Ig superfamily (IgSF):* although these proteins are found in diverse tissues, they all appear to share a common primordial origin.

B Lymphocytes

B cell precursors, the first cells to be appraised in ontogenesis, originate from hemopoietic bone marrow stem cells and complete developmental and maturative processes that began in the fetal liver. Briefly, the earliest distinguishable cells in the B repertoire are known as pro-B cells and pre-B cells with no surface Igs but with surrogate light (L) chains. At this stage the cells express RAG-1 and RAG-2 (recombination-activating genes),

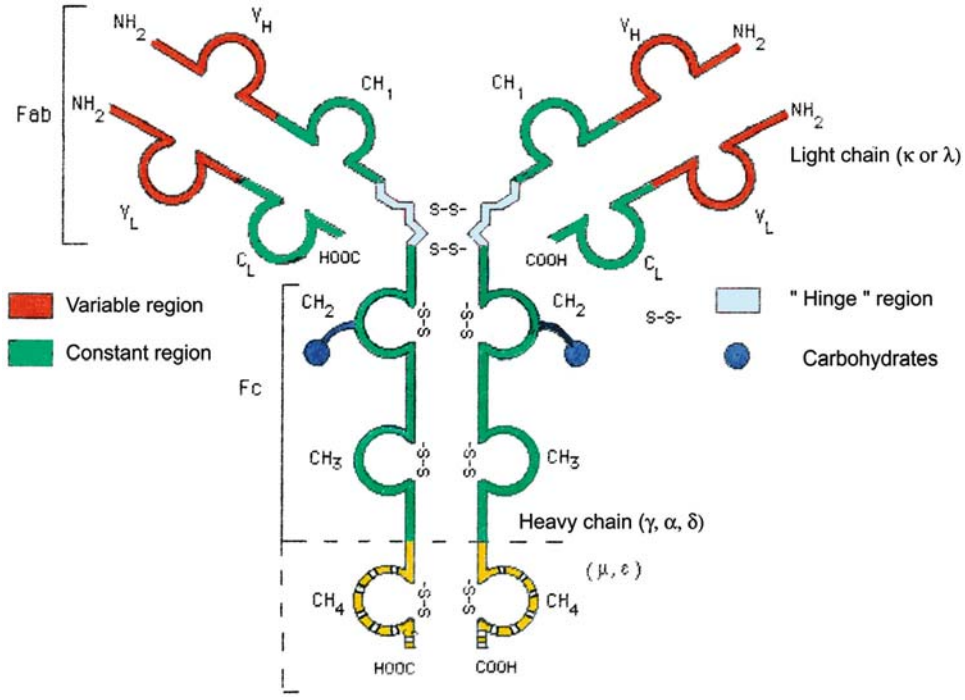


Fig. 1.6. Immunoglobulin basic structure. VH + VL Antigen binding site, which is located in the V (variable) domain. CH_1 + CL Covalent and noncovalent binding between the H (heavy and L (light) chains; spacer between the antigen binding site and effector functions. Intrachain -S-S bonds regulate the molecular flexibility and determine the spatial conformation. CH_2 C1q fixing site (complement activation). CH_2 + CH_3 Binding site for Fc receptor of monocytes, macrophages, neutrophils, streptococcus A protein, syncytial trophoblasts (placenta crossing). CH_3 binding site for Fc receptor of lymphocytes and mast cells

whose deficiency is responsible for one form of SCID; affected patients are unable to produce functional lymphocytes bearing antigen receptors [508]. The next cells in the B-cell lineage bearing monomeric surface IgM (IgMs or mμ) are referred to as immature B cells. In the following stages of maturation, a mature B cell expresses IgM, IgD, and Fc receptors (FcR) for IgGs. Up to this point, all maturative steps take place in the bone marrow and are antigen-independent (Chap. 2). It has been suggested that bursal epithelial cells might produce polypeptides, triggering maturation of lymphoid stem cells into immunocompetent B cells, similarly to what occurs in the thymus. These short-lived lymphocytes colonize typical zones of lymph nodes and the spleen. Each cellular clone synthesized in a GC produces isotypes of the same class, specific for a given antigen. During the evolution of immune responses, there is a switch in Ig classes from IgM to IgG, IgA and IgE. If IgG and IgA antibodies are produced last, this could account for an associated defect of the two Ig classes. Babies with X-linked Hyper-IgM Syndrome (HIgMS) have little or no IgG and IgA [96, 544].

The Igs are structurally similar. A typical antibody molecule has a Y-shaped configuration when viewed schematically (Fig. 1.6) and consists of a basic unit of four polypeptide chains, two identical L and two identical H (heavy) held together by interchain -S-S bonds also present on single chains and by hydrogen bonds. As the figure shows, NH_2 and COOH indicate amino and carboxy terminals, respectively. L chains are common to the diverse Igs, which instead differ from their H chains that determine class type, hence IgA H chains are α, those of IgG are γ, etc. Each L chain is made up of two domains: a V region that varies from antibody to antibody and a constant (C) region, essentially identical among L chains of a given type (Fig. 1.7). For this reason, the molecules are practically identical, except in amino-terminal domains where variability differentiating V domains of both L and H chains is most pronounced [18]. In man, C regions are encoded by one of four C_H genes for H chains and by one of two C_L genes for L chains (Lλ and Lκ). H chains therefore consist of four C and one V domains and L chains of one C and one V region. Both chains, organized in sequence, are distinguished by their succession of amino acids: L chains consist of 211–217 amino acids and H chains of 450 for γ and α chains, and of 614 for μ and ε chains [3]. One noteworthy characterization of Igs lies in their *bifunctional aspects,* in that they bind antigens and, in addition, elicit biological processes that are independent of antibody specificity. Each of these functions is localized to a different part of protein: C domains have various effector activities (complement activation, opsonization, etc.) via IgG, IgE and IgG_4 FcRs, while V do-

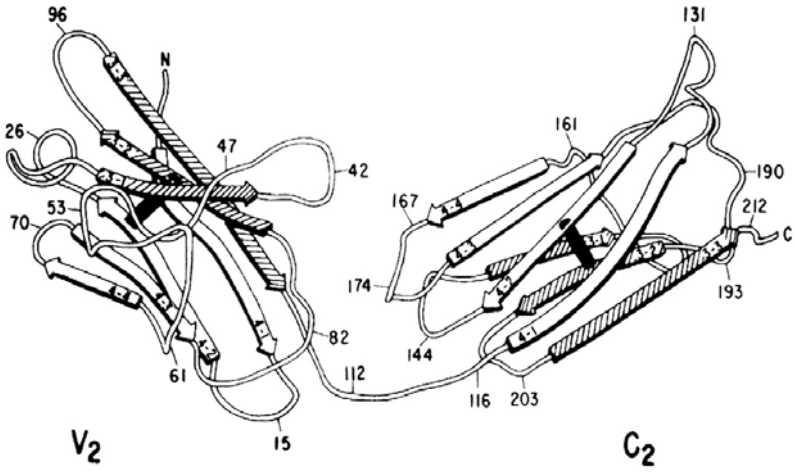


Fig. 1.7. Structure of V and C domains of a light chain. The -S-S bridges in each domain are indicated by *thick black lines*. The hypervariable regions that form the antibody combining site or paratope correspond to the loops around positions 26, 53 and 96

mains are committed to the specificity of antigen recognition. V regions within any group are not uniformly variable across their whole 110 amino acid span. Instead, the greatest amount of variability of V domains of each chain (genes V_H and V_L) is concentrated in three regions of both L and H chains called hypervariable regions: the less variable stretches interspersed within these hypervariable regions are called *framework regions* (FR) of 15–30 amino acids. The six hypervariable regions, which are each 9–12 amino acids long, participate in antigen binding and form a region that is complementary in structure to antigen epitopes, accordingly designated *complementarity determining regions* (CDRs) principally involved in antigen contact, three in V_H domains and three in V_L domains [86]. H chains γ, α and δ are formed by four C_H (C_H1, C_H2, C_H3 end C_H4) domains, μ and ε by five. Only γ, α and δ chains have a hinge region located between two C_H domains; however, for the other two chains there is the C_H2 region. The C domain of H chains is dictated by one gene (C_γ, C_μ, C_ε, etc.) dictating isotypes [474].

To understand the diversity of antibody repertoires, recent evidence shows that coding information of a typical human gene, that is the sequences coding regions to be transduced into amino acid sequences, does not exist as intact, functional genes. Therefore a single, uninterrupted DNA stretch in germ cells is broken up along the chromosome and dispersed along the DNA strand into multiple, shorter discrete entities (exons), widely separated by noncoding DNA regions (introns). Eventually, exons coding for V domains may be broken down in still smaller segments, each lacking some features needed for proper RNA splicing and unable to function separately [470]. Consequently, a V domain of a V_L chain is encoded by two separate DNA gene segments, exons separated by introns: the first 95 amino acids of V_κ domains are dictated by a V exon, the shorter exon J_κ (joining) codes the remaining 13 amino acids (96–108) of C-terminal domains [414]. H chains are dictated by V_H, J_H, D_H (diversity) and C_H exons; the D exon, absent in L chains, dictates a few amino acids and is in charge of the higher H chains variability compared to L chains [3]. An L chain gene is assembled from three types of gene segments: V_L and J_L are transferred on their genome (total inherited DNA) and integrated with a C_L segment. This machinery, known as *gene rearrangement*, necessary for transcription of gene information (mRNA synthesis) is employed only for genes dictating Ig L and H chains or TcR. After genes are transcribed into RNA, introns are removed from transcripts and exons are joined together by RNA splicing. As will be seen later, this rearrangement is the core of the capability shown by the immune system to recognize an incredible variety of antigen structures in nature [18].

Genetic Rearrangements for Ig Synthesis

Antibody molecules are encoded by three independent groups of genes. Two genes dictate λ chains: one comprises V_λ and C_λ genes, κ comprises V_κ and C_κ, genes, while the third group dictates H chains and has V_H and C_H genes. Before synthesizing Ig chains, however, Ig genes must be assembled within a genome of differentiating cells (rearrangement). Briefly, a V region gene can be located in a DNA position of an inherited chromosome (*germline*), and can then move to another position on the chromosome during lymphocyte differentiation. For example, five J segments of a κ chain are clustered near C exon, whereas at least 30–35 different V segments lie scattered widely over many DNA kilobases (kb). Rearrangements follow an order fixed in advance: H chain first, then L κ, finally L λ, encoded by chromosomes *14q32*, *2p12* and *22q12*, respectively (Fig. 1.8), with resulting deletion of intervening genetic materials and consequent realignment according to a configuration calling for Ig generation [186].

In the rearrangement of H chains, selection of one each from ten D, five J genes, and ≈200 or more V regions will produce a tripartite gene complex VDJ recombination for V_H regions. VDJ recombination is the process by which the V region exons encoding the antigen recogni-

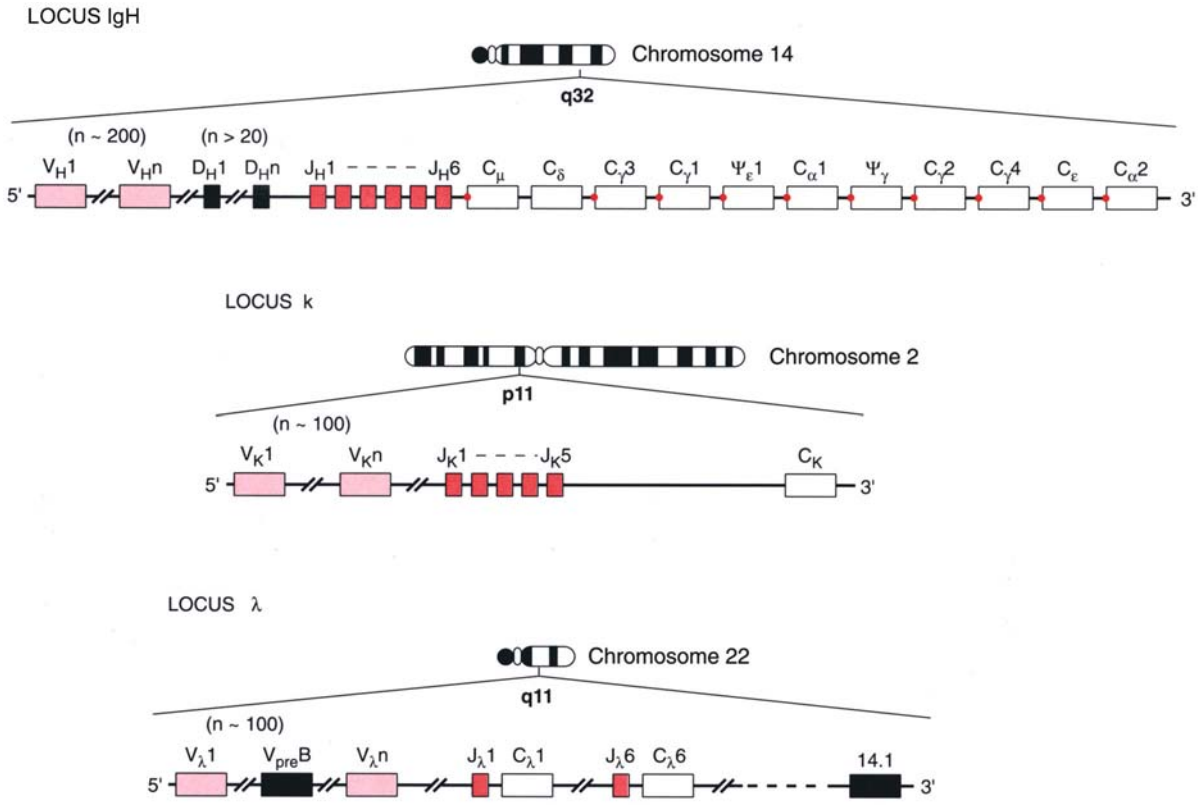


Fig. 1.8. Schematic representation of chromosomes 14, 2 and 22

tion sites of receptors expressed on B and T lymphocytes are generated during early development via somatic assembly of component gene segments [128]. During maturation of B and T lymphocytes, the first rearrangement involves H *locus* with the juxtaposition of D and J genes (DJ rearrangement) by the deletion of intervening DNA. This rearrangement occurs in a very early stage of maturation, when precursors are not yet clearly committed to B lineage; indeed it is also observed in 20% of T cells [474]. The second rearrangement involves subsequent translocation of DJ segments to selected V region genes (VDJ rearrangement) (Fig. 1.9), which occurs in pre-B cells, already committed to B lymphocyte family. As a consequence, the VDJ segment joins the C_H gene [591]. However, recombination can be somewhat imprecise, since during this process several nucleotides may be removed from or added to a junction, so combination of VDJ segments of H chains results in a large number (10 D_H × 4 J_H × 200 V_H) of possible sequences (that is, antibodies). This reshuffling process, known as *combinatorial joining,* is the prevalent source of protein diversity. For example, when organizing κ genes, as there is only one Cκ exon, all κ proteins must have identical C domain sequences, while cells can choose among several Vκ and Jκ segments, with the result of joining such segments in diverse combinations. Thus, a large number of different V region sequences can result and when combined with 50 κ chain V domains could form 400,000 different V_H genes. Considering a necessary heterogeneity of gene segments and combining 400,000 with 200 V_κ and four J_κ genes (200×4) × 10 equal to flexibility of VJ rearrangement, we have *at least* $3{\times}10^8$ *different antigen-binding sites*. Such an outstanding recombinant capacity is further increased by somatic mutations to 10^{10}–10^{15} [18]. Nonetheless, even using a relatively small amount of gene segments, the immune system can secrete an impressive antibody diversity through combinatorial joining [414].

In the most common scenario, a VDJ segment joins first with C_μ genes, and subsequently with C_γ, C_ε, C_α genes, with synthesis of a complete H IgM chain, etc. Without an associated L chain, surface expression is not possible and only cytoplasmic μ is found (pre-B cells). Isotype switching requires DNA rearrangements. Consequently, a single V gene is associated in successive phases with different C genes on the same chromosome, thereby allowing each cell to produce antibodies of different isotypes with the same paratope. Several components that are involved in this process have been identified. Among them, a complex system of enzymes and other proteins jointly known as *recombinase activity* appear to be necessary for gene rearrangements. Thus the enzyme VDJ recombinase catalyzes recombination processes, during which intervening DNA is usually excised. However, since similar sequences are found also

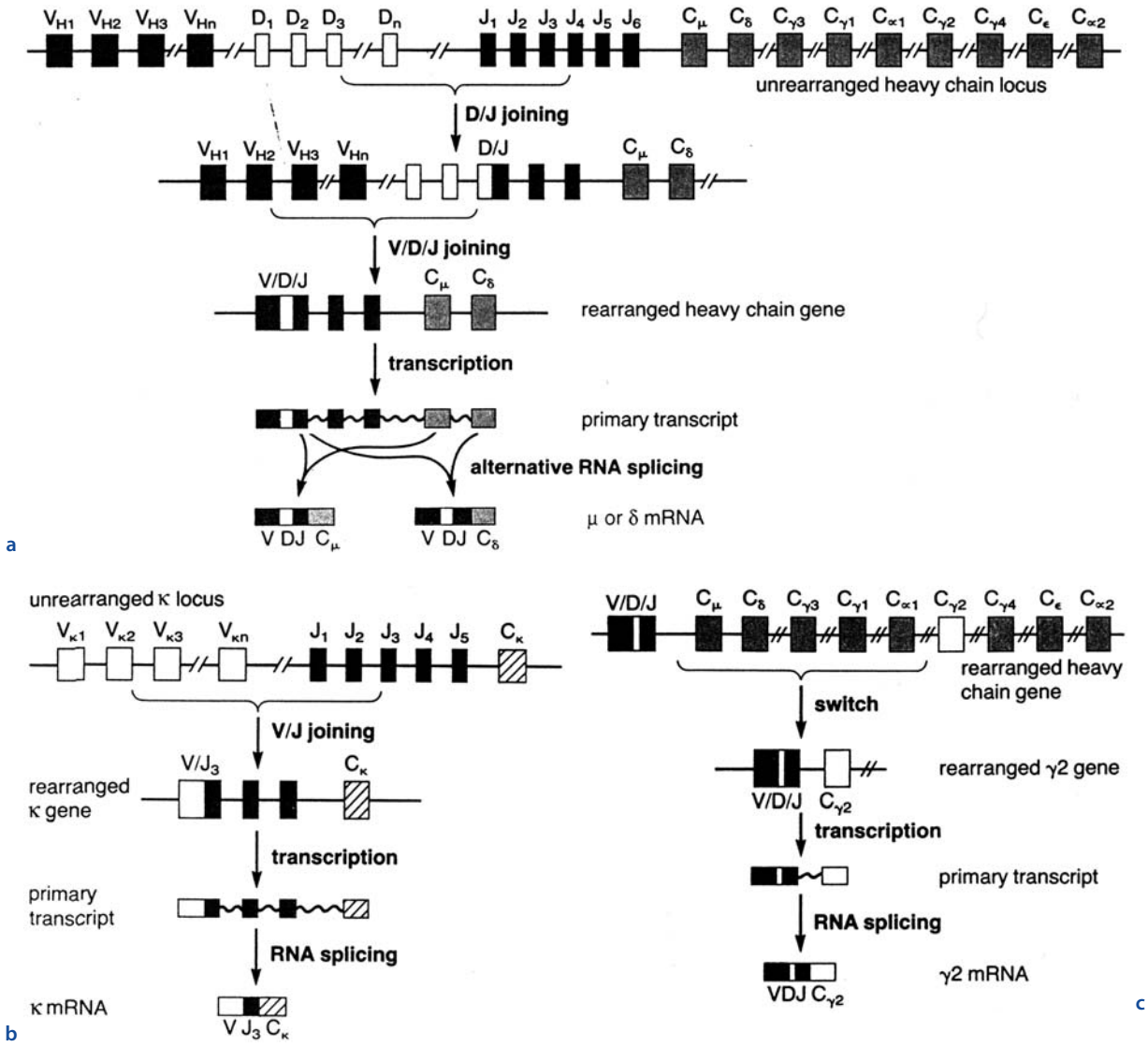


Fig. 1.9. **a** Schematic depiction of transcription, rearrangement and splicing of an H chain (Hμ or Hδ). **b** Schematic depiction of transcription, rearrangement and splicing of an L chain (Lκ or Lλ). **c** H-chain isotype switching

in TcR T-cell regions, it appears that VDJ recombinase is used by both B and T cells. VDJ recombination proceeds via precise DNA cleavage initiated by the RAG proteins at short conserved signal sequences [128]. Whatever their precise role, the coordinated expression in pre-B and pre-T cells is essential for the rearrangement of Ig genes and TcR αβ chains, but RAG activity is switched off in mature lymphocytes [299]. Mutations of such genes consisting in imprecise gene segment joining of coding sequences during VDJ recombination appear to cause SCID with B and T cells severely impaired [299].

After hierarchical H-chain rearrangement, L-chain rearrangement occurs (Fig. 1.9): when a D segment is absent, V domain is encoded by VJ genes (VJ rearrangement), with subsequent juxtaposition to respective C segment; λ genes are rearranged only in the event of a nonproductive κ rearrangement. This procedure continues until efficient V genes are generated for H and L chains and ends with synthesis of functional polypeptides.

Rearrangements are carefully orchestrated, following the principle of *allelic exclusion,* that is, in each B cell is transcribed the gene product of only one of each chromosome pair. The gene located on the second chromosome typically is not used, to prevent contemporary synthesis of H chains with differing V domains in any given cell; it is an *allele*, a term designating two genes or two or more alternate forms of a single gene occupying the same *locus* [591]. The gene on the second chromosome is not rearranged unless a nonproductive rearrangement occurs; when rearrangements are nonproductive on both chromosomes, cell death ensues, there-

by clarifying why the same B lymphocytes within their entire life span can produce only a single type of L chain (κ or λ) [474].

Precombination of gene segments implies further elements of diversity, due to either an imprecise DNA rearrangement of nucleotides that are believed to be inserted at V–J junction, which increases junctional diversity of L chains, or of H chains by mechanisms amplifying diversity, including the possible joining of two D genes, with or without inversion of one rearranging gene segment, as well as disparities in recombination of chromosome 14 [263]. Another very important mechanism generating variations is somatic mutations, which are able to introduce a heterogeneity even higher in nucleotide sequences. Insertions of such nucleotides are carried out by the enzyme terminal-deoxynucleotidyl-transferase (TdT), a terminal-independent DNA polymerase expressed in B lymphocytes early during their development, but also in thymocytes of all species. The variations in gene sequences resulting from imprecise joining or from insertion of N regions, via addition of nucleotide sequences, absent in germline, provide a supplemental diversity at VDJ junctions designated "N-region diversity." Moreover, these processes affect sequences within each VDJ exon coding for CDR3 of L- or H-chain V domain. Hence, TdT can be viewed as another component of the VDJ recombinase system [263]. This implies that all these mechanisms engendering a disproportionate antibody diversity leave an unknown fraction of B cells to aberrant Ig gene rearrangements, probably >50% [47]. Recent data show that TcR and Ig V, D, and J gene segments are flanked by conserved *recombination signal sequences* (RSS), consisting of a heptamer and a nonamer separated by a nonconserved spacer of either 12 or 23 nucleotides. The 12–23 base pair rule first postulated to explain Ig gene rearrangement also governs TcR gene recombination. Virtually, during a rearrangement, a gene with a flanking sequence containing a 12-base pair spacer can only join to a gene whose flanking sequence has a 23-base pair spacer and vice versa, thereby elucidating the precise order of Ig gene transcriptions [337].

In conclusion, because of the very efficient DNA usage displayed in gene rearrangements, as well as RNA transcription and joining machinery described above, B lymphocytes can produce an isotype switching conserving antigen specificity, although utilizing an assembly of about 300 gene segments, there would be several million different antibodies in the same subject [3]. Once the recombination process is completed, C region genes of both H and L chains are associated with the VDJ or VJ complex. In this way, the VDJ complex becomes joined to C region genes of other isotypes located downstream on the same chromosome. The conclusion of such rearrangements is the production of different isotypes of the same antibody. Such antibodies capable of recognizing a specific antigen will be on the whole clone, on the whole progeny originating from a single B cell precursor. In that way, each lymphocyte clone can respond only to antigens able to bind to its unique pair of H and L chains, and all antibodies synthesized by an activated clone are directed against that particular antigen, as in the case of B lymphocytes, or has receptors ready for the antigen, as in the case of T lymphocytes [470].

On the other hand, papain digestion splits Ig molecules yielding three fragments of roughly similar size (50 kD), two monovalent Fab (fragment antigen binding) fragments with a whole L chain and half H chain (retaining ability to combine with antigen), and an Fc fragment (fragment crystallizable) comprising carboxy-terminal portions of both H chains. On B cell membrane, Igs are disposed as follows: the tail of Y is the Fc fragment, while the arms correspond to Fab fragment (Fig. 1.6).

Pepsin digestion yields a single large fragment called $F(ab)'_2$, roughly corresponding to two -S-S linked Fab fragments with bivalent antigen-binding activity. Antibody ability to fix on several cell types with Fc region of H chains takes place via FcRs, present on different cells. FcR molecules appear to exemplify a point of contact and cooperation between humoral and CMI. The main FcR for H chains of different Igs are summarized in Table 1.3 [186, 486].

In addition to the variability related to paratope diversity for antigen molecules, under appropriate circumstances antibodies deliver three main forms of Ig epitopes as defined by their location on antibody molecules, that is isotype, allotype, and idiotype determinants [18].

The term *"isotype"* is often used as equivalent to the terms "class" and "subclass;" isotypes are Ig epitopes codified by identical gene segments found in all healthy individuals of the same species. Individuals of a different species have different isotypes, since each isotype is located on a distinct gene locus on the pertinent genome. Antibody isotypes are defined principally in relation to H- or L-chain C regions and in some instances to V_H and V_L invariable regions, as are Ig epitopes that characterize classes, subclasses and types of H and L chains. As a general rule, isotypes are recognized by antibodies produced by different species.

The *allotypes,* or genetic markers, a form of variation in Ig structure, are allelic forms of the same protein as a result of different forms of the same gene at a given locus. They are located in C regions of both L and H chains: allotypes of L chains are named Km, those of H chains Gm, Am, and Mm. Inherited as dominant mendelian traits, they stem from genetic differences in amino acid sequences of C genes (*genetic polymorphism,* GPM). Allotypes are accepted in some countries as legal evidence in paternity disputes. As in other allelic systems, variants are not present in all healthy subjects and therefore distinguish Igs of one subject from Igs of another, as do blood groups.

The *idiotypes* (from Greek "ιδιος," personal or private), are as many as B-cell clones, 10^8 in adults [186], and are formed by single idiotopes building a given paratope with its characteristics and binding idiotypes

Table 1.3. Immunoglobulin (*Ig*) receptors

Receptors	CD	Immunoglobulins	Cells
FcαR	89	IgA	Neutrophils, lymphocytes, monocyte-macrophages, eosinophils
FcεRI	–	IgE	Mast cells, basophils, eosinophils, LC
FcεRIIa	23	IgE	B cells
FcεRIIb	23	IgE	B and T cells, eosinophils, LCs, NK cells, platelets
FcγRI	64	IgG_1 +++, IgG_3 +++, IgG_4 ++	LCs, monocytes, neutrophils
FcγRII	32	IgG_1 +, IgG_3 +	B, T and NK cells, monocytes, macrophages, eosinophils, neutrophils, platelets, basophils, LC, FDC, epithelial and stromal cells
FcγRIII a, b	16	IgG_1 +, IgG_3 +	T and NK cells, neutrophils, macrophages

FcαR consists of an α chain linked to IgA and a dimeric chain FcγR for transduction of signals [470].
Modified from [186, 470].
LC Langerhans' cells, *FDC* follicular dendritic cells.

to antibody specificity [469]. Being determined by antibody V regions in hypervariable regions, *they are not linked to the class*: each idiotype is defined by association of V_H and V_L domains and therefore distinguishes one V domain from the others. There are two types, those associated with paratopes are located very near CDRs and those recognizing structures outside the binding site are carried by hypervariable and V regions, respectively. Based on such definitions, we can see two ways to evaluate V regions: by serologic aspects (idiotype) or by antigen-binding properties (paratope) [58].

The IgSF (Table 1.4) [4, 23, 34, 109, 139, 186, 323, 557, 693] (Fig. 1.10) is formed by polygenic groups of genes and single genes, structurally correlated but not necessarily functionally, but whose structural features recall those of Igs; they belong to families sharing an evolutionary homology, probably originating from a few ancestral genes. It is presumed that V regions of such molecules may have a role in intercellular interactions, or as receptors for soluble ligands [186].

T Lymphocytes

T cells especially, but also B cells and other immune cells, synthesize ILs, proteins with a molecular weight (MW) of 15–25 kD governing intensity and duration of immune reactions, mediating cellular proliferation and maturation, and also wound healing (Tables 1.5–1.8) [4, 14, 34, 45, 50, 52, 55, 66, 68, 78, 92, 98, 118, 119, 126, 128–130, 140, 141, 152–154, 156, 158, 167, 172, 181, 213, 219, 220, 240, 245, 249, 258, 272, 278, 318, 326, 372, 377, 383, 387, 398, 399, 412, 413, 421, 426, 437, 455, 461, 474, 480, 481, 487, 517, 523, 526, 528, 532, 534, 560, 562, 607, 641, 669, 693]. In parallel, there is a leukocyte superfamily (Table 1.9) [27], less extensively represented as the IgSF.

Table 1.4. Constituents of the IgSF

H and L chains
Class I and II HLA molecules (correlated)
β_2-Microglobulin
TcR, CD3, CD2, CD7, CD4 and CD8
BcR, CD79a, CD79b
Poly-IgR (receptor of IgM and IgA antibodies), a fragment of poly-IgR constitute the SC of sIgA
Adhesion molecules, integrins, selectins, etc. (see below)
Additional molecules: CD1, CD16, CD19, CD22, CD28, CD32, CD47, CD48, CD58, CD64, CD66a-e, CD80, CD83, CD86, CD89, CD90, CD96, CD101, CD117, CDw150, CD152, CD155, CD158 a, b, CD166
Receptors of: IL_1 (IL_1R, I and .II, CD121a and 121b) IL_6 (IL_6R, CD126) GM-CSF (GM-CSFR, CD116) M-CSF (M-CSFR, CD115) PDGF, etc.

Table 1.4. (Continued)

Adhesion molecules as part of the IgSF		
Molecules	**Ligands**	**Distribution**
CD1	?	Thymocytes and DCs
CD2	CD58 (LFA-3], CD48, CD59	T and NK cells, endothelial and epithelial cells
CD3/TcR	Nominal antigen	T cells
CD4	HLA-II	T cells, monocytes
CD7	?	T and NK cells
CD8	HLA-I	T and NK cells
CD22	CD45RO	B cells
CD28	CD80, CD86	T cells and plasma cells
CD31 (PECAM-1])	CD31, heparin	T cells, endothelial cells, monocytes, platelets, neutrophils
CD48	CD2	Hematopoietic and not hematopoietic cells
CD50 (ICAM-3)	CD11a/CD18	T cells, monocytes, neutrophils
CD54 (ICAM-1)	CD11a/CD18, CD11b/CD18, CD43	Activated T and parenchymal cells, NK cells, endothelial and epithelial cells, fibroblasts, monocytes, chondrocytes, DCs
CD56 (N-CAM)	CD56	T, B, NK cells, endothelial and epithelial cells, keratinocytes, DCs
CD102 (ICAM-2])	CD11a/CD18	Endothelial cells (high expression), epithelial, T, and NK cells, monocytes, DCs, platelets (low expression)
CD106 (VCAM-1)	CD49d/CD29, $\alpha_4\beta_7$	Endothelial cells, monocytes, DCs, fibroblasts, stromal medullary cells, myoblasts
HLA-I	CD8	APCs
HLA-II	CD4	APCs
Ig	Nominal antigen	B cells
MAdCAM-1	CD62L	Mucosal and lymphoid HEVs

CD2, CD48, CD58 form a family, although they are located in pericentric loci of two different chromosomes: 1p13 for the first two and *1q21–23* for the third one; a correlated family is CEA, with CD66b (ex CD67) and CD66e (NCA).
Data from [4, 6, 23, 34, 109, 139, 186, 323, 557, 693] and International Workshops on Human Leukocyte Differentiation Antigens [501].
APCs antigen-presenting cells, *CEA* carcinoembryonic antigen, *DCs* dendritic cells, *ICAM-1, 2, 3* intracellular adhesion molecules-1, 2, 3, *IgSF* immunoglobulin superfamily, *LFA-3* lymphocyte function-associated antigen-3, *MAdCAM-1* mucosal addressin cell adhesion molecule-1, *N-CAM* neural cell adhesion molecule, *PECAM-1* platelet endothelial cell adhesion molecule, *SC* secretory component, *VCAM-1* vascular cell adhesion molecule-1.

A tentative nomenclature may divide ILs into five main types:
a) Interleukins (IL_1–IL_{33})
b) Hematopoietic growth factors (CSF)
c) IFN
d) TNF
e) Growth factors: EGF (epidermal growth factor), FGF (fibroblast growth factor), PDGF (platelet derived growth factor), TGF, etc.
ILs are released from several inflammatory effector cells [151] (Table 1.5):
1) From *Th1 T cells* IL_2, IL_3, IL_{12}, IL_{18}, IL_{22}, IL_{23} IL_{27}, IL_{32}, TNF-α (tumor necrosis factor-α) and -β, IFN-γ, and GM-CSF
2) From *Th2 T cells* IL_3, IL_4, IL_5, IL_9, IL_{10}, IL_{11} IL_{13}, IL_{18}, IL_{25}, IL_{27}, IL_{31}, GM-CSF, whose genes are associated with the chromosome 5 gene cluster in region q31–q33, together with IL_6, IL_9, IL_{12}, IL_{13}, M-CSF and CD14 (IL_4, IL_5, IL_9, IL_{11}, IL_{13}, IL_{16}, IL_{17}, IL_{25} induce asthma)
3) From Th1 and Th2 lymphocytes IL_{14}, IL_{28}, IL_{29}, GM-CSF, IFN-γ, BaDF, NGF
4) From B lymphocytes IL_{10}, IL_{12}, IL_{14}, IL_{19}
5) From endothelial cells GM-CSF, TNF, IL_1, IL_3, IL_6
6) From fibroblasts GM-CSF, G-CSF, M-CSF, IL_1, IL_3, IL_6, IL_{15}
7) From hemopoietic cells IL_{23}
8) From mast cells IL_3–IL_6, GM-CSF and TNF-α
9) From monocyte-macrophages IL_1, IL_6, IL_8, IL_{10}, IL_{12}, IL_{15}, IL_{18}, IL_{19}, GM-CSF and TNF-α
10) From NK cells GM-CSF, M-CSF, IFN-γ, IL_2, IL_{15} and TNF-α
11) From PBMCs IL_{24}
12) From skin and trachea IL_{20}
13) From thymus and medullary stromal cells IL_7, IL_{11}.

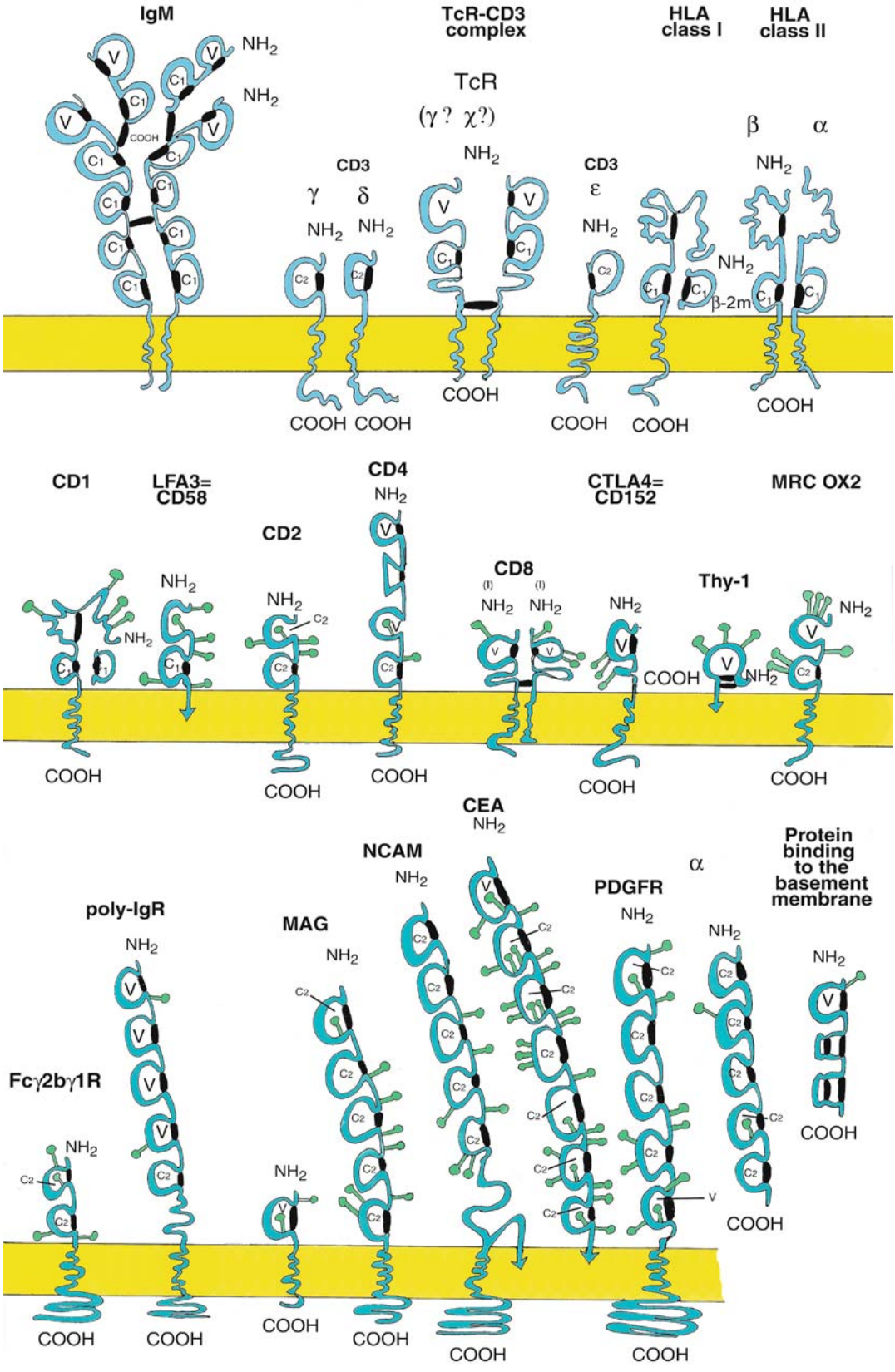


Fig. 1.10. Immunoglobulin superfamily (IgSF). Protein binding to the basement membrane. A model is shown for each molecular type from one species, the *circles* show sequence segments that form Ig domains. α *1Bgp* non-cell surface glycoprotein, β_2-*m* β-microglobulin, *CEA* carcinoembryonic antigen, *MAG* myelin associated glycoprotein, *poly-IgR* immunoglobulin receptors, *NCAM* neural adhesion molecule, *PDGFR* platelet-derived growth factor receptor = CD140, *Po* myelin protein, *Thy-1* and *MRC OX2* brain/lymphoid antigens

Table 1.5. Main characteristics of interleukins (*ILs*; cytokines)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_1	α and β (17 and 15–17; *2q13–21*) and IL_1RA [27] with a structure similar to that of IL_1, however, competes for binding of type I receptors $IL_1α$ Sources: Several different cell types, especially monocytes and macrophages, T and B cells, NK cells pluripotent stem cells: proliferation T lymphocytes, primarily Th2, but also Th1: stimulate to express IL_2 B and NK cells: activation, differentiation and proliferation Macrophages: activation Basophils: differentiation $IL_1β$ Sources: T and B lymphocytes, dendritic cells (DCs), endothelial, and epithelial cells, fibroblasts, etc. Effects: Induces acute-phase responses of liver (thus playing an important pro-inflammatory role) Stimulates the production of IL_2, IL_3, IL_6, IFN-γ by activated, antigen-specific T lymphocytes IL_1 and IL_{18} have an analogue signaling pathway, are key molecules in both the innate and adaptive immunity, and are members of a larger family of related receptors, some of which contribute to host defense TRAF6 participates in IL_1 signaling
IL_1R	$IL_1α$ and β bind with about the same affinity to the same cell surface receptors IL_1R-activating kinase (IRAK), leads to translocation of NF-κB
IL_1R type-I	(80) T and endothelial cells, fibroblasts, hepatocytes and other cells, has an extended amino acid cytoplasmic tail and after binding IL_1 transmits signals intracellularly
IL_1R type-II	(6) Defined as inactive, can function as a precursors of soluble forms binding IL_1
IL_2	(15–20; *4q26–28*) Sources: Exclusively Th1, NK, B cells, and mast cells Effects: Stimulates antigen activation of TcR T lymphocytes stimulated by IL_1 (CD4 and cytotoxic cells = TCT, NK cells and TCT IL-activated cells = LAK): Growth, activation, and differentiation B lymphocytes a) Proliferation and differentiation b) Isotypic selection of IgG_2 Basophils and macrophages: differentiation, increase of TNF-α expression and/or production of IL_3, IL_4, GM-CSF (colony-stimulating factor) and IFN-γ
IL_2R	Formed by three chains: α (55; 10) one affinity-modulating subunit, and β (75; 22) and γ (64; X), two essential signaling subunits Mediates the functions and activities of IL_2 (see above); resting NK cells express its β and γc receptor; the β chain associates with JAK1 and γc associates with JAK3 JAK1/JAK3 (and STAT3/STAT5): induce IL_{15}-like activities $IL_2Rγ$ gene (*Xq13*) encodes the γc-chain of the IL_2, IL_4, IL_7, IL_9, IL_{13}, IL_{15} receptors that are essential for the development of T and NK lymphocyte subsets
IL_3	(14–30; *5q31–33*, where linkage to human asthma has been demonstrated) Sources: Bone marrow stromal cells, activated Th cells, mast cells, keratinocytes Effects: Hematopoietic stem cells (all lines): growth factor and differentiation B and T cell early precursors: growth factor Basophils and mast cells (mostly of mucosal type): proliferation, differentiation, activation, degranulation Basophils: mediator release NK cells and LAK: stimulation Eosinophils: pro-inflammatory alterations

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_3R	Has α and β chains, the β chain is common to IL_5R and GM-CSFR
IL_4	(18–20; *5q31–33* on gene cluster) Sources: Progenitor cells, B and Th2 subsets, and mast cells Effects: Hematopoietic stem cells: differentiation, proliferation B and T lymphocytes: activation, proliferation and differentiation B lymphocytes a) Isotype switching of IgG_1 and IgE antibodies b) Up-regulation of HLA class II and of FcεRI for IgE antibodies c) Induction of ε-germline transcription in B cells T lymphocytes: a) Differentiation of naive T cells to Th2 cells producing Th2-like ILs b) Proliferation of Th2 cells c) Stimulation of antigen activation of TcR Th1 lymphocytes: inhibits switch of T cell differentiation from Th0 to Th1 and IFN-γ production Eosinophils: transendothelial migration Macrophages: inhibit activation Mast cells: growth factor (in experimental animals, in synergy with IL_3), up-regulates FcεRI and CD54 Basophils: up-regulates FcεRI Mononuclear phagocytes: up-regulates FcεRII and HLA class II Neutrophils: activation (see also IL_{13}) Fibroblasts: up-regulates chemokine production Epithelial cells: up-regulates mucin gene expression Endothelial cells: up-regulates CD106 Additional effects: Is thought to exert its effects through the FcεRI present on several cell lines (see text) Induces the expression of FceRII by eosinophils, monocytes and platelets Down-regulates the production of IL_8 by monocytes, of $IL_1α$, IL_6 and TNF-α by macrophages and of IL synthesis by Th1 subsets Activates STAT3 and STAT6
IL_4R	(CD124) (*16p12*) hematopoietic precursors, B and T cells, fibroblasts The receptors signaling triggers the activation of JAK-1 (via the receptor α) and JAK-3 (via the common γc chain, part of this receptor Stimulates T-cell growth, and B-cell activation to promote IgE isotype switching and T–B interactions, induces CD8 cells to produce IL_4
IL_5	(25–50; *5q31–33* on gene cluster) Has α and β chains, the β chain is shared by IL_3 and GM-CSF receptors, explaining the overlapping biological activities Sources: Th2 subsets, mast cells and eosinophils Effects: Activated B lymphocytes: a) Differentiation b) IgA and secretory IgM (sIgM) production c) Expression of IL_6 receptors T lymphocytes: a) Cytotoxic activity b) Expression of IL_2 receptors c) Antigen activation of TcR Eosinophils: a key role in augmenting activation, differentiation, vascular adhesion (CD11b), life span *in vitro* (eosinophilopoietic IL) and IgA-mediated degranulation Basophils: histamine release
IL_5R	(60) The α chain (CD125) (60 kD) has non-binding β chain (CD131) (95 kD) shared with IL_3R and GM-CSFR α chains to form the high-affinity IL_5R [62] Distribution: eosinophils and basophils Binding to IL_5 promotes growth and differentiation of eosinophil precursors, activating mature cells

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_6	(21–28; *7q15–21*) Pleiotropic cytokine induced by IL_1, IL_2, TNF, PDGF and IFN and inhibited by IL_4 and IL_{13} Forms a family with IL_{11}; they have overlapping effector profiles and multimeric receptor complexes in which the promiscuous gp130 molecule serves as a signaling subunit Sources: T and B lymphocytes, monocytes, macrophages, fibroblasts, keratinocytes, hepatocytes, epithelial and endothelial cells Effects: Th2 cells: synergize with IL_1 or TNF to promote growth of immature thymocytes and activation of mature T cells B cells: a) Growth and differentiation b) Ig production: synthesis of IgA, of IgG subclasses, and of IgE induced by IL_4 NK cells: activation TCT: differentiation Additional effects: Antiviral activity Hepatocytes: synthesis of acute phase proteins up-regulating the gene transcription Interacts with CSFs in the proliferation and cell differentiation of pluripotent stem cells Inhibits the inducer and effector phases of delayed-type hypersensitivity
$IL_6R\alpha, \beta$	The α chain is CD126 (80 kD), and the β chain CD130 (130 kD) (gp130), high-affinity IL_6R is formed with CD130 Distribution: resting or activated B cells, plasma cells, T cells, monocytes, fibroblasts, hepatocytes, neural cells $IL_6R\beta$ has signaling chains for receptors for IL_{11}, keratinocytes, LIF, oncostatin M (OSM), and ciliary neutrophil factor (CNF) Overlapping actions: induction of differentiation and proliferation of hematopoietic precursors, and of acute phase proteins
IL_7	(25; *8q12–13*) Sources: Chiefly thymic, bone marrow and stromal cells, but also human keratinocytes Effects: T precursors: proliferation and differentiation of early thymocytes Mature T cells: enhancement to produce IL_2 and IL_2R IL_2 and IL_6 synergize with IL_7 effects on T cells TCT and LAK cells: generation, proliferation and activation Pro- and pre-B lymphocytes: proliferation and differentiation of progenitor cells Megakaryocytes: maturation Monocytes: IL_1, IL_6 and TNF secretion $\gamma\delta$: Proliferation
IL_7R	CDw127 (68 kD) associated with the γ common chain of CD132 (64 kD) to form the high-affinity IL_7R The common γc chain is part of this receptor Distribution: immature thymocytes, pre-B cells, mature T cells, monocytes, $\gamma\delta$ IEL Induction/promotion of immature T-cell growth, expression of CD25 on T cells, proliferation of pre-B/B cells, and monocyte activation, critical for T lineage but not for B lineage development
IL_8	(8–10; *4q12–21*), neutrophil-activating peptide (NAP) Sources: Activated monocytes and macrophages, in addition to monocytes, endothelial and epithelial cells, T lymphocytes, fibroblasts, keratinocytes, hepatocytes, and chondrocytes IL_8 production is stimulated by LPS, IL_1, TNF and virus

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Effects: Neutrophils: a) Potent chemoattractant b) Activation and induction of degranulation, respiratory burst and adhesion to endothelium via CD11b/CD18 T lymphocytes: chemotactic activity and directed migration Basophils: inhibits histamine release
	Additional effects: Together with TGF-β inhibits IgE synthesis Promotes angiogenesis by blood vessels
IL_8R	(44–59/67–70; 2) (CDw128)
	Distribution: CD8 T cells, neutrophils, monocytes, NK-cell subsets
	Receptor of α chemokines
IL_9	(32–39; *5q31–33*) growth factor for clones of CD4 cells which also produce it
	Sources: T lymphocytes (especially Th2, but not CD8) and B lymphocytes, mast cells, and eosinophils, and potentially involved in allergy and asthma
	Effects: Mast cells, megakaryocytes: enhances proliferation Basophils: proliferation, increases the sensitivity to IL_3 B lymphocytes: enhances IL_4-induced production of IgE antibodies
	Additional effects: Enhances the synthesis of IL_6 Induces IL_{22} activity Substantial promoter of myeloid and erythroid precursor
IL_9R	(CD129) T and B cells, macrophages, megakaryoblasts
	The common γc chain is part of this receptor
IL_{10}	(19; *1q32*), several IL synthesis inhibitory factor (CSIF)
	The genes for IL_{10}, IL_{19}, IL_{20}, IL_{22}, IL_{24}, IL_{26} are found within a 200-kb region of chromosome 1q32, whereas genes encoding the two other IL_{10} family members, IL_{22} and IL_{26}, are found within 30 kb of each other and less than 100 kb from the IFN gene on chromosome 12q15; the family can be further divided into two groups. IL_{19}, IL_{20}, and IL_{24} belong to one group, whereas IL_{10}, IL_{22}, and IL_{26} form another group. Moreover, IL_{19}, IL_{20}, and IL_{24} use the common $IL_{20}R2$ chain for signaling, and IL_{10}, IL_{22}, and IL_{26} may also share a common R2 chain
	Pleiotropic IL with important immunoregulatory functions whose actions influence activities of many of the cell types in the immune system
	Sources: $IL_1β$, IL_6, IL_8, IL_{12}, G-CSF, GM-CSF, IFN-γ, TNF-α produced by activated monocytes, IL_4 and IL_5 by Th2 subsets, leukocytes and skin
	IL_2 and IFN-γ produced by Th1 subsets, thus eliciting the generation of IL_4
	$IL_1α$ and β and TNF-α by Th1 and NK cells
	IFN-γ and TNF-α by NK cells
	Negatively regulates its own synthesis by monocytes
	IFN-γ inhibition results in the switch of T cell differentiation from Th0 to Th2
	B cells, Th1, Th2 and suppressor T subsets, DCs, mast cells, monocyte-macrophages, eosinophils and keratinocytes
	Effects: Macrophages: inhibition of differentiation and expression of HLA class II and adhesion molecules Monocytes: inhibition of differentiation into macrophages CD4 lymphocytes: Inhibits IL_8-induced migration Down-regulates Th1 responses, thus suggesting an anti-inflammatory activity

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Suppressor CD8 T cells: Favors differentiation and chemotaxis Inhibitory factor at the maternofetal interface (Chap. 2) TCT precursors: enhances the number and the function B lymphocytes: has a significant effect opposite to the SCIF activity stimulating: a) Growth of progenitors of the erythroblast series and of mast cells b) Differentiation and expression of HLA class II c) Isotype switching to IgA_1 and IgA_2 secretion along with TGF-β, and to IgG_1, IgG_3 and IgG_4 On IL synthesis, in particular inducing IFN-γ on NK cells by IL_{12} On APCs function down-regulating HLA class II expression, an effect reversed by IL_4 On production of reactive oxygen and nitrogen species d) Isotype switching to IgE e) Modulation of IL_4-induced B-cell IgE production in favor of IgG_4 f) Inhibition of IgE-dependent mast cell activation ILs: inhibits $IL_1\alpha$, $IL_1\beta$, IL_2, IL_6, IL_6, IL_{18}, TNF-α Langerhans' cells (LCs): tolerogen effects in addition to suppressing their APC function STAT1, 3, 5: activation, such as IL_{22}
$IL_{10}R1$	(110 kD) Distribution: thymocytes, B cells, mast cell and macrophage cell lines After binding to B cells induces B-cell proliferation, differentiation and isotype switching to IgA secretion with CD40L = CD154 and TGF-β
$IL_{10}R2$	Intact second chain of the human IL_{10} R complex, which may be shared by receptors for the other IL_{10} homologs A distinct feature of the $IL_{10}R$ complex is that both chains can independently bind ligand, whereas in the IL_{10} and IFN–R complexes, only one chain (the R1 chain) can bind ligand in the absence of the other: in all of these receptors, the second (R2) chains are necessary for signaling through the JAK-STAT complex Recognizes IL_{10} and IL_{22}
IL_{11}	(23; *19q13.3–q13.4* and *7* centromeric region) Sources: Bone marrow stromal cells Effects: Hematopoietic progenitor cells of megakaryocytes and macrophages/monocytes: growth factor B lymphocytes: increases Ig secretion independently of T lymphocytes Hepatocytes: synthesis of acute phase proteins cooperating with IL_1 and IL_6 Modulates Th1/Th2 IL production from activated $CD4^+$ T cells, and augments T-cell IL_{10} elaboration Additional effects: Acts synergically with IL_3 in megakaryocyte differentiation Sharing several biological activities with IL_6, it is likely that they use similar signal transduction mechanisms
$IL_{11}R$	Belongs to the receptor family, also including IL_6R, etc.; IL_{11} initiates signaling via binding to a unique IL_{11} receptor chain ($IL_{11}R$). Isoforms of $IL_{11}R$ have been described that contain $IL_{11}R1$ and do not contain $IL_{11}R2$, a cytoplasmic domain. The binding of IL_{11} to $IL_{11}R$ forms a complex that binds to and induces gp130 molecule homodimerization, resulting in the assembly of the active $IL_{11}R$ trimer
IL_{12}	(*5q31–33*) Heterodimeric IL manifests some effects almost reciprocal to those of IL_{10}; an IL_{12}-family is composed of IL_{23} and IL_{27} Has two covalently bound 35- and 40-kD chains (and thus designated p35 and p40 subunits), encoded by two separate genes regulated independently and inactive if expressed separately – the p40 subunit is expessed in large excess of the p35 subunit – p35 is among the early inducers of Th1 responses, while it inhibits Th2 lymphocytes – when p35 associates with p40, a soluble member of the cytokine receptor superfamily, it forms IL_{12}, which induces Th1 differentiation and the release of IFN from Th1 and NK cells – CD40 cross-linking up-regulates IL_{12} p40 mRNA in monocytic and B-cell lines and in human monocyte-derived DCs – the two subunits act as signal transducer by providing a cytoplasmic STAT4 binding sit which enables STAT4-mediated responses to IL_{12} to occur DCs; The gene encoding the p40 subunit is located in the region of chromosome 5 – the duo $IL_{12}p35/IL_{12}p40$ is called IL_{12} p70

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Sources: B cells and monocyte-macrophages (via CD40–CD154 interactions) Effects: Allergen-specific T cells Crucial role in selective switch of T cell differentiation into Th0 or Th1 Stimulation of adhesion molecules Th1-inducing and Th1-maintaining B lymphocytes Induction of STAT1 activation, T-bet expression and IgG2α class switching NK cells a) Activation, proliferation and cytotoxicity b) Expression of CD2, CD56 and CD11a c) Stimulates production of IFN-γ from Th1 cells and NK cells NK cells, macrophages and Th1 subsets: production of IFN-γ – p40 is expressed by some cells such as nonprofessional APCs and only after activation Has homology with the soluble receptor of IL_6 Costimulates proliferation of peripheral lymphocytes Promotes cell-mediated immunity (CMI) Protection against infectious diseases
$IL_{12}R$	(α chain or R1, 120 kD, and β chain or R2, 140 kD) Sources: CD4, CD8 activated T cells, NK cells, and B cells Effects: Stimulates their proliferation as well as the peripheral hematopoiesis R2 is a key Th1 commitment step when naive Th precursor cells commence differentiation into Th1 cells
$IL_{12}Rβ1$	(*5q31–33*) Encodes the p40 subunit
IL_{13}	(9–17; *5q31*) The gene encoding IL_{13} is only 25 kb upstream of the IL_4 gene and in the same orientation Has a receptor different from that of IL_4 possibly a subunit in common with IL_4R Sources: CD4 (Th 0, 1 and 2) and CD8 T cells stimulated by antigens, monocytes and mast cells Effects: Up-regulates HLA class II and adhesion molecules such as CD11b, 11c, 18, 28, 49e Down-regulates CD16, CD31, and CD 64 Manifests no growth effects on T cells, unlike IL_4 Human B lymphocytes: a) Expression of CD23 (also on monocytes), CD71, CD72, sIgM and HLA class II b) Class switching to IgE and IgG_4 c) Expression of ε-germline transcripts independently of IL_4 even if two to five times less powerful Monocyte-macrophages: IL_4-like effects (inhibits HIV replication in monocytes and ADCC in both cells) Additional effects: Has a persisting activity unlike IL_4, which is activated only 8–12 h Together with IL_4 induces VCAM-1 = CD106 on endothelial cells Its polymorphism is associated with high total serum IgE levels and increased risk of atopic asthma
$IL_{13}R$	Appears to be heterotrimer, and formed by $IL_4Rα$, $IL_2Rγ$ and $IL_{13}Rα1$- or $IL_{13}Rα2$-binding Associates with CD124 to form the $IL_{13}R$ complex; the common γc chain is part of this receptor Distributed to human B cells, endothelial cells, several non-hematopoietic cells, including monocyte-macrophage populations, B cells, basophils, eosinophils, mast cells, fibroblasts, smooth muscle, and airway epithelium Crucial role in inducing human B-cell proliferation and class switching to IgE in presence of CD40; suppresses the induction of inflammatory ILs, activates STAT6

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_{14}	(53) Sources: T and B lymphocytes Effects: Activated B lymphocytes: a) Expands clones b) Suppresses antibody secretion c) Enhances selected subpopulations d) Promotes long term growth in vitro Monocytes: release of ILs
$IL_{14}R$	Binding of IL_{14} increases expression of $IL_{14}R$, as well as in intracellular cAMP, DAG and Ca^{++} levels
IL_{15}	(*15*; *4q31*) Sources: Stromal cells, monocyte-macrophages, fibroblast cell lines, epithelial cells, and in various tissues such as muscle, placenta, etc. Effects: T lymphocytes: growth factor Powerful chemoattractant Stimulates proliferation sharing its functions with IL_2R β and γ chains and the only α chain possessed T- and NK-cell clones: proliferation LAK, NK and CTL cells: activation and differentiation B cells: proliferation and differentiation Human tonsillar B cells stimulated by CD154: proliferation and Ig synthesis γδ subpopulations: proliferation and differentiation Mast cells: stimulation STAT3/STAT5 and JAK1/JAK3: phosphorylation Additional effects: IL_{15} presence in several fetal tissues suggests a role in differentiation and maturation of fetal immune system Shows several biological activities similar to IL_2, for example the capacity to stimulate T-cell proliferation and induction of cytotoxic T lymphocyte and IL-activated killer cells
$IL_{15}R$	(58–60) Forms a new family with CD25; the common γc chain is part of this receptor Sources: T-cell lines, macrophage lines, diffused in non-lymphoid cells and tissues Induces proliferation and differentiation of activated B cells
IL_{16}	(50–60) Sources: Activated CD8 lymphocytes, airway epithelial cells Effects: T lymphocytes: chemotactic action binding to CD4 receptor Eosinophils: chemotaxis Additional effects: Expression of HLA class II and IL_2R Suppresses HIV replication
IL_{17}	Has 5 members, $IL_{17}A$, $IL_{17}B$, $IL_{17}C$, $IL_{17}E$ (IL_{25}), and $IL_{17}F$ (ML-1) Sources: a) CD4 memory and CD8 lymphocytes and TcR-αβ b) Spleen $CD4^+$ cells and neutrophils c) IL_{15} able to induce IL_{17} release from purified spleen $CD4^+$ cells and airway neutrophils and could lead to IL_{17} production following bacterial infection Effects: T cells: proliferation Fibroblast: proliferation Stimulates production and expression of ILs by macrophages, epithelial and endothelial cells, and fibroblasts

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
$IL_{17}R$	(98, 22) Sources: Triple negative thymocytes, T-cell clones and cell lines Effects: Enhances T-cell proliferation induced by PHA, activation of NF-κB, IL_6, IL_8 and expression of CD54; the soluble form instead inhibits T-cell proliferation and IL_2 production
IL_{18}	(24; *11q22.2–22.3*) Initially described as an IFN-γ-inducing factor; is closely related to the IL_1 family in terms of structure and pro-inflammatory properties Sources: Activated macrophages, airway epithelial cells, Kupffer cells, dermal keratinocytes, osteoblasts, adrenal cortex cells, and intestinal epithelial cells Effects: Activation of both CCL and CXCL chemokines Activates NF-κB, expresses Fas ligand, induces human HIV Synergizes with IL_{12} and anti-CD40 to induce B cells to express IFN-γ Stimulates T cells for IFN-γ production and growth promotion, also in the absence of T cell antigen receptor engagement Stimulates T-cell proliferation and potentiates the Th1-driving capacity of IL_{12}p70 With IL_1 is a key molecule in both the innate and adaptive immunity and is member of a larger family of related receptors, some of which contribute to host defense Also enhances Th2 cytokine production (IL_4 and IL_{13}) and regulates IgE production in vivo in the absence of allergen
$IL_{18}R$	Recruits IRAK and IL_1R-activating kinase and NF-κB-inducing kinase (see above) One of the IL_{18}R chains is the IL_1R-related protein, a member of the IL_1R/Toll-like receptor (TLR) superfamily
IL_{19}	(*1q32*) Shares 21% amino acid identity with IL_{10} and binds to IL_{20}R Signals through a receptor complex that is also utilized by IL_{20} and IL_{24} Two distinct IL_{19} mRNA species differ in their 5′-sequences IL_{19}, IL_{20}, IL_{22}, and probably also IL_{24} could form a single subfamily of helical ILs Sources: B cells and monocytes Effects: Signaling via STAT3, induce JAK-STAT signal transduction pathway through a specific receptor complex IL_4 and IL_{13} potentiate IL_{19} gene expression in LPS-stimulated monocytes GM-CSF directly induces IL_{19} gene expression in monocytes
$IL_{19}R$	
IL_{20}	(*1q32*) a new member of the IL_{10} family Sources: Skin (where its overexpression leads to skin abnormalities) and trachea Effects: Activates a STAT3-containing signal transduction pathway Skin differentiation and keratin expression Pro-inflammatory effects on keratinocytes and possible central role in the epidermal response to inflammation Stimulates chemotaxis and antimicrobial activity of myeloid cells
$IL_{20}R1$	Both its subunits are expressed in keratinocytes throughout the epidermis, recognizes IL_{19} and IL_{24}
$IL_{20}R2$	Also on certain endothelial and mononuclear cells, recognizes IL_{10} and IL_{22}
IL_{21}	(4q26–q27), IL_9-induced factor maps to the same locus as the IL_2 gene, separated by roughly 180 kb Sources: Activated peripheral T cells

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
	Effects: Proliferation and maturation of NK cells populations from bone marrow Proliferation of mature B-cell populations costimulated with anti-CD40 Proliferation of T cells with or without anti-CD3 costimulation in concert with IL_2, IL_{15} and, to a lesser extent, with IL_7
$IL_{21}R$	(*16p11*) With the first exon situated just 39 kb from the $IL_4R\alpha$ locus, the common γc chain is part of this receptor Sources: Stromal cells in the bone marrow or thymus Resting B cells Cell lines of B, T, and NK lineages Human $CD23^+$ and $CD56^+$ Associated with JAK1, JAK3 and Tyk2
IL_{22}	(*12q15*), or IL-TIF, another member of the family of IL_{10} homologs, located on human chromosome *12q*, where several *loci* potentially linked to asthma and atopy have been identified by genetic studies, particularly in the *12q13.12–q23.3* region. More precisely, it is located on chromosome *12q15*, at 90 kb from the IFN-γ gene, and at 27 kb from the IL_{26} gene, which codes for another IL_{10}-related cytokine. In the mouse, the IL_{22} gene is located on chromosome 10, also in the same region as the IFN-γ gene Sources: Th1 cells, IL_9 Pleiotropic effects: Activates STAT1, 3, 5; as IL_{10}, stimulates monocytes to make TNF Can activate IFN-γ-like biological responses such as HLA class I induction and STAT activation Target tissue is the liver, where it induces acute-phase reactants and is active in the innate immunity In keratinocytes IL_{22} activated STAT3 and directly increased the expression of β-defensin and β-defensin 3
$IL_{22}R$	(1) Complex composed of two subunits, the $IL_{22}R\alpha$ chain and the second $IL_{10}R\beta$ chain: both chains are required for signaling, each chain alone is capable of binding IL_{22}
$IL_{22}BP$	(*6q24.1–25.2*). This receptor, named IL_{22} binding protein, inhibits IL_{22} activity by binding IL_{22} and preventing its interaction with the functional $IL_{22}R$ complex, but $IL_{22}BP$ often fails to block IL_{22} activity Both chains of the $IL_{22}R$ complex are ligand binding chains; however, none of them is capable of transducing IL_{22}-signaling alone. Both chains are necessary to assemble the functional receptor complex able to induce signaling after binding IL_{22}
IL_{23}	A p19-p40 heterodimer structurally related to IL_6, G-CSF, and the p35 subunit of IL_{12} sharing homology with members of the IL_6/IL_{12} family of ILs, also combining the IL_{12p40} subunit with its subunit p19, requires interaction with $IL_{12}R1$ and $IL_{23}R$ with a cytoplasmic STAT4 binding domain Source: Hemopoietic cells, bone-marrow-derived DCs Effects: Th1 activation T-cell memory Activates $IL_{17}E$ and STAT4 in blast T cells Induces exclusively the proliferation of naive and CD45RO memory T cells and of related IFN-γ
$IL_{23}R$	Associates with $IL_{12}R\beta1$ in a combined deficiency
IL_{24}	(*1q32*) (melanoma differentiation-associated factor MDA-7) Sources: PBMCs and melanoma (MDA-7) Induced by: IFN-β and mezerein Effects: Signaling by STAT3 Targets: tumor cells Biological effects: Tumor and apoptosis inhibition

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_{25}	Also called $IL_{17}E$ Sources: Th2 cells Effects: Induces Th2-like IL_4, IL_5, and IL_{13} gene expression through a subset of APCs expressing high levels of HLA class II Amplifies systemic and localized allergic-type inflammatory responses by its actions on several cell types Increases serum IgE, IgG, and IgA levels, induces blood eosinophilia and pathological changes in the lungs and digestive tract
IL_{26}	(*12q15*) (= AK155) (a factor discovered after overexpression in human T lymphocytes after transformation by the simian rhadinovirus herpesvirus saimiri) Sources: T cells Mast cells after FcεRI cross-linkage Effects: IL_{26} and IL_{19} bind to the $IL_{20}R$ complex, composed of cytokine receptor family 2–8/$IL_{20}R\alpha$ and $IL_{20}R\beta$ (type I). IL_{26} or $IL_{10}R$ 2 chains and $IL_{20}R1$, but not IL_{21}, bind to the receptor complex, composed of $IL_{22}R$ and $IL_{20}R\beta$ (type II) Enhances secretion of IL_8 and IL_{10}, and cell surface expression of CD54 Activates the JAK/STAT signaling pathway, inducing a rapid tyrosine phosphorylation of STAT1 and STAT3 in cells expressing $IL_{20}R1$ and $IL_{10}R2$ IL_{26} may play a role in local mechanisms of mucosal and cutaneous immunity
$IL_{26}R$	Formed by a combination of two receptor subunits, $IL_{20}R1$ and $IL_{10}R2$, which dimerize to generate the receptor engagement and results in phosphorylation of STAT1 and STAT3
IL_{27}	p28 protein, a further member of the IL_{12} family involved in Th1 initiation A heterodimer composed of two chains, p28 (IL_{30}) and EBI3, analogous to $IL_{12}p40$ and $IL_{12}p35$ (p28-related protein), respectively, and a p40-related protein The heterodimer is expressed by APCs Sources: Activated APCs, macrophages and DCs Effects: Involved in Th1 initiation Triggers clonal proliferation of antigen-specific naïve but not memory $CD4^+$ T cells and synergizes with IL_{12} in IFN-γ production by naïve $CD4^+$ T cells Activates STAT-1, -2, -3, and -5, JAK-1 and -2 in naïve $CD4^+$ T cells Promotes polarization towards a Th1 phenotype with expression of IFN-γ IL_{27} stimulation induced phosphorylation of STAT-1 and expression of T-bet and $IL_{12}RB2$ in naïve $CD4^+$ T cells. Together with IL_{12}, IL_{27} augmented IFN-γ secretion in naïve $CD4^+$ T cells Suppresses $CD4^+$ T cell proliferation and Th2-II-like production Activates Th0 and Th1 cells Activates STAT-1 and induces T-bet expression and IgG2a in stimulated B cells, together with WSX-1 limits innate and adaptive components of type 2 immunity at mucosal sites
$IL_{27}R$	One subunit is WSX-1 Its engagement results in IFN-γ production
$IL_{28}A$	(19) IFN-λ2 mediates antiviral activity in cells in response to viral infection
$IL_{28}R$	Includes receptors for type I and type II IFNs (IFN-αR1, IFN-αR2, IFN-γR1, and IFN-γR2) and receptors for $IL_{10}R$, $IL_{20}R$, and $IL_{22}R$
$IL_{28}B$	(19) IFN-λ3 mediates antiviral activity in cells in response to viral infection
IL_{29}	(19) IFN-λ1 mediates antiviral activity in cells in response to viral infection
IL_{30}	(16p11) a member of the long-chain 4-helix bundle IL family, and EBI3, form the IL_{27} heterodimer Termed p28 because of its molecular mass

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IL_{31}	The oncostatin M receptor (OSMR) is part of receptor complexes for OSM and IL_{31} a four-helix bundle IL Sources: Preferentially Th2 cells Effects: Skin tropism Signaling events are triggered by JAKs constitutively binding to membrane-proximal receptor regions
$IL_{31}R$	Gp130-like receptor (GPL) recruits JAK1, JAK2, STAT-1, STAT-3, STAT-5 signaling pathways, as well as the Pi3 kinase/AKt cascade
IL_{32}	Exists as four splice variants Sources: Human peripheral lymphocyte cells after mitogen stimulation, human epithelial cells by IFN-γ, and NK cells after exposure to the combination of IL_{12} and IL_{18} Effects: Induces human TNF-α, and IL_8 in THP (Th precursors) monocytic cells Activates nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK); may play a role in inflammatory/autoimmune diseases
IL_{33}	Sources: Member of the IL_1 family Effects: Mediated via IL_1R ST 2 Activates NF-κB and MAP kinases Drives production of Th2-associated ILs
G-CSF	(18–22; *17q11–21*) Granulocyte colony-stimulating factor Favors growth of myeloid progenitor cells
GM-CSF	(22; *5q31–33*) Sources: Activated T cells, monocytes, macrophages, fibroblasts, bone marrow stromal cells Effects: Stimulates growth of hematopoietic precursor cells, mostly of granulocytes/monocytes; modifies the functions of mature granulocytes Enhances neutrophils (inhibiting migration), macrophages and eosinophils (paraeosinophil IL) Induces phagocytosis, production of eicosanoids, ADCC, platelet production by megakaryocytes Promotes growth and maturation of LCs to APCs
IFN α, β, γ (interferons) produced by Th1 cells;	Common properties Hematopoietic stem cell growth B cells: proliferation NK cells, TCT: activation Macrophages: phagocytosis and accessory activities (IFN-γ > IFN-α, β) Additional effects: IL_1 and IL_2 synthesis (IFN-γ > IFN-α, β) Induction of HLA molecules of class I (IFN-γ > IFN-α, β) and class II Production of FcR Production of Igs Antiviral activity (IFN-γ < IFN-α, β): inhibition of viral replication and of tumor growth Differentiation of leukemia cells of promyelocytic and monoblastic origin Additional actions (IFN-γ > IFN-α, β) Differentiation of erythroleukemia cells Antibody production Influence on CMI
IFN-α	(16–27) (*9p22*) Sources: Mainly monocytes, macrophages, and lymphocytes, secondarily B cells, NK cells Stimulates macrophages and B differentiation Has activities similar to IL_{12} inducing the differentiation of allergen-specific T cells into Th0 or Th1 cells Inhibits virus-infected cells

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
IFN-β	(20) Sources: Macrophages and granulocytes, and also fibroblasts Effects: On B proliferation
IFN-γ	(20×25; *12q22–24*) Sources: Th1 and T γδ lymphocytes, CD8 cells, activated NK cells and macrophages Has a central role in the immune response, since stimuli activating T lymphocytes induce IFN-γ synthesis Effects: T lymphocytes a) Inhibits Th2 proliferation inducing their shift to Th1 b) TCT and NK cells: induction and proliferation B lymphocytes a) Differentiation inhibiting IgE antibodies production either directly or down-regulating FcεRI expression by B cells and of CD23 by IL_4 b) Expression of IgG Fc Activated B lymphocytes: proliferation and differentiation, in synergy with IL_2 Additional cells: Monocytes, macrophages, mast cells, DCs, fibroblasts, and T lymphocytes: induces or increases the expression of HLA class II Eosinophils: increases the cytotoxic activity and the adhesion to endothelium via the expression of CD54 Macrophages and neutrophils: activates and enhances their phagocytic and bactericidal activities Macrophages: induces chemotaxis and increases survival Mast cells: prolongs the life span Neutrophils, monocytes and macrophages: activation to promote ADCC reactions NK cells: activation Pluripotent stem cells: growth and activation Additional effects: Antagonizes the action of IL_4 and inhibits the IL_4-induced IgE production by B lymphocytes Inhibits secretion of IL_{10}
IFN-κ	Expressed in epidermal keratinocytes Sources: Resting DCs and monocytes Effects: Release of several cytokines from both monocytes and DCs Inhibits inducible IL_{12} release from monocytes
IFN-λ 1–3	Correspond to $IL_{28}A$, $IL_{28}B$ and IL_{29}
IFN-λR1	One of the two receptors utilized by all three IFN-λ proteins, the other is $IL_{10}R2$
IFN-τ	Suppression of proliferation and inhibition of IgE production
IFN-ω limitin	Sources: Mature T lymphocytes in spleen and thymus Bronchial epithelial and salivary duct cells Effects: Induces apoptosis in pre-B cell lines Reduces the proportion of CD45R-positive cells Enhances the antigen-induced cytotoxic lymphocytes Suppresses the antigen-induced T-cell proliferation
IFN-αβR	Consists of two subunits, IFNAR-1 and IFNAR-2
IFN-γR	Receptors 1 (*6q23–24*) and 2 Expressed on nearly all cell types Coupled to the JAK-STAT signaling pathway Mice lacking this receptor or STAT1 display a profound disruption of both innate and adaptive immunity

Table 1.5. (Continued)

Name	MW (kD); chromosomes, primary sources and targets, principal biological effects
M-CSF	(45–70; *5q31–33*) Monocyte/macrophage-colony stimulating factor Promotes proliferation and differentiation of monocyte/macrophages and the activation of NK cells
TGF-α	(5, 6) transforming growth factor, eosinophil generation
TGF-β	(12.5–25) the TGF-β family contains five homologous members, TGF-β 1 and 3 are encoded by single genes on chromosomes 19.1, and 14, respectively; TGF-β may be a Th3 IL Sources: Activated B and T cells, macrophages, platelets; has opposing actions: a) Promotes proliferation of fibroblasts and collagen synthesis, increases IgA production by B cells b) Inhibits almost all other cells, including T and B lymphocytes, blocks IgM and IgG synthesis, IL_1R production and HLA molecules expression c) Eosinophil generation
TGF-βRI	Recognizes Smad-2 and -3 that transduces TGF-β-triggered signals together with Smad-TGF-βR
TGF-βRII	Causes recruitment and phosphorylation of TGF-βRI and formation of a receptor complex
TGFR-1	CD120a
TGFR-2	CD120b
TNF α, β	Tumor necrosis factors Effects: Express HLA class I and II and manifest antiviral activity Epithelial cells: induce the proliferation by G-CSF Endothelial cells: interact to produce CD54 Monocytes: stimulate both motility and production of IL_6 and IL_8 Neutrophils: potent activators and adhesion-inducing, promote chemotaxis and degranulation B cells: modulate immune responses mediated by IL_4
TNF-α	(17–51) (*6p21.3*) Sources: Monocyte-macrophages, mast cells, PMNs, endothelial, NK and activated T cells Effects: Macrophages, neutrophils, eosinophils: activation and expression of HLA class I and adhesion molecules B cells: activation Hepatocytes: synthesis of acute phase proteins Muscle cells: induces endotoxic shock Additional effects: Favors delayed-type and contact hypersensitivity Manifests an inhibiting effect on LCs
TNF-β	(20–25) Sources: Activated B and T (Th1] lymphocytes Effects: On neutrophils and on the proliferation and differentiation of B lymphocytes Favors the expression of adhesion molecules
TNFR-SF	Includes CD30, CD40, CD95, CD97

Several other molecules have been described: *BaDF* basophil differentiation activating factor, *EAF* eosinophil activating factor, *ECEF* eosinophil cytotoxicity enhancing factor, *EGF* epidermal growth factor, FGF fibroblast growth factor; *MIF* (monocytes) migration inhibiting factor, *NGF* nerve growth factor, *PDGF* (30–32) platelet derived growth factor, *SCF* stem cell factor.
IL_4R, IL_7R, IL_9R, $IL_{13}R$, $IL_{15}R$, and $IL_{21}R$ have the common γc chain; cytokine receptors are dealt with also in Table 1.2.
EBI3 Epstein-Barr virus-induced gene 3, * chemokines, see below, *WXS-1* WSXWS (Trp-Ser-X-Trp-Ser).
Data from [4, 14, 34, 45, 50, 52, 55, 66, 68, 78, 92, 99, 118, 119, 126, 128–130, 140, 141, 152–154, 156, 158, 167, 172, 181, 213, 219, 220, 240, 245, 249, 258, 272, 278, 318, 326, 372, 377, 383, 387, 398, 399, 412, 413, 421, 426, 437, 455, 461, 474, 480, 481, 487, 517, 523, 526, 528, 532, 534, 560, 562, 607, 641, 669, 693] and PubMed ID: 14764690.

Table 1.6. Regulation of isotype switching and HLA expression by some ILs

ILs	Immunoglobulins	HLA class
IL_3		II
IL_4	IgG_1 and IgE	II
IL_5	IgA	I
IL_{10}		II
IL_{12}	IgE	
IFN-α and -β		I/II
IFN-γ	$IgG_{2α}$	I/II
TGF-β	IgA	II
TNF-α and -β		I

Table 1.7. Synergic activities of ILs

Synergy	Cytokine synergic effects
IL_1, IL_3, IL_5	Production of granulocytes, eosinophilopoiesis
IL_2, IL_4	Enhancement of T lymphocytes
IL_2, IL_5, IL_6	Synergy with IL_4 and IL_{13} in promoting IgE production
IL_2, IL_{12}	Generation of TCT and LAK
IL_3, IL_{11}	Megakaryocytopoiesis
IL_3, IL_4, IL_{10}	Differentiation of B lymphocytes, production of mast cells
IL_3, IL_6	Hemopoiesis
IL_3, IL_4, IL_{11}	Cooperation in several functions
IL_3, IL_9	Promotion of the growth of some mast cell lines
IL_4, IL_6	Hemopoiesis
IL_4, IL_{13}	Isotype switching ε
IL_5, NGF	Production of basophils/mast cells and eosinophils
IL_{10}, IL_2, IL_4	Growth of immature thymocytes
GM-CSF, NGF	Production of granulocytes

Table 1.8. Activity of ILs and chemokines in atopic diseases

Effects	Cytokines and chemokines	Activity
IgE regulation	IL_4, IL_{13}	ε Isotype switching
	IL_4	Generation of IL_4 producing CD4
	IL_2, IL_5, IL_6	Synergy with IL_4 and IL_{13}
	IFN-γ, TGF-β	Inhibit IL_4 and IL_{13}
	IL_{12}	Enhances production of IFN-γ by T cells and NK cells
IgA regulation	TGF-β	α Isotype switching
Eosinophils	IL_3, IL_5, GM-CSF, RANTES*, MIP-1α*, eotaxin, MCP-3* IL_1, TNF	Eosinophilopoiesis Eosinophil chemotaxis and activation Eosinophil activation
Mast cells: development and activation of GM-CSF	IL_3, IL_9, IL_{10}, NGF, H-CSF	Mast cell growth factors Inhibits mast cell proliferation
	MIP-1α*, MCP-1*, MCP-3*, RANTES*	Basophil chemotaxis, histamine release
	IL_8	Inhibition of histamine release
Inflammation	IL_1, IL_4, IL_6, IL_8, GM-CSF, G-CSF, TNF, IFN-γ	Activation of neutrophils
	IL_1, IL_3, IL_5, TNF, GM-CSF	Activation of eosinophils
	IL_1–IL_4, GM-CSF, M-CSF, TNF, IFN-γ	Activation of macrophages
Anti-inflammatory	IL_{10}, TGF-β	Inhibit IL production and T cell and/or monocyte function

EBI3 Epstein-Barr virus-induced gene 3, * chemokines, see below.
Data from [4, 14, 34, 45, 50, 52, 55, 66, 68, 78, 92, 99, 118, 119, 126, 128–130, 140, 141, 152–154, 156, 158, 167, 172, 181, 213, 219, 220, 240, 245, 249, 258, 272, 278, 318, 326, 372, 377, 383, 387, 398, 399, 412, 413, 421, 426, 437, 455, 461, 474, 480, 481, 487, 517, 523, 476, 528, 532, 534, 560, 562, 607, 641, 669, 693] and PubMed ID: 14764690.

Table 1.9. Leukocyte surface molecule superfamilies

Superfamily domains	Examples	Common functions in immune system
Complement control proteins	CD21, CD35, CD62P	Control of complement cascade
IL receptors	$IL_2R\beta$ (CD122), $IL_6R\alpha$ (CD126)	Growth factor receptors
Epidermal growth factor (EGF)	CD62L, CD62P	Cell surface ligand binding
Fibronectin type II	Mannose receptor	Polyvalent functions
Fibronectin type III	Integrin β4 (CD104), IL_7R (CDw127)	Polyvalent functions
Immunoglobulin V set	IgV, TcRV, CDw90	Adhesion, recognition
Immunoglobulin C1 set	β_2M, HLA class I α3 domain	Adhesion, recognition
Immunoglobulin C2 set	CD2 domain 2, CD3ε	Adhesion, recognition
Integrins	CD11/CD18, CD49	Adhesion
Lectin C-type	Mannose receptor, CD23, CD62L	Carbohydrate binding
Lectin S-type	CD11b/CD18	Carbohydrate binding
Leucine-rich glycoprotein repeats	CD42a, CD42b	Protein–protein or –lipid interactions
Link	CD44	Hyaluronic acid/chondroitin sulfate binding site
LDL receptor	LDL receptor	Lipoprotein binding, NN function
Ly-6	CD59	NN
HLA	Class I α1, α2, II α1, β1 domains	Recognition
NGF receptor	CD27, CD40	NN
Rhodopsin (serpentine receptor)	IL_8R (CDw128), C5aR, CD5, CD6	G-protein coupled receptor
Somatomedin	PC-1	NN
Transmembrane 4 pass	CD9, CD37, CD53	NN
Phosphotyrosine phosphatase	CD45	Signal transduction
Tyrosine kinase	M-CSFR, c-kit (CD117), lck	Signal transduction

Modified from [27].
IL interleukins, *LDL* low-density lipoproteins, *M-CSFR* Monocyte/macrophage-colony stimulating factor receptor, *NN* not known, *PC-1* plasma cell surface antigen-1.

PubMed ID: 12734330 has given a full description of IL_{30}: according to the HUGO Gene Nomenclature Committee, the symbol p28 (IL_{27}) should be used. It was finally identified as one subunit of $IL_{27}R$ [664].

Recently, the IFN family has been enriched by several new acquisitions (Table 1.5): type I IFN family contains IFN-α, IFN-β, IFN-κ, IFN-λ, IFN-ω, IFN-τ and IFN-ζ. Limitin is IFN-ω, an IFN-like IL that has approximately 30% sequence identity with IFN-α, IFN-β, and IFN-τ [398, 399]. IFN-κ, like IFN-β, induced the release of several ILs such as IL_{28} and IL_{29} from both monocytes and DCs, without the requirement of a costimulatory signal [390]. TLR-9 (Toll-like receptor-9) stimulation by CpG (cytosine-phosphate guanine) DNA induced the expression of all IFN-α, -β, -ω and -λ subtypes in PDCs (plasmacytoid dendritic cells), whereas TLR-4 stimulation by LPS (lipopolysaccharide), or TLR-3 stimulation by poly I:C, induced only IFN-β and IFN-λ gene expression [88]. A new IFN, STAT1-induced FLN29, might be involved in the termination of LPS signaling [335]. Three genes on human chromosome 19 have been found to encode distinct but similar proteins, which are called IFN-λ1, IFN-λ2 and IFN-λ3, and are designated as $IL_{28}A$, $IL_{28}B$ and IL_{29}, respectively. It is suggested that these ILs are functionally referred to as type III IFNs because of their unique primary sequence homology and receptor usage [88]. A distinct receptor complex is utilized by all three IFN-λ proteins for signaling and is composed of two subunits, a receptor designated as IFN-λR1 and a second known as $IL_{10}R2$, which signal through the JAK-STAT pathway [274, 519].

This receptor mediates the tyrosine phosphorylation of STAT1, STAT2, STAT3, and STAT5 (signal transducers and activators of transcriptions). Activation of this receptor by IFN-λ can also inhibit cell proliferation and induce STAT4 phosphorylation, further extending functional similarities with type I IFNs [88]. T lymphocyte origin is from pluripotent stem cells, initially arising during embryonic development from hemopoietic tissues of the yolk sac, then the early stages continue in the

fetal liver. Unlike B cells, maturation does not occur *in situ,* but from bone marrow-derived progenitors undergoing maturation in the thymus, where the greater part of cells multiply and differentiate into immunocompetent lymphocytes. In the thymus, functionally competent cells are exported into peripheral lymphoid compartments in accordance either with thymus ability to produce different soluble factors or with particular intercellular interactions. Early in development, thymocytes express several cell surface molecules, including CD2 and CD7 (CD7 deficiency is described in Chap. 22), but lack both DN CD4/CD8 and CD3. Precisely in the thymus, via a series of intermediate steps, DN cells change into DP $CD4^+8^+$ thymocytes. As thymocytes mature into T cells, they express increasing levels of TcR, finally turning into $CD4^+8^-$ and $CD4^-8^+$, becoming MP. Therefore, during early stages of thymus phases, thymocytes express indifferently both CD4 and CD8; the association of either subset with TcR will show whether cells recognize MHC class I or II molecules, thus regulating subsequent differentiations [255]. IKK (inhibitor of κB kinase)-induced NF-κB (nuclear factor κB) activation, mediated by either IKK1 or IKK2, is a pivoral factor for the generation and survival of mature T cells, and IKK2 has a crucial role in regulatory and memory T cell development [503]. NF-κB is also activated by IL_{32} [258].

T cells have been identified by their ability to bind sheep erythrocytes to form E rosettes (red cells), and more sensitive binding of monoclonal antibodies (mABs) identifies their TcR, referred to as CD2. However, the most commonly used marker for T cells is CD3 associated with TcR. Further definition of cell surface protein antigens derives from rDNA technology. The molecules expressed on lymphocyte membranes, characterizing diverse phases of the differentiation process of T subsets, allow their identification and are overall assayed by means of lymphocyte differentiation antigens designated with CD terminology (CD1–CD342) defining cellular antigens. Such markers also distinguish nonhematopoietic cells involved in both innate and acquired immunity, permitting their recognition via analysis of cell surface molecules expressed on lymphocytes during stages of cell development and/or activation. CDs constitute a group still under definition and classification [470, 501] and recently revisited [4, 262, 343, 604]. These CD cell surface markers have been grouped into T cells, B cells, and NK cells, among others. The grouping is somewhat arbitrary because essentially none of such CD markers is restricted to a single cellular lineage.

Uniformity of *nomenclature* is assessed via monoclonal antibody technology, which unlike polyclonal ones prepared from immunized animals all have the same antigen specificity, and are immunologically completely homogeneous since each antibody is synthesized from cells derived from a single clone. Briefly, a hybridoma can readily be formed by fusing a single normal B cell suspension from immunized mice to cells of continuously replicating tumor cells: hybrid cells so obtained show a unique association of antibody specificity and proliferate indefinitely. The cells can also be grown as individually cloned and screened to produce antibodies with desired specificity [414]. Periodically specialists meet in international workshops to compare specific reagents.

The CD4–CD8 dichotomy is considered out-of-date, because a few CD4 have suppressor/cytotoxic and a few CD8 have helper functions [30]. We refer to CD4 or CD8 cells to specify either helper or suppressor/cytotoxic functions, unless otherwise specified [30]. CD4 cells express a CD4 surface antigen, a monomer of 60 kD with four extracellular Ig-like domains, while CD8 is associated with CD8 molecules, αα or αβ dimers each of 34 kD, linked by -S-S bonds, both cells with a short cytoplasmic tail interacting with $p56^{lck}$ [4]. CD4 and CD8 are encoded not by MHC, but by genes on chromosomes 12 and 2, respectively; quantitative and qualitative differences make it possible to distinguish CD4 from CD8 cells present in the bloodstream with a 2:1 ratio [481]. However, CD4 and CD8 functions are at least twofold, since their extracellular portions bind to MHC molecules on the APC surface, thus acting as adhesion molecules. Another major function of CD4 and CD8 is to act as signal transducers in T cells due to their intracellular portions linked to specific kinases; thus CD4 and CD8 are phosphorylated following antigen binding to TcR [36].

Unlike B cells, T cells can interact with antigens directly, even in solution. Antigen recognition by T lymphocytes occurs only when antigens are inside or on the surface of a cell, more precisely when antigens are presented by APCs associated in man with HLA (human leukocyte antigens). Such *double* recognition of both antigens and HLA molecules is critical for T-cell activation, whether immunoregulatory or cytotoxic. We can therefore conclude that in the thymus T lymphocytes are committed to recognizing HLA antigens, and also that tolerance starts in the thymus following TcR affinity for HLA molecules, attributing to HLA a crucial role in the *recognition* process.

Originally described in mice, two phenotypes were defined showing that T dichotomy is actually the heterogeneity of CD4 cells. However in man there are three subpopulations (Tables 1.10–1.12) [52, 69, 130, 158, 220, 249, 455, 470, 650], divided into three helper (h) subsets based on different patterns of ILs they secrete [643].

These subsets are: Th1 T cells predominantly involved in DTH reactions, Th2 T cells apparently specialized in IgE-mediated reactions, and Th0 T cells [159, 455]. Th0 cells represent a heterogeneous population of effector cells, mostly naive lymphocytes that in the absence of signals clearly driving differentiation into Th1/Th2 T cells have never before been activated and thus have not acquired the ability to secrete a mature profile of ILs, modulating their effects with respect to

Table 1.10. Functional characteristics of human CD4 Th0, Th1 and Th2 lymphocytes

	Th1	Th2	Th0
IL_2	+++	–	+++
IL_3	+	++	+
IL_4	–	+++	+
IL_5	–	+++	+
IL_6	+	++	+
IL_{10}	–	++	+
IL_{11}	–	++	
IL_{12}	+++	–	?
IL_{13}	+	+++	+
$IL_{17}E$	–	++	–
IL_{18}	++	+++	
IL_{23}	++	–	
IL_{25}	–	++	?
IL_{27}	++	–	+
IL_{31}	–	++	?
IL_{32}	++	++	?
GM-CSF	+	++	+
IFN-γ	+++	–	+++
TNF-α	+++	++	++
TNF-β	+++	–	+
Necessary for development	IFN–γ	IL_4	?
Cytolytic activity	+++	±	++
Total Ig levels	+	+++	?
IgE levels	–	+++	±
IgA, IgG, IgM levels			
Relationship T/B ↓	++	++	++
Relationship T/B ↑	–	+++	±
Activation of eosinophils/mast cells	–	+++	+
Activation of macrophages	+++	–	?
Delayed-type hypersensitivity	+++	–	–
Positive immune responses	Atopic diseases, Virosis, Leishmaniasis, Leprosy	Pregnancy, Autoimmunity, Arthritis, Helminthiasis	
Negative immune responses	Autoimmunity, Arthritis, Helminthiasis	Atopic diseases, Virosis, Leishmaniasis, Leprosy	
Response to proliferation and/or production of cytokines			
IL_2	↑	↑	↑
IL_4	=	↑	?
IL_{10}	↓	↓	↓
IL_{12}	↑	↓	?
IFN-γ	=	↓	?
CD30 phenotype	–	+++	+

Data from [52, 69, 105, 119, 129, 130, 158, 220, 249, 455, 470, 650].

Table 1.11. Mechanisms of Th1/Th2 differentiation

Genetic factors	Familial predisposition toward atopy development
Allergen-specific factors	Allergens vs antigens, allergenic epitopes, physiochemical factors, dose, route of administration
Antigen processing presentation	Antigen processing pathways and cells, expression of adhesion, accessory, or homing molecules
HLA restriction/V regions used by TcR	
Pattern of cytokines	IL_4, IL_{12}

Data from [105].

Table 1.12. Differentiated production of cytokines (ng/ml) by Th1 and Th2 clones activated by CD3 and/or CD28

Clone	Activation	IL_4	IFN-γ	IL_5
Th2	–	<0.1	<0.1	<3.0
	+	31.9	0.2	39.9
Th1	–	<0.1	<0.1	<3.0
	+	<0.1	24.8	0.8

Data from [642].

the type and quantity of ILs produced in the microenvironment and the nature of responsive cells [643].

The conventional definition of a Th1 or Th2 cell depends strictly on the secretion of IFN-γ or IL_4. Th1 cells secrete IFN-γ but do not secrete IL_4, whereas Th2 cells secrete IL_4 but not IFN-γ. T cells secreting neither IFN-γ nor IL_4 are neither Th1 nor Th2 cells [470]. A subset of $CD4^+$ T-cell lines, which secrete TGF-β but not IL_4 or IFN-γ, has been termed a Th3 cell [470]. In addition to Smad-2/4 of TGF-βRI, there is Smad-7 which blocks activated receptors and interferes with phosphorylation of both Smad-2 and Smad-3 (G. Monteleone, pers. comm. 15. 2. 2005) [156]. An IL_{10}-producing subset has been termed Tr1 (T-regulatory 1) [470]. Another family, that of T-cell Ig domain and mucin domain (TIM) proteins, is identified to be expressed on T cells [320]. The quantitative difference of ILs synthesized by T cells is remarkable, as demonstrated either by production of high levels of IL_4 and IL_5 by Th2 T cells (GATA-3 is critical for expression of the IL_5 gene in Th2 cells) [678], while that of IFN-γ is significantly lower, or by nearly specular IL secretion by γδ Th1 T cells [665]. Thus, either GATA-3 or single-nucleotide polymorphisms in the IL_{18} gene might be relevant in inducing the very broad Th2 phenotype observed in atopic subjects [278]. A specific factor triggering differentiation into Th1 lymphocytes has been identified in IL_{12}, which in a dose-dependent manner increases IFN-γ levels in TcR of antigen-specific T cells while antagonizing an IL_4-induced B-cell switch to IgE production. It also appears that IFN-γ IL_{12}-mediated production is necessary to stimulate complete expression of Th1 phenotype [105]. For the outcome of CD4 T cell differentiation, the balance between T-bet (TFs T-box expressed in T cells) and GATA-3 is critical [665]. However, T-bet initiates Th1 lineage development from naive *Thp (Th precursors)* cells, both by activating Th1 genetic programs and by repressing the opposing Th2 programs [556].

CD4 T cells coming from thymus, although they can differentiate either into Th1 or Th2 T cells, are not predestined to IL production [644]: the current theory is that only at the moment of antigen presentation do uncommitted cells begin to secrete ILs, with polarization of immune processes into a model of type I or IV reactions [290]. As yet, it is not clear how CD4 T cells distinguish the antigens they encounter, because there is no proof that such antigens are endowed with specific structural features allowing CD4 to recognize them [469]. Since T-cell antigen specificity is prearranged in the thymus apparently at random, it is challenging to imagine how CD4 cells on the point of recognizing a given antigen can be programmed at this stage of development, so that they can express a defined set of ILs at the subsequent encounter. To undo the Gordian knot, the present dogma is that in any inflammatory site both Th1 and Th2 lymphocytes differentiate from common post-thymic antigen-specific ThP, while an intermediate point between ThP from one side and Th1/Th2 T cells from the other is represented by Th0 [473]. Hitherto, cell surface marker analysis for Th1-Th2 T-cell subset identification has yielded unconvincing results. Recent studies show that Th1-Th2 T cells differentiate in conformity with CD30 expression, with CD27, CD40, CD95/Fas, OX40 and other molecules belonging to the receptor superfamily of TNF/NGF (TNF-R = CD120a, CD120b and NGF-R), with low or hardly noticeable levels of Th1 and high levels of Th2 cells (and of CD8) [388]. CD30 is therefore viewed as a specific marker and if stimulated as a cofactor of T lymphocyte differentiation, preferentially toward the Th2 T-cell phenotype, which expresses CD30 on its membrane [113]. An interesting observation is that a biologically significant number of CD30 markers are present in the bloodstream of patients allergic to grasses (and not in controls) in concomitance with pollination periods [113]. A soluble CD30 (sCD30) (88 kD) is increased in serum of HIV (human immunodeficiency virus) seropositive patients [4]. Recent data show that the LAG-3 molecule (lymphocyte activation gene-3), an IgSF member binding to the nonpolymorphic part of HLA class II molecules, is selectively transcribed into activated Th1 and NK cells, since its expression is correlated with IFN-γ production by Th1 cells and not with that of IL_4 [457]. Of particular

interest is the role of the STAT family of molecules, including SH2 and SH3 (src homology 2, 3) [248], activated by an additional TF, PTK (protein tyrosine kinase) [224]. Two classes of SH2-containing inhibitory signaling effector molecules have been identified: the tyrosine phosphatase SHP-1 and the inositol phosphatase SHIP (SH2-containing inositol polyphosphate 5-phosphatase) interacting with the immunoreceptor tyrosine based inhibitory motif (ITIM) [446]. *STAT6,* activated by IL_4 and IL_{13}, *plays a crucial function related to genes regulating differentiation into Th2 T cells* [248, 523], similarly to *STAT4 phosphorylated* by IL_{12} *for Th1 T cells* [523]. Th2 T cells are thought to have a defect of STAT4 phosphorylation [401]. Thereby STAT4 and STAT6 appear to control Th1 T-cell differentiation [523], while IL_2 and IL_{15} activate phosphorylating STAT3 and STAT5, and IL_4 and IL_{12} activate phosphorylating STAT3 [240].

CD 8 T cells. IL_7 and IL_{15} are crucial for the development of $CD8^-$ T cells within the thymus, and SOCS1 (supressor of cytokine signaling-1) regulates this process by regulating both ILs [441]. CD8 T cells are the main type of effector T lymphocytes, as suppressor and cytotoxic cells. In the first case, they bind one HLA class I/peptide molecule also contacting β_2-microglobulin (β_2-m). A flexible loop of the α3 HLA domain is clamped between the CDR-like loops of two CD8 subunits in the classic manner of an antibody-antigen interaction, precluding the binding of a second HLA molecule [170]. The binding of CD8 to HLA can prime CD8 T-cell precursors that depend on IL_2 (cytotoxic T-cell differentiation factor) (Table 1.5) produced by Th1 cells to be transformed into active cells [349]. CD8 T cells thus activated secrete soluble factors suppressing both Th1 and Th2 T cells, as well as growth of Th2 T cells enhanced by IL_4, thereby down-regulating immune responses and inhibiting activation of B cells [255]. Consequently, eliminating CD8 T cells in immunized mice, the synthesis of IgE antibodies was amplified, whereas it was inhibited when CD8 T cells were deleted prior to treatment, suggesting that there are two different subsets of T suppressors, also because IL_4 inhibits CD8 T cells producing IL_2 and IL_6 and stimulates those secreting IL_4 and IL_5 [255]. CD8 T cells have two distinct patterns with different effects: *type 1 of Th1 T cells (Tc1)* and *type 2 of Th2 T cells (Tc2)* [456, 469]. CD8 Tc1 produces IFN-γ, so the highest production of IL_4 is supported by CD8 absence and anti-IFN-γ presence [349] and IL_4 may encourage Tc2 [470]. Animal data suggest that functionally distinct subsets of CD8 Tc2 T cells may play a significant role in IgE regulation [255, 349]; also in humans, such CD8 trigger isotype switching of B cells to IgE antibodies [105]. In addition, virus-specific CD8 can eventually generate IL_5 with an implicit increase of eosinophilia in airways [100], thus providing key elements to explain clinical links between viral infections and asthmatic exacerbations [105]. As we shall discuss subsequently, infantile atopy is associated with a deficiency of T suppressor cells [142] (Chap. 4).

Once activated by antigens or lectins, *cytotoxic T CD8 lymphocytes* (CTLs) play a prominent role when antibodies are unable to block cytolytic viruses, circulating or present on cell membranes. CTLs associated with HLA class I molecules via a specific receptor recognize viral peptides expressed on target cell surfaces, lysing them independently of antibodies or complement [123]. A first signal causes translocation and secretion of cytotoxic cell granules containing proteins named cytolysins, also known as *perforins* (which perforate target cell membranes) and granzymes also present in NK cell cytoplasmic granules: secretion of such Ca++-dependent proteins leads to cell lysis [322]. Typical is the granule speed when they are directionally released to make contact with target cells [189]. A second signal may induce death via a self-destructive mechanism mediated by Fas/Apo-1 (apoptosine-1) (CD95) [559], activated by contact between effector and target cells [322]. Fas and Apo-1 are identical molecules localized on murine and human cells, respectively [559]. Both are encoded by TNFRSF6 on chromosome *10q24.1* and are based on TcR-mediated recognition of target cells [189] and on increased membrane permeability of target cells: the increase in intracellular fluid polarizes to lysis [421]. The lytic process can be outlined in four steps:

- *TcR-CD8-mediated recognition* in the context of HLA class I molecules, CTL adhesion to antibody coated plasma membrane of target cells forming a CTL-target cell conjugate persisting for a few minutes and mediated by CD11a/CD18/LFA-1 (lymphocyte function-associated antigen-1) interactions with pertinent ligands CD54/ICAM-1 (intercellular adhesion molecule-1) and CD58/LFA-3 and possibly CD2/CD80 molecules
- *Irreversible programming* of lytic equipment with release of perforins, which, when in the contact area of conjugates, form 5- to 16-nm pores, upon which mobilized granules deposit their contents into intercellular clefts by exocytosis
- *Lethal hit* in unidirectional fashion mediated by the release of granule contents
- *Cellular death* by osmotic lysis, or more likely by enhanced fragmentation of nuclear DNA, a process characteristic of programmed cell death (PCD) or ritual suicide, or apoptosis [421]

The lytic process is in perspective unremitting, because cell recycling is activated by new TcR stimulations [189].

NK (natural killer) *cells* (non-T non-B) are morphologically similar to lymphocytes, but somewhat larger with a reniform nucleolus and a cytoplasm rich in large granules, assuming therefore an LGL phenotype [460]. NK cells make up about 15% of peripheral blood lymphocytes, and 3%–4% of splenic lymphocytes, while notable amounts are found in lung interstices, gut mucosa, and liver. IL_{21} and $IL_{21}R$ play a role in NK cell proliferation and maturation from bone marrow [412]. Deriving from precursor cells common to T lymphocytes residing in the fetal liver and bone marrow [193],

together with HLA-restricted CTLs, naturally occurring NK cells lyse a variety of targets with no prior specific sensitization of the host (unlike T cells), regardless of HLA gene restrictions and of antibody or complement. NK cells are thus enabled, along with macrophages and leukocytes, to act as a *first line of defense* playing a critical role in natural resistance against a variety of infectious diseases [289]. NK cells and CTLs are mutually self-regulated: NK-cell limitation of viral replication is higher in the first 3 days, contrary to CD8 lymphocytes developing from their precursors within 5–6 days. Accordingly, there is a complementary balance between innate and acquired immunity, since NK-cell cytotoxicity is temporally accomplished and replaced by that of CD8 T cells [273]. NK cells share with cytolytic cells of lower vertebrates and invertebrates both killing activity and lack of TcR and memory, thus suggesting that they are innate and primitive components of the vertebrate immune system [193]. Identified in humans, several CDs possess Fc receptors (FcR) that bind IgG (FcγR) and are activated by membrane receptors such as CD2R, CD16 (FcγRIII), CD39, CD69, and NKRP-1 (natural killer receptor = CD161) and CD94, a product of a gene in human chromosome *12p12–p13*, both members of C-type lectin superfamily [225, 320, 460, 470]. In addition to displaying cytotoxicity against autologous tumor cells, triggered by IFN-α, IL_2, IL_{12} and IL_{15}, NK cells release GM-CSF, M-CSF, IFN-γ, and TNF-α. Both activated IFN-γ and TNF-α are important for immune resistance against pathogens and regulation of hemopoiesis and immune responses, and IFN-γ also generated by other cells in response to microorganisms enhances NK cell cytolytic activity [25, 339, 460]. Increasing evidence shows that NK cells play a cardinal role in the control of microbial agents and with natural cytotoxicity of viral infections, also in absence of T and B cells, as in SCID mice [25, 130]. More precisely, the NK cell kills the abnormal target cell by inserting the pore-forming molecule perforin into the membrane of the target cell, and then injecting it with cytotoxic granzymes [115]. Furthermore, NK cells produce IL_2, necessary for their proliferation, IL_{12} [77], IFN-γ induced by IL_{15} generated by activated human monocytes [68], and VLA-4/VLA-5 (very late antigens 4 and 5) (CD49d/CD29 and CD49e/CD29) mediating NK-cell adhesion to fibronectin (FN) [64]. IL_{12} (NK cell stimulatory factor) primes in vitro Th1-specific immune responses and inhibits development of IL_4-producing Th cells [339]. IL_2 increases expression of adhesion molecules on their cell membranes [193]. Lastly, the MCP chemokines (monocyte chemotactic protein) are NK-cell major attractants, also inducing their chemotaxis [317].

One of the more intriguing aspects of NK-cell function is its possible recognition of foreign substances as non-self, allowing their recognition system to discriminate between self and non-self, to start cytotoxic activities and to produce ILs [289]. This theory is based on two models: an HLA-independent system of recognition triggered by IL_2 [289], or the *missing receptor* hypothesis focusing on immune surveillance with consequent elimination of cells failing to express HLA class I molecules [313]. The second theory has been confirmed by several experimental studies; however, at present how NK cells interact with target cells remains unanswered [667]. According to prevailing hypotheses, expression of HLA molecules could deal with a protective role from NK-mediated lysis [381]. In agreement with this model is the presence on NK cells of ≈20 receptors (NKR, natural killer receptor) specific for HLA class I antigens [4, 667]. NKRs are divided into NKAR (NK activating receptor) and NKIR (NK inhibitory receptor), among which are p50 1–3 (NKAR) and p58, p70, and p140 (NKIR), associated with IgSF, activators of HLA-C (p50 and p58), HLA-B, and HLA-A molecules, respectively [4, 381], and CD94 associated with proteins (94AP) phosphorylated by tyrosine, all implicated in HLA antigen recognition [427]. There are additional NKRs specific for HLA class I antigens, also IgSF membrane proteins and encoded by genes in human chromosome *19q13.4* [91], including the NKAT 1–4 (NK associated transcripts 1–4) family encoding transmembrane (TM) proteins with an extracellular region characterized by 2–3 IgSF domains and one cytoplasmic associated with ARAM (antigen recognition activation motif) [91].

HLA class I molecules may deliver negative signals protecting from lysis cells attacked by NK cells, and may resume cytotoxicity if target cells have reduced class I expression or abnormal peptide-HLA complexes impair recognition [320]. Thereby, NK cells are subjected to a delicate balance between activatory and inhibitory signals: only upon HLA molecule loss or reduction in number is inhibition dampened and killing enhanced [443]. A system of recognition that is characteristic of NK cells relies on the NKARs and NKIRs of these cells. The NKARs recognize a number of different molecules present on the surface of all nucleated cells, whereas the NKIRs recognize MHC class I molecules, which are also usually present on all nucleated cells. If the NKARs are engaged, a "kill" instruction is issued to the NK cell, but this signal is normally abrogated by an inhibitory signal sent by the NKIR on recognition of MHC class I molecules [287, 365]. Even if several issues remain to be adequately explained, for example, regarding specific functions of receptors so far identified and distribution of their activities, it is likely that different receptors may be used depending on the NK-cell activation state, thus releasing a negative signal that recognizes polymorphic HLA class I determinants on potential targets [667]. NK cells and CTLs may regulate functions of each other, in addition to the role of NK cells in CTL differentiation [273], but CTLs down-regulate NK-cell action expressing their inhibitor NKB1 [428], also complemented by TcR-αβ [273]. However, NK cells can express inhibitory receptors such as C-type lectins (CD94), while rodent NK cells may express those of IgSF [320].

The *LAK (lymphokine-activated killer)* cells are additional cells possibly with a cardinal role in discrimination between self and non-self [470]. LAKs display selective cytotoxicity for tumor cells not killed by NK cells with a broader spectrum, synergically expressed by IL_2 and IL_{12} and activated by IL_{15} [557, 693].

In the lytic process mediated by NK cells, we can distinguish four stages similar to those described for cytotoxic CD8 cells, the main difference regarding a shorter length of the whole procedure (4–6 h) compared to the longer one of antigen-specific CD8 lymphocytes. According to recent evidence, CTLs and NK cells not only deliver lytic messages to targets, but also bring about a substantial DNA fragmentation, a feature of CD8 cells via several secreted membrane molecules [190]. Recent reports of an adolescent and two children [43, 273] with life-threatening and relapsing infections, a quantitative and qualitative defect of NK cells [43] or no cells with NK-cell phenotype [273] and a reduced total number of CD8 T cells but with antibodies to several viruses [43], emphasize that NK cells *hold a key position in the response to infections* [460]. Dysfunctions of NK cells have been reported in Chédiak-Higashi syndrome with absence of cytolysis due to a secretory deficiency impairing granule secretion [21], in PID such as SCID, suggesting in this PID a combined deficiency of both NK and T cells, in X-linked lymphoproliferative syndrome (XLP), X-linked agammaglobulinemia (XLA), common variable ID (CVID), LAD (leukocyte adhesion deficiency), and in chronic fatigue syndrome.

K (killer) *cells,* morphologically and functionally similar to lymphocytes, are in practice null cells since they lack markers of both B and T lymphocytes. Not HLA-restricted, they have receptors for complement but not for surface Igs, and can recognize and kill target cells coated with specific antibodies by binding and being triggered via their FcRs: when target cells are killed but not phagocytosed, the process is called antibody-dependent cell-mediated cytotoxicity (ADCC). It appears that the NK-K cell system is actually one heterogeneous subpopulation also comprising LGLs, in which killing is alternatively mediated by a mechanism of NK or ADCC type [460].

A small subset of human γδ T cells fulfills in vitro either nonspecific killing or cytotoxic activities, possibly associated with CD1c molecules, with some similarity to HLA class I molecules [337]. Some activated γδ T cells can lyse infected phagocytes, being powerful K cells [110].

Lymphocyte Recirculation

T-cell recirculation illustrates the elegance of immune cell regulated migrations. Stem cells, after reaching the thymus or bone marrow via the bloodstream and maturing into B or T cells, enter the systemic circulation, peripheral lymphoid organs, and MALT, never traveling in opposite directions. Both phenotypes migrate electively between corresponding thymus-dependent and thymus-independent areas. Memory T cells leave the bloodstream via peripheral efferent vessels, especially to inflamed sites, and subsequently enter regional afferent lymphatic channels that eventually direct cells to a draining regional lymph node, circulating by lymphatics or via cortical venules into lymph node parenchyme (hence the high percentage of memory T cells) [634]. In contrast, naive cells move across afferent postcapillary HEVs into lymph nodes, directly through the HEV barrier. HEVs originate from pre-existing capillary venules (Fig. 1.3), differentiating under the influence of IFN-γ secreted by local Th1 T cells [226]. A particular feature of T lymphocytes is that they selectively bind to specific HEVs in lymphoid tissues and appear to completely ignore normal vascular endothelium, unlike in inflamed sites [226]. Moreover, between HEVs and distinct subsets of T cells there is a selective binding of finer specificity, further regulating lymphocyte homing into various lymphoid and nonlymphoid tissues [522]. In lymph nodes, encounters take place between novel antigens coming from afferent draining tissues and virgin cells. Lymphocyte traffic is not a random mixing of cells in varying tissues of the body. Yet this distinctive migration is directed by lymphocyte cell surface molecules that are receptors for ligands expressed on endothelial structures on HEV cell membranes (*homing receptors*) (Figs. 1.5, 1.11) [62]. As a result of these interactions, T lymphocytes transmigrate into mucosal tissues with immune memory of antigen sensitization, hence regulating local immunity [326]. Leaving secondary lymphoid organs and entering efferent lymphatics, lymphocytes of either phenotype recirculate via mesenteric lymph nodes and return to the blood via the thoracic duct, emptying into the superior vena cava, and crossing HEV endothelial vascular linings; they are eventually exported back to mucosal sites as memory T cells via the subclavian veins [591]. It is believed that lymphocytes migrate nonrandomly to their home: for example, naive T cells expressing CD62E that binds HEV can also migrate into skin T cells, as well as memory T cells, if they are equipped with CLA (cutaneous lymphocyte-associated antigen), which binds to CD62E on endothelium of skin venules [634]. The contribution of adhesion molecules should be pointed out: for example, $\alpha_E\beta_7$ integrin is equal to CD103 inducible by TGF-β1 (transforming growth factor β1), binding to E cadherin directs T lymphocytes to the IEL (intraepithelial lymphocytes) group, >95% of which expresses it, thus mediating lymphocyte adhesion to mucosal epithelial cells [76]. These meaningful data provide an immune basis to analyze reactions triggered in distant target organs, so that lymphoid cells stimulated, for instance in GALT, migrate into the bloodstream and then to the gut lamina propria: *this traffic is very important for maintaining the immune surveillance within the MALT system* [326]. This also elucidates why some T lymphocytes appear to

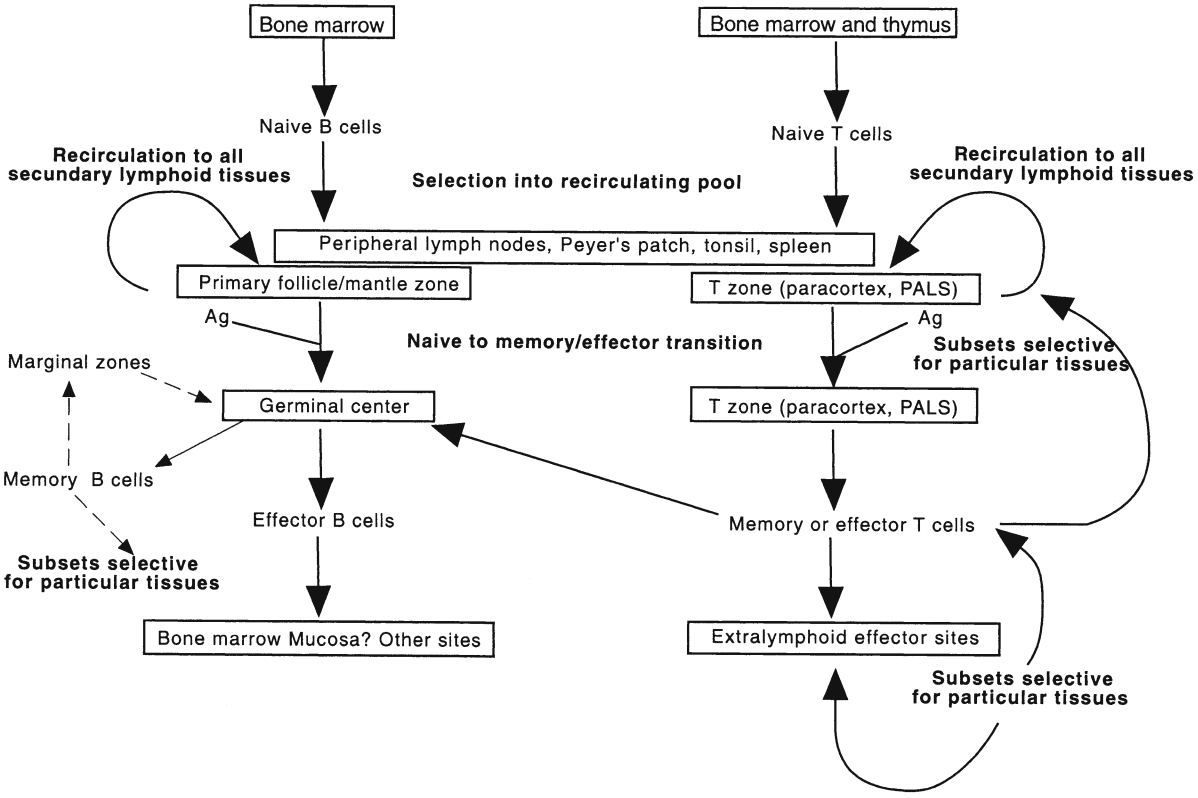


Fig. 1.11. Recirculation of lymphocytes: differential distribution of naive versus memory cells. Unlike naive cells, memory and effector T (and probably B) cells can efficiently extravasate in tertiary or extralymphoid tissues (see text). Antigen-activated B cells may home to specialized environments in the outer T zone during primary responses, or may colonize germinal center sites of hypermutation, affinity maturation, and memory cell differentiation. Less numerous, specialized lymphocyte subsets, such as γδ T cells or IELs, may be targeted from their origin in the thymus or bone marrow directly to reproductive, cutaneous, intestinal, or other tertiary sites. Extralymphoid tissue sites of selective homing include skin, lung, intestinal lamina propria, and synovial tissues. *Ag* antigen, *IEL* intraepithelial lymphocytes, *PALS* periarteriolar lymphoid sheath. (Modified from [62])

patrol throughout the body (*recirculation*) and their traffic is greatly enhanced if an inflammatory response develops. The toxic action carried out by viruses can alter both homing and recirculation of T cells until inactivation, notably of lymphocytes specific for that particular virus (Chap. 22). The immunocompetent memory cells recirculate uninterruptedly, since encounters with particular antigens and triggering of immune reactions are facilitated on the one hand, and on the other localization of specific cells in elective areas is supported [634]. While naive cells recirculate almost exclusively via lymph nodes and other secondary lymphoid tissues, where priming may take place, traffic of memory and effector lymphocytes is also directed to nonlymphoid tissues, including gut lamina propria, lung tissues, as well as inflamed skin, and joints (Fig. 1.11). More precisely, naive cells are excluded from effector mucosal sites because their HEVs lack MAdCAM-1, expressing inadequate levels of $\alpha_4\beta_7$ to bind it [62]. Sophisticated studies on animal models have observed that homing receptors direct traffic with a specific migratory behavior of the cells: those coming from GALT, for example, from PPs, have a preferential migration into the gut, mediated by β_7 integrins [615], and to a lower extent into other sites, and likewise for other preferential localizations (Fig. 1.12) [534]. Memory cells, once activated in specific tissues, depend on unending antigen stimulation and with remarkable selection home in to tissues related to initial exposure to foreign antigens [226]. The selective pattern of recirculation has a prerequisite of preventing inappropriate competitions between T and B cells and migrations into nonlymphoid organs [62]. Similar discrepancies involve specialized subsets making use of preferential routes, such as γδ and IEL cells, which can migrate into skin and/or gut [62]. Studies have hypothesized that an important role may be entrusted to gut-derived IELs homing preferentially to mucosal lung tissue, also effective in preventing airway infections [454]. Because of lymphocyte circulation, B_{IgA} cells of PPs following antigen sensitization home in to regional lymph nodes, where they differentiate, activate, and then return to colonize electively the lamina propria of various compartments [548], including mammary glands, where they transform into IgA-se-

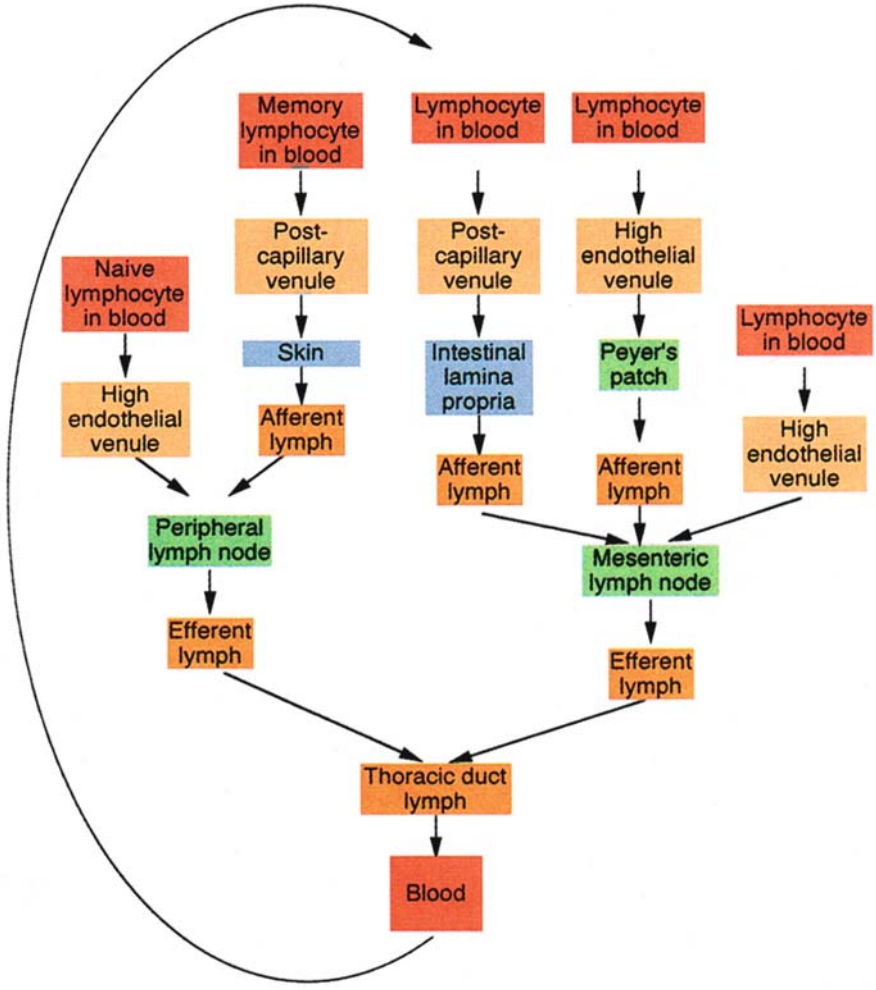


Fig. 1.12. Lymphocyte circulation routes. Patrolling the body in search of foreign antigens, lymphocytes follow circuits through both lymphoid and non-lymphoid tissues

creting plasma cells (*enteromammary axis;* Chap. 2). Analysis of B lymphocytes is limited: naive B cells are the great majority of cells recirculating between blood and lymphoid tissues [635]. As regards the volume of cell traffic, human circulating blood contains $\approx 10^{10}$ lymphocytes, with a blood transit time of 25±6 min, resulting in a 40-fold daily exchange ($\approx 5\times10^{11}$ lymphocytes) [635]. In the human thoracic duct, there is a recirculation of only $\approx 3\times10^{10}$ lymphocytes, that is about 6%–7% of the daily migration via peripheral lymphoid tissues: in rats the rate of naive and memory cells is nearly equivalent [636].

T and B Cell Receptors

T and B lymphocytes can recognize an extremely wide range of disparate foreign antigens because of the high variability of their membrane receptors, stemming from the ability of developing cells to arrange and modify genes encoding antigen receptors, even if single B cells express only one type of specificity, and the same is probably also true for T cells [225]. There are $\approx 10^{14}$ TcR and 10^{18} BcR [353] (Fig. 1.13) playing a central role in antigen recognition and activation, with structures complementary to those of specific antigens; epitope recognition by receptors is a critical event triggering immune responses. In contrast, the human genome contains 75,000–100,000 genes, most of which have no role in immune recognition [353]. Molecules coded for by HLA molecules regulate B- and T-cell differentiation, because they are involved in the host's ability to set humoral or CMI responses. More prominently, HLA molecules are necessary to signaling and their intercellular interactions contribute to distinguishing between self and non-self [470]. Specificity dominates in the molecules induced in every individual *in one or two versions,* dictated by maternal and paternal genes, whereas TcR and BcR are produced in *millions of copies* that, although adhering to a common structural plan, are different in V domains [115]. If each lymphocyte bears receptors with a unique specificity, coordinately expressed by progenitor stem cells via processes of genetic recombination, there must necessarily exist a great variety calling on the immune system to recognize an extensive spectrum of antigens. Doubtless, clonal selection interferes to naturally eliminate receptors destined to die via PCD [276, 510]. Both TcR and BcR are able to recognize a given antigen due to their unique molecular structures, which are spatially and chemically complemen-

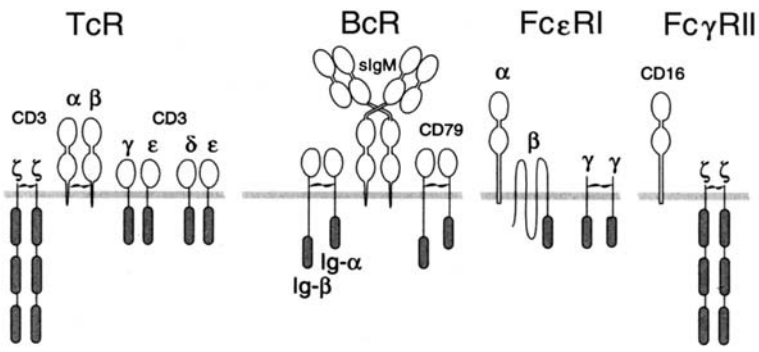


Fig. 1.13. Receptors of hemopoietic cells

tary to one another, fitting together in a "lock-and-key" relationship, thus giving the starting signal to cellular replication. The progeny of cells derived from any naive cell originate as a lymphocyte clone with cells morphologically identical to one another in nearly all respects, while each BcR or TcR expressed by cells of a given clone are also identical [185]. Lymphocytes, already triggered by binding to their specific antigens and activated, proliferate and deliver specific effector functions, thereby recognizing the intruder and successfully inactivating or eliminating it: evidence exists that cells with a specific receptor that fits better to binding sites may differentiate selectively, thereby predominating over other cells [47]. Receptor molecules mediating specific recognition have heterodimeric structures: L and H Ig polypeptides behave as BcR, while α and β chains are related to TcR. L, H, α and β chains possess V domains arranged in a specific spatial contour on available antigen surfaces and also C domains interacting with receptors on host tissues and transmitting signals to cell cytoplasm.

Therefore, BcR and TcR genes share many features and both undergo similar DNA rearrangements [18]. In addition, genes coding for BcR and TcR use a unique strategy to achieve the degree of diversity required. The set of human BcR and TcR genes is complete for all the seven *loci* – the three Ig *loci:* IgH (after the H chain), IgK (after the κ L chain) and IgL (after the λ L chain); and the four TcR *loci:* TcRα/δ on chromosome 14, TcRβ (on chromosome 7, and TcRγ on chromosome 7. The IgH cluster (431 human genes and 798 alleles) corresponds to 4 types of gene segments: V, D, J, and C. The Igκ and Igλ clusters lack D segments. All these segments contain multiple genes; in the IgH cluster, for example, there are ≈50 functional V segments. The *TcR genes* have a similar organization and in the 4 TcR *loci* there are 242 human genes and 443 allels [115, 183]. In contrast to the TcRβ and TcRδ *loci,* the TcRα and TcRγ *loci* do not contain D segments. And, as in the case of Ig genes, each TcR *locus* contains multiple V, D, and J genes; on TcRα, for example, there are 70 to 80 V genes and ≈60 J genes [115]. To such genes is committed a molecular control to increase variability via somatic and evolutive processes, but inheritable chromosomes, as discussed earlier, contain no Ig genes at all: otherwise there would be several million of these genes. For example, spermatozoon and ovum contain gene fragments that via subsequent phases of rearrangement, reorganize genome sequences so that *new genes can be created in immune cells* to continue the cycle [18]. The basic principles governing the genetic mechanism to create such a variety can be focused on DNA somatic rearrangement, versatile and casual, accompanied by deletion and an evolutionary mechanism with reference to active engagement and frequent combinations [185]. Antibodies and T-cell clones thus achieved are specific not only for antigens present now in the microenvironment, but are also able to develop different specificity to antigens not yet present, to be encountered in the future [326].

T-Cell Antigen Receptors

While nearly all developing TcRαβ thymocytes express a single TcRβ protein, many thymocytes rearrange and express two different TcRα chains and, thus, display two αβTcRs on the cell surface. The number of such dual TcR-expressing cells is surprisingly lower among the mature T cells [284]. The immature TcR consists of a β chain identical to that found in the mature TcR and a pre-T chain that contains only a C region. This segment is replaced by an α chain to form the mature TcR, and each chain consists of a V and a C region [115]. The PTK signaling and coreceptor involvement may be operating in normal thymocytes [284]. *Antigen-specific T cells have a TcR similar to membrane Igs (mIg) of B cells* and also contain V, D, J, and C segments. *CD3-TcR complex is the unity of mature T cells: TcR is able to recognize and discriminate among different foreign antigens, CD3 polypeptides have long intracytolastic tails necessary to TM transduction of activation signals* [222]. *Each TcR* molecule *is formed by two pairs of heterodimers, αβ and γδ,* each α, β, γ, and δ chain containing a V domain and a C domain, and a *CD3 complex by three distinct invariable chains known as γ, δ, ε correlated between them.* Antigen-specific T cells have a TcR similar to membrane Igs (mIg) of B cells. CD3-TcR complex is the unity of mature T cells: TcR is able to recognize and discriminate among different foreign antigens, CD3 polypeptides have long intracytoplasmic tails necessary to TM transduction of activation signals [225]. Each TcR molecule is

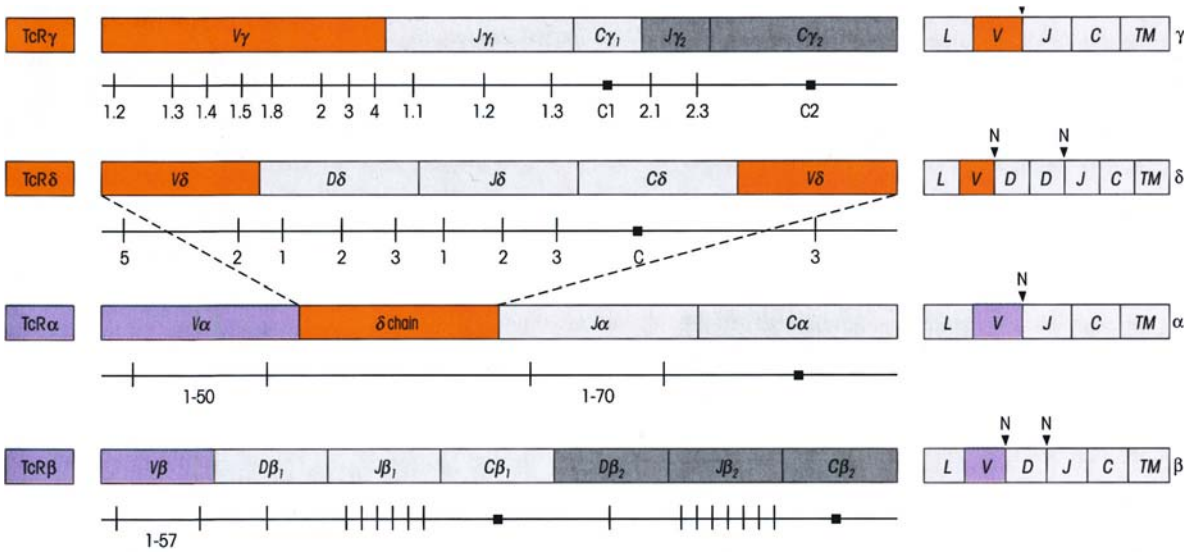


Fig. 1.14. Genes coding for TcR (for details see text)

formed by two pairs of heterodimers, αβ and γδ, each α, β, γ, and δ chain containing a V domain and a C domain. CD3 complex by three distinct invariable chains known as γ, δ, ε correlated between them. As in the antibody molecule, the V domains contain three CDRs, where the highest degree of amino acid variability is concentrated. The CDRs in the case of the αβ TcR recognize a complex formed by a peptide seated within the groove of an MHC molecule [171]. Each TcR has a single extracellular Ig-like C domain, a TM segment, a cytoplasmic tail (44–81 amino acids in length), and is completed by ζ and/or η chains. ζη chains also are invariable molecules, derived by conjunction of alternative transcripts of the same gene, and form homodimers linked by a -S-S (ζ-ζ and/or η-η) bond, or heterodimers (ζ-η), not covalently associated with five subunits (αβγδε) to form the heptameric complex. The human chain MWs are as follows: γ 25–28 kD, δ 21 kD, ε 20 kD, ζ 16 kD and η 22 kD [4].

Two T structures recognize antigen peptides bound to HLA molecules:

- In 90%–95% of T lymphocytes is found a TcR formed by two polypeptide chains α and β (αβ) (MW of 45 and 40 kD) expressed on the cell membrane in the form of 90-kD heterodimers and linked by -S-S bonds, associated on the cell surface with CD3, a clonally invariable protein, and encoded by gene segments organized in a discontinuous way on chromosomes (V, D, J and C); hence gene rearrangements are necessary. V regions are present in both chains; in addition there are J and C for α chain, and D, J and C for β chain. The α and β molecules, membrane gps belonging to IgSF (Table 1.4), are encoded by genes located on chromosome 14 (region *q11*) for α chains and on chromosome 7 (region *q32*) for β chains [337]. Use of Vα and Vβ regions of TcR from T lymphocytes is very stable, as TcR gene expression is controlled by DNA sequences, among others.
- In 5%–10% of TcRs a second heterodimeric receptor formed by a γ (45 kD) and a δ chain (40 kD) (γδ) linked by S-S bonds is expressed. The γ chain locus is on the short arm of chromosome 7 (region *p15*) and the δ chain on chromosome 14 is embedded within the α locus [222, 337] (Fig. 1.14) [470]. Human genes encoding γ chains contain exons for V, J and C regions, and gene exons for δ chain encode for V, D, J and C regions, similarly to α and β chains [18]. In humans, γ chains have two different Cγ2 exons, one encoding a cysteine residue. The γδ cells are about 80%–90% $CD8^+$ and the remaining cells $CD4^+$ [337]. In humans, both receptors are present in IELs, but αβ T cells prefer to differentiate mostly in normal epithelia homing lymphocytes [525].

The same basic principles of gene rearrangement described for Ig apply for TcRs. Also, genes encoding V and C regions of TcR chains are found on DNA varying segments, and therefore should be rearranged. However, repertoires of different TcR are believed to be at least as large as Ig molecule repertoires, also regarding antigen specificity. A remarkable degree of independence seems to dominate in the generation of αβ and γδ lineage cells from progenitor cells that, in theory, could simultaneously express a TCRγδ and a pre-TCR [180]. In peripheral lymphocytes, STAT5 is primarily required for the generation and/or maintenance of γδ T cells and TCRγδ(+) IEL [246]. Both γ and δ chains exhibit typical *fetal rearrangements* because there is essentially no N region diversity at VJ or VDJ junctions of rearranged γ and δ genes [525]. A remarkable difference between genes coding for αβ and γδ chains of TcR is a programmed regulation of their activation during ontogenesis [229], in that comprehensive rearrangements at γδ loci appear before those at αβ loci [525] in reciprocal independence [229]. *The γδ TcR is the first receptor expressed by thymocytes in utero* [279]: until day 17 of gestation, γδ cells are mainly TcR^+ subsets in murine

thymus [263]. Such TcRs appear to predominate compared to αβ TcRs in murine skin and gut epithelia [582], where γδ could play a leading role in *first-line* defense. The much earlier activation and conversion to memory of the γδ T cells is a significant differnce between the γδ and αβ T cell lineages, and illustrates the central role that γδ T cells have in adressing Ag challenge from birth onward [111], substituting for αβ cells that are scarce in mice [587], and protecting epithelia from damage caused by inflammations via selective suppression of IgE responses [279]. This effect is endorsed by normal levels of γδ cells [80]: reduction to only 500 cells [350] or suppression of γδ cells, as reported in atopic babies [495], could imply an increase in IgE levels [80, 350, 495]. As γδ TcRs develop normally in mice deficient in HLA class I and II molecules, these cells could be present in HLA defects. In addition, studies related to CDR3 of γ, δ, and α TcR chains and Ig H and L chains suggest that γδ cells may act as Igs in antigen recognition without a prior processing and presentation by professional APCs [466, 500].

In children with PIDs, studies reported CD3 γ, ε, or ζ deficiency, with TcR expression reduced on the cell membrane by about 50%–90% of T lymphocytes; ε defect is much more severe than γ defect as regards TcR expression or complete conformation, thus resulting in mature and immature T cells [263]. According to recent data, it does not seem necessary that parts of TcR αβ, γδε and ζ be present all grouped together in one complex to be expressed on the cell membrane as a functional unit: in the absence of functional γ chains, a healthy child shows that in vivo T cells may use fewer receptors than is expected (see "CD3γ Deficiency," Chap. 22). *ζ chain deficiency seems to be more severe* because of the high reduction of CD4, CD8 and TcR-αβ levels and absence of CD44 and CD25 [102].

The three CD3 chains, γ, δ, and ε, have homologous sequences of amino acids and their cytoplasmic domains comprise ITAM (immunoreceptor tyrosine-based activation motif) or ARAM, which contain tyrosine residues necessary to induce tyrosine phosphorylation in activated TcRs [3289]. TcR complex is not expressed by mature T cells and exists at their surface to carry out the whole set of T-cell functions, showing several similarities with Ig structures: it is formed by two instead of four polypeptide chains that are different from one another, linked by -S-S bonds, and consist of both V and C regions. The two polypeptide chains (similarly to Ig chains) are delineated by a C-terminal C region, the same for each TcR, and an N-terminal V region, different for each TcR. Such wide variability enables various TcR clones *to recognize a practically infinite number of invaders* [225]. On lymphocytes and in the bloodstream, there is a substantial number of TcR and BcR with different V regions; variability is acquired during somatic rearrangements between gene segments V, D, and J linked together in respective chromosomal regions. *Diversity* is generated by multiplicity of independently assorted components of V regions; however, TcRs have a greater tendency to use N-region diversity and frame shifts, whereas Igs prefer to use somatic hypermutation. TcR diversity is amplified by multiple V genes in germline, random combination, and junctional and insertional variability, thereby ensuring generation of a noteworthy structural diversity to deal with a practically unlimited universe of non-self antigens [36].

As a corollary of data discussed above, self/non-self discrimination by TcR is HLA-restricted, in that T cells and APCs must be complementary to HLA molecules: following this assumption, CTL $CD4^+$ recognize class II antigens as highly preponderant T $CD4^+$ cells, and CTL $CD8^+$ (the majority) recognize class I antigens. Therefore interactions between CD4 and CD8 and respective HLA class molecules increase efficiency of T-cell and APC interactions. CD4 and CD8, IgSF members, adhesion molecules, and signal transducers could act as receptors for nonpolymorphic HLA epitopes, contrary to TcR interacting with polymorphic epitopes [463].

B-Cell Antigen Receptors

The events following transduction of activation signals are better understood for T than for B cells. The immature pre-B cells (and pre-T cells) express preliminary versions of the antigen receptor. At this stage, the BcR comprises a pair of H chains, each with a V and a Cμ region identical to those found in the mature receptor, and a pair of surrogate L chains, termed Vpre-B and γ5. As the B cell develops, the surrogate L chains are replaced by conventional L chains, each with a V and a C region [115]. BcR consists of mIgs, more common proteins critical for antigen recognition, differing from antibodies only for an extra sequence of amino acids at C-terminal domains of H chains [214]. During embryonic and fetal life, mIgs of different isotypes alternate in B-cell populations; similarly, this occurs during clonal maturation in their adult life [3]. Igs of all classes can exist in either membrane-bound or secreted forms. Membrane forms combine with Ig L chains to make mIg; however, mIgs are retained in ER, unless associated with Igα and Igβ [414] . B cells comprise:

- *Specific mIgs:* IgM (5%–20%) and IgD (5%–10%) more frequently than IgG (1%–7%) and IgA (<5%) antibodies. These specific molecules facilitate B cell identification by immunofluorescence (IF) techniques, using antisera specific for each isotype.
- *Membrane receptors* for the Fc fragment of IgG (FcγR) [18], found also on non-B cells and monocytes (Table 1.3), possible false positivities due to IgG passive fixation on such receptors.
- *Receptors for complement* components C3d and C3b with rosette formation; receptors recognize complement when bound to either IgG or IgM antibodies (EAC rosettes).

- *CD21* is the receptor for C3d complement fragment and EBV (Epstein-Barr virus). This latter event allows microorganisms or their products to link to B cells, thus giving activating signals. The mature IgM molecule acts as the BcR for antigen, usually together with IgD BcR with the same antigen specificity. The V regions of the H and L chains each contain three CDRs. The CDRs make contact with the antigen [115]. When IgMs (with low affinity bond) play a BcR role, membrane signaling requires binding to CD45, equal to LCA (leukocyte common antigen), an important amplifier of BcR-mediated signals contained in intracytoplasmic tail domains with phosphatase activity (PTPase) [103], with several isoforms indifferently distributed between B and T cells [395].
- *Additional receptors* of mature B cells include IFN, IL_4 and IL_2. B cells present unrestricted surface antigens to cells of B lineage such as CD9, CD10 and CD23 (FcεRII).
- *B cells* have a high density of HLA class II molecules and express CD71 [96].

BcR consists of tetrameric H chains (IgH) with five isoforms: μ (73 kD), δ (67 kD), γ (50 kD), α (55 kD), and ε (70 kD), and L (IgL) with two isoforms: κ (26 kD) and λ (26 kD) [4]. mIgs of all types associate noncovalently with two heterodimers, each formed by a pair of polypeptidic chains, α and β, Igα (CD79a) and Igβ (CD79b), -S-S-linked to each other. In addition, two TM gps have an extracellular domain of Ig type (included in the IgSF), and an intracytoplasmic tail of 61 amino acids for α chains and of 48 for β chains in close contact with mIgM (mμ) [453]. Interestingly, a BcR without the T-cell CD3 complex has these proteins structurally resembling it [214]. More precisely, CD79a and CD79b cytoplasmic tails contain an amino acid motif with two tyrosine residues such as ITAM (see activation of lymphocytes), representing an important point of communication between BcRs and two types of PTKs: *src-family kinases and spleen tyrosine kinase syk* [453]. The nonreceptor PTK syk is widely expressed and has an important role in intracellular signal transduction in hemopoietic cells. It displays a leading role in BcR spectrum of activities, as demonstrated by BcR complete activation via PLCγ1 (phospholipase Cγ1) linking. In addition, syk controls signaling pathways between the two, whereas its deficiency blocks B-cell development at pre-B stages [446, 589]. A member of the src family is Bruton tyrosine kinase (btk) underlying XLA (Chap. 22). As an illustration of the SHP-1-dependent inhibition pathway, recruitment of SHP-1 to the B cell inhibitory receptor PIR-B attenuates BcR-triggered activation responses [446]. Extra BcR functional interactions are with B-cell-specific surface proteins CD19 and CD22 [453], two ligands, CD80 and CD86 (Table 1.2), also common to T cells, DCs, macrophages, etc. Their activation increases CD80 and CD86 levels [453]. CD80 expression peaks after several days, whereas CD86 expression peaks within 24 h of activation; thus higher levels are stimulated [242]. Two pairs of TM BcR-associated proteins, *BAP32/BAP37* and *BAP29/BAP31,* have been recently mapped, associated with IgM BcR and IgD BcR, respectively, in a class-specific fashion [453].

In conclusion, there are evident analogies in gene organization and structure of both receptors that suggest a common evolution from ancestral genes. All genes belong to IgSF (Table 1.4).

Immunogens, Antigens and Allergens

Definitions. Briefly, immunogens, antigens and allergens [1, 185, 428] can be defined as:

- *Immunogen:* any antigen that in a particular host can elicit immune responses and react with the relative products
- *Immunogenicity:* the capacity of provoking an immune response (B- or T-mediated)
- *Antigen:* anti(body) gen(erator), any substance recognized by TcRs or antibodies, that can trigger immune responses
- *Antibody:* selected Ig molecule containing a specific sequence of amino acids and binding specifically to antigens, inducing its synthesis
- *Antigenicity:* an epitope capacity to be recognized by specific receptors of the immune system
- *Allergen:* aller(gy) gen(erator), any foreign substance able to activate IgE synthesis
- *Allergenicity:* the ability of an allergen to elicit an IgE-mediated reaction in sensitized patients
- *Sensitization:* natural or artificial induction of an immune response, notably when it causes allergy in the host. In subsequent contacts with the same immunogen there is a quicker onset and a more severe immune response [185]
- *Panallergen:* molecule with properties shared by different species, for example, a protein with a conserved IgE-binding epitopes across species that cross-react with foods, plants, and pollen
- *Epitope:* antigenic determinant; an *allergenic epitope* marks a specific peptide domain associated with allergenic potential
- *Paratope:* antibody-combining site for epitope

Strictly speaking, all immunogens are also antigens, but not all antigens are naturally immunogens: the immune system may recognize an antigen, although it does not respond to an antigen unless it is also an immunogen. However, the terms "antigen" and "immunogen" are often employed interchangeably [185].

Immunogenicity *of a given protein* depends on epitopes present on its molecule triggering sIgE synthesis (specific IgE antibodies), thus in turn triggering allergic reactions. It also relies on the existence on the protein surface of hydrophil amino acids such as lysine, arginine, and aspartic and glutamic acids. That a non-immunogen molecule could become the target of immune responses if attached to an immunogen protein is evident with compounds with low MW, called *haptens*

(from the Greek "απτειν," to fasten) or ligands, small molecules with a restricted number of identical epitopes. Haptens are therefore antigenic but not immunogenic, and can combine with only one type of antibody, but are unable by themselves to elicit immune responses, owing to their low MW. To become an immunogen, the free hapten must bind a *carrier,* either serum proteins or epidermal proteins: for a single hapten more than one carrier may exist [185]. Anti-hapten antibody responses require cooperation between subsets of B and T cells first recognizing the hapten and second the carrier. Current examples are drugs, metal contaminants such as Ni, Cr, Cu and β-lactamines, natural constituents of vegetable origin, including balsams, fucomarins, lactones and terpenes, to be found in fruits, vegetables and aromatic plants [18].

Antigens are soluble or corpuscular substances, mostly made up of proteins, each formed by one, two or more chains composed of different combinations of about 20 amino acids linked to one another. Every chain is rolled and folded; hence when it is extended, parts distant from each other come into contact when protein structures fold [12]. The term "antigen specificity" refers to antigen binding only to antibodies thereof activated. This specificity has a crucial value in the immune system: notwithstanding complexity of molecular structure, any given antibody including IgE will recognize and bind not to the whole molecule, but to the epitope, a limited portion of such a molecule formed by a few amino acids arranged in sequence or close by because of chain folding. Antigen–antibody reactions always denote a primary, dynamic fixation, based on noncovalent forces; hence they are relatively low and dependent on steric complementarity between epitopes and antibody. Epitopes often behave as haptens [18].

Epitopes and Paratopes

The *epitope* is a molecular structure with a diameter of 2–3 nm representing *the antigen part electively recognized by* a given antibody (that is *the paratope*) or TcR, thereby determining immune reaction specificity. Chemically, it is composed of five to seven amino acids active in molecules of globular proteins and glucide units of lateral chains of polysaccharides (PS). A part of the epitope reacts with Fab of L chain, the other with antibody H chain, being the fundamental part of a protein molecule recognized by binding either to T cells or Fab fragments [1]. Another molecule, the *agretope,* also formed by amino acid residues interspersed in primary sequences, binds to both TcR and paratope [11] and to a HLA-DR molecule of new synthesis localized in a hypervariable β1 region, the *desetope* (Fig. 1.15) [577]. Each TcR chain supplies three CDRs [in particular CDR3 with (D) J sequences as Igs interact with epitopes] contributing to the antibody combining site, the paratope, three-dimensional space defined by the folding of new

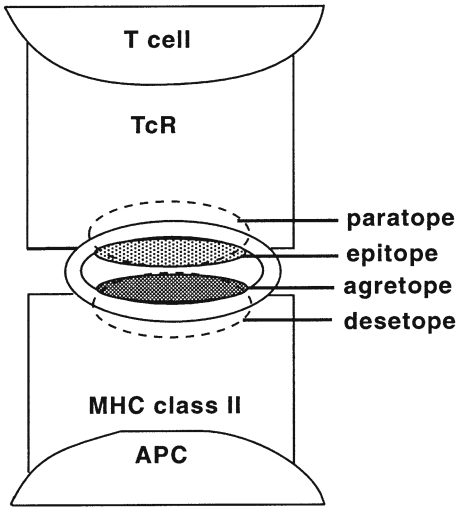


Fig. 1.15. Interactions between T cell, TcR and APC before antibody production. The epitope of the immunogenic peptide binds to TcR (and the paratope region), while the agretope binds to the Ia antigens of APC. The desetope is the part of HLA molecule to which the agretope binds. *APC* antigen-presenting cell, *TcR* T cell receptor

polypeptide chains. H chain regions corresponding with V_H and C_H1 domains and whole L chains forming Fab (Fig. 1.6) are closely located to form paratope walls. In conclusion, one H and one L chain together form a paratope, therefore a single four-chain unit is bivalent, since it contains two combining sites [469]. The paratope is positioned in the NH_2-terminal Fab region, whose shape changes to recognize and ensure a close fit between antibodies and complementary epitopes [459]. A *paratope* is a molecule portion determining antibody specificity, making contact with an antigen-related part, a binding pocket allowing peptides to be accommodated, thus suggesting the picture of a *key in a lock* [1, 470] (Fig. 1.16). Paratope specificity depends on amino acid sequences of hypervariable loops, an effect of minute variations of genes encoding for this antibody portion. Analyses of antibody terminal parts have demonstrated a three-dimensional flexibility of the paratope calling antigens for binding, with spatial conformational changes made on peptide binding, absent in unliganded Fabs. As Fig.1.17 illustrates, in liganded Fabs a prominent groove connected to a deep pocket is formed to fit peptides, adding a prominent channel to encompass extended portions of bound peptides [459]. Affinity between these two structures depends on the strength of attraction and repulsion existing between them. Mutation of an amino acid residue forming part of an epitope or paratope can greatly increase or weaken such affinity [459]. Structurally, there is also flexibility in epitopes, permitting antigens to interact with antibodies, thus widening the likelihood of antigen–antibody encounters of sufficient affinity to generate immune responses [459]. The immune system utilizes such properties to

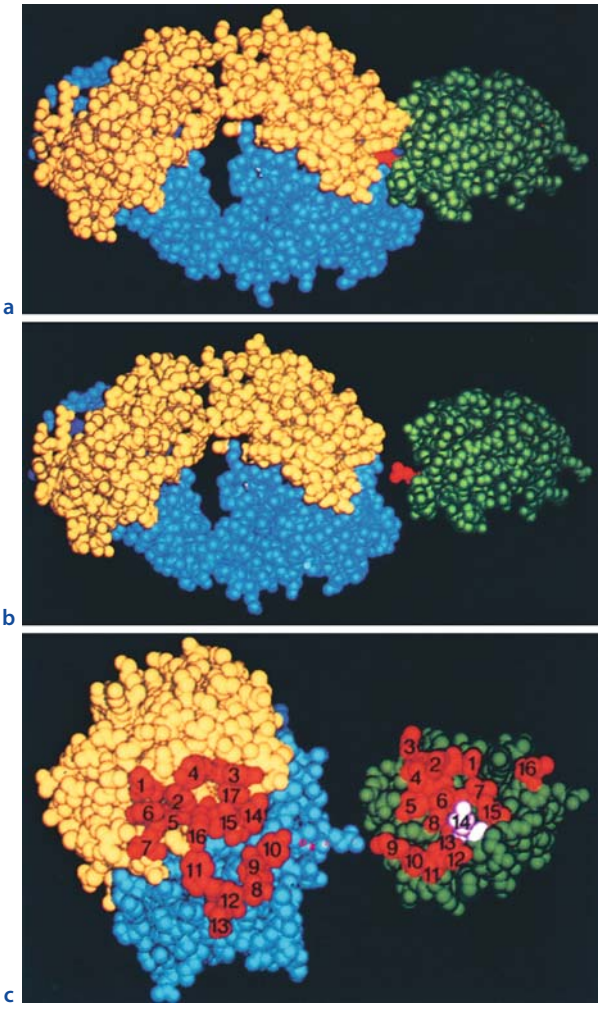


Fig. 1.16 a–c. Structure of the antigen-antibody interface. **a** Space-filling model showing Fab and lysozyme molecules fitting closely together. Their interactions form an antigen–antibody complex. The antibody H chain is shown in *blue*, the L chain in *yellow*, lysozyme in *green*, and glutamine 121 in *red*. **b** The Fab and lysozyme models have been pulled apart to show that protuberances and depressions of each are complementary to each other. **c** End-on views of the paratope (*left*) and lysozyme epitope recognized by antibody (*right*), formed from **b**, by rotating each molecule approximately 90° around a vertical axis. Contact residues on both antigen and antibody are shown in *red*, except for glutamine 121 in *light purple*

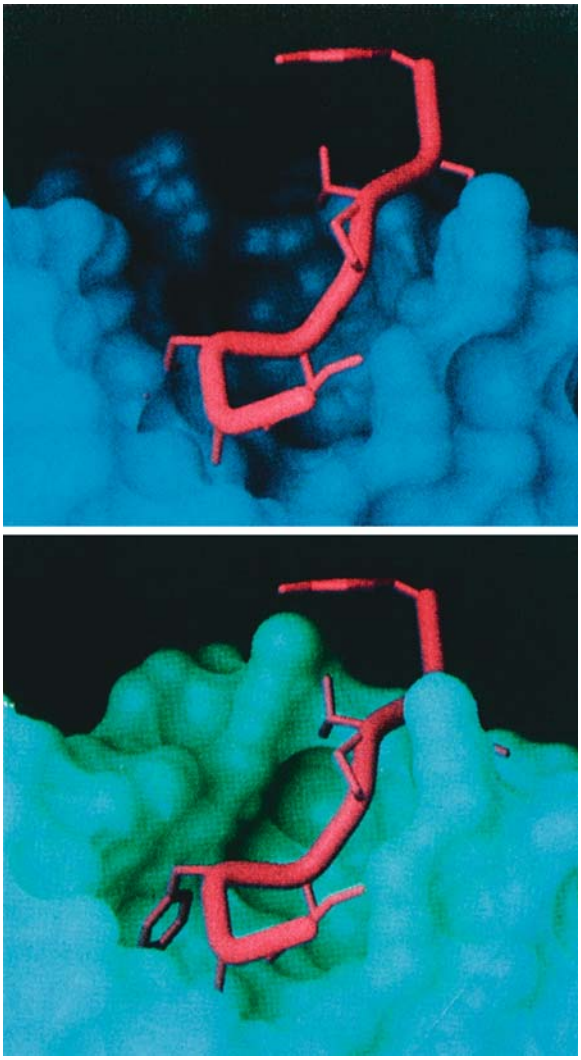


Fig. 1.17. Shape of the binding pockets of the unliganded (*above*) and liganded Fab (*below*). The unliganded Fab appears as an open, basin-shaped pocket, and in the liganded Fab a prominent groove connected to a deep pocket is formed to fit the peptide antigen

amplify antibody affinity during immune responses (affinity maturation and antigen selection). However, microorganisms may use such changes to evade host immune recognition by a variety of strategies, including shifts in critical surface antigens, as is the case of HIV, of influenza virus, etc. [337].

Epitopes are classified into *B- or T-reactive epitopes*. *B-epitopes* are recognized by B cells and binding sites of specific antibodies, whereas T-epitopes correspond to small peptide fragments (8–12 amino acids) recognized by TcR in the context of HLA molecules on antigen linear surfaces [368]. Several B- and T-epitopes have been characterized, but antigen-specific T cells appear to be distinct from those individuated by IgE antibodies [672]. The total number of separate B or T epitopes on antigen molecules, identical or different, is called *valence*, overall proportional to foreignness, molecular size and chemical complexity, which represent the factors influencing immunogenicity of a given antigen [185]. We shall see that an immune response also depends on the dose and mode by which foreign invaders enter the body. As a rule, macromolecules are the strongest immunogens, usually having more epitopes; however, in a given subject and under the influx of particular circumstances, only one or a few are recognized by BcRs and/or TcRs, since they are targets of immune responses. Macromolecules with one type of epitope are

named monospecific, but their greatest part has many independent epitopes with different specificities. Some large proteins have been found to contain as many as 50 separate T-cell epitopes. However, even small molecules can have bivalent structures: for example, the human hormone glucagon, which is only 29 amino acids long, contains separate B- and T-cell epitopes [185]. In addition to the events described, T-cell epitopes show the exquisite specificity required to activate CD4 T cells, which in turn are needed for B-cell responses against nearly all antigens. The first requirement for a molecule to be an immunogen is to contain at least one T-cell epitope: consequently, molecules comprising only B-cell epitopes (such as haptens or amino-terminal parts of glucagon) may serve as targets for antibody responses, being unable to elicit such responses autonomously [185]. Even if, in theory, T-cell epitopes do not bind to IgE antibodies, small peptides may do so and subsequently block (as monovalent haptens) or stimulate basophils [496]. These observations are of substantial importance, since identification of IgE-binding B-cell epitopes may:

1. Increase both specificity and sensitivity of diagnostic tests by including molecules with epitopes of this type
2. Distinguish B-cell from T-cell epitopes
3. Elucidate trigger mechanisms of IgE-sensitized metachromatic cells [496]

The epitopes of a protein are of two types [12, 185]:

- *Sequential,* or linear, or segmental epitopes (usually T-cell epitopes), determined by primary structures, wholly positioned sequentially in linear sequences of a protein or PS antigen, a segment of amino acid sequences: immunogenicity is the result of covalent linkage of these residues to one another, which cannot move significantly apart. By definition, such epitopes are more resistant and generally remain unchanged following heat or enzyme denaturation and may be left untouched by enzymes not specific for amino acid bonds present within epitopes.
- *Conformational,* or discontinuous epitopes (usually B-cell epitopes), are far apart in primary sequences and critical residues are brought close together via folding of antigen chains in normal three-dimensional steric configurations, so that they may encompass residues widely distant from one another along protein sequences, positioned also nearby, but juxtaposed in tertiary structures. Immunogenicity is due to molecular configuration. Normally, such epitopes are scarcely resistant; hence they are lost when antigens are denatured and fail to refold appropriately [13, 414] (Fig. 1.18).

Consequently, since B epitopes are mostly conformational, they can be more easily eliminated, while sequential T epitopes are more resistant. Epitopes of this type have acquired relevance for their use in specific immunotherapy (SIT) and preparation of protein hydrolysates. *Spatial conformation* plays a key role, since *accessibility* of epitopes is a prerequisite for binding to immunocompetent cells and for immunogenicity. In the case of atopy, IgE antibodies recognize one specific B-cell epitope in peptide tertiary structures, whose three-dimensional conformation can mask additional epitopes that will not bind to antibodies. On the contrary, T lymphocytes recognize sequential epitopes with a few amino acids located on antigen linear sequences [368]. T- and B-cell epitopes are distinct by their topographical position being located at different points, with T-cell epitopes positioned in primary structures, whereas B-cell epitopes lie in secondary and tertiary structures. Due to such differences, following heat denaturation, conformational shifts may eliminate certain epitopes on a protein, not affecting the integrity of sequential ones, and enzyme hydrolysis may alter both types. Physicochemical manipulations therefore do not alter all conformational epitopes, yet they may unmask epitopes hidden within a native protein three-dimensional structure, which may become immunogenic and accessible to IgE antibodies for binding while causing new ones to form [1, 12]. Actually, polypeptides without flanking amino acids can originate from hydrolysis, apt to combine with antibodies [591]. Instead, hydrolytic reduction of -S-S bonds of β-lactoglobulin (βLG) does not influence reactivity with IgE antibodies of native molecules or of fragments of tryptic hydrolysis, but reduces IgG reactivity [351]. These studies have revealed the structural bases of βLG antigenicity and immunogenicity: βLG immunogen epitopes are comprised in a relatively restricted part of the molecule, while those reactive with IgG appear to be correlated with conformational sites [351]. However, the distinction between immunogen and antigen epitopes is as yet scarcely known [402]. Individual residues within single epitopes, due to characteristics of either antigens or those specific to patients, are called *immunodominant,* thus are recognized

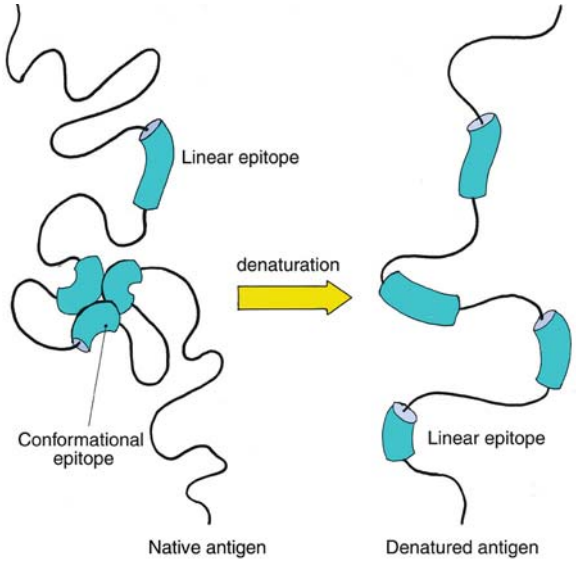


Fig. 1.18. Conformational and linear epitopes

Table 1.13. Main properties of human immunoglobulins (*Ig*)

Ig class	Heavy chains	Light chains	MW (kD)	Serum level (mg/dl)	Presence in secretions	Serum half-life (days)
IgA	α	κ/λ	160 (sIgA 390)	200±50	+	6
IgD	δ	κ/λ	175	3–4	–	3
IgE	ε	κ/λ	190	0.03	±	2
IgG	γ	κ/λ	150	1,250±300	–	23
IgM	μ	κ/λ	900	150±50	±	5

by most T or B lymphocytes induced by whole proteins. However, certain immunodominant, linear epitopes are represented by peptides with four to nine amino acids [511]. A single antibody may also react with an antigen other than the one causing its formation, as well with *different epitopes on the same molecule or carried by different molecules*: this is called cross-reactivity. Consequently, *allergenicity does not depend on the size of a given protein, but on the number of immunoreactive epitopes, similar or not* [185]. In practice, the same molecule can bind several different epitopes, either two combining with and cross-linking the same IgE molecule, or six, eight, or even ten nonidentical epitopes; whereas sIgE can bind more strongly to an allergen different from the one that stimulated its synthesis, but with characteristics similar to the first one [1].

In this context, children sensitive to Der p (*Dermatophagoides pteronyssinus*), for example, may produce sIgE binding to allergens of a species they have never met in the environment. Accordingly, following exposure to only one species of mites, children recognizing these common epitopes may produce antibodies that will recognize and respond to other species with this epitope, even if such species are absent in the environment where children live [671]. Additional studies support that different proteins may have common epitopes, thereby explaining cross-reactivities between different proteins within a given food as well as between different related foods, or between pollens, vegetables and fruits [1] (see p. 164). Likewise, if there are epitopes common to different substances with the same amino group in *p* (para) position on the benzene ring, patients sensitive to a drug with such a group should prudentially avoid other substances within the same chemical group. In conclusion, *epitopes identical or nearly identical can be found on antigen molecules of different origin or species*: an epitope common to two or more allergens triggers cross-reactions between substances with a similar structure, or between metabolites alike from an immunochemical point of view. As a consequence all molecules containing an epitope provoke allergic reactions in patients sensitive to another, even in the absence of a previous exposure and/or sensitization.

Antibodies

Immune responses are characterized by Ig production (Fig. 1.6): there are nine different isotypes correlated with nine C_H genes and two C_L genes: IgA_1, IgA_2, IgD, IgE, IgG_1, IgG_2, IgG_3, IgG_4, IgM, belonging to five major classes [3]. All Igs are glycoproteins and contain 3% to 13% of carbohydrates, depending on the class of antibody. The carbohydrate is essential in maintaining the Ig structure [115]. Tables 1.13 and 1.14 [544] summarize their main characteristics, including MW and half-life, and Table 1.15 [545] presents Ig levels at various ages compared with adult values; other authors prefer geometric means (GM). Isotype switching has an indubitable final significance, with every isotype having specialized biological properties, as follows:

- *IgG* comprise 75%–80% of serum Igs, being the only class crossing the placenta from mother to fetus, where it is found and is responsible for neonate protection during the first months of life. The levels decrease progressively, leading to a transient hypogammaglobulinemia at approximately 4–6 months of age, while normal adult values are attained only at about 4–6 years. Molecules are further divided into four subclasses: IgG_1 (70%), IgG_2 (20%), IgG_3 (8%), IgG_4 (5% of total). Besides the Hγ chain, subclasses have different properties, regarding for example, binding to serum complement and activating its alternative pathway, and adhesion to macrophages. IgG_2 has a lower placental passage, and IgG_4 is the only IgG unable to fix complement by the classic pathway, in addition to having a poor affinity for phagocytes. Abnormal levels of one or more IgG subclasses have been reported in children with severe chronic asthma, or recurrent respiratory infections (RRI) whether associated with asthma or not, of allergic type or not, with selective IgA deficiency (IgASD) or other PIDs, but not in otherwise healthy subjects (Chap. 22). Therefore, IgG antibodies represent the more differentiated phase of antibody responses with variations of subclasses according to encountered pathogens (or persistence of antigen stimuli). IgG is also an opsonizing antibody, although with lesser potency than IgM, reacting with epitopes on microorganisms via its Fab portions. However, the Fc portion for which many phagocytes bear

Table 1.14. Main biological properties of human Ig classes and IgG subclasses

	IgG_1	IgG_2	IgG_3	IgG_4	IgM	IgA	sIgA	IgE
Placental transport	+	+	+	+	–	–	–	–
Complement activation								
Via classic pathway	++	+	+++	–	+++	–	–	–
Via alternate pathway	–	–	–	–	–	+	?	?
Presence in secretions	–	–	–	–	–	–	++	+
Agglutination	+	+	+	+	++	–	–	–
Opsonization	+	+	+	+	++	–	–	–
Virus neutralization	+	+	+	+	+	–	+	–
Hemolysis	+	+	+	+	++	–	–	–
Bacterial lysis	–	–	–	–	+	–	–	–
Degranulation of MC	–	–	–	?	–	–	–	+++
Fixation to macrophages	+	–	+	–	–	–	–	–

Data from [544].
MC metachromatic cells.

Table 1.15. Levels of IgG, IgM, IgA and total immunoglobulins (mean±1 SD) in sera of normal subjects by age (mg/dl)

Age	IgG	% of Adult level	IgM	% of Adult level	IgA	% of Adult level	Total Ig
Newborn	1,031±200 (645–1,244)	89±17	11±5 (5–30)	11±5	2±3 (0–11)	1±2	1,044±201 (660–1,439)
1–3 Months	430±119 (272–762)	37±10	30±11 (16–67)	30±11	21±13 (6–56)	11±7	481±127 (324–699)
4–6 Months	427±186 (206–1,125)	37±16	43±17 (10–83)	43±17	28±18 (8–93)	14±9	498±204 (228–1,232)
7–12 Months	661±219 (279–1,533)	58±19	54±23 (22–147)	55±23	37±18 (16–98)	19±9	752±242 (327–1,287)
13–24 Months	762±209 (258–1,393)	66±18	58±23 (14–144)	59±23	50±24 (19–119)	25±12	870±258 (398–1,586)
25–36 Months	892±183 (419–1,274)	77±16	61±19 (28–113)	62±19	71±37 (19–235)	36±19	1,024±205 (499–1,418)
3–5 Years	929±228 (569–1,597)	80±20	56±18 (22–100)	57±18	93±27 (55–152)	47±14	1,078±245 (730–1,771)
6–8 Years	923±256 (559–1,492)	80±22	65±25 (27–118)	66±25	124±45 (54–221)	62±23	1,112±293 (640–1,725)
9–11 Years	1,124±235 (779–1,456)	97±20	79±33 (35–132)	80±33	131±60 (12–208)	66±30	1,334±254 (966–1,639)
12–16 Years	946±124 (726–1,085)	82±11	59±20 (35–72)	60±20	148±63 (70–229)	74±32	1,153±169 (833–1,284)
Adults	1,158±305 (569–1,919)		99±27 (47–147)		200±61 (61–330)		1,457±353 (730–2,365)

Levels of total serum IgE are in given in Chap. 6.
Modified from [545].

receptors delivers opsonizing properties; similarly it plays a critical role in ADCC, focusing NK cells on their targets [36]. To sIgGs (specific IgGs) and STS-IgGs (short-term sensitizing IgGs) has been credited a sensitizing activity mostly belonging to IgG_4, that turned out to mediate *similar-reaginic reactions but not of the IgE*

type, competing with IgE antibodies to bind to allergens [514]. In allergen molecules, IgE antibody binds to sIgE epitopes, while IgG antibody can bind to IgG-specific epitopes localized in the same point of the molecule or elsewhere: namely, IgG may react with the same epitope as IgE antibodies or the two Igs may react with two quite different epitopes on the same molecule [401]. IgGs are erroneously called *blocking antibodies* since they inhibit effects of IgE antibodies in the Prausnitz-Küstner test [1]: IgGs probably act as such, without interfering with sIgE epitopes to provoke an in vivo synthesis of IgE antibodies to a given allergen. However, not being reagins, IgG antibodies cause diagnostic perplexities due either to negativity of allergic tests in individuals with typical symptoms of immediate hypersensitivity or to a paradoxical situation of the same antibody acting in pathogenic and protective ways [1], an issue to be dismissed among the hypotheses put forward [13]. In summary, no direct challenge can reproduce clinical disease or tissue reactivity ascribed solely to specific IgG_4 antibodies to challenged allergens [13]. From a clinical point of view, in FA diagnosis measurement of IgG antibodies is superfluous because healthy subjects following a prolonged allergen exposure also have frequent elevations of serum IgG antibodies [13], while in respiratory allergy such levels are identical in patients whether they be atopic or not (Chap. 11). Characteristically, IgGs increase during SIT, although correlations between their rise and clinical results are unstable (Chap. 13). A high amount of IgG_4 was found in complexed IgG anti-IgE antibodies, recognizing at least two epitopes located within Cε2-Cε3 and Cε4 domains. IgG antibodies binding FcεRI located within interdomain regions might potentially cross-link IgE bound to FcεRI and blocking it, since only one of two ε sequences binds to FcεRI [514]. That an effector function of such weight is recognized by anti-IgE allows us to conclude with good reliability that anti-IgE antibodies play a modulator role, so essential during activation of metachromatic cells as to stimulate basophils to release histamine, a fact related not to IgG antibody levels or to subclasses, but to epitope specificity [513]. Administered intravenously (IV), Ig are highly beneficial to children with PIDs and several other affections.

- *IgA* antibodies prominent in the MALT are the sole antibodies in secretions (aside from a small percentage of IgM), hence it is called sIgA. IgA occurs as monomers, dimers, trimers and polymers. IgA monomers have no agglutinating properties (unlike sIgA) but can bind to antigens, predominant in serum, while dimeric IgA is quantitatively the most abundant in secretions. Two dimers are held together by the same J chain and associated with a polypeptide, called *secretory component* (SC), localized on the long arm of chromosome 1. SC is a fragment of epithelial cell poly Ig receptors and a component of high significance because it protects mucosal sIgA from proteolytic degradation, literally wrapping round $C_\alpha 2$ domains of both sIgA subunities, even if -S-S bonds are for only one subunit [186]. The two subclasses, IgA_1 and IgA_2, represent 12% and 3% of total serum Igs, respectively. sIgA is actively transported across epithelial cells, *covering as a film* and protecting mucosal surfaces from microorganisms, preventing organism attachment to cells and clearing them off by phagocytosis. IgA can activate complement via the alternative pathway, not inducing bacterial lysis mediated by the classic pathway, and possess bactericidal activity against Gram– organisms, but only when lysozyme is present, interestingly, in the same secretions containing sIgA [36]. Table 1.15 shows IgA levels slowly ascending, and sIgA levels quickly produced. IgA antibodies present in colostrum and breast milk protect at-risk infants (Chaps. 2 and 24). IgASD is the most frequent PID.
- *IgE* (in which the E is erythema), also termed reagins with a half-life of 2–4 weeks on metachromatic cell membranes, are normally present in serum at the lowest concentration, <0.001%, of total circulating Igs. For this reason, IgE concentration is expressed in international units (IU), one IU being equal to 2.4 ng/ml. IgE levels increase considerably in atopic diseases, hyper-IgE syndrome (HIgES), infections with parasites [471], pediatric AIDS, and other diseases. High levels saturate mast cell receptors and pass into the circulation, where detectable levels are extremely elevated compared to normal subjects [471]. IgE antibodies come from plasma cells distributed primarily in lymphoid tissues of respiratory and gastrointestinal tracts, derived from pre-B cells from a differential lineage originating from IgM B lymphocytes and via the following passages: $B_{IgM-IgD}$ – $B_{IgM-IgD-IgE}$ become IgE B lymphocytes. Like other Igs, IgE has four polypeptidic chains, but two chief immunobiological characteristics: an ε chain 11 kD greater than homologous chains, with five domains, one V and four C, one more than other Igs, and is cytophilic, having a propensity to bind to metachromatic cells with Fc fragment, of which FcεRI is a receptor (Table 1.3). FcεRI extends from the C-terminal part of Cε2 to the N-terminal sequence of Cε3 domains, while the FcεRII binding site, the second receptor, is localized in the N-terminal part of the Cε3 domain close to FcεRI [553]. Since IgE molecules have a bent form, their convex surface nearest the membrane (Cε3) binds to FcεRI, thereby *making remote a binding site for another receptor* (Fig. 1.19, showing also FcεRII). This explains why FcεRI has only an α chain, its affinity for only an ε chain, and why there is only one receptor [553]. IgE molecules have no subclasses, similarly to IgM and IgD molecules [186].
- *IgM* is present early in primary immune responses to most antigens. Small IgM amounts in secretions (sIgM) also contain SCs. Being a pentamer, covalently linked by -S-S bonds with J chain, it *does not pass through the placenta*. Moreover, the IgM molecule is the most efficient agglutinating and complement-fixing Ig (by classic pathway). Because of their pentameric form, IgM antibodies can form macromolecular bridges between epitopes that may be too distant from each other to be

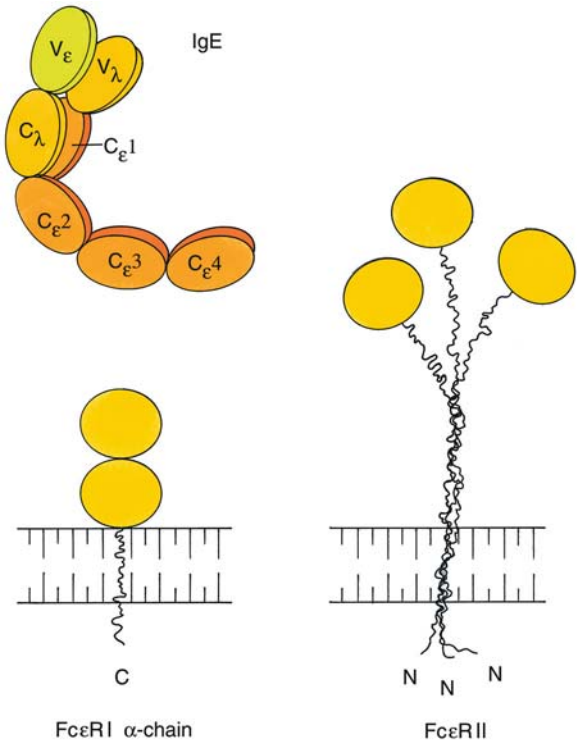


Fig. 1.19. IgE and its receptors. FcεRI α-chain. Schematic representation of IgE and its receptors FcεRI and FcεRII drawn to the same scale. IgE is illustrated as a drawn molecule, whereas the FcεRI α-chain is drawn erect. The other chains have been omitted to simplify the diagram

bridged by monomeric IgA antibodies. Also, because of their multiple valences, they are best suited to combine with antigens containing more epitopes, as related to PS or cellular antigens [36]. Together with IgD, IgM is the most common Ig expressed on B cell surfaces (and virgin B cells). IgM is the first Ig class synthesized by both the fetus and newborn: high fetal IgM levels are indicative of congenital or perinatal infections [186]. IgM is the isotype synthesized in noticeable amounts by children and adults as a primary antibody response after immunization or exposure to T-dependent and T-independent antigens; therefore a role of IgM as regulator of immune responses via a specific receptor of Th lymphocytes has been postulated. IgM antibodies are significantly increased in IgASD and particularly in HIgMS.

- *IgD* is present in serum in very small amounts. Its presence on B-cell membranes during certain stages of development may suggest either an involvement in B cell maturation or a prominent role in immune tolerance. However, IgD functions are mainly unknown 186].

Idiotypes and Anti-idiotypes

V regions of antibodies may have two different functions:

- *Recognizing, with the paratope,* one or more epitopes present on an antigen or on different antigens
- *Acting as an antigen* with an idiotype leading to anti-idiotype antibodies [18]

Such antigen functions are committed to idiotypes, present in V_H and V_L regions of antibody molecules.

Definitions

- *Public or cross-reacting idiotypes,* expressed by antibodies produced by all individuals of the same race, are directed against a single epitope or against nonidentical antigens. V gene mutations altering paratopes without modifying idiotypes determine the sharing of a public idiotype from antibodies with different specificity.
- *Private or individual idiotypes,* present in a single clone, expressed irregularly by subjects of the same species, therefore exclusive of a given individual, directed against a single epitope.
- *Anti-idiotypic antibodies* are complementary antibodies, directed against the structure of an antigen-recognizing antibody, which can prospectively trigger production of a network of anti-anti-idiotypic antibodies and so on.
- The *internal image of the epitope* is a structurally identical idiotype cross-reactive with epitopes of foreign antigens.
- *Regulatory idiotype,* present on a relatively high number of Ig molecules and/or T lymphocytes, where it can function as a base of a regulatory receptor-specific system.

In other words, a V region of an antibody contributing to paratope expression and antigen recognition has idiotypes against which the organism reacts, instinctively inducing anti-idiotype antibodies against sIgE; anti-idiotype antibodies are normally formed during immune responses. In some instances, a fraction of an anti-idiotype may exemplify a facsimile (or an internal image) of the nominal epitope that triggered the original reaction [47]. Figure 1.20 summarizes a wide panorama of idiotype activities.

In Network Theory, the Nobel laureate Niels Jerne [238] proposed a network of idiotype–anti-idiotype interactions as a regulatory mechanism of immune responses, the expression of each idiotype being suppressed by complementary anti-idiotypes. The foreign antigen approach, disturbing pre-existing homeostasis sustained by an equilibrium between idiotype and anti-idiotype, generates an immune response by T and B cells, activating *anti-idiotype responses* [238], either humoral (antibodies against idiotypes of soluble Igs) or CMI (T cells with TcR specific for hypervariable regions of both TcR and BcR) [474] (Fig. 1.20). Some anti-idiotype antibodies may competitively block binding to paratopes of corresponding antigens, interacting with epitopes or directly with paratopes (*associated idiotypes*), thus eliciting an antigen–antibody reaction wholly similar to classic reactions induced by antigens [454]. Other anti-idiotypes, without such selective inhibition,

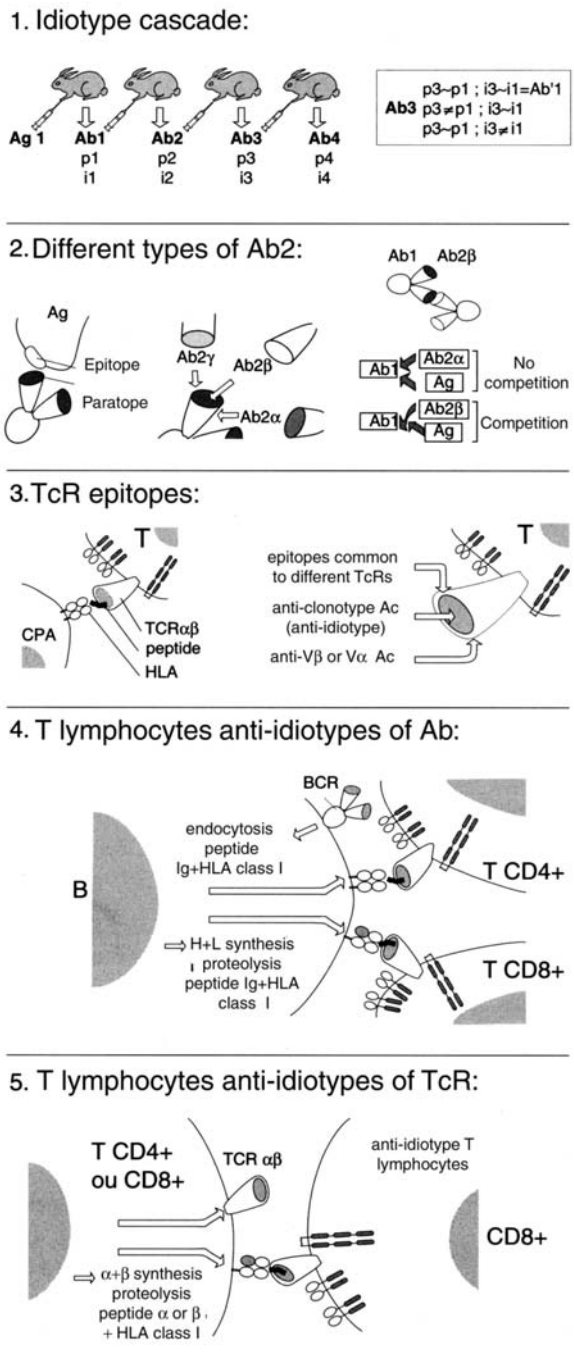


Fig. 1.20. *1*: Idiotype cascade. It was observed that antibodies with different specificities can share the same idiotypes. *Ag* antigen, *Ab* antibody. *2*: Different types of Ab2α. Ab2βs, internal images of Ag, bind to Ab1 at a site corresponding to the paratope, thus inhibiting Ab1-Ag binding. *3*: TcR epitopes. The anti-clonotype Abs are directed against the hypervariable regions of Vα and Vβ domains forming the TcR binding-site: anti-Vα or Vβ Abs recognize epitopes defined by sequences common to members of the same family, while other Abs recognize epitopes common to TcRs with different specificity. *4*: T lymphocytes anti-idiotypes of Ab. *5*: T lymphocytes anti-idiotypes of TcR

may instead bind to amino acid sequences of the paratope FR region, which do not contribute to antigen binding (*not associated idiotypes*) [454]. As it was demonstrated, anti-idiotype antibodies can bind directly to Ig idiotype determinants, regulating their expression [58]. Also for T cells there is evidence of T-cell idiotypes (clonotype markers) and of interactions between B-cell and T-cell idiotypes [58]. In addition, anti-idiotype antibodies specific for TcR clonotype markers have been observed, in turn interrupting the network of anti-idiotype antibodies produced in certain diseases during a normal immune response: since T cells can have varying phenotypes, a potential exists for augmenting or suppressing immune responses via idiotype networks involving T cells [58]. For example, T-cell idiotypes may be recognized by B cells (antibodies) or by peptides associated with T cells (TcR) presented by HLA class I or II molecules. Similarly, T cells can recognize B idiotypes presented as HLA-associated peptides [236] (Fig. 1.20). Additionally, although antibody responses to target antigens are characterized by formation of several hundred different antigen–antibody molecules, idiotype–anti-idiotype reactions are much more restricted depending on common amino acid sequences, in close contact with paratopes of different antibodies. According to these studies, cross-reactivity is frequently observed among various antibodies in anti-idiotype responses to target antigens. In other words, if paratopes able to recognize a given idiotype exist, classic epitopes should present it together with idiotypes already existing in the molecule [236].

Anti-idiotypes can regulate sIgE responses but cannot cause antibody responses; however, miming functionally original epitopes, the so-called idiotypes *internal image* of antigen *or network antigens* (in the sense given by Kohler) [268] can behave as *surrogate* antigens, or as epitopes in terms of structural affinity, like antigen molecules, thus increasing antibody responses. Figure 1.21 [58] shows another of Jerne's theories, in which idiotypes can mimic structures of apparently unrelated antigen molecules, binding to receptors specific for that antigen and inhibiting immune responses or, on the contrary, up-regulating them by replacing some antigen functions. The paratope of antibody 1 is complementary to a structure on the immunizing antigen; in turn the paratope of antibody 2 is complementary to that of antibody 1, and thus antibody 2 can resemble a structure on the immunizing antigen. In the case of insulin and of anti-insulin antibodies (Fig. 1.21), the paratope of antibody 1 is complementary to the epitope, antibody 2 is an anti-idiotype vs anti-insulin antibodies (antibody 1), and can bind to the insulin receptor and even stimulate glycolysis [58].

The circuit thus far described may have envisioned an idiotype network. Examining part A1 of the figure, we consider two B cells, B1 and B2, whose complementary surface Igs form an idiotype–anti-idiotype pair. Thereby, B1 cell binds to an Ig V region on the B2 cell surface,

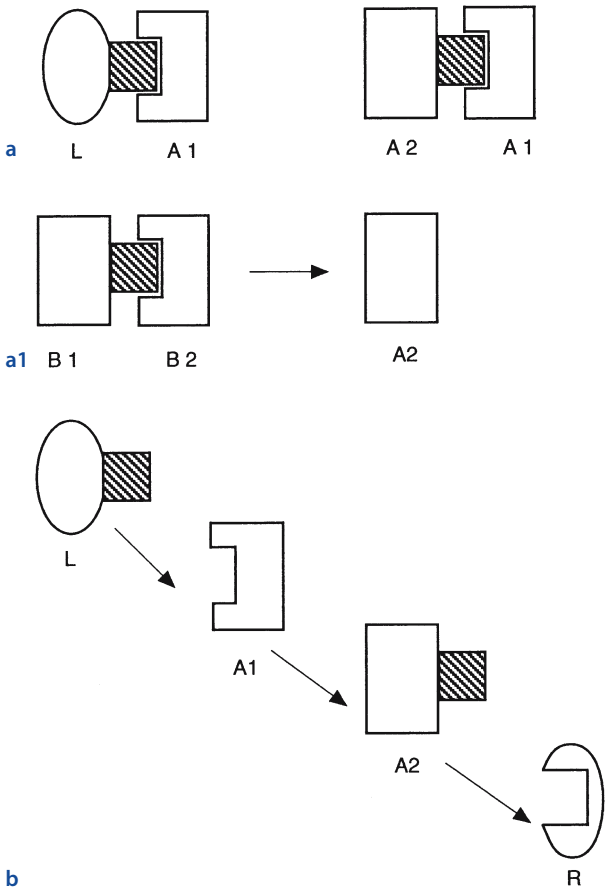


Fig. 1.21. **a** Diagram of the complementary relation between antibody 1 (*A1*, idiotype) and antibody 2 (anti-idiotype). **b** Because of the complementarity with the ligand (*L*), *A2* can bind to the receptor (*R*) for L. (Modified from [58])

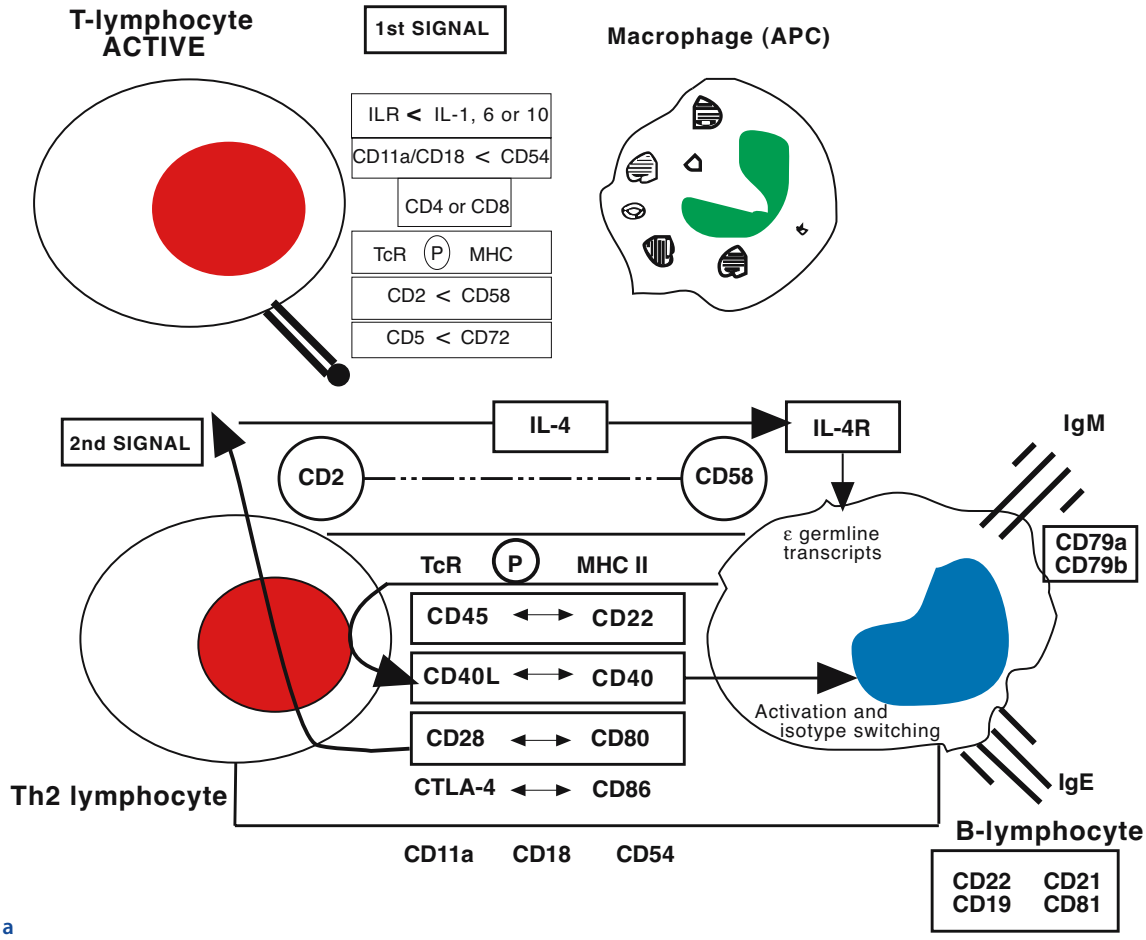
stimulating the cell to secrete antibody 2: in this way an idiotype secreted during immune response to a given antigen can yield a corresponding anti-idiotype. In several idiotype–anti-idiotype systems, anti-idiotypes can act as anti-receptor antibodies. Among hormones, besides insulin, we find thyroid-stimulating hormone, prolactin, glucagon, etc.; among neurotransmitters are found acetylcholine, catecholamines, endorphins, reovirus, etc. In all these instances, the V region of antibody 2 represents the internal image of the external antigen. It may seem odd that an antibody is disguised as an antigen, but among 10^8 three-dimensional V regions there must be a kind of mimicry of other molecules [58].

Interesting applications of these studies are observed in laboratory animals: the basic principle is that in different species immune responses elicit formation of antibodies expressing a common idiotype. For example, oral immunization with heterologous antibodies modulates both systemic and mucosal anti-Ig responses: after administering murine IgA antibodies to rabbit females, murine anti-idiotype anti-antibodies are found in the serum and colostrum [683]. It is striking that murine monoclonal anti-idiotype antibodies, specific for the rye grass pollen allergen *Lol p 4*, are able to inhibit binding of murine, rabbit, and even human antiserum. Since *anti-Lol p* antiserum can inhibit idiotype–anti-idiotype interactions, accordingly the monoclonal antibody in question has been characterized as an internal image anti-idiotype of the antigen [683]. Further experiments have demonstrated that immunizing adult mice with anti-idiotype antibodies against poliovirus are activated antibodies that effectively neutralize upcoming viruses. An interesting study (Chap. 2) following this line of research has revealed that the CB of offspring of agammaglobulinemic mothers or with IgASD was able to inhibit binding of poliovirus antigens to anti-poliovirus antibodies: a suggested rationale is a probable reactivity against CB anti-idiotype anti-antigen antibodies.

Current acquisitions on immune system regulations are still a matter of debate. At least two control systems are concerned, one provided by the suppressor cell network and the other by the idiotype cell network. The first network can be activated by original immune stimuli that regulated the system. CD8 T cells may be specific for a given clone or may down-regulate T-cell responses in a nonspecific way. Consequently, the same complex molecule can trigger helper T cells, effector T cells, and specific or nonspecific suppressor T-cell responses. Several ILs can also contribute to these results, modulating CD4 or CD8 T cells [469]. Idiotype regulation depends on recognition by the immune system that an immune reaction took place. In this event, antibody production in response to an antigen stimulus and pertinent antigen–antibody interactions trigger a response by a second series of IgE-producing B cells. The second-generation antibodies react with idiotypes on the original paratope producing the first-generation antibody and block its production. Anti-TcR antibodies are also formed and down-regulate T-cell responses in a similar way, while CD8 T cells can operate against idiotypes. Such intervention of cells and responses is essential to avoid that *a failure, even partial, of any regulatory systems may result in active immune responses* [469].

Apoptosis or PCD

If a thymocyte fails to produce any functional α chain, it cannot be selected and eventually dies of apoptosis; if a T-cell cannot be activated because the second signal is absent, it may be a target of a distinctive event known as *clonal anergy* (Fig. 1.22a) [138, 160, 177, 457]. Consequently, mature lymphocytes are rendered functionally unresponsive, resulting in *tolerance,* for example because CD28/CD80–CD86 [446] or CD40–CD154 (CD40L) costimulatory signals are lacking, which instead amplify such signals [37, 160]. Signaling via the CD122 γ chain can prevent induction of T-cell anergy



a

Fig. 1.22. **a** Schematic representation of the interactions arising during antigen presentation and the effects of T and B cells in the lymph node cortex (T zone) (see text). *CTLA-4* CD152, *CD40L* = CD154. (Modified from [138, 160, 177, 436]). (Fig. 1.22 b, c see next page)

[4]. *Clonal deletion* applies to particular clones of autoreactive, immature T cells that are physically destroyed during ontogeny to maintain self-tolerance [30]. Figure 1.22b shows that a CD4 T-cell associated with a HLA molecule recognizes a self-antigen and is eliminated instead of being activated (PCD) [138, 185]. Costimulatory signals are crucial also for T naive cells: when such signals are absent, the first stimulation by TcR renders them ignorant, refractory to further stimuli, and following a likely second stimulation, they are exposed to an inappropriate activation; therefore they may remain functionally inactive (anergy) or die (PCD) (Fig. 1.22c). Clonal deletion and negative selection also take place in immature B lymphocytes. For example, *limitin* (IFN-ω) (Table 1.5) produced by mature T lymphocytes in spleen and thymus as well as by bronchial epithelial and salivary duct cells suppresses the proliferation of pre-B cells [402]. Figure 1.23 shows positive and negative selections developing in GCs [309]. PCD occurs during embryogenesis, in the thymus during cell immune maturation, and at the end stage of immune responses [115, 276, 510]. It was also shown that PCD is an active form of genetically programmed cell suicide, not provoking inflammatory reactions [192]. More precisely, PCD is a physiological apparatus essential for normal development and homeostasis of multicellular organisms, a sophisticated defense mechanism to remove potentially dangerous cells, including self-reactive cells, virus-infected cells, and tumor cells, aiming at restoring a previous equilibrium [542]. CD4 and CD8 T cells, after their maturation into effector cells, die in 95% of cases, not only for precise regulation of cell numbers, or to maintain cellular homeostasis, but to protect T cells from continued secretion of potentially harmful amounts of ILs [400] and to leave a stable pool of long-lived memory cells [7]. Apoptosis occurs by *ex novo* activation of specific genes acting as PCD inducers/activators, or whose expression coincides with cell entry into apoptotic pathways (Tables 1.16, 1.17) [192, 400]. Here Fas/Apo 1 (CD95)-induced apoptosis is an important

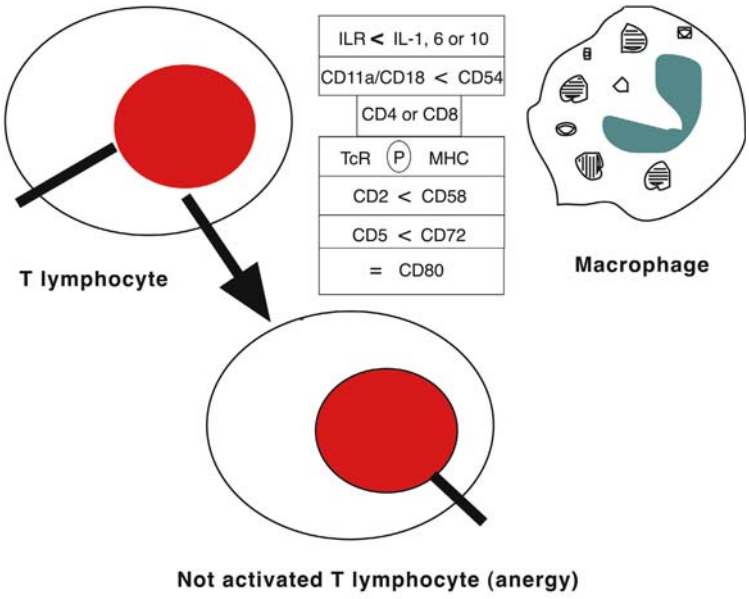
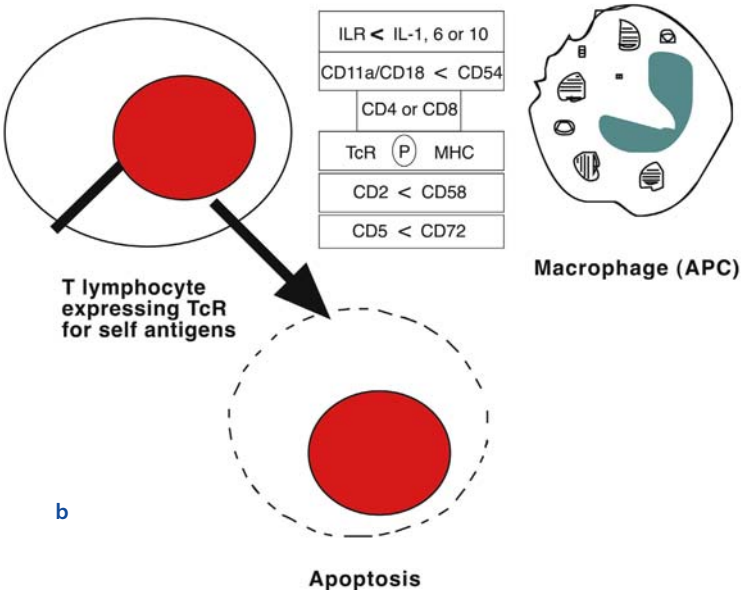


Fig. 1.22. b *Upper part*: Clonal anergy. CD4 T cell recognizes the antigen presented by APC; however, there is no CD80–CD28 interaction and it remains inactivated. *Lower part*: Clonal deletion. CD4 T cell recognizes a self-antigen; however, instead of being activated it is eliminated (apoptosis). **c** Effect of the intervention or not of costimulatory signals: on the *left* there is normal binding between peptide-HLA-TcR associated with the binding of a costimulatory ligand (*CL*) to a costimulatory receptor (*CR*), on the *right*, ignorance, anergy and apoptosis result because of the lack of costimulation. (Modified from [138, 160, 177, 457])



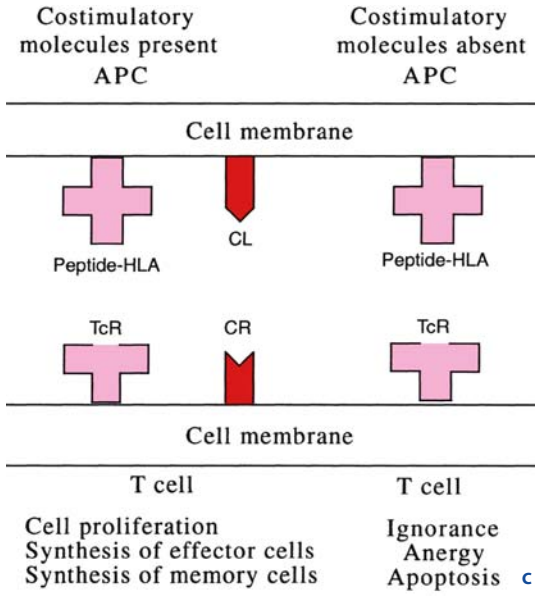


Table 1.16. Genes of the apoptotic pathway, either induced or expressed during apoptosis

Gene	Gene products	Probable function
c-*fos*	Transcriptional factor	Induces apoptosis when continuously expressed
c-*myc*	Transcriptional factor	Activates an apoptotic program
Fas/APO-1	Membrane receptor	Ligand binding induces apoptosis
Grb-3	Transduction factor	Expressed in some tissues during apoptosis
ICE/pr ICE	Cysteine protease	Initiates the active phases of apoptosis
nur 77	Nuclear receptor	Expression during apoptosis starts gene transcription
p-53	Transcriptional factor	Induces apoptosis upon DNA damage or loss of Rb function
RP2	Membrane receptor	Expressed during early stages of apoptosis
TG	Transglutaminase	Accumulates in apoptotic cells
TRP M2	Clusterin, SGP-2	Expressed in some tissues during apoptosis

Modified from [192].

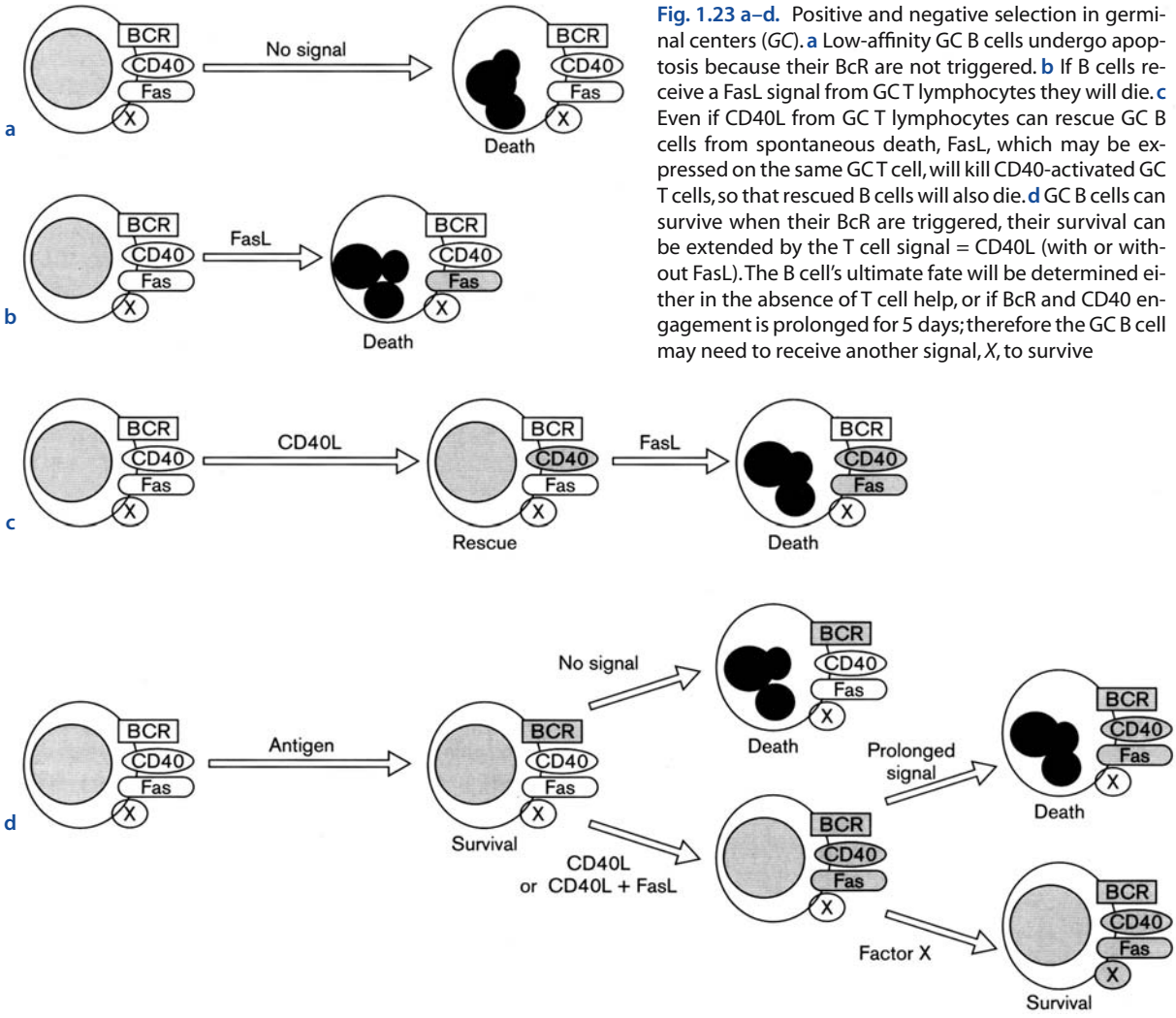


Fig. 1.23 a–d. Positive and negative selection in germinal centers (*GC*). **a** Low-affinity GC B cells undergo apoptosis because their BcR are not triggered. **b** If B cells receive a FasL signal from GC T lymphocytes they will die. **c** Even if CD40L from GC T lymphocytes can rescue GC B cells from spontaneous death, FasL, which may be expressed on the same GC T cell, will kill CD40-activated GC T cells, so that rescued B cells will also die. **d** GC B cells can survive when their BcR are triggered, their survival can be extended by the T cell signal = CD40L (with or without FasL). The B cell's ultimate fate will be determined either in the absence of T cell help, or if BcR and CD40 engagement is prolonged for 5 days; therefore the GC B cell may need to receive another signal, *X*, to survive

Table 1.17. Genes interacting with Fas

Gene	Probable function
CAP-3	Signal transducer of Fas
CAP-4	Signal transducer of Fas
FADD/MORT1/CAP1,2	Signal transducer of Fas
RIP	Signal transducer of Fas

Modified from [415].

regulatory mechanism in T cells. CD95 is in the first place as well as additionally involved proteins, including ICE ($IL_1\beta$-converting enzyme), and other proteases of the family, which may be common effectors of cell death [400]. Other genes code for products preventing cell entry into PCD pathways or regulate cell survival (Table 1.18) [192]. A third option is the inhibition or induction mediated by viral genes (Table 1.19) [192], clearly a strategy to ensure virus survival until the genome has replicated to establish a successful infection. More recently, the protein kinase RIP (receptor-interacting protein) has been included. Although the best role of RIP is in TNF signaling and NF-κB activation, it contains a death domain and it is capable of causing apoptosis upon cleavage [26]. From the picture shown by the tables, this multiform apoptosis pattern is unfolded: at variance with necrosis, cells shrink, both nucleus and cytoplasm condense, dying cells often release intercellular elements bound to membrane fragments, rapidly phagocytosed and engulfed by neighboring cells to remove possible noxious contents, and activated nucleases extensively degrade chromosomal DNA into small oligonucleotide fragments [542].

Studying cell-death defective (ced) species, three genes were shown to play a central part in PCD: *ced-3, ced-4 and ced-9*. The first two promote cell suicide; instead, ced-9 prevents the process started by ced-3 and ced-4, thus inhibiting apoptosis [542]. The mammalian counterpart of ced-3 is ICE, and that of ced-9 is proto-oncogene *bcl-2* (B cell lymphoma-2), mainly located within outer mitochondrial membranes, the ER, and nuclear membranes [267]. *The bcl-2/ced-9 gene prevents*

1.18. Genes of the apoptotic pathway: inhibitors and survival regulators

Genes	Gene products	Probable function
bcl-2 (ced 9)	Radical trap (?)	Inhibits apoptotic program(s)
Regulators of bcl-2 (with structural homology with bcl-2)		
A1	bcl-2-Related protein	Presumed inhibitory function
bax	bcl-2-Related protein	Inhibits bcl-2 activity
bcl-xB	bcl-2-Related protein	Inhibits apoptotic program(s)
bcl-xL	bcl-2-Related protein	Inhibits apoptotic program(s)
bcl-xS	bcl-2-Related protein	Inhibits bcl-2 activity
MCL-1	bcl-2-Related protein	Presumed inhibitory function
abl	Protein tyrosine kinase	Inhibits apoptotic program(s)

Modified from [193].

C cytochrome mitochondrial release, necessary to start apoptosis, and interacts with ced-4 inhibiting its function [661]. Expression of gene product par-4 correlates with severe impairment of cell proliferation and apoptosis via inhibition of PKCζ (protein kinase Cζ) enzymatic activity more probably of atypical PKC isoforms, also impairing MAP kinase (mitogen-activated protein) activation [655], whereas PI3K (PI-3-kinase) *generates survival signals* [412, 684]. The PKC family consists of serine/threonine-specific protein kinases that transduce a cascade of signals especially derived from the hydrolysis of PIP2 (phosphatidylinositol-bisphosphate). The classical PKC (cPKC) α, βI, βII and γ isoforms require diacylglycerol (DAG) and Ca^{++} for activation, whereas PKC δ ε γ and η isoforms, along with the related protein PKCμ, need DAG but do not require Ca^{++} [114]. In a recent study, CD43 ligation led to membrane translocation and boosted the levels of membrane-bound PKC isoenzymes, mainly of the PKCζ, PKCα/β, PKCε and θ, and PKCμ isoforms. Following CD43 ligation PKCθ activation induced CD69 up-regulation via an ERK (extracellular signal-regulated kinase)-dependent kinase pathway, promoted the AP-1, NF-κB activation and an ERK independent pathway promoting NFAT (nuclear factor of activated T cells) activation. Consequently, PKCθ was found to play a key role in the co-stimulatory functions of CD43 in human T cells [114]. bcl-2 was identified as a mammalian homolog to the antiapoptotic ced-9 in *Caenorhabditis elegans,* but mutations in the bcl-2-like gene ced-9 as well as DRP-1 (dynamin-related protein-1) and BH3-only protein EGL-1 may block its mitochondrial fragmentation [235]. Studies on this nematode by the 2002 Nobel laureates have shown that it has a fixed number of cells, 959, and if the number is altered, it is because too many cells die or, conversely, too many proliferate. In humans, AIDS could be provoked by excess PCD and autoimmune disease by insufficient PCD. At least 19 bcl-2 family members have been identified in mammalian cells, which possess at least one of *four conserved motifs* (BH1–BH4) [684]. The bcl-2 family members can be subdivided into three categories according to their function and structure: *antiapoptotic members,* such as bcl-2, bcl-XL, bcl-w, Mc1–1, and A1 (Bf1–1); *proapoptotic molecules,* such as Bax, Bak, and Bok (Mtd); and the BH3-only proteins, Bid, Bad, and Bim, which are called BH3-only proteins because of 4 bcl-2 homology regions, and share only the third [684]. *Galectin-1* (Gal-1) and *galectin-3* (Gal-3) are

Table 1.19. Viral genes whose expression inhibits or induces apoptosis

Genes	Gene products	Probable function
Inhibition		
BHRF-1 (EBV)	bcl-2 Related protein	Inhibits apoptosis
LMW5-HL (swine fever)	bcl-2 Related protein	Inhibits apoptosis
E1B (adenovirus)	p19 K	Inhibits apoptosis
E1B (adenovirus)	p55 K	Inactivates p53
crmA (cowpox virus)	Protease inhibitor	Inhibits ICE
p35/IAP (baculovirus)	p35/IAP	Inhibits apoptosis
ICP 34.5 (herpes simplex)	ICP 34.5	Inhibits apoptosis
E6 (papilloma virus)	E6	Inactivates p53
Induction		
E1A (adenovirus)	E1A	Inhibits RB
E7 (papilloma virus)	E7	Inhibits RB

Modified from [193].

β-galactoside-binding proteins with pro- and anti-apoptotic properties, respectively (Chap. 18). Some CTLs play a role of effector cells during apoptosis, as we have seen: this is the result of perforin action [69] or of activation and concurrent transcription of Fas and its counterreceptor CD178 or Fas ligand [388]. In humans, Fas gene is located on the long arm of chromosome 10, spans 12 kb, comprises nine exons, and in mice is expressed in tissues enriched by mature lymphocytes, except for DN [388]. A further study documented a TNF role in the apoptosis of activated CD8s [681], thereby confirming the higher inclination of Th1 T cells to effectively modulate killing by Fas binding [69]. A main pathway involves the signaling pathway of TNFR/CD95 activating both PCD and TF nuclear factor κB (NF-κB) (also activated by IL_1), the two events occurring independently [599], via recruitment of multifunctional FADD (Fas-associated death domain) and TRADD (TNFR-1-associated death domain) molecules [82]. PCD provides death receptor 3 (DR3) that most likely participates in lymphocyte homeostasis [82]. Activation of NF-κB is also regulated by the NF-κB phosphorylation via the IKK complex. The IKK complex, consisting of two kinases, IKK1/α and IKK2/β, and the NF-κB essential modulator (NEMO)/IKKγ regulatory subunit, mediates NF-κB activation by most known stimuli [503]. Another essential component of NF-κB activation is NIK (NF-κB-inducing kinase) whose interacting protein, TNAP (TRAF or TNFR-associated factor and NIK-associated protein) specifically inhibits NF-κB activation induced by TNF-α, TNFR1, TRADD, RIP, TRAF2, and NIK but does not affect IKK1- and IKK2-mediated NF-κB activation [217]. Overexpression of a new molecule, NIBP (NIK and IKKβ-binding protein), potentiates TNF-α-induced NF-κB activation [218]. TRAF6 interacts with TIR domain-containing adaptor inducing IFN-β (TRIF) through the TRAF domain of TRAF6. However, disruption of TRAF6-binding motifs of TRIF impaired its association with TRAF6, thus resulting in a reduction in the TRIF-induced activation of NF-κB [491]. TRAFs as components of the IL_1 signaling pathway mediate signaling by interacting with TNFR, rather than with TLR, thus playing a role in cellular processes such as apoptosis [327]. The scenario is not complete, since TRAF2 and TRAF3 have been shown to play opposing roles: a positive one in the standard pathway that activates NF-κB through IKKβ, but a negative role in the uneven pathway that activates NF-κB via IKKα, roles of TRAF proteins possibly linked to their ability to synthesize different forms of polyubiquitin chains [656]. The apoptosis–associated induction of the ubiquitin–proteasome pathway components and the proteasome activity shows that the proteasome plays an important role in the successful execution of apoptosis. Inhibiting either the proteasome activity or the increase in proteasome 26S gene expression or its upstream PI3 kinase activity results in an inhibition of NF-κB translocation thereby suppressing apoptosis [527]. There are many Fas variants: soluble Fas (sFas) can block apoptosis induction [600] as well as crmA – inhibiting ICE/ced-3 (Table 1.19). A second pathway depends on mitochondrial participation by releasing apoptogenic factors: cytochrome c catalyzes the oligomerization of APAF-1 (apoptotic protease activating factor 1), which recruits and promotes the activation of apoptosis proteins (IAPs) such as procaspase-9. These proteins interact via CARD-CARD (caspase recruitment domain interactions) [599]. Procaspase-9, in turn, activates procaspase-3, leading to apoptosis, but is prevented by members of the bcl-2 family, but cells also contain natural IAP inhibitors, the caspases (aspartate-specific cysteine protease), a family of cysteine proteases, which were found both in baculovirus and in human cells (XIAP, c-IAP1, and c-IAP2). IAPs can act as direct inhibitors of the two death effectors, caspase-3 and caspase-7, and are able to suppress the activation of two initiator caspases, caspase-8 and caspase-9 [418, 599]. Studies performed on the activity of effector caspase 3 and on the initiator caspases 2, 8, and 9 revealed that, in the absence of RIP, the activity of these caspases decreases, indicating that RIP-associated apoptosis is caspase-dependent [26]. Thus, Fas, TNF-related apoptosis-inducing ligand (TRAIL) and TNFRs can initiate cell death by two alternative pathways, one based on caspase-8 and the other dependent on the RIP kinase [211]. NF-κB is an essential regulator of immune cell survival, critical for the activation of T and B lymphocytes, and is a central coordinator of innate and adaptive immunity [470].

The apoptosis machinery in B lymphocytes may be different, generally immature cells with IgMs or IgDs or cells that have formed an extra paratope [37]. Also, B cells with poor affinity for antigens, or autoreactive, are destined to a rapid apoptosis and are phagocytosed by macrophages leaving nuclear residues forming tingible bodies [254]. The *bcl-2/ced-9 gene blocks PCD* in B cells and *cells provided with bcl-2* [400]. CD23 may prevent apoptosis of GC B cells [4]. Mounting evidence suggests that autoimmune diseases and viral infections, for example, may be associated with failure to undergo PCD, as well as others characterized by inappropriate cell destruction, AIDS as a first example [542].

The HLA System

The HLA system is the human version of the MHC called H2 in mice. HLA, first discovered in the 1950s, was recognized by its major influence in transplant rejection. Subsequent studies have revealed that HLA is in mammals a single gene region with a pivotal role in antigen recognition and control of immune response (Ir); some Ir genes are also mapped within its structure [329]. The chromosome region containing genes coding for self/non-self discrimination is a highly polymorphic complex region of about 4,000 kb located on *chromosome 6 short arm* (6 in region *p21.3*) (Fig. 1.24). HLA is

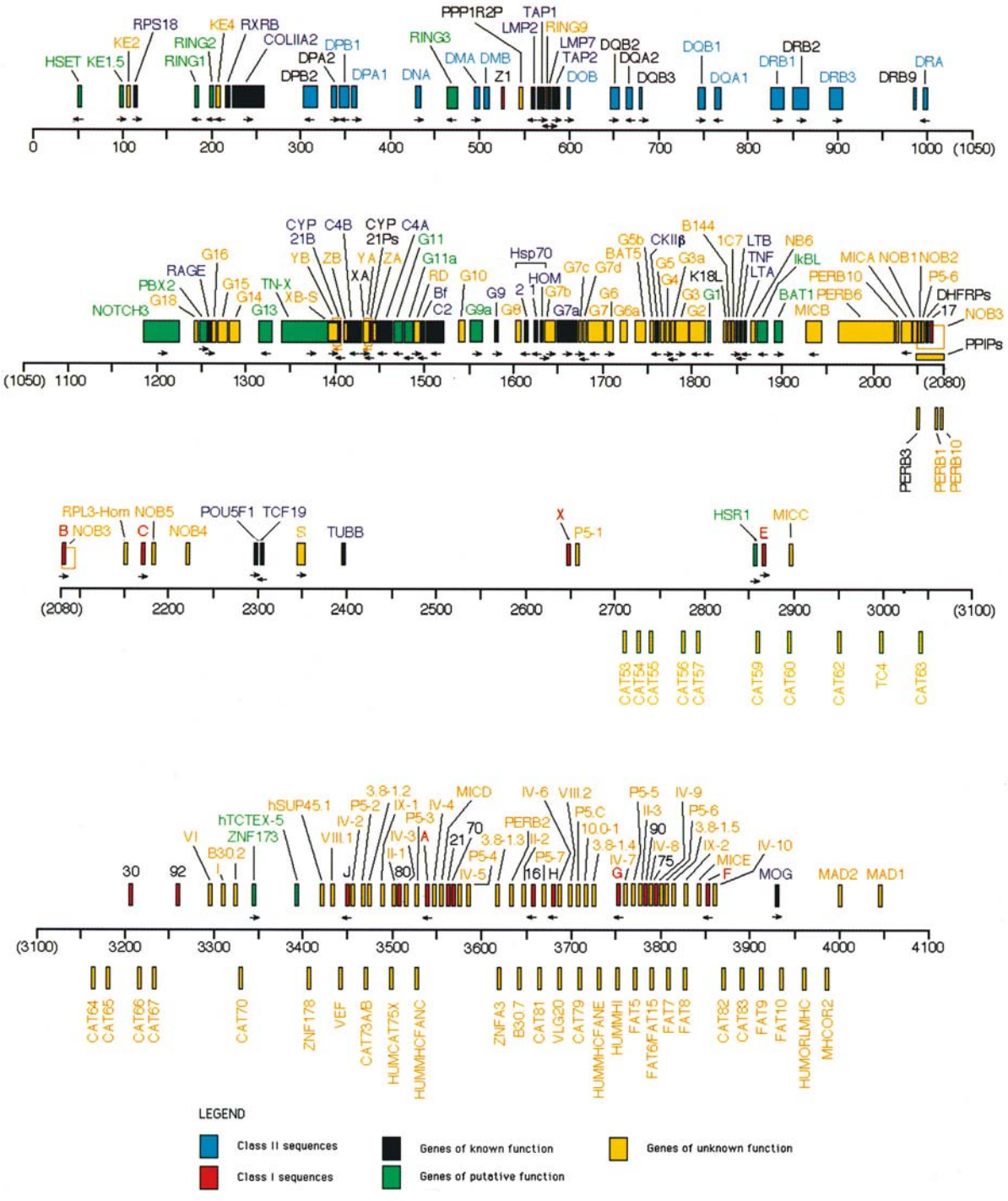


Fig. 1.24. Scheme of human HLA

referred to as complex because its genes are located on a single chromosome, and all genes can therefore be transmitted to children as one unit [18]. In humans this is the best known histocompatibility system as defined at the genetic level, comprising hundreds of genes connected together (>40% of which encode leukocyte antigens) and characterized by structural and functional diversions [264]. *Histocompatibility* is the property of accepting cells or tissue grafts between individuals [329]. Because of their close linkage, the combination of alleles at each locus on a single chromosome is usually inherited as a unit, except for infrequent cases of recombination, and is referred to as the *haplotype*. Although during evolution there may have been variations, the haplotypes of various mammals and birds hitherto studied are fundamentally similar. A key task

carried on by these molecules is to present antigens or peptides deriving from enzyme cleavage to TcRs. That peptides associate with HLA molecules and are recognized as a single entity demonstrates either the phenomenon of restriction [688] or the genetic control mediated by products of HLA genes present on all nucleated cells. HLA class II genes, expressed in a constitutive way in some cells, including B and T cells, macrophages, and DCs, stimulated by specific activators such as IFN-γ in endothelial and epithelial cells, form a trait with high genetic variability, so that proteins encoded by the above-mentioned genes usually differ from one individual to another [329].

In mice, chromosomal segments controlling immune phenomena are divided into five regions: K and D regions encode serologically defined (SD) antigens, found in all nucleated cells and called *HLA class I molecules.* Ir genes (in mice antigen-associated with the I region of the H2 complex and in man DR- and D-related) are present on APCs, more restricted as regards their distribution and defined as *HLA class II molecules;* the S region controls synthesis of complement components. K and D regions are recognized by CD8 T cells during transplant rejection. It was also shown that following a viral infection, murine CD8 cells will lyse only infected target cells derived from a line that is genetically identical with the same K- and D-region molecules as the original stimulating cells [33].

HLA includes *loci* A, B, C (class I region) and D (class II region); lymphocyte-defined (LD) antigens are called HLA-DR, -DP and -DQ. Each *locus* is composed by a series of alleles determining the corresponding gene products (HLA antigens) on cell membranes. According to recent nomenclature, alleles with confirmed sequences of amino acids or nucleotides have a specific designation, including the name of *locus* and four arabic numbers: the first two designate more closely related specificity and the last two the allele number (Appendix 1.1) [640]. The number is preceded by an asterisk: e.g., HLA-DRB*0401 stands for allelic variant of 0401 of gene 1 [264]. The nomenclature of different HLA specificities has grown very large, including more or less completely defined *locus* numbers of alleles: 287 HLA-A, 527 HLA-B, 147 HLA-C, 6 HLA-E, 1 HLA-E, and 15 HLA-G, making a total of 980 class I alleles, 454 HLA-DR, 79 HLA-DQ, 128 HLA-DP, 10 HLA-DM, 16 HLA-DO, for a total of 649 class II alleles [640]. Some genes HLA-C, HLA-D, HLA-E, HLA-F, HLA-G are less characterized [640]. There are 54 class I genes and MICA pseudogenes; TAP 1 and 2 (transporter associated with antigen presentation 1 and 2) are 10 in number [640], while MICB, MICC, MICD and MICE do not yet have a fixed function [469]. MICA and MICB are ligands for the activating CD94: CD94R may inhibit class I molecules [329]. As Appendix 1.1 shows, not all alleles have serologically defined specificities. Inclusion of different designations of HLA alleles and specificities comes from using serologic methods instead of molecular reactions recently employed for typing such as *in situ* hybridization, PCR (polymerase chain reaction), RFLP (restriction fragment length polymorphism), etc. For example, HLA-B27 was associated 30 years ago with several autoimmune disorders [329], now encompasses several subtypes, from B*2701 to B*2709 [640].

HLA and encoding genes form three categories, classes I, II and III, membrane gps expressed on all nucleated cells. Class I and II molecules are members of IgSF (Figs. 1.25–1.27) [454]. Class I and II molecules can be differentiated according to their structure, tissue distribution, and function [329]:

- *Class I genes,* located in the more distal region from centromere, encoded by main HLA-A, HLA-B and HLA-C *loci,* include a polymorphic H chain, a 45-kD α chain, in close, noncovalent association on the membrane with a nonpolymorphic 12-kD L chain, a β_2-m encoded by a single gene located on chromosome 15. A molecular three-dimensional structure shows H chains divided into six regions: three extracellular globular domains, α1, α2, an Ig-like domain, α3, a short extracellular connecting peptide N-terminal, a hydrophobic TM region (25 amino acids), and a hydrophilic intracytoplasmic C-terminal tail (30 amino acids), while L chain forms only an extracytoplasmic Ig-like domain. A molecular part furthest from the membrane embraces a deep groove or cleft, *the peptide-binding site,* made up of segments of α1 and α2 domains with α-helical sides and an irregular β-sheet base. A single α3 domain has sequences interacting with CD8 cells.
- *Class II genes,* positioned in a more proximal region to the centromere, controlled by at least three HLA-DR, HLA-DP, HLA-DQ subloci, consist of two polypeptide domains, a 33-kD α and 28-kD β, noncovalently linked. This pattern resembles that of class I, but with five domains, since each chain contains two extracellular globular regions (α1 and α2, or β1 and β2), not covalently linked. The peptide binding groove has a structure similar to that of class I molecules. Also in this case the cleft chemical surface, distinct from class II molecule GPM, determines specificity of antigen binding. Class II is restricted to immunocompetent cells presenting processed antigens to CD4 cells and is necessary for interactions between immunocompetent cells. Additional class II-related molecules are eight HLA-DOA alleles [469]. The designation of class II *loci* on chromosome 6 consists of three letters: the first (D) indicates the class, the second (M, O, P, Q, or R) the family and the third (A or B) the chain (α or β, respectively).
- *Class III genes,* placed between classes I and II loci, encompass a heterogeneous mixture of genes, including classic pathway complement components (C2, C4a, C4b), properdin factor P of the alternate pathway, enzyme β-21 hydroxylase, molecules of the heat shock protein family (HSP70-1 and -2), and TNF-α and TNF-β. Many class III genes are involved neither as transplant antigens nor in antigen presentation [329, 337].

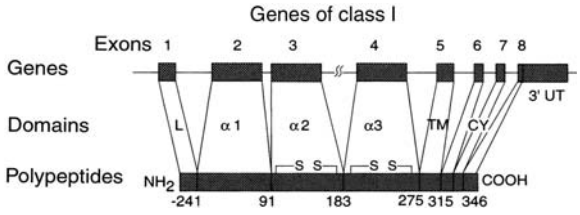


Fig. 1.25. Diagrammatic representation of HLA class I genes and molecules

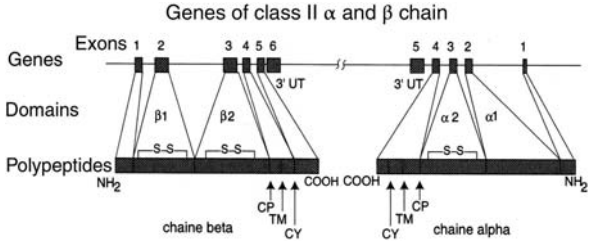
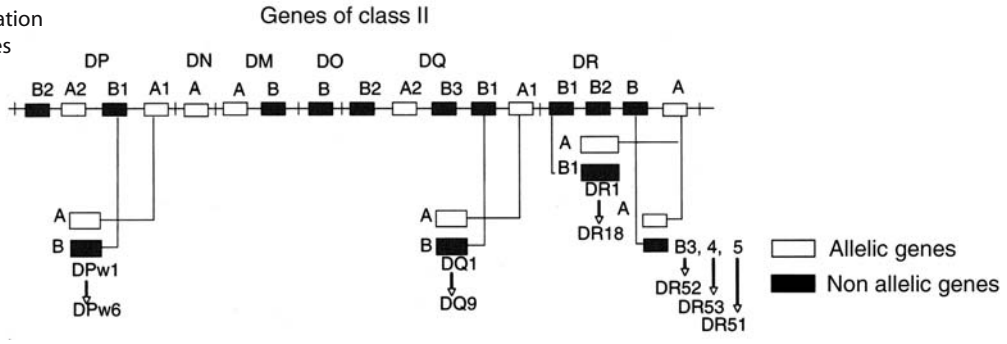


Fig. 1.26. Diagrammatic representation of the genes of α and β chain of HLA class II molecules

Fig. 1.27. Organization of HLA class II genes



Other genes encoding proteins involved in antigen processing machinery have been identified interspersed among class II genes, between HLA-DQ and HLA-DP, such as six TAP-1 and four TAP-2 alleles (Appendix 1.1), and two LMP2 and LMP7 genes (low-MW polypeptides 2 and 7) [329].

The Ir genes, mostly mapped to HLA antigens, encode a synthesis of class II molecules, that is HLA-restricting elements recognized by CD4 cells. Owing to allelism of the above-mentioned molecules, different individuals respond differently to the same antigen when responses are HLA-restricted, that is, only when antigen presentation is in the context of class II specific molecules. In the absence of Ir alleles, there is no production of antibodies against specific antigens; hence individuals have inherited HLA antigens that in peptide presentation to TcR are markedly effective in eliciting synthesis of antibodies different from IgE antibodies. It was speculated that Ir genes are expressed on macrophages and not on T cells. Actually, the genetic control related to interactions between T cells and macrophages is localized in HLA I regions and products encoded by genes of such regions are defined as HLA-DR [198]. Only 10%–50% of macrophages isolated from peritoneum and spleen are provided with these antigens, and it seems that only HLA^+ cells look after antigen exposure [12]. To better define *genetic restriction,* it refers to different specificity for different HLA molecules, since not all T-cell subsets use the same HLA molecules, each having a capacity of response limited to some HLA components, since both class I and II contain recognition sites for CD8 and CD4 coreceptors on T cells [463].

According to the restriction concept, the HLA molecule has a key role in binding to immunogenic peptides deriving from processing foreign proteins: bimolecular HLA–peptide complexes expressed on APC membranes are the ligand recognized by T cells. CD4 cells recognize only peptides expressed on membranes of actively phagocytic APCs (10^5 molecules/cell) [337] associated with HLA class II antigens. Suppressor-CTLs are restricted to associating with HLA class I molecules, expressed by most nucleated cells of the body (between 10^4 and 5×10^5 molecules/cell), while the HLA C region contacts CD8 coreceptor [463]. HLA class II antigens bind to peptides with sufficient affinity by means of amino acid sequences of their hypervariable parts situated in α-1 and β-1 domains; similarly, TcRs recognize HLA–peptide complexes. Instead, T cells, with a γδ TcR that is often DN, represent an exception to restriction in that, in addition to class II antigens on DCs [558], they recognize bacterial proteins as well as heat shock proteins (HSP) in an HLA-independent way [279]. Centrality of HLA molecules in tripartite interactions with processed antigens and TcRs is exemplified by the repertoire of antigen specificities recognized by T lymphocytes.

Main features distinguishing class I and II molecules are GPM, association and codominance [18, 329]:

- *The major part* of HLA genes is characterized by an almost unique GPM, which has been shown to be even more extensive by rDNA technology. Each gene has multiple alleles, leading to a great number of likely combinations on each chromosome (haplotype): the number of genotype combinations is $>10^{10}$. Besides these alleles, there are many variants in the general population, so

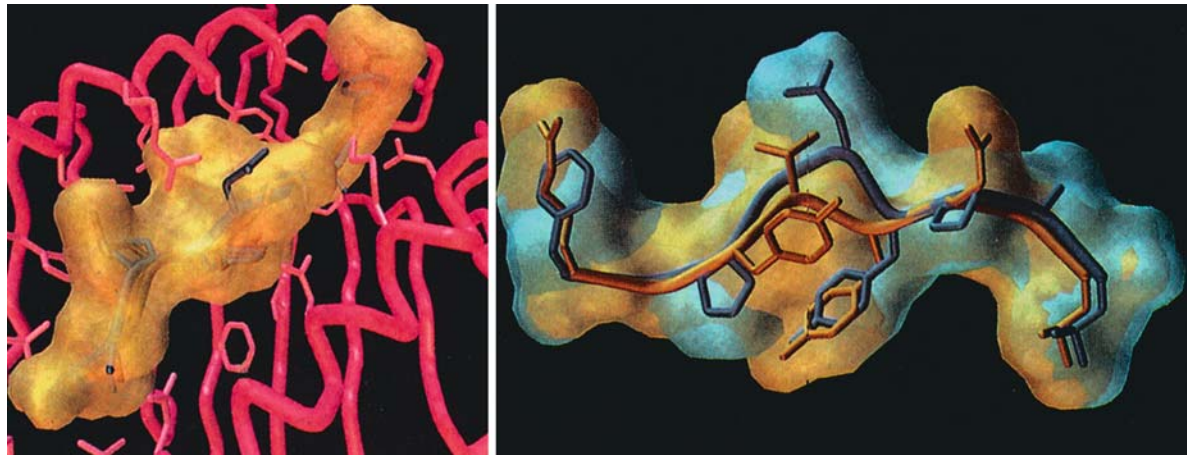


Fig. 1.28 a,b. A viral peptide buried in a HLA class I molecule (**a**), and together with another peptide (**b**) in the extended configuration they assume when complexed with HLA class I molecule [161]

that the majority of subjects are heterozygous (HZ) for different *loci*. Therefore, an extensive GPM makes wholly different HLA molecules expressed by genetically distinct individuals, that is each one has his own genetic set of HLA molecules, shared only by a few individuals of the same population. GPM is also the basis for rapid graft rejection between genetically different individuals.

- *Association* means that all genes are transmitted en bloc by HZ parents to their children. When paternal haplotypes are a and b and maternal ones c and d, each child inherits from both haplotypes, that is ab, ac, bc and bd; thus the chances of a child having the same two haplotypes are = 25 %. A recombination between two haplotypes (cross-over) can be in germ cells, paternal or maternal, creating a new haplotype. The recombination rate is 1 % in HLA-A and -B and between -B and -D.
- *Codominance* means that each cell expresses HLA proteins transcribed from paternal and maternal chromosomes. One point worth noting is that only one chromosome is used to form Ig and TcR molecules, the unique occurrence of allelic exclusion. Each individual is commonly HZ for each *locus* and her/his HLA group is composed by several letters as described above, plus two numbers corresponding schematically to the alleles transmitted by both parents.
- Moreover, *some gene combinations* can have an unexpected frequency, because individuals of a heterogeneous population can have two genes segregating together, with a frequency markedly different (higher or lower) than the predicted frequency. *Linkage disequilibrium* is a phenomenon common to almost all complex genetic systems, the tendency of specific alleles of linked genetic *loci* to be inherited together (as a unit or haplotype) on the same chromosomal region far exceeding that expected by chance association, functionally interacting between themselves.
- *GPM* is a strategy for generating diversity of HLA molecules; hence it contrasts with the unique strategy of gene rearrangement of TcR and BcR. Accordingly, each lymphocyte expresses only slightly different receptors, whereas every cell of an individual expresses the same HLA molecules, but different from HLA molecules expressed by genetically unmatched subjects. GPM also contrasts with principles of allelic exclusion and thus with the genetic principles governing receptors: class I and II molecules are codominant, that is, each cell expresses HLA proteins transcribed from both maternal and paternal chromosomes, consequently confirming GPM [36].
- As regards the respective functions and properties, class I and II molecules have a similar general structure, although not identical: both seem to have a wide GPM and bind to peptide fragments subsequently recognized by TcRs. Perhaps due to different types of processing, class II molecules appear to present a heterogeneous group of peptides for a given epitope, instead of only one well-defined epitope as is normal for class I molecules.
- GPM of HLA *loci* and some alleles of different *loci* tend to associate between themselves; this inclination has been understood as necessary for human immune system diversity or functioning [463].

Several ILs can stimulate HLA molecules: IFN-γ is one of their most potent activators, whereas TNF-α, TGF-β and IFN-α and -β functions remain obscure [579]. IFN-γ induces activatory signals for CIITA (class II transactivator) expression [539], which is more of an essential regulator for expression of HLA class II genes than their direct modulator: CIITA is defective in a form of primary HLA deficiency [539].

Many peptides bound to HLA molecules may not be invariably presented to TcRs, in part because cells expressing TcRs reacting with peptides derived from a given individual are often removed during T-cell development in the thymus, either clonally deleted, or dying by apoptosis as formerly alluded to [579]. The set of HLA alleles inherited by an individual is exceedingly tiny compared to diverse TcR repertoires. In theory, TcRs are

restricted to recognize a very limited subset, while HLA molecules can bind to a large collection of foreign and self-derived peptides: this issue appears to be paradoxical since HLA molecules can bind a multitude of peptide fragments in a very restricted way, with an affinity at least 1,000-fold higher than that of TcR. Of course, each HLA molecule could bind this large group of peptides, but what governs the ability of a peptide to bind one HLA haplotype rather than another? [18]. It might also be that a very small number of HLA–peptide complexes are required at the APC surface to generate immune responses [36]. As we see from Fig.1.28 [161], class I molecules in concomitance with the encounter with molecules to be scrutinized present, as previously described, the special conformation with binding grooves destined to reception of already processed peptide fragments [161]. In particular, as evidenced by crystallographic studies, conserved pockets at both ends of the peptide-binding groove accommodate their N-terminals via extensive H_2 binding, thus warranting a correct orientation of peptide-binding and its closed nature at both ends [264]. In the middle of the groove there is a deep pocket providing structural complementarity (like a gem in a ring) for one of the peptide amino acids called the *anchor* (the amino acid residue recurring more frequently). The peptide antigen appears to be anchored by residues either at or near the end of the peptide, thus enhancing the specificity of HLA–peptide binding [344]. The single substitution of an amino acid of an exposed peptide residue, although changes do not seem to be critical for the above binding, is sufficient to abrogate TcR recognition. Since HLA molecules are very unstable, only peptides with an allele-specific anchor residue may provide sufficient stability, while a small decrease in affinity has the wide biological effect of increasing the dissociation rate of bound peptides, hence preventing adequate time for presentation to T cells [161]. The pocket of class II molecules is open-ended, allowing larger peptides to bind [264].

Initial Phase of the Immune Response

Functions of HLA Molecules and Antigen Processing

Antigen presentation is defined as processing or cleavage of foreign antigens consisting in transformation from a native form to a nonnative form to yield peptides complexing with HLA molecules, thereby associating HLA products only with fragments of presenting antigens, and not with intact antigens themselves, drawing attention to the analysis of proteolytic events lying upstream of presentation events.

A remarkable contribution to understanding this step of immune responses comes from studies on related viral attitudes. As discussed in the preceding section, HLA class II molecules interact with CD4 cells, class I molecules with cytotoxic CD8 cells (Fig. 1.29) [591]. These mechanisms entail recognition of peptide fragments deriving from intracellular microbial antigens, which sometimes expose native proteins to the host surface, and thus to a cytolytic attack [591]. Some viruses in particular can infect every type of human cell, so that to respond to aggressions T cells must recognize viral peptides associated with class I molecules. When other pathogens degraded via phagocytosis are active, this process implies T cells being reactive with exogenous peptides associated with class II molecules [337]. Such programming has a remote ontogenetic origin, since TcRs were planned by the immune system to recognize even small fragments of viral antigens, sometimes of only eight to ten amino acids, hence virtually indistinguishable from the related peptides of human cells. Consequently TcR was diversified to react with such fragments only if associated with HLA products [457]. Prerequisites are therefore processing outside peptides and assembling molecules for one or more HLA alleles. To degrade a protein into small fragments, both endogenous and exogenous antigens should be first broken down into vesicles with acid pH, to be associated with HLA alleles on the cell surface. Processing clarifies why T cells, unlike B cells, fail to recognize protein conformational epitopes, but only linear ones, and why they cannot distinguish between native and denatured proteins of the same antigen [474].

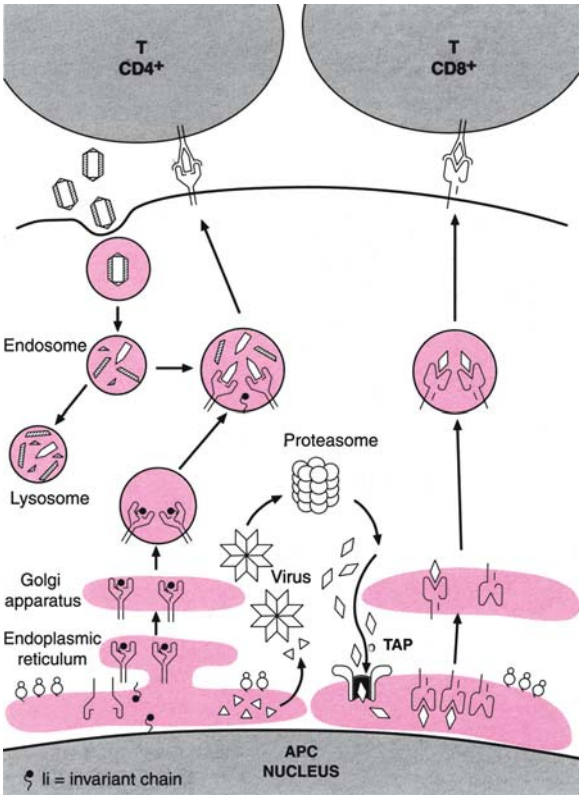


Fig. 1.29. Antigen processing and presentation to T lymphocytes

Various methods are employed by cells for antigen processing as follows:

- *Phagocytosis* delineates the most prominent function of mononuclear phagocytes at the point of assuming such denomination, and antigen processing has a pivotal role in inducing immune responses: during the different steps, among fragments subjected to processing, those cleaving in the endosomes and with affinity for HLA molecules escape a complete denaturation, bind to HLA alleles, and are then transported to macrophage surfaces [540]. On the contrary, after an exhaustive proteolytic cleavage in *lysosomes,* peptides with no HLA association are excreted outside the cell surface through a process of exocytosis.
- *DCs and B lymphocytes* cannot use phagocytosis, but a more common process called pinocytosis, by which fluids or very small particles (diameter <10 nm = 100 Å) are taken into the cell.
- *B cells* can employ antigen-specific Igs (IgMs or IgDs), a much more effective procedure because the antigen concentration necessary is 1,000-fold less than that required by pinocytosis. However, despite the advantage of close linking with B lymphocytes, this process is really restricted to a comparatively limited number of these cells [384].

Exogenous peptides generated in acid vesicles bind to class II restricted T cells [391], whereas endogenous peptides synthesized by cytosol and ER are recognized by class I restricted T cells [372]. When processing of a non-virus-infected cell (modified, for example, in a vaccine) takes place in acid vesicles, the resulting peptides are presented to CD4 lymphocytes associated with class II molecule. However, if the same antigen infects the cell, processing moves over the cell cytosol/ER, then antigens are presented in association with CD8 lymphocytes and HLA class I molecules. Following these two different procedures, CD4 and CD8 lymphocytes of an individual can recognize different epitopes of a given antigen [47]. Moreover, as a number of experiments have demonstrated, the structural characteristics of peptides do not determine the binding to one or to another HLA class, but it can be hypothesized that distinctions arise from two different ways of processing and presentation [369, 391].

Now the way by which HLA molecules acquire peptides is the center of attention, rather than the origin of peptides presented by HLA products. As regards class I molecules, before entering an exocytic system, processing of antigen material in the cytosol is usually attributed to *proteasomes,* an ATP-dependent complex of peptidases, proteolytic enzymes encoded by LMP2 and LMP7, a pathway probably operative in converting native antigens into peptide fragments then translocated into the ER to associate with class I molecules [377]. Unfolded or worn-out polypeptides in the ER are retrotranslocated into the cytosol where the SC factor (SCF) (Fbs1, 2) proteins distinguish native from unfolded glycoproteins and ubiquitin targets the worn-out proteins for dumping [648]. These proteins unfold with the help of other specialized molecules, the chaperones, and the polypeptide chains are then fed into the proteasomes, where unassembled or defective proteins in the cytosol are degraded into peptides [264]. The ubiquitin-proteasome system is a fundamental machinery in the cell [520] and has been shown to be involved in the virion budding process of several viruses, particularly of rhabdoviruses [198]. In the presence of the proteasome inhibitor MG132, the entering viruses accumulated in both the endosome and denser lysosome, suggesting that the ubiquitin-proteasome system is involved in the virus transfer from the endosome to cytoplasm during the virus entry step. Understanding the sensitivity to the ubiquitin-proteasome inhibitors may be used to distinguish the early steps of viral entry [656]. Studies of *Thermoplasma acidophilum* [316] have shown that proteasomes, with a barrel-shaped structure, consist of two inner rings, each consisting of seven β subunits, and two outer rings, each made up of seven α subunits, all different [316]. Proteasomes interact with either ER membranes or TAP-1 and TAP-2 [293] encoded by polymorphic genes of class II and associated with H chain α1 and α2 domains [443] (Fig. 1.30). TAP-1 and TAP-2, closely located to genes encoding LMP2 and LMP7 inducible by IFN-γ [293], import into the ER *lumen* selected peptides, which may attach to a newly synthesized β chain of the class I molecule encoded by the β_2-m gene [263]. Possibly the peptide NH_2 terminus is also trimmed [361]. The peptides are necessary for a correct assembly of HLA class I molecules. Class I molecules first assume a peptide-receptive conformation, then release peptides to be delivered to the cell surface for antigen presentation to CTLs with CD8 markers [337]. However, subsequent analyses have shown the polymorphism of TAP and LMP genes that map between DPB1 and DQB1 within class II genes [372]. So it seems reasonable to imagine that different epitopes of the same antigen are presented to T cells of different subjects. Support for this hypothesis will lead to evident implications, such as individual differences in immune responses to specific antigens [28], in addition to differences in tolerance induction, predisposition to autoimmunity, and disorders associated with HLA molecules [28]. HLA class I molecules could also bind to exogenous antigens loaded by macrophages and DCs [39]. The principal routes are as follows: release of antigens acquired from endocytic or phagocytic vesicles returning immediately to normal pathways, or antigen digestion in a vesicle by using normal class I mechanisms; pertinent examples in support of both hypotheses derive from inhibitors of processing pathways such as brefeldin A and chloroquine [39]. When TAP-2 is deficient, assembly of class I molecules cannot be completed.

As reported by studies on the routes of class II molecule intracellular traffic, newly synthesized HLA products are collected in the ER where they are delayed for

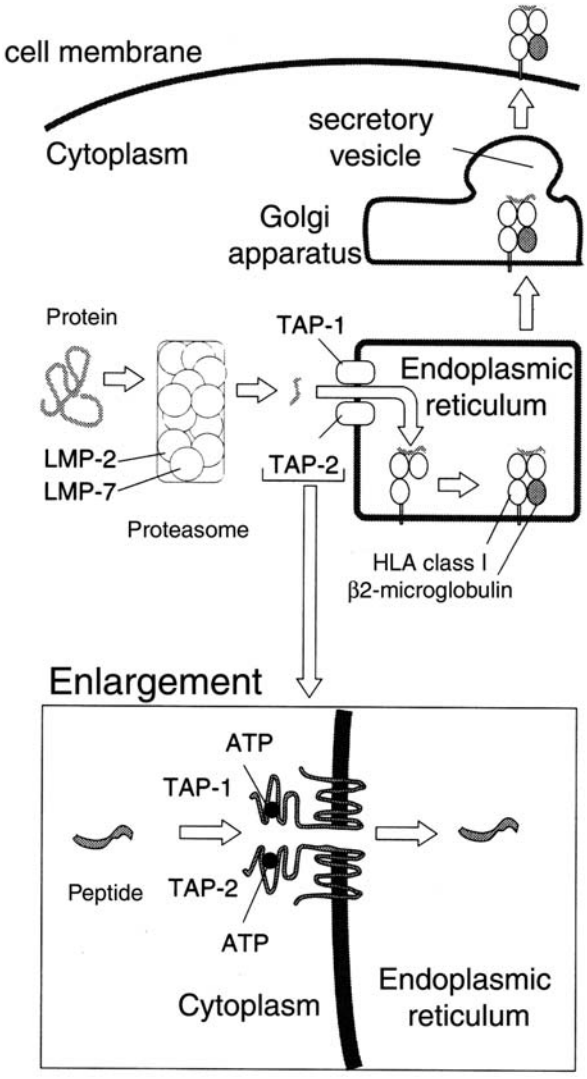


Fig. 1.30. Biosynthesis of HLA class I molecules

1–3 h, apparently not for recycling back to the cell surface, but for peptide loading to take place. Molecules leave it only when linked with three pairs of αβ heterodimers of the same ER, transiently aggregated to three copies of the TM invariant chain (li=CD74) [61]. The agglomeration of nine chains (nonamer) without contacting peptides in the ER is transported across trans-Golgi and post-Golgi networks to an endocytic compartment, MIIC (MHC class II-loading compartment) [443]. The li chain appears to protect class II dimers from binding cytosolic peptides imported into the ER for class I binding [61]. The li region, important in binding class II molecules and in preventing premature peptide binding, corresponds to CLIP (class II associated invariant chain peptide), which binds HLA-DR3 in sites normally occupied by peptide antigens [61]. In *Golgi networks,* after glycosylation peptides are internalized into the cell by endocytosis, an APC encounter with foreign peptides occurs in endosomal or prelysosomal compartments where peptides are internalized, then extruded in intra- and extracellular compartments [378]. HLA class II peptide presentation can be inhibited by lysosomotropic agents such as chloroquine and NH_4Cl, which raise the pH, and by inhibitors of endosomal proteases, with no effect on class I endogenous peptides [378]. While in transit to endocytic routes, the li chain subjected to the compartment acid pH is degraded by endosomal proteases and CLIP is removed from DR3 by HLA-DM [182]. As the intact li chain, CLIP inactivates the groove by binding directly to it [61]. Consequently, HLA molecules can bind processed antigen peptides, and peptide–class II-αβ complexes can be transported to cell surfaces: HLA molecule antigen binding assures sufficient stability, allowing it to reach cell membranes to activate the correct T cells [372].

In conclusion, the intracellular routes followed by HLA class I molecules and respective antigens traverse structures different from those crossed by both antigens and HLA class II products, thus avoiding their reciprocal encounter.

Antigens presented in association with class I molecules bind preferentially to peptides usually 8–11 residues long (their grooves are restricted in length and are closed at both ends) [337], whereas class II molecules can accommodate much longer peptides, with 11–17, and up to 25 amino acids [337]; therefore both ends of a class II molecule-bound peptide have been assumed *to protrude out of the groove* [482], thus showing a higher heterogeneity at NH_2 and COOH termini [337]. Accordingly, peptides with 13–17 amino acids associated with class II molecules can stimulate T-specific clones [482], considering that the theoretical number of different peptides formed by nine amino acids is 5.1×10^{11} [337]. Selectivity of HLA molecules implies that only some of them have grooves with sufficient length to hold processed peptides [482]. It is critical that a different structure is appointed for trimming of longer peptides, which could be:

- In *ER precursors,* associated with class I molecules [144]
- In accordance with *binding sites,* or the same peptide, not associated with class I molecules [482]
- *Within the cytosol,* where peptides may be immediately re-exported [361], or where there may be envisaged a proteolytic structure specialized in reducing peptides to fitting size for HLA before transport in the ER
- *Stress protein gp96* may function as a chaperone for peptides not fitting HLA molecules [286]

On the contrary, class I molecules need no trimming, being unable to hold longer peptides.

Antigen processing also has particular features: if amino acid chains or peptides are rolled up and folded, when the chain is extended, distant parts can be brought together via folding of protein structures, leading to conformational epitopes. Furthermore, unlike B cells directly interacting with antigens, being able to recog-

nize epitopes contained in intact and normally folded protein molecules, T cells recognize only epitopes present in denatured and linear molecules. Besides splitting, in macrophage intracellular vacuoles an unfolding takes place, which makes accessible previously hidden, totally or partially, immunogen peptide parts [443]. Small amino acid sequences equipped with the structural characteristics essential for complex formation with HLA molecules are often found inside of rising protein molecules and become available for binding to HLA products only after *unfolding of protein structures* is accomplished: fragments containing the critical amino acid sequences are transferred into the macrophage membrane surface, ready for subsequent antigen presentation [12].

Therefore, if antigen processing consists in the *conversion of an antigen from the native to a non-native form*, a process carried out by APCs expressing HLA antigens, peptides must make contact with TcR as well as bind to an HLA molecule. A most likely hypothesis is that processing involves changes into antigens provided with a conformational freedom to form a secondary structure permitting both epitope and agretope to form. Both are composed of small amino acid sequences, at least *two or three residues* interspersed into the primary sequence of native proteins: *recognition is facilitated* by an α-helical conformation of peptide chains, in other words the helicoidal spatial arrangement. Residues making contact with T cells and HLA molecules, respectively, during enzyme splitting segregate to opposite sites of the α-helix one group forming the epitope and the other the agretope. However, although in many instances the whole amino acid sequence of several allergens was successfully disclosed, it is poorly understood which sequences are responsible for IgE binding, probably because IgE antibodies have different requirements as regards the conformation of the peptides to bind [12].

Subsequent studies have proposed a new type of antigen processing, a noncytosolic pathway, typical of class I molecules, also suggesting that peptides internalized into phagosomes are hydrolyzed by proteinases in endocytic compartments, but it is uncertain how these peptides bind to class I molecules, thus more mechanisms may be operative [467]:

- In the first type, HLA class I molecules from plasma membranes or newly synthesized may enter the phagosome, bind peptides and transport them to the cell.
- In the second type, class I molecules in macrophages may recycle between the cell surface and endocytic compartments.
- In the third type, the li, although commonly used in class II transport, can associate with newly synthesized class I molecules and direct their transport into endocytic vesicles [467].

The role of this exogenous pathway may be in innate immunity, again with three different types:

- In the first type, the immune system may have the privilege to detect and monitor pathogens surviving in phagosomes, not eliminated by CD4, permitting CTLs to destroy infected cells, in turn CTLs can secrete IFN-γ, which would stimulate macrophages to kill the microbes.
- In the second type, antigens are imported from somatic cells, poor stimulants of naive T cells lacking CD80, CD86 and CD54, into professional APCs; such exogenous pathway APCs can trigger primary T-cell responses since they express high levels of HLA class I and II molecules, in addition to costimulatory and adhesion ligands, and trafficking into lymphoid organs at that time.
- In the third type, the exogenous pathway potentially stimulates CTL immunity using protein-based vaccines: as a rule antigens in extracellular fluids are not presented in association with class I molecules on mast cells [467].

Cells of the Immune System Participating in Immune Responses

Phagocytes, so called for their ability to ingest and digest living or inert particles, have a common origin in the bone marrow, from progenitor cells, or colony-forming unit-spleen (CFU-S), or -thymus (CFU-T). CFU-S can generate myeloid, lymphoid and erythroid cells (Fig. 1.31) and, in the presence of stimulating factors (M-CSF, GM-CSF) (Table 1.5), mixed colonies of polymorphonuclear leukocytes or PMNs and macrophages. Mononucleated phagocytes, having achieved their maturation in the bone marrow, pass into the bloodstream for a short period of time, which leave to enter the tissues through capillary walls by diapedesis in response to chemotactic factors released in inflammatory processes (see "Innate Immunity", p. 152). Strictly speaking, phagocytes are granulocytes and monocyte macrophages with the particular uptake of vital strains, as numerous other cells are widely distributed throughout the body (macrophages of alveoli, spleen, lymphoid tissue, and histiocytes, Kupffer cells, osteoclasts, chondroclasts, mesangial cells of the kidney and microglial cells of the brain), and constitute the reticuloendothelial system (RES), comprising other phagocytes in a broad sense (Fig. 1.32) [470].

Monocytes (Fig. 1.32, a–c) derive from bone marrow promonocytes under the action of specific mediators (CSF). They have a diameter of 9–15 µm, and a round, oval or indented nucleus in an eccentric position with one or two prominent nucleoli [609]. The first cells to circulate in blood, but only for about 1–2 days, they migrate via blood-vessel walls, then are discharged into various tissues where, because of the stimuli of differentiation signals typical of each tissue, differentiate into resident macrophages. In certain tissues they become fixed or may enter internal cavities. During this period, the resting cells are activated by IFN-γ, a process also increasing the transcription of new genes [474]. Moreover,

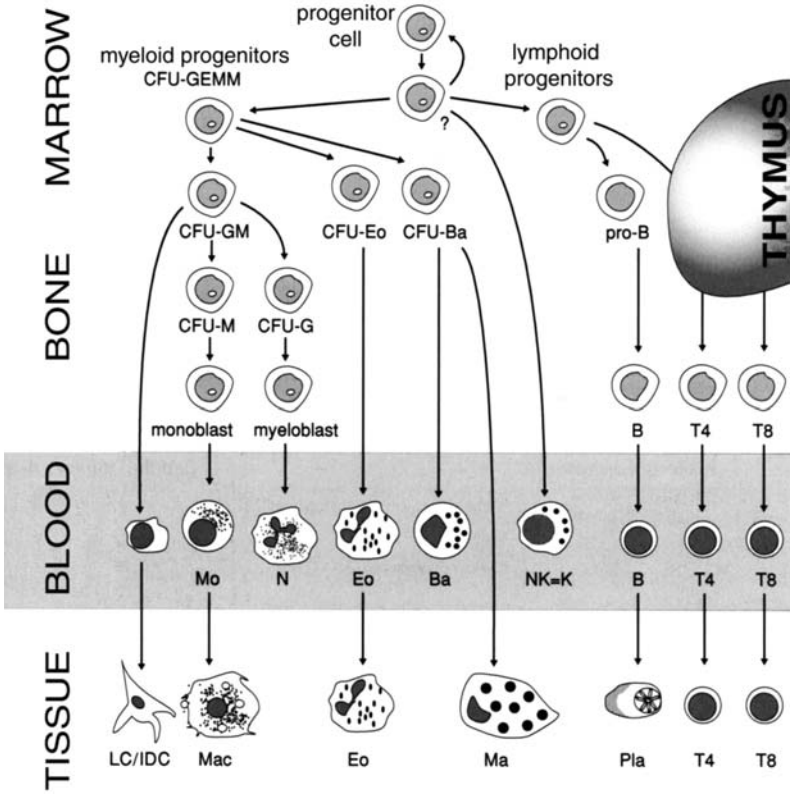


Fig. 1.31. Hemopoiesis: myeloid and lymphoid differentiation and the tissue compartment in which they occur. *Ba* Basophils, *IDC* interdigitating dendritic cells, *LC* Langerhans' cells, *CFU* colony forming unit, *Eo* eosinophils, *CFU-GM* colony forming unit-granulocyte-macrophage, *Mac* macrophages, *Mo* monocytes, *N* neutrophils, *Pla* plasma cells, *T4* CD4, *T8* CD8

IFN-γ-primed monocytes exposed to LPS showed enhanced phosphorylation of IRAK (IL_1R-associated kinase) and increased NF-κB DNA binding activity [49]. Mature cells, before differentiating lose CD34, an early marker of hematopoietic progenitor cells and of other immune cells, and CD62L ligand, whose delayed maturation could be, for example, an SCID marker. Monocytes express normal levels of CD13 and CD33, and high levels of $CD14^+/CD11c^+$ useful for their identification, in addition to a greater number of membrane receptors for IgG Fc fragments. CD14 engagement on monocytes could also inhibit human Ig synthesis, including IgE antibodies [608]. The phenotype and function of monocytes are modulated by several ILs and include IL_1RA, IL_2, IL_3, IL_4, IL_7, IL_8, IL_{10}, IL_{12}, IL_{13}, IL_{15}, IL_{16}, IL_{18}, IL_{19}, GM-CSF, TNF-α and -β, IFN-γ [73, 609]. The TNF and IL_{12} induction was dramatically increased in IFN-γ-primed monocytes [49]. The release of these ILs by monocytes can be modulated by different infectious and noninfectious stimuli. Monocytes also yield MCP 1–3 (see later) several ILs, such as IL_1RA, IL_8, TNF-α and -β [73], and MIF (monocyte migration inhibiting factor) [73, 609]. As IgE antibodies bind to their receptors, they acquire bactericidal and cytotoxic properties producing $O_2^{\bullet -}$ (superoxide), apparently due to activation of NADPH (reduced nicotinamide-adenine dinucleotide phosphate)-oxidase [115]. They are endowed with CD23 [115] and FcεRI, with a stronger binding to IgE [347], also becoming capable of engulfing complexed IgE-peptides subsequently presented to T lymphocytes [347]. In atopic patients they provide the RNA with IL_{13} transcripts [219].

Macrophages [540, 593] (Fig. 1.32, d, e) have variable dimensions (15–50 μm) and probably a structural and functional heterogeneity like mast cells. They are particularly active, together with PMNs, in defense against infections, playing a pivotal role in immune responses via a wide range of functions performed (Table 1.20) [68, 540, 609]. Although referred to by a variety of names depending on the location, they have mostly common features: in addition to being APCs, they are avid phagocytes engulfing any bacteria, cellular debris, or foreign particulate materials in the area. Stimulated by the ILs produced during the recognition phase, they are attracted into a site of injury and acquire the phagocyte's typical aspect. Macrophages undergo several morphological, functional and metabolic changes, with a parallel increase in size, adhesion, endocytosis (both pinocytosis and phagocytosis), lysosomal enzymes and chemotaxis, as well biochemical-metabolic activities, enhancing their equipment of enzymes of the respiratory chain, the Emden-Meyerhof way, and hexose-monophosphate shunt [540]. Thus positioned along capillaries, macrophages readily make contact with and engulf invading antigens and pathogens, which are broken down with the aid of the cited enzymes into simple amino acids, glucides and other substances, for a subsequent excretion or recycling, and eventually remove them from circulation [47]. These cells also express HLA class I and II molecules for CD4 and CD8. Due to receptors for IgG

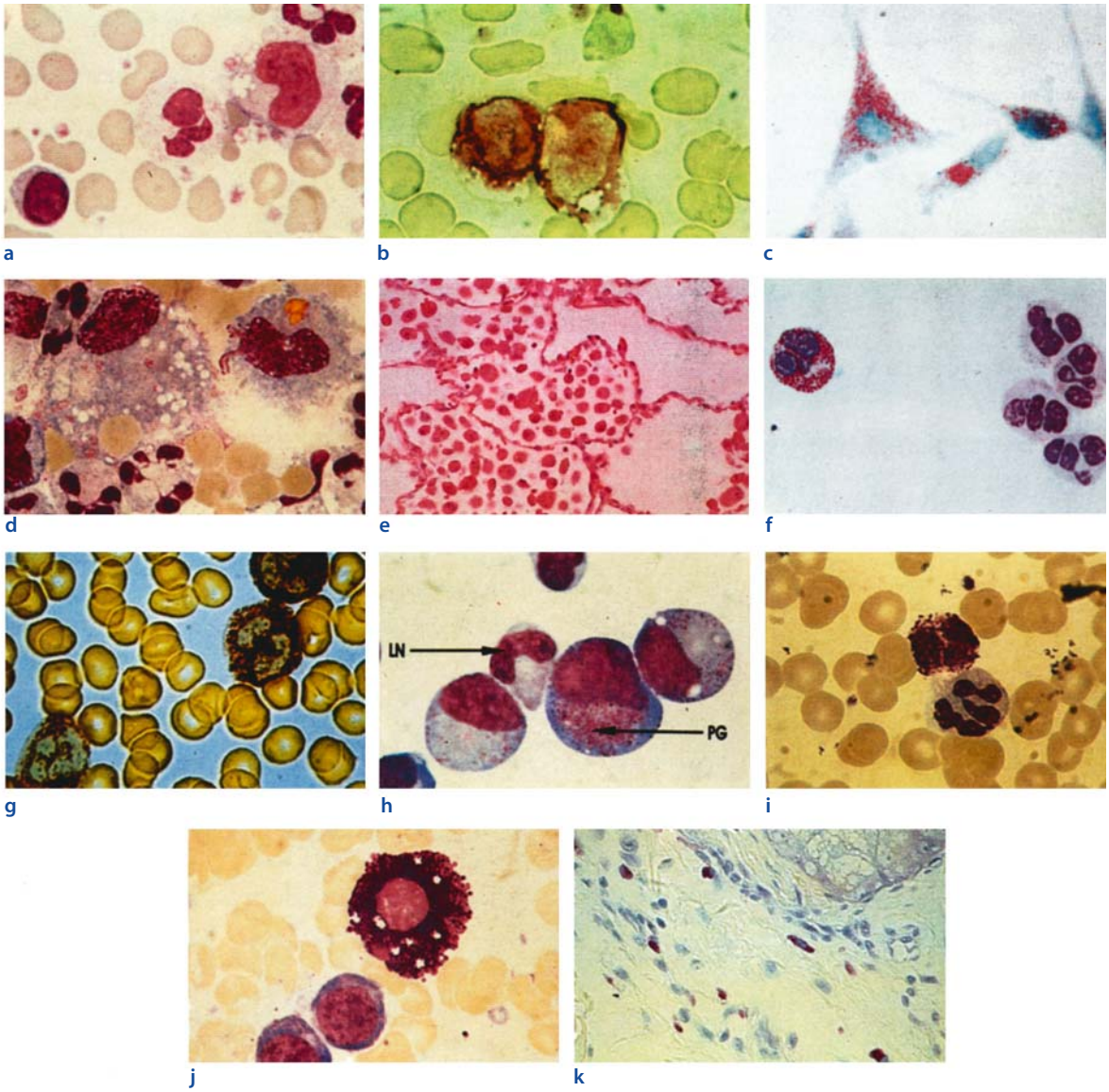


Fig. 1.32 a–k. Cells of the immune system. **a** Monocyte showing a horseshoe-shaped nucleus and moderately abundant pale cytoplasm. Note the three multilobed polymorphonuclear neutrophils (*PMNs*) and the small lymphocyte (*bottom left*). **b** Two monocytes with vacuolated cytoplasm. The small cell with focal staining at the top is a T lymphocyte. **c** Monocytes in monolayer cultures after phagocytosis of mycobacteria (stained *red*). **d** Inflammatory cells showing a large active macrophage in the *center* and phagocytosed red cells and prominent vacuoles. To the *right* is a monocyte with horseshoe-shaped nucleus and cytoplasmic bilirubin crystals (hematoidin); several multilobed PMNs are clearly delineated. **e** Numerous plump alveolar macrophages within air spaces in the lung. **f** Four PMNs (neutrophils) and one eosinophil. The multilobed nuclei and the cytoplasmic granules are clearly shown, those of the eosinophil being heavily stained. **g** A PMN neutrophil showing cytoplasmic granules. **h** Early neutrophils in bone marrow. The primary azurophilic granules (*PG*) originally clustered near the nucleus move toward the periphery and as the cell matures, the neutrophil-specific granules are generated by the Golgi apparatus. The nucleus gradually becomes lobular (*LN*). **i** Basophil with heavily stained granules compared with a neutrophil (*bottom*). **j** Mast cell from bone marrow with a round central nucleus surrounded by large darkly stained granules. Two small red cell precursors are shown in the *bottom*. **k** Tissue mast cell in skin stained with toluidine *blue*. The intracellular granules are metachromatic and stain *reddish purple*. Note the clustering in relation to dermal capillaries

Table 1.20. Secretory products of macrophages

Enzymes
Arginase
Angiotensin converting enzyme
Lipoproteinlipase
Lysozyme
Neutral proteinases Alveolar macrophage elastolytic metalloproteinase Collagenase specific for collagen of basal membrane (type IV) Collagenase specific for interstitial collagen (types I–III) Collagenase specific for pericellular collagen (gelatinase) (type V) Cytolytic proteinase Elastases metallo-dependent Plasminogen activator Stromelysin
Acid hydrolases Glycosidases Lipases Nucleases Phosphatases Proteases and peptidases Others
Plasma proteins
α_2-Macroglobulin
Apolipoprotein E
Fibronectin
Inhibitor of α_1-proteinase
Tissue inhibitor of metalloproteins
Transcobalamin II
Coagulation factors Factors V, VII, IX, X Thromboplastin
Complement components C1–C9 Factor B Factor D Factor H (β_1H) (C3 convertase inactivator) Factor I (C3b inactivator) Properdin
Reactive O_2 species
H_2O_2
Superoxide anion
Others
Bioactive lipids
6-Ketoprostaglandin $F_{1\alpha}$
12-Hydroxyeicosotetranoic acid
LTC
PGE_2
Tromboxane B_2
Others

Table 1.20. (Continued)

Nucleotides
Adenosine
cAMP
Guanosine
Thymidine
Uracil
Factors regulating cell functions
IL and IL-like
Activin
Erythropoietin
Fibroblast growth factor
Insulin-like growth factor
Heparin-binding growth factor
Inhibiting factor for leukemic cells
Platelet factor 4
G-CSF
GM-CSF
IFN-α and -β
IL_1
IL_6
IL_8
IL_{10}
IL_{12}
IL_{15}
IL_{18}
M-CSF
MCP, MIP 1 and 2 and other chemokines
PDGF
CCL chemokine receptor 1
Receptor of IL_1 antagonist
TGF-α and - β
TNF-α
Factors promoting the proliferation of: B cells Endothelial cells T cells Fibroblasts
Factors inhibiting the proliferation of: *Listeria monocytogenes* Tumor cells

Data from [68, 540, 609].

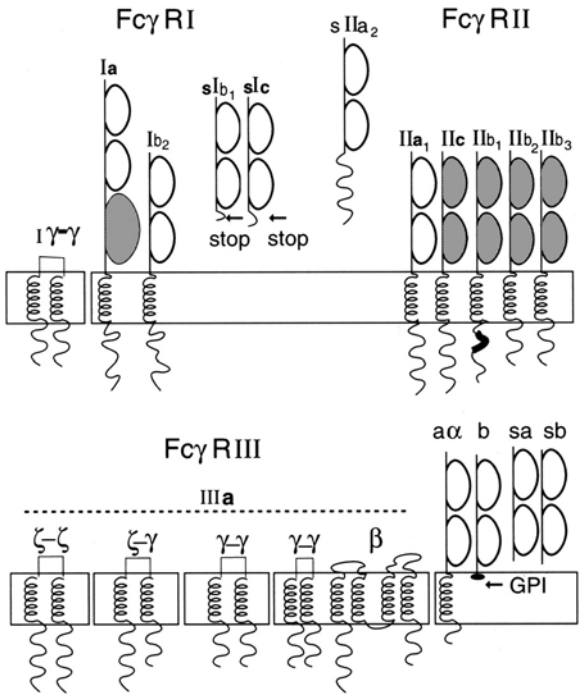


Fig. 1.33. Structure and properties of Fcγ receptors. *R* receptor

Fc fragments, they attract potential targets to be processed later, modified by enzyme digestion and subsequently presented to T cells. They possess membrane receptors for [540, 609]:

- *Igs:* IgG = FcγR, with three distinct types, FcγRI (CD64), FcγRII (CD32), and a low-affinity receptor (FcγRIIb) (Table 1.3; Fig. 1.33), in addition to the specific Fc receptor, which promotes phagocytosis of particulate antigens such as antibody-coated bacterial antigens, a phenomenon called opsonization.
- *Complement* (C3b, C5a).
- *Hematopoietic growth factors* (M-CSF, GM-CSF), lipoproteins, peptides and PS.
- *Membrane receptors* the best characterized of which are FcεRII (bind to IgH chains) and CR3/CD11b (fix iC3b) [445].

Macrophages are versatile secretory cells: they have ≈ 100 receptors on their surface, including complement proteins, active O_2 radicals, bioactive lipids such as PGE_2, PAF (platelet-activating factor), nucleotides and arachidonate metabolites and several enzymes including lysozyme, acid hydrolases, neutral proteases, and enzyme inhibitors (Table 1.20). Macrophages generate CCL chemokines, including MIP-1α and -1β (macrophage inflammatory protein-1α and -1β) and MCP-1 [540, 609]. Like monocytes, they are activated by IFN-γ and MIF, in certain conditions acquiring cytotoxic or suppressive properties, in addition to contributing to lymphocyte responses as accessory cells, releasing ILs such as TNF, IL_6, IFN-γ via IL_1 and IL_{12}, as well as IFN-α and TGF-β [488]. Macrophages synthesize IL_{18} as an IFN-γ induction factor [669]. In turn, IFN-γ induces in macrophages IL_{12} and $IL_{12}R$, whose activity depends on Th1 T-cell activation [124]. IL_{15} can also be a macrophage endogenous product [67]. Four ILs instead deactivate these cells: IL_4, IL_{10}, IL_{13} and TGF-β [124]. Correspondingly, macrophages are pluripotent cells supporting opposite actions depending on their local microenvironment: for example, the response to IL_2 can activate T-cell proliferation or suppression and CMI [124]. Probably, CD28 expression by IL_{12}-producing macrophages can lead to generation of Th1 T-cell responses [387], anticipating that CD28 binding to CD80 represents a costimulatory signal for T lymphocytes. Because the number of monocytes and macrophages can increase 3- to 100-fold at a site of inflammation, the regulatory and effector roles of these cells become even more prominent after an inflammatory response has begun. Also prominent is the portfolio of chemokines that attract these cells to a site of inflammation, which include CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22 [87].

Granulocytes live only a few days; have the main function of ingesting and kill any non-self substance. C/EBPα is essential for their development [678]. Cells of granulocyte lineage, PMNs, eosinophils, and basophils are distinct according to cytoplasmic granule staining. Granulocyte surface markers are shown in Table 1.21 [470] and compared with metachromatic cells.

Neutrophils (Fig. 1.32, d, f, g, h) are the most numerous leukocytes in the bloodstream (half-life, 4–10 h), with a normal adult having >100 billion PMNs present daily, a number that can increase to almost 1 trillion when the host is stressed by infections. PMNs carry on

Table 1.21. Surface markers of neutrophils or PMN and eosinophils compared with metachromatic cells

Cells	Cell markers								
	CD88	CD35	CD11a	CD11b	CD49d	CD32	CD16	FcεRI	FcεRII (CD23)
Neutrophils	+	+	+	+	+	+	+	–	–
Eosinophils	+	+	+	+	+	+	±	+	+
Basophils	+	+	+	+	+	–	+	+	–
Mast cells	+	+	+	+	–	–	+	+	–

Modified from [470].

the following activities: chemotaxis, phagocytosis, degranulation, and opsonization [360]. PMNs are involved in the immune inflammation in concert with eosinophils and platelets, also determining within such processes the delayed phase of allergic reactions. Prolonged ADAM17 (a disintegrin and a metalloproteinase 17) expression during neutrophil effector functions and apoptosis may play a role in both the induction and downregulation of neutrophil activity [617]. Primed PMNs in the presence of tissue injury due to microbial agents produce factor(s) which inhibit some of the cell's antimicrobial functions contributing to immune dysfunction, while the factor(s) produced by unprimed PMNs facilitate antimicrobial countermeasures [407]. High mobility group box 1 (HMGB1) protein increases the nuclear translocation of NF-κB and enhances the expression of proinflammatory ILs in human PMNs. These effects appear to involve the p38 MAPK, PI3K (phosphatidylinositol 3-kinase), and ERK1/2 pathways. However, the mechanisms of HMGB1-induced neutrophil activation are distinct from endotoxin-induced signals [407]. They have receptors for:

- *Igs:* IgG=FcγRII and FcγRIII (CD16) and IgA=FcαR (CD89), while PMNs appear to lack IgE receptors.
- *Complement:* C3b, C3a, C5a; C5a is different in structure and affinity from that of eosinophils.
- *LTB_4* (LTB_4), GM-CSF, and G-CSF.

As APCs, PMNs have only limited potential as they synthesize only HLA class I molecules. PMNs have a rich equipment of proteins and mediators, and release several immunoregulatory ILs, modulating both cellular or humoral immunity (Table 1.22) [73, 315]. During acute inflammations, IL_1 enhances T-cell activation, also inducing IL_6, IL_8 and GM-CSF. Recruited early to injured sites, PMNs release M-CSF, hence activating the more slowly invading monocytes [315].

PMNs are activated by NAP (neutrophil activating factor)/IL_8 released by PBMCs (peripheral blood mononuclear cells) and then recruited to inflamed sites by NCF (neutrophil chemotactic factor) and LTB_4, utilizing CD11/CD18 integrin ligands of CD54 (ICAM-1) and CD102 (ICAM-2, intercellular adhesion molecule 1,2) to adhere to vascular endothelium (margination) [23]. PMNs infiltrate injured sites ingesting whatever foreign protein or cellular debris they encounter, including bacteria and CIC (circulating immune complexes)-IgG by virtue of specific receptors, releasing enzymes with lytic action responsible for the maintenance of immune inflammation [135]. When activated, the enzyme NADPH oxidase attached to the cytoplasmic membrane produces monovalent hydroxyl radicals with oxidant properties and oxidized metabolites [663]. During phagocytosis and especially when antigens adhere to the PMN surface and are therefore not phagocytosed, PMNs undergo a respiratory burst, degranulate and fully mobilize their secretory vesicles. Their cytoplasmic membrane fuses with the intracellular granules, finally emptying their enzyme content into the cell lumen

Table 1.22. Proteins and other mediators produced by PMNs

Efferent mediators
CR1
CD11b/CD18
FcR
Class II MHC
Plasminogen activator
IFN-α
PAF
LTB_2
Heat shock proteins
Fibronectin
PGE_2
Afferent mediators
M-CSF
$IL_1\beta$
IL_1RA
IL_3
IL_6
IL_8
GM-CSF
IFN-α
TGF-β
TNF-α

Modified from [73, 315].
CR complement receptor, *IL_1RA* IL_1 receptor antagonist.

[135]. The secondary granule proteins (SGP) are secreted in a hierarchical manner: first gelatinase-containing granules, then specific granules, and last azurophilic ones [135] (Table 1.23 [315], Fig. 1.32 g). In the later stages of myeloid development, the SGP genes are coordinately upregulated, and members of the C/EBP family of TFs, in particular C/EBPα and C/EBPη, play specific and unique roles in upregulating their expression [257]. Among the many granule enzymes (>20), MPO (myeloperoxidase), elastase, collagenase and gelatinase play a prominent role in cytotoxicity, also attacking and degrading above all connective tissues [630]. The mature granules also contain Igs, complement proteins, clotting factors, cationic proteins and defensins (see "Innate Immunity" p. 152). The CCAAT-enhancer binding protein (C/EBP) family of nuclear TFs is implicated in the regulation of terminal myeloid differentation. In particular, C/EBPα and C/EBPη play specific and unique roles in upregulating SGP expression [257, 646]. The abnormal PMN accumulation in states of acute inflammation consists of several processes taking shape in sequence:

Table 1.23. Constituents of specific granules and azurophil granules of human neutrophils

Specific granules	Azurophil granules	
Histaminase	Myeloperoxidase	Elastase
Collagenases	Acid phosphatase	Histonase
Binding vitamin B_{12} protein	β-Glucosaminidase	Lysozyme
Laminin receptor	5′-Nucleotidase	Cationic proteins
C3b receptor	α-Mannosidase	BPI
Receptor of formylated peptides	Arylsulfatase	Defensins
Cytochrome b558	α-Fucosidase	Glycosaminoglycans
Lactoferrin	Neuraminidase	Chondroitin sulfate
Flavoproteins	Cathepsin D	Heparin sulfate
Lysozyme	Cathepsin G	

Modified from [315].
BPI bactericidal/permeability inducing protein.

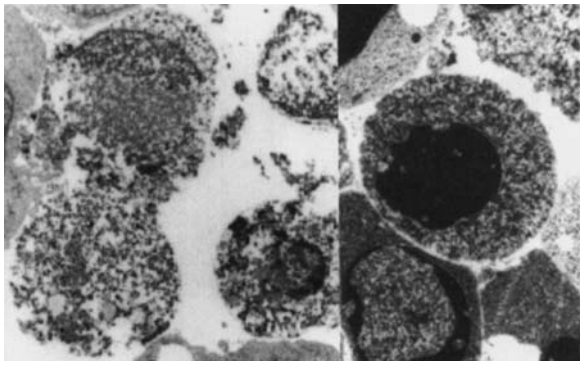


Fig. 1.34. Necrosis and apoptosis: ultrastructural aspects. Cells undergoing necrosis (*left*) or apoptosis (*right*)

endothelium and PMN activation, adhesion, diapedesis and phagocytosis [315]. Besides their essential defensive functions, PMNs are coming under scrutiny whereby they may cause excessive injury to host cells, since a potent cytotoxicity in acute inflammation is potentially damaging. PMN persistent accumulation involves an excessive secretion of O_2 toxic radicals and proteases, with amplification of tissue injury following LTB_4, LTC_4 (leukotriene C_4) and TXA_2 (thromboxane A_2) synthesis [630]. It has been suggested that such negative regulation is favored by the density reduction observed in allergic subjects [364]. Aged neutrophils undergo *apoptosis* [276]: endogenous activations of endonucleases allow PMNs to be recognized and engulfed intact by macrophages to avoid dispersion of cytotoxic products [510] (Fig. 1.34). Early neutrophil influx into the airways after allergen challenge is mediated by IL_1, IL_{18} and p38 MAPK and can be reduced by inhibiting either IL or p38 MAPK. However, both the neutralization of these ILs and reduction of PMN number do not modify the later development of eosinophilic airway inflammation or the BHR (bronchial hyperreactivity) insurgence, thus suggesting that the early and transient neutrophil response fails to play a direct role in the development of allergen-induced BHR. The effects of inhibiting p38 MAPK in decreasing BHR indicate activities independent of its prevention of PMN accumulation [569].

Eosinophils (Fig. 1.32f, 1.35a) [680], present in peripheral blood at a mean concentration of 300–400 cells/m^2 from 0 to 2 years and of 200–250 cells/m^2 from 4 to 21 years, survive longer than other granulocytes (half-life, 6–12 h). Eosinophils migrate into the thymus during the neonatal period, localize to the corticomedullary region and attain maximum levels by 2 weeks of age. A second influx of eosinophils to the medullary region is observed at 16 weeks of age, when the thymus begins to involute [477]. Even after leaving the bone marrow together with CD34, they continue the synthesis process and can return into the bloodstream from tissues; however, compared to PMNs, they are inconclusive as phagocytes and less efficient at intracellular killing [631]. Eosinophils are present in allergic, immunological, parasitic disorders and hypereosinophilic syndromes. They can be activated either *in vivo* or *in vitro* gy diverse agonists, such as Igs, lipid mediators, and ILs [371] and from CD69, CD44, and CD54 [670]. On activation, they probably kill parasites mainly by releasing cationic proteins and reactive O_2 metabolites into the extracellular fluid [623]. Eotaxin induces a rapid concentration-dependent activation of ERK2 and p38 in eosinophils, and the activation of these kinases is required for eotaxin-induced eosinophil chemotaxis, actin polymerization, and degranulation. It is therefore proved that eotaxin plays an integral role in the development of eosinophilic inflammation in asthma and allergic inflammatory diseases [247]. Key eosinophil regulatory ILs such as IL_5 and the eotaxin subfamily of chemokines regulate eosinophil production and localization at baseline and during inflammatory responses

Table 1.24. Surface molecules of human eosinophils (excluding Ig receptors and complement components)

Adhesion molecules
CD11a/CD18
CD11b/CD18
CD11c/CD18
CD15
CD31
CD44
CD49a/29
CD49b/29
CD49c/29
CD49d/29
CD49e/29
CD49f/29
CD62E
CD62L
CD62P
CDw65

Ig supergenes
CD9
CD13
CD24
CD43
CD45
CD50
CD54
CD102

Ig supergenes (Continued)
CD46
CD47
CD50
CD59

Inducible molecules
CD4
CD16
CD23
CD25
CD54
CD64

Receptors
Adenosine
β_2-Adrenoreceptor
FMLP
GM-CSF
IFN-γR
IL_3R
IL_5R
IL_8R
LTB_4
PAF
TNF-αR

Modified from [197].
FMLP formylmethionyl leucylphenylalanin.

[477]. *In vitro* studies and those on BALF (bronchoalveolar lavage fluid) show that the regulation of eosinophil production is highly dependent on GM-CSF, IL_3 and IL_5 [550]. Such ILs play a pivotal role in promoting eosinophil maturation, remarkably influencing both differentiation in the bone marrow and activation in the tissues [61]. GM-CSF and IL_3 particularly increase the number of precursor cells prolonging their survival, IL_5 acting primarily as a selective chemotactic factor mediates their maturation and triggers degranulation and adhesion [34]. GM-CSF and IL_5 further activate eosinophil cytotoxicity and oxidative metabolism and, added to culture with CD34, increment notably their number, making the cells functionally mature on the 21st day [515]. Since during intense responses eosinophils can undergo cytolysis, the detection of cationic proteins is a useful marker of their involvement (Chap. 7). Eosinophils can be engaged to express HLA class II molecules and act as APCs [632]. Eosinophils also express CD48, CD58, CD84, CD244/2B4, but not CD [371] on their surface. Table 1.24 [197] summarizes the most significant data on surface molecules and Tables 1.5–1.8 detail IL effects on eosinophils playing a vital part in inflamed lesions of allergic disorders. Accordingly, eosinophils yield [631]:

- Fc receptors for Igs: both IgE receptors, FcεRI and FcεRIIb, present chiefly on the hypodense phenotype, with an affinity comparable to that of FcγRII (CD32) for IgG antibodies and FcαR; IL_4 and other unidentified factors amplify FcεRI α chain expression on eosinophils [573], making them capable of diffusing their cationic proteins.

- Receptors for complement components: C1q, C3a, C3b/C4b (CR1=CD35), iC3b (CR1, CR3) and C5 are different from neutrophil receptors. C3a, C4a and C5a are called *anaphylatoxins,* a term derived from anaphylaxis-like responses produced when such peptides were injected in experimental animals [159].
- Receptors for GM-CSF, IL_3, IL_5 and IL_8 are further potential sources of IL_1, IL_4, IL_6, TGF-α, TNF-α and MIP-1α [46, 477]. Signal-transduction molecules implicated in these IL-mediated priming responses include Lyn, JAK2, PTK, and p21 [46].
- A functional CD244R cross-linking on the surface of eosinophils which elicits ERK, activates NK cells, and causes eosinophils to release EPO, IL_4 and IFN-ɣ can contribute to eosinophil effector functions in both Th1- and Th2-like responses, thus indicating a broader role for eosinophils in health and disease [255].
- Receptors for chemokines, including eotaxin, MCP1–4, MIP-1α and β (now called CCL3, CCL4), and RANTES (regulated on activation normal T expressed and secreted), now called CCL5 [687].

Whether eosinophils generate additional ILs is unclear, since circulating cells, unlike BALF cells, fail to express ILs [550]; however, they can secrete, as no other cell does, the *CD40–CD154 couple* [175], with substantial repercussions on B cell isotype switching to IgE phenotype, in addition to synthesizing IL_4, especially in the airways [397].

Eosinophils contain substantial intracellular quantities of several granule- and vesicle-associated IL receptors, including IL_4R, IL_6R, and $IL_{13}R$ as well as CCR3. A temporal coincidence of $IL_4Rα$ and IL_4 mobilization from granules into the vesicles was combined with a clear association of IL_4 with secretory vesicle membranes, thus suggesting that eotaxin-mobilized $IL_4Rα$ functions as a transporter for IL_4 via the secretory pathway. The intracellular ILRs localization possiby extended to granules of innate immune system cells. Mast cells and neutrophils may play a role in the secretion of granule-derived ILs from both these cells comparable with the ILs recognized in eosinophils. This suggests that several additional ligand-binding receptor chains such as an ILR chain specific for eosinophil-derived ILs and chemokine secretion may provide a crucial component of the regulatory mechanisms governing specificity of rapid, stimulus-induced release of preformed immunomodulatory ILs from human eosinophils, as well as other innate immune cells containing granule stores of preformed ILs and chemokines [532].

Eosinophils also express:

- $β_2$ integrins facilitating their migration from blood into normal and inflamed tissues such as CD11c (p 150,95), the adhesion molecules CD54, its receptor CD11a/CD18 and VCAM-1 (vascular cell adhesion molecule), CD106, and IL-induced endothelial protein (appears 6–12 h after stimulation), playing a critical role in eosinophil adhesion to activated vascular endothelium and epithelium, their extravasation, tissue localization and interplay with other cells [197].
- The surface molecule CD4 identified on T4 lymphocytes binding to HIV-1 glycoprotein 120 (HIV-1 gp120). So far the role of CD4 is less clear; however, it might act as a signal transducer, as demonstrated by the capacity of its three ligands (HIV-1 gp120, bivalent monoclonal anti-CD4 antibody and chemotactic factor of leukocytes) to trigger eosinophil migration but neither their degranulation nor their superoxide formation [593].
- GM-CSF, IL_3, IL_5, TNF-α, and RANTES positively regulating the last two properties [210].
- HLA-DR class II synthesized by mature cells stimulated by GM-CSF, IL_3, IL_4, IFN-ɣ [594], thereby mediating interactions of other APCs with $CD4^+$.
- Eicosanoids, released in particular conditions from membrane phospholipids, including LTB_4, LTC_4 prostaglandins (PG) such as PGE_2, and PAF, substances with a well-known bronchoconstrictor action, bradykinin, H_2O_2, $O_2^{\bullet -}$ and several enzymes. They include peroxidase and additional enzymes with oxidoreductive power, among which histaminase (inactivating histamine) is the best known, digestive enzymes (proteases, nucleases, other hydrolases such as kininase), anti-inflammatory enzymes, aryl sulfatase B specifically inactivating LTs, one of the main mediators of bronchospasm and immune inflammation, phospholipase D (PLD) inactivating PAF, catalase, collagenase, β-glucuronidase, and non-enzymatic molecules of which plasminogen is the most notable [470].

Eosinophils are also active in immune reactions with a *destructive armamentarium at the tissue level,* initiating highly damaging actions committed to cationic proteins and O_2 radicals. Histamine released by skin mast cells and other chemotactic factors, PAF in the first line, C5a, IL_{16} and various chemokines such as eotaxins 1, 2, 3 [435] selectively recruit eosinophils to the site of inflammation where they release cationic proteins and other mediators [581]. Whether cells are activated during their migration is not clear, but certainly the involvement of chemotactic factors and interplay with the extracellular matrix (ECM) expedite their progression, including signals transmitted from adhesion molecule receptors, which, all findings considered, involve cell activation and mediator release [625].

Diversely from other granulocytes, eosinophils have typical secondary granules, containing four distinctive cationic proteins. All of them yield toxic actions at the cell level, *directly damaging host cells and tissues.* MBP (MBP-1 and MBP-2; major basic protein) of 14 kD, elaborated also by basophils and rich in reactive sulfydryl groups, has no inflammatory property, but via a direct cytotoxic mechanism, has adverse effects extended to mono- and multicellular parasites and to human cells, including bronchial epithelium. MBP produces pomphoid reactions, *induces ciliostasis,* activates neutrophils and platelets and neutralizes heparin, pro-

vokes degranulation of metachromatic cells and histamine release, making a relevant contribution to the perpetuation of inflammation. A likely cause of its accumulation in AD lesions is the IgE-mediated delayed reaction [593]. In asthmatic patients, it provokes *BHR*, inhibited in vivo in animal studies by a specific antiserum [294]. MBP is the crystalloid core of secondary granules, while the matrix surrounding it contains other cytotoxic proteins: all are among the more destructive mediators, not released by extrusion from the entire granule (as in the case of mast cell reaction), but following a process of granule exocytosis, unlike PMNs, to kill foreign substances internally [337]. About 90% of granule proteins are represented by EDN (eosinophil-derived neurotoxin), or EPX, of 18.6 kD, cytotoxic and neurotoxic, interfering with CMI. EPO (eosinophil peroxidase), consisting of two 15- to 55-kD polypeptides, carries on the same routine as EDN, interferes with coagulation and fibrinolysis, degranulates metachromatic cells and has ribonuclease properties. ECP (eosinophil cationic protein), 18–21 kD, more cytotoxic than EDN and up to tenfold more potent than MBP, degranulates mast cells and has a peroxidase activity [631]. Release of such proteins is selective, because EPO release takes place subsequently to the others, perhaps in different stages of activation. The remaining 10% of proteins are formed by hexagonal and bipyramidal Charcot-Leyden crystals (CLC), present also in basophils. CLCs, first observed in 1872, belong to the type-S lectin superfamily; they can neutralize natural lung surfactants, causing atelectasis [631]. Both eosinophil activation and release of cationic proteins are IgA-mediated [581], via production of IgA antibodies endorsed by IL_5, SC binding, and consequent IgA-mediated eosinophil degranulation [572]. If ECP reduces IgA production and alters oral tolerance this could confirm that eosinophil detrimental activity is a prominent pathological feature at the expense of mucosal surfaces. Evidence suggests that eosinophil damaging activity is also committed to their oxidative products, such as reactive O_2 radicals, triggered by PAF and to a higher extent by C5a, while EPO will oxidize a variety of substrates in the presence of H_2O_2 (Fig. 1.35b) [680], with resulting production of other potent O_2 radicals, among which is singlet O_2 ($^1O_2$) [680]. PAF deriving from T lymphocytes is also active on mature cells and on the most potent chemotactic factor for eosinophils also due to O_2 uptake, $O_2^{\cdot -}$ release and iC3b increase in binding capacities [673].

Recent studies have critically revisited eosinophil phenotypic changes, showing that peripheral cells may be distinguished on the basis of their *hypodensity* (of sedimentation), chiefly under GM-CSF, IL_3, IL_5 [631], C5a and PAF effects [680] (Fig. 1.35c), but such cells are also found in normal nonatopic subjects [649]. Furthermore, in addition to speed cell survival, the hypodense phenotype can more easily bind to IgE antibodies, thereby appearing more metabolically active with IL_3 intervention and above all with IL_5 [367]. Certainly such change is not synonymous with activation [680], of which a more sensible parameter is LTC_4 synthesis under GM-CSF, IL_3 and IL_5 stimulation [631]. Eosino-

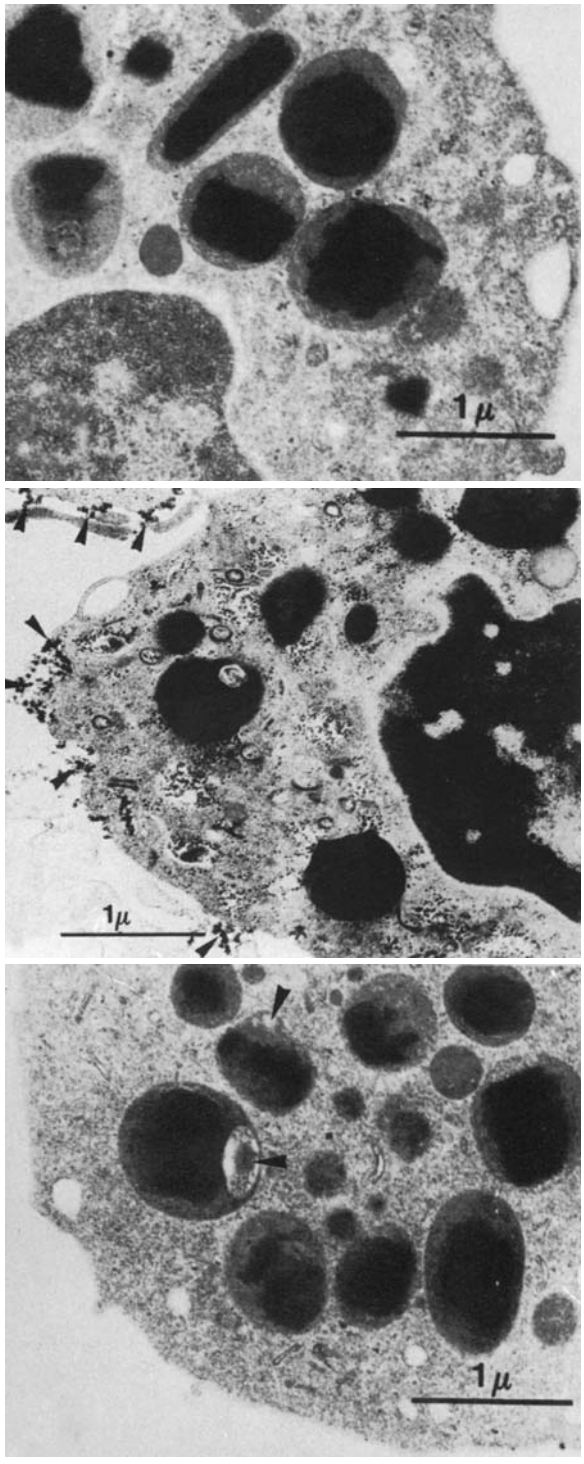


Fig. 1.35 a–c. Ultrastructural aspects of eosinophil activation (electron microscopy, EM). **a** Unstimulated eosinophils. **b** Eosinophils stimulated with PAF: *arrowheads* indicate production of H_2O_2. **c** Eosinophils stimulated with PAF and C5a: *arrowheads* indicate *hypodense cells*

phil exposure to activating ILs leads to the development of these hypodense eosinophils, with a specific gravity of <1.085 g/ml. Increased numbers of hypodense eosinophils are found in many allergic disorders, including AR and asthma [46]. In children these hypodense cells may be *truly immature* (Chap. 11): these findings could explain why ECP levels, activation markers, are not augmented in children compared to adults [367]. Studies have given rise to the hypothesis that hypodense cells are of two types, one with features similar to normodense cells [631].

Mast cells and basophils (*metachromatic cells*), cells prominently involved in immediate and late reactions, have in common cytoplasm granules containing histamine, heparin, serotonin or 5-hydroxytryptamine (5-HT) and kinins. Immature progenitors of human mast cells (SCF=c-kit=CD117) are present in fetal liver cells, bone marrow and CB, enter a peripheral tissue still without secretary granules and cell surface FcεR1, and then complete their differentiation probably with multipotential capabilities in connective tissues and mature beneath epithelial tissues and in areas adjacent to blood and lymph vessels, and near or within nerves [168, 509]. Basophils differentiate and mature in the bone marrow and circulate in the bloodstream (CDw17), but only rarely in connective tissues [341]. Basophils develop largely under the influence of IL_3, a process that is increased by TGF-α [509]. IL_3 present in cultures with CD34 promotes basophil and mast cell development up to 16%–28.5%; however, addition to the medium of GM-CSF and IL_5 reduces the rate to 3%–15% in favor of eosinophils [515]. Mast cells, which Ehrlich called "overfed cells" because their cytoplasm is filled with granules [136], abound in lymphoid organs, connective tissues of most organs, in particular on the epithelium and airway lumen, sensitive to IgE-dependent stimuli, while in blood and lymph there are instead basophils [593]. Mast cells and basophils migrate toward the chemokine gradient at a site of inflammation but stop and accumulate at the site where allergen concentration is high [580]. Both cell types, real biochemical "powder-kegs," interact with allergens, degranulate and release mediators with different pharmacological actions, and provoke clinical manifestations of immediate hypersensitivity, such as vascular permeability increase, smooth muscle contraction, epithelial mucus secretion, chemotactic action on eosinophils, and platelet aggregation. Costimulation via FcεRI engagement with IgE/antigen and CCR1 engagement with human rCCL3 synergistically enhance the degranulation of metachromatic cells at the site where the cells accumulate, thus playing important roles in the orchestration and focusing of the allergic response. The progression toward chemoattractants requires actin cytoskelton rearrangement and polarization such as formation of leading edge and membrane ruffles [127]. However, FcεRI engagement affects CCL3-mediated actin reorganization and chemotaxis of CCR1 cells [580]. Moreover, Rac signaling and/or phosphatidylinositol (PI)4,5-bisphosphate synthesis by PI 4-phosphate 5-kinase I was required for membrane ruffling [127]. Only inflammatory cells provided with cytoplasm granules containing histamine and FcεRI [358], together with other cells, also express FcεRII a and b and can up-regulate and aggravate tissue inflammation [509]. Both cells are important sources of CD16 (FcγRIII), C5aR and CD35 [470]. In concert with adhesion molecules and receptors, they mediate the binding to other cells and to ECM gps, including CD49d and 49e/CD29 (β1 integrins), basophils add CD11a, CD11b, CD11c/CD18 (β2), and mast cells CD51/61 (β3) [196]. Metachromatic cells possess high-affinity receptors for IgE (FcεRI) and thereby become coated with IgE antiboidies [175]. *Activated metachromatic cells produce IL_4 and express CD154, with the immediate consequence that IgE antibody synthesis may also occur in peripheral tissues* [174], even independently of T cells. Therefore these cells are important in AD, asthma, and AR in which allergen binding to the IgE cross-links the FcεRI [347]. Several differences call for distinguishing these cells from functional, ontogenetic, histochemical points of view, and the quantity and quality of released mediators: Table 1.25 [166, 168, 340, 567] shows that differences prevail over analogies also regarding the immune stimuli activating them (Table 1.26) [340].

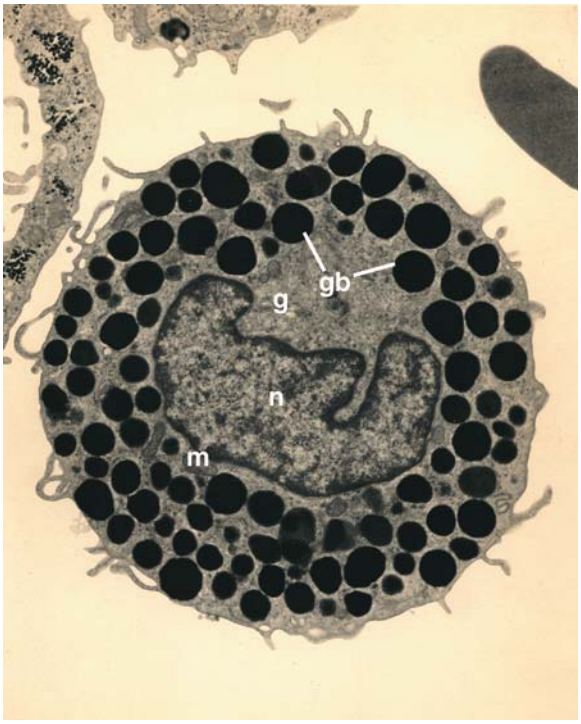


Fig. 1.36. Basophil, EM (×18,500). *n* Multilobed nucleus, *m* mitochondrion, *gb* basophil granules, *g* Golgi apparatus

Circulating *basophils* (Fig. 1.32 i, 1.36) have concentrations of 20–45 cells/mm^3, = 0.3%–0.6% of leukocytes, 6–60×10^5 FcεRI/cell (twice as much as mast cells), 0.04 pg/cell of tryptase [166, 168] (250- to 875-fold less

Table 1.25. Differences among mast cells and basophils

Characteristics	Mast cells	Basophils
Origin of precursors	Bone marrow	Bone marrow
Site of maturation	Connective tissue	Bone marrow
Mature cells in the circulation	No	Yes (<1 % of blood leukocytes)
Mature cells recruited from the circulation	No	Yes (immune inflammation)
Mature cells normally residing in connective tissues	Yes	No
Form	Round	Irregular
Cell diameter	10–15 μm	5–7 μm
Nucleus	Round or oval; eccentric	Bilobed or multilobed
Granulations	0.1–0.5 μm	10–15 μm
Cytoplasmic membrane	Less regular	More regular
Cell surface	Cytoplasmic protrusions	Smooth
Life span	Weeks or months	Days
Preliminaries to degranulation	The granules fuse intracellularly	Fusion of individual granules
Degranulation procedure	Chains of connected granules are released via newly formed channels to cell membrane	Fusion to cell membrane with extrusion of the granule
Cytoplasmic granules	Numerous and relatively small granules	Relatively few and large granules
Major granule contents	Histamine, chondroitin sulfate, neutral acid hydrolases, heparin, MBP	Histamine, chondroitin sulfate, proteases, neutral proteases, MBP and CLC
Mediators and other molecules	PGD_2, TNF-α, thromboxane A_2 (TXA_2), LTB_4, LTC_4, 5-HETE, PAF, NCF, ECF	PGD_2, LTC_4, LTC_4, TXA_2
LTC_4	60 ng/10^6 cells	60 ng/10^6 cells
PGD_2	60 ng/10^6 cells	0.006 ng/10^6 cells
TXA_2	5 ng/10^6 cells	0.005 ng/10^6 cells

CD	Receptors	Mast cells	Basophils
Interleukins			
25	IL_2R	–	+
116	GM-CSFR	–	±
117	SCFR, c-KIT	++	–
119	IFN-γR	?	±
121b	IL_1RII	–	+
123	IL_3R	–	+
124	IL_4R	–	+
125	IL_5R	–	+
128	IL_8R	–	±
Immunoglobulins			
NC	FcεRI	+	+
32	FcγRII	–	+

Table 1.25. (Continued)

CD	Receptors	Mast cells	Basophils
Complement			
11b	CR3	–	+
11c	CR4	–	+
35	CR1	–	+
88	C5aR	–	+
Adhesion molecules			
09		++	++
11a		–	+
18		–	+
29		+	+
31		–	+
41		+	–
43		++	++
44		++	++
49c, d, e/29		+	+
50		±	+
51		+	–
54		±	+
58		+	+
61		+	–
102		±	±
104		–	+
Additional receptors			
13		–	+
45		–	+
47		–	+

The receptors/antigens not expressed by both cell types are not shown.
Modified from [166, 168, 331, 376, 567].
NC not classified.

than TC and T mast cells) and ≈1 pg/cell of histamine, 25% of mast cells [341]. Although basophils account for only 0.5% to 1% of peripheral leukocytes their participation is emphasized in all allergic diseases, and the presence in tissues is correlated with affection severity. Despite their short life span and their reduced percentage, they characteristically increase in number in delayed-type responses, matching the rise of histaminemia [46, 332]. Circulating human basophils co-operate with eosinophils by playing a significant role in promoting allergic inflammation through the release of pro-inflammatory mediators [including ECP, MBP, histamine-releasing factor (HRF), IFN-γ, IL_4, and IL_{13}], capable of potentiating or priming histamine and LTC_4 release [46], as well as CD203c on blood cells exposed to recombinant allergens (RAs) [200] and represents a basis for a sensitive novel allergy test (Chap. 6). Basophils have several receptors (Tables 1.25, 1.26), and ILs as well as adhesion molecules regulate their chemotaxis [567]. Several chemokines induced chemotaxis at different potencies: eotaxin > SDF-1 > RANTES MCP-1 >> MIP-1 [239]. Their passage from blood vessels to inflamed tissues is correlated with the availability of specialized chemotactic factors, among which are factors of leukocyte derivation, kallikrein, C5a, specific antigens [528] and CD62L [625]. CD44 and CD54 are constitutively expressed on basophils, CD69 expression is preferentially and strongly upregulated by IL_3 [670]. Exposure of basophils to priming stimuli increases their sensitivity to FcεRI mediated-activation [46]. Basophil recruitment and activation may be facilitated by the CXCR4-SDR-1 receptor ligand pair [239]. The action of

Table 1.26. Immune stimuli activating human basophils and mast cells (*MC*)

Stimuli	Basophils	Lung MC	Skin MC
IgE-mediated			
Antigens	++	+	+
Anti-IgE	++	+	+
Anti-FcεRI	++	+	+
HRF	+	–	–
A protein	++	–	–
Fv protein	++	+	+
L protein	++	+	+
Not IgE-mediated			
C5a	+	–	+
IL-3	+	–	–
SCF	–	+	+
TNF-α	–	–	+
MBP	+	–	–
MCP-2	+	–	–
MCP-3	+	–	–
MCP-4	+	–	–
MIP-1α	+	–	–

Modified from [331].

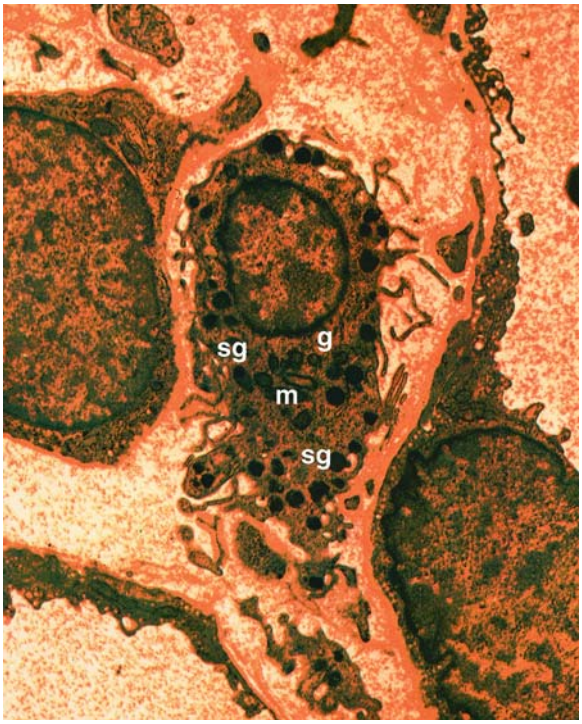


Fig. 1.37. Mast cell, EM (×26,000). *g* Golgi apparatus, *m* mitochondrion, *sg* secretory granules

anti-IgE, and in sequence by growth factors including GM-CSF, IL_3, IL_5, NGF, IL_8, IL_{18}, IFN, PAF and C3a, drive basophils to release histamine [46, 567, 669]. IL_{18} also stimulates IL_4 by basophils [669]. Histamine release was induced by MCP-1 SDF-1 > eotaxin > RANTES > MIP-1 [239]. In this process, the PAF role is Ca^{++}-dependent and -independent of GM-CSF and IL_3, which up-regulate PAF activity [92]. IL_3 increases adhesion to endothelium and induces basophils either to produce IL_4 priming these cells at the level of membrane IgE [55], even if there are measurable IL_4 levels in cells devoid of IL_3, although tenfold less [506], or activating MCP-1 triggering their degranulation with dose-dependent histamine release [22], an effect inhibited by several CXCL and CCL chemokines [281]. The tendency to release histamine is genetically controlled, but in a way different from IgE production: it may be particular of allergic subjects, in whom it should be considered as a biological feature favoring the progression to chronic inflammation [331]. Several disease states also result in a concordant relationship between serum IgE and basophil FcεRI expression [485]. Studies have focused on new aspects of releasability (Chap. 11), a parameter not yet defined from a biochemical point of view, although it regulates proinflammatory mediator release and IL release from effector cells, including mast cells and eosinophils [332]. *Basophils represent a prominent source of IL_4, above all considering that IL_4 stimulates peripheral monocytes to synthesize IgE antibodies* and maybe also additional ILs regulating immune responses of other cells, thus amplifying inflammation. The role of IL_4 produced by basophils is also reflected at the Th2 T-cell level [507]. IL_4 present on endothelial surfaces participates in regulating eosinophil adhesion and selective transmigration as well; therefore eosinophil accumulation in inflammatory sites can be propitiated by activated basophils [506]. A recent study shows that the ability of TLR2 ligands to target basophils not only for IL_4 but also for IL_{13} secretion could have relevance to *in vivo* findings [41], *yet IL_{13} early in ontogeny* [182] so that they could play an important role in promoting and amplifying the Th2-dependent responses manifest in allergic disease [506]. The best characterized TRL2 ligand, peptidoglycan, directly activated basophils for IL_4 and IL_{13} secretion and greatly increased IL and mediator release in response to IgE-dependent activation [41]. Increased spontaneous basophil histamine release improves with food avoidance in children with FA (Chap. 10).

Each mast cell (Figs. 1.32 j, k, 1.37) bears on its surfaces $10–30\times10^5$ FcεRI receptors able to bind to Fc fragments, leaving free the Fab one, which is provided with the binding site for antigens, probably within the Cε3 domain [168]. An IgE molecule binds to one FcεRI receptor and vice versa: as a result, parallel to a high number of receptors, *only one or a few ng of IgE are enough to start mast cell activation.* Mast cells are also capable

of presenting antigens to T cells, resulting in their activation, in either an HLA class I or class II-restricted and polarizing T cells toward the Th2 phenotype via the effects of IL_4 and IL_{13} [347]. Mast cell precursors arise from pluripotent bone marrow-derived stem cells, circulate in the blood and lymphatics, and migrate into tissues, in which they reach phenotypic maturation [347]. Mast cells are stategically located at perivascular sites to trigger inflammatory responses [343]. Increased vascular permeability induced by mast cell-derived tryptase and chymase, and degradation of ECM components by enzymes such as matrix metalloproteinase 9 (MMP-9) which has been shown to be released from mast cells on activation by T cells also following a possible autocrine regulation by mast cell TNF-α, may further expedite cell migration through barriers including the vascular wall and the blood–brain barrier [347]. These cells, usually absent from blood, are scattered in connective tissue sites throughout the body, and especially around blood vessels and nerve endings in a variable number, between 7,000 and 20,000 cells/mm^3 of tissue [168]. Molecules such as HLA class I and II, CD28, CD54 (ICAM-1), CD154, β_2-integrins, and TLRs (TLR1 to TLR4, TLR6 and TLR9 [333]) allow mast cells to interact with other inflammatory cells, thus orchestrating an immune response [347]. MIP-1α may be a costimulatory signal operating via CCR1 for mast cell-mediated immediate hypersensitivity reactions [357]. A unique role for mast cells is to produce and release a vast array of mediators such as vasoactive amines, products of arachidonic acid (AA) metabolism, and several proinflammatory, chemoattractive, and immunomodulatory ILs that may contribute to immune reactions by affecting cell growth, recruitment, and function [476] (see also Innate Immunity). By using complementary DNA microarrays 1–2 hours after cross-linking with FcεRI, 2,530 genes exhibited 2–200-fold changes in expression and mast cells were shown to produce 18 ILs, including 130–529 pg of $IL_{11}/10^6$ cells and TNF-α, 13 chemokines, including two CXCL (IL_8, Gro2) and lymphotactin), ten CCL chemokines and several adhesion molecules [494]. Every human cell isolated from nose, lung, skin and intestine contains on average 2, 2.5–10, 4.6 and 1–2 pg of histamine, respectively [402]. Mast cells have been identified as a potential source of MBP [376]. Moreover, IL_{11} mRNA was co-localized with MBP in inflammatory cells in the subepithelial layer of the airway in subjects with severe asthma [354]. This raises the possibility that both mast cells and eosinophils represent sources of IL_{11} in asthma [354, 494]. Studies in rodents have revealed two mast cell phenotypes, called T or TC, being associated either with mucosal and connective tissue or with both tryptase and chymase (35 and 4 pg/cell) or, respectively, only tryptase (10 pg/cell) [168]. Apparently, T mast cell maturation, but not the TC phenotype, requires the help of IL_3 and IL_4 generated by T lymphocytes. Mast cells produce several ILs in response to cross-linking with FcεRI (Tables 1.25, 1.26), including TNF-α and IL_4 [509] and in vitro IL_1, IL_3-IL_6, GM-CSF, IFN-ɣ, which do not elicit histamine release as for basophils, as well as chemokines such as MIP-1α and -1β [168]. IL_1, IL_3, GM-CSF, MCAF (monocyte chemotactic and activating factor), = MCP-1 and RANTES; IL_4 and TNF-α induce adhesion molecules on vascular endothelium in injured sites, a first step for inflammatory cell migration such as lymphocytes and granulocytes [420]. Also, the expression on the mast cell surface of integrins linked to the FN receptor causes their activation [420]: FN binds to β1 integrins including CD49a, CD49c, CD49d, CD49e, and CD49f/CD29, playing a fundamental role when both IgE and antigen levels are low in local microenvironments [420], whereas FcεRI aggregation to cells adherent to FN specifically amplifies the phosphorylation of such proteins [195]. Signal transduction obtained in such a way by FcεRI looks like that of TcR/CD3 in T cells. We mention that antigen binding to FcεRI-IgE brings about the degranulation of metachromatic cells, thus initiating reactions of immediate hypersensitivity; however, mast cells can undergo forms of non-IgE-mediated signals from their environment (Chap. 10).

Table 1.27. Tissue prevalence (%) of T and TC mast cells

	T mast cells	TC mast cells
Skin	12	88
Conjunctiva and nasal epithelium	100	0
Small intestine mucosa	98	2
Small intestine submucosa	13	87
Bronchial epithelium	99	1
Bronchial subepithelium	77	23
Alveoli	90	10

Modified from [227].

Table 1.27 [227] summarizes several differences between T and TC mast cells on the basis of tissue prevalence. In humans, somewhat different phenotypes have been recognized; in addition, mast cells residing in the airways contain mostly tryptase, and those of other locations both proteases [227]. Their predilection to occupy tissues that interface the external environment makes them well represented in inflamed tissues under T-cell functional control through IL_3 (proliferation) and IL_4 (maturation) [593]. Under the influence of IL_3, IL_4 and NGF, T mast cells may assume characteristics of the TC phenotype; therefore, one may consider the distinction into two types nearly obsolete, with both phenotypes potentially coexisting in the same site, although in different proportions. Furthermore, it has been suggested that mast cells in unrelated locations respond to allergens with the same pattern of mediators [227]. As a consequence, mast cells are strategically positioned to

detect rapidly inhaled or ingested allergens, expressing a chronic array of proinflammatory mediators, without neglecting PAF and chemotactic factors such as LTB_4, NCF, and ECF (eosinophil chemotactic factor).

Appendix 1.2 summarizes receptors and surface molecules expressed from eosinophils, basophils and mast cells [593].

Platelets too have a virtual role in the pathogenesis of allergic disease [405], in addition to a typical role in coagulation processes. Classically thought to originate in the bone marrow from cytoplasm of megakaryocytes, it has recently been suggested that actually megakaryocytes travel to lung vessels, where they are physically fragmented into small clumps of granules, each of which is a platelet. These are the smallest blood cells, anucleated (2 µm in diameter), with a half-life of about 10 days. Platelets have been shown to express HLA class I molecules on their surface, IgG receptors (CD32), IgE (CD23), vitronectin (CD51), CD9, CD17, CD31 (GPIIa), CD36 (GPIIIb), CD41a (GPIIb/IIIa), CD42a–d (platelet antigens), CD49f, CD60, CD61 and CD63 (Table 1.2). Human platelet antigens (HPA) are at least 15: PHA 1–15 [298]. When activated, together with aggregation they undergo morphological modifications, secrete PAF, cytotoxic cationic proteins and free radicals. Moreover, platelets release 5-HT from dense bodies, which like histamine produce contraction of smooth muscles, increase vascular permeability and produce proteolytic enzymes and cationic substances from α granules with equal effects on blood vessels, in addition to chemotactic factors including PF4 (platelet factor 4), PDGF, 12-HETE, NO (nitric oxide), TGF-α and -β, albumin, β-thromboglobulin, eicosanoids (PGG_2, PGH_2, TXA_2, and CD62P), allowing binding to fibrinogen, FN and CD51. Consequently, platelets, although confined in the vascular compartment, also acting by diapedesis, can release mediators active in inflammatory extravascular foci. However, their specific role in inflammatory reactions is not well defined as it is for other cells: if activated, they also have chemotactic and phagocytic properties and contribute to immune reactions, releasing growth and coagulation factors, vasoactive amines and lipids as well as acid and neutral hydrolases. Following platelet aggregation, abnormal agglomerates develop and recruiting and entrapping leukocytes may contribute to the start of an endovascular occlusion. The human PMN adhesion to vascular endothelial cells was increased by the platelet presence. This effect was endothelial cell dependent and involved platelet activation. Thus platelet participation in cell recruitment occurs at the circulation level and might involve leukocyte priming for subsequent adhesion and transmigration into tissues [430]. Platelets produce enzymes cleaving C5, thus resulting in C5a, with a marked chemotactic activity for neutrophils: this is most likely the establishment of an active form of cooperation for the production of new mediators. C5a can prime mast cell degradation, while C5b-9 participate in the non-lytic platelet activation [680]. Platelets interact with the immune system via FcεRII (in 10%–30% of healthy and in 50%–60% of atopic subjects), FcγRII (CD32), the VLA-2, -5 and -6 integrins, ensuring adhesion mechanisms among immunocompetent cells and CD62P [65]. Activation of FcεRII elicits PAF production and an IgE-dependent platelet activation, which is not expressed as the classic aggregation, but by secretion of O_2 toxic radicals, triggered by SP, CRP (C-reactive protein), IFN-γ and TNF. Also prominent is the portfolio of chemokines that attract these cells to a site of inflammation, such as α-chemokines (CXCL), β-family (CCL), including eotaxin, Gro-α, RANTES, TARC, macrophage-derived chemokine (MDC), and SC-derived factor 1 (SCDF1), and chemokine receptors, such as CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22, activate platelets to give Ca(++) signals, aggregation, and release of granule content [87]. The inappropriate platelet activation materializes with eosinophil recruitment on the sites of immune inflammation, TGF-β secretion with mitogen activity towards bronchial smooth muscles, PF4 released during asthmatic attacks and PDGF triggering fibroblast proliferation. So platelets can be involved in the onset and perpetuation of structural alterations underlying subepithelial fibrosis, which contribute to emphasize BHR. Two series of experiments account for what we discussed earlier: platelets from patients with asthma from ASA (acetylsalicylic acid) or NSAIDs (nonsteroidal anti-inflammatory drugs) incubated with SP, PCR, IFN-γ and TNF start the release of O_2 radicals. Thrombocytopenia is congenital in Wiskott-Aldrich syndrome (Chap. 22) [405].

Additional Cells

The principal *APCs* expressing class II determinants [541] are DCs (in the skin LCs), macrophages, Kupffer cells, endothelial cell, enterocytes, monocytes with FcεRI [384], and B cells. All these cells, provided with HLA class II molecules, constitutive or inducible by bacteria and macrophage IFN-γ, collaborate with T lymphocytes (as well as among themselves), in different procedures according to the microenvironment and antigen type. DCs present antigens and virus in extralymphoid tissues and B cell toxins, virus, and bacteria in the spleen, while macrophages focus their attention on intracellular pathogens [49]. A differential type I IFN gene transcription was induced in monocyte-derived DCs and PDCs stimulated by specific TLR agonists. TLR-9 stimulation by CpG DNA induced the expression of all IFN-α, -β, -ω and -λ subtypes in PDCs [88]. Activated TcR-γδ can secrete ILs efficient in the activation of several cell families, also inducing a functional maturation of professional APCs with the accessory aid of several molecules, among which is CD154, hence facilitating the recruitment of antigen-specific TcR-αβ [110].

Dendritic Cells

Myeloid DCs are crucial APCs for primary T-cell responses: tissue-resident immature DCs are excellent at internalizing and processing antigen, but they exhibit a low ability to stimulate naive T cells [88]. DCs encompass a heterogeneous group of cells present either in lymphoid tissues such as thymic DCs and FDCs or in parenchymal organs such as IDCs, circulating and/or cutaneous LCs. Moreover, several chemokine receptors in $CD4^+$ lymphocytes are primed by DCs (see Chemokines) [88]. LCs arise from bone marrow precursors that colonize peripheral tissues through the blood or lymph, according to recent data, a line common to macrophages [424], which modulated by GM-CSF and TNF-α [221, 546] leads to two precursors identified by CD1a and CD14, both maturating into LCs and, respectively, DCs or macrophages depending on IL influence [74]. Activation causes DCs to up-regulate the CSMs expression (CD80 and CD86) on their surface. CSMs provide the signals necessary for lymphocyte activation in addition to those provided through the antigen receptor [115]. Circulating conventinal DCs coexpress and IFN-γ most potently favors activating CD32a, whereas soluble anti-inflammatory concentrations of monomeric IgG express inhibitory CD32b, both isoforms of IgG FcγR II (CD32). Ligating complexed human IgG to CD32a *matures and activates DCs* in proportion to the frequency of CD32a expression. However, coligation of CD32b significantly abrogates all of these immunogenic functions. These findings have important implications for understanding the pathophysiology of CIC disease and for optimizing the efficacy of therapeutic mAbs [48]. DCs produce a wealth of ILs: immature DCs exhibited higher amounts of IL_1, TNF, TGF-1, and MIF mRNA/protein than mature DCs. After differentiation, DCs up-regulated the levels of IL_6 and IL_{15} mRNA/protein and synthesized *de novo* mRNA/protein for IL_{12} p_{30} and p_{40} and IL_{18}. CD1a precursors generate cells expressing Birbeck granules and E cadherin characteristic of LCs, while CD14 progenitors mature into CD2, CD9, CD14, CD68 and factor XIIIa, specific of dermal DCs [74]. DCs, described as localized in the suprabasal layer of epidermis, represent in the adult only 2%–8% of epidermal cells, sharing with dendritic lymph nodes several phenotypic and functional features [454]. Although immature bone-marrow-derived DCs did not stimulate naive allogenic T cells, mature DCs elicited a mixed population of Th1 (mainly) and Th2 cells. The DC subset may contribute significant polarizing influence on Th differentiation and the CD subset 1 may exert Th1 polarization by IL_{12} production and STAT4 activation [366]. Growth and differentiation of LCs and their migration delineate a crucial step in the immune surveillance of foreign antigens invading the host [221, 546]. LCs via afferent lymphatics reach the paracortical areas of regional lymph nodes as FDC with APC function; thereby they are the first cells to trap antigens, which are

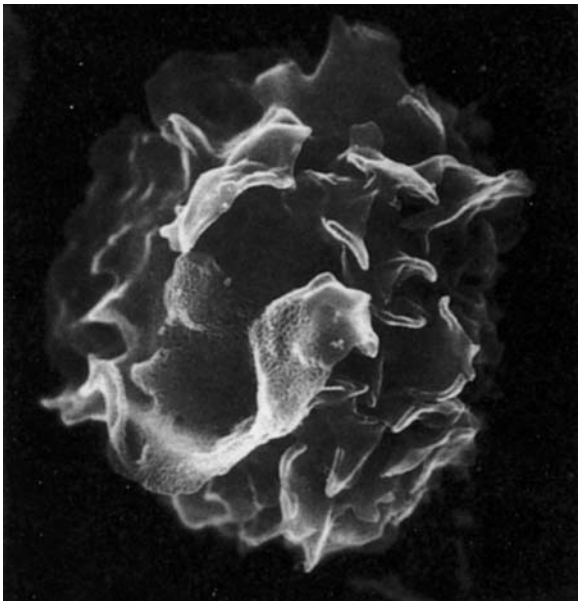


Fig. 1.38. Langerhans' cells appearing as veiled cells

then internalized and processed at the level of target cells [541], then presented in draining lymph nodes to upcoming T cells stimulated by the same DCs [546]. In comparison, DCs are strategically located below the M cells of PPs, thus sampling antigens in vivo and migrating to T-cell areas of the same PP or mesenteric lymph nodes, where they present antigen to naive T lymphocytes [253]. LCs migrate quite rapidly after having taken up a peptide, present a rounded phenotype with long cytoplasmic protrusions rhythmically moved, hence assuming the aspect of *veiled cells* [541] (Fig. 1.38). FDCs returned in paracortical areas as APCs, having a poor expression of HLA class II molecules, present the same peptide processed over several days [541] or months [37], also contributing to long-term maintenance of memory B cells [37]. FDC maturation due to an increased presence of CD80 or CD86, and enhanced by CD40, is stimulated until FDCs encounter T cells [424]. To understand the role of DCs in antigen presentation and processing, we mention that DCs select potential antigens taking up microbial glycoconjugates by means of specialized receptors. An in vitro model has demonstrated that PBMCs, in GM-CSF and IL_4-dependent cultures, develop into DCs that are extremely efficient as APCs, a property lost when treated with TNF-α [487]. LCs are thought to play a key role in enhancing immunogenicity since their first identification, because they *express FcεRI binding to IgE,* and pick up antigens in vivo even before presentation, and like they other APCs process antigens, degrading them into peptides that become approximately six to eight amino acids in size with a low MW [384]. The evidence that *LCs also possess FcεRII* [40] implies a major role in view of their significant activity shown in atopic diseases [546]. Table 1.28 [208, 424] summarizes their markers, denot-

Table 1.28. Surface markers of LC

Markers	Skin
CD1a	+/++
CD2	
CD4	
CD8	
CD11a	
CD11c	
CD14	–
CD15s	
CD18	
CD23	
CD29	
CD32	
CD34	
CD40	+++
CD45	
CD49f	
CD50	
CD54	
CD59	
CD80	+
CD86	++
CGRP	
FcεRI	
HLA-DP	++
HLA-DQ	++
HLA-DR	+++

See CDs in Table 1.2.
Data from [208], the skin markers from [424].
CGRP calcitonin gene-related peptide.

ing the skin LCs [424]. The LC ability to stimulate primary responses is up-regulated in the epidermis where they migrate in association with CD15s and CD62E and express *in loco* the E cadherin to bind to keratinocytes [566]. Cells very sensitive to UV action lose the capacity of presenting antigens to T cells after irradiation, an effect modulated by IL_{10} secreted by keratinocytes [461].

Afferent Phase of Immune Response

Antigen Processing and Presentation

Antigen processing and presentation are among the key events between a foreign protein penetration into the host via mucosal, skin or blood routes and its recognition by immunocompetent cells. The immune response results from a complex network of subpopulations of different cells interacting via soluble proteins, the ILs, most of which are involved in either inactivating or activating the expression of immune effector functions (cytokine cascade) [34]. This picture is integrated by a variety of actively trafficking cells such as lymphocytes, APCs, adhesion molecules, etc. [28]. T lymphocytes cooperate in the induction of immune responses influencing the up-regulation of B cell progenitors of IgE-secreting cells (Fig. 1.39). Antigen recognition and activation are neither consequent processes nor are they homologous: antigens can be recognized by the immune system without inducing mandatory immune responses, as in nonatopic subjects who instead yield Th1-cell clones [601, 606].

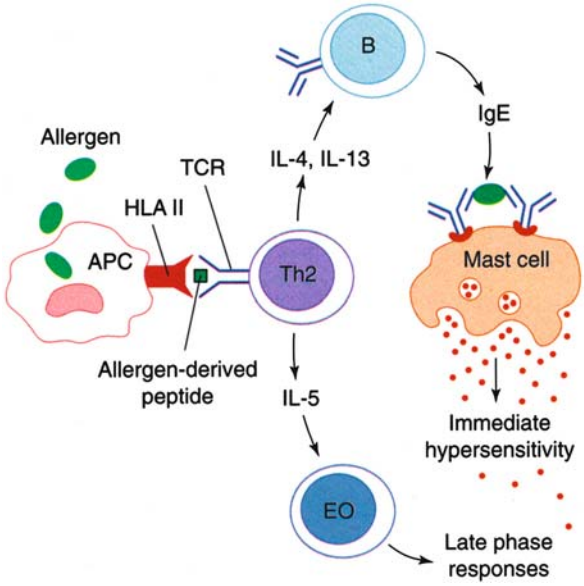


Fig. 1.39. Role of CD4+ cells in the immune response. Upon entry in the body, allergens are taken up and processed by APC, after presentation, HLA class Ii restriction and TcR usage of allergen-specific Th2-like cells, B cell progenitors of IgE-secreting cells are up-regulated. If IgE are produced, an immediate Th1-type response may ensue, but Th2-like cells may activate eosinophils, thus resulting in a late-phase response. *APC* antigen presenting cell, *B* B cell, *EO* eosinophil, *Th2* Th2 T cell, *TcR* T-cell receptor

CD4 cells do not recognize intact antigens, but interact only with previously processed native, exogenous antigens associated with class II HLA molecules, unlike CD8 and B cells recognizing endogenous peptides associated with class I molecules [12, 372]. γδ T cells recognize antigens differently, especially small molecules, but the functional consequences remain to be elucidated [109]. CD1 comprising five different proteins (Table 1.2) present lipids or glycolipids of microbial origin to T lymphocytes. However, their role in vivo is not yet clear [115]. HLA-E, HLA-F, HLA-G, HLA class I-like, representing differentiation antigens, have a limited tissue

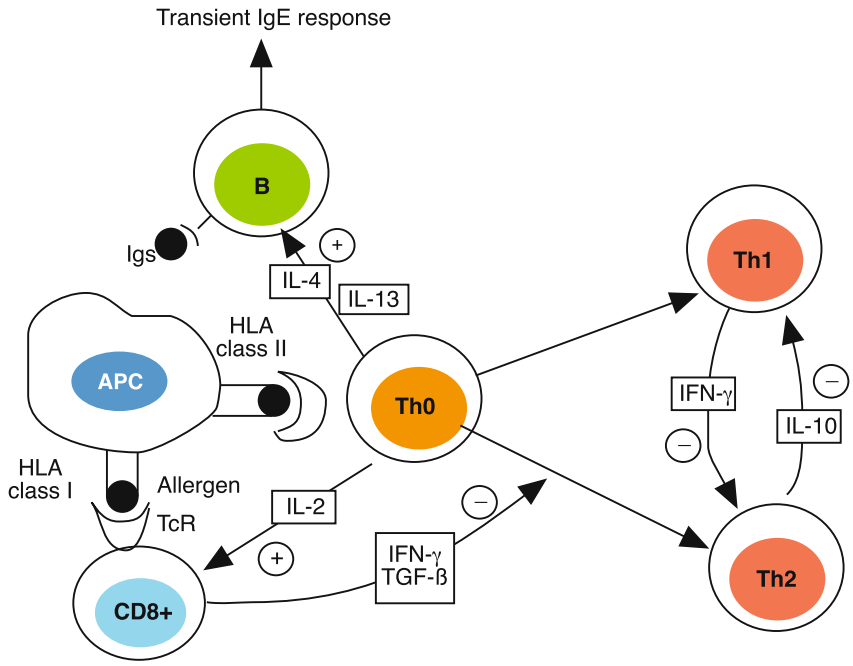


Fig. 1.40. Selection of Th1-mediated protective immunity toward allergens during primary immune response via class I $CD8^+$ HLA-restricted immunodeviation. Cell shading reflects the presence of peptides bound to class I or II HLA presented to $CD8^+$ or Th0 T cells, respectively. *Igs* surface immunoglobulins. (Modified from [212])

distribution and polymorphism [47]. HLA-G has been recently characterized as TAP-associated, which directs its expression and binding to nonameric peptides [293]; HLA-G expression on target cells protects from NK-mediated lysis interacting with NKR [419], of special significance pertaining to maternofetal tolerance.

Classic studies have shown that immune responses consist in the production of antibodies, up-regulated stimulating CD4 lymphocyte activation, but may be down-regulated when CD8 cells predominate. Non-self substances that have gained access to sites patrolled by the immune system enter automatically in contact with it, thus activating a complex mechanism of cellular activity aimed at its destruction and at restoration of preexistent homeostasis. Therefore, immunogen peptides encountering lymphocytes of an atopic individual for the first time trigger a multiple pathway of cell interactions ensuring that B lymphocytes differentiate into antibody-secreting cells [601]. As soon as an invader is identified by two different immune effector mechanisms, antibodies (humoral effector limb) and TcR (cell-mediated effector limb), the foreign antigen is captured by APCs, the peptide-HLA complex is recognized by TcR, then internalized and processed in fragments subsequently exposed on the cell surface in association with class II HLA molecules. Thus the interaction between antigen-specific T and B cells (cognate interaction) and consequent IL release result in the activation of lymphocytes [290]. A theory of two signals is suggested also for the T cells: accordingly, Th0 T cells receive the first signal from the TcR triggered by pathogen-derived antigenic peptides bound to HLA class II molecules on APCs, which indicates the peptide molecular identity. Signal 2 delivered from costimulatory molecules (CSM) comprises contact-dependent and humoral signals and transmits the information about the DC-activating property of invading pathogens, reflecting its pathogenic potential. The combination of signal 1 and signal 2 results in Ag-specific activation of naive Th cells and their development into effector/memory cells [290]. A Th0 signal might provide a further refining cadence, whereby the IL milieu produced by DCs provides naive T helper cells with a Th1- or Th2-polarizing signal at the time of priming [212]. Activated T cells differentiate into Th2 T-cell clones, which secrete low amounts of IL_4, thought to play a crucial role in IgE isotype switching. The resulting IgE low levels can be captured by high-affinity receptors on the mast cell surface [290]. Thereby the first exposure to an immunogen leads to the production of antigen-specific IgE and priming of the immune system (*primary immune response*), an event preventing a second encounter via the $CD8^+$ class I HLA-restricted immune deviation (Fig. 1.40) [212]. In immunologically healthy neonates and infants, such initial responses, an integral part of normal immune responses, are self-limited and gradually resolve, after weeks or months, despite continuous allergen exposures, due to the development of tolerance [250]. In healthy, uncommitted subjects, membrane-bound IgG forms immune complexes with allergens. IgG is tethered to the membrane by binding the Fc fragment of FcεRIIb. When an allergen binds both IgE and IgG, the activating FcεRI is brought together with the inhibitory FcεRIIb, thereby silencing the FcεRI-mediated activation pathway [259]. According to this model, following subsequent exposures, CD4 clones from nonatopic individuals have a Th1 profile, whereas in atopic patients they can lead to an immediate hypersensitivity reaction (*secondary immune response*). Antigen persistence or reexposure leads to ongoing antibody production, which is outstanding, rapid, more specific

and enduring with different functional features, dominated by Th2 T cells and IgE, an expression of immune memory [212]. When receptor-bound IgE is cross-linked, release of potent biochemical mediators and further IL_4 production induce uncommitted T cells recruited at a site of allergen re-entry to differentiate into a Th2 phenotype, hence amplifying immune reactions [250]. Th2 T cells, IL_4-derived IgE production and IL_5-triggered serum and tissue eosinophilia result in *a vigorous IgE response* and a severe clinical response [212]. T cells, eosinophils, metachromatic cells provided with IL_4 and CD40L, ILs and adhesion molecules and their interactions are the major players around which atopic diseases evolve [177].

Antigen Capture and Processing

Antigen capture by APCs can occur via three distinct mechanisms [488]. The first is macropinocytosis [555], a type of fluid phase endocytosis, uptake of large vesicles (1–3 μm) mediated by membrane ruffling driven by actin cytoskeleton [488]. In DCs this constitutive mechanism calls for a continuous internalization of large volumes of fluid (1,000–1,500 μm^3, a volume close to one cell/h), whereas macrophages and epithelial cells need to be stimulated by growth factors [555]. The second mechanism is mediated via the mannose receptor (MR), a 175-kD C-type lectin, which on human cultured DCs modulates endocytosis of $>10^5$ molecules of mannosylated proteins per cell/h [488]. Furthermore, a membrane protein of murine DCs, structurally homologous to macrophage MR, internalizes peptides, delivering them to a multivesicular endosomal compartment provided with HLA class II molecules, of which DCs synthesize elevated levels: in this model, the signaling process initiated by T cells is up to 100-fold more efficient [488]. The third mechanism is mediated by FcγRII, also expressed by DCs [290]. B cell clones bear high-affinity mIgM and mIgD on their membranes ready for antigen epitopes and antibodies and their BcRs fulfill two functions: signal release leading to B cell activation and antigen uptake and delivery to processing compartments [290]. In addition, nonprofessional receptors are surface molecules able to occasionally capture antigens and effectively present viral proteins bound to surface receptors [396].

Presentation and Recognition

Antigen recognition from T cells with a TcR complementary to peptide-HLA association triggers the first phase of *T-cell activation and consequently the immune response* [28]. For this purpose, both the epitope and agretope that bind to an HLA molecule are critical (Fig. 1.15). The peptide–HLA complex exposed to the CD3–TcR complex of antigen-specific T cells is expressed on the cell membrane in the *fitting pocket* of HLA class II molecules (a *genetic restriction* mechanism) (Fig. 1.22). Such complexes are as firm and as high as the peptide affinity for the hypervariable part of HLA molecules; such adhesion is mediated by CD2, CD11a/CD18, CD54, CD58, and other integrins and selectins [534]. The cells expose peptide fragments assembled with HLA class I or II molecules, so that they are examined by circulating T cells, which, although relatively few in view of the great number of potential antigens, and of their great diversity, are conditioned to recognize those they encounter [34]. The affinity for peptides depends on the amino acid sequence of hypervariable regions and consequently on the HLA molecules that everybody inherits. TcR and HLA interactions are characterized by a high-sensitivity and low-affinity paradox, which is only a small number of TcRs that interact with APCs [595]. On the contrary, APCs are fit for almost any foreign invader encountered by the immune system, thus raising a very intriguing question of how so few receptors can transduce an activation signal [225]. It remains to be elucidated how as few as 80–100 HLA–foreign peptide complexes on the cell surface (which may express as many as 10^5 HLA molecules) are sufficient to trigger a T-cell response [47]: the answer lies in the capacity of a single peptide–HLA complex to serially engage and trigger up to ≈ 200 TcR, amplifying the signal according to T-cell biological responses [595]. C3b plays a critical role in at least two phases of recognition, as shown by the T clone response to presentation of C3b–Ig complexes. The uptake of such complexes is helped by the interactions with complement receptors virtually present on all APCs; furthermore, C3 covalent binding to specific antigen peptides can define, during the processing, which part of the molecule is selected as epitope presented to T cells [359].

Recognition of immunogenic proteins in their natural configuration is not sufficient to stimulate B lymphocytes to differentiate into plasma cell IgE, since switching steps requires T lymphocyte cooperation, which materializes via IL_2 production; so B cells and APC interaction within TcR presentation of peptide–HLA complexes involve antigen specificity and consequently B lymphocyte capacity to bind to and present peptides, even if their extracellular number is reduced. At the level of peripheral lymphoid tissues, where antigen concentration is greater, other APCs are involved in antigen processing and presentation background. The first step of B-cell activation requires signals generated upon recognition of antigen by the BcR as well as additional signals provided by cognate interaction with T cells, including the CD40–CD154 interaction [201]. Following peptide–HLA complex recognition on the B-cell surface by TcR, T cells deliver appropriate activating signals to B cells (*cognate help*); hence T cell–B cell cooperation begins, class II-restricted and antigen-specific, with formation of tightly associated conjugates [457]. Such conjugates result from peptide–HLA complex presentation from B cells; fur-

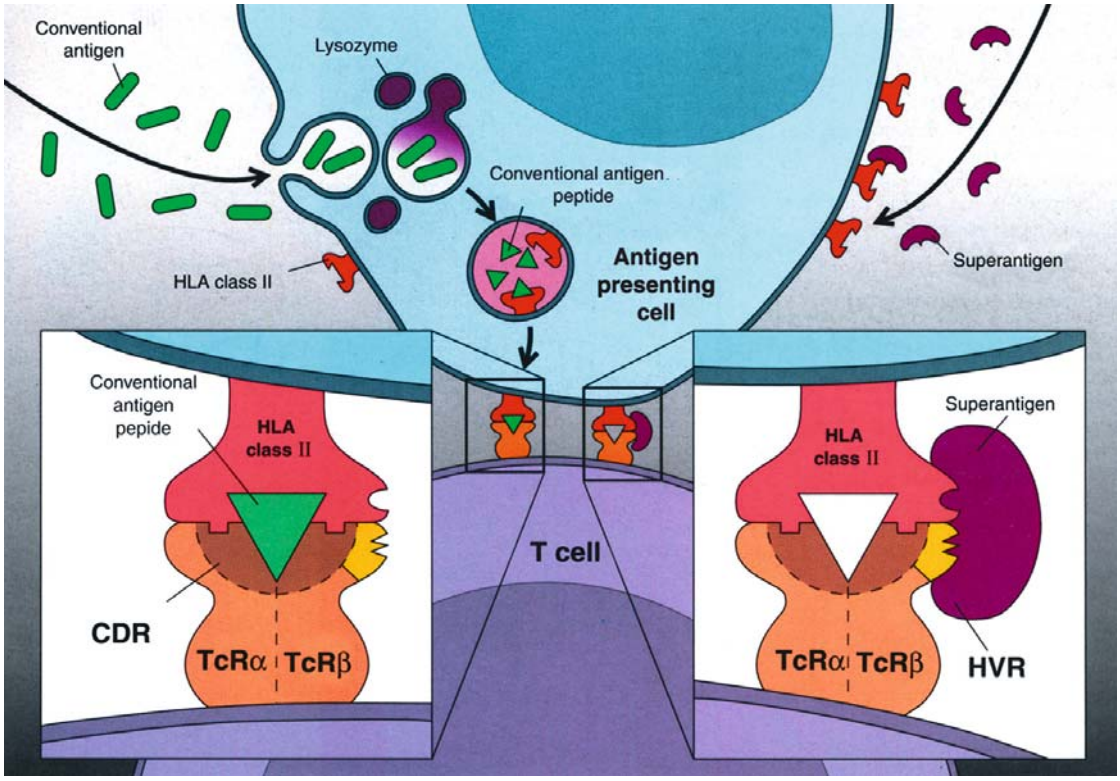


Fig. 1.41. Superantigen abbreviated presentation. Superantigens sidestep the usual pathways of antigen presentation (*left*), but are presented intact on the outside of the HLA peptide-binding groove (*right*) and activate T cells. Superantigen is recognized by a side face of TcR-Vβ, which encompasses a HVR that has been designated HVβ-4. *CDR* complementarity determining regions, *HVR* hypervariable region

Table 1.29. Bacterial superantigens

Superantigen	Toxin (name and abbreviation)	MW (kD)
Staphylococcus aureus	Enterotoxin A=SEA	27.8
	Enterotoxin B=SEB	28.3
	Enterotoxin C1=SEC1	26
	Enterotoxin C2=SEC2	26
	Enterotoxin C3=SEC3	28.9
	Enterotoxin D=SED	27.3
	Enterotoxin E=SEE	29.6
	Toxic shock syndrome toxin-1=TSST-1	22
	Exfoliating toxin A=ExFTA	26.9
	Exfoliating toxin B=ExFTB	27.3
Streptococcus pyogenes	Erythrogenic toxin A=SPEA	29.2
	Erythrogenic toxin B=SPEB	27
	Erythrogenic toxin C=SPEC	24.3
	Protein M	22
Mycoplasma arthritidis	Mycoplasma arthritidis mitogen=MAM	26
Pseudomonas aeruginosa	Exotoxin A	66
Clostridium perfringens	Clostridium perfringens toxin=CPET	34

Modified from [474].

Table 1.30. Prominent characteristics of superantigens (*SAs*) interacting with B lymphocytes (B superantigens, *BSA*)

SAs activate a large percentage of B lymphocytes, about 40% of human polyclonal IgM binds to SPA
SAs interact with the major part of components of V_H– gene family: SPA binds to a high rate of V_H3+IgM
SAs trigger B lymphocytes in vitro; SPA delivers activation signals to IgM V_H3+, thus triggering Ig differentiation; HIV-1 gp120 selectively induces Ig secretion by V_H3+ IgM
SAs also induce in vivo changes in B lymphocytes; it has been suggested that during HIV-1 infection the B V_H3+ cells are initially up-regulated, and then highly down-regulated
SAs interact with regions of the V_H gene domain; for SPA binding a motif between residues 75 and 84 of FR3 is involved, outside the conventional paratope
SA binding activity experiences age-related alterations

Modified from [692].
FR framework region, *SPA Staphylococcus aureus* protein A.

ther enhancement of cognate interactions depends on CD54 binding to CD11a/CD18 and CD4 to monomorphic domains of class II proteins [391]. Engagement of both the BcR and CD40 results in synergistic activation of B cells [201]. CD4 T cells bind to antigen-specific B lymphocytes; the associative recognition induces B-cell activation, clonal expansion, and differentiation, while cell division goes on as long as T cells stimulate it. Mature plasma cells are generated and secrete specific receptors, the mIgs, which bind to antigens present in the bloodstream [457]. Previous studies suggested that binding to TRAF2 and/or TRAF3 but not TRAF6 is essential for CD40 isotype switching and activation in B cells [232]. More recently a model was presented in which Btk contributes to the enhancement of the CD40 response by TRAF2 in a BCR-activated protein kinase D (PKD)-dependent manner [201].

Superantigens (SA) are antigens able to select subsets of T cells during thymic ontogenesis, playing an important role in T cell development: an example is given by bacterial toxins, some of which can be mitogen for some T cell subsets. Such SAs bypass key antigen processing and recognition steps in T cell activation, by binding more or less exclusively to lateral exposed surfaces of HLA class II molecules and TcR determinants of the Vβ region (HVβ-4), that is, not to a normal paratope. As a result of this sidestep they are able to activate greater proportions of lymphocytes, not $1/10^4$ or $1/10^5$, as with usual antigens, but whole clones up to 30% of T lymphocytes, thus amplifying their activity, and functioning as a bridge between T cells–HLA and accessory cells [275]. Figure 1.41 shows a polyclonal activation of T cells, which recognize both conventional peptides with Vα and Vβ regions, and SAs essentially with an area of the Vβ region [275]. Table 1.29 [474] details the different types of microbial SAs. NKB1 inhibitor receptor, expressed by many T cell clones and engaged by their HLA class I ligands on potential target cells, protects against cytotoxicity induced by bacterial SAs [427]. An additional means of interaction between T and B cells can occur, whereby molecules termed B-SAs (BSA) can bind directly to human BcR of a given variable V gene family [647]. This mechanism requires contributions from the FR loop away from CDRs; hence this loop is less favorably placed for antigen contact and has a greater potential for unconventional binding (Table 1.30) [605].

Lymphocyte Activation

Like many other cells of the body, T and B cells exist for most of their life span in a quiescent state or a G_0 state. To proliferate, the cells must re-enter the G_1 phase, where several proteins undergo a substantial process of biosynthesis, so these cells grow in size and prepare for DNA synthesis. In the S stage, DNA synthesis and replication of each chromosome bring about two matching sister chromatids. The subsequent G_2 and M (mitotic) phases involve the two sister separations, generation of two new nuclei, and final division of the cytoplasm to produce two daughter cells: growth factors and different environmental stimuli are required for cell cycle progression, depending on the cell type [337]. There are several *functional differences* between T and B cells and recent work has focused on their mutual interactions: T lymphocytes have a variety of signals allowing them to leave the circulation and enter tissues to reach the site of antigen exposure, both because they constitute the prevalent portion of peripheral lymphocytes and they have the central feature to recirculate. Instead, B cells encounter preferably native macromolecules *in situ,* in specialized organs and tissues; however, in an antigen-independent phase of B-cell development, it is likely that B cells do not require interactions with antigens, which will be ultimately recognized by soluble antibodies subsequently synthesized [481]. Both T and B cells need to be stimulated before acquiring the capacity of responding to specific antigens, T cells by their clonally restricted TcR and B cells by Igs, or in T-independent polyclonal systems or molecules with mitogenic properties, both experimentally and physiologically [326]. We also note some biochemical similarities in B and T cell activation: as an antigen binds to an APC, a series of defined events occur over a period of several hours. Within a few seconds, the phosphorylation of cell proteins takes place, mostly associated with CD3ε and ζ and CD79a and b receptors and membrane phospholipid cleavage. A cascade of protein activation in regulated sequence and the rise of Ca^{++} levels occur. As a result of these early activation events, TFs such as NFAT 1, 2 and NF-κB are activated to enter the B cell nucleus and promote transcription of nontranscribed specific genes. In

T cells, the most important genes include ILs and IL receptors, while B cells start to transcribe Ig genes. Within about 48 h, DNA is synthesized and cells undergo division [36, 47].

Role of T Lymphocytes

CD3, the nonpolymorphic part of the TcR complex, is a signal transducer in T cells whose activation with IL_1 contribution elicits both proliferation and activation of cell subsets. CD4 stimulation by HLA molecules and IL_1 drives IL_2 and IL_6, an intervention defined as a *synergistic promoter* [34], and additional metabolic processes lead to a final activation and proliferation of CD4 cells, of cytotoxic CD8 and, as a result, of B lymphocytes, which become antibody-secreting cells [206]. For definitive proliferation, CD3 must be escorted by accessory stimuli, one expressed by BcR, the membrane protein CD80, recognized by CD28/CD152 receptor = CTLA-4 (CTL-associated antigen-4) [242]: we stress that when appropriate signals are absent, clonal anergy ensues (Fig. 1.22 a, b).

During the processes of presentation and activation, the trimolecular complex made up of TcR-αβ/CD4 and peptide–HLA transmits signals to cells, as discussed earlier. Due to TcR binding to extracellular V regions, modulated by CD3ζ, also following second-messenger generation, TcR transduces signals initiating biochemical and conformational changes. Intracellular signals generated by TcR and transmitted to T cells appear, therefore, to be critical for proper T cell maturation and activation [276]. Transduction of activating signals by CD3-CD28 costimulation initiates multiple signaling cascades that lead to the activation of several TFs, including the activation of NF-κB family members [276]. TcR-CD3 stimulation alone is not sufficient to optimally activate NF-κB because its requires *Bc110, a CARD* associated with CARMA1 (CARD11) [504], a member of the *CARD family* also including CARMA2 (CARD14) and CARMA3 (CARD10) [621]. The CARD family also encompasses CARD/NOD a member of the *ced-4 superfamily* also including APAF-1, mammalian NOD-LRR (leucine rich repeat) proteins and CARD15/NOD2, which in turn act in LPS recognition and activate NF-κB [79] that is depressed in patients with Crohn's disease [621]. Prominent in this context is *G protein* participation with a manifold role in cellular signal transduction coupling an array of receptors at the cell surface with a variety of intracellular effectors exposed to the plasma membrane's inner surface that couple a large family of receptors to effectors, such as adenylcyclase, PLC, and ion channels [292]. The large heterotrimer G proteins have 21 Gα subunits, which are related to small G proteins, plus five Gβ and six Gγ that exist as a single complex (Gβγ). Gαs are stimulated by GAPs (GTPase-activating proteins) and Gβγ by RGS (regulators of G protein signaling) [231]. In the resting state, guanosine diphosphate (GDP) is tightly bound to Gα. When a membrane receptor is activated by binding of a first messenger, this causes GDP to dissociate from Gα and be rapidly replaced by guanosine triphosphate (GTP). GPT binding leads the Gα subunit to dissociate from Gβγ, each of them can independently transmit signals, hence activating effector cells (active state). In a subsequent phase, hydrolysis of GTP to GDP inactivates Gα, allowing it to reassociate with Gβγ (inactive state) and reset stable heterotrimers. Gβγ subunit binding to several components of the Gα subfamily could open up a new *communication pathway among second messengers* [292]. Considering this activity, G proteins oscillate between GPT- and/or GDP-bound states, and regulate diverse processes, including signal transduction [24]. G proteins also belong to a superfamily comprising a number of receptors; however, the amount of G proteins bound by a given receptor is reduced, practically restricting to one G protein signaling to one receptor. One of the best characterized among signal transduction systems is an increased formation and accumulation of intracellular cAMP (cyclic adenosine monophosphate) as a result of β-adrenergic receptor stimulation; further receptor/ligand interactions enhance Gα3 activity due to Gα3/GTP dissociation from Gβγ. Correspondingly, the activation of the enzyme chain of membrane adenylyl cyclase catalyzes cAMP synthesis in Mg ion presence. Returning G protein to its initial conformation the enzyme is inactivated, while cAMP is converted to noncyclic inactive 5-AMP by cAMP-PDE (phosphodiesterase) constitutive activity; otherwise other receptors activate another G protein, Gi, which binds to adenylyl cyclase to block enzyme activity [326].

Direct evidence suggests that, depending on the type of related cells, cAMP, a second messenger present only inside the cells, plays a role in enzyme phosphorylation, Ca++ levels increase, also affecting both gene expression and further endocellular processes [292]. In addition, the CD3ζ cytoplasmic domain interacts with two families of tyrosine kinases such as *PTK of the src and syk* (intracytoplasmic) *families* (Tables 1.31, 1.32) [157, 214, 222, 240]. Some PTK src are associated via the SH2 domain with ARAM or ITAM sequences of intracellular regions of γ δ ε chains of CD3 as well as α and β (CD79a and CD79b) of BcR and ζ or γ of CD16. To fulfill G protein effects on CD4/CD8 T cells [292], within a few seconds GTP is hydrolyzed to GDP; a cytoplasmic tyrosine kinase, ZAP70 (ζ-associated protein 70), belonging to the syk family, becomes active upon attachment to the TcR–CD3 complex [213] and in turn activates PLCγ1 [462]. The important result of PKC is PLCγ1 activation, which then acts to hydrolyze PIP2 into IP3 (inositoltrisphosphate) and DAG [462]. IP3 and DAG serve as second messengers of the T-cell activation process: IP3 with a short half-life rapidly increases cytoplasmic Ca++ levels; however, in the absence of additional signals there is no activation [337]. DAG has been shown to activate PKC, a process leading to its translocation to the

Table 1.31. Kinases and receptors (*R*)

Receptors associated with kinase domains	
Tyrosine kinase	CSF-R, EGF-R, M-CSFR, PDGF-R, SCF-R, insulin-R
Serine/ threonine kinase	Activin-R eg:TGF-β-R
Receptors associated with cytoplasmic kinases	
Src-family kinases (blk, fgr, fyn, hck, lck, lyn, src)	TcR (fyn, lck); BcR (blk, fyn, lck, lyn); FcR (fgr, lyn); CD4 (lck); CD8 (lck); CD19 (lyn)
Syk-family kinases (syk, ZAP70)	TcR (ZAP70), BcR (syk); FcR (syk)
PI 3 kinase	CD28
Tec-family kinases (btk, itk, tec)	CD28 (itk); BcR, pre-BcR (btk?)
JAK-family kinases (JAK 1, 2, 3, tyk 1, 2)	Receptors of all IL (except IL_1, IL_8, IL_{11}, IL_{14}); EGF, G-CSF, GM-CSF, all IFN; M-CSF; PDGF, growth hormones, erythropoietin

Data from [157, 214, 222, 240].

Table 1.32. Cytokines, receptors and signaling

Receptors	Activated JAK	Activated STAT
IL subfamily sharing the γ chain		
IL_2R	JAK1, JAK3	STAT3, STAT5
IL_4R	JAK1, JAK3	STAT6
IL_7R	JAK1, JAK3	STAT5
IL_9R	JAK1, JAK3	?
$IL_{13}R$	JAK1, JAK3	STAT6
$IL_{15}R$	JAK1, JAK3	STAT5
$IL_{21}R$	JAK1, JAK3	?
Subfamily of GM-CSF receptor sharing the gp140β chain		
IL_2	JAK1, JAK2	STAT5, STAT6
IL_5	JAK1, JAK2	STAT5
GM-CSF	JAK1, JAK2	STAT5
Subfamily of IL_6 sharing gp130		
IL_6	JAK1, JAK2, Tyk2	STAT1, STAT3
IL_{11}	?	?
CNTF	JAK1, JAK2, Tyk2	STAT1, STAT3
LIF	JAK1, JAK2, Tyk2	STAT1, STAT3
Oncostatin M		
IL_{12}	JAK2, Tyk2	STAT3, STAT4
IFN receptors		
IL_{10}	JAK2, Tyk2	
IFN-α/β STAT3	JAK1, Tyk2	STAT1, STAT2,
IFN-γ	JAK1, Tyk2	STAT1α/STAT1β
Receptors with single chain		
G-CSF	JAK1, JAK2	STAT3
GM-CSF	JAK2	STAT5α
EGF	JAK1	STAT3
EpoR	JAK2	STAT5
TpoR	JAK2	STAT5
EpoR	JAK2	STAT5
GH	JAK2	

Data from [157, 214, 222, 240].

cell surface and to a cascade of downstream events, also resembling the biochemical way employed for EGF transduction signals, which activates phospholipase C (PLC) instead of PLCγ1 [454].

The increase in Ca++ concentrations by the second messenger cascade activates calmodulin, which regulates several protein kinases and phosphatases, including calcineurin (CN). This event, together with PKC phosphorylation of serine residues of the CD3 γ chain, and of tyrosine residues of the ζ chain, plays a major role in T cell activation as well as in gene transcription coding the IL_2R α chain (G_1 phase). CN also regulates NFAT activity and contains a binding site for one of its components (Fig. 1.42) [457]. In particular, CD3ζ phosphorylation appears to be *the signal for ZAP70 binding to CD3 ARAM* [462]. The significance of ZAP70 in such processes is demonstrated by its deficiency in a form of SCID characterized by the absence of CD8 T cells [544], emphasizing its prominence in T lymphocyte intrathymic selection and not only in mature cell activation. CD45 (CLA) is associated with T- and B-cell activation processes: for example, CD4-mediated signals are enhanced by cross-linking to CD45, with a rapid rise in Ca++ levels, an effect mediated by Ca++-independent PTPase activity of two domains within the CD45 cytoplasmic tail [584]. On the contrary, CD45 direct interactions with the TcR–CD3 complex could lead to dephosphorylation of CD3ζ and a down-regulation of the response [337]. CD45 is also able to dephosphorylate and activate members of the src family of kinases, a likely basis for the requirements of antigen-induced receptor signaling [395, 584]. CD45 activation of the src-family members before stimulation is consistent with the belief that src are the first tyrosine kinases required for antigen-induced signaling [395].

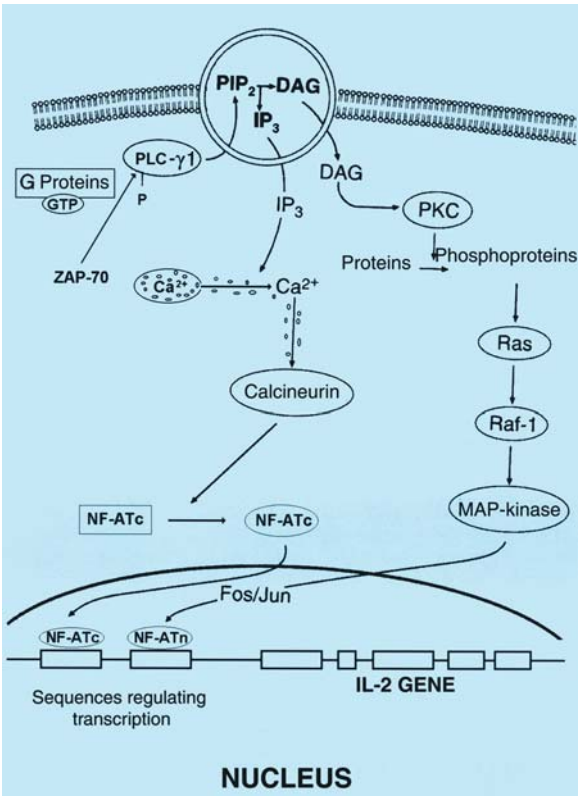


Fig. 1.42. Schematic representation of the morphofunctional aspects of T and B lymphocyte activation signals

Costimulatory Molecules

Recently stressed is the part played by CSMs such as CD2 present on T cells and its ligand CD58 on B cells. CD2 enhances antigen recognition drawing TcRs into zones of cell–cell contacts also arranging the opposing cell membranes of both T cells and APCs at the optimal distance, thus promoting TcR–peptide-HLA interactions. Thus CD2 can allow lower affinity TcRs to be utilized, thereby increasing the size of the mature T-cell repertoire [109]. CD58 for B cells stimulated by IL_4 and CD2 for T cells can provide a second signal for isotype switching to IgE; CD58 can only cross-bind to anti-CD58 lower than that of CD40 [243]. CD2 could also mediate an alternative pathway of T activation if aggregated to the TcR–CD3 complex [109]: it is postulated that molecules different from CD40L are involved in IgE synthesis. Further molecules are CD28 (expressed by the B-cell majority and CD4 as well as by ≈ 50 % of CD8 T cells) and CD152 is = CTLA-4 (present only on activated T cells), whose interactions with CD80 and CD86, main ligands for CD28/CD152, represent a very important costimulatory membrane signal for T-cell activation and are crucial for both proliferation and differentiation of T-cell effector functions [243]. Studies have also hypothesized that both ligands could follow a different model in regulating T-cell differentiation, CD86 to Th2 T cell production with IL_4 predominance and CD80 to Th1 T cell phenotype [279]. Skin CSMs are keratinocytes coming on stage when external factors shift the immunological balance toward an epithelial sphere of influence. Cells expressing CD80 can have a pivotal role in triggering immune responses, delivering costimulatory signals to resting T cells and modulating their maturation into Th2 T cells [279]. Lack of such a strategy can depend on intrinsic differences of class II HLA molecules (that is, li reduction), rather than on defects of CSM potential [381].

Role of B Lymphocytes

At first glance, the B cell first stage of activation is in the T cell area, the splenic PALS, then proliferating cells form GCs (Fig. 1.43). In the superficial cortical zone there are primary follicles, lymphoid aggregates of uniform cellular density, with mature resting B lymphocytes probably not yet stimulated by antigens, and secondary follicles containing GCs, proliferated in response to antigen stimulation. GCs are characterized by a *central dark zone* proximal to T areas, containing many rapidly dividing B cells failing to express surface Igs, the centroblasts [37], and a *basal light zone* filled with centrocytes, non-dividing B Ig^+ lymphocytes [254]. The GC reaction reaches a peak volume by day 10–12 after immunization, when PALS (Fig. 1.11) start to decline; without further antigen stimulation GCs also gradually regress until they wane around 4 weeks after immunization [37]. In GCs there are macrophages with phagocytic activity and IDCs deriving from tissue homologous cells, among which are also DCs. Naive B cells come into contact with antigens presented by DCs in the GC light zone: within a few hours B cells interact with specific CD4 cells; their proliferation reaches the apex by day 5, followed by their migration into lymphoid follicles or other peripheral sites [454]. Exponential proliferation of a given B clone leads to thousands of antibodies/min in 3–4 days that are secreted outside GCs [254]. During the course of primary response, isotype switching occurs in centroblasts, and somatic mutations accumulate in V_H and V_L regions [63]; by day 10 of the response, GCs are clearly divided into dark and light zones [37]. In the dark zone, at about 2 days B blasts differentiate into mIg-negative centroblasts which collect at one pole adjacent to the FDC network, which a little later fills up with centrocytes [37]. Studies suggested that in the apical light zone centroblasts differentiate into memory cells (small lymphocytes) or plasma cells with T-cell cooperation [256]. When FDCs form their protrusions embracing B cells to present bound antigens to BcR, the process is exhausted and plasmocytes migrate into the medulla and eventually the bone marrow, where they undergo a terminal differentiation [256]. Interestingly, light zone centrocytes re-enter the dark zone, join the centroblast population and reinitiate proliferation,

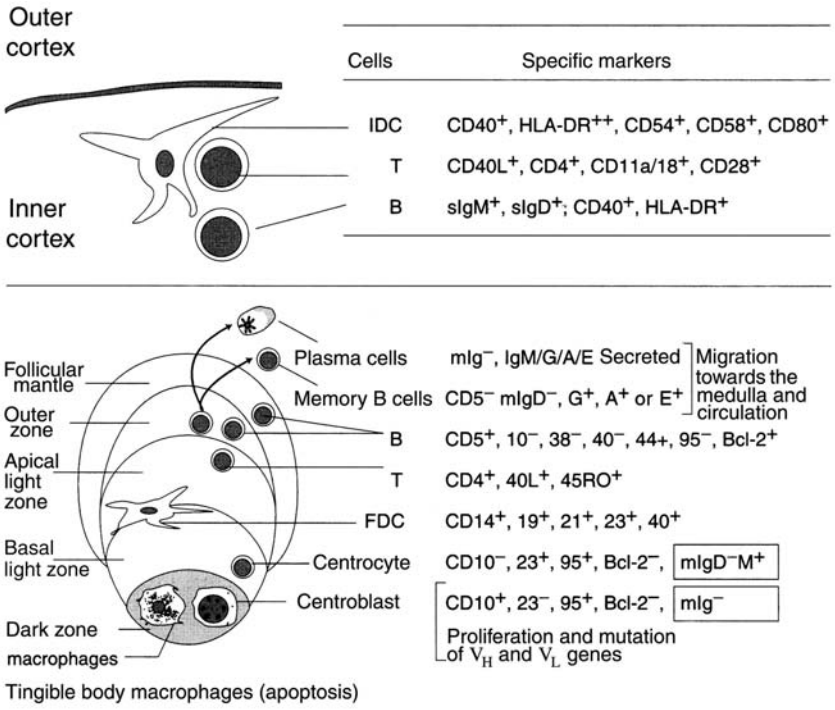


Fig. 1.43. Formation and structure of germinal centers (GC). Tingible body macrophages (apoptosis). *FDC* follicular dendritic cells, *IDC* interdigitating dendritic cells

whereas T–B collaboration in the light zone is necessary to maintain active GC reactions [256]. A principal purpose of GC formation is to direct VDJ rearrangement, and mutated Igs are first observed on day 7–10 of primary responses, coincident with GC polarization and CD86 expression on centrocytes [254]. T-cell–B-cell interactions involve signaling via CD40 and CD154 (CD40L), found in the outer zone of tonsillar GCs [71]. Inhibition of this signaling pathway also impairs GC formation [71]. Markers and/or participants in the activation process are CD19 and CD20 expressed at all stages of differentiation, CD21 (CR2) and CD22 expressed only by mature B cells; IgE^+ antibodies instead express CD5 ligand of CD72, CD32, CD38, CD45RA and CD45RO (Table 1.2). CD19 interactions with BcR markedly lower the threshold (100 antigen receptors per cell, 0.03% of total) to enable B cell activation [70], as they are consequently processed, degraded into peptides, and transported to the cell surface associated with HLA molecules [384]. Independently of signals mediated or not by T cells, BcR internalization does not require ITAM participation [453]. B cell activation by CD79a and CD79b involves triggering src and syk, which form molecular mechanisms able to transduce activation signals generated by interactions between antigen and epitope [408]. However, so that the B cell functions as an effective APC, CD80 and CD86 coexpression is necessary, while it is absent in resting B cells. Upon BcR cross-linking, PTK src are activated, tyrosine residues are phosphorylated in the ITAM, while syk is required for BcR communication with PLCγ1, IP3 generated via PIK3 activation, and Ca^{++} release [453]. Analogous to ZAP70, in addition to tyrosine kinase regions, src and syk carry a SH2 domain with high affinity for phosphorylate tyrosine residues, binding those from CD79a and CD79b [453]. Two pathways are involved in IL_4-mediated Cε transcription: the one associated with PLCγ1, leading to PKCδ translocation with cooperation of IP3, PIP2 and DAG, and the other based on PIK3 and PKCζ [659]. A growing body of evidence indicates that FcγR in B cells inhibits their activation and Ig production [337]. If properly glycosylated, CD45 may interact with CD22, an important step for cell–cell adhesion [395], which has been shown to regulate the B cell phosphatases, also regulating T cell activation. As a result of this sequence of events, 12 h after the antigenic stimulation, the blasts increase in size and, *if they receive appropriate signals from T cells,* proliferate and differentiate in plasma cells [36]. As we have mentioned, B cells can be at the center of *antigen-independent* responses, which occur early in the B cell developmental pathway and can be induced by the association of H chains with CD79b in B cells that develop to the pre-B cell stage even in the absence of L chain synthesis [408].

Expression of Genes and Transcriptional Activity

Phosphorylation of several membrane and cytoplasmic proteins corresponds to a transient stage during which both translocation of TFs and expression of new genes are modulated. It plays an important role in intercellular transduction of signals: studies on animal mast cells have shown that phosphorylation of tyrosine residues is an essential component of the signals deriving from

Table 1.33. Cytokine usage of JAK and STAT proteins

ILs	JAK1	JAK2	JAK3	Tyk2	STAT1	STAT2	STAT3	STAT4	STAT5	STAT 6
Antigen (BcR)					+		+			+
Angiotensin		+		+	+	+				
IL_1										
IL_2	+		+				+		+	
IL_3		+							+	
IL_4	+		+							+
IL_5		+							+	
IL_6	+	+		+	±		+			
IL_7	+	+							+	
IL_8										
IL_9	+		+	+	+					
IL_{10}	+			+	±		+		+	
IL_{11}										
IL_{12}	+			+				+		
IL_{13}	+		+	+						
IL_{14}										
IL_{15}	+	+	+				+		+	
IL_{16}										
IL_{17}										
IL_{18}										
IL_{19}							+			
IL_{20}							+			
$IL_{21}R$	+		+	+						
IL_{22}					+	+	+		+	
$IL_{22}R$							+			
CNTF	+	+		+	+		+			
CSF1					+				+	
EGF	+				+		+			
Epo		+							+	
G-CSF	+	+			+		+			
GM-CSF		+							+	
HGH		+			+		+			
IFN-α	+		+	+	+		+			
IFN-β				+	+	+	+			
IFN-γ	+	+		+	+		+			
LIF	+	+		+	+		+			
OsM	+	+		+	+		+			
PDGF	+	+		+	+		+			
Thrombopoietin	+			+	+				+	

Data from ICI.

IL_6R is homologous to the p40 subunit of IL_{12}, which in turn may produce gp130 dimers; however, signaling takes place above all through the activation of a peptide homologous to gp130. G-CSFR is homologous to the p130 chain of IL_6. EpoR has a high degree of homology with the$IL_2R\beta$ chain. Updated from the Institute of Clinical Immunology.

CNTF ciliary neurotropic factor, *EGF* epidermal growth factor, *Epo* erythropoietin, *GH* growth hormone, *IL* interleukin, *LIF* leukemia inhibitory factor, *PDGF* platelet derived growth factor, *Tpo* thrombopoietin.

FcεRI [35, 195]. Aggregation of polyvalent antigens to the FcεRI–IgE complex results in tyrosine phosphorylation of several protein substrates, including β and γ subunits of FcεRI, and proteins such as $p72^{syk}$, $p53/56^{syn}$, $pp60^{sc\text{-}src}$, PKCγ, $p95^{svav}$, paxilline, pp105–115 and $pp125^{FAK}$: FN adhesion of cells of basophil lineage, when FcεRI is absent, reduces phosphorylation to only the last three proteins [195, 196]. Another example is NF-κB associated with IκB-α (inhibitory κBα), an inhibitor and multiform protein induced by Bc110 [24, 621], probably to prevent inadvertent tissue detriments [303]. In vitro studies show that IκB-α phosphorylation by PKCζ [118], since it does not lead to protein degradation [224], allows the NF-κB–IκB-α complex dissociation and NF-κB activation by PKCζ translocation [118] into the nucleus and fixation on κB regulatory sequences [54]. TRAF6 is thought to activate a member of the MAPK family, which directly or indirectly leads to the activation of IKK1 and IKK2. Both kinases phosphorylate IκB on serine residues, thus targeting IκB for degradation and releasing NF-κB [345]. A TF of the NFAT family, including c-*jun* and c-*fos* dimers that together form a potent transcriptional activation complex, binds via NF-κB to specific DNA-regulatory sites of many IL genes in T cells [252]. CN then dephosphorylates NF-κB, passing from a pre-existing state, NFATp, to the cytosolic state, NFATc, beyond which there are NFAT3, NFAT4 genes, etc. [207]. NFATc is then translocated into the nucleus and binds to regulatory sequences in position 5' of the promoter region of some genes, for example of IL_2 [252]. During the 30 min following ligand interactions with membrane receptors, there is the expression of proto-oncogene c-*fos*, c-*jun* and c-*myc*: their products bind to regulatory structures [454]. The association of c-*jun* and c-*fos* dimers, products of *immediate-early genes,* generates the heterodimer TF AP-1 (activating protein-1) [252]. At the T-cell level, the coordinated fixation of several TFs on regulatory elements, such as NFATc, NF-κB, the fos/jun proteins and AP-1 disposed upon a site from −300 to −63 bp upstream of the IL_2 promoter leads to the pertinent gene transcription and IL synthesis [444]. IL_2–IL_2R binding yields a progression signal allowing the complex internalization and lymphocyte progression from G_1 stage to S stage of cell cycle and DNA replication, accompanied with CD71 and HLA-DR expression [444]. Similar processes regulate IL_2R α chain and other receptor transcription, IL_4, IL_7, IL_9, IL_{13}, IL_{15}, *whose α chain is a functional component of* IL_2*-Rγ* [99, 269, 395, 483, 693] and of IL_5, IL_6, IL_{10}, the IFNs, GM-CSF and TNF-α [222, 444]. In particular IL_2, IL_4, IL_5 and TNF-α are under NFAT influx stimulated by calmodulin [588]. Recently, the GATA family of transcription factors has been characterized, which bind to DNA sequences through a highly conserved C4 zinc finger domain. Six members (GATA-1–GATA-6) of this family have been identified in avians, with homologs in mammals and amphibians [342]. Based on their expression profile, the GATA proteins may be classified functionally as hemopoietic (GATA-1–GATA-3) or non-hemopoietic (GATA-4–GATA-6) [342]. GATA-3 is expressed primarily in T lymphocytes and in the embryonic brain. Functionally important GATA-3-binding sites have been identified in TcR genes and the CD8 gene [342]. Significantly, Th2 cells contain GATA-3 protein in a constitutive fashion, which increases upon stimulation of the cells by antigen or cAMP, whereas Th1 cells express very little or no GATA-3 at the basal level [678]. T-bet, a TF member of the T-box family expressed in T cells is necessary to induce T cells to differentiate into Th1 cells and for Th1 cells to produce IFN-γ. Since IFN-γ induces T-bet expression, it is possible that IFN-γ affects T-bet expression by Th1 cells. Mice lacking T-bet do not have a functional Th1 response in vivo [556], and recent studies stress that T-bet expression is down-regulated in asthmatic patients [155]. Further, T-bet enhances IFN-γ secretion and suppresses IL_4 secretion in γδ cells, and GATA-3 fails to counterbalance T-bet-mediated IFN-γ production, accounting for the default synthesis of IFN-γ by these T lymphocytes [665].

Signal Transduction

The importance of post-receptor signals transduced by members of the IL receptor superfamily attached to JAK (Janus-family kinase) 1–3 and Tyk-2 activated family members connecting the receptors with the STAT factors is now apparent [214]. The STAT family in turn also includes erythropoietin, G-CSF, and the IFNs [214]. In the Tables 1.32, 1.33 we show two aspects of IL interactions with both JAK and STAT proteins. STAT specificity, phosphorylated and activated by different ILs, is mediated by STATs present within target cells and by affinity of such proteins to the JAK–Tyk complex [222]. In addition, IL_4-mediated expression of pertinent genes activates the JAK modulating STAT phosphorylation, three exons of which dictate the SH2 region necessary to its activation, of IL_4-NFAT (NFAT activated by IL_4), and other signaling pathways: in particular STAT6 binds to DNA sequences controlling the expression of IL-4-induced Th2 T cell response [562] (Fig. 1.44) [457], absent in mice with disrupted STAT6 genes [523]. Mice devoid of STAT6 fail to produce both IgE antibodies and Th2 lymphocytes in response to IL_4 or IL_{13} [248]. A differential expression of the IL_4 gene in Th1 and Th2 T cells is associated with a diverse regulation of NFAT binding to IL_4 CLE0 (consensus lymphokine element-0), mediated via a different TF regulation in Th1 and Th2 lymphocytes [642]. GATA-3 protein is expressed in both immature and mature T cells, but in a constitutive fashion in Th2 cells, which increases upon stimulation of the cells by Ag or cAMP. In contrast, GATA-3 is selectively suppressed in Th1 cells; thus *GATA-3 may function as a more general regulator of Th2 ILs expression* [678]. An alternative signal transduction system in activated T cells, formed by JAK-1, JAK-3, STAT3 and STAT5, is asso-

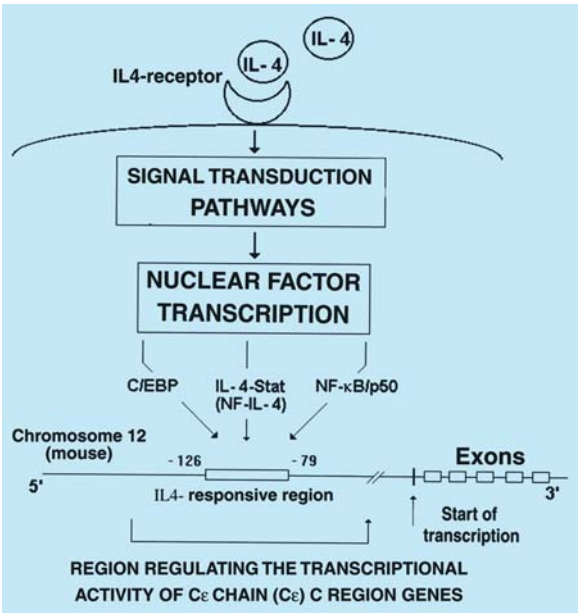


Fig. 1.44. Schematic representation of IL_4-mediated regulation of genes controlling the Cε chain synthesis of IgE antibodies

ciated with IL_2 and IL_{15} [240], JAK1 and -2, STAT1, -2, -3 and -5 by IL_{27} [245], also including IL_2R, $IL_{15}R\alpha$ and -β [557] as well as the receptors sharing the IL_2R γ chain [404]. Studying intracellular signals has led to the identification of three immunosuppressors of T lymphocyte activation: cyclosporine A (CsA), FK-506 (tacrolimus), and rapamycin (RAP) [505]. CsA and FK-506 bind, respectively, to a cyclophilin and the proteins binding to FK-506 (FKBP): the complexes thus formed bind to CN-dependent phosphatase 2B activity [505]. The CsA/FKBP/RAP-mediated inhibition of NFAT-dependent IL_2 gene transcription is overcome by CN overexpression [341]. CN is therefore essential in the lymphocyte signal transduction pathway, also leading to metachromatic cell degranulation [341].

Activation and Immunosuppression of B and T Lymphocytes and of Other Cells

The *lectins,* carbohydrate-binding gps, are active either substituting IgE antibodies on mIgs or cross-linking their H chains to carbohydrates expressed on various cells, and activating mast cell degranulation by an aspecific binding, and are also known as mitogens (proliferation inducers). Mitogen in vitro stimulation of lymphocytes is believed to mimic fairly closely specific antigen stimulation. B and T cells are activated by different mitogens: mouse B cells by LPS (lipopolysaccharide), human B and T cells by PWM (pokeweed mitogen), and human and mouse T cells by Con-A (concanavalin A) and PHA (phytohemagglutinin) [470].

Table 1.34. Absolute size of the main, age-related lymphocyte subpopulations (median + 5th–95th percentiles)

Lymphocyte	Neonate	7 d–2 m	2–5 m	5–9 m	9–15 m	15–24 m	2–5 y	5–10 y	10–16 y
Lymphocyte Absolute size	4.8 (0.7–7.3)	6.7 (3.5–13)	5.9 (3.7–9.6)	6.0 (3.8–9.9)	5.5 (2.6–10.4)	5.6 (2.7–11.9)	3.3 (1.7–6.9)	2.8 (1.1–5.9)	2.2 (1.0–5.3)
CD19 (%)	12 (5–22)	15 (94–260)	24 (14–29)	21 (13–35)	25 (15–39)	28 (17–41)	24 (14–44)	18 (10–31	16 (8–24)
Absolute size	0.6 (0.04–1.1)	1.0 (0.6–1.9)	1.3 (0.6–3.0)	1.3 (0.7–2.5)	1.4 (0.6–2.7)	1.3 (0.6–3.1)	0.8 (0.2–2.1)	0.5 (0.2–1.6)	0.3 (0.2–0.6)
CD3 (%)	62 (28–76)	72 (60–85)	63 (48–75)	66 (50–77)	65 (54–76)	64 (39–73)	64 (43–76)	69 (55–78)	72 (55–83)
Absolute size	2.8 (0.6–5.0)	4.6 (2.3–7.0)	3.6 (2.3–6.5)	3.8 (2.4–6.9)	3.4 (1.6–6.7)	3.5 (1.4–8.0)	2.3 (0.9–4.5)	1.9 (0.7–4.2)	1.5 (0.8–3.5)
CD3/CD4 (%)	41 (17–52)	55 (41–68)	45 (33–58)	45 (33–58)	44 (31–54)	41 (25–50)	37 (23–48)	35 (27–53)	39 (25–48)
Absolute size	1.9 (0.4–3.5)	3.5 (1.7–5.3)	2.5 (1.5–5.0)	2.8 (1.4–5.1)	2.3 (1.0–4.6)	2.2 (0.9–5.5)	1.3 (0.5–2.4)	1.0 (0.3–2.0)	0.8 (0.4–2.1)
CD3/CD8 (%)	24 (10–41)	16 (9–23)	17 (11–25)	18 (12–28)	18 (13–26)	20 (11–32)	24 (14–33)	28 (19–34)	23 (9–35)
Absolute size	1.1 (0.2–19)	1.0 (0.4–1.7)	1.0 (0.5–1.6)	1.1 (0.5–2.2)	1.1 (0.4–2.1)	1.2 (0.4–2.3)	0.8 (0.3–1.6)	0.8 (0.3–1.8)	0.4 (0.2–1.2)
CD4/CD8	1.8 (1.0–2.6)	3.8 (1.3–6.3)	2.7 (1.7–3.9)	2.5 (1.6–3.8)	2.4 (1.3–3.9)	1.9 (0.9–3.7)	1.6 (0.9–2.9)	1.2 (0.9–2.6)	1.7 (0.9–3.4)
CD3/HLA-DR (%)	2 (1–6)	5 (1–38)	3 (1–9)	3 (1–7)	4 (2–8)	6 (3–12)	6 (3–13)	7 (3–14)	4 (1–8)
Absolute size	0.09 (0.03–0.4)	0.3 (0.03–3.4)	0.2 (0.07–0.5)	0.2 (0.07–0.5)	0.2 (0.1–0.6)	0.3 (0.1–0.7)	0.2 (0.08–0.4)	0.2 (0.05–0.7)	0.06 (0.02–02)
CD3/CD16/56 (%)	20 (6–58	8 (3–23)	6 (2–14)	5 (2–13)	7 (3–17)	8 (3–16)	10 (4–23)	12 (4–26)	15 (6–27)
Absolute size	1.0 (0.1–1.9)	0.5 (0.2–1.4)	0.3 (0.1–1.3)	0.3 (0.1–1.0)	0.4 (0.2–1.2)	0.4 (0.1–1.4)	0.4 (0.1–1.0)	0.3 (0.09–0.9)	0.3 (0.07–1.2)

Absolute counts ($\times 10^3$ cells/mm^3). Data from [93]. *d* days, *m* months, *y* years.

Table 1.35. Changes in lymphocyte major subsets and analysis as a function of age (median + 25th and 75th percentile)

Lymphocytes	Cord blood	2 Days to 11 months	1–6 Years	7–17 Years
Lymphocyte count Absolute count	12 (10–15)	9.0 (6.4–11)	7.8 (6.8–10)	6.0 (4.7–7.3)
Lymphocytes (%) Absolute count	41 (35–47) 5.4 (4.2–6.9)	47 (39–59) 4.1 (2.7–5.4)	46 (38–53) 3.6 (2.9–5.1)	40 (36–43) 2.4 (2.0–2.7)
T lymphocytes (%) Absolute count	55 (49–62) 3.1 (2.4–3.7)	64 (58–67) 64 2.5 (1.7–3.6)	(62–69) 2.5 (1.8–3.0)	70 (66–76) 1.8 (1.4–2.0)
B lymphocytes (%) Absolute count	20 (14–23) 1.0 (0.7–1.5)	23 (19–31) 0.9 (0.5–1.5)	24 (21–28) 0.9 (0.7–1.3)	16 (12–22) 0.4 (0.3–0.5)
NK cells (%) Absolute count	20 (14–30) 0.9 (0.8–1.8)	11 (8–17) 0.5 (0.3–0.7)	11 (8–15) 0.4 (0.2–0.6)	12 (9–16) 0.3 (0.2–0.4)
T cells (%)				
HLA-DR in CD3	2.0 (2.0–3.0)	7.5 (4.0–9.0)	9.0 (6.0–16) 12	(9.5–17)
IL_2R in CD3	8.0 (5.5–10)	9.0 (7.0–12)	11 (8.0–12)	13 (10–16)
CD57 in CD3	0.0 (0.0–0.0)	1.5 (0.0–2.5)	3.0 (2.0–5.0)	5.5 (3.0–10)
T cells $CD4^+$ (%) Absolute count	35 (28–42) 1.9 (1.5–2.4)	41 (38–50) 2.2 (1.7–2.8)	37 (30–40) 1.6 (1–1.8)	37 (33–41) 0.8 (0.7–1.1)
$CD45RA^+$ in CD4 (%)	91 (82–97)	81 (66–88)	71 (66–77)	61 (55–67)
Leu-8+ in CD4 (%)	91 (85–95)	90 (88–98)	91 (84–95)	87 (81–89)
T cells $CD8^+$ (%) Absolute count	29 (26–33) 1.5 (1.2–2.0)	21 (18–25) 0.9 (0.8–1.2)	29 (25–32) 0.9 (0.8–1.5)	30 (27–35) 0.8 (0.8–0.9)
$CD57^+$ in CD8 (%)	0.0 (0.0–1.0)	7.0 (4.0–9.5)	10 (6–15)	17 (12–24)
CD4/CD8 ratio	1.2 (0.8–1.8)	1.9 (1.5–2.9)	1.3 (1–1.6)	1.3 (1.3–1.4)
B cells ($CD5^+CD20^+$) Absolute count	0.5 (0.4–1.0)	0.5 (0.2–1.1)	0.5 (0.3–0.8)	0.2 (0.1–0.3)
$CD5^+$ in CD20 (%)	72 (58–79)	68 (47–76)	64 (53–77)	56 (44–64)
$CD23^+$ in CD20 (%)	35 (30–50)	50 (44–66)	61 (53–70)	63 (52–73)
Leu-8^+ in CD20 (%)	57 (23–68)	66 (40–90)	79 (57–89)	90 (83–94)
$CDw78^+$ in CD19 (%)	49 (37–64)	22 (15–37)	30 (17–45)	32 (19–50)

The absolute counts are $\times 10^3$ cells/mm^3.
Data from [142].

T cells expressing low CN levels are more sensitive to the action of CsA and/or FK-506; similarly immunosuppressive activity of CsA or of its analogs correlates with CN phosphatase 2B activity inhibition [341]. In addition, in healthy volunteers CsA caused a rapid inhibition of histamine release from basophils or a 30%–60% inhibition of their releasability [72].

Mean Values of Lymphocyte Populations and Subpopulations and of Other Immune Cells

Immunophenotyping of blood lymphocytes has become an important tool in the diagnosis of pediatric PIDs and AIDS. The increased prevalence of these disorders, as well as of pediatric asthma, frequently makes a determination of lymphocyte subsets and of pediatric BALF necessary.

Blood values determined in normal children as a function of age are reported in Tables 1.34, 1.35 [93, 142] and 1.36–1.39 [203, 440, 458]. Lymphocyte values include the median and 25th–75th percentiles, apart from one study with a 5th–95th percentile range (Table 1.34; Appendix 1.3) [93]. Table 1.35 [142] and especially in Tables 1.36–1.39 outline relative and absolute values of several lymphocyte subsets. The age range is extended to 16 years (Table 1.34), 17 years (Table 1.35) and compared to adults (Table 1.36). In a recent study the age range is extended up to 18 years [516]. We do not agree that CB values at 5 days after birth of healthy neonates (Table 1.38) [440] can serve as a reference range in the evaluation of probable PIDs and HIV infection. We recommend determining chiefly the ab-

Table 1.36. Percentage values of lymphocyte subpopulations in children at various ages and in adults (mean + 25th–75th percentile)

Age	Cord blood	1.28 (0.63–3.06)	4.25 (3.92–4.84)	9.7 (7.7–10.6)	Adults
B-lineage markers					
CD19	12.0 (3.0–29.0)	14.5 (6.0–33.0)	17.0 (4.0–38.0)	9.0 (7.0–27.0)	4.5 (2.0–6.0)
CD20	8.0 (0.0–23.0)	4.0 (0.0–47.0)	8.0 (0.0–19.0)	2.0 (0.0–8.0)	1.0 (0.0–2.0)
CD21	2.0 (0.0–10.0)	5.0 (0.0–14.0)	3.0 (0.0–28.0)	1.0 (0.0–6.0)	1.0 (0.0–2.0)
CD22	6.0 (2.0–23.0)	8.5 (1.0–30.0)	10.5 (0.0–30.0)	7.0 (2.0–13.0)	2.0 (1.0–5.0)
CD23	1.0 (0.0–7.0)	1.0 (0.0–3.0)	0.0 (0.0–2.0)	0.0 (0.0–2.0)	0.5 (0.0–6.0)
CD24	3.0 (0.0–8.0)	7.0 (1.0–14.0)	6.5 (2.0–12.0)	5.5 (2.0–22.0)	2.0 (1.0–4.0)
CD37	13.0 (4.0–29.0)	13.5 (3.0–31.0	14.5 (4.0–35.0)	10.5 (1.0–24.0)	5.0 (2.0–11.0)
CD39	1.0 (1.0–6.0)	3.0 (0.0–9.0)	4.5 (0.0–37.0)	1.5 (0.0–4.0)	1.0 (0.0–2.0)
CD40	12.0 (1.0–28.0)	14.5 (8.0–24.0)	18.0 (5.0–39.0)	9.5 (5.0–15.0)	4.0 (1.0–7.0)
HLA-DR	14.0 (8.0–29.0)	19.0 (8.0–47.0)	18.0 (10.0–35.0)	12.0 (5.0–21.0)	5.5 (3.0–8.0)
FMC7	10.0 (1.0–29.0)	10.0 (3.0–24.0)	17.5 (7.0–39.0)	11.0 (4.0–33.0)	4.0 (2.0–7.0)
IgD	7.0 (3.0–26.0)	9.5 (2.0–21.0)	6.5 (1.0–25.0)	5.0 (3.0–16.0)	1.5 (0.0–2.0)
IgG	1.5 (0.0–28.0)	2.0 (0.0–17.0)	1.0 (0.0–3.0)	0.0 (0.0–1.0)	1.5 (0.0–7.0)
IgM	11.0 (3.0–26.0)	15.4 (5.0–23.0)	14.5 (5.0–34.0)	8.5 (4.0–28.0)	3.0 (1.0–6.0)
T-lineage markers					
CD2	69.0 (36.0–81.0)	75.0 (25.0–87.0)	66.0 (42.0–82.0)	76.0 (50.0–84.0)	86.5 (71.0–92.0)
CD3	63.0 (21.0–73.0)	67.0 (53.0–84.0)	62.0 (39.0–74.0)	71.0 (58.0–78.0)	75.0 (53.0–81.0)
CD4	49.0 (16.0–58.0)	46.0 (22.0–87.0)	37.5 (29.0–51.0)	43.5 (28.0–55.0)	40.5 (29.0–62.0)
CD7	77.0 (55.0–86.0)	63.5 (44.0–83.0)	63.5 (47.0–72.0)	68.0 (58.0–84.0)	70.0 (41.0–87.0)
CD8	19.0 (13.0–29.0)	18.0 (12.0–52.0)	22.0 (11.0–33.0)	21.5 (17.0–31.0)	25.5 (20.0–43.0)
CD4/8	2.5 (0.8–4.0)	2.65 (0.4–5.4)	1.75 (1.2–4.6)	2.1 (1.0–3.2)	1.55 (0.9–3.1)
CD26	11.0 (2.0–59.0)	2.5 (0.0–10.0)	5.5 (2.0–10.0)	3.0 (0.0–7.0)	5.0 (0.0–10.0)
NK series					
CD16	15.0 (4.0–30.0)	3.5 (0.0–13.0)	6.5 (4.0–12.0)	4.5 (2.0–10.0)	6.5 (2.0–25.0)
Non-lineage marker					
CD38	75.0 (40.0–88.0)	34.0 (12.0–75.0)	24.5 (16.0–34.0)	12.5 (11.0–25.0)	8.5 (2.0–21.0)
Leukocyte common markers					
CD45	75.0 (54.0–87.0)	74.0 (12.0–81.0)	68.0 (62.0–74.0)	66.0 (41.0–91.0)	48.5 (29.0–71.0)
CD45R	68.0 (46.0–85.0)	76.0 (39.0–85.0)	63.5 (46.0–79.0)	71.0 (60.0–92.0)	54.0 (34.0–82.0)

Data from [203].

solute values, even if we also report relative values of lymphocyte subsets to allow a complete evaluation. As regards age variations, lymphocyte values decrease from 66% to 50% between 2–3 months and 5 years of age, but remain substantially stable [117], whereas CD4 values are constantly higher than CD8 values, with a reversed CD4/CD8 ratio returning to normal in adolescents when CD8 cells increase. Only one study [142] found that the CD4/CD8 ratio remained unchanged with age, a result limited to the 5- to 13-year age range [464], or was disputed [613]. *In the 1st year of life, CD8 cells are less than 50% of CD4 cells (41%) and B cells 22.5%* [142], with evidently negative consequences [647].

BALF CD4/CD8 ratios are lower than in adults [445] due to an increase in CD8 cells with a reversed CD4/CD8 ratio, which has not been observed in healthy adults [445, 458]. The case reports published (Tables 1.40, 1.41) [202, 445, 458] regard nonatopic children without acute respiratory infections aged 3 months to 10 years (mean 31 months) [458], or 3–16 years (mean, 8±3 years)

Table 1.37. Absolute values of lymphocyte subsets in children at various ages and in adults: (mean + 25th–75th percentile) $\times 10^3$ cells/mm^3

Age	0.63 (1.29–3.06)	4.08 (4.28–4.83)	7.66 (9.66–10.59)	Adults
Leukocytes	6.60 (4.50–12.80)	7.20 (5.50–8.80)	5.55 (3.00–7.20)	5.60 (3.80–9.10)
Monocytes	4.75 (3.00–9.30)	3.20 (2.00–5.60)	2.80 (1.70–4.50)	2.20 (1.20–4.80)
B-lineage markers				
CD19	0.76 (0.18–1.62)	0.58 (0.12–2.05)	0.31 (0.15–1.22)	0.08 (0.05–0.29)
CD20	0.20 (0.03–2.26)	0.26 (0.03–1.03)	0.05 (0.00–0.36)	0.02 (0.00–0.10)
CD21	0.21 (0.00–0.60)	0.06 (0.00–0.32)	0.02 (0.00–0.18)	0.02 (0.00–0.10)
CD22	0.41 (0.12–1.47)	0.38 (0.00–1.62)	0.22 (0.03–0.43)	0.05 (0.02–0.19)
CD23	0.05 (0.00–0.18)	0.00 (0.00–0.03)	0.00 (0.00–0.05)	0.00 (0.00–0.14)
CD24	0.37 (0.18–0.67)	0.16 (0.06–0.65)	0.16 (0.05–0.99)	0.04 (0.03–0.19)
CD37	0.80 (0.24–1.52)	0.56 (0.12–1.89)	0.28 (0.04–1.08)	0.09 (0.05–0.34)
CD39	0.14 (0.00–0.41)	0.06 (0.00–2.00)	0.05 (0.00–0.13)	0.01 (0.00–0.05)
CD40	0.81 (1.30–1.21)	0.74 (0.15–2.11)	0.30 (0.09–0.68)	0.09 (0.03–0.34)
HLA-DR	0.81 (0.33–1.30)	0.65 (0.24–1.35)	0.34 (0.14–0.95)	0.12 (0.07–0.38)
FMC7	0.58 (0.14–1.18)	0.64 (0.29–2.10)	0.28 (0.10–1.49)	0.09 (0.03–0.19)
IgD	0.44 (0.09–1.03)	0.17 (0.03–0.42)	0.13 (0.07–0.72)	0.03 (0.00–0.06)
IgG	0.05 (0.00–0.75)	0.00 (0.00–0.10)	0.00 (0.00–0.03)	0.02 (0.00–0.06)
IgM	0.75 (0.18–1.13)	0.39 (0.14–1.09)	0.22 (0.09–1.26)	0.06 (0.02–0.13)
T-lineage markers				
CD2	2.85 (1.28–7.16)	2.02 (1.22–4.14)	2.07 (1.41–3.70)	1.98 (1.02–3.98)
CD3	2.83 (1.83–6.60)	1.94 (1.14–4.09)	2.04 (1.22–2.86)	1.65 (0.85–3.60)
CD4	2.00 (0.94–5.05)	1.25 (0.76–2.24)	1.21 (0.77–1.80)	0.87 (0.50–1.82)
CD7	2.89 (1.77–5.21)	1.97 (1.30–4.03)	1.99 (0.99–3.08)	1.49 (0.50–2.44)
CD8	0.86 (0.51–1.86)	0.59 (0.36–1.34)	0.56 (0.32–1.36)	0.75 (0.28–1.54)
CD4/8	2.80 (1.00–5.44)	1.79 (1.50–4.64)	2.03 (1.00–3.18)	1.50 (0.85–3.10)
CD26	0.12 (0.00–0.46)	0.16 (0.06–0.22)	0.09 (0.00–0.22)	0.08 (0.00–0.29)
NK marker				
CD16	0.17 (0.00–0.47)	0.22 (0.14–0.39)	0.13 (0.05–0.45)	0.13 (0.03–0.29)
Non-lineage marker				
CD38	1.49 (0.61–3.36)	0.90 (0.38–1.19)	0.38 (0.20–0.81)	0.23 (0.03–0.42)
Leukocyte common markers				
CD45	3.42 (0.61–6.88)	2.18 (1.24–3.92)	1.82 (0.94–3.06)	0.99 (0.52–2.21)
CD45R	3.64 (2.25–7.72)	2.21 (1.18–3.47)	1.94 (1.38–3.29)	1.38 (0.55–2.40)

Data from [203].

[445] and healthy children aged 5 months to 14.6 years (median, 7.2 years) [202]. Such data derive from pooled aliquots and age-corrected volumes, where the first sample showed more ciliated cells than subsequent ones [458]; among the aliquots there is not always a significant difference [445, 458]. The analysis of the reduced CD4/CD8 ratio suggests a possible influence of the highest frequency of viral infections in the younger age groups [445].

The BALF pediatric levels of other immune cells, including macrophages, granulocytes, mast cells, etc., are summarized in Tables 1.40 and 1.41. The study in healthy children [202] examined the cells with a less invasive procedure, using a neonatal catheter (external diameter 2.6 mm), inserted prior to the start of surgery, without noting significant differences. These studies are of invaluable use in asthmatic and immodeficient children.

Table 1.38. Absolute and relative values of lymphocyte subpopulations in cord blood and venous blood (day 5): (mean + 25th–75th percentile)

CD markers	Cord blood	5 days
CD1 (%)	0.4 (0.3–0.7)	0.4 (0.2–0.6)
Absolute count	0.02 (0.01–0.03)	0.01 (0.01–0.03)
CD2 (%)	64.9 (57.0–72.8)	74.3 (65.1–87.5)
Absolute count	2.77 (2.19–3.78)	2.97 (2.49–3.95)
CD3 (%)	59.1 (52.9–67.9)	73.4 (65.6–82.9)
Absolute count	2.61 (2.01–3.36)	3.03 (2.38–3.95)
$CD3^+/CD16^+ + CD56^+$ (%)	0.1 (0.1–0.2)	0.1 (0.1–0.2)
$CD3^-/CD16^+ + CD56^+$ (%)	12.2 (7.3–17.2)	4.8 (2.3–7.0)
Absolute count	0.50 (0.26–0.88)	0.18 (0.09–0.30
CD4 (%)	44.2 (39.3–51.4)	56.9 (52.2–64.0)
Absolute count	1.93 (1.49–2.59)	2.35 (1.97–3.4)
$CD4^+/CD45RA^+$ (%)	31.8 (27.5–38.8)	47.0 (41.0–52.7)
$CD4^+/CD29^+$ (%)	8.5 (6.2–10.6)	6.6 (4.8–9.2)
Absolute count	0.92 (0.70–1.30)	0.91 (0.70–1.11)
$CD5^+/CD19^-$ (%)	61.9 (55.3–68.9)	77.8 (71.6–87.4)
Absolute count	2.68 (2.17–3.54)	3.18 (2.61–4.11)
CD7 (%)	76.8 (70.2–82.6)	81.4 (74.1–89.5)
Absolute count	3.26 (2.57–4.54)	3.38 (2.75–4.24)
CD8 (%)	21.6 (18.6–26.0)	21.2 (18.0–24.4)
Absolute count	0.92 (0.70–1.30)	0.91 (0.70–1.11)
$CD8^+/S6F1^+$ (%)	8.8 (6.0–11.7)	5.3 (3.8–8.7)
$CD8^+/S6F1^-$ (%)	12.5 (9.0–18.0)	14.9 (11.9–18.7)
CD4/CD8 ratio	1.97 (1.62–2.46)	2.74 (2.34–3.26)
CD10 (%)	0.6 (0.4–1.1)	0.3 (0.1–0.5)
CD19 (%)	12.8 (9.2–17.4)	16.0 (1.4–10.4)
Absolute count	0.62 (0.34–0.91)	0.21 (0.06–0.43)
$CD19^+/CD5^+$ (%)	0.9 (0.6–1.3)	0.6 (0.3–1.1)
CD20 (%)	13.8 (9.8–18.0)	6.1 (1.4–11.1)
Absolute count	0.62 (0.37–0.98)	0.24 (0.05–0.44)
CD22 (%)	11.6 (8.5–16.5)	5.5 (1.4–10.2)
CD23 (%)	0.6 (0.4–1.0)	0.4 (0.3–0.8)
CD57 (%)	0.1 (0.1–0.2)	0.1 (0.1–0.3)

Values for absolute cell counts are expressed as $\times 10^3$ cells/mm^3.
Data from [440].
$CD8^+/S6F1^+$ killer effector cells, *$CD8^+/S6F1^-$* suppressor effector cells.

Table 1.39. Mean and percent values of CD (lymphocyte surface antigens) in cord blood

	Percent values		Absolute values (cells/μl)	
	Mean	Range	Mean	Range
T- and NK-cell lineage				
CD1	3.8	2.3– 5.8	173	110–262
CD2	60.9	52.4–66.8	2803	1,821–3,514
CD3	57.5	50.5–63.3	2,477	1,820–3,371
TcR-αβ	57.7	48.1–61.0	2,573	1,557–3,287
CD4	36.0	28.0–42.6	1,780	904–2,320
CD8	23.0	20.0–27.4	967	673–1,248
B-cell lineage				
CD19	12.1	8.6–14.8	424	214–633
CD20	11.1	6.7–15.5	485	93–877
Activation markers and others				
CD11a	56.3	46.3–68.5	2,704	1,876–3,804
CD25	2.6	2.1– 4.5	140	75–197
CDw52	61.0	51.2–76.1	2,740	1,851–4,145
CD71	5.2	3.1– 9.3	228	164–289

Values expressed as cells/μl; 1 μl=10^6 cells/l.
Data from [269].

Table 1.40. BALF lymphocyte subpopulations from pediatric studies (BALF cells – pooled samples – % of total lymphocytes)

CD	Mean +25th–75th percentile[a]	Mean±SD[b]	CD	Mean +25th–75th percentile[a]	Mean±SD[b]
CD3 (T)	81.0 (75.5–88.0)	85.8 ±4.9	CD19	5.0 (4.0–9.5)	
CD4	27.0 (22.0–32.0)	33.1 ±12.8	CD20 (B)		0.9±1.5
CD8	45.0 (33.8–57.0)	56.8 ±13.1	CD25	2.0 (0.0–3.0)	1.9±1.3
CD4/CD8	0.6 (0.4–1.0)	0.68 ±0.44	CD57 (NK)		7.8±8.2
CD16+CD56	4.0 (1.5–7.5)		HLA-DR		1.4±1.7

[a] Data from [458]. [b] Data from [445].

Table 1.41. BALF differential cytology from pediatric studies (BALF cells – pooled samples – % of total lymphocytes)

Cell types	Mean + 25th–75th percentile[a]	Mean and range[b]	Mean and range[c]
Macrophages	91.0 (84.2–94.0)	83 (47–90)	70.07 (29.0–96.3)
Granulocytes		1.4 (0.2–11)	0.09 (0.0–0.9)
Eosinophils	0.2 (0.0–0.3)		
Neutrophils	1.7 (0.6–3.5)		0.2 (0.4–34.4)
Mast cells			0.2 (0.0–0.8)
Epithelial cells			13.4 (0.3–64.7)

[a] Data from [458]. [b] Data from [445]. [c] Data from [202].

Central Phase of the Immune Response: Synthesis of IgE Antibodies

A Two-Signal Model for Induction of IgE Synthesis

According to the two-signal theory [606], we can distinguish a first signal delivered by IL_4 and a second one provided by interactions between $TCD4^+$ and B lymphocytes [172].

First Signal

IL_4 production by Th2 lymphocytes is for B lymphocytes a crucial factor for isotype switching to IgE plasma cells. IL_4, similarly to IL_{13} [437] is sufficient to trigger in B cells the expression of ε germline transcripts containing one exon located upstream of the ε switch region, and spliced to the C_H region codified by Cε genes [176]. According to this model, such transcripts are thought to play a critical role in IgE isotype switching by increasing the ε locus opening, which thereby becomes accessible to recombination enzymes [296]. Furthermore, IL_4 acts by altering the chromatin structure of the Sγ1 region inducing γ1 and ε transcript accumulation, and B cells activate TFs binding DNA in the region 179 bp upstream of germline ε. IL_4 can therefore be sufficient to regulate some events of recombination machinery at the B lymphocyte level [177], initiating with IL_{13} transcription of the $C_ε$ gene of Ig H chain [250]. Besides, IL_4 contributes to Fcε receptor increase on B cells and LC, increases the expression of class II HLA molecules on macrophages, inhibits their IL_1 production and primes them to differentiate into DCs [296]. IL_4R (CD140), of which $IL_{13}R$ is a component [64], assists with appropriate signals inducing Cε germline transcripts and increasing their role via PLCγ1 and PI3K, which also modulates isotype switching leading to mature Cε transcription and to IgE synthesis [659]. Moreover, CD140 evokes Th2 T cell differentiation and IL_4 production mediated by CD8 lymphocytes [4]. IL_4 and IL_{13} are in some way independent, since anti-IL_4 abrogates the effects charged to IL_4, but not those that are IL_{13}-triggered [125]. Likewise, immune responses to IL_{13} can also be mediated by IL_4, whereas the contrary is not known [64]; however, cells not expressing the γc chain, as is the case of X-SCID, respond to both IL_4 and IL_{13} [64].

Role of ILs [205, 473, 606]. Besides IL_4, several ILs take part in IgE synthesis [34, 296]. When an antigen is encountered, both macrophages and accessory cells following a signal delivered by IFN-γ release ILs starting to secrete IL_1, with a substantial effect on thymocyte proliferation stimulated by a lectin, further enhancing T-lymphocyte increase, chiefly Th2 T cells. However, on antigen recognition, IL_1 binds to Th1 T-cell-specific receptors, priming them to produce IL_2 growth factor for T cells and express mRNA for IL_2 molecules [457], although IL_7 is more powerful than IL_1 and IL_6 [470].

IL_2 receptors (IL_2-R) appear on the CD4 T-cell surface. At this stage, the role of IL_2 is played by its receptor equipped with a high, mean and low affinity: the high-affinity receptor, necessary for IL_2 proliferation, consists of three subunits: α, β and γ, of 55, 75 and 64 kD, respectively [172]. The α subunit (CD25), not expressed on resting cells, with a synthesis that does not depend on antigen signals, amplified from IL_1, TNF and IL_5, controls the production of the high-affinity receptor on T cells; preventing its synthesis is inhibited by IL_2 proliferation [34, 172]. Picomolar IL_1 levels are sufficient to drive IL_2 transcription, synthesis and secretion, as well as expressing membrane receptors. IL deficiency underlying SCID demonstrates IL_2-Rγ's crucial role in intrathymic development of human T cells.

Activated T lymphocytes produce IL_3 and IFN-γ, which stimulate APC induction, while IL_4–IL_6 and IL_{10} may drive B-cell production, maturation and isotype switching; similar mechanisms on activated cells are ensured by IFN-γ synergizing with IL_2 [480]. T lymphocytes generate IL_4 but their signaling totally depends on IL_4; otherwise mast cells and/or basophils provide autonomously for its production [174].

Several ILs may play a role in modulating or contrasting IL_4-dependent IgE synthesis [177] (Fig. 1.22).

IL_6, with powerful amplifying effects on IgE responses, acts on B lymphocytes as a main factor of effector function development, with no isotype preference except IgG secretion [28]; it also plays an obligatory role in IL_4-induced human IgE production [607].

IL_2, IL_5, TNF-α and CD23 have similar effects, CD23 recognizes FcεRII, which, interacting with CD21 ligand, plays an essential role in IgE synthesis modulation [16].

IL_5 and IL_6 up-regulate IgE production, especially when IL_4 levels are suboptimal, although these ILs do not stimulate IgE synthesis together [296].

IL_8, IL_{12}, IFN-γ, IFN-α and TGF-β seem to act at different levels:

IL_8 and TGF-β inhibit IgE synthesis either in T cell-dependent or T cell-independent systems, thereby acting directly on B cells. CD14 operates with a similar mechanism [608]. TGF-β blocks ε germline expression at a transcriptional level, while inhibition by IL_8 is isotype-specific.

IFN-γ, IFN-α and IL_{12} work only in T cell-dependent systems, thus showing an indirect mechanism of suppression: all three ILs inhibit mature Cε transcript expression, whereas only IFN-γ and IFN-α have such an effect on ε germline in PBMC cultures [606].

IL_{12} mediates specific Th1 T cell immune responses and inhibits development of IL_4 producing Th2 cells [339].

PAF blocks both ε germline and mature Cε transcripts [606].

IFN-γ is a major IL_4 antagonist and suppresses IgE production by normal human lymphocytes induced by IL_4, either directly or by reducing FcR expression for IgE antibodies from B lymphocytes. IFN-α and PGE_2 also block IL_4-induced IgE production in a dose-dependent way [421]. The IFN-γ suppressive mechanism is indirect, since no inhibition of ε germline transcripts has been reported [296], therefore suggesting that IFN-γ may prevent recombination events without affecting ε-mRNA transcript expression [205]. IL_4 down-regulates IFN-γ production, but when this is driven by T cells stimulated with allogenic cells, and in mixed lymphocyte cultures, IL_4 fails to initiate IgE synthesis. By contrast adding IL_4 early to the culture, there is IgE synthesis and suppression of IFN-γ synthesis. So the selection of isotype switching is fixed by a chronological order of secretion of diverse ILs [332].

IL Role in T-Cell Preferential Activation. Also in humans, IL_4 and IFN-γ production is under the influx of a preferential activation of Th1, Th2 T cells (and Th0) cells [644] (Table 1.10) and directed, in addition to the genetic background of the individual, by antigen nature and concentration, individual APCs, and ILs produced in the microenvironment by different cells and antigen dose. IFN-γ, IFN-α, TGF-β, IL_1 and IL_{12} evoke antigen-specific T-cell differentiation into Th0 or Th1 T cells [401], whereas IFN-γ absence or low levels and IL_4 promote Th2 T-cell expansion, while IL_2 supports all three subsets [606]. T-cell stimulation directed by IL_{12} is IFN-γ- or IFN-α–dependent [401]; similarly DCs drive Th1 T-cell expansion from virgin DC^+ in an IL_{12}-dependent scenery, in the absence of IL_4 [387], with the help of CD80 [401] or CD16 [339]. IL_1 directs T cells stimulated by SEB (staphylococcal enterotoxin B) SA to differentiate into Th1 T cells [504]. Instead, IL_4, IL_{10}, and IL_{13} not only inhibit Th1 T cell growth, but also considerably reduce IL_{12} production from macrophages [606], while IL_{10} blocks IL production from Th1 lymphocytes at a transcriptional level and induces LC toleration [139]. The IL local setting therefore takes a decisive role in selecting the predominant subset: IL_4 modulates Th2 T cell differentiation, IFN-γ that of Th1 cells [644], taking into account that NK-cell deficiency, quantitative or functional, can promote poor IFN-γ production [77]. In this setting, *the Th2 T cell prevalence could be the key of aberrant and increased IL_4 and IL_5 production, and of high IgE levels* specific to severe atopic subjects [413]. This approach is confirmed by IL_{13} presence only in atopic individuals [219]. However, severity of atopic disease is not a necessary prerequisite, since the same dichotomy is observed in patients with less severe disease [219, 318], but only in patients with high IgE levels [318]. A rationale could be the CLE0, CLE1, and CLE2 presence shared by IL_3–IL_5 and GM-CSF genes expressed coordinately after antigen stimulation [588, 645]. We stress that in the CB of at-risk newborn babies an IFN-γ deficient differentiation is operative, paralleling an excessive IL_4 production (Chap. 3): this etiopathological mechanism involves the IL_3–IL_5 trio spreading aimed at activating basophils, hence emphasizing B lymphocyte transformation in plasma cells-IgE and activating eosinophils as well [473].

Therefore, in humans, a greater part of Th1 T cells could be active within DTH reactions, while Th2 T cells could promote preferential IgE, IgA, IgG_1 and IgM production (with B-cell help) and stimulate IgE antibodies with IL_4, mast cells with IL_3 and IL_4 and eosinophils with IL_5. Th1 clones carry on even cytotoxic activities against APCs, including B cells: these data suggest that such Th1 T cells eliminate not only B cells functioning as APCs, but also Ig production, hence demonstrating poor helper activity. The studies discussed above have been further emphasized by recent observations in healthy individuals on Der p 1-specific Th1 clones' cytolytic potential, but not in Th2 clones from atopic subjects [456]. The data implicit from an increasing amount of experimental results underline that activated T cells produce factors useful for B-cell growth and differentiation and a parade of ILs *crucial in immune responses* [421]. As a consequence, the selection of ILs to be secreted and T subsets to be involved is determined by the pathway of immune response. These studies appear to suggest that chronic allergen stimulations may select IL_4 predominant intervention under allergen-specific Th2 cells influx in individuals whose T cells are intrinsically prone to secreting large amounts of IL_4 on activation [176]. IL_4, strongly supported by highly IL_4-producing CD4 clones, directs B lymphocyte isotype switching to IgE production, inducing in B cells the gene recombination that represents the critical premise for B-cell differentiating activation within IgE-secreting cells (Fig. 1.22). Allergen concentration can control Th1 or Th2 phenotype development from Th0 T cells [643]: actually, in animal models low-mean doses of peptides determine IFN-γ production from Th1 T cells and almost nonexistent IL_4 levels, whereas *increasing the doses implies IFN-γ vanishing and IL_4-producing cell release* [215]. Therefore, in allergen-specific T cells there will be a fine balance between APCs (DCs) producing IL_{12} and Th2 T cells of IL_4: the antigen dose will result in critically establishing the outcome to either IFN-γ or IL_4 [401].

IgE-BF (IgE Binding Factors). According to previous explanations, several gps with affinity for IgE and the capacity to regulate its synthesis (IgE-BF) could be implied, such as the inducer factor stimulating T cells to IgE-BF synthesis, EFA (enhancing factor of allergy), analogous to GEF (glycosylation enhancing factor), SFA (suppressive factor of allergy), IgE-PF (IgE potentiating factor) and IgE-SF (IgE suppressive factor). Apparently, lymphoid cells should be able to synthesize and release IgE-BF and to develop an isotype-specific regulatory function, acting directly on B cells. IgE-PFs demonstrate affinity for lectins, for their content in mannose-rich oligosaccharides, while IgE-SFs do not have affinity for

lectins, but bind peanut agglutinins. IgE-PFs are produced by T cells when GEF is present and IgE-SFs when GIF (glycosylation inhibiting factor) is present: indeed the serum of healthy, nonatopic individuals contains IgE-SFs, while IgE-PFs were found in patients with HIgES. Post-transductional glycosylation is regulated by two factors: GIF, released by CD8 cells, which inhibits both glycosylation and IgE synthesis, and GEF, released by CD4 cells, with opposite actions. GEF is a 25-kD peptide kallikrein-like enzyme, released after stimulation with *Bordetella pertussis*, Al salts or parasite antigens, and produces a kinin activating IgE-BF production. GIF is a 15-kD derivative of phosphorylated lipomodulin capable of inhibiting PLA_2 (phospholipase A2), produced by T lymphocytes stimulated in the presence of CSs (corticosteroids) or after treatment with Freund's complete adjuvant, inhibiting glycosylation of IgE-BFs secreted from T lymphocytes expressing, therefore, an antagonist activity for GEFs. GEF should be present in atopic subjects and GIF in healthy, nonatopic subjects, a surplus of IgE-PFs and a lack of IgE-SFs foster IgE production. IgE-PF is detected when GEF is formed, with which GIF competes, whereas IgE-SF counters with IgE-PF for lymphocyte differentiation with membrane IgE in B cell IgE producers. IgE-PFs were described in the murine model, primed by T cell FcεRII$^+$: in humans they can be produced by B-lymphocyte-FcεRII$^+$, breakdown products of FcεRII (soluble FcεRII or sFcεRII) [469]. Glycosylation does not appear to be a crucial moment for the binding of FcεRII-IgE but, on the contrary, nonglycosylated IgE bind to FcεRII with an affinity tenfold greater than that of native IgE. Also, receptor glycosylation influences its activity, because carbohydrates interfere with sFcεRII release from proteases. IgE glycosylation and its affinity with FcεRII seem to act in the same direction in precluding the receptor state of solubility: IgE glycosylation interferes with its binding to FcεRII, preventing proteolytic enzyme intervention, receptor glycosylation disguises its binding site; therefore IgE glycosylation appears to be in vivo a heterogeneous process, probably subjected to regulative laws present in rodents [553].

FcεRI and FcεRII/CD23. *FcεRI* (Fig. 1.19) is a high-affinity receptor for IgE antibodies, with 325 amino acid residues, thus another key player. A tetrameric complex (4 TM polypeptides), it consists of four chains, one α, mainly extracellular containing two domains characteristic of IgSF and most closely resembling that of FcγR, FcαR and poly-Ig receptor, one β with four TM segments and two identical γ domains, mostly intracellular with evident analogies with CD3 ζ chain [553]. The cytoplasmic regions of α and β chains contain, as the ζ chain, more binding sites with tyrosine kinases, the α chain binds IgE, the β and γ are membrane proteins; the γ chains are required for signal transduction and metachromatic cell activation after their interactions with IgE. FcεRI is monovalent, each one binds only one IgE molecule and such binding triggers mast cell and basophil degranulation and release of mediators responsible for immediate hypersensitivity reactions [176, 358]. PBMCs of nonatopic individuals express the receptor in, on average, 18% of non-IgE-binding cases; the reverse is true for atopics: such differences depend on IgE levels [452]. Zhu et al [684] have reported a strategy that takes advantage of the natural capacity of FcεRIIb to inhibit the allergenic activity of FcεRI. The FcεRI-mediated activation pathways are modulated by an inhibitory receptor such as the IgG receptor FcεRIIb. The allergen-specific IgGs produced in response to immunization have formed complexes with allergens, which can, in turn, form a bridge between FcεRI and FcεRIIb with 320 amino acid residues. Both receptors are expressed on mast cells and basophils; the Fc fragment of IgG in the immune complex binds to FcεRIIb, whereas the allergen binds to IgE, which is already bound to adjacent FcεRI. The formation of this bridge induces the aggregation of activating FcεRI with inhibitory FcεRIIb, which inhibits the activation pathways activated by FcεRI [259, 684].

FcεRII (Fig. 1.19) or CD23 [259], a counter receptor of CD21 [16], is a *low-affinity receptor* different from FcεRI for its structure, MW (70–83 kD) and affinity of binding IgE antibodies; it is the only known antibody receptor not belonging to IgSF. The TM receptor for IgE antibodies, with the support of CD21, can perform different functions, either IgE-mediated, above all IgE synthesis regulation, or non-IgE-mediated such as B-cell survival in GCs, maturation of pre-thymocytes, proliferation of myeloid precursors, antigen presentation to T lymphocytes in association with class II HLA molecules [16] and B-cell activation [259]. CD23 is included among the cytotoxicity mechanisms and the IgE-dependent release of inflammatory mediators from eosinophils, macrophages and platelets [259]. It can also be involved in adhesion interactions with epithelial cells, due to CD62E and CD62P selectins. The latter is a membrane protein associated with granules and has a domain with analogies with liver lectins [341]. Among the ligands there are platelet CD41, CD21 and IFN-α [349]. Interacting with CD11b/CD18 of monocyte-macrophage CD21, and EBV of B cells, CD23 can increase its central role in positive IgE regulation and consequently in inflammatory diseases [16]. The two diverse molecular forms, FcεRIIa and FcεRIIb, dictated by distinct exons, differ in their intracytoplasmic portion by 6/7 N-terminal amino acids [4]. *FcεRIIa* is expressed on mature B cells and FcεRIIb on IL_4-activated cells (Table 1.3) and on monocytes of nonatopic donors [452]. CD23 is able to form dimers and/or trimers, which may account for the increased IgE avidity [384]. Its C-type lectin structure does not bind to IgE molecule carbohydrates [553].

FcεRIIa, inducible also by IL_{13} [437], seems to play a role in IgE regulation with obvious effects on atopic disease [553]. Such a receptor confined to mIgM and mIgD is no longer expressed by B lymphocytes that have undergone isotype switching.

Soluble fragments of FcεRII (*sFcεRII*), 37 kD, can be involved in IgE regulation, thus initiating both humoral and CMI responses, while fragments containing the C-terminal tail behave as IgE-BFs, and sFcεRII may provide a mechanism for sIgE activation on committed B lymphocytes activated in a maturational stage subsequent to that of IL_4. All sFcεRII retain binding specificity for IgE, but smaller fragments bind with lower affinity than intact molecules: they can stimulate growth and differentiation of several cell precursors such as plasma cells, T cells, basophils regulation and thymocyte maturation [47].

Demonstration of type II receptors has shown that allergic reactions can be due to interactions between allergens and *IgE present not only on mast cells and basophils, but also on several additional cells* [174]. We stress that a common problem of atopic patients is an unremitting overproduction of IgE and, as soon as they replete high-affinity receptors, they also occupy low-affinity receptors, as evidenced by the FcεRIIb increase observed in such patients [174].

In the animal model, IgE synthesis can be enhanced by a variety of factors, including adjuvants, insoluble molecules such as $AlOH_3$, SiO_2, certain organisms including *Bordetella pertussis* and mycobacteria, parasite extracts and perhaps gasoline residues, in addition more specifically to massive doses of X-rays, ablation of the thymus and spleen and immunosuppressive drugs. On the contrary, low doses of X irradiation or of radiomimetic drugs have the paradoxical effect of enhancing IgE synthesis by interfering with CD8 production, whereas complete Freund's adjuvant, employed to accelerate isotype switch from IgM to IgG, has little effect on IgE. However, if antigens are coupled with mycobacteria, it may even suppress IgE antibodies [45].

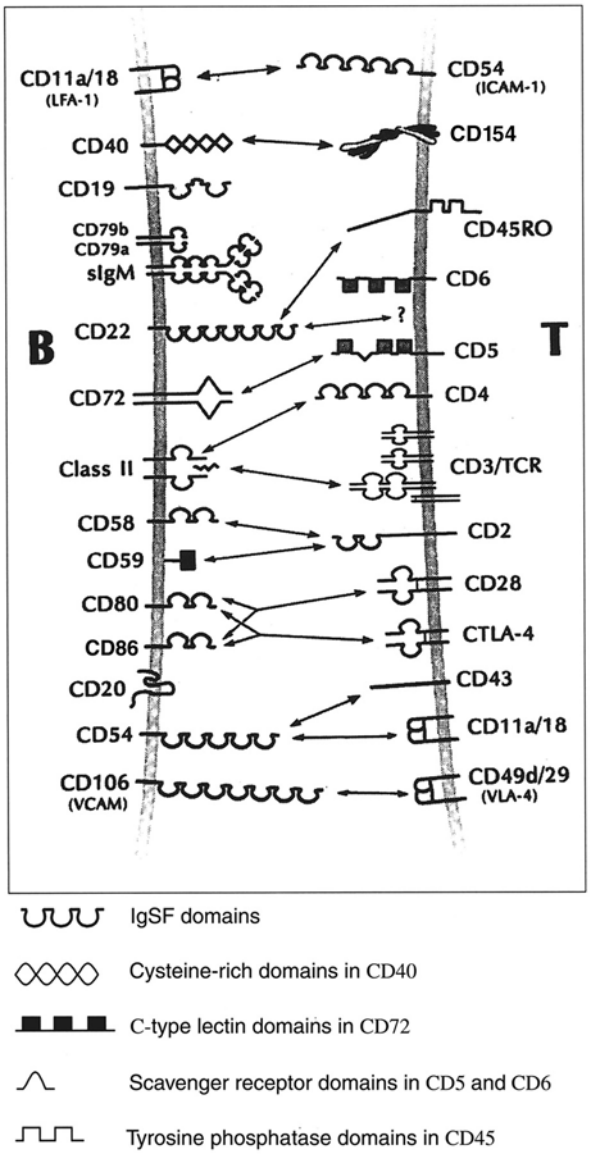


Fig. 1.45. Membrane interactions between CD4 T lymphocytes and activated B cells. *IgSF* immunoglobulin superfamily

Second Signal

The T-cell to B-cell *cognate interaction* or *associative recognition* promotes B-cell activation, proliferation and differentiation in plasma cells–IgE secretion (Fig. 1.58): T lymphocyte intervention in IgE production can be inhibited by IFN-ɣ as demonstrated in vivo from anti-IL_4 and anti IFN-ɣ antibodies [456, 473]. In this sense, activated T cells provide help for B cells stimulated by antigen molecules in two ways, either secreting ILs that regulate B-cell differentiation or resulting from an associative recognition, the HLA-dependent *cell–cell cognate interaction* (Fig. 1.45). A significant series of studies has been conducted on a tangible aspect of cooperation between T and B cells: the IgE synthesis elicited by plasma cells and IgE has led to the formation of 2,000 IgE epitope-specific/s over a few days. T lymphocytes are indispensable for IgE production and control, because B cells alone do not produce IgE, not even if stimulated [456, 473]. When *conjugated T and B lymphocytes* are cultured in close contact in the same compartment, the induction of IgE synthesis involves an associative recognition between T and B cells through a tripartite complex, allowing locus C_H rearrangement and mRNA intervention, leading to H chain formation so that IgE protein expresses ε germlines. Before this interaction, an IL profile including IL_4 in the microenvironment cannot stimulate B_{IgM} precursors to switch into B_{IgE}. However, conjugated T-B cells increase IL_4 receptor expression on B cell membranes. Consequently, Th2 lymphocytes release IL_4 and other ILs, inducing B lymphocytes to synthesize IgE [473]. The role of T cells in IgE synthesis has been further confirmed, because, in atopic patients, clones of Der p 1-specific $CD4^+$ lymphocytes occasion high IL_4 levels with a powerful amplifying effect on IgE synthesis in comparison with non-Der p

1-specific clones or with healthy individuals, similarly to CD4 clones obtained from patients with parasitosis [473]. In perspective, chronic stimulation by specific antigens seems to be able to select antigen-specific T cells in patients with T repertoires predominantly secreting high IL_4 concentrations during their activation [456].

More specific interactions between T and B cells occur following the expression of CD40L T-cell ligand, = CD154 for the CD40 molecule, surface gp of 50 kD (Fig. 1.22a), homologous to NGF and TNF-α receptors, expressed on membranes of all B cells, macrophages, FDCs and other cells able to evoke responses of activated T lymphocytes, monocytes, basophils, endothelial and epithelial cells, fibroblasts, DCs and other T cells [600]. CD154 or CD40L is similar to a 30- to 39-kD membrane gp, formed by 263 amino acids, homologous to TNF and expressed by activated CD4 T cells [446]. Subsequently to this interaction, activated B cells proliferate and acquire APC activity, generating CD80 and CD86 surface molecules, counter receptors of CD28 of T cells, which in turn also express CD152 [243]. However, CD86, because of an earlier expression, its higher levels and pro-Th2 T cells and -IL_4 orientation, may play a key part in IL_4 production. *CD40–CD154 binding amplified by CD80 and CD86 binding* [446] *is the prime stimulus for the second signal,* which plays a crucial role in responsive B cells in IgE synthesis. The ensuing IL_4 secretion and BcR binding directs gene transcription and isotype switching from IgM to IgE [132]: if CD40–CD154 are expressed on activated T cells, IL_{12} is also synthesized [524]. After the significant identification that mutations of the gene encoding CD154 on chromosome *Xq26.3–q27.1* are responsible for HIgMS, with absent IgE synthesis in the presence of IL_4 [442], studies have shown that decreased expression of CD154 inhibits the switching from IgM to IgG in 77% of patients with CVID [147] and HIgMS [442]. CD2–CD58 binding, as described above, fulfills a role in IgE induction independent of CD40–CD154: indeed the role of this pair assumes great importance in IgE production at the level of the lamina propria, where T and B lymphocytes have a major expression of CD2 and, respectively, of CD58 in comparison with their subsets in peripheral blood [600].

Second-type B-cell activating signals act in synergy with IL_4 and T cell-independent systems in the induction of IgE synthesis [205, 383, 437].

- *IL_4-dependent IgE synthesis* by *non-cognate* T–B-cell interactions has been reported in which TcR fails to recognize the HLA–peptide complex, for example, an inducible molecule associated with membranes of T $CD4^+$ clones is apparently capable of directing the B lymphocyte differentiating process toward IgE synthesis [455]. However, a latency time of 2–4 days is necessary for T-cell-activated IL_4 to deliver the signal to undergo class switching to B cells, which can drive B cells to secrete IgE independently of T cells [205]. Such data indicate that metachromatic cells select ILs able to stimulate B cells activated by T cells to produce other ILs as well as Th2 lymphocytes. Similarly, splenic non-T–non B cells produce IL_4 as a result of cross-binding to FcεRI or of exposure to IL_3 [205]. The T-cell role has been emphasized by their ability to produce ILs, thus stimulating IgE-mediated reactions by a direct action on metachromatic cells [496].
- *Stimulation with EBV and IL_4* has been shown to elicit IgE synthesis in human B cells. B_{IgE} cells obtained by activation with EBV and IL_4 contain both mature and germline Cε transcripts, whereas IL_4 alone yields only sterile μ transcripts [233].
- *IgE production* can also be promoted by direct activators of B cells, which, costimulated with rIL_4 and CD40, or anti-CD40 mABs mimicking in vitro CD154 interactions, are able to synthesize high IgE levels [233]. In nonatopic subjects, IgE synthesis is stimulated after addition of anti-CD40 and rIL_4 [294] and accelerated by adding IL_{10} simultaneously to IL_6 production [590]. However, IL_4 and anti-CD40 alone produce IgE in modest amounts (<0.1 ng/ml) [590], and IL_4 antagonizes IgE synthesis induced by B cells activated by anti-CD40 [400]. Proliferation of preactivated B cells is positively affected by IL_{10} also inhibiting Th1 cells and related ILs [480]: such processes, independent of gene restriction, could explain polyclonal IgE formation, in addition to sIgE during, for example, strong allergic reactions [176]. CD40 effects are likely to be important under physiological conditions, considering that soluble CD40 (sCD40) inhibits in vitro T-cell-dependent IgE synthesis after an appropriate second signal [132], while CD154 drives IgE production in IL_4-treated B cells [132] (Fig. 1.46) [456]. In this scenario, crucial data appear to play a significant role, since it is well known that CD154 expressed by circulating basophils and mast cells also of skin and lungs [174] and from eosinophils [175] together with CD40 [407] modulate *IgE synthesis* with the immediate implication that it takes place *not only in germinal centers of lymph nodes, but also in peripheral tissues.*
- *IgE may also be involved* in indirect mechanisms such as enhancement of APC antigen-capturing capacity: IgE-mediated presentation of a great number of allergens by interacting with FcεRI and FcεRII can activate the immune system in a continuous way, even with low allergen concentrations, thus leading to an overproduction of both IgE and Th2 cells, which in turn will induce more B cells to a switch recombination, also explaining why certain atopic patients worsen from a monosensitization to a multiple sensitization [383].
- *PBMCs* in the presence of IL_4 and hydrocortisone trigger IgE synthesis [205] and by means of FcεRI can be involved in an IgE-mediated presentation with a T-independent mechanism [384].
- *IL_{13}* is known to enhance IgE synthesis even in the absence of IL_4 [437], thus demonstrating that additional soluble mediators, as yet not well defined, are potentially involved in IgE regulation.

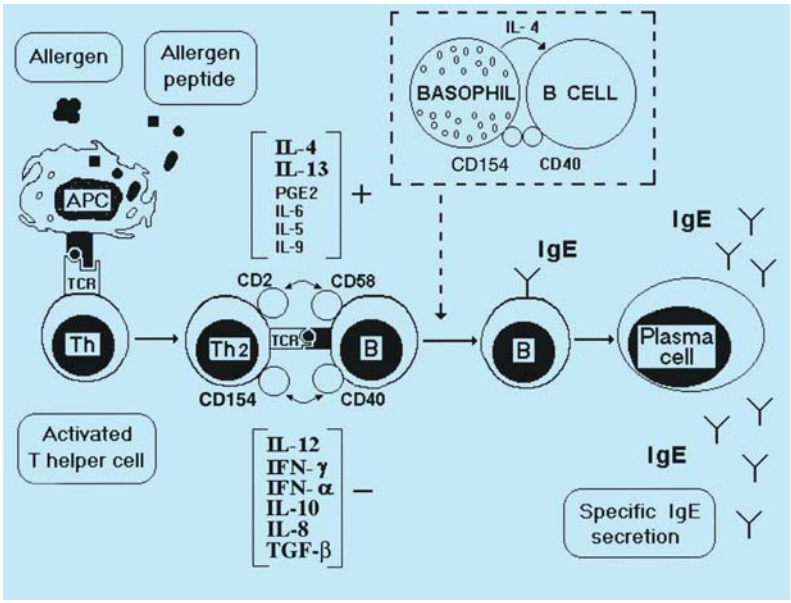


Fig. 1.46. Cellular and molecular interactions involved in the modulation of IgE antibody production. Schematic representation of the physiological aspects of IgE synthesis. (Modified from [456])

Further signals leading to isotype conversion with expression of high IgE levels are modulated by IL_5, a growth factor particularly of eosinophils, and IL_6. In a speculative way these findings suggest that IgE production can also be driven independently of T cells and that the above messengers have in common the capacity of activating the isotype switching machinery in B lymphocytes whose ε gene was activated by a transcriptional process and/or made accessible by IL_4 [176].

Immune Memory

There is wide agreement with the belief that lymphocytes preserve the memory of their first antigen encounter: when antigen is encountered again, IgE response will be more rapid and vigorous, even after decades. This reaction is due to specific CD45RO deriving from the proliferation of a specific clone following the priming, an event which induces a further expansion of production in children without an atopic background of IgG and IgA antibodies [101, 688]; such an event also explains why *vaccine booster doses* are effective. About 50%–60% of CD45 isoforms are associated with the 180-kD form, CD45RO, memory cells, and 40%–50% with the 220-kD form, CD45RA, virgin cells that are functionally inactive in the absence of antigen-driven stimulation. The RA isoform of CD45 is expressed on newly formed T cells that have not yet encountered specific antigen; during activation by exposure to specific antigen, the RA isoform is stably modulated to RO, a process that involves differential, post-transcriptional processing of the CD45 mRNA turning into CD45RO after IL_2-dependent T-cell activation. However, CD45RA and CD45RO, rather than being markers for distinct naive and memory Th cell populations [627], are probably respective markers for reversible resting and activated states; CD29 is expressed two to four times more on several adhesion molecules in addition to CD45RO cell surface than on CD45RA [23]. This cellular basis consists in antigen-specific lymphocyte expansion during primary responses, so that sensitized cells play a specific function, announcing in the bloodstream the occurred recognition, leaving a greater number of T and B cells [101]. Other memory cells undergo a rapid turnover: activated after stimulation by antigen-specific $CD4^+$ T cells are engaged to respond to a wide spectrum of APCs, and to secrete ILs and high IgG levels [326]. Memory T and B cells recirculate via peripheral blood and lymphoid organs (Fig. 1.11), and besides some cells with a short life span there are others with a long life span: their characteristics are summarized in Table 1.42 [688]. Since cytopathic viruses cause damage to fetuses and neonates when the immune protection necessary to survive is absent, particularly during a critical immunoincompetent period, mothers protect their babies passively transferring neutralizing antibodies [688]. Which signals lead to memory cell development at birth vs effector cells? One possible answer is that large antigen amounts plus costimulation in an environment dominated by inflammation may result in differentiation to effector cells, whereas the absence of an inflammatory milieu or ILs favor memory cell generation: according to this model, T cells activated in early stages of an infection would differentiate into effectors, whereas during later stages with a reduced antigen load memory T cells would prevail [7].

One theory on long-lived memory cells admits a constant stimulation of T-cell clones, according to three procedures: contact with antigens (and/or pathogens), new encounter with antigens, then with antigens bound to DCs with reconstitution of supplies [101]. According

Table 1.42. Characteristics of memory B and T lymphocytes

Characteristics	Memory B lymphocytes		Memory T lymphocytes	
	Resting	Activated	Resting	Activated
Location/migration	Recirculation blood → LN (via HEV)	Associated with persisting antigens in lymphoid organs and bone marrow	Spleen and blood possible migration blood → LN (via HEV)	Migration through tissues
Function	Secondary reaction (challenge)	Maintenance of memory, IgG levels	Secondary reaction (challenge)	Immediate killing of infected cells in periphery
Type of response	Delayed	Immediate	Delayed	Immediate
Proliferation	No	Yes	No	Perhaps yes
Site of proliferation		Germinal center		Perhaps in lymphoid organs associated with antigens
Antigen dependence	No (poor)	Yes	No	Yes
Site of antigen persistence	FDC		Unknown	

Modified from [688].
FDC follicular dendritic cells, *LN* lymph nodes.

to another hypothesis, which is gradually being widely received, memory cells do not require further contacts for their survival [7, 56, 216, 385]; hence, if a memory cell prerequisite is a persistent stimulation, one could argue that T-cell memory may not exist [56]. As a consequence, these studies have not proved that there are two types of memory, one short-term and one long-term memory, even if in 1847 a measles epidemic spared the aged infected 65 before years are the Faroe Islands [407], thus confirming earlier observations made by Thucydides. New findings are supported by demonstrations that memory CD8 CTLs persist indefinitely when associated with CD44 homing receptor, ensuring long-type memory also independently of steady antigen stimulation [291]. CD8 CTLs from a previous reaction to a given virus may in the future be reactivated and contribute to natural resistance by cross-reacting to another putatively unrelated infectious agent [512]. CD8 T-cell memory stimulation, long persisting also in the absence of the original antigen, can thereby depend not on a new encounter with known antigens, but with wholly different antigens, only if they have a cross-reaction with the first ones. Therefore CD8 long-term memory persists without antigen stimulation [216, 385]. Differential expression of memory phenotype markers CD44, CD122, and Ly6C by SOCS-1 (suppressor of cytokine signaling) IL_{15}^{-} $CD8^{+}$ lymphocytes suggests that multiple signals contributed to the memory cell differentiation program. However, the acquisition of the memory phenotype by SOCS-1-deficient $CD8^{+}$ lymphocytes does not require prior antigenic stimulation, but requires the presence of activated T cells [441].

Another particularity of CD45RO cells is to be exposed to an antigen universe precluded to virgin CD45RA cells, which can respond to antigen presented by APCs since they express increased levels of adhesion molecules [101], or on B cells exceedingly efficient at capturing even low-density antigens, unable to stimulate virgin cells [345]. Secondary immune responses are faster, with more Igs and higher affinity, provided that they are T-dependent [310]. B lymphocytes, after differentiation into effector cells or IgE producers with CD154 cooperation [71], return to a quiescent state, constituting immune memory, with mIgs and properties slightly unrelated to their ascendant with which they can interact, at the time of activation, although in a different stage of maturation; their long-term survival depends on the persistence of antigens retained in CICs by FDC networks [337].

Recent data support the so-called *decreasing potential hypothesis,* suggesting that memory T and B cells, activated after each round of stimulation, generate fewer memory cells undergoing terminal differentiation into effector cells, a phenomenon that in B lymphocytes could be more accentuated in mature cells (Fig. 1.47) [309]. Consequently, the immune system will have the resources to quickly generate large numbers of effector cells to successfully eliminate pathogens [309]. Immune memory works such that infections elicit, in most cases, a state of disease only on the first contagion, whereas subsequent contacts with the same pathogens resolve without any pathological manifestation, thanks to microorganism elimination in a rapid and sound way [474]. Another area of increasing interest suggests that it is tempting to hypothesize that *endotoxin* exposure can be used as an essential adjuvant in the induction of antigen-specific T-cell memory [303].

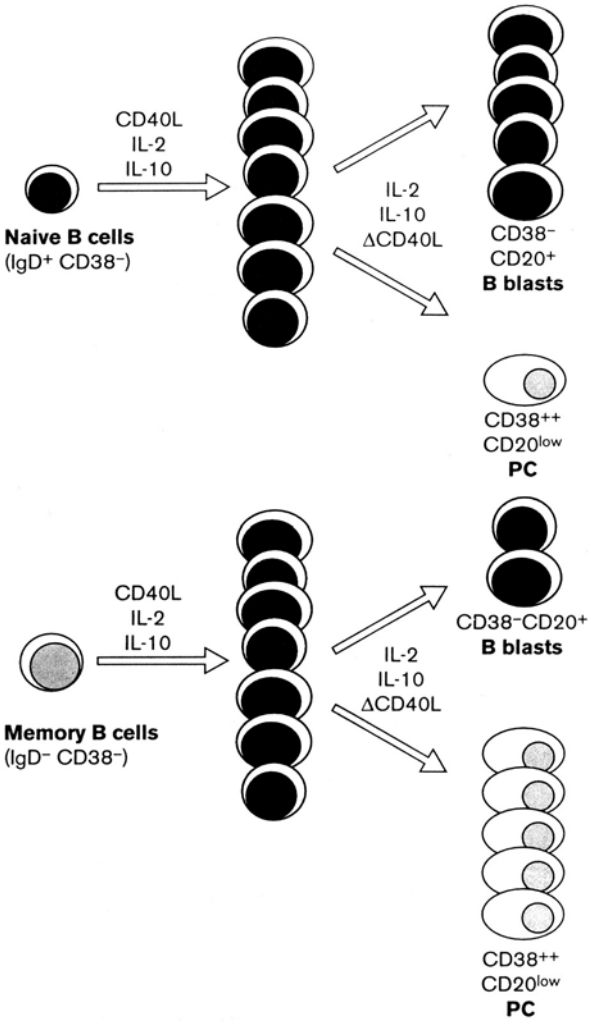


Fig. 1.47. Terminal differentiation of memory and naive B cells. Memory B cells but not naive B cells undergo terminal differentiation into plasma cells (*PC*) upon activation in vitro

Immune Responses

Not surprisingly, immunity, instead of protecting against endogenous or exogenous antigens, thus contributing to maintenance of immune homeostasis, exposes individuals to immune reactions, whose ultimate result is to damage tissues, producing clinical illness, even severe or fatal (Fig. 1.48) [34]. Such reactions are characterized in the first place by allergens according to a commonly accepted classification. Hence, hypersensitivity is a form of abnormal reactivity of the host against a non-self agent (allergen), innocuous for the majority of healthy subjects exposed to similar doses of a matching allergen. A fundamental stage of a cognitive process was the Gell and Coombs *classification system of immune reactions* [179], useful from a practical point of view for exemplification reasons. Immune reactions are particular to atopic disease and are divided into four classes, I, II, III, IV [34, 179], divided into immediate or anaphylactic, cytotoxic, IC-mediated and CMI reactions. Otherwise, the four types of reactions can overlap and almost no atopic disease is limited to only one mechanism. For example, penicillin can provoke anaphylactic shock (type I), immune hemolysis (type II), serum sickness (type III), or contact dermatitis (type IV) [470].

Immediate and Delayed Reactions

Specific studies done over the years have encouraged revisiting the Gell and Coombs historical classification. In reality, immune reactions can be subdivided into two types, immediate and delayed, according to the latency period from the appearance of allergic manifestations following the re-stimulation with the same sensitizing antigen: immediate reactions start after minutes and are IgE-mediated, while delayed reactions start after hours or days and are T-cell mediated. The main differences between these reactions, which take place expressing Th2-like ILs and, respectively, Th0 or Th1-like ILs [585], are shown in Fig. 1.49 [104]. Some perplexities have recently been aroused by focusing attention on the role of IgE antibody in DTH reactions and multifold IL interactions within inflammatory reactions. Actually, in addition to the role of IgE-mediated immediate reactions marked by effects of histamine released by mediators, recent reports have highlighted the role of DTH, characterized by release of biologically active mediators induced by IgE. The picture occurring in patients can be schematized as follows: 1. The immediate reaction; 2. A phase of relative quiescence; 3. The delayed reaction.

We underline that such distinctions have a more theoretical than practical nature, as evidenced by an almost constant association or overlapping of these two phases, not permitting a clear-cut division from a clinical point of view. Molecular studies led to recent reports on the role played by ILs, with a pattern wholly different according to reaction chronology: in acute reactions the IL_4 mRNA is very early and selectively expressed, whereas in delayed reactions IL_4, IL_5 and IFN-γ are positively regulated [105]. The practical result of the above studies is that reactions developing >24 h after allergen challenge allow for an increased number of antigen-specific T cells to invade peripheral tissues. These current investigations emphasize delayed reactions in the immune system and make it possible to elucidate the mechanisms at the base of chronic inflammation, indicating IgE heterogeneity among atopics and, indirectly, a genetically determined GPM.

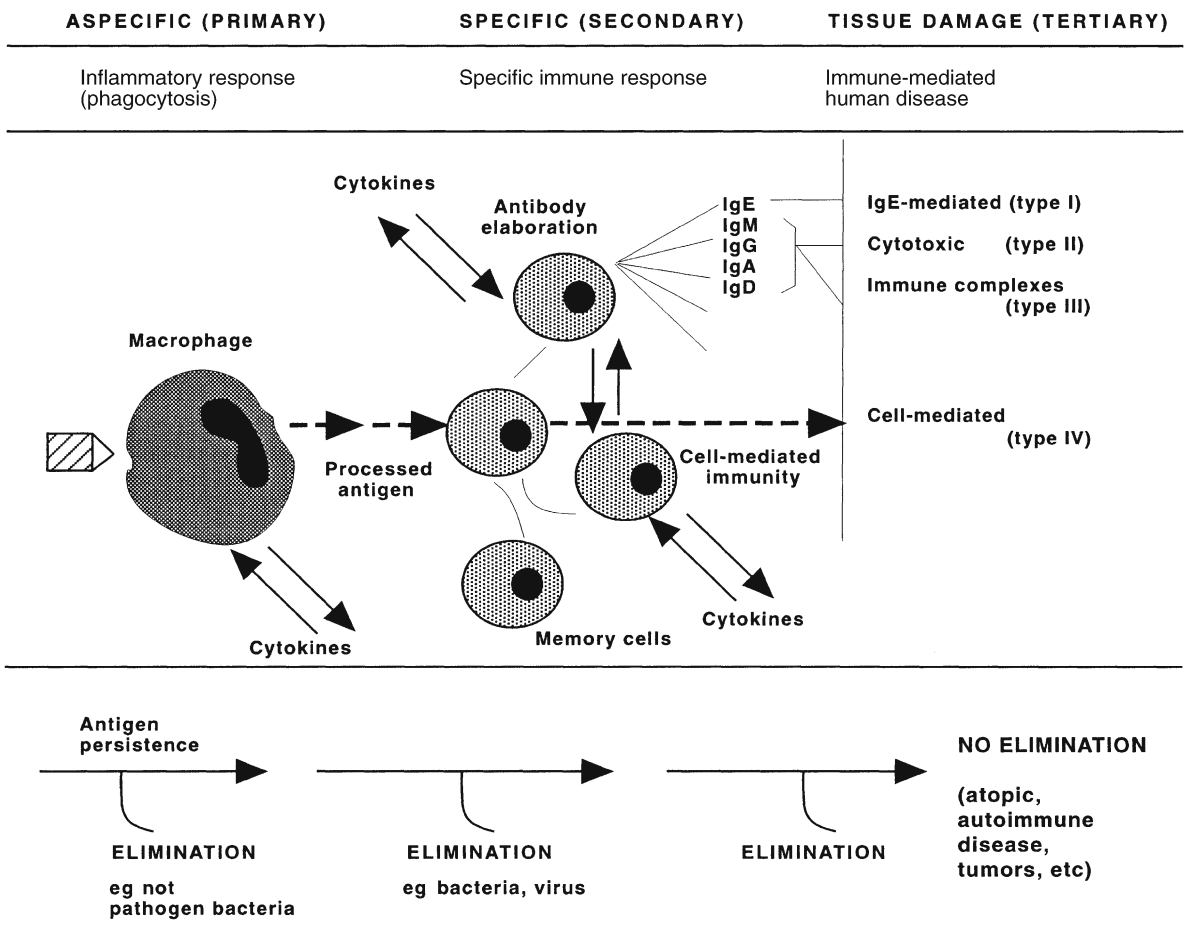


Fig. 1.48. Schematic representation of the immune reactions and the body immune capacity of eliminating foreign material. The *lower part* shows that interactions between foreign agents and the immune system may lead, depending on the antigen nature or the genetic constitution of the individual, either to elimination or persistence of the antigen with resulting alterations of immune homeostasis. (Modified from [34])

Hypersensitivity Reactions

Type I Hypersensitivity Reaction, IgE-Mediated – Anaphylactic Reactions

An immediate hypersensitivity reaction is characterized by a rapid development of clinical symptoms (a few minutes or more) when the allergen to which the patient is sensitized cross-links IgE bound to FcεRI on tissue mast cells and circulating basophils, with consequent degranulation, but IgE indosable levels are sufficient to initiate an immune reaction [367]. The sensitizing allergen, variably penetrated in the host, binds IgE fixed on the mast cell external surface. *IgE binding to FcεRI* is not wholly effective to stimulate mast cells without allergen intervention. The triggering signal of an impending immune reaction requires that the antigen is bivalent, so that it can bridge the Fab of two adjacent IgE molecules on the cell surface [349]. Signals are then activated, which, on the one hand induce transcription processes resulting in IL production, and on the other hand trigger a cascade of intracellular metabolic events, able to augment the local blood flow and to recruit a series of cells drawn to the reaction site by specific chemotactic factors.

Signaling is initiated by *mast cell activation* started by transduction signals, allowing cells to perceive changes in the extracellular environment that translate into an intracellular biochemical signal that causes an appropriate cell response (Fig. 1.50). Broad structural modifications of phospholipid components of the cell membrane follow. Biochemical events may be summarized as follows [210, 528]:

1. FcεRI cross-linking:
Interactions with cytoskeleton
2. Signal transduction:
Serine esterase activation
GPT-binding proteins
Membrane depolarization/repolarization

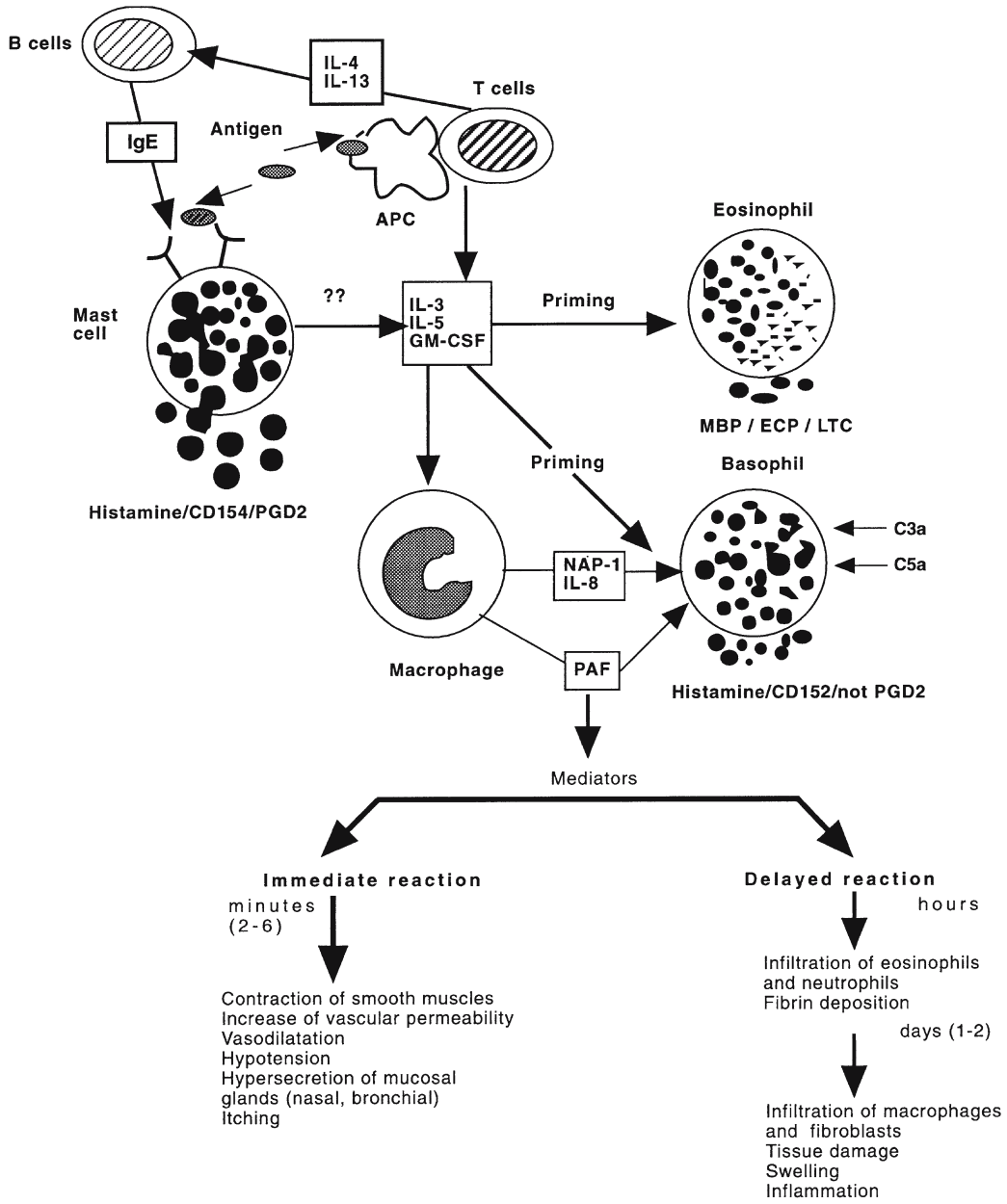


Fig. 1.49. Current hypotheses regarding the pathogenesis of immediate and late-phase reactions. Consequences of mast cell degranulation and principal differences between these reactions. *APC* antigen-presenting cell, *ECP* eosinophil cationic protein, *MBP* major basic protein, *NAP* neutrophil-activating peptide, *PAF* platelet-activating factor. (Modified from [104])

3. Signal translation and amplification (Fig. 1.51):
Development of second messengers
PLC, PLD, AA/eicosanoids, adenylate cyclase (activated by G proteins) (Fig. 1.52) with an increase in other messengers such as cAMP, cGMP (cyclic guanosine monophosphate), PKA (protein kinase A), IP3, DAG (Fig. 1.53), ion transport Ca^{++}

4. Activation of second messengers (target/effector proteins) or Ca^{++}-dependent responses:
Activation of PLC, which causes PIP2 hydrolysis, a membrane phospholipid, whose breakdown generates IP3 and DAG
Activation of PLA with AA release
Release of Ca^{++} from intracellular stores (Fig. 1.54)
5. Role of intermediate second messengers:
Activation of Ca^{++} channels and mobilization of intracellular Ca partly regulated by IP3

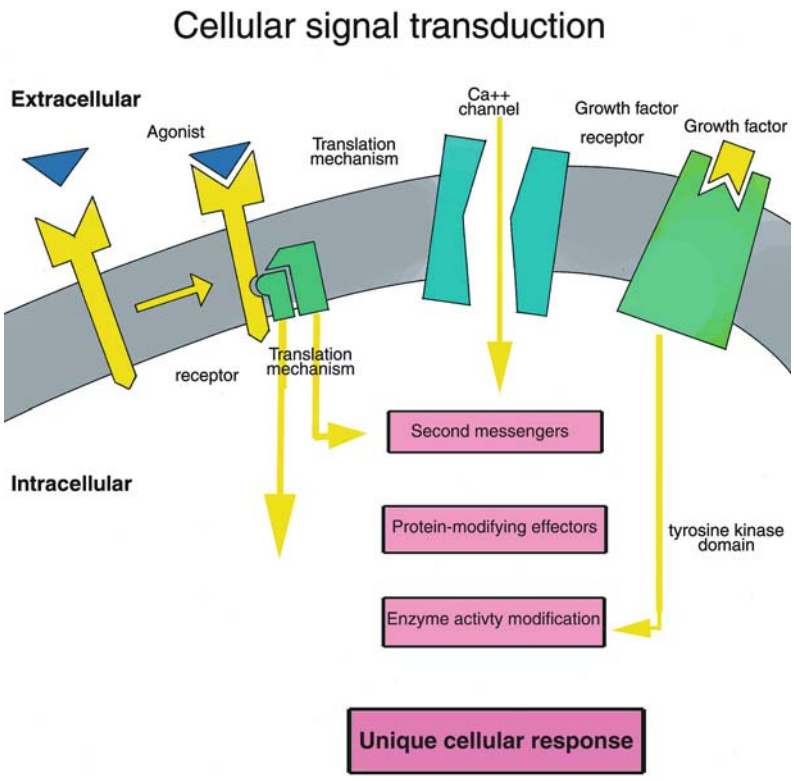


Fig. 1.50. Receptor-initiated intracellular signal transduction. Cellular signal transduction

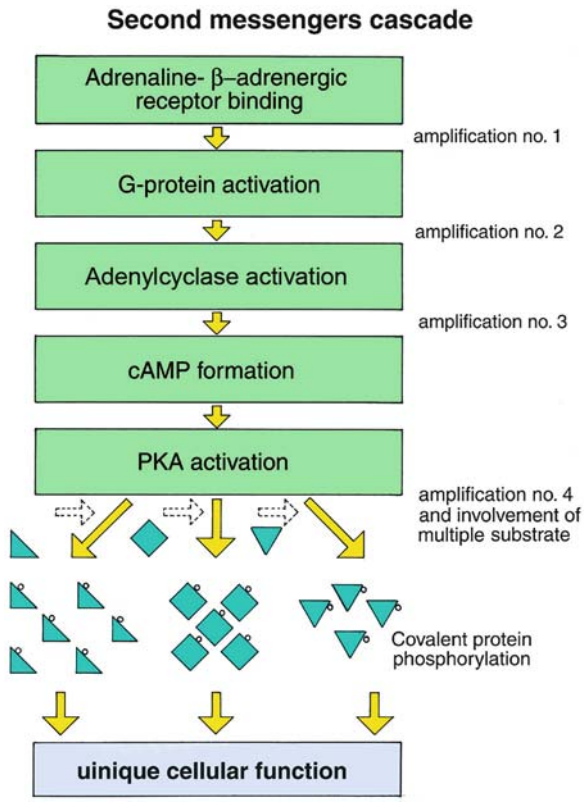


Fig. 1.51. Model of second messengers cascade. *PKA* protein kinase A

6. Effects of second messengers:
Phosphorylation and activation
Of protein kinases A and C (due to interaction with DAG)
Of calmodulin and Ca^{++}-dependent proteins altered polymerization of F-actin
7. Cellular responses:
Ca release leads to activation of glycolysis responsible for assembly of microtubules and of an ATP-dependent energy pathway due to contraction of microfilaments.
Microtubule aggregation promotes movements to the cell surface of preformed granules whose membranes fuse with plasma membranes.
Granules are then released with a mechanism of exocytosis not resulting in the loss of integrity of either the plasma membrane or granule membrane, with the support of specialized lipids called fusogens.
Granule opening leads to preformed mediator release [210, 528].

Granule release does not imply cell lysis or death. Degranulated cells regenerate and, once granule content has been revived with a de novo synthesis, are ready to resume their function.

Ca channels are of three types [29]:

- VOC (voltage-operated Ca channels), divided into L (long-lasting), T (rapid) and N (neuron) activated by different voltages
- ROC (receptor-operated Ca channels), opening in response to activation of receptors associated with these channels

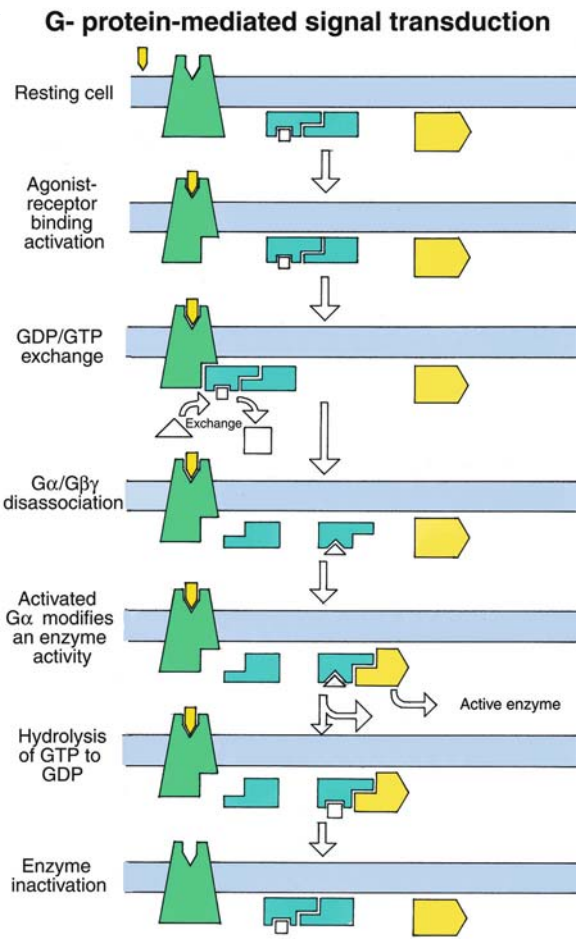


Fig. 1.52. Mechanism of G-protein-mediated signal transduction. The disassociation of the Gα/enzyme leaves Gα free to reassociate to Gβγ. *GDP* guanosine diphosphate, *GTP* guanosine triphosphate

- SMOC (second messenger-operated Ca channels)

FcεRI may activate, instead of IP3, sphingosine kinase and then sphingosine-1-phosphate, an alternative second messenger to mobilize Ca [84]. The released chemical mediators trigger the immune reaction immediate phase; meanwhile mast cells, in addition to histamine, PGs and LTs, release ILs, PAF and mediators able to induce a DTH.

Mast cell activation can also be elicited by other mechanisms [470]:

- *Immunological mechanisms:* specific IgM and IgG antibodies, anti-IgE antibodies and anaphylatoxins, MBP, IL_3, IL_5, GM-CSF, chemokines.
- *Non-IgE-mediated mechanisms* (for direct action on mast cells), such as activation of PGs, CIC-IgE, aspecific bridging due to lectins, SP, foods, drugs and a variety of chemical substances and physical agents. Mention should also be made of the degranulation effected by HRF(s) via IgE-dependent and -independent mechanisms. Non-IgE-mediated degranulation is characterized by lesser influx of Ca ions, a higher velocity (<20 s vs >5 min), a smaller LT (<18-fold) and PG (<21-fold) release and an equal histamine release in comparison with IgE-dependent degranulation [469]. However, recent results indicate that IgE-reactivity to immunoblotted human protein and IgE-dependent HRF activity

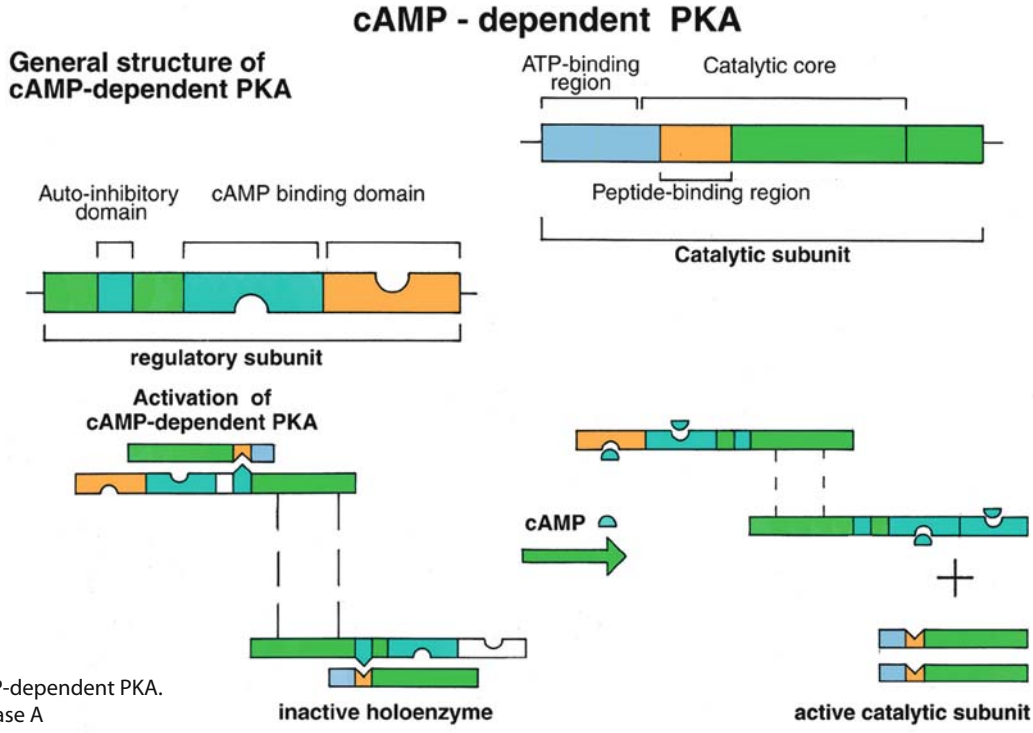


Fig. 1.53. cAMP-dependent PKA. *PKA* protein kinase A

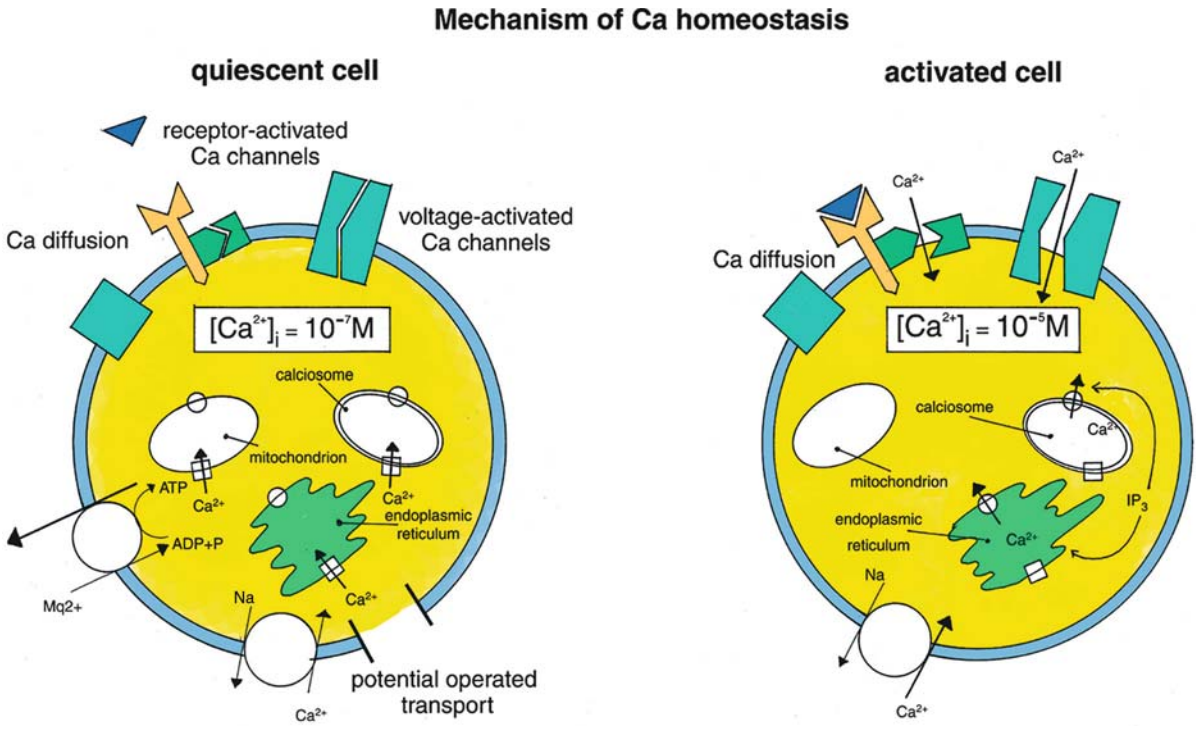


Fig. 1.54. Basic mechanism of intracellular Ca homeostasis

(IgE$^+$) are distinct entities that may co-occur in atopic patients since IgE$^+$ might be a structurally different IgE molecule which is not polymeric IgE [57].

The serum of an allergic individual can sensitize a nonallergic individual following passive transfer or Prausnitz–Küstner reaction; local hypersensitivity persists for 3–5 weeks.

Type II Hypersensitivity Reaction: Cytolytic or Cytotoxic Reactions

In type II hypersensitivity, IgG or IgM antibodies bind antigens present on target cell membranes or transported by certain molecules; antigens can be expressed on a specific cell surface (such as erythrocytes, platelets), or on exogenous (such as drugs, foods), or on haptens attached to the cell surface (CTLs and NK cells). Cytotoxic effects can be accomplished involving several effector mechanisms:

- *Activation of the whole complement cascade,* with consequent damage in various tissues, direct or indirect and subsequent cytolysis, with likely NK-cell intervention with receptors for both complement and Fc fragment of IgG. Examples include hyperacute graft rejection when a transplant recipient is preimmunized, formation of anti-erythrocyte antibodies provoking hemolysis, neutralizing enzymes, inhibiting cell receptors, etc.
- *By enhancing phagocytic cell activity* with receptors for Fc fragment of IgG, and for activated C3b are able to phagocyte target cells, such as circulating blood cells.
- *On clinical grounds* the type of reaction depends on antigen localization: on erythrocytes, hemolytic disease of the newborn from maternofetal Rh incompatibility, autoimmune or drug-induced hemolytic anemia; on several forms of leukocytes, on neutropenia, on platelet autoimmune thrombocytopenic purpura and CM-associated or drug-induced thrombocytopenia, and on other blood cell reactions due to isoimmunization.
- *The cytotoxic reaction* known as ADCC (Fig. 1.55) is performed by NK cells, macrophages and PMNs: antibodies bind to target cells through the paratope and are recognized by cytotoxic cells with FcR specific for IgG. Clinical manifestations are transfusion reactions, by Rh incompatibility, autoimmune and drug-induced [18, 454].

Type III Hypersensitivity Reaction: Immune Complex-Mediated Reactions

Circulating immune complexes of antigens and antibodies (mainly IgG) or CIC can be formed in tissues or in blood vessel walls (with an eventual deposition in the organs via the phagocyte system), overall found in antigen excess (for example, streptococcal proteins or HBsAg), with subsequent complement fixation and

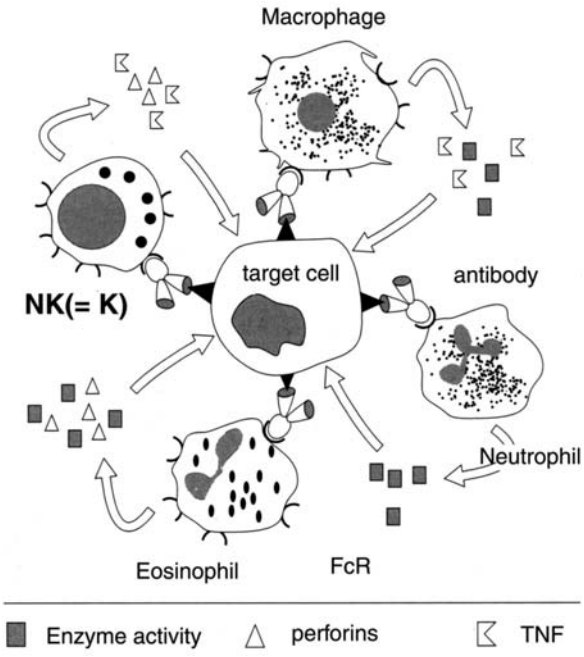


Fig. 1.55. Antibody-dependent cell-mediated cytotoxicity. *TNF* tumor necrosis factor

formation of microprecipitates at the level of small vessels. Onset of clinical manifestations is 7–14 days after primary antigen contact; following a repeated contact, the developing reaction reaches a peak intensity even in 4–6 h. When CICs are cleared in perivascular spaces, microprecipitates are formed, and if deposited in vessel walls CICs provoke vasculitis. A classic example of experimental vasculitis is the *Arthus reaction*: soluble antigen is injected subcutaneously into an animal previously sensitized to the same antigen, the antigen–antibody reaction results in high CIC local levels activating both complement and PMNs, thus provoking local inflammation. Tissue damage caused by this mechanism is in proportion to the CIC quantities and presence of vasoactive amines, which increase vascular permeability, favoring CIC deposition in tissues. First of all, there is the complement activation also increasing vascular permeability, via C2b action to activation of anaphylatoxins, and release of leukocyte lysosomal enzymes recruited by C3a, C5a, C6 and C7; then protein aggregation and lysis with vasoactive amine release; lastly activation of the prekallikrein–kallikrein system with vasoactive kinin release including bradykinin. Later complement-derived chemotactic factors recruit PMNs on the site of reaction, promoting phagocytosis and tissue damage [18].

A classic manifestation of type III immune reaction is *serum sickness,* whose relevance is presently limited, apart from some drug-induced sporadic cases (Chap. 19) or following vaccinations or sting bites. Type III reactions are also seen in infantile infections, viral (prodromic of *adenovirus* and *rhinovirus, Coxsackie,* EBV infections, infectious mononucleosis, *cytomegalovirus,* etc.), abscesses (tonsillar and abdominal abscesses, sinusitis, etc.), bacterial (mainly staphylococcus and streptococcus, mycobacteria), or chronic, in *urticarial vasculitis,* which reveals CIC with complement activation and PMN infiltration, resulting in a necrotizing vasculitis, often included in autoimmunity. We further mention polyarteritis nodosa, cryoglobulinemia, genetically determined complement deficiencies, extrinsic allergic alveolitis, allergic vasculitis such as Schönlein-Henoch purpura, nephrites and reactive syndromes from drugs and foods. Since CICs are seen both in physiological and pathological states, one cannot establish correlations between disease state and CIC levels, thus re-evaluating their pathogenetic role [18].

Type IV Hypersensitivity Reaction: CMI Reaction

A classic LPR with delayed symptoms takes up to 24–48 h after allergen contact to develop: this characteristic is different from Arthus reaction, which instead reaches the apex after a few hours. Such responses begin with lymphocytes reacting with antigens presented by APCs in association with HLA molecules. Appropriately sensitized T-lymphocytes migrate to the place where they encounter the antigen and react with target cells; while also following activation by APCs carrying antigen, other lymphocytes produce a number of ILs, often facilitating the immune response resulting in tissue damage. In reality, the scope of LPR should be that of protecting the host from intracellular parasites, such as viruses, bacteria, mycetes; however, the nature of LPR and mediator release provoke DTH.

Schematically, type I, III and IV reactions are involved in different ways in causing FA, type IV immune reaction is present in AD and in allergic contact dermatitis, type I and IV reactions in respiratory allergy, and all four in drug allergy and intolerance.

Mediators

In recent times, considerable attention has been focussed on the effector mechanisms of inflammation and has enabled us to recognize the interactions of mediators, each with potent proinflammatory properties, able to rapidly elicit the pathophysiological effects of the type I reaction. Such mediators released following mast cell degranulation are divided into primary (preformed and newly formed) and secondary (of cellular and extracellular origin) mediators [104, 210, 528, 626].

Table 1.43. Primary mediators of immediate hypersensitivity

Mediators	Structure	Origin	Functions/effects/symptoms
Preformed			
Histamine		Mast cells, basophils, platelets	VD, VP, BC, production of mucus, itching
Serotonin	5-Hydroxy-tryptamine	Mast cells, platelets, enterochromaffin cells	Specific broncho- and vaso-active effects
NCF	750 kD	Mast cells	Neutrophil chemotaxis
ECF	Tetrapeptide	Mast cells	Eosinophil chemotaxis
Proteases	Protein	Mast cells	Tissue damage, production of anaphylotoxins
Heparin	Mucopolysaccharide	Mast cells	Anticoagulant
Hydrolases	Protein	Mast cells	Function not yet clear
Newly synthesized			
PGD_2, PGE_2	AA derivatives (cyclo-oxygenase pathway)	Leukocytes, monocyte-macrophages	VD, VP, BC
LTB_4, LTC_4, LTD_4, LTE_4	AA derivatives (lipoxygenase pathway)	Leukocytes, monocyte-macrophages	Extended BC, VP
TXA_2	AA derivative (cyclo-oxygenase pathway)	Leukocytes, monocyte-macrophages	VC, BC, platelet aggregation
PAF	Phospholipid (1,000 D)	Leukocytes, macrophages, neutrophils	Tissue damage, production of anaphylotoxins

Data from [470, 626].
AA arachidonic acid, *BC* bronchoconstriction, *VC* vasoconstriction, *VD* vasodilatation, *VP* vascular permeability.

Primary Mediators

Preformed mediators, which are granule-associated with quick release, are implicated in the immediate phase of immune reaction. Among them are included: histamine, serotonin, chemotactic factors of eosinophils and neutrophils (ECF and NCF); enzymes: chymase, tryptase, callicrein, arylsulfatase, chymotrypsin, etc.; proteoglycans: heparin, etc.; acid hydrolases: β-exoglucosidase (β-glucuronidase, β-hexosaminidase, β-galactosidase), etc. (Table 1.43) [470, 626].

Histamine, from the Greek "ιστος," meaning tissue, because of its ubiquitous nature, is a dibasic vasoactive amine synthesized in metachromatic cell Golgi apparatus by decarboxylation of histidine, from where it is stored in cell granules. Normal histamine concentrations are ≤5 nmol/l in plasma, 6–24 μg/cm^3 in skin, 15–40 μg/cm^3 in lungs, and >100 mmol/l inside mast cell granules. However, histaminemia is not a valid routine test, due to its release either by basophils or mast cells and its rapid tissue turnover. Histamine has wide-ranging biological activities with an indubitable influence on allergic disease: after it is slowly dissociated from the solubilized granular matrix, histamine exercises its biological effects by binding to receptors and increases vascular permeability with subsequent fluid and plasma leakage containing C3. This is followed by tryptase and kallikrein release. Last, it drives the leukocyte diapedesis toward the site of immune reaction, where several chemotactic factors are also produced [528].

Figure 1.56 [145] shows histamine stimulated by IL_3, IL_5 and IL_8, as well as by IL_1 with consequent IL_6 production. Through negative feedback, the IL_1 increase can have an inhibiting effect on TNF-α and itself; IL_1 further potentiates histamine action on PGs and 15-HETE released by epithelial cells, while IL_3 triggers the production of histamine, IL_4 and IL_6, but has no effect on IL_2 and IFN-γ, a pattern similar to that of Th2 lymphocytes [145]. Histamine negatively influences the proliferation elicited by antigens, production of ILs and antibodies and cytolytic activity, whereas it enhances the activity of NK cells and release of mediators by other cells.

Following its release from mast cells, histamine slowly dissociates from the granule matrix to exert its large variety of effects by binding to its three receptors [210]: H_1 receptors mediate vasodilation, increase microvascular permeability acting on contractility of postcapillary venules, cause constriction of airway smooth musculature and IgE-mediated release of PGE_2 and $PGF_{2\alpha}$, generate itching, inhibit basophil chemotactic responses, while promoting that of neutrophils and eosinophils, with an increase in cAMP. Classically, *H_1 receptors* provoke the triple response consisting of *flushing, flaring*

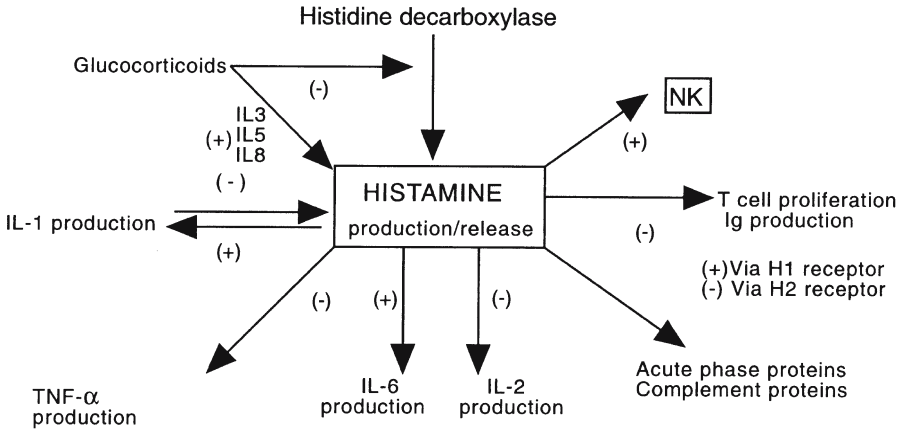


Fig. 1.56. Histamine interactions with interleukins. *Ig* immunoglobulins, *NK* NK cells. (Modified from [145])

and whealing: an intradermal injection of picomolar quantities of histamine within a few seconds yields vasodilation (flushing) by a direct effect of histamine on the microvasculature. Over the next 5–10 min the vasodilation gradually spreads away from the injection site (flaring) by an axon reflex mechanism involving antidromic neural conduction in neuropeptide-containing primary afferent nerves activated by histamine either injected or released by dermal mast cells. The final phase is the development of edema (whealing), as a consequence of contraction of endothelial cells of postcapillary venules with resulting increase of microvascular leakage, mainly due to the direct effect on blood vessel peptides released by afferent nerves.

Histamine acts on *H_2 receptors* to produce a number of negative feedback effects, including the addition of vasodilation to the triple response, reduction of C2 synthesis by monocytes and of C3 stimulated through H_1 receptors (bidirectional modulation), suppression of granulocyte-induced lysosomal enzyme release, H_2O_2 and superoxide production, and chemotactic responses from both neutrophils and eosinophils. H_2 receptors mediate gastric acid and mucus hypersecretion in the airways, the increase in cAMP cellular levels of CD8 lymphocytes and of plasma fluid leakage, as well as suppression of lymphocyte toxicity and inhibition of T-cell responses.

The concurrent stimulation of both receptors results in vasodilation, erythema, itching, bronchoconstriction, hypotension, tachycardia and headache, in addition to inhibition of chemotaxis and histamine release from basophils, and secretion of complement proteins from monocytes. However, histamine plays a positive part with H_1 receptors and a negative part with H_2 receptors, as in the paradigmatic case of APP.

H_3 receptor inhibits both histamine synthesis and release from skin and airways. Moreover, it seems that it regulates interactions between inflammatory cells and the autonomic nervous system. This receptor could play a role by inhibiting the airway cholinergic system and, in the animal model, in the control of nonadrenergic and noncholinergic bronchoconstriction, an immunoregulating action inhibiting the release of involved neurotransmitters [145].

In summary, histamine has multiple effects on various organs:

- Skin: urticaria and erythema
- Mucosa: periorbital edema, nasal congestion and itching, angioedema, pallor and cyanosis
- Upper airways: edema of oral cavity, tongue and larynx, hoarseness, stridor, sneezing, rhinorrhea
- Lower airways (with LTs and PAF): dyspnea, acute emphysema, air trapping (bronchospasm and bronchorrhea)

Among the preformed mediators there is *serotonin* (5-HT), a tryptophan metabolite and well-recognized neurotransmitter, which has effects similar to those of histamine, increasing vascular permeability and inducing airway smooth muscle constriction; 5-HT has been incriminated in the pathogenesis of migraine and has a marked pruritogenic action only if associated with PGE_2, the chemotactic mediators are the following (Table 1.43):

- NCF, distinct in two forms, one heat-stable and one heat-labile, released from mast cell granules, functions as a chemotactic factor, persisting for quite a long time, thus extending the inflammatory effects of immune reactions.
- ECF is not the principal mast cell factor and probably represents a cleavage product of a heterogeneous group of more complex oligopeptides that exhibit such activity. A more potent factor is phosphorylcholine acetyl glyceryl ether and the granule-associated enzymes.

Granule-associated enzymes include:

- *Neutral proteases,* including tryptase with a MW of 134 kD and chymase (Table 1.27). In particular TC mast cell *tryptase,* released together with histamine, cleaves C3 to yield C3a, an anaphylatoxin with degranulatory activity; the determination of tryptase levels is employed in nasal studies (Chap. 12) and in anaphylaxis diagnosis (Chap. 20). *Chymase,* at variance with tryptase, is rapidly inhibited in the extracellular environment. The evidence that it converts angiotensin I to angiotensin II and degrades bradykinin and many neu-

ropeptides suggests a role for chymase in the local control of microcirculation.

- *Additional enzymes* include kallikrein, also generated by TC mast cells, which releases several kinins with inflammatory activity from kininogen proteins including bradykinin (see "Type III Immune Reaction", p. 128), eliciting powerful effects on vasodilation, vascular permeability and airway smooth muscle. Intradermal injection provokes a triple response of a lesser degree compared to histamine not inhibited by antihistamines, equally to chymotrypsin and trypsin. Arylsulfatase has a limited capacity of regulating mediator release [418].

Proteoglycans, also of mast cell origin, found in secretory granules of many hemopoietic cells, essentially include *heparin,* with the following biological activities, in addition to the classic anticoagulant activity:

- Amplifies complement alternative pathway.
- Inhibits complement classic pathway activation.
- Stabilizes and enhances mast cell tryptase, which can express its catalytic activity.
- Potentiates C1-esterase inhibitor.
- Plays important anti-inflammatory roles by inhibiting eosinophil basic proteins provoking respiratory mucosa basement membrane thickening and airway smooth muscle hypertrophy, essential aspects of chronic asthma, whereas the inhibition caused by a powerful antidegranulating action exerted on mast cells by β_2-agonists probably leads to a breaking point in the equilibrium between inflammatory and anti-inflammatory factors (Chap. 11). Furthermore, in scarring areas, where mast cells are hyperplastic, increased heparin release improves tissue repair and the fibroblast action of structural remodeling, augments neutrophil elastase activity and recruits capillary endothelial cells into the area.

The *acid hydrolases,* present also in lysosomal granules of other inflammatory cells, include β-galactosidase, β-glucuronidase and β-hexosaminidase.

Newly synthesized mediators (membrane-derived) include [210]: PAF, PGs and LTs to which, together with TX, are ascribed DTH symptoms. Returning to mast cell degranulation, phosphatidylcholine is metabolized into lipophosphatidylcholine by PLA_2 activated by Ca, with subsequent AA release which gives rise to metabolic derivatives, the *eicosanoids.* AA is metabolized either by the 6-lipoxygenase pathway leading to LT synthesis, or by the cyclooxygenase pathway, generating PGs, PGI_2 (prostacyclin) and TX. Figure 1.57 shows how PGs and LTs derive from AA metabolism.

Biological activities of the following products of the *cyclooxygenase pathway* (Table 1.43) include:

- $PGD_{2\alpha}$: airway smooth muscle constriction, inhibition of platelet adherence and aggregation, activation of adenylate cyclase, virtual induction and increase in basophil mediator release, bronchoconstriction, facial erythema, nasal congestion
- PGD_2/I_2: vasodilation and increase in microvascular permeability, suppression of leukocyte functions
- PGE_2: bronchodilation, peripheral vasodilation and increase in microvascular permeability, inhibition of metachromatic cell mediator release, functional suppression of lymphocytes and PMNs, stimulation of pituitary function with rapid release of luteinizing hormone (LH), and conditioning of primary afferent neurones
- $PGF_{2\alpha}$: airway smooth muscle constriction, small vessel and pulmonary vasculature constriction, and stimulation of airway mucus hypersecretion
- PGI_2: pulmonary vasodilation, suppression of platelet aggregation in collaboration with NO, activation of adenylate cyclase and airway smooth muscle release, tachycardia, hypotension, facial erythema
- TX (A_2): constriction of microvasculature, stimulation of platelet adherence and aggregation [626]

Primary actions of diverse products of the *5-lipoxygenase pathway:*

- LTB_4: stimulation of leukocyte chemotaxis and activation, increased leukocyte adhesion to endothelium, functional suppression of T lymphocytes, activation of NK cells, mobilization of CA^{++} deposits, stimulation of $O_2^{\cdot -}$ synthesis, eosinophil chemotaxis and keratinocyte proliferation, infiltration of neutrophils into skin
- Cysteinyl products, powerful spasmogens of airway smooth muscles:

– LTC_4, LTD_4 and LTE_4: airway smooth muscle constriction, bronchoconstriction 200-fold to 20,000-fold more potent than histamine, vasodilation and increased microvascular permeability, mucus hypersecretion and electrolyte secretion, stimulation of gastric acid secretion, depression of myocardial contractility, mucociliary clearance impairment, inflammatory cell recruitment, and potent eosinophil chemoattractant, increased IL_1 production by monocytes, peripheral contraction, skin wheal and flare reactions, as well as LTC_4 initiation of LH release and LTE_4, BHR induction [528].

PAF, a vasoactive mediator released by numerous cells including mast cells, monocyte-macrophages, eosinophils, neutrophils, fibroblasts, vascular endothelial cells and platelets, causes a rapid sequestration of airway platelets, with TXA_2 secretion. PAF has a bronchoconstrictor activity, by recruiting and activating eosinophils and neutrophils, also because they can bind to their receptors, amplifying CD11a–CD18 and CD11b–CD18 expression and consequently both cells' adhesion to endothelium. Other biological effects include an increase in microvascular permeability, cytotoxicity, generation of $O_2^{\cdot -}$, as well as of cationic proteins and potent mediators. PAF therefore plays a prominent role in the pathogenesis of atopic disease, in addition to rapidly inducing hypotension and depressing myocardial contractility [626]. The proof of PAF pathogenicity and immunogenicity is shown by the complete defect of PAF-acetyl hydrolase, due to a mutation of exon 9, in 4% of the Japanese population, 27% of which has the heterozygote trait [535].

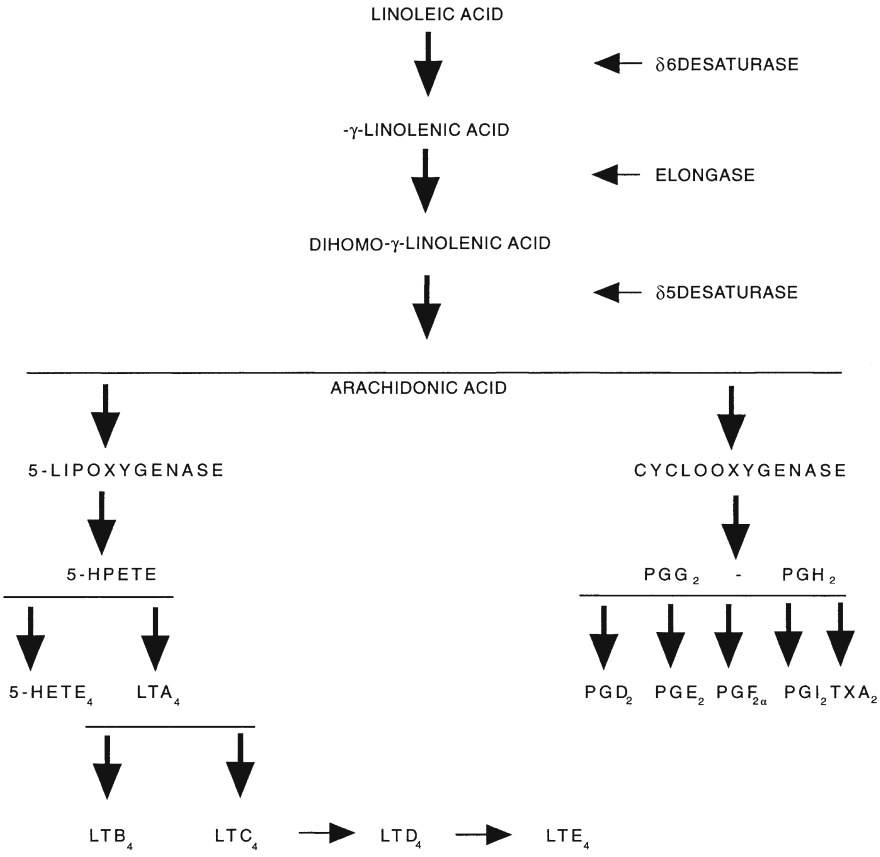


Fig. 1.57. Metabolic pathways of arachidonic acid. *HPETE* 12-hydroperoxy-eicosatetraenoic acid, *HETE* 12-hydroxyeicosatetraenoic acid, *PG* prostaglandin, *LT* leukotriene, *TX* thromboxane

Recent studies suggest that SP, a polypeptide with 11 amino acid residues, augments vascular leakage, stimulates mucus secretion in human bronchi, contracts bronchiolar smooth muscles, degranulates mast cells and is 100-fold more potent than histamine in eliciting wheal and flare reactions when injected subcutaneously [104].

Secondary Mediators

Secondary mediators are often released from primary mediators:

- *Mediators of cellular origin* released from neutrophils (toxic products of O_2, TXA_2, HETEs, LTB_4, LTC_4), eosinophils (MBP, ECP, PGE_2, HETEs, LTB_4, LTC_4), platelets (PF4, TXA_2), monocyte-macrophages, etc.
- *Mediators of extracellular origin,* from activation of complement, and of the following systems: coagulation, fibrinolytic, and kininogen-kinins

The inflammatory mediators can be also divided into:

- *Vasoactive mediators* (histamine, PGs, PAF, LTC_4, LTD_4 and LTE_4) active in the immediate phase.
- *Chemotactic mediators* (ECF-A, NCF-A, 5-HETE, LTB_4) active in the late phase (with recruitment of neutrophils, etc.) and inflammatory phase (with recruitment of eosinophils and monocytes) [626].

Several drugs inhibit mast cell degranulation and/or AA metabolism (Fig. 1.57), with very significant implications in the treatment of atopic disease [643]:

- CSs inhibit the ILs and, via lipomodulin (GIF is a fragment), inhibit PLA_2 and as a consequence AA separation from phospholipids.
- ASA and NSAIDS inhibit processes regulated by cyclo-oxygenase (they enter the cycle before the enzyme), thus stopping PG and TX formation, but not that of LTs since they augment the lipoxygenase pathway by compensation.
- Theophylline inhibits PDE-induced cAMP increase, thus down-regulating mast cell degranulation.
- β-Mimetic agents awaken the same effects because they increase cAMP levels.

Mechanisms of Cell Adhesion: Interleukins and Adhesion Molecules

As many as 51 different mostly T-cell-derived ILs of which 33 are ILs with their receptors have been identified so far (Table 1.5) as well IL receptors and signaling (Table 1.32). They comprise a heterogeneous group of intercellular regulatory proteins, with a MW of 8–140 kD ($IL_{12}R$ chain), often glycosylated, characterized by a very different biological activity and pleiotropic functions, produced singularly or associated. They were

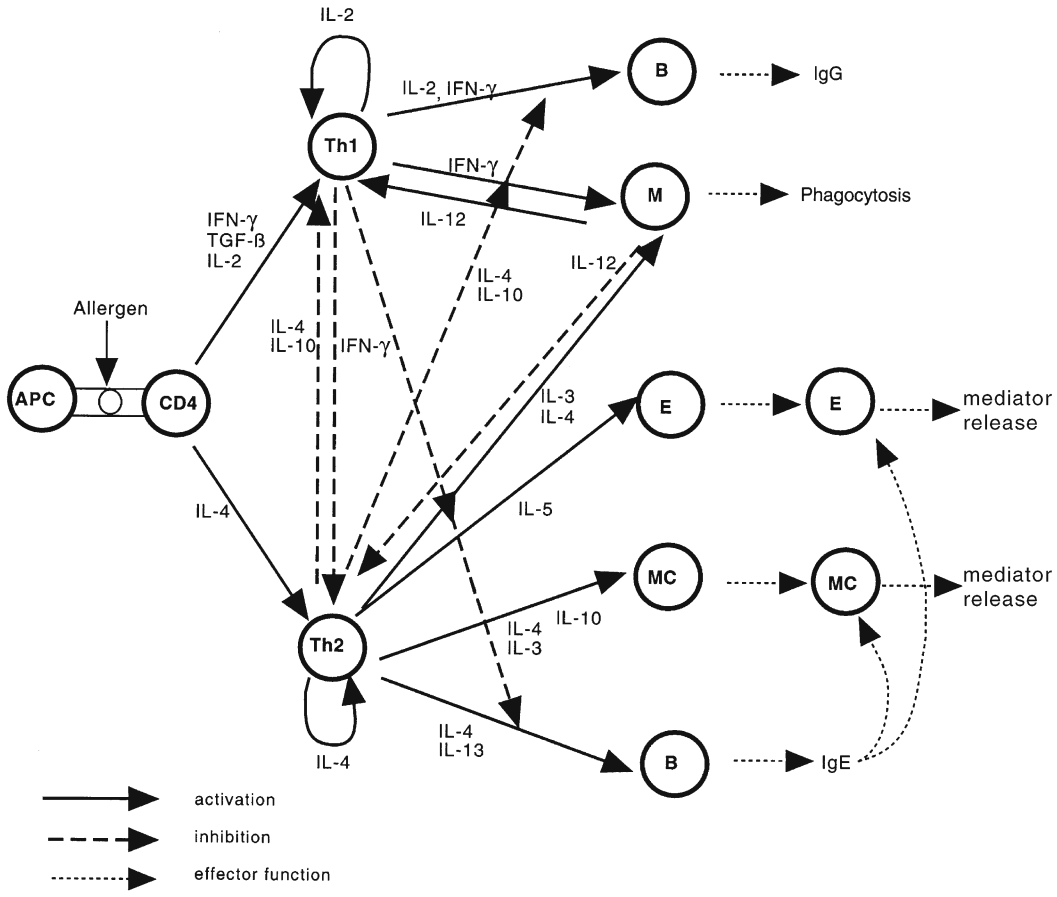


Fig. 1.58. Schematic diagram showing IL production and their activity during immune responses. *APC* antigen-presenting cells, *B* B lymphocytes, *E* eosinophils, *IL* interleukins, *M* macrophages, *MC* mast cells, *Th1*, *Th2* lymphocytes. (Modified from [619])

initially described as communication signals among leukocytes (inter-leukocytes), or soluble factors with a cellular derivation, with functions of messengers and activators of the immune system, and hence immunomodulators, regulating the growth and differentiation of immunocytes and/or many other cell types. ILs can exert a real hemopoiesis, that is, an action also on the growth and differentiation of hematopoietic cell progenitors in the area of immune inflammation where they are released, assuming a role of endogenous mediators. ILs are produced by cells exposed to inflammatory stimuli or bacterial products, or even not belonging to the immune system. They are involved in diverse stages of immune response and inflammatory reactions, greatly regulating their amplitude and duration, interacting at the cell surface with membrane receptors for which ILs have a high affinity, which transduce signals inside the cells. ILs act at picomolar concentrations and are therefore very potent, while their action is of short duration and self-limited. *A second IL cluster* has been localized on chromosome *1q32* including IL_{10}, IL_{19} and IL_{20} [45]. To IL_{12} is credited a contra-regulatory role of allergic inflammation, while a similar role could be shared by IL_{10} since it inhibits eosinophil survival [125]: these two ILs are also a pathognomonic example of opposing roles identified in the pathogenesis of atopic disease. In addition to several other ILs, the IL_{16} gene is associated with asthma, Crohn disease, and allergic contact dermatitis [59]. The numbers of IL_{16}-immunoreactive cells are increased in bronchial mucosa from atopic asthmatic patients 24 h after antigen provocation in vivo, and in allergen-induced late-phase nasal responses [283]. We have described above the relationships of human CD4 Th1 and Th2 T cells with several ILs. Recently the IL_{10} family has been shown to include IL_{19}, IL_{20}, IL_{24} (localized on chromosome *1q32*), IL_{22}, and IL_{26} (localized on chromosome *12q16*) [153]; some cardinal functions in atopic disease [457] are outlined in Fig.1.58 [619].

Recently SOCS, such as SOCS-3 induced by IL_{27} [403] and SOCS-1 and SOCS-3 genes stimulated by IL_{21} [547], have been reported to play a critical role in the inhibitory effect in a negative feedback mechanism [403]. SOCS are known to suppress DC functions and interfere with TLR4 signaling [547]. Both SOCS-1 and SOCS-3 can desensitize primary bone marrow-derived macrophages to IFN-ɣ and IL_6 signaling, respectively

[651]. Pretreatment of DC with IL_{21} inhibited LPS-induced expression of CD86 and HLA class II. Moreover, LPS-induced TNF-α, IL_{12}, CCL5, and CXCL10 production was clearly reduced in IL_{21}-pretreated cells [547]. IL_{27} inhibits CD28-mediated IL_2 production and also IL_2 responses [403].

Adhesion of immunocompetent cells between themselves, to target cells or to the intercellular matrix is a phenomenon of crucial significance in multiple immune reactions and mechanisms directing leukocyte distribution and localization. In this respect it is helpful to consider the *relationships between ILs and adhesion molecules,* which are based on vascular endothelial cells: indeed the leukocytes, when guided into circulation, must interact with such cells, which regulate leukocytes and are regulated by them. Leukocytes adhere to endothelial cells via a binding increase between leukocyte surface receptors and their *co-receptors or ligands* on the endothelial cell surface, which therefore plays a fundamental role in leukocyte migration from vascular to inflammatory sites in interstitial tissues [65]. Leukocyte adhesion to endothelial walls, chiefly of lymphocytes, is also of great magnitude for their compartmentalization and recirculation [209, 328, 534], as shown by Figs. 1.5, 1.11, and 1.58. Apart from new insights on the connections between ILs and leukocytes, the bidirectional relationships between ILs and endothelial cells are intriguing. Indeed, in addition to being targets of the action of soluble mediators of immunity, activating in vascular endothelium a large spectrum of functional interactions, endothelial cells are able to produce several ILs, some of which direct leukocyte extravasation from the circulatory system [534]. High vascular cells, the HEVs, are specialized for lymphocyte extravascular emigration into peripheral lymph nodes: lymphocytes bind selectively to HEVs, as shown by studies on the inhibition of lymphocyte–HEV adhesion done in the animal model and in humans by means of monoclonal antibodies. Therefore, it was possible to point out the role of surface structures present on HEVs. Such molecules, CD44, CD49d and others called addressins, then *selectins,* endothelial coreceptors considered to be specific for lymphatic tissues and involved in lymphocyte adhesion to endothelial cells, would bind to lymphocyte homing receptors, but with affinity for selectins present on lymphocytes [534].

Mechanisms of Cellular Adhesion

Adhesion molecules are a group of surface molecules crucial for immune system functions. In addition to promoting adhesion to the cellular matrix and modulating leukocyte adhesion to vascular epithelium and/or to ECM, they are able to direct leukocyte migration from vascular compartments to extracellular tissues, prime antigen-specific recognition by T lymphocytes, provide costimulatory signals for T-cell activation, stimulate the effector machinery for activated lymphocytes and regulate their proliferation and growth [23]. At least 70 soluble molecules and ILs are involved in lymphocyte activation and differentiation after antigen presentation. There are two different antigen-independent adhesion mechanisms between T cells on the one hand and APCs and/or target cells on the other, where CD2 (LFA-2), CD4, CD7, CD8, CD43 and CD45RO interact, as well as CD11a/CD18 adhesion molecules on T cells [328], more often Th2 T cells than Th1/Th0 cells [143]. In the first mechanism, CD2 binds CD58 and CD59 on APCs and/or B cells. In the second mechanism, CD11a/CD18 binds CD54 and CD102 expressed by APCs, where CD58 binds T-cell CD2: so both mechanisms integrate each other [23]. The largest group of these manifold and multidirectional *cell–cell adhesion molecules* encompasses integrins, selectins, etc., expressed on all surfaces and allowing cell contacts. The important molecules in the AD and asthma pathogenesis are outlined in Table 1.44 [378]: there we note the correspondence between the

Table 1.44. Adhesion molecules in AD and asthma

Adhesion molecules	AD	Asthma
Selectin superfamily		
CD62E	+	+
CD62L	+	+
CD62P	+	+
IgSF		
CD54	+	+
CD102	+	+
CD106	+	+
Integrin superfamily		
β1 Integrins		
CD49d/CD29 (VLA-4)	+	+
CD49e/CD29 (VLA-5)	–	–
β2 Integrins		
CD11a/CD18	+	+
CD11b/CD18	+	+
β3 Integrins		
GPIIb/IIIa	–	–
VNR	–	–
Others		
CD15 s (sLe^x)	+	+
CD36 (GPIIIb)	–	–
CD44 (H-CAM)	–	–
Thrombospondin	+	?
CLA	+	–

Modified from [378].

Table 1.45. β1 Integrins

Molecules	MW (kD)	Ligands	Distribution
$\alpha_1\beta_1$=CD49a/CD29 (VLA-1)	200–210	Laminin, collagen I, IV	T and NK cells, activated T, capillary E, F, HS
$\alpha_2\beta_1$=CD49b/CD29 (VLA-2)	155–165	Laminin, collagen I-IV	T and B cells, E, F, platelets, keratinocytes
$\alpha_3\beta_1$=CD49c/CD29 (VLA-3)	145–150	Laminin, fibronectin, collagen I–IV	B cells, E, F, keratinocytes
$\alpha_4\beta_1$=CD49d/CD29 (VLA-4)	150	Fibronectin, CD106, MAdCAM-1, PP	T and B cells, monocytes, NK, DC, eosinophils
$\alpha_5\beta_1$=CD49e/CD29 (VLA-5)	160	Fibronectin	Lymphocytes, monocytes, E, F, MC, basophils
$\alpha_6\beta_1$=CD49f/CD29 (VLA-6)	150–130	Laminin	T cells, platelets, eosinophils, E, monocytes
$\alpha_7\beta_1$=(VLA-7)		Laminin	Widespread
$\alpha_8\beta_1$			Widespread
$\alpha_9\beta_1$			
$\alpha_{10}\beta_1$			Thymic epithelium, endothelium, epidermis
$\alpha_V\beta_1$		Fibronectin, vitronectin	Thymic epithelium, endothelium, epidermis

Data from [4, 23, 226].
DC dendritic cells, *E* endothelial cells, *F* fibroblasts, *HS* hepatic sinusoid, *MC* metachromatic cells, *PP* Peyer's plaques.

two atopic diseases. Molecules of intracellular adhesion belonging to IgSF are listed in Table 1.4. CD54, a 90-kD gp controlled by IL_1 and TNF-α and -γ, plays a role of unquestionable importance in atopic disease. Of particular relevance is that CD11a/CD18 and CD11b/CD18 support leukocyte adhesion mechanisms binding to counter receptors of endothelial cells, including precisely CD54 and CD102 [23].

Integrins

A new chapter on the knowledge of surface molecules involved in adhesion mechanisms and leukocyte migration to tissue sites of inflammation was opened following the characterization of these gps with a heterodimeric structure made up of α and β chains, noncovalently associated, of 1,100 and 750 amino acids, respectively, important in the adherence of immunocompetent cells to tissues and cell surfaces. This preliminary stage, definite and representative, is related to antigen recognition from CD4 T cells (adhesion to APCs) or to effector activities (adhesion to target cells). Eight subfamilies of leukocyte integrins are as yet known, each made up of 24 different α subunits and a common β chain with 8 subunits distinct in β1–β8, noncovalently linked, forming many combinations [65, 534]. The α integrins play a key role in inflammatory processes, including leukocyte adhesion and migration. Their genes are located on the p arm of chromosome 16 [164].

β1 Integrins (CD29) are heterodimers composed of a common β chain, which can bind to variable α chains. They are the VLA proteins, very late antigens, because the first two appear on lymphocytes 2–4 weeks after antigen priming and are variably expressed on the leukocyte surface. VLAs are divided into various types, as is seen in Table 1.45 [4, 23, 226]. They are chiefly receptors of ECM components such as FN (VLA-5), fibrinogen, laminin (VLA-6), collagen (VLA-2), vitronectin, von Willebrand factor (vWF) and IgSF members [65, 534]. VLAs interact with a wide range of other cells: epithelial (VLA-3), mesangial (VLA-1), activated T (VLA-1, VLA-2), skin fibroblasts (VLA-1), hemopoietic lines (VLA-3), thymus, bone marrow, monocytes, leukocytes (VLA-4 and VLA-5), basement membranes (VLA-3), platelets (VLA-2, VLA-5), and liver sinusoids (VLA-1). VLAs are expressed on circulating lymphocytes and monocytes, like other VLAs on subsets and resting cells of the T phenotype. Thus they are important in lymphocyte homing, transendothelial migration to sites of inflammation, and interactions with ECM [226]. VLA-4 has distinct sites for VLA-5 and CD106, while $\alpha_4\beta_1$ could mediate pro-thymocyte migration from bone marrow to thymus [614].

β2 Integrins (CD29), characterized by a β2 common chain, and a higher immunological and immunopathological interest, are heterodimers formed by two covalently bound peptide chains expressed on PBMCs for the most part; hence they are called leukocyte integrins and regulate adhesion for PBMCs [226]. β2 Integrins have four receptors, each with two chains, α distinct and β common (Table 1.46) [4, 23], while $\alpha_d\beta_2$ is expressed by tissue macrophages [209]. However, a 30%–50% homology has been found between the two chains, denoting a common ancestral gene [23]. β2 Integrins recognize polypeptides with an Arg-Gli-Asp amino acid sequence, likely to be capable of blocking neutrophil adhesion to endothelial cells by a competitive mechanism. CD11b/CD18 and CD11c/CD18 (CR3 and CR4) mediate

Table 1.46. β2 Integrins

Molecules	MW	Ligands	Distribution
$\alpha_L\beta_2$=CD11a/CD18	180	CD50, CD54, CD102, CD62E	Widespread on all leukocytes
$\alpha_M\beta_2$=CD11b/CD18	170	CD54, iC3b, fibronectin, LPS	Monocyte-macrophages, neutrophils, NK cells
$\alpha_X\beta_2$=CD11c/CD18	150	CD54, iC3b, fibrinogen	Monocyte-macrophages, activated T cells, NK cells
$\alpha_d\beta2$	125	CD50>CD54	Macrophages, granulocytes, foam cells

Data from [4, 23].

Table 1.47. β3 Integrins

Molecules	Ligands	Distribution, effects
$\alpha_{II}\beta_3$=CD41/CD61, GP IIb/IIIa	Vitronectin, fibrinogen, fibronectin, vWF	Platelets, megakaryocytes
$\alpha_V\beta_3$=CD51/CD61	Vitronectin, fibrinogen, vWF	Endothelial cells, monocytes, platelets, T cells, LAK cells, mast cells, some B cells
VNR	Vitronectin, fibrinogen, fibronectin, vWF, leukocytes, platelets, laminin, thrombospondin	Endothelial cells

Data from [4, 11].
vWF von Willebrand factor, *VNR* vitronectin receptor.

Table 1.48. β4–β8 Integrins

Molecules	Ligands	Distribution, effects
$\alpha_6\beta_4$=CD49f/CD104	Laminin	Basal cell layer of stratified thymic epithelium
$\alpha_V\beta_5$=CD51/–	Fibronectin, vitronectin	
$\alpha_V\beta_6$=CD51/–	Fibronectin	
$\alpha_4\beta_7$ (LPAM-1)	MAdCAM-1, CD106, HEV	MALT, T-cell homing molecule, T, memory
$\alpha_E\beta_7$ (HML-1)=CD49d/β7 (CD103)	E cadherin	IEL T cells, lamina propria lymphocytes
$\alpha_V\beta_8$	CD31 ?	Cellular
$\alpha_6\beta_p$=VLA-4	Fibronectin, CD106	Cells of murine leukemia

Data from [11, 19, 226].
HML human mucosal lymphocytes, *IEL* intraepithelial lymphocytes.

phagocytosis binding CICs coated with iC3b and iC4b [337]. CD11a/CD18 (CR1) promotes CTL adhesion to target cells, CD11b/CD18 (CR3) functions as a receptor for iC3b, which has a domain with the same sequence [23], and finally CD11c/CD18 (CR4) binds to CD54 and iC3b. CD11a/CD18 and CD11b/CD18 are also involved in the LAD syndrome, fatal if not corrected by a bone marrow transplantation (BMT) [564]: five different types have been reported (Chap. 22).

β2 Integrins are distributed on a larger spectrum of cells:

- NK cells (CD11b/CD18 and CD11c/CD18)
- Leukocytes (CD11a/CD18 all, CD11b/CD18 and some CD11c/CD18)
- Monocyte-macrophages (CD11b/CD18 and CD11c/CD18) [610]

β3 Integrins (CD61) or cytoadhesins are given in Table 1.47 [4, 11]. Among *β4-β8 integrins* (Table 1.48) [11, 19, 226] is included CD104 expressed on thymocytes [166]. There are two β7: $\alpha_4\beta_7$ (CD49d/β7), which binds to MAdCAM-1, with two N-terminal domains with homology for CD54 and CD106, followed by a mucin-like region between domains 2 and 3 ending with an IgA-like domain. MAdCAM-1 further binds to both CD62L and $\alpha_4\beta_7$ [209], also mediating lymphocyte rolling and adhesion to epithelium, performing a double function of both a selectin and an integrin ligand [534]. Another β7, $\alpha_E\beta_7$, is the first to recognize E cadherin [76]. Thus, two *β7 integrins* may be important in GALT formation, since they provide lymphocyte trafficking to PPs and lamina propria [615]. Additional unclassified adhesion molecules (Table 1.49) [4, 11] are CD44 (ligand hyaluronic

Table 1.49. Additional adhesion molecules not classified among families

Molecules	Characteristics	Ligands	Distribution, effects
CD26	See Table 1.2	Fibronectin, collagen	Thymocytes, T and NK cells
CD35	See Table 1.2	C3b, C4b	E, B and T cells, phagocytes, splenic follicular CDs; on CDs possibly role in presentation of allergen, eosinophils
CD36	See Table 1.2	Thrombospondin	Monocytes, megakaryocytes, small vessel endothelium, platelets, reticulocytes
CD42a/d	See Table 1.2	Thrombin, von Willebrand factor	Platelets, megakaryocytes
CD44	See Table 1.2	Hyaluronic ac, collagen, laminin, fibronectin	B and T cells, E precursors, glial cells, monocytes, neutrophils, fibroblasts, myocytes, epithelia
CD73	See Table 1.2		Epithelial and endothelial cells
Laminin	140–400 kD	See "Integrins"	Integrity of basement membranes (embryogenesis, development)
Fibronectin	250–235 kD	Gelatin, fibrin, heparin, integrins (see "Integrins")	Adhesive and migratory events (embryogenesis, angiogenesis)
CD134	See Table 1.2	gp34	Adhesion of T cells to vascular endothelial cells, costimulation
Sialoadhesin	185 kD	Sialyl proteins	Contacts macrophage-granulocytes during hematopoiesis
VAP-1	?	?	Endothelial and dendritic cells, HEV of lymph nodes

Data from [4, 11].
E erythrocytes, *VAP* vascular adhesion protein.

Table 1.50. Selectins

Molecules	Ligands	Distribution
CD62E (E selectin)	ESL-1, CD15s, CD62L, CLA	Leukocytes, activated endothelia, epithelia, HEV of lymph nodes
CD62L (L selectin)	CD34, CD15s, GlyCAM	Resting leukocytes, peripheral lymph nodes, MALT
CD62P (P selectin)	PSGL-1, CD15s	Activated endothelial cells and platelets

All selectins mediate tethering and rolling of various cells (see text for details).
Data from [23, 610].
CLA cutaneous lymphocyte-associated antigen, ESL-1 E-selectin ligand 1, *GlyCAM* glycosylation-dependent cell adhesion molecule 1, *PSGL-1* P-selectin glycoprotein ligand 1=CD162.

acid), a homing receptor active in leukocyte extravasation to inflamed tissues [23], CD35, CD36, CD42, and others shown in the table.

Selectins

The *selectin family* is independent of integrins, mostly expressed on lymphocytes and neutrophils, and involved in the adhesion process with vascular endothelial cells; three molecules have been characterized so far, distinct by sequence and function homology (Table 1.50) [23, 610]. All belong to the C-type lectin family and share domains with regulatory complement proteins; CD62L also shares an EGF-like domain [4]. To these molecules with a slow expression (their peak is 4–6 h after IL stimulation, in TNF or IL_1, for example, the levels return to basal values after 12–24 h) also belong two molecules with a structure different from CD62E and CD62P, that is, CD54 and CD106 [610]. Selectins are typical lymphocyte homing molecules, to lymph nodes as well as to sites of inflammation [226]. CD62L expressed by 70% of circulating leukocytes is in part responsible for neutrophil in vivo recruitment to inflamed tissues. CD62E, a receptor of mononucleated cells, is on HEVs at sites of immune inflammation: IL-activated, both mediate adhesion to endothelium [4]. CD62P expressed by platelet α granules has the same preference of CD62E for endothelial cells, which express it after agonist stimulation (histamine, thrombin, etc.), returning to basal levels after 20–60 min. β2 integrins and CD62L can act in agreement with guide neutrophils to inflamed

Table 1.51. Cadherin classification

Molecules	Ligands	Distribution
Uvomorulin	Homophilic	
LCAM	Homophilic	
E-cadherin	Homophilic $\alpha_E\beta_7$	Epithelial cells (IEL)
N-cadherin	Homophilic	Neural cells
P-cadherin	Homophilic	Placental cells
V-cadherin	Homophilic	Vasal cells
VE-cadherin (CD144)		β-Cadherin

Modified from [65].
LCAM liver cell adhesion molecule.

tissues [348]. High-affinity selectin ligands are ESL-1 (E-selectin ligand 1), PSGL-1 (P-selectin glycoprotein ligand 1=CD162) related to FGF and GlyCAM-1 (glycosylation-dependent cell adhesion molecule 1) for CD62L [209]. Particularly, ESL-1 and CD162 mediate myeloid cells binding to the two selectins; CD162 also mediates CD62P-associated leukocyte binding and rolling [4].

The *cadherins* (Table 1.51) [65] are distributed in different tissues, and with their desmosomes serve as anchoring sites for the cytoskeleton to the point of Ca-dependent adhesion between adjacent cells and junction formation through which bundles of actin filaments run among cells [162]. Cadherin homophilic adhesion plays a key role in segregating embryonic tissues and in cell migration and tissue differentiation [162].

The cadherin superfamily is wide: ≈80 cadherins have been isolated. Most members are expressed in the CNS. These are homophilic adhesion molecules, and for their homophilic interactions, the ectodomains (EC) play a crucial role [657]. Over recent years *protocadherins* have emerged as a growing superfamily of molecules, with a complex picture of their structure and con-

Table 1.52. Adhesion between leukocytes and endothelial cells at the molecular level

Cells	Adhesion molecules	Ligands on endothelium
T, B, monocyte-macrophages, neutrophils, NK cells	CD11a/CD18	CD50, CD54, CD102
Monocyte-macrophages, neutrophils, eosinophils	CD11b/CD18	CD54
Monocyte-macrophages	CD62E, CD62P	CD15 s
Monocyte-macrophages, neutrophils, NK cells	CD11c/CD18, CD11d/CD18	CD106
Lymphocytes, monocytes, eosinophils	CD49d/CD29	CD106
Lymphocytes, monocytes, neutrophils, eosinophils	CD62L	CD34, GlyCAM-1

Modified from [130]. *T*, *B*, T cells, B cells.

Table 1.53. Adhesion between leukocytes and endothelial cells at the organ level

Adhesion steps	Peripheral Lymph node HEV		Peyer's plaque HEV		Gut		Skin	
Tethering								
Lymphocytes	CD62L		CD62L		CD62L		CLA	
Endothelium	CD34, GlyCAM-1?		MAdCAM, CD34?		MAdCAM, CD34?		CD62E	
Triggering								
Lymphocytes	$G\alpha_1$-coupled receptors	?	$G\alpha_1$-coupled receptors	?	$G\alpha_1$-coupled receptors	CD31/GAG	$G\alpha_1$-coupled receptors	CD31/GAG
Endothelium	chemo-attractant?	CD31?	chemo-attractant?	CD31?	MCP-1? HGF ? MIP-1?	CD31?	MCP-1? HGF ? MIP-1?	CD31?
Strong adhesion								
Lymphocytes	$\alpha_L\beta_2$		$\alpha_4\beta_7$	$\alpha_L\beta_2$	$\alpha_4\beta_7$		$\alpha_4\beta_7$	$\alpha_L\beta_2$
Endothelium	CD54–CD102		MAdCAM	CD54–CD102	MAdCAM		MAdCAM	CD54/102

GAG glycosaminoglycan.
Data from references [226, 534].

stitute the largest subgroup within the cadherin family of Ca^{++}-dependent cell–cell adhesion molecules [657]. This new family is large, 52 novel protocadherins were identified on human chromosome 5q31 [653]. Subsequently, 66 protocadherins were identified in cluster genes arrayed into two clusters [380]. Protocadherins are organized into α, β, and γ. The β genes in man are 19 and in mouse 22 [605]. The γ-protocadherins are expressed exclusively in the CNS [629]. A fourth group, δ, includes δ 1-protocadherins (comprising protocadherin-1, -7, -9 and -11 or -X/Y) and δ 2-protocadherins (comprising protocadherin-8, -10, -17, -18 and -19). Some δ-protocadherins appear to mediate weak cell–cell adhesion in vitro and cell sorting in vivo [451].

Tables 1.52 and 1.53 summarize the adhesion molecules involved in interactions between lymphocytes and endothelial cells at the molecular [226] and organ levels [226, 534].

Relationships Between ILs and Adhesion Molecules

Several ILs, mainly those with a tendency for inflammation, activate on endothelial cell membranes the expression of adhesion molecules for leukocytes and/or other circulating cells:

- IL_1, TNF-α and IFN-γ increase the expression of CD54 and CD102, CD62E, CD106, etc.; the first two molecules induce adhesion on all granulocytes, the last one only on eosinophils and basophils.
- IL_2 activates CD54.
- IL_3 fosters basophil adhesion to endothelial cells and CD11b/CD18 expression on basophils.
- IL_4 induces lymphocyte adhesion and can make up for lack of CD106 modulating its expression in the absence of CD54, CD102 and CD62E.
- IL_5 promotes CD11/CD18 expression on eosinophils.
- IL_6 expresses CD11b/CD18 on pro-monocytes, further CD11c and CD54.
- IL_7 primes CD11b/CD18 expression from T lymphocytes and of CD54 from T and B lymphocytes.
- IL_8 activates CD11b/CD18 and CD11c/CD18 on PMNs.
- GM-CSF provides neutrophils and granulocytes with CD11b and endothelial cells with CD18 [23, 323].

Chemokines

Chemokines [22, 179, 247] are more than 65 small protein molecules (8–10 kD) generated by leukocytes, monocyte-macrophages and platelets and activated endothelium, which can thus regulate leukocyte trafficking and that related to IL_8 identified as analogous to NAP-1. As their name suggests, *chemo*attractant cyto*kines* play the role of chemotactic molecules interested in integrin activation. Chemokines may be defined as small but potent leukocyte pro-inflammatory chemoattractants, cellular activating factors, and HRFs, that bind to specific G-protein-coupled seven-span TM receptors present on plasma membranes of target cells, which makes them particularly important in the pathogenesis of allergic inflammation. Chemokines are major regulators of cell trafficking and may also modulate cell survival and growth [280, 687]. Chemokines IL_8, MIP-1, MCP-1 and RANTES are known to recruit neutrophils, eosinophils, macrophages and T lymphocytes to the site of inflammation. MIP-1, MCP-1 and RANTES are direct chemoattractants of Th1 cells. These chemokines are promiscuously used by several receptors, but all three are ligands for CCR5, which is preferably expressed in type 1 cells. Therefore, it is of interest to note that the CCL-chemokines and their receptors are coexpressed in Th1 cells. The coexpression is not only Th1-cell-specific, but the similar expression pattern applies to Th2 cells that express CCR4 bearing thymus and activation-regulated chemokine (TARC) receptor. Structurally related, chemokines are divided into three groups based on the *chromosomes by which they are coded:* α = 4, β = 17, depending on whether the first two cysteine residues are separated by an amino acid (CXCL) or are adjacent (CCL), γ = 1, and δ is an exception on chromosome 16, interacting with receptors different from G proteins [9, 22, 178] (Tables 1.54, 1.55) [2, 9, 19, 22, 178, 247, 479, 574]. The proposed chemokine nomenclature is based on the nomenclature currently in use, derived from the one already assigned to the gene encoding the 4 families of chemokines [687], and includes CC, CXC, XC, or CX3C followed by L (ligand) or R (receptor) and then a number [667]:

- *CXCL α chemokines:* for neutrophils there are many specificities (Table 1.54), GROs are specific even for basophils [178]. Moreover, NAP-1/IL_8 chemoattractant of eosinophils can activate also basophils (Fig. 1.49) and act as a potent inducer of T-cell chemotactic and activating responses. From PBP (platelet basic protein) β-TG, CTAP-III and NAP-2 originate [19]. A further subdivision might be introduced, since mig, IP-10 and PF-4 lack the E-L-R (glutamic acid-leucine-arginine) residues [19].
- *CCL β chemokines:* (Table 1.55) RANTES is produced by fibroblasts to attract and activate eosinophils. LPS is known to induce RANTES and cause protein tyrosine phosphorylation [658]. RANTES is a chemoattractant selective for activated and resting T cells, including memory cells [552]. CD45RO, macrophages and eosinophils. MIP-1α and MIP-1β and MCP-1 are chemotactic for T subsets, macrophages, eosinophils and basophils; while macrophages in turn elicit several CCL chemokines [540]. In particular, MIP-1α favors activated B cells and CD8 migration and MIP-1β-activated T cell infiltration; both direct T-cell adhesion to endothelial cells, the former of CD8 and the latter of CD4 T cells. Basophils and eosinophils are among the more sensitive cells, mostly to CCLs, while MCP-3 combines the properties of RANTES and MCP-1 [247, 396].

Table 1.54. Main properties of α chemokines or CXCL chemokines

Name	Source(s)	Target cells and (biological effects)
BCA-1	Secondary lymphoid organs	B lymphocytes (chemotaxis)
ß-TG	Monocyte-macrophages, platelets	Monocytes, platelets, fibroblasts (growth), neutrophils (chemotaxis)
CK-α1		
CTAP-III	Platelets, monocytes	Fibroblasts (activation), neutrophils (chemotaxis)
ENA-78	Monocytes, neutrophils, NK and endothelial cells	T cells, basophils, neutrophils (activation and degranulation)
GCP-2	Osteosarcoma cell line	Neutrophils (chemotaxis)
GRO α, β, γ	monocyte-macrophages, fibroblasts, synovial cells and epithelial cells, hepatocytes, keratinocytes, neutrophils, T lymphocytes	neutrophils (degranulation, adhesion, activation, endothelial chemotaxis), basophils (in a reduced way), fibroblasts (growth), endothelial cells (angiogenesis)
IL_8/NAP-1	Osteoblasts, endothelial/epithelial cells, fibroblasts, keratinocytes, smooth muscle cells, astrocytes, B and T lymphocytes, monocyte-macrophages, hepatocytes, melanoma cells	Neutrophils (activation, chemotaxis, adhesion, killing), lymphocytes and NK cells (chemotaxis), basophils (chemotaxis, histamine release), endothelial cells (angiogenesis), keratinocytes (mitogenesis)
IP-10	Monocytes, endothelial cells, fibroblasts, keratinocytes, macrophages	T cells (activation, chemotaxis, integrin expression by T cells), NK cells (chemotaxis, cytolytic activity), endothelial cells (angiogenesis inhibition)
I-TAC	Monocytes, neutrophils, epithelial cells	T lymphocytes, Th1 and NK cells (chemotaxis)
MAD-2		
mig	Monocyte-macrophages, NK cells	T cells (activation and chemotaxis)
NAP-2	Platelets	Neutrophils (activation and chemotaxis)
PBP	Platelets, lymphocytes	
PF-4	Platelets, megakaryocytes, T lymphocytes	Fibroblasts, neutrophils, monocytes and T cells (endothelial cells adhesion, angiogenesis inhibition)
SDF-1α, β	Several tissues	Lymphocytes, monocytes (chemotaxis)

β-TG β-thromboglobulin, *CK-a1* α1 chemokine, *CTAP-III* connective tissue activating protein-III, *DC* dendritic cells, *ELC* EBI1 ligand chemokine, *ENA-78* epithelial cell-derived neutrophil-activating protein, *GCP-2* granulocyte chemotactic protein 2, *GRO-α, GRO-β, GRO-γ* growth-related gene, *IP-10* Inflammatory protein-10, *I-TAC* interferon inducible T-cell alpha chemoattractant, *MAD-2* monocyte adhesion dependent protein-2, *MCP 1/5* monocyte chemotactic protein-1/5, *mig* monokine inducible by IFN-γ, *MIP-1α, -1β, 2* macrophage inflammatory protein-1α, -1β, 2, *NAP-1* neutrophil activating factor-1, *PBP* platelet basic protein, *PF4* platelet factor 4, *SDF-1a/1β* stromal cell-derived factor.
Data from [2, 9, 19, 22, 178, 380, 459, 574].

MCP-1, MCP-2 and MCP-3 are also major attractants for monocytes, $CD4^+$ and $CD8^+$ T cells [317, 609]. MCP-1–4 bind to specific G-protein-coupled receptors, initiating a signal cascade within the cell. CCR2 is considered the major G-protein-coupled receptor for MCP-1 [616]. MCP-5, a 9.2-kD peptide that consists of 82 amino acid residues, has been identified in the mouse [614]. MCP-1 is efficacious nearly as much as C5a and, in conjunction with RANTES and MIP-1α, stimulates basophils to release histamine, even more so if IL_3, IL_5 and GM-CSF interfere. Furthermore, MCP-1 turns into a powerful chemoattractant of eosinophils, similarly to IL_8 if it loses the N-terminal region [628]. MCP 1–3 are active chemoattractants for NK cells [316], MCP-1 also for T cells of memory phenotype [67], whereas MCP-3 appears to be involved in the regulation of early responses to specific allergens [666]. Parallel to eotaxins [435], RANTES has an effect on eosinophil local recruitment, by acting on their locomotion similarly to C5a, and 2- to 3-fold more powerful than that of MIP-1α [658]. RANTES in IL_5-stimulated cells is an effective inducer of eosinophil transendothelial migration by a single mechanism, CD49d/CD106-dependent [133]. Although RANTES and IL_5 are elevated 24 h after antigen challenge, eosinophil recruitment and degranulation is associated only with IL_5 [552]. Unprimed mast cells are influenced to migrate by MCP-1 and RANTES, while IgE-activated cells respond to MIP-1α and PF4, but do not degranulate in response to chemokines [568]. C10, with homology to CCF-18, seems to involve only T-cell chemotaxis [19]. Positive correlations link the level of Syk expression and

Table 1.55. Main properties of chemokines: CCL, XCL and CX3CL chemokines

Name	Source(s)	Target cells and (biological effects)
β or CCL chemokines		
ACT-2		
AMAC	Activated macrophages	Naive T cells (chemotaxis)
C10		
CKβ1, 3, 4, 6–11		
DC-CK1	Dendritic cells	$CD45RA^+$ T cells, naive T cells (chemotaxis)
ELC/MIP-3β		T cells (selective)
Eotaxin-1	Nasal epithelium	Eosinophils (chemotaxis)
Eotaxin-2	Activated monocytes, basophils, myeloid progenitors	Resting T cells (chemotaxis), eosinophils, basophils
Eotaxin-3	Eosinophils	
Exodus-1/MIP-3α/LARC	DCs (regulation and migration)	
Exodus-2	Lymph nodes, airways, appendix, spleen	
FIC		
HCC-1	Normal tissues, $CD34^+$ myeloid progenitors	Monocytes (chemotaxis)
HCC-2	Liver	Monocytes (chemotaxis)
HCC-4		T lymphocytes
I-309	Monocytes, mast cells, activated T cells	Monocytes (chemotaxis)
MCP-1	Monocytes, endothelial/epithelial cells, fibroblasts, keratinocytes, smooth muscle cells, mast cells, mesothelial cells	Monocytes (chemotaxis, adhesion, phagocytosis, killing, superoxide release, arachidonic acid activity) metachromatic cells (chemotaxis, degranulation, histamine release), basophils (LCT synthesis), T cells (chemotaxis), eosinophils; macrophages (activation, secretory activity, chronic inflammation), stem cells (colony formation inhibition)
MCP-2	Monocytes, osteosarcoma cells, fibroblasts	Has half of MCP-1 activity: monocytes, T cells, eosinophils (chemotaxis), mast cells (chemotaxis, histamine release)
MCP-3	Osteosarcoma cells	Combines MCP-1 and RANTES properties: monocytes, T cells, eosinophils (chemotaxis)
MCP-4	Endothelial/epithelial cells	Lymphocytes, monocytes, eosinophils (chemotaxis)
MCP-5	Monocytes	Peritoneal macrophages (chemotaxis)
MDC/STCP-1	Macrophages, monocyte-derived DCs	Monocytes and derived DCs, activated T cells, thymic T cells, CD8 T cells, IL_2-activated NK cells (chemotaxis)
MIP-1α now CCL3	B and T lymphocytes, monocytes, fibroblasts	B and T lymphocytes, neutrophils, monocytes, NK cells, eosinophils, DC (chemotaxis); T cells (adhesion, collagenase release, integrin expression mostly by CD8 T cells), eosinophils (cationic protein release), metachromatic cells (chemotaxis and histamine release), stem cells (colony formation inhibition)
MIP-1β now CCL4	B and T lymphocytes, monocytes, fibroblasts	Monocytes (chemotaxis), T lymphocytes (chemotaxis, adhesion, integrin expression mostly by CD4 T cells), stem cells (antagonizes MIP-1α effects)

Table 1.55. (Continued)

Name	Source(s)	Target cells and (biological effects)
MIP-3α	Liver, monocytes, lymphocytes	Mononuclear, dendritic, and T cells (chemotaxis)
MIP-3β	Lymphoid tissue, activated B lymphocytes	Activated T lymphocytes (chemotaxis)
MIP-5	Liver, intestine, lymphocytes (airways)	Monocytes, T lymphocytes (chemotaxis)
MIPF-1		Resting T cells, monocytes, neutrophils
PARC	T cells (selective), lung, lymphoid tissue	T lymphocytes (chemotaxis)
RANTES now CCL5	T cells, platelets, macrophages, endothelial cells	T cells (chemotaxis, adhesion, integrin expression mostly by CD4 T cells), monocytes, DC and NK cells (chemotaxis) specific for eosinophils (cationic protein release), basophils (chemotaxis and histamine release); additional actions are similar to those of MCP-1
SLC	Lymphoid tissue, activated macrophages	T lymphocytes (chemotaxis)
TARC	Lymphoid tissue, mononuclear cells	T lymphocytes, Th2 T cells (chemotaxis)
TCA3		
TECK	Thymic dendritic cells, small intestine, liver	Activated macrophages, dendritic cells (chemotaxis)
γ or XCL chemokines		
Lymphotactin	$CD8^+$ T lymphocyte, thymocytes, NK cells	T cells (chemotaxis)
ATAC		
SCM-1	Mononuclear cells, spleen	Lymphocytes (chemotaxis, activation)
CX3CL chemokines		
Fractalkine	Endothelial cells, monocytes	T cells and monocytes (chemotaxis)

When the chemokines are without indications, the sources and the target cells are as yet unknown.
From [2, 9, 19, 22, 178, 247, 459, 574].
ACT-2 immunoactivating cytokine-2, *β-TG* β-thromboglobulin, *CK-α1*, *3 CK-β1*chemokines α1 and -β1, *DC-CK1* dendritic cell chemokine-1, *FIC* fibroblast-induced chemokine, *HCC-1* hemofiltrate CC chemokine-1, *I-309* I-309 protein, *LARC* liver and activation-regulated chemokine, *MCP-1* monocyte chemotactic protein-1, *MCAF* monocyte chemotactic and activating factor, *MIP-1α* and *MIP-β* macrophage inflammatory protein-1α and -1β, *MIPF-1*, *MIPF-2* myeloid inhibitory factor-1, -2, *PARC* pulmonary and activation-regulated chemokine, *RANTES* regulated on activation normal T expressed and secreted, *SDF-1* stromal cell-derived factor, *SLC* secondary lymphoid tissue chemokine, *STCP-1* stimulated T cell chemotactic protein-1, *TARC* thymus and activation-regulated chemokine, *TCA3* T cell activation gene 3, *TECK* thymus-expressed chemokine.

RANTES production induced by LPS. RANTES production from nasal fibroblasts stimulated with LPS is enhanced by overexpression of wild-type Syk gene transfer [658]. Several CXCL and CCL chemokines alone or together release 11 %–43 % of basophil histamine [283] and have similar activity: for example, GRO α, γ and MCP-1 are constitutively produced in human airway epithelium and bronchoalveolar macrophages [32], while IL_8 and CCL chemokines manipulate IL_2-activated NK-cell chemotaxis [334]. The *eotaxins* have a great significance in the *atopic march*: STAT-6 is required to up-regulate eotaxin-1 and eotaxin-2 expression by chronic IL_4 stimulation [687]. Multiple other STAT molecules (STAT-1, STAT-2, and STAT-3) can be recruited to various other chemokine receptors after JAK recruitment. These activated STAT molecules can then translocate into the nucleus of the chemokine-stimulated cell and directly activate (and sometimes repress) gene expression [396]. TARC, constitutively expressed in the thymus and produced by monocyte-derived DCs and endothelial cells, is a ligand for CCR4 and CCR8 and serves for the recruitment and migration of these receptor-expressing cells, and is thus responsible for the selective trafficking of Th2 lymphocytes into sites of allergic inflammation [393]. More chemokine receptors are rapidly up-regulated following T cell activation. Th1 cells express CXCR3 and Th2 CCR3, CCR4, CCR8 [687].

- *XCL γ chemokines:* only ATAC, and a lymphotactine specific for T cells are known to date [2, 9].
- *CX3CL chemokine:* only fractalkine (Table 1.55).

Table 1.56 [2, 19, 380, 396, 574, 687] summarizes the chemokine interactions.

Table 1.57 [2, 6, 247, 396, 690] shows correspondence of chromosome location, chemokine receptors, and

Table 1.56. Summary of chemokines and their interactions

Chemokines	Neutrophils	Monocytes	NK	T cells	Basophils	Eosinophils	Endothelia	Mast cells	FB	DC
CXCL										
β-TG	+									
CTAP-III	+				+				+	
ENA78	+									
Exodus-1/MIP-3α/LARC										
GCP-2	+									
GROα, β, γ	+						+			
IP-10			+	+			+			
NAP-1/IL_8	++		+	+	+		+			
NAP-2	+				+				+	
PF4	+	+							+	
SDF-1α/1β		+		+						
CCL										
C10				+						
CCF-18										
ELC/MIP-3β				++						
Eotaxin					++					
Eotaxin-2/MIPF-2				++	+	++				
I-309		+								
MCP-1		++		+	++			+		
MCP-2		++		+	+	+		+		
MCP-3		++		+		+		+		+
MCP-4		+		+		+				
MCP-5		+								
MDC/STCP-1		+	+	+						
MIP-1α	+	+	+	+ (CD8)	+	+		+		+
MIP-1β		+		+ (CD4)						
MIP-5		+		+						
MIPF-1	+	+		+						
PARC/DC-CK1				++						
RANTES		+	+	++	+	+				
XCL										
Lymphotactin				+						
CX3CL										
Fractalkine			+	+						

ELC/MIP-3β and PARC/DC-CK1 are selective for T cells, eotaxin-2/MIPF-2 and MIPF-1 act only on resting T cells, not on activated T cells, TECK is specific for T-cell development in thymus.
Data from [2, 19, 380, 396, 687].
DC dendritic cells, *FB* fibroblasts, *NK* NK cells.

Table 1.57. CXCL, CCL and CX3CL chemokine/receptor families

Systematic name	Human ligand	Human chromosome	Chemokine receptors
CXCL chemokines/receptor family			
CXCL1	GRO-α/MGSA-α	4q12-q13	CXCR2 > CXCR1
CXCL2	GRO-β/MGSA-β	4q12-q13	CXCR2
CXCL3	GRO-γ/MGSA-γ	4q12-q13	CXCR2
CXCL4 (fusin)	PF4	4q12-q13	–
CXCL5	ENA-78	4q12-q13	CXCR2
CXCL6	GCP-2	4q12-q13	CXCR1, CXCR2
CXCL7	NAP-3	4q12-q13	CXCR2
CXCL8	IL-8	4q12-q13	CXCR1, CXCR2
CXCL9	Mig	4q21.21	CXCR3
CXCL10	IP-10	4q21.21	CXCR3
CXCL11	I-TAC	4q21.21	CXCR3
CXCL12	SDF-10 /,8	10q11 .1	CXCR4
CXCL13	BLC/BCA-1	4q21	CXCR5
CXCL14	BRAK/bolekine	–	–
CXCL15	SR-PSOX	–	CXCR6
CXCL16	–	–	CXCR6
CCL chemokines/receptor family			
CCL1	I-309	17q11.2	CCR8
CCL2	MCP-1, MCAF	17q11.2	CCR2
CCL3	MIP-1α	17q11.2	CCR1, CCR5
CCL4	MIP-1β	17q11.2	CCR5
CCL5	RANTES	17q11.2	CCR1, CCR3, CCR5
CCL6	Exodus-1, LARC	17q11.2	–
CCL7	MCP-3	1 7q11 .2	CCR1, CCR2, CCR3
CCL8	MCP-2	1 7q11 .2	CCR3
CCL11	Eotaxin	17q11.2	CCR3, CCR5
CCL13	MCP-4	1 7q11.2	CCR3
CCL14	HCC-1	17q11.2	CCR2
CCL15	HCC-2/Lkn-1/MIP-1β	17q11.2	CCR2, CCR3
CCL16	HCC-4/LEC	1 6q13	CCR1
CCL17	TARC	17q11.2	CCR1, CCR3
CCL18	DC-CK1/PARC AMAC-1	9p13	CCR1
CCL19	MIP-3δ/ELC/exodus-3	2q33-q37	CCR4
CCL20	MIP-3cr/LARC/exodus-1	9p13	CCR6
CCL21	6Ckine/SLC/exodus-2	1 6q13	CCR4
CCL22	MDC/STCP-1/ABCD-1	1 7q11 .2	CCR1
CCL23	MPIF-1	7q11.23	CCR3
CCL24	MPIF-2/Eotaxin-2	1 9p13.2	CCR9
CCL25	TECK	7q11.23	CCR3
CCL26	Scya26/Eotaxin-3	9p13	CCR10

Table 1.57. (Continued)

Systematic name	Human ligand	Human chromosome	Chemokine receptors
CCL27	CTACK/ILC	9p13	CCR10
CCL28	MEC	–	CCR3, CCR10
C chemokine /receptor family			
XCL1	Lymphotactin/SCM-10/ATAC	1q23	XCR1
XCL2	SCM-10	1q23	XCR1
CX3C chemokine /receptor family			
CX3CL1	Fractalkine	16q13	

Systematic name	Human ligand	Expression
Chemokine receptor		
DARC	IL_8, GROα, NAP-2, ENA-78, MCP-1, MCP-3, RANTES	Endothelia (spleen, lungs, brain, kidneys), lymphocytes (CD45RA), Purkinje cells, erythrocytes
Viral receptors		
CMV US28	MIP-1α and -1β, MCP-1, RANTES	
HSV saimiri	IL_8	
Lipidic autacoid		
PAFR	PAF	Myeloid cells and smooth muscle cells, lymphocytes, CNS
Anaphylatoxin-formyl-peptide		
C5aR	C5a	Myeloid cells, microglia, astrocytes, mucosal epithelia, mast cells, hepatocytes
C3aR	C3a	Myeloid cells, heart, lungs, brain, intestine, some lymphocytes ?

Cell types, receptors found and known ligands	
Neutrophil	
CXCR1	IL_8, GCP-2
CXCR2	IL_8, GCP-2, GRO-α, GRO-β, GRO-γ, ENA-78, NAP-2, LIX
Eosinophil	
CCR1	MCP-3, MCP-4, MIP-1α, RANTES
CCR3	MCP-3, MCP-4, eotaxin-1, eotaxin-2, RANTES
Basophil	
CCR2	MCP-1, -2, -3, -4, -5
CCR3	MCP-3, MCP-4, eotaxin-1, eotaxin-2, RANTES
Monocyte	
CCR1	IL_8, GCP-2
CCR2	MCP-1, -2, -3, -4, -5
CCR5	MIP-1α, MIP-1β, RANTES
CCR8	I-309
	MDC, HCC-1, TECK
CX3CR1	Fractalkine
CXCR4	SDF-1

Table 1.57. (Continued)

Cell types, receptors found and known ligands	
Dendritic cell	
CCR1	IL_8, GCP-2
CCR2	MCP-1, -2, -3, -4, -5
CCR3	MCP-3, MCP-4, eotaxin-1, eotaxin-2, RANTES
CCR4	TARC
CCR5	MIP-1α, MIP-1β, RANTES
CCR6	MIP-3α (LARC, Exodus-1) MDC, TECK
CXCR4l	SDF-1
Resting T Lymphocyte	
	PARC, DC-CK-1
	Lymphotactin
CXCR4	SDF-1
Activated T lymphocyte	
CCR1	IL_8, GCP-2
CCR2	MCP-1, -2, -3, -4, -5
CCR4	TARC
CCR5	MIP-1α, MIP-1β, RANTES
CCR7	MIP-3β (ELC) PARC, SLC, 6CKine (Exodus-2)
CX3CR1	Fractalkine
CXCR3	IP-10, MIG, I-TAC
Natural killer cell	
CCR2	MCP-1, -2, -3, -4, -5
CCR5	MIP-1α, MIP-1b, RANTES
CX3CR1	Fractalkine
CXCR3	IP-10, MIG, I-TAC

CCR5 favors HIV-1 entry into target cells [82] (see Chapter 23).
DARC Duffy antigen receptor complex, *MGSA* Melanocyte growth stimulating activity , *PAFR* PAF receptor.
Data from references [2, 4, 246, 396, 690].

their different ligands: 15 for CXCL (CXCL 1–18), 28 for CCL (CCL 1–28), 1 for CX3CL chemokines and other unclassified ones [2, 4, 690]. All have seven G-protein-linked TM-spanning domains and their signaling can be typically inhibited by pertussis toxin [2], in addition to two virally encoded chemokine receptors that may be used, together with IL_8 R and IFN-R, by viruses to subvert the host immune system [2] by altering the local conditions in favor of viral persistence and replication [11]. The inhibition of chemokine receptors by pertussis toxin (PT) suggests that Gi proteins are key to the transduction of signals. Gi proteins physically associate with multiple chemokine receptors, but there is also evidence that other PT-resistant Gi proteins, such as Gq or G16, might also associate with certain receptors [465]. AA release driven by PLA2 is important for optimal cell movement toward a chemokine gradient. The roles for activated PLD are as yet unclear. Recent studies have clearly demonstrated a key role for PI3K in chemokine receptor signaling [396, 554]. This leads in turn to activation of PIP-specific PLC, PKC, small GTP-ases, Src-related tyrosine kinase, PI3K, and PKB. PLA delivers two secondary messengers, inositol- 1,4,5 triphosphate, which releases intracellular calcium, and DAG which activates PKC. Multiple phosphorylation events are triggered by chemokines. PIP-OH-kinase can be activated by the βγ subunit of G protein, small GTP-ase or Src-related tyrosine kinases [687]. The membrane tyrosine phosphatase CD45 has also been shown to regulate CXCR4-mediated activation and phosphorylation of TcR downstream effectors Lck, ZAP-70, and

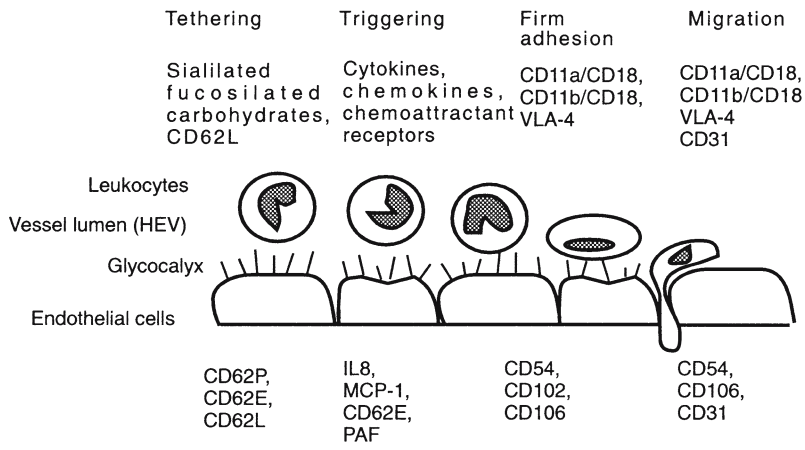


Fig. 1.59. Leukocyte trafficking and adhesion cascade. Sequential steps in leukocyte recruitment, their adhesion to endothelium and transendothelial migration (see text). (Modified from [209, 230, 328])

SLP-76. Activation of the RAFTK (related adhesion focal tyrosine kinase), a member of the related kinase family, has been shown to be induced by signaling via CXCL12 binding to CXCR4 [151]. Mitogen-activated protein kinases have also been shown to be phosphorylated and activated within 1 min after exposure of leukocytes to CCR3 ligands [247]. CXCR4 is predominantly expressed on inactivated naive T lymphocytes, B lymphocytes, DCs, and endothelial cells. SDF-1 is the only known ligand for CXCR4 [239].

The principal biological function of chemokines is contributing to leukocyte recruitment, firstly by activating integrins, as described above, and secondly by promoting leukocyte migration across endothelium and through ECM [2]. Additional functions consist in the antiviral immunity (innate immunity), hematopoiesis and angiogenesis regulation, growth and cellular metabolism [9]. The existence of so many characterized chemokines with overlapping targets is not surprising, since it is also possible that certain chemokines may have a restricted tissue expression, although their large number can actually result in a degree of redundancy [2]. However, the strong expression of IL_8 and MCP-1 by epithelial cells could also suggest that they might be key factors in leukocyte recruitment to counteract invading pathogens [141]. This notion agrees with the priming of cytotoxic CD8 T cells and NK cells by RANTES and MIP-1α [569], which can provide substantial costimulatory signals for T-cell proliferation and promotion of effector functions, also by enhancing CD80 and CD86 expression on APCs and IL_2 production from activated T lymphocytes [20]. In addition, CCL chemokines direct basophil and eosinophil migration for activation and response at DTH sites, where ILs of Th2 T cells surround and in turn stimulate the above cells, amplifying their negative effects. Nonetheless, the presently available data, even if suggestive, need to be thoroughly analyzed in the context of the pathogenetic hypotheses currently discussed.

More to the point, chemokines act in conjunction with HRFs which, like antigens, are able to trigger histamine release and to activate a large number of cells (mast cells, basophils, lymphocytes, eosinophils, macrophages, monocytes and platelets) and possibly B and T lymphocytes. By binding to sIgE, monocytes and basophils, HRFs can perpetuate histamine release, thereby inducing allergic reactions too late or too prolonged to be classified as IgE-mediated reactions. The observation that IgE of atopic subjects bind HRFs at variance with the IgE of nonatopic individuals suggests that these molecules have an unequivocal clinical weight. There is a factor inhibiting HRF (HRIF) in relation with NAP-1/IL_8 (at the same time an HRF and an HRIF), a protein of 8,000 Da derived from PBMCs, B and T lymphocytes and possibly alveolar macrophages, whose generation is augmented by normal histamine concentrations, hence suggesting a feedback mechanism inhibiting the histamine itself, which can thus regulate HRF activities. CCL chemokines are HRFs, if nothing else due to the similarity of conditions in which they are generated [687].

Leukocyte Trafficking and Migration

Adhesion processes, mediated by proteins adherent to endothelial membranes and correspondent receptors on leukocyte membranes, acquire a new impetus due to specific interactions between HEVs and lymphocytes. Transendothelial migration of activated lymphocytes from the blood into the tissues is an essential step for immune functions, which may be arrested by Zap-70 deficiency (Chap. 22). Preceded by inflammatory mediator release, causing vasodilation and blood flow deceleration, this preliminary step allowing the cascade to proceed, as mentioned in the preceding section via HEVs, influences *leukocyte rolling* [23]. Up-regulated CD62s, mainly CD62P, with a long molecular structure that extends above the surrounding glycocalyx, have the task of capturing passing leukocytes expressing appropriate receptors (Fig. 1.59) [209, 230, 328]; moreover, CD62L is localized on the tips of microvilli, a first point of contact with the endothelium [348]. Ensuing passages are orchestrated by chemokines and integrins, preceded by T-cell rolling with selectin interactions (CD15, CD62L

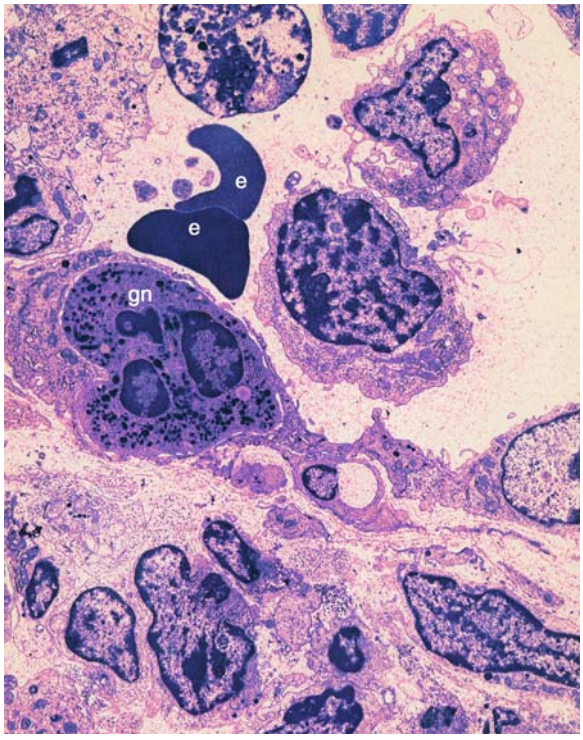


Fig. 1.60. Diapedesis. *e* Two red cells in the vessel lumen, *gn* granulocyte neutrophil while passing through the endothelial wall by diapedesis

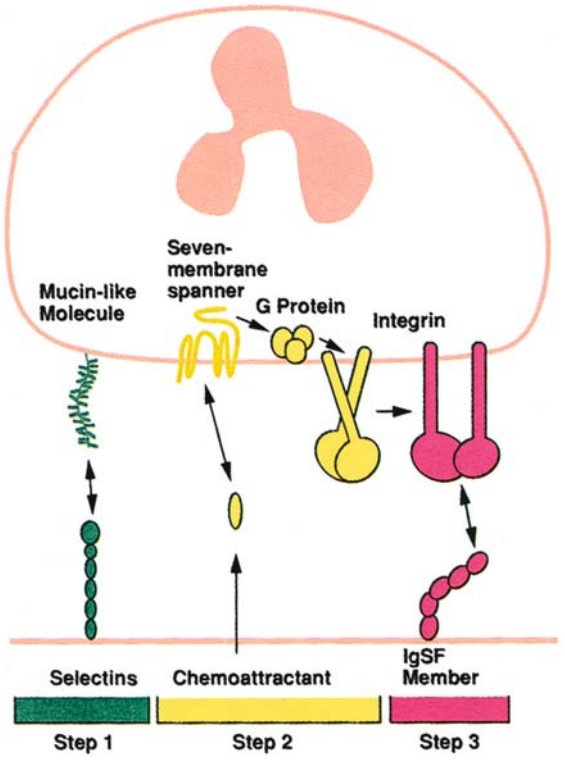


Fig. 1.61. Springer's three-step theory: how to provide the traffic signals that regulate leukocyte localization in the vasculature (for details see text). *IgSF* immunoglobulin superfamily

and CLA of lymphocytes, CD62P and CD62E on endothelium) and by integrin stimulation (activated by CD31 and chemokines). Functional activation of integrin receptors on lymphocytes, above all CD2, CD28 and PDGF, and signal transduction regulating adhesion point to a PI3K primary role, in turn activated by chemokines and receptors sensitive to G proteins [521]. After conformational modifications of the cells and integrins ensure a strong adhesiveness, lymphocytes undergo diapedesis (Fig. 1.60), and their migration between endothelial cells in extravascular spaces is followed by directional cues from chemoattractants (Fig. 1.61) [534]. The process continues within the inflamed tissues, generating additional mediators and ILs. The initiation of endothelial activation, which modulates selectins binding carbohydrate ligands, often displayed on mucin-like molecules, is responsible for the initial tethering of a flowing lymphocyte to the vessel wall and for a transient rolling along the endothelial cells (step 1). Tethering contributes to chemoattractant-mediated adhesion, resulting in integrin triggering and binding to endothelial ligands (step 2). The sound T-cell adhesion is modulated by G proteins, whose signals activate strong integrin adhesiveness, which binds IgSF on endothelium (step 3) and the transendothelial migration (step 4). Eventually T lymphocytes spread via endothelial cell–cell junctions, cross the basement membrane and migrate across lymphoid tissues [522]. A recent study indicated that activation of integrin avidity to endothelial ligands by endothelium-displayed chemoattractants (or chemokines) can take place within fractions of a seconds and can promote both reversible rolling adhesions or immediate conversion of leukocyte rolling to firm arrest on vascular ligands [187]. Obviously the adhesion required is not too strong, otherwise it would lead to cell immobilization [226] (Fig. 1.61). In step 4, lymphocytes cross intercellular HEV junctions, allowing them to enter HEVs: the entire process of lymphocyte sticking to HEVs takes only 1–3 s and step 4 10 min [454]. In humans, as many as 5×10^6 lymphocytes leak from blood via HEVs every second [454]. Even if the equilibrium among variables stabilizes with speed, relatively small changes in integrin expression or integrin–ligand affinity, or cell-substratum adhesiveness, can lead to substantial changes in migration speed [406]. Several integrins govern interactions: the initial ones are mediated by CD62L recognizing CD34 and GlyCAM-1 (Table 1.50). After activation, lymphocytes firmly adhere to endothelial cells since β2 integrins interact with IgSF members on both endothelium and G-protein-coupled receptors [162]. During stage 3, CD11a/CD18 inhibit lymphocyte migration via CD54 and CD102 HEV counter-receptors, playing the major role [534]. Then lymphocytes migrate, using β1 integrins, to

Table 1.58. Molecules active in the homing, memory and inflammatory responses of lymphocytes

Lymphocyte receptors	Ligands	Distribution	Activity
$\alpha_E\beta_7$=CD103	E-cadherin	Epithelium (unknown)	Homing
$\alpha_4\beta_7$=CD49d/CD29	MAdCAM-1	MALT, HEV	Homing
CD62L	CD34		Homing, memory
CLA	CD62E	Skin	Homing, memory
ESL-1	Unknown		Inflammation
PSGL-1	CD62P		Inflammation
CD11a/CD18	CD54, CD102		Inflammation
CD11b/CD18	Fibrinogen, CD54		Inflammation
CD11c/CD18	Fibrinogen		
CD49d/CD29	CD106, fibronectin		Inflammation

Data from [61, 209].
CLA Cutaneous lymphocyte-associated antigen, *ESL-1* CD62E ligand 1, *PSGL-1* CD62P glycoprotein ligand 1.

a chemotactic stimulus (such as a bacterial invasion) [163], while step 4 is CD31-modulated [230]. The pathways used by lymphocytes to bind endothelium depend on the site and nature of stimuli activating it:

- *IL_1 and TNF-α,* by increasing CD54 and CD106 expression on endothelium in vitro, allow lymphocytes to bind either β1 or β2 integrins [540]: IL_4 primes only CD106 expression, VCAM, hence restricting the field to β1 integrins.

There are differences regarding the involved site: the umbilical vein endothelium stimulated by IL_1 or TNF-α expresses both CD54 and CD106, whereas cutaneous microvascular endothelium responds much better to TNF-α.

Such processes are regulated by proteoglycans well expressed on endothelium, which stimulate granulocyte and T-cell adhesion by means of the chemokines IL_8 (via β2 integrins) and MIP-1β (via β1 integrins), respectively [565].

Lymphocyte recirculation is also influenced by adhesion molecules in specific tissues: one such molecule is MAdCAM-1, an IgSF member, largely restricted to gut epithelium (HEV of PPs, mesenteric lymph nodes, and endothelium of enteric mucosa) [328], where it mediates binding of a specific subset of $CD45RO^+$ cells expressing $\alpha_4\beta_1$ integrin [38]; wherever MAdCAM-1 is mentioned, its localization is in MALT [209].

CD62E mediates T-cell binding to lymph node HEVs. Such binding to mucosal HEVs is promoted by another integrin, LPAM-1, with an α chain homologous to an α4 CD49d/29 chain, in turn a CD106 receptor.

Additional molecules involved in lymphocyte homing include CLA expressed on T cells with an exclusive tropism for binding to CD62E on skin endothelium, and VAP-1 (vascular adhesion protein) also with an endothelial origin that mediates lymphocyte binding to lymph nodes and synovial membranes [328].

Lymphocyte migration into tissues has slightly diverse phases depending either on peripheral lymph node HEV, or on PPs, or skin, or intestine (Fig. 1.5). However, the CD106/receptor CD49d/29 duo plays a leading role in binding both lymphocytes and monocytes [85]. In Table 1.58 [62, 209] are shown the molecules involved in homing (and related memory) and in lymphocyte inflammatory responses: also CD62L, CD11a, b, c/CD18, $\alpha_4\beta_1$ = CD49d/CD29 (VLA-4) and $\alpha_4\beta_7$ = CD49d are included [209].

As regards *leukocytes,* the same selectivity comes on the scene: the involved molecules are CD62E and CD106, which also promotes adhesion of *eosinophils* expressing CD49d/CD29 at the surface, unlike neutrophils [624]. CD62E governs PAF production, which together with other endothelial cells with activating properties, expresses IL_1 and integrins such as CD11a/CD18, CD11b/CD18, etc. [65]. As a consequence, the CD106/receptor CD49d/29 duo represents the greater association of adhesive molecules in eosinophil recruitment and extravasation, to be directed to in vivo inflamed sites [624]. Eosinophils, following what is outlined by Fig. 1.59, supported by CD49d/CD29, adhere to endothelial cell membranes supplied with CD106 and are activated; expressing CD11a/CD18 bind to CD54 and CD102, then transmigrate into the underlying mucosa. In brief, the key points are as follows [230]:

- *Cell recruitment* mediated by T cells by means of IL_4, IL_5, RANTES and MIP-1α.
- *Selectin* (CD62E, CD62P and CD62L) and ligand (CD34, GlyCAM-1) selective expression on endothelial cells produced by ILs and mediators.
- *Integrin* (CD11a/CD18, CD11b/CD18, CD49d/CD29) selective activation induced by ILs, CCL and CXCL chemokines and chemoattractants.
- *IgSF* (CD54, CD102, CD106) selective expression induced by ILs: above all CD106 specifically expressed by

IL_4 on epithelial cells is an important regulatory moment in eosinophil adhesion to epithelium and antigen-driven migration [578].

- *TNF-α* has been demonstrated to have an important role in the expression of adhesion molecules that induce transendothelial migration of eosinophils [534].

Neutrophils in normal conditions are absent or poorly represented on endothelium, being expressed after activation. These cells in the early phase roll on activated endothelial vessel walls, accompanied by a mast cell histamine-modulated increase in vascular permeability, a process mediated by CD62P expressed on neutrophils [154]. PAF is released at these sites and in perspective can elicit, together with SP, the chemotactic movement of parallel activated neutrophils, which strongly adhere to endothelium and proceed to transendothelial migration. Lipoxin A4 modulates transmigration of human neutrophils across intestinal epithelial monolayers [90] (see also Chap. 11). In the late phase, LPS and ILs (IL_1, IFN, TNF, etc.) with inflammatory action stimulate CD62P on endothelial cells, expression of CD54, IL_8 release, and CD62L and CD15s activation on neutrophils which, via CD15s, CD62E and CD62P specific binding, continue their rolling along vessel walls, adhesion modulated by CD11b/CD18/CD54, activation and diapedesis [19, 154]. However, cell migration can be different as a consequence of an eicosanoid exposure, although neutrophil retention in a specific anatomical site could play an important role in mucosal defense [90]. Selectins mediate the initial rolling contacts of *monocytes* with the endothelium. Firm adhesion to the endothelium is the second step and involves other adhesion molecules such as CD11a, CD11b, CD18, and VLA-4 on monocyteds and CD54 and VCAM-1 on endothelial cells. The adherence to endothelial cells is modulated by endothelin-1 via the involvement of Src (p60src), JAK1-like kinases, and vascular endothelium growth factor by an increased flt-1 expression by monocyes. The final step is monocyte transmigration into the subendothelial space [609].

A primary function of *epithelial cells* is to maintain vascular integrity. Vascular injury promptly promotes epithelial cells to release their granule content (CD62P, vWF), which is quickly deposited on ECM, where it plays a crucial role in platelet adhesion to damaged sites. *Platelet degranulation* and activation of their $\alpha_{II}\beta_3$ integrin = CD41/CD61 drives further accumulation of platelets (aggregation) and modulates neutrophil and monocyte recruitment, which participate in the repair of damaged tissues. Thus platelet rolling, analogous to lymphocyte rolling, may represent an initial step in hemostasis [163].

Although knowledge on the role effectively played by such different molecules in human physiopathology is still lacking and far from yielding logically linked reference patterns, such acquisition suggests that some molecules may play a central role in normal conditions regarding homeostasis and the pathogenesis of some morbid conditions.

Interrelations with Other Organs

Several observations clearly show that the immune system interacts with other communication systems of our organism such as the endocrine apparatus and central nervous system (CNS). There are important correlations between ILs and neuropeptides: after its release from nervous tissue termination, SP is able to increase transcriptions at the gene level and synthesize and secrete IL_1, IL_6 and TNF-α, with an effect on stromal cells, GM-CSF, G-CSF and M-CSF, and consequent neutrophil, basophil, macrophage and eosinophil adhesion and activation, thus modulating the effector stages of type I and type IV reactions. Interconnections with neuroendocrine circuits are complex: SP and VIP (vasoactive intestinal peptide) are involved in the control of IL_2 production by T lymphocytes, while IL_1 and IL_1R are present in neurones and endocrine glands. IL_2 and IL_6 alter the proliferation pattern of anterior pituitary cells, as well as GH, ACTH and prolactin secretion, while IL_5, IL_7, IL_9 and TGF-β are involved in the regulation of neurodifferentiation [493]. Indirectly, an initial event such as an infection or a tissue lesion of less impact can trigger polysaccharide or ECM protein release, which stimulates tissue macrophages to release TNF-α. TNF-α-exposed endothelial cells express adhesion molecules to attract first PBMCs and then T cells to recognize APC-expressed antigens. TNF-α acting on these cells mediates IL_1 start-up, inducing phagocytes, T lymphocytes and endothelial cells to produce IL_6 [326]. The integrin-linked kinase (ILK), colocalizing with the β1-integrin subunit, is expressed in various regions of the CNS. ILK staining revealed that it is enriched in neurons and is an important effector in NGF-mediated neurite outgrowth [353]. Some ILs induce the acute-phase response (APR) in the liver, and CSs stimulate APP production, whereas they block TNF-α, IL_1 and IL_6 actions. These ILs act independently on the hypothalamus-hypophysis-adrenal (HPA) axis, but also display synergic effects: TNF-α and IL_6 act on the hypothalamus inducing fever. In addition IL_6 causes a concentration of cortisol and corticotrophin that is higher than the levels obtained with the greatest stimulation of corticotrophin-releasing hormone (CRH). How IL_6 can reach the CRH hypothalamic neurones is not known; however, it is possible that IL_6 is produced by endothelial and glial cells stimulated by TNF-α and IL_1. CNS and the endocrine system also mount inflammatory responses: for example, they generate β-endorphin endowed with a local analgesic action. CRH and probably also arginine-vasopressin have a pro-inflammatory activity; in particular CRH is found in inflamed areas, most likely produced locally by postganglionic sympathetic neurones [326]. Appendix 1.4 [326] concludes the issue of neuroendocrine interactions with the immune system: for example, prolactin amplifies IL_2 production, β-endorphins, and melanotonin, which are active on T and B lymphocytes.

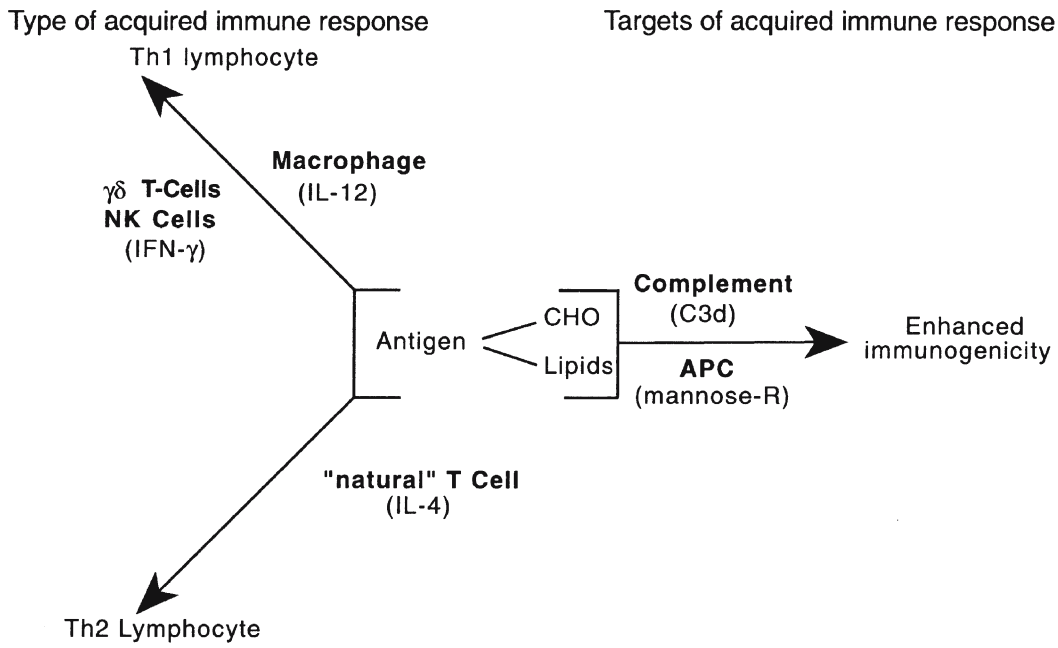


Fig. 1.62. Components of innate immunity (*in bold*), which recognizes carbohydrates (*CHO*) and lipids and instructs the acquired immune response to the antigens with which they are associated. *APC* antigen-presenting cell, *R* receptor. (Modified from [148])

Innate Immunity

Innate, or natural immunity, substantially aspecific and independent of previous contacts with pathogen agents, has evolved as the first line of defense against the constant threat of myriad microorganisms in the surrounding environment. This immunity is based on the genetic constitution of individuals. To avoid infections and prevent access of pathogens or potentially pathogenic microorganisms, present in the environment and on the body surface lying in direct contact with the environment, the host has evolved a series of *sophisticated defense systems,* closely integrated among themselves. The achievement of protective immunity against invading pathogens relies on sensing specific molecular features expressed in microorganisms and depends on the ability to elicit the pertinent type of immune response to fight a specific pathogen. Germline-encoded receptor molecules enable the cells of the innate immune system to recognize structural components conserved among classes of microorganisms [449]. The recognition of microbial products by the host innate immune system rapidly triggers appropriate responses to contain the infection and regulate the development of adaptive immunity. The exposure to microbial antigens primes several ILs and proinflammatory mediators that affect T cell differentiation and are rapidly produced by APCs, such as DCs and macrophages [366, 447]. Recent data show that IL_1R and $IL_{18}R$ are key molecules in both the innate and acquired immunity and are members of a larger family of related receptors, some of which contribute to host defense [526]. Table 1.1 shows the differences between innate and acquired immunity, which is instructed by soluble and cellular components of innate immunity to select the appropriate antigens coincidentally with strategies devised for their elimination (Fig. 1.62) [148]. Natural immunity is realized by skin, mucosal barriers and secretion protective effects, phagocyte cells, complement, and additional biological activity of nonspecific factors. Among them there are proteins coded in the germline, employed by innate immunity to recognize potentially noxious substances [148]. Such proteins are either soluble or have the form of surface receptors: for example, macrophage phagocyte particles or soluble glycoconjugates linked with the mannose receptor, a C-type lectin with a wide specificity for carbohydrates, and in addition an LPS receptor, LBP (lipopolysaccharide-binding protein) [536]. Bacteria coated with innate immune surfactants or MBP (mannose-binding protein), often including complement activated by the alternate (innate immune) pathway, are opsonized and more readily phagocytosed. In this process the receptors for antibodies and complement of macrophages and neutrophils are important, so that the coating of microorganisms with antibodies, complement, or both enhances phagocytosis. The engulfed microorganisms are subjected to a wide range of toxic intracellular molecules, including $O_2^{\bullet -}$, hydroxyl radicals, hypochlorous acid, NO, antimicrobial cationic proteins and peptides, and lysozyme [115]. LCs become activated and behave as APCs when pattern-recognition receptors on their surface recognize distinctive pathogen-associated molecular patterns on the surface

of microorganisms [345]. Such common constituents of Gram– bacteria external membrane signal infectious agents triggering IL_1, IL_6, IL_{12} and TNF-α synthesis, thus eliciting an APR [31], stimulate macrophages and other cells, as well as CD4 differentiation and growth [148]. Current data show that IL_1R and $IL_{18}R$ are key molecules in both the innate and adaptive immunity, and are members of a larger family of related receptors, some of which contribute to host defense [525] and that IL_{22} directly promotes the innate, nonspecific immunity of tissues [650]. IL_{22} induces APRs in the liver, which suggests that IL_{22} plays a role in innate immunity via induction of an inflammatory process [129]. Attractive data support the concept that potential prophylactic and therapeutic results are expected from $IL_{17}A$ and $IL_{17}F$ in host defense against bacterial infection. The IL_{25} activity associated with systemic and localized Th2 responses offers an experimental basis to modulate Th2-associated allergic diseases [249]. Some NK cells posses lectin-like membrane receptors involved in the recognition of target cells destined to cytolysis [289]. Complement is activated when the alternative pathway interacts with particles rich in carbohydrates but lacking sialic acid, or the classic pathway is stimulated by *collectins,* which bind to specific carbohydrates [210]. It is understandable why, unlike acquired immunity, *its innate immunity has a more complex organization:* constituents are soluble cells and factors with different structure and function, sometimes acting on different targets, but on the whole often become in turn integrated [148]. Recent studies have individuated the TcRγδ, which also in natural immunity precedes an αβ in antigen responses, thus influencing αβ-expressed IL pattern, discriminating between pathogens [152]. In this way, γδ could begin to supervise αβ responses to infectious agents due to their much earlier activation and conversion to memory [210]. A similar control could be extended to NK cells [287].

The natural defenses, the skin and mucosal barriers, are strengthened by mucosal secretions containing sIgA, able to complex with antigens and to identify antibodies capable of their recognition, and to complex in turn with antigens. Activation of the complement cascade can lead to directly destroying undesired hosts or to facilitating their phagocytosis. Complement and phagocytes are the first to activate when an infection approaches, supplying host defenses with notable contributions by means of a nonspecific protection against invasive pathogens, also without antibodies and/or T CTLs intervening [531]. Therefore specific immune mechanisms act in concert with those of innate immunity. We will examine:

1. Anatomic barriers: skin, mucus, and secretions
2. Proteins with anti-infectious activity (PAA):

a) Lysozyme
b) IFN
c) Complement
d) APP or pentraxins

3. Phagocytes: neutrophils and macrophages

1. *Anatomic barriers,* or physicochemical barriers, offer not only a mechanical, but also chemical protection, performed via production of biological molecules with antibacterial activity, including lactoperoxidase, lysozyme, lipase, spermine and fatty acids with bactericidal power. *Skin* is provided with the *stratum corneum,* which by means of its physiological desquamation resists penetration of a great number of microbes. In addition, it is normally impermeable to the greater part of infectious agents, because of bacteriostatic, bactericidal and fungicidal effects of triglycerides, free fatty acids and lactic acid, present in sebaceous secretions and sweat, which also contains lysozyme. As a compensation of the thinner epithelial stratum, mucus-capturing microorganisms are present on *mucosal surfaces,* subsequently removed from airways by the mucociliary apparatus. The secreted mucus layer overlays the epithelium in the respiratory, gastrointestinal, and genitourinary tracts, and the epithelial cilia sweep away this mucus layer, permitting it to be constantly refreshed after it has been contaminated with inhaled or ingested particles. Invaders are transported with a continuous cycle from bronchioles to the pharynx, a protective action that extends to the stomach HCl with bactericidal action. Saliva, swallowing, peristalsis and defecation mechanically expel microorganisms from the gastrointestinal system [353]. Salivation, lacrimation, and coughing, are further mechanisms that are very effective in reducing bacterial assaults: patients presenting with severe changes in lacrimation and salivation (Sjögren syndrome) suffer from severe eye infections and tooth caries. Nasal secretion and saliva contribution are significant, provided with mucopolysaccharides inactivating some viruses of anaerobic germs associated with the normal intestinal bacterial flora, both preventing pathogen attachment via competition for essential nutrients and/or production of inhibiting substances as well as urinary flow ensuring unremitting cleansing. Basic proteins, such as lysine, arginine, spermine, spermidine and particular gps, among others transferrin, make additional contributions [47].

2. *PAA,* important associates of innate immunity, integrate mechanisms that rapidly come into play, with a notably crucial result when the innate system faces the first encounter with infectious agents, since it will take from 4 to 15 days before elaborating antibodies and cytotoxic cells. Not all germs are assaulted with identical means and proportions: in biological fluids and interstitial spaces, the intervention of factors bound to antibodies and complement is more germane, unlike parenchyma where phenomena of cell immunity prevail (ILs, cytotoxic cells).

2a. *Lysozyme,* produced by macrophages and neutrophils, is found in salivary, lacrimal, nasal secretions, intestinal and respiratory mucus, lymph nodes and spleen. It is able to split β-glycoside bindings, in particular β 1–4 links between *N*-acetylglucosamine and

Table 1.59. Anti-infectious and anti-inflammatory activities of complement

Component(s)	Function in host defense
C1, C4, C4a, C4b	Neutralization of viruses ↑
C1q	Opsonization and phagocytosis ↑, antibody formation ↑, binding to CICs, cytotoxicity mediation
C2 kinin	Vascular permeability ↓
C3 fragment (C3e)	Release of granulocytes from bone marrow ↑
C3a	Antibody formation, antigen-induced T-cell proliferation, cytotoxicity mediated by T and NK cells ↑
C3a, C4a, C5a	Release of histamine and other mediators from mast cells ↑
C3b	Opsonization, IC phagocytosis, B-cell growth, IL_2-dependent T-cell growth, killing mediated by T and NK cells ↓, antigen presentation, clearance of CICs, antigen localization in lymphoid tissues
C3b soluble	Antigen-induced T-cell proliferation, cytokine production ↑
iC3b	Opsonization, phagocytosis, ADCC ↑
C3d, C3dg	B-cell growth↑
C3d, C3dg soluble	B-cell growth, IL_2-dependent T-cell growth ↓
C5a	Chemotaxis of PMNs inducing the influx into inflammatory sites, stimulation of phagocytes to release cytokines (TNF, IL_1),granule enzymes and O_2 metabolites, antigen-induced T-cell proliferation, antibody formation ↑
C5b6789 (MAC)	Lysis of bacteria, fungi, protozoa, viruses and virus-infected cells
Factor Ba	B-cell proliferation
Factor Bb	B-cell growth and differentiation ↑
Factor H	Growth of murine lymphocytes

Modified from [241].
IC immune complexes, ↑ increase/up-regulation, ↓ decrease/down-regulation, *PMN* polymorphonucleates.

N-acetylmuramic acid, a normal constituent of several bacterial cell walls.

2b. *IFN α, β, γ,* have several effects, including a timely antiviral activity, especially IFN-λ1–3 (*IL_{28a}*, *IL_{28b}*, *IL_{29}*) [377]. All IFN-α, -β, -ω, and -λ subtypes are expressed in influenza-virus-infected monocyte-derived DCs and PDCs [88]. It is significant that IFN-γ induce in murine B cells the IgG_1 isotype switch that increases phagocytosis, activating the complement classic pathway and linking macrophage Fc receptors [148].

2c. *Complement* [241, 633] represents, together with antibodies, the main component of the humoral defense system against microorganisms. *If activated it participates in host protection* in a specific and nonspecific way, intervening, besides phagocytosis, with functions divided into lytic and nonlytic, such as chemotaxis, opsonization and anaphylactogen activity (Table 1.59) [241]. In addition, with its receptors, it acts on B-cell antibody synthesis, immune memory, and CIC solubilization and clearance [159] (Tables 1.60, 1.61) [241, 260, 474]. From a simplistic viewpoint among immune reactions, complement is comparable to a motor vehicle, while antibody is an ignition key: indeed, once antibody has recognized the non-self molecule, it has specific functions, including complement activation and its fixation on the cell surface [633]. Complement components, provided with defensive and immunoregulatory properties, are normally present in the bloodstream in an inactive state and all act in concert, but each must be sequentially activated and in suitable conditions (a relatively small starting signal is sufficient), so that the typical mechanism of cascade reaction is triggered [633]. The primary source of such proteins is the liver, with smaller contributions from tissue macrophages, epithelial cells of the gastroenteric tract, and PBMCs. Like Igs, it is hypothesized that they arose late in evolution and are found only in vertebrates [36]. Complement is formed by >25 serum proteins interacting with nine functional components, designated C1–C9, reflecting the orderly sequence of their activation, with the exception of C4, which is activated after C1 and before C2. As regards nomenclature, a horizontal bar over a component denotes active enzyme activity of either a protein or a protein complex; proteins that become inactivated either by enzymatic cleavage or internal rearrangement are prefixed a small i (iC3); small postscripts a and b (C3a, C3b) indicate the biologically active fragments of a component; C1 subunits are designated q, r, s. The C1 complex consists of C1q, two molecules of C1r, and two molecules of C1s which bind to antibodies bound to an anti-

Table 1.60. Receptors binding complement components

Receptor(s)	Ligands	Major functional results of binding	Cell distribution
CR1 (CD35)	C3b, C4b, iC3b	Phagocytosis ↑ IC clearance, BC activation, antigen presentation, cofactor for cleavage of C3b or C4b	M, N, B, E, BC, CD4, ER, CD
CR3 (CD11b/18)	iC3b	Phagocytosis ↑ cell adhesion ↑	M, N, NK/K cells, CD
CR2 (CD21)	C3d, C3dg iC3b, C3b, EBV	Primary antibody response ↑ BC activation, also of BC memory, receptor for EBV infection	BC, CD, immature Epithelial cells
CR4 (CD11c/18)	C3dg, C3d	Phagocytosis mediated or not by FcR ↑	M, N, NK/K cells, CD
C4a/C3aR	C4α, C3α	Anaphylotoxin (see text)	M, B
C5aR	C5a, C5a des arg	Chemotaxis muscle and endothelial cells	MC, B, N, E, M, smooth
C1qR	C1q	Anaphylotoxin (see text) phagocytosis ↑ chemotaxis ↑	M, B, N, E, BC, endothelial cells, fibroblasts

Modified from [241].
R receptor, *M* mononucleates, *N* neutrophils, *B* basophils, *E* eosinophils, *BC* B cells, *ER* erythrocytes, *CD* follicular dendritic cells, *MC* mast cells, *IC* immune complexes, ↑ increase, *ND* not defined.

Table 1.61. Regulatory proteins of the complement system

Proteins	Target(s)	Biological functions
Soluble proteins		
C1 INH	C1r, C1s	Inhibits the serine proteases, binds to C1r, C1s inhibiting their participation in the classic pathway, binds to C1 inactive preventing its activation, inhibits kallikrein, plasmin and factors XIa and XIIa
C4bp	C4b	Increases decay of C3 classic convertase, cofactor of C4b cleavage mediated by factor I
Factor H	C3b	Up-regulates decay of C3 alternative convertase, cofactor of C3b cleavage mediated by factor I
Factor I	C4b, C3b	Cleaves and inactivates C4b, C3b using as cofactors C4bp, factor H, MCP
Properdin (P)		
Protein S or vitronectin	C5b-7	Binds to C5b-7 complex and prevents MAC insertion into cell membranes
SP40/40	C5b-9	Modulates MAC formation
Membrane proteins		
CR1 (CD35)	C3b, C4b, iC3b	Up-regulates decay of C3 classic and alternative convertase
DAF (CD55)	C4b2B, CebBb	Up-regulates decay of C3 classic and alternative convertase
HRF o C8bp	C8	Inhibits complement lysis
	C9	Blocks the binding of C9 to C8, preventing both MAC insertion into lipid membranes of autologous cells and complement lysis
MACIF (CD59)	C8	Blocks the binding of C7, C8 to C5b, C6, preventing MAC development and complement lysis
MCP (CD46)	C3b, C4b	Assembly and decay of C3b and C4b mediated by factor I

Modified from [260, 474].
C1 Inh C1 Inhibitor, *C4bp* protein binding C4, *C8bp* protein binding C8, *HRF* homologous restriction factor, *MAC* membrane attack complex, *MACIF* membrane attack complex inhibitory factor.

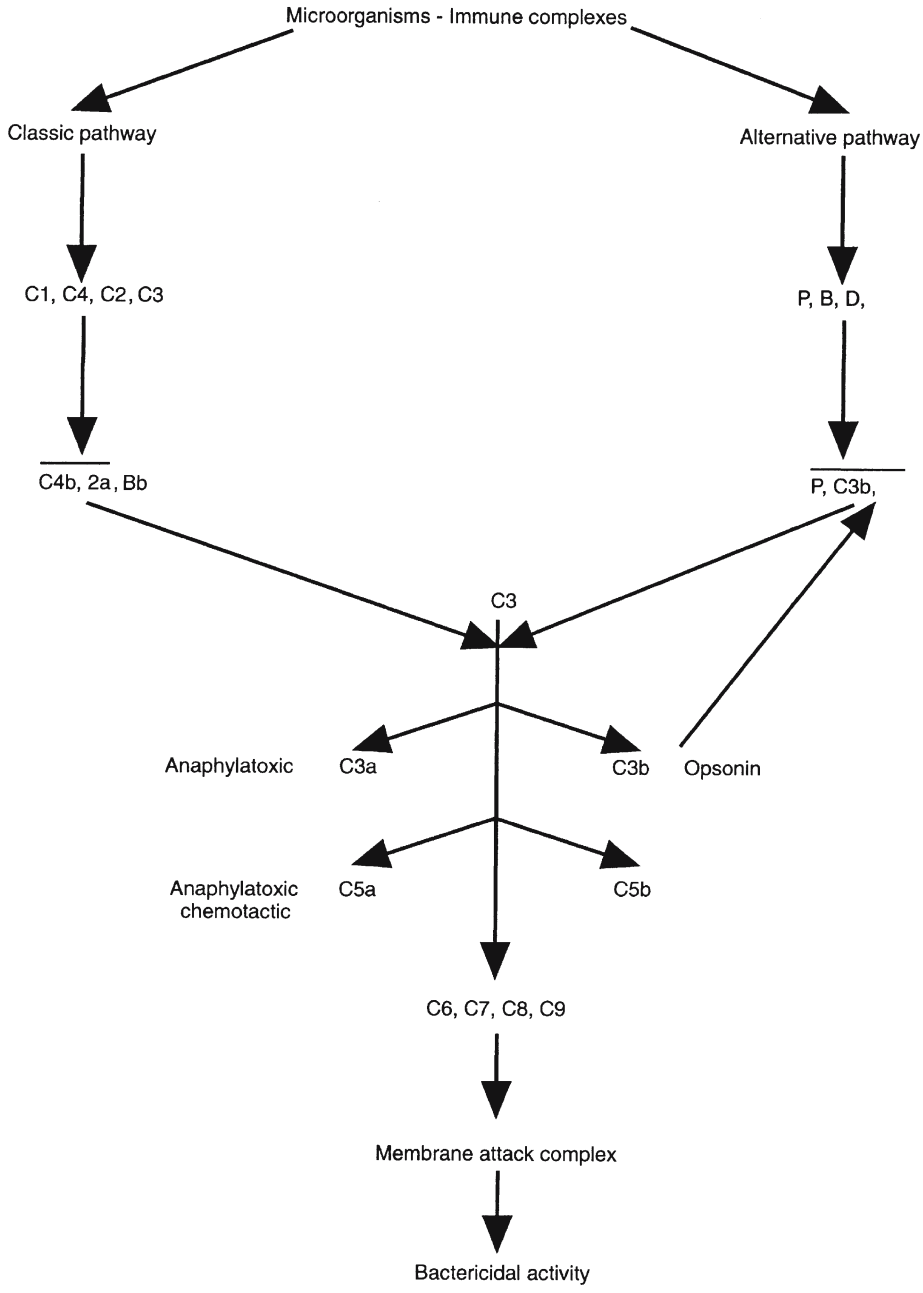


Fig. 1.63. A schematic representation of the complement system. *B* B fragment, *Bb* larger fragment of B, *D* D factor, *P* properdin. (Modified from [648])

gen on the surface of a bacterial cell [345]. Letters of the Latin alphabet, P, B, D (initials of properdin, factor B, factor D), designate the alternative pathway; factor B is divided into a small fragment (Ba) and a larger one (Bb) [648]. Proteins are activated by two pathways: the classic and alternative pathways (Fig. 1.63) [648].

The *classic pathway* is activated by antigen–antibody complexes, the alternative pathway by microbial-cell walls, and the lectin pathway by the interaction of microbial carbohydrates with MBP in the plasma [618]. This pathway is so called because it was described first, it is more effective, but to be activated requires the presence of acquired immunity. The initial seed is C1q, the first component, with a MW of 400 kD, which interacts with antigen–antibody complexes, or with IgM or IgG_{1-3}. To activate IgM, with a much higher MW, a single pentameric IgM is sufficient, with IgG_3, IgG_1 and IgG_2 following in an orderly fashion. IgA, IgD, IgE, and IgG_4 cannot bind to C1q; consequently no such antibody is able to activate this pathway. Complement interactions with natural antibodies are of crucial significance for the host, being inhibited complement-mediated autoimmune reactions [350]. More rarely, activation may be mounted by various substances, including bacterial LPS, CRP, certain viruses, etc. Activated C1q activates C1r, which, in turn, activates C1s, with MWs of 95 and 85 kD, respectively, to form a C1 complex. C1s, if activated, is able to act on its natural substrates, namely C4 and C2 [633].

C4 is a 180-kD gp synthesized by macrophages; activated C1s results in C4 cleavage into anaphylotoxin, C4a, and a larger fragment, C4b. C4b possesses several functions, especially that of binding both to molecules adjacent to the antigen–antibody complex that has initiated the cascade and the next component, C2.

C2 is a 115-kD gp; more than by C4b, it is activated by molecules next to C1s, but remains bound to a complex with C4b to form the C3/5 convertase (C4b2a), which in turn splits and activates both C3 and C5 [345].

C3 (1.2 g/l) has a central role in the complement cascade: it consists of two S-S-linked α and β chains. When C3 is activated by the convertase, two highly active biological forms produce a small peptide, C3a cleaved from the α chain, and a larger C3b fragment [159]. C3 splitting is due to C3/5 convertase, secreted through both pathways [648].

The *alternative pathway* was discovered more recently, but is phylogenetically the earlier pathway. It includes C3 and factors B and D. Activation results also from non-immunological mechanisms and yields physiologically active substances, thus achieving the complement bactericidal and opsonic effects in the absence of bound antibodies for initiation. The pathway is triggered by contact of complement proteins with LPS from cell walls of bacteria, virus, yeasts, parasites, a factor present in cobra venom, and most likely by aggregated IgA not activating the classic pathway [241].

The antibody-independent activation calls for an extreme instability of internal bonds of the native C3 molecule. Based on such potentialities, C3b binds factor B, thereby forming the C3bB complex, further activated by factor D, which cleaves factor B while bound to C3b to generate the enzymatic complex C3bBb. This complex acts as a C3 convertase and, similarly to the classic pathway, releases C3a and C3b from C3, allowing C3b to resume its properties, increasing C3 convertase and C3 activated levels. A closed circuit is established, where the alternative pathway acts as a positive feedback loop with active amplification; therefore more substrate is cleft, more C3b results. If this mechanism remains uncontrolled, it could rapidly consume the entire C3 and the subsequent components of the cascade. Consequently, this amplification is balanced by a rapid C3bBb complex dissociation. The P binding stabilizes this enzyme. C3b is also largely inactivated by factor H, which competes with factor B to bind to C3b, practically preventing C3bBb formation, and by C3b inactivator eliciting a further C3b degradation to C3c and C3dg fragments. Certainly, factor H and the proteins linked to its binding site contribute to the protection of healthy host cells, regulating C3 activity [689].

Notably, C3b is present in trace amounts in normal serum, probably because there are low concentrations of factors B and D. It is also postulated that LPS of Gram+ bacteria and other substances that trigger the alternative pathway amplification loop somehow protect the small C3b amounts from total inactivation, so that the above substances initiate the alternative pathway. There is evidence that thioesters are present in the native forms of complement proteins C3 and C4 and that their molecular conformational changes dramatically on activation [122]. C3b is a 77 amino acid residue polypeptide and following the above-mentioned changes exposes the thioester bond, which is very reactive, and interacts with amine ($-NH_2$) and hydroxyl (–OH) on proteins and carbohydrates, allowing C3b a rapid covalent link to other biomolecules, since the thioester half-life is 60 µs while that of native C3 is >200 h [470]. Therefore, thioester hydrolysis ensures that activated molecules do not diffuse away from the activation site to bystander cells of the host [122]. As is seen in Tables 1.60, 1.61, C3 is distributed to different receptors (CR1, CR3, CR2) through its ligands (C3b, iC3b, C3dg), thus being capable of interacting with different cell types and bringing about a large spectrum of biological functions (Table 1.59), considering that C3b also has opsonizing properties [18].

When the *common final pathway* is activated, C5–C9 assembly and activation constitute the lytic activity of complement on target cells. C5–C7 are globular proteins with a MW of 180, 130 and 120 kD, respectively. C3b acts as an acceptor site for C5, which is cleft to form two anaphylotoxins, a small fragment, C5a, and a larger, C5b, which binds C6 and C7 to form a C5b67 or C567 complex on the cell membrane, which in turn, through conformational changes, modulates C8 and C9 activation, two 160- and 80-kD proteins, respectively [633].

The final act is the polymerization of perforin-like C9, around the C5b678 complex, which links six C9 molecules involving the assembly of MAC (*membrane attack complex*) (Fig. 1.63) with a MW of $\approx 10^6$ D resembling perforins, since it is a molecule forming pores on membranes with walls constituted by C9; in this way there is Na influx and K outflow, with a consequent increase in membrane permeability and subsequent target cell lysis [648].

The finding that MBL (mannose-binding lectin) residues binding to mannose can initiate complement actiation was followed by the discovery of the MBL-associated serine protease (MASP) enzymes. MBL activates complement by interacting with two serine proteases 1 and 2 (MASP1 and MASP2). MBL binding to its microbial ligands activates MASP1 and MASP2. MASP2 cleaves and activates the complement components C2 and C4 and MASP1 may cleave C3 directly [338]. The cleavage products C2a and C4b then form a C3 convertase, which initiates the complement cascade by cleaving the C3 protein. The MBL complex and its proteases functions similarly to the C1 complex of the classic complement cascade [345]. These components of the complement system have been named the MBL pathway [633].

Activation of the whole cascade elicits the formation of several products with different biological activity: some adhere to external cells, altering their properties

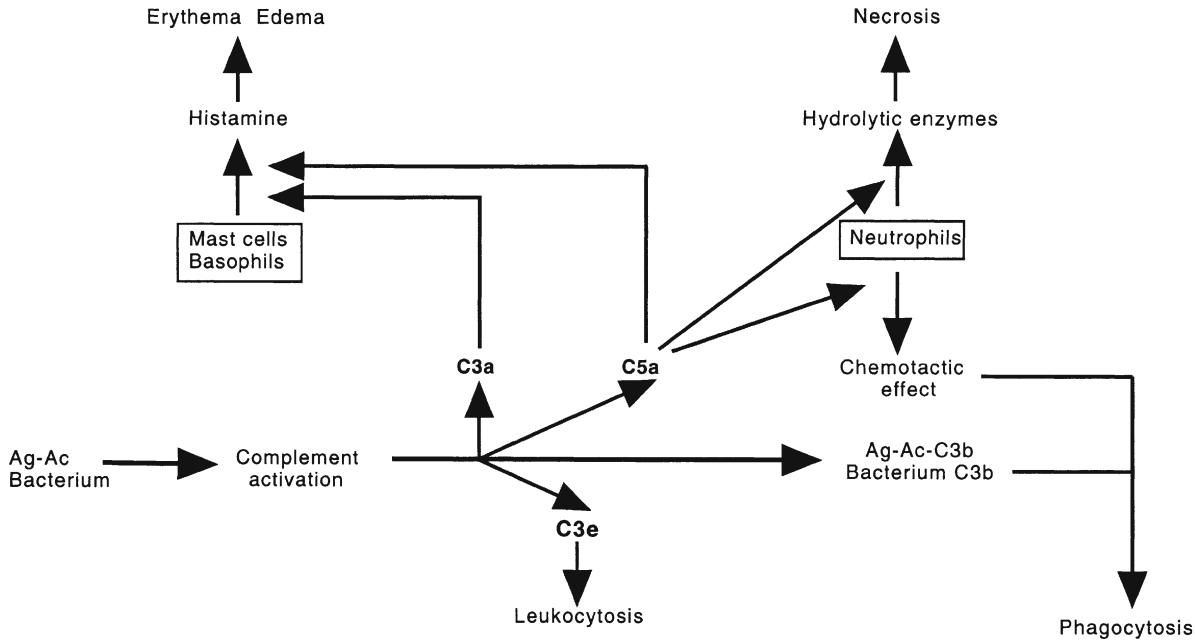


Fig. 1.64. Role of complement in inflammation. (Modified from [18])

and determining the lysis, for example, of infecting microorganisms; others provoke a local inflammatory reaction [260]. Complement thereby plays an essential role in each humoral defense system against external aggressions. A particular characteristic of this system is the lack of action specificity and intervention against harmful agents of varying nature, although antibody responses form a substantial way of activation; however, such aspecificity, if on the one hand it allows notable savings of selective defense systems, on the other hand it can foster an aggressive response against host components [159]. Table 1.59 and Fig.1.64 [18] document how complement actively participates in host defense and inflammation, and Tables 1.60 and 1.61 summarize both features and functions of complement receptors and regulatory proteins. Several cells are expressed, namely, known integrins including CR3 (CD11b/18) and CR4 (CD11c/18) [53], CR2 (CD21), able to activate B lymphocytes, and CR1 (CD35) distributed to human red cells. Some proteins act as regulators, just to control undesired activities: CR1, DAF (decay accelerating factor), CD55 and MCP (membrane cofactor protein), or CD46, prevent the formation of a full C3-splitting enzyme and related biological effects; CD59 or MACIF (membrane attack complex inhibitory factor) and C8 bp (protein binding C8) or HRF (homologous restriction factor) preclude a full MAC development [241]. During the activation C3a, C4a, C5 anaphylotoxins are up-regulated, genetically correlated, but also efficient in different ways (C5a≫C3a≫C4a), for which phagocytes, endothelial cells and smooth muscle cells express receptors, also mediating infectious germ contact with antibodies, complement and phagocytes. C5a, generated by activation of both complement pathways, is the most chemotactic for PMNs, also inducing their microbicidal activity [648]. Anaphylotoxins are active but with a negative role in inflammation, causing aspecific release of histamine and other mediators from metachromatic cells and their consequent degranulation, increasing vascular permeability. C5a is much more active in provoking bronchoconstriction and smooth muscle contraction, it binds to specific receptors on bronchial and alveolar epithelial cells, vascular smooth cells, endothelium, etc., delivering an unexpected up-regulation to various target cells. C5a also couples with a G protein transducing signals to the cell [637]. Additional active complement fragments deliver biological activity: macrophage distension by Bb fragment, Ba fragment is chemotactic, C2 kinin (C2 fragment) increases vascular permeability, and C3e (Table 1.59) increases circulating leukocyte titers mobilizing medullary reserves [18].

To confirm such collaboration of two levels of immunity, we mention that CD19, a component of acquired immunity, is associated with CD21 receptor of C3d and thus is part of innate immunity [571]. CD19 is necessary for normal antibody responses to antigens, being dependent on B–T interactions, and hence it amplifies signal transduction [571]: for this purpose a cross-linking with mIgs and a complement covalently linking carbohydrates to C3d fragment and PSAs are necessary [122]. Regarding infectious diseases, recent studies have demonstrated the substantial defense capabilities of C3 and C4. Fixation of PSAs, with C3d support, to FDCs and B cells of the marginal zone equipped with CD21 on their surface shows that an important anti-infective function is played by the spleen [423], scarce in babies

aged <2 years and absent in C3-deficient individuals [633]. C3d deposition on external membranes of noxious cells is able to perforate such cells, followed by signaling to immune cells and increasing immune stimulation; therefore a molecular adjuvant of innate immunity may select antigens for recognition by acquired immunity [116]. These results should be complemented, in this context, with pathogenetic implications, such as HIV part opsonization with C3, which widens virus diffusion, and usage of CD55 as a receptor for *Escherichia coli*, CD35 and CD21 (CR1 and 2) from virus and mycobacteria, CD46 from measles virus and M protein CD46 and/or H factor from *Staphylococcal pyogenes* for adhesion to keratinocytes [415].

Another machinery put forward by bacteria is a mimicry to hold the products of complement activation off the cell walls to resist their attack. Further capsules containing sialic acid are very poor activators of the alternative pathway in the absence of antibody, in addition to representing an effective defense barrier [159]. *Endotoxin* (an LPS) and its receptor CD14 [537] is a cell-wall component of the outer membrane of Gram– bacteria, which can thus be protected, either because they activate complement at a safe distance from their outer walls, disposing of long lateral polysaccharide chains, or because they are released directly from bacterial walls in a soluble form [97]. Early in life, endotoxin *promotes the development of Th1 CD4⁺ T cells* at the expense of proallergic Th2 $CD4^+$ T cells. Evidence suggests that the lack of endotoxin in the environment might lead to a higher incidence of asthma [537]. A further protective technique employed by bacteria is to release enzymes activating complement proteins adherent to their walls, or borrowing molecules regulatory of their function from host cells, namely, CD55 and CD59, utilized for protection from lysis, as is the case of HIV isolated from cells of patients with current AIDS [484]. There is also a chance that bacteria, virus and parasites are able to inhibit complement activation and elaborate factors with immunoregulatory properties or with a wide spectrum of noxious actions (Table 1.29), proteins with IL-like activity and molecules activating metachromatic cells [168]. Such data could justify a more focused vaccine preparation, thus suggesting that in the future strengthening the antimicrobial therapy inhibiting complement activation may be successful [415]. *Genetic defects of complement components* evaluated on ≈550 patients are marked by recurring infections that are mostly systemic, autoimmune diseases and vasculitis, in addition to pyogenic infections caused by C3 deficiency [632]. *Early in life* severe infections may be complicated with glomerulonephritis [633]. These defects represent 2% of PIDs [544]; the deficiency of the C1-INH control protein is discussed in Chap. 8.

The *liver* increases APP production during the acute phase. These proteins are host protectors since they eliminate reactive O_2 radicals, control serine proteases, activate complement [271], enhance resistance to infections, promote the repair of damaged tissue, and thus participate in the humoral machinery that makes up a second defensive strategy. AAP is a heterogeneous group of proteins such as CRP, α_2M, α_1-antitrypsin, serum amyloid P component (SAP), fibrinogen, ceruloplasmin, C3, etc. (Table 1.62) [148], several depending on NF-κB for an efficient transcription [270]. Inducible by macrophages activated together with platelets, they quickly mobilize as soon as a danger to tissue integrity is announced, including wounds, trauma, and microorganisms, to limit local damage and rapidly enhance host homeostasis, favoring both resolution and repair of damaged tissues [538]. During this process, IL_8 and MCP are synthesized by endothelium, two chemoattractants for neutrophils and monocytes, respectively, while TXA_2 and diverse PGs act on the vascular tone and mediate vasoconstriction and vasodilation, and LTB_4 has the task of attracting phagocytes. APPs are divided into two groups depending on their induction by human hepatocytes [31]:

- By IL_1, IL_6 or TNF, serum amyloid A protein (SAA), α_1 acid gp, C3 and CRP
- By IL_6 fibrinogen, α1-antichymotrypsin, haptoglobin, ceruloplasmin, hemopexin

2d. *Pentraxin* levels substantially increase in response to tissue injury or inflammation (Fig. 1.65) [31].

As is seen in Table 1.5, IL_1 especially regulates CRP, while IL_6 acts on the liver to increase APP synthesis, resulting in a reduction in albuminemia. CRP has the ability to bind to several microorganisms, containing phosphorylcholine in the membrane, the complex having the property of activating complement by the classic pathway. This enhances complement-dependent bacteriolysis, phagocytosis and production of biologically active peptides [538]. ILs raise body temperature (endogenous pyrogens) through PGE_2, also inducing EGF production, and through angiogenic activity. IL_1 and IL_6 in turn act on the adrenocortical axis with resulting ACTH production and thereby cortisol production. Negative repercussions on the acute phase are mediated by IL_4 and IL_{10}, which block the reaction within 24–48 h [31], and by SAA and SAP, which, being IL-induced, allow the adoption of strategies to reduce their levels during chronic inflammations [538].

Structurally and functionally correlated to C1q protein of the classic pathway are the *collectins* [140, 211] (Table 1.62), belonging to the lectin family, and another group of molecules active in first-line defense [140, 490]. Collectins are so named because they consist of a collagenous domain linked to the calcium-dependent lectin domain [345]. According to a fascinating hypothesis, they bind to a wide spectrum of microbes, to interact in perspective with cells or with complement as an active part in the antimicrobial defense [211, 490]. Among them we note the MBL, a plasma protein with many attributes including a crucial role in the above defense, being a member of the Ca-dependent lectin

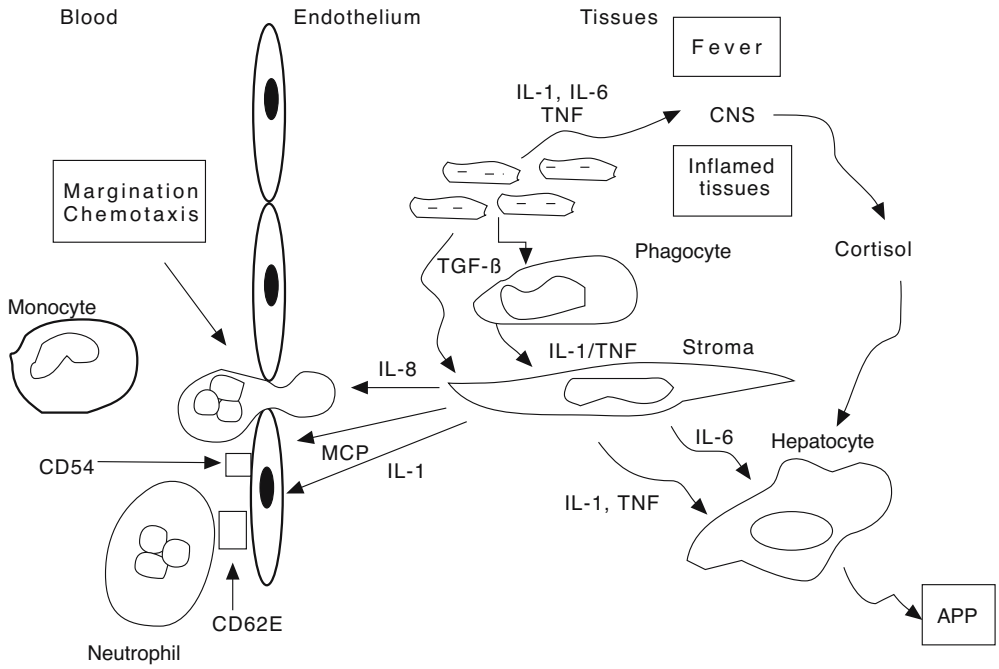


Fig. 1.65. Interactions between cells and ILs in the acute-phase response. Platelets and phagocytes release in the site of tissue lesion the first ILs, activating the adjacent stroma and endothelium, which in turn releases additional ILs. The hepatic response is activated following production of ACTH and cortisol. *MCP* monocyte chemotactic protein. For other abbreviations, see the list. (Modified from [31])

Table 1.62. Main recognition molecules of the innate immunity system

Molecules	Structure	Localization	Ligands	Function
Humoral receptors				
CRP	Pentraxin; Ca^{++}-dependent lectin	Liver synthesis; APR; increases (1 µg/ml to >1 mg/ml) in plasma	Microbial PSs, phosphatidyl-choline	Activates complement and opsonization, enhances phagocytosis
SAP	Pentraxin; Ca^{++}-dependent lectin	Liver synthesis; NL=30 µg/ml	ECM protein; microbial cell wall CHO	Enhances phagocytosis and opsonization, stabilizes ECM proteins
MBL	Collectin; has 18 CRD sites/ molecule on helical collage-nous domains	Liver synthesis; NL up to 10 µg/ml, varies with allelic variants	Microbial cell wall saccharides	Binds C1q collectin receptor; activates complement; enhances phagocytosis; modulates CD14-induced cytokine synthesis
LBP	Lipid transferase	Liver synthesis; NL=<5 µg/ml; increase to 50 µg/ml with APR to serum LPs	Transfers LPSs to CD14 and from CD14 LPSs; has bactericidal power	Enhances sensitivity to LPSs; system for inactivating LPSs; has bactericidal power
sCD14	Leucine-rich protein	Plasma protein; NL=3 µg/ml; origin perhaps from myelo-monocytic cells (unknown)	LPS; several microbial cell wall components	Enhances sensitivity to LPS 100- to 10,000-fold, complex with LPS binds to receptors on endothelium, PMNs and macrophages
C3	S_2-linked dimer	Liver synthesis; NL=1 µg/ml; induced by APR	Forms ester linkage to OH-groups on CHOs and proteins	Attachment of ligand for receptors such as CD21 and CD35

Table 1.62. (Continued)

Molecules	Structure	Localization	Ligands	Function
Cellular receptors				
Mannose receptors				
Macrophage MR	8 CRDs	Tissue macrophages; endothelial hepatic cells	Multiple CHO	Potentially targets antigens bound to class II molecules
DEC 205	10 CRDs; mannose-type receptor	Dendritic cells; thymic epithelium	Multiple CHO	Potentially targets antigens bound to class II molecules
Scavenger receptors				
Type I	Type II trimeric transmembrane protein with endothelial helical collagenous and terminal SRCR domains	Tissue macrophages; hepatic cells	Bacterial and yeast cell walls	Clearance of LPS and microbes; adhesion
Type II	Alternatively form missing terminal SRCR domain			
MARCO	Extended form similar to type I	Marginal zone of spleen; medullary lymph nodes; macrophages	Bacterial cell wall	Bacterial clearance
LPSR CD14	Leucine-rich protein; lipid-anchored glycoprotein	Monocyte-macrophages; PMNs	LPS; several microbial cell wall components	LPS sensitivity; microbial clearance; pro-inflammatory cytokine induction
Complement receptors				
CD35 (CR1)	30 SCR	Monocyte-macrophages; PMNs; lymphocytes	C3b, C4b	Enhances cleavage of C3b and C4b
CD21 (CR2)	15 SCR	B lymphocytes; FDC	iC3b, C3dg, C3d	Increases B-cell activation by antigens
CD11b, CD18 (CR3)	Integrin	Monocyte-macrophages; PMNs; NK cells	iC3b, fibrinogen, LPS	Adhesion; LPS clearance

Modified from [148].
APR acute phase response, *CHO* carbohydrates, *CRD* Ca^{++}-dependent carbohydrate recognition domain, *CRP* C-reactive protein, *ECM* extracellular matrix, *LPS* lipopolysaccharide, *LBP* lipopolysaccharide binding protein, *MBL* mannose binding lectin, *MR* mannose receptor, *NL* normal levels, *PSs* polysaccharides, *SAP* serum amyloid protein, *SCR* short consensus repeat, *SRCR* scavenger receptor cysteine-rich domain.

family that binds to microbial carbohydrates to initiate the lectin pathway of complement activation [345]. MBL is formed by a head region that binds to mannose residues and a tail with a triple helix mimicking C1q and thus activates several complement fractions, eventually killing and opsonizing the invader [490]. In particular, MBL appears to be a potent mediator of innate immunity in infants aged 6–18 months, in whom passively transferred maternal antibodies have disappeared and the immune system has not yet matured [575]. MBL levels are comparable to adult levels at 3 months of life, with a wide range (from 10 ng/ml to 10 mg/ml) that increases during APR [575]. Chapter 22 reports a susceptibility to infections caused by MBL defect; however, this phenomenon is diversely interpreted related to large interindividual variation in MBL levels [313]. Additional collectins have sparked interest, such as bovine conglutinin, another opsonin inducing Gram– bacteria eradication by coating them with C3b, which can activate opsonization [648]. The family has further expanded to include SP-A and -D (surfactant protein A and D), able to stimulate phagocytosis and agglutination of Gram–

Table 1.63. Phagocytic cells

Circulating phagocytes	
Granulocytes	Neutrophils (polymorphonuclear)
	Eosinophils
	Basophils
Mononucleates	Monocytes
Mononuclear phagocytes	
Macrophages	Tissue macrophages
	Alveolar macrophages
	Serosal macrophages
	Spleen and lymph node sinus macrophages
	Mesangial macrophages
Kupffer's cells	
Astroglial cells	

Modified from [352].

bacteria and virus, indicating a role in the removal of particulate material from the airways [211, 490], bovine collectin-43 and two novel proteases associated with MBL (MASP) [140].

3. Phagocytes fulfill basilar functions of nonspecific defense: one of their main assignments is to present selected antigens to T and B lymphocytes. The cells are divided into circulating, including granulocytes, macrophages and eosinophils, and nondividing, the macrophages (Fig.1.32 c, d, e) distributed throughout body tissues and assuming specialized structures and functions related to the different locations where they operate such as Kupffer's cells and splenic macrophages in the red pulp (clearance of corpuscular elements and soluble factors), LCs and splenic macrophages in the white pulp and lymph nodes (processing and presentation of the antigen to T cells), and finally bone marrow macrophages (growth and differentiation of hemopoietic cells) (Table 1.63) [352]. IFN-α sensitizes macrophages to microbial recognition by up-regulating the TLR3, TLR4 expression also enhancing the MyD88 (myeloid differentiation protein gene), TIR (Toll/IL_1R), TIR domain-containing adaptor inducing IFN-β (TRIF), IKKη, RIP-1, IL_{28} and IL_{29} genes expression [529]. Mast cells are provided with phagocytic activity [348] and therefore able to orchestrate, for host protection, inflammatory responses against invading microbes, in addition to tissue damage by antibody-independent activation [168]. Mast cells are to-day seen as a sentinel in host defense against bacterial infections, and evidence for their involvement in early responses to viral and fungal pathogens is growing. Mast cells are activated during innate immune responses by multiple mechanisms, including well-established responses to complement components. Changes in vascular permeability induced by *early release of preformed mediators* and lipid mediators enhance the availability of complement components and some initial inflammatory cells to the site of infection [332, 347]. Mast cell proteases have also been demonstrated to play critical roles in vivo in the recruitment of both neutrophils and eosinophils to sites of inflammation [502] and an *immune complex hypersensitivity reaction in the synovium* [347].

Circulating and nondividing cells, *PMNs* (Fig. 1.32) have CR1, CR3 receptors, for IgG Fc fragments and several others (Table 1.64) [18]. PMNs recruited to the site of injury ingest the intruders, bacteria, foreign materials or damaged tissues: IgGs and C3b are dedicated to coating infecting particles, promoting both opsonization and subsequent phagocytosis. Prominent is the role played by monocytes, numerically inferior to PMNs, but with the advantage of not being terminal cells and changing into tissue macrophages, two to three times larger than PMNs, readily recruited during local inflammatory or degenerative processes [345]. When the inflammatory process initiates, macrophages instigate a bit of a delayed maneuver, whereas PMNs, more mobile, are the first to accrue, perhaps facilitated by IL_5 augmenting IgA production (Table 1.5), but above all by IAPs (integrin associated proteins, CD47), which activate PMNs, even in extravascular sites [302]. Such cells are also termed *professional phagocytes,* because they are provided with membrane receptors for IgG_1 and IgG_3 Fc fragments and C3b fragment. Nonspecific inflammatory reactions are the first components of innate immunity to appear in the phylogenesis, and it seems likely that such primitive responses have influenced the immune responses' evolution. In this sense, Th response polarization could have been an evolutive consequence of nonspecific responses of a divergent sign directed to counteract different types of infection [470]. *Macrophages* and NK cells exemplify the primitive limb of IFN-γ-dependent immune responses required for defense against infection with virus and other intracellular invaders, whereas metachromatic cells could have evolved to combat extracellular parasites [470]. Macrophages, perhaps the phylogenetically more ancient immune cells, can secrete IFN-γ due to their IL_{18} synthesis [669]. Stimulated, moreover, by bacterial and viral contact and primed by CD14 a receptor that functions with TLR4 in LPS responses [194] and γδ T cells [111], in the first stages, macrophages release TNF-α and IL_{12}, which synergize with T-cell generated IL_2 and macrophage-generated IL_{15} [68], and both prime *NK cells* [583] *to express IFN-γ* [146]. IFN-γ and IL_{18} form two potent stimuli to suppress IgE responses [421, 669], enhancing macrophages' microbicidal power. This power is necessary for macrophages to combat invasions by endocellular facultative microorganisms, particularly resistant to the phagocyte microbicidal action despite Igs opsonization. Microorganisms duplicate within the macrophages; however, the trapped intruders are not killed:

Table 1.64. Principal ligands of phagocyte membrane receptors

Opsonins
Fc IgG (diverse), IgA, IgE
iC3b
C3d
C1q
Coagulation factors
Fibrinogen
Thrombospondin
Thrombin
Factors VII and VIIa
Urokinase
Molecules of cellular matrix
Fibronectin
Laminin
Sulfate polysaccharides
Hyaluronic acid
Adhesion and recognition molecules
Sheep erythrocytes
Erythroblasts
CD54
CD62E
CD11a/CD18
Endocytosis and transport additional molecules
Fucosylated glycoconjugate
Galactosylated particles
β-Glucan
Native and modified LDL lipoproteins
Modified aldehydes
Transferrin
Lactoferrin
Ceruloplasmin
α_2-Macroglobulin
Hemopexin
Glycoprotein mannose-6-phosphate

Growth factors, ILs, polypeptides
CSF-1
GM-CSF
IL_1
IL_2
IL_3
IL_4
TNF
IFN-γ
IFN-α, IFN-β
Insulin
Glucagon
Somatomedin
Vasopressin
Parathormone
TGF-β
C5a
Peptides and mediators of inflammation
f-Met-Leu-Phe
Angiotensin
Bradykinin
VIP
Tuftsin
LTB_4
Neurotensin
Drug substances
β_2-Adrenergics
Nicotinics
Cholinergics
Histamine
Serotonin
Benzodiazepine
Phorbol esters
Adenosine

Modified from [18].

their intracellular growth can be blocked only by an IL intervention [251].

During the direct antibacterial activity supplemented by macrophages, NK cells and, while the response progresses, Th1-like ILs prime CSMs and HLA class II molecule expression on macrophages, thus preferentially triggering Th1 T cell expansion in concert with IL_{12}, whereas Th2 T cell response is limited by IFN-γ counterregulation [251]. However, during a chronic infection intracellular bacteria may prime HLA class II molecule expression on macrophages when CSMs are absent, thus provoking Th1 T cell anergy [251]. Th2 T cells dominate only because counterbalancing counterparts are missing and consequently play a large part in the Th1 T cell reduction in numbers [251]. Therefore NK cells and macrophages cooperate to eliminate microbial infections [25]; on the contrary, Th2-like ILs sanction their progression [365]. However, it is possible that endogenous IFN-α/β reduce IL_{12} expression, also stimulating CD8 T cells, as if NK-cell and IFN-γ protective activity should be hampered to avoid unwelcome effects for the host [43].

To adhere to microbes, *neutrophils* engage CD11b/CD18, potentially playing a vital role in cell activation and triggering the oxidative and nonoxidative apparatus in an attempt to destroy the invader (or the host tissues), while adhesion mechanisms provide an environment where killing can progress protected by tissue fluids [154]. From this viewpoint, the degranulation has three important roles in host defense with release of granules (Table 1.23):

- Azurophil is able to potentiate the digestive and microbicidal activities of phagocytes
- Secondary or specific granules enable the cells to regulate inflammation
- Tertiary, associated with translocation of receptors for CR1, CR3, FMLP (formylmethionyl leucylphenylalanin), laminin, NADPH oxidase and cyto-

chrome c and subsequent interaction with these receptors, helping neutrophils in their phagocytic activity [315, 663].

The process of phagocytosis schematically includes six different stages:

1. Adhesion to foreign particles
2. Chemotaxis leading to contact with particles
3. Opsonization mediated by IgGs interacting with peptides and enhancing their digestion
4. Ingestion
5. Metabolic activation
6. Lysis

1. Adhesion. Adhesion is mediated by cytophilic IgGs present on the surface of particles to be phagocytosed, and by their Fc receptors on phagocyte membranes. Part of the cells remain in the bone marrow as part of a large reserve pool (*fixed cells*). Two PMN pools are included in the circulation, the circulating PMN pool and marginating pool along the postcapillary of vascular endothelium. As soon as the first signs of injury or infection are manifested, fixed and circulating PMNs are mobilized and increase their adhesion to the epithelial lining of local blood vessels, supported by endothelial cells and mediated by IL_1 and other mediators of inflammation. Even if clear-cut mechanisms are not fully understood, it is likely that PMN adhesion to cells is mediated by surface glycoproteins such as iC3b, β integrins and interactions between CD11b/CD18 and iC3b for complement-coated bacteria or endothelial cells [663], which in turn synthesize CD62E. In addition, PMNs possess receptors for ECM components, laminin and FN, facilitating their adhesion to host tissues or bacteria walls [311].

2. Chemotaxis. Chemotaxis is the start of directional migration of organisms or other cells provided with motility, able to travel up to a concentration gradient of *chemotactic factors* or *chemotaxins* (chemo + "ταξις," distribution). Cell response is directed to a large extent by signals released from chemotactic factors, or cell locomotion is piloted toward sites where signals are more expanded. Differently from tissue eosinophils, present at the level of epithelial surfaces and in contact with the environment, fixed and circulating PMNs are absent from tissues until they are recruited from the bloodstream as a result of tissue damage. To meet this demand, a variety of chemotactic factors can potentially regulate PMN recruitment and migration to the site of inflammation: these stimuli yield chemotaxin production and, in ever greater concentrations, attract circulating PMNs to capillaries around the site of injury, where they actively insinuate themselves between endothelial cells. Studies have verified that if PMN migration is delayed as little as 2 h, their capacity to localize the inflammatory process is severely jeopardized [311]. Among the more known chemotactic factors, we enlist FMLP, LTB_4 and C5a, which certainly play the most important role

Table 1.65. Chemotaxic defects

Chemotaxic defects due to insufficient production of chemoattractant substances
Complement components
C1r C2 C3 C5
Agammaglobulinemia
Coagulation/fibrinolytic pathway
Prekallikrein Hageman factor
Cellular
Mucocutaneous chronic candidosis Wiskott-Aldrich syndrome
Chemotactic defects due to defects of cellular functions
Primary immunodeficiencies
Chédiak-Higashi syndrome Chronic granulomatous disease (CGD) Hyper-IgE syndrome Leukocyte adhesion disorder (LAD) Shwachman syndrome Specific granule deficiency
Additional affections or physiological states
α-Mannosidase deficit Bone marrow graft Congenital ichthyosis Diabetes mellitus Drugs Hypophosphatemia Neonatal neutrophils Neutrophil actin dysfunction Tuftsin deficit

Modified from [663].

[345]. Others derive from cell debris, the bloodstream (C567, fibrin or collagen fragments), lipids (LTB_4, PAF), peptides containing *N*-formyl-methionine (from bacterial or mitochondrial protein breakdown), including N-FMLP with specific receptors on PMN membrane, and ILs such as GM-CSF and IL_5 [663]. Apart from bacteria, macrophages, lymphocytes, platelets, and mast cells can also attract PMNs; certain chemotaxins such as C5a, LTB_4 and PAF are specific for varying types of leukocytes and some *chemokines* for PMNs (Table 1.57). PMNs with nanomolar amounts of chemotactic factors respond by increasing both adhesion and the number of receptors specific for such factors. Binding of chemoattractants and activation of G proteins and their effector enzymes result in the cell orientation toward the higher concentration of the stimulus. Cell shape changes from a round to a triangular structure with the cell front at the triangle base. Accordingly,

rugosities are formed on the cell surface and following AA metabolism activation, membrane polarity is altered because rugosities are rapidly concentrated on a pole: here contraction waves begin and the cells utilize microactin filaments, which provide the contractile forces required for cell movements. Microtubules composed of tubulin allow the locomotion, and actin alternation of polymerization and depolymerization may be a critical regulator of microfilament contractility [311]. The HIgES shows the close correlation between efficient chemotaxis and a normal response to infection, as well as the possible influence of histamine and additional amines on PMN mobility. Table 1.65 [663] outlines the defects of chemotaxis depending on their origin: chemoattractants or cell substance deficiency (the phagocyte defects may have an 18% prevalence within PIDs) [544].

3. Opsonization. Bacteria expressing capsular polysaccharides pose a unique problem to phagocytosis inhibited by the charge and hydrophilia typical of such bacteria, especially the polysaccharide capsule of pneumococci, a virulence factor expressing a strong negative chemotactic activity. *Opsonization* (from the Greek "οφωνειν," acquire foods and therefore prepare for eating) is a not immunological contact between cells and bacteria, mediated by the *opsonin* family comprising Igs, CR3, CR4, and FN. Igs contribute chiefly via IgM, IgG_1 and IgG_3 with CD23 or CD64, interacting with epitopes on bacteria via its Fab portions, while C3b and iC3b – induced by both classic and alternative pathways – adhere to the bacterial (or other intruder) surface to approach neutrophils. Antibody fixation also allows activation of the complement classic pathway so that the Fc portion, Clq and C3 act synergically to facilitate the capture of bacteria from phagocytes. Complement proteins exercise an important conclusion of Ig-mediated opsonization, because CR and FcR concurrent involvement often fulfills a marked synergic action in activating phagocytes and stimulating their functions. In this context, addition of complement to Ig-opsonized bacteria promotes phagocytosis, in parallel reducing the Ig number necessary for an efficient ingestion of bacteria [345]. An additional, potent opsonizing stimulus is supplied by *tuftsin,* a tetrapeptide (Thr-Lys-Pro-Arg) produced by the spleen. Its action appears to be indispensable, as demonstrated in patients with familial deficiency of this peptide and in splenectomized patients, who suffer from severe infections occurring with *Candida, S. aureus,* and *Staphylococcal pneumoniae* [311]. Macrophages, IgA and IgE receptors also play a striking role in opsonization. Lectins have been identified on different cell membranes, mainly macrophages, as receptors of mannose-fucose, galactose binding laminin, sialic acid or sialoadhesine, and β-glucan with specificity for CD11b/CD18. However, there are bacteria blocking opsonization or deviating C3b covalent binding and MAC adhesion to a position on the cell surface distant from the cell membrane where no defense mechanism can be exploited, or resisting opsonization by expressing capsular polysaccharides, as mentioned above: C3b causes their breakdown by binding Factor H instead of Factor B [159].

4. Ingestion. The concerted action of two receptors multiplies hundreds of times the ingestion of phagocytosed particles previously trapped within vacuoles. The cell membrane binds and invaginates engulfed particles adhering to its surface, thus forming a *phagosome,* subsequently approximating cytoplasm and fusing with lysosomes to create the structure called *phagolysosome,* with reduced pH. The final transformation therefore takes place in phagolysosomes, membrane-bound intracellular vesicles containing a rich supply of proteolytic enzymes operating degradation, thus completing the reduction to constituents of amino acid fragments: a portion of antigen fragments thus produced is expelled and transported to the cell surface where it is expressed, but the major portion is stored internally in endosome [540].

5. Metabolic Activation. Phagocyte exposure to varying stimuli increases cell respiration and, consequently, O_2 generates reactive metabolites within the phagosome to clear target cells from the body, that is, $O_2^{\cdot -}$ then reduced to H_2O_2, as described below. Such O_2 products are highly toxic since they oxidize cell components of parasites, tumor cells and other tissues [159].

6. Lysis. After a preventive opsonization with formation of pseudopods, by virtue of FcεRII and CR3, the cells can bind more tightly to antigens, facilitating antigen uptake and internalization (endocytosis). Following lysis carried on by the C5b-9 terminal complex, the final disruption of imprisoned bacteria ensues, also mediated by MPO catalyzing H_2O_2 transformation into HOCl with bactericidal activity. Phagocytosis is the end point, therefore any interruption of the process compromises host defenses [540].

Microbicidal Activity

O_2-Dependent Mechanisms

The anti-microbial PMN activity depends on O_2-dependent or O_2-independent microbicidal mechanisms. A paradigmatic example of the lacking activation of O_2-dependent microbicidal mechanisms is *chronic granulomatous disease* (CGD) resulting from a genetically determined enzyme deficiency of leukocyte intracellular bactericidal function, thus being unable to kill ingested bacteria or fungi, particularly catalase-producing bacteria. CGD especially results from genetic defects in the various components of NADPH-oxidase, a potent enzyme that is central in the respiratory burst. This process reduces O_2 to superoxide and then to H_2O_2, thus leading to NADP formation. Activation of glucose oxidation during phagocytosis starts trouble for each

invader. A roughly tenfold increase in the activity of the hexose monophosphate shunt is provided by an increase in the NADP/NADPH ratio, determined in turn by activity of the same oxidase and a chain of redox reactions involving glutathione. This enzyme system has the goal of transforming into H_2O and O_2 the H_2O_2 produced in excess and potentially toxic for cells. Catalase-positive bacteria cannot be killed by the phagocyte MPO/halide system, since they synthesize catalase destroying any excess H_2O_2 they produce, whereas catalase-negative bacteria are killed, since they fail to stop H_2O_2 production and trigger the MPO/halide system: for this reason in CGD patients they play the role of scavenger bacteria. Two oxidoreductive groups involved in the electron transport chain, namely flavine and cytochrome b558, are defective in the X-linked from (≈ 60% of cases), but not in autosomal recessive CGD (≈ 30%). *Glucose-6-phosphate-dehydrogenase deficiency* (G6PD), inherited as an X-linked disorder, also leads to impaired intracellular killing via the reduced glucose oxidation. *MPO deficiency,* inherited with an autosomal recessive pattern, is associated with susceptibility to systemic candidiasis, since intracellular killing is reduced and/or delayed (also greatly) as a result of impaired halide system function. MPO deficiency has a greater incidence than in the past, frequently as a casual laboratory finding in healthy subjects. The underlying basis of such defects is that the oxidative metabolism requires a notable O_2 consumption: $O_2^{\bullet -}$ is generated, reacts with two NADPH molecules and acquires an electron; the reaction takes a few seconds and is followed by bacteria adhesion and phagocytosis via PMNs. The speed highlighting this reaction shows that the oxidase could be localized on PMN membrane [663].

Therefore, an unremitting influx of O_2 radicals is also achieved around and beyond the plasma membrane of phagocytosed intruders, thus intensifying the toxic effects at their expense. One product of this metabolic process, H_2O_2, carries on microbicidal activities, and because of its oxidant properties reacts with MPO stored in azurophil granules and released into phagosomes during PMN degranulation. This highly oxidant enzyme reacts in turn with halide (predominantly iodine and chloride) ions present in phagocytic vacuoles, catalyzing the halogenate reaction (iodination or chloruration), which is as toxic for microbial wall proteins. It should be noted that PNMs undergo apoptosis following an excessive *necrotizing activity* [630] (Fig. 1.34). During oxidoreductive metabolism, additional O_2 radicals are generated (normally imprisoned within phagolysosomes), including $^1O_2$ and OH radicals. *$O_2^{\bullet -}$ toxicity* for bacteria is demonstrated by the high concentrations of superoxide dismutase (SOD) (converting $O_2^{\bullet -}$ into O_2 and H_2O_2), possessed by several O_2-resistant aerobic microorganisms, whereas O_2-sensitive anaerobic organisms are devoid of SOD. Human beings are provided with low SOD levels localized in the cytosol, whose activity does not interfere with $O_2^{\bullet -}$ microbicidal activity within phagosomes, thus protecting cytoplasm from highly diffusible toxic radicals [663].

The following reaction is produced:

1) $2\ \mathrm{NADPH} \xrightarrow[\mathrm{SOD}]{\text{NADPH oxidase}} H_2O_2 + 2\ \mathrm{NADP};$

2) $2\ O_2^{\bullet -} + 2\ H_2 \rightarrow O_2 + H_2O_2;$

3) $O_2^{\bullet -} + 2\ H_2\ O_2 \rightarrow {}^1O_2 + \mathrm{OH} + \mathrm{OH}^-$

$^1O_2$ is an O_2 molecule produced by neutrophils during phagocytosis; it is highly reactive and unstable and emits luminosity (chemiluminescence) as it returns to the ground state. It is formed by spontaneous $O_2^{\bullet -}$ dismutation, or by MPO, hypochlorite and H_2O_2 reactions. This microbicidal action could depend on the ability of splitting double C atom bonds of bacterial membranes. The role played by *hydroxyl radicals* in microbicidal actions is demonstrated by their inhibition of bactericidal killing by means of hydroxyl scavengers, benzoate, ethanol and mannitol [420].

O_2-Independent Mechanisms

In addition to producing *toxic O_2 compounds,* phagocyte cells express bactericidal mechanisms, assuring a relevant defense function, eminently via bactericidal proteins of azurophil granules (Table 1.23), especially considering Chédiak-Higashi syndrome. These proteins bind lipid A and the core oligosaccharides of LPS, thus altering bacterial cell wall permeability and amino acid uptake. Such mechanisms can be particularly important in patients with CGD or MPO deficiency. For example, NO is credited with a powerful antimicrobial action. In a potential biodefense strategy *cathelicidins and defensins* are major families of antimicrobial peptides in mammals with a broad spectrum of antimicrobial activity [169]. Both peptides disrupt the integrity of the microbial membrane, which is mediated by their cationic and amphipathic properties, which enable them to bind to negatively charged microbes and insert into their membranes [342]. An emphasis was given to azurophil granules, delivering *defensins,* which cleave an array of substances, including peptidoglycans of Gram+ bacteria, cationic proteins acting at an alkaline pH, BPI fixing LPS of Gram+ bacteria perforating their membrane, proteins binding the B_{12} vitamin, lysozyme, lactoferrin, an iron-binding cationic protein that blocks the activity of Fe-dependent bacterial enzymes, neutral hydrolases (proteases, nucleases) acting at an acid pH within phagosomes, neutral proteases, etc.

Defensins (α and β) are activated by MMP-7 [118]. To date, 6 α-defensins have been identified; 4 of them are known as α-defensins 1, 2, 3, and 4, the other 2 α-defensins are known as human defensins 5 and 6. There are 14 β-defensins, however, in human beings, 4 types of β-defensins have been identified as yet, plus the ϑ defensins [169]. In keratinocytes IL_{22} activated STAT3 and directly and transcriptionally increased the expression of β-defensin 2 and β-defensin 3 [650]. Defensins form 5%–10% of neutrophil total protein content, have antibacterial or

cytotoxic properties, and are more effective at an alkaline pH, therefore before phagolysosome acidification. These disinfectants against a wide spectrum of Gram+ and Gram– bacteria and *fungi* apparently insert themselves into microbial membranes and inhibit their growth, thus activating host cell processes involved in immune defense and repair [169]. By using chemokine receptors on DCs and T cells, defensins are believed to contribute to the regulation of host adaptive immunity against microbial and viral (HIV) invasion: their killing is consequent to disruption of microbial membrane [660]. Human defensins stimulate IL_1, IL_8, TNF-α release and decrease IL_{10} release [489].

Thus, like the cathelicidins, defensins can participate in immune defense in at least 2 ways, both killing bacteria and influencing the cellular innate and adaptive immune response [169].

Cathelicidins are a class of small cationic peptides that are an active component of mammalian innate immunity and are expressed at high levels in neutrophils, in skin and in other epithelial cells and can act as natural antibiotics by directly killing a wide range of microorganisms [118, 676]. About 30 different cathelicidins have been described in mammals, but so for only one has been identified in humans and one in mice. The human cathelicidin (LL-37) and the murine cathelicidin-related antimicrobial peptide (CRAMP) are both expressed by mast cells [118]. Recent findings suggest that their function is to disrupt the integrity of the microbial membrane, which is mediated by their cationic and amphipathic properties, which enable them to bind to negatively charged microbes and insert into their membranes [118]. Thus, after proteolytic cleavage the cathelicidin-like domain can contribute to innate host defense via inhibition of bacterial growth and limitation of cysteine-proteinase-mediated tissue damage, both functions being complementary to LL-37. LL-37 also represents a multifunctional effector molecule for innate immune defense of the skin [676] and for the local treatment of pulmonary infections [622]. In addition to the antimicrobial effects, LL-37 has been shown to have chemotactic effects on mast cells, which might aid in the migration and accumulation of mast cells at the site of inflammation in several diseases [382].

A defensive role could be assigned to *scavenger receptors,* defined by their ability to carry on the following functions:

- *Macrophage* activation and homing
- *Clearance* of LPS, microbes and toxic substances
- *Phagocytosis* of cells undergoing apoptosis
- *Antigen uptake and/or processing* in the acquired immunity [420].

Macrophages use CD36, vitronectin and thrombospondin receptors to engulf cells undergoing apoptosis [165]. *Eosinophils* are additional cells active in innate immunity, since they arm against parasites and some forms of tumor cells the cytolytic activities of MBP and ECP their cationic proteins. ECP is able to perforate the membrane of a target cell, similarly to the perforins of both CTLs and NK cells.

For completeness or an alternative to antimicrobial defense, we should mention another potent defense establishing a link between innate and acquired immune systems, represented from *DCs* [352], which assure a system of sentinel receptors activated by microenvironmental cellular and tissue damage [221]. A key element supporting this hypothesis is that the innate immune system has evolved an evolutionary strategy, developing several parallel mechanisms, oriented to an immunoregulatory direction, but *sensitive to local injury.* In this context, PLA_2 which in patients with rheumatoid arthritis (RA) is associated with propagation of inflammation, also favored DC maturation and PLA_2-generated DCs stimulated IFN-γ secretion by allogeneic T cells. These effects were correlated with the activation of NF-κB, AP-1 and NFAT. Thus a transient increase in PLA_2 activity generates signals that promote transition of innate to adaptive immunity during the APR [422]. We have discussed complement, NK cells, T-cell TcR and phagocytes. DCs exemplify the pathway joining these mechanisms: activation of any of these mechanisms may be associated with a switch from DC precursors to the mature phenotype [251]. At this level, DCs act as APCs, activated by GM-CSF and TNF-α in synergy with IL_1, IL_6 and IL_{12}: their role is focused on local tissue repair and therefore amplifies a self-limiting control mechanism, to shift the balance of the response back to tolerance. When tissue injury is present, NK cells could provide a negative-feedback effect on antigen presentation [221]. Furthermore, DCs could play a triple role:

- *In viral infections* as potent inducers of CTL cells, also presenting antigens on HLA class I molecules.
- *Immune responses* triggered by the association of allergenic and toxic molecules would prime pollution-activated APC DCs to provide the necessary costimulatory signals to activate T cells specific for those allergens.
- Action *as sensitive sensory receptors,* since DC peripheral sentinels might be regarded as a kind of sensory nerve ending, susceptible to local chemical signals, and designed to monitor tissue damage [221].

The DC defensive effect is probably amplified during acute inflammation, in particular presenting viral antigens to T cells, which may accordingly protect the host against concomitant viral infection [352], or inducing T-cell-mediated tolerance to inhaled antigens, since DCs are the only cells present in airway epithelial cells expressing surface HLA antigens, a prerequisite for antigen presentation [467].

Recent studies have shed light on the pivotal role played by memory B and T lymphocytes, critical for host protection from secondary viral infections (Table 1.42) and ILs within innate immunity, which is entitled to the regulation of several of the nonspecific mechanisms discussed so far, with the objective of clearing infections [688]. Purposely we point out the inhibi-

tion of apoptosis mediated by viral genes (Table 1.19), to guarantee the species survival until genome replication and to counteract host responses to infection [192]. Several viral genes have binding sites either for NF-κB, which can assure their replication, as demonstrated for HTLV-I (human T-cell leukemia virus), or for IL and chemokine receptors (Table 1.57) [688], interfering with their activity and modulating virus evasion strategies [11]. Poxvirus proteins are able to block different arms of the host response against infection (complement activation, IL function, antigen presentation to T cells) or mimic host growth factors [11]. Thus, members of the family activating NF-κB have a key role in the induction of immune responses in mammals, including TLRs [353]. More positively, pathogen recognition, whether mediated via the TLRs or via the antigen-specific TcR and BcR, initiates the activation of distinct signal transduction pathways that activate NF-κB. Activation of NF-κB by these pathways is necessary for lymphocyte activation, expansion, and effector function in response to infection. However, IL_{10} and CSs inhibit NF-κB [470]. *Chemokines* play a versatile role in the defense against infections, even viral: a rapid inflammatory response is expected to counteract bacterial and fungal pathogens, and in this context the fast chemokine induction was observed in several animal models and in clinical studies. Deletion of endogenous genes for MIP-1α in knockout mice provoke a great delay in influenza episode resolution, as well as a reduced CD8 T cell recruitment in infected airways [95]. Above all, CCL3, CCL4 and CCL5 are in the first line against AIDS, highly expressed in the lymph nodes of these patients [9], and when some receptors (CXCR = α and CCR of β chemokine receptor) act as cofactors for HIV entry to macrophages (CCR3 and CCR5) and T cells (CXCR-4) [9, 83, 150], chemokines have an anti-HIV effect primarily competing for receptor binding; thus MIP-1α, MIP-1β and RANTES bind to CCR5 and SDF-1 CXCR-4 [405].

Human TLRs belong to a family of pattern recognition receptors (PRRs) that aid to recognize microbial products derived from several classes of microbes, as well as endogenous ligands that represent a danger signal [448]. Transcripts for TLR4 and TLR2 were expressed in whole tissue extracts of fetal gut and skin [243]. The TLR7-, TLR8-, and TLR9-dependent induction of IFN-α/β and -λ is strictly IRAK-4 dependent and plays an important role in protective immunity to most viruses in humans. IRAK-4-deficient patients may control viral infections by TLR3- and TLR4-dependent and/or TLR-independent production of IFNs. Thus 5 TLRs seem to play a crucial role in this innate immune response [662]. However, *children who had defective signaling* in the molecule IRAK-4 had a greatly increased risk for pyogenic infections [431]. TLRs are structurally characterized by a cytoplasmic TIR domain that, via the signal transduction factor essential for several TLR-mediated responses MyD88, connects the receptor to the intracellular signaling machinery shared by IL_1 and IL_{18} [448]. *LPS are associated with TLRs.* TLR4 and TLR3 recognize LPS from Gram– bacteria [345]. The recognition of microbial components by TLRs leads to activation of innate immunity: progress in elucidating the molecular mechanisms for LPS tolerance has been made through the analysis of TLR-mediated signaling pathways [491, 561]. *So far the TLR family includes* 11 *members that have been identified* [677], *which bind to microbial products, activating host defense responses.* These TLRs and their signaling pathways are represented in such diverse creatures as mammals, fruit flies, and plants, and several of them appear to recognize specific microbial products, including LPS, bacterial DNA. TLR signaling represents a key component of the innate immune response to microbial infection [299]. The analysis of DC and PBMC activation has shown that TLR2 agonists are able to block the induction of IP-10, $IL_{12}p35$, and IFN-γ, but not IL_{15} and IFN-β by TLR3 and TLR4. TLR2 stimulation led to rapid release of IL_{10} that is responsible for inhibition of IP-10 and $IL_{12}p35$ induction [448]. TLR4 agonist specifically promoted the production of the Th1-inducing $IL_{12}p70$ and IP10, which is also associated to Th1 responses. Instead, TLR2 stimulation failed to induce $IL_{12}p70$ and IP-10 but resulted in the release of the IL_{12} inhibitory p40 homodimer, producing conditions that are predicted to favor Th2 development. TLR2 stimulation also resulted in preferential induction of IL_8 and p_{19}/IL_{23} [449]. Thus TLR primary responses to their agonist may be modified by cross-talk between different TLRs [448]. The receptor superfamily also includes $IL_{18}R$ [131]. *Ligands* and chromosomes [537] *for* 10 TLRs, such as TLR1 (4p14), TLR2 (4q32), TLR3 (4q35), TLR4 (9q32-33), TLR5 (1q32-33), TLR6 (4p14), TLR7 (Xp22.3), TLR8 (Xp22) TLR9 (3p21.3) and TLR10 (4p14), *have been characterized* [50, 191, 333, 448]. Stimulation of resting B cells with anti-μ and anti-CD40 antibodies increased expression of TLR9 and TLR10 [50].

The newest member of the TLR family to be identified is TLR11; however, it is not clear whether humans express TLR11, as the murine Ser119 residue appears to be replaced by a stop codon in humans [187]. TLR10 is notably expressed in lung tissue, where involvement in the innate immune responses of the lung to common respirable exposures such as allergens may be a *potential asthma candidate gene* [285], thus suppression of TLR responses may reduce excessive inflammation in chronic diseases [185]. All TLRs with the exception of TLR3 can signal via MyD88, TLRs 2 and 4 utilize MyD88, and TLR3 and 4 can engage TRIF and TRAM (TRIF-related adaptor molecule) under certain circumstances [188, 658]. TLR4 recognizes LPSs and lipoteichoic acids from Gram– and Gram+ bacteria, respectively, and TLR9 is critical for recognizing bacterial DNAs [345]. A CpG oligonucleotide, a TLR9 agonist, also stimulated TLR9 expression in B cells [50]. TLR4 and TRAF6 [374] were shown to induce the activation of the NF-κB signaling pathway: by this pathway, TLR4 activation

induces the expression of a variety of ILs and CSMs [345]. IFN-γ up-regulates TLR4 expression, and was shown to counteract the LPS-induced down-regulation of TLR4 [50]. Recently the TIR domain-containing adaptors have increased in number: they include MyD88, TIRAP (TIR domain-containing adaptor protein), TRIF, and TRAM [491, 560, 658]. These TIR play essential roles in TLR signaling. MyD88 is essential for inflammatory IL production via all TLRs, whereas TRIF is involved in TLR3- and TLR4-mediated MyD88-independent induction of IFN-β [560]. These findings confirmed that TLRs may function as receptors of the innate immune system [345, 560]. Moreover, the activated TLR4 recruits the *MYD88/IRAK signaling* pathway. Further downstream, IRAK and IRAK-2 interact with the adapter molecule TNRF6 that bridges them to the protein kinases TAK1 (transforming growth factor-β-activated kinase) and NIK [261, 372]. Once activated, the MyD88/IRAK signaling cascade bifurcates and leads to the activation of a TF NF-κB and c-jun N-terminal kinase (JNK), which initiate the transcription of proinflammatory IL genes that subsequently drive the transcriptional induction of several IL genes (see above) [374, 563]. However, a dominant-negative version of MyD88 specifically inhibited TLR4-induced NF-κB activation, lending functional credence to the interaction occurring between TLR4 and MyD88 [373, 374]. Genetic, gene transfer, and dominant-negative approaches have involved TLRs 2 and 4 in LPS recognition and signaling [374]. The new IFN, FLN29, is a negative feedback regulator of TLR signaling in innate immunity; FLN29 may thus play an important role in endotoxin tolerance [335]. Two members of the NOD family, NOD1 and NOD2, together with TLRs, have also been shown to be involved in the innate immune response as sensors of specific bacterial components [429]. Genetic variation in the genes encoding the NOD-LRR proteins, and NOD2 in humans and Naip5 (neuronal apoptosis inhibitory protein 5) in mice, is associated with inflammatory disease or increased susceptibility to bacterial infections [79]. NALPs [NACHT (neuronal apoptosis inhibitor protein)], LRR- and pyrin domain (PYD)-containing proteins 1–14 have been defined in the human genome. NALP1 protein forms a large, signal-induced molecular platform, the inflammasome, *resulting in the activation of proinflammatory caspases* [582]. Instead, IL_{32} synergized with the NOD1- and NOD2-specific muropeptides of peptidoglycans for the release of $IL_1\beta$ and IL_6 via a caspase 1-dependent mechanism; the marked expression of IL_{32} in colon mucosa suggests a role of IL_{32} in the pathogenesis of Crohn's disease (CD) [379]. NOD2-S is preferentially expressed in the human colon and is up-regulated by the antiinflammatory IL_{10}. Overexpression of NOD2-S down-regulates NOD2-induced NF-κB activation and IL_8 release. NOD2-S also interferes with the maturation and secretion of pro-$IL_1\beta$ downstream of NOD2 and its adaptor molecule receptor-interacting protein kinase 2. Increased levels of NOD2-S can abolish residual NOD2 signaling by the CD-associated R702W variant. However, interfering with the delicate balance of NOD2 signaling in inflammatory bowel disease, e.g., by changing the ratio of NOD2 splice variants, may have detrimental effects on the CD course [475]. Until recently, most TLR/ligand interactions had been shown to favor Th1-like responses rather than promoting the Th2 responses commonly associated with allergic disease. However, this concept was recently challenged in rodent models, in which both TLR ligand concentration and the route of immunization were shown to induce *a Th2 immune response* [450]. How might this shift be achieved? [537]. Depending on the early environment of the individual, this might lead to Th cells that are dominated by allergy-prone Th2 cells, which support the production of antigen-specific B cells. The increase in the Th2 cells comes at the expense of Th1 lymphocytes [450]. The synergistic interactions of TLR ligands and antigen might have relevance to the exacerbation of IgE-mediated allergic diseases by infectious agents [438]. In addition to host defense against pathogens, the TLR-dependent pathways are involved in a variety of immune responses [560]. Therefore, an important challenge for the future will be to develop suitable suppression of TLR responses to reduce excessive inflammation in chronic diseases [187].

A prominent role in innate immunity is played by *keratinocytes* expressing both TLR2 and TLR4 at the mRNA and protein levels; TLR2 and TLR4 are present in the normal human epidermis in vivo and their expression is regulated by microbial components [432]. The expression of MyD88 has been demonstrated in keratinocytes. LPS and IFN-γ increased the expression of TLR2 and TLR4 50-fold and fivefold, respectively [432].

A prevalent dogma is that a key defense mechanism belongs to Th1 and Th2 T cells mediating killing, Th1 T cells of intracellular and Th2 of extracellular parasites. However, everything can be modulated by ILs, their number [31] and chronology of intervention. Th1 and Th2 T cells are potentially activated by signals deriving from phagocytes and NK-CMI. Moreover, we speculate that Th2 T cells act only in immune reactions independent of phagocyte cells: several ILs generated by both T cells inhibit macrophage functions potentially provoking severe damage to the host [471]. IL_{12} establishes a bridge with acquired immunity and provides defense against infections at different levels: it influences rapid IFN-γ production from T and NK cells, modulates virgin T-cell differentiation into Th1 lymphocytes due to their capacity for *up-regulating IFN-γ titers and reducing IL_4 production* from virgin T cells, and is also necessary for the optimal proliferation of IFN-γ producing Th1 cells in response to antigens and APCs [181]. Thus, IFN-γ and IL_{12} form a positive feedback system amplifying IFN-γ levels to activate macrophages and IL_{12} to promote proliferation and activation of NK and Th1 cells [181]. Recently, the primary function of IL_{12} has been shown to up-regulate adhesion molecules, and IL_{28} and IL_{29} medi-

Table 1.66. Primary antibody-mediated defenses against pathogen microorganisms

Immunologic function	Pathogens affected	Main Ig involved	Nonspecific cofactors required
Opsonization	V, B, M	IgG, IgM	Phagocytes and complement (in some cases)
Neutralization	V	IgG, IgM, IgA	Complement (in some cases)
Neutralization	B	IgG	–
Inhibition of binding	B, M (?)	IgA	–
Cytolysis	V[a], B, P	IgG, IgM	Complement
Enzyme inhibition	V, B (?)	IgG	–
ADCC	V, B (?), M (?)	IgG	–
Growth inhibition	Mycoplasma B	IgG, IgA IgA	– Lactoferrin

Modified from [360].
V Viruses, *B* Bacteria, *M* Mycetes, – none.
[a] Lysis of virus-infected cells.

Table 1.67. Critical immunological effector mechanisms for protection against secondary virus infections

Viruses		CTL (perforin)		T (cytokines)		Antibodies	Neonatal antibody protection
		Resting	Activated	Resting	Activated		
Cytopathics	IV	–	–	–	–	++	+
	Periphery	–	–	–	++	+	±
Noncytopathics	IV	+	+	–	–	+	+ (NN)
	Periphery (antiviral)	–	++	–	–	–	– (NN)
	Periphery (immunopathology)	–	++	–	–	–[a]	–

The combinations shown with "+" or "++" mark the critical memory effector mechanisms for protection.
Modified from [688].
IV intravenously, *NN* not necessary.
[a] Can enhance immunopathology.

ate antiviral activity in cells in response to viral infection [519]. In addition, activated T cells generate additional ILs with a multitude of functions, including the widening of antimicrobial activities of immune cells, macrophages, neutrophils and NK cells [299]. Most CTLs are CD8 and also a CD4 T cell subset and HLA class I or II restricted. Similarly, T cells can directly kill microorganisms not expressing HLA molecules: this direct antimicrobial activity can also occur when HLA restrictions are absent [299]. CD8 T cells, unlike NK cells, also attack *Candida albicans* and show a direct antiparasite activity, although several of their activities are played together with NK cells [299]. Loss of direct T-cell activity could contribute to AIDS and other severe PID infections [299]. *Mice lacking T-bet* fail to control a Th1-dependent infection, yet interesting evidence suggests a pathogenic role of Th1 cells in autoimmunity [556]. Also suggestive is an antibacterial defense achieved via the innate immune system, such as innate opsonins, complement components, and certain antimicrobial peptides, leading to a polyvalent machinery formation presumably yielding a lymphocyte pathway of recognition and activation [194]. For example, opsonins, by binding to microbial cell walls and flagging them for recognition by the complement system and phagocytes, could be effective antibacterial agents [345]. As a corollary, pathogens with components capable of inducing phagocytes to produce IL_{12} already in the early events of infection elicit Th1-like responses [106]. Instead, the optimal responses against organisms with a complex structure, for example, intestinal nematodes cannot be rapidly killed because they are so large, are thus susceptible to active attack by Th2 T cells, which directly inhibit parasite growth without killing them

[592], also trying to prevent Th1 responses and bypassing macrophage activation. Tables 1.66 and 1.67 [360, 688] include antibody-mediated defenses against pathogen microorganisms related to memory effector mechanisms critical for newborn protection (Table 1.67).

Because innate immune stimulation is thought of as a broadly applicable strategy for biodefense and potentially a boon for vulnerable special populations, such as the *very young*, as well as *children* and *adolescents* whose adaptive immune systems are impaired by chemotherapy or HIV, innate immune function in different age groups and disease conditions requires that the exciting possibility that powerful inborn defenses against infection is manipulated to provide a counterweapon against broad classes of bioterror agents and newly emerging infectious diseases [194]. Such defenses are more realistic because of a growing understanding of the *pathogen genomes* [183].

In *human neonates,* despite normal basal expression of TLRs and membrane CD14, innate immune responses of mononuclear cells to LPS are characterized by a strikingly reduced release of the pro-inflammatory Th1-like TNF-α and IFN-γ with relative preservation of antiinflammatory Th2-polarizing ILs. These differences extend to a range of TLR agonists, including bacterial lipopeptides (BLPs), and are due to differences in soluble factors present in blood plasma. The suppressed TLR-induced TNF-α release from monocytes by soluble factors in neonatal blood plasma is likely to alter both innate and adaptive immune responses in neonates profoundly. Thus, neonates are at increased risk of overwhelming infection, yet the mechanisms underlying this susceptibility are incompletely defined [297]. Moreover, exposure to reduced levels of sCD14 in the fetal and neonatal gut is associated with the development of atopy, eczema, or both. A supply of sCD14 [of which breast milk is rich (Chap. 2)] could affect disease outcome, although the IgE- and non-IgE-dependent consequences of this require elucidation [243]. In contrast to the protective effects of TLR9, immunization with Ag in the context of TLR2 ligands, can result in experimental asthma [450]. Furthermore, TLR10 may be a potential asthma candidate gene: TLR10 is notably expressed in lung tissue, where a *potential asthma candidate gene* my be involved in the innate immune responses [289].

Neonatal deficiency of innate cellular immunity includes a reduced IFN-γ production by neonatal lymphocytes, hyporesponsiveness of neonatal macrophages to IFN-γ activation, a reduced production of IL_{12} by CB mononuclear cells, defective STAT-1 phosphorylation in CB monocytes, a high IL_{13} production by neonatal T cells, a hyper-methylation at CpG sites within the IFN-γ promoter region, a defective production of IL_{18} by CB mononuclear cells, a reduced Myd88 expression in newborn monocytes, and an impaired response by neonatal monocytes to multiple TLR ligands [329].

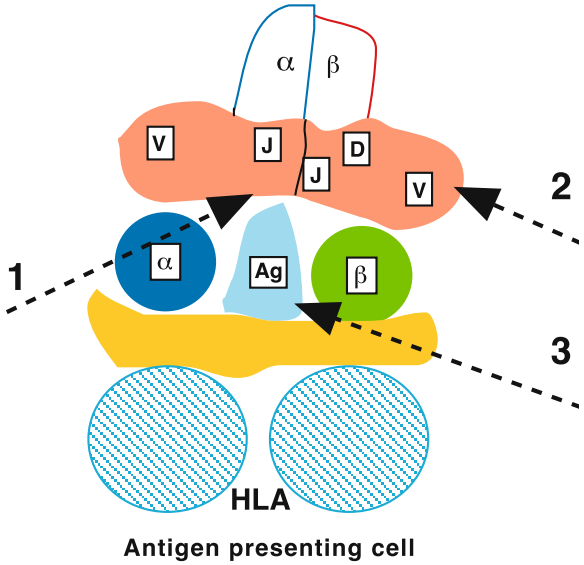


Fig. 1.66. Sites of potential peptide intervention. For details see text. (Modified from [404])

Therapeutic Perspectives

Following the leitmotif of the unabated flood of data that has taken us from the early studies on immune cells to some of the most complex areas of research in today's basic immunology, the important advances in the range of strategies heralding possible and interesting developments, which have been or will be focussed to hit specific targets, in particular allergic inflammation. We note that the task appears to be ever more challenging as the intricacy of immune mechanisms unravels.

Substantial evidence shows that *synthetic peptides* (Chap. 13) are able to disarm potentially harmful allergen-reactive T cells and/or inhibit IgE binding to allergens and/or compete with IgE antibodies for receptor binding. Logically a census of all relevant epitopes present on allergenic molecules is presently inconceivable, and certainly cross-reactivity imposes a further but not indifferent obstacle; however, this arm of immunology is making rapid progresses, as is shown in Fig.1.66 [404]. The functional inactivation can be selectively referred to as specific clones of T lymphocytes (*arrow 1*) or to a subset of T cells with different specificities, and TcRs with particular features but reciprocally common (*arrow 2*), or lastly to the substitution of epitopes bound to the disease with inert peptides at the level of the peptide–HLA complex combination site (*arrow 3*). Another option, devoid of toxic effects for human beings, is that of employing synthetic peptides corresponding to linear sequences of HLA class I molecules (residues 56–69, 60–84, 99–113 and 222–235) to cause T-cell anergy, increasing the influx of intracellular Ca and interrupting the normal pathway of signal transduction [277]. To these strategies down-regulating T cell modulation to

promote apoptosis or, on the contrary, to render T cells unable to respond to IgE synthesis, experiments have offered new insights into the inhibition of IgE binding to metachromatic cells or, alternatively, IgE conjugated with toxins able to dampen mast cells, or synthetic IgE fragments with deletion of specific responses.

Characterization of IgE receptors can provide specific clues to potentially blocking IgE binding to other cells: the use of anti-IgE IgGs with a double epitope specificity could modulate IgE-producing B lymphocytes, either bridging IgE bound to FcεRI or blocking IgE binding to its receptor, increasing or, respectively, abolishing their activity [358, 514].

Atopic individuals have *natural anti-IgE antibodies* directed against the IgE Fc region, different from anti-idiotype antibodies, which are not directed against allergen-specific IgE and therefore possess an immunoregulatory effect on IgE antibodies. Furthermore, anti-idiotype antibodies cannot establish a cross-linking with basophil IgE inducing their degranulation, thus blocking allergen binding to cells expressing IgE receptors [638]. However, to accomplish a therapeutic intervention, the degranulation impact should be very limited, but in view of the scarce number of metachromatic cells expressing the corresponding idiotypes, several authors deem this goal feasible [638]. Moreover, taking into account the particular convex structure of IgE antibodies [553], a strategy blocking IgE bound to FcεRI without triggering mast cell degranulation should be based on natural autoantibodies directed toward an IgE region (residues 305–313) across the FcεRI *locus* (residues 330–345) [513].

As an alternative, *IgE synthesis could be directly inhibited* (as well as synthesis of other Igs); incrementing CD14 engagement on monocytes increases monocyte activation, since the exerted block is at the B cell level, which is conceivable in either T-dependent or T-independent systems. CD14 delivers a negative signal that terminates T-cell proliferation, the repercussions of an event about which too little is as yet known [608].

What is most likely to be successful is the attempt to inhabit IL_4, the factor triggering IgE synthesis:

- In a *mutant IL_4*, substituting the aspartic acid of a protein with a tyrosine residue in position 124 (IL-4.Y124D) blocks IL_4R activation while leaving the binding capacity, and a IL_4- and IL_{13}-induced dose-dependent inhibition of IgE synthesis. By increasing the dose 100-fold, the inhibition increases up to 95% [437]. Moreover, the addition of an IL_4 binding protein, a sIL_4R, blocked the IgE enhancement by $CD8^+$ T cells [346]. However, if the peripheral blood of atopic patients contains long-lived B cells with a previous isotype switch to IgE, these antibodies cannot be directly influenced by ILs [125].
- The intervention of a *potential suppressive factor* of transcriptional events, inversely regulating IL_4 transcription of a not yet identified protein, determines a high capacity of binding to Th1 T cells at the moment of T-cell activation, coupled with a virtual absence of IL_4 after 16–24 h; instead, the high binding capacity of Th2 T cells is expressed with not yet stimulated cells. However, it is decreased when Th2 are activated. Such events are correlated with high inductive signals for IL_4 after the same time span [206].
- Increasingly, soluble *sIL_4R* may be able to block in vitro IL_4-mediated B cell activation, proliferation and differentiation in human PBMCs and therefore both IL_4-dependent IgE synthesis and CD23 induction on B cells. The clinical basis of using sIL_4R is the location in the blood, rendering sIL_4R a potentially stimulating candidate. Moreover, IL_4-deficient mice obtained via gene targeting do not display immune abnormalities, except for their inability to produce IgE [172]. A contrary effect has been assessed [492].
- An interesting subject is the demonstration that *anti-CD23 antibodies* block IgE synthesis in an isotype-restricted fashion, that is, inhibiting B lymphocyte differentiation in IgE-producing cells under the IL_4 influx [16].
- STAT6 is phosphorylated by IL_4, so any agents that *block STAT6 function* may be useful for treating atopic disease [562].
- Even more interesting is the likelihood of inducing a *peptide-mediated IL_4 anergy*. Using a clone of T cells incubated in a culture medium or with a specific peptide in either an immunogenic or nonimmunogenic dose, and stimulating again with a nonimmunogenic specific peptide and the related APCs, it was elucidated that T cells becoming unresponsive completely lose the ability of secreting IL_4, but fully maintain the ability of producing IFN-γ [138].
- In the animal model, the concerted action of IL_{12} together with IL_{18} and anti-CD40 *inhibits IL_4 and IgE synthesis* by induction of IFN-γ production from activated B cells, however, without inhibiting B cell proliferative response [669].
- A significant series of experiments in the animal model with synthetic peptides assure relevant implications for SIT. Administering a peptide along with an adjuvant in APCs induces T-cell proliferation and development of immunity. If a peptide able to activate T cells is administered, but disregarding the adjuvant (or CD28/CD80 interaction is missing), the *2nd signal is abrogated*. T-cell *clonal anergy* ensues as does the eventual apoptosis that drives to tolerance, as discussed in "Apoptosis" [138] (Fig. 1.22c). A similar option could prove successful in atopic patients [276].
- Ongoing studies on single amino acid substitutions have paved the way to assemble a peptide with a T epitope possibly able to *interrupt T/B interactions* and the consequent isotype switch to IgE, exciting IFN-γ production or inhibiting CD4 proliferation. However, skin prick tests (SPTs) with a modified allergen failed to drive immediate reactions [223].
- A potentially promising candidate is *clonal deletion* [138], a process of negative selection following, for

Table 1.68. Partial list of the agents inhibiting apoptosis

Inhibitors		
Physiological inhibitors	**Viral genes**	**Pharmacological agents**
Androgens	Virus LMW5-HL Herpesvirus γ1 34.5	
CD154	Baculovirus IAP	Tumor promoters
Estrogens	African swine fever	α-Hexachlorocyclohexane
Extracellular matrix (EC)	Baculovirus p35	Cysteine protease inhibitors
Growth factors (GF)	Adenovirus E1B	Calpain inhibitors
Neutral amino acids	Cowpox virus crmA	PMA
Zn	EBV BHRF1, LMP-1	Phenobarbital

Data from [576].

example, a self-HLA–antigen complex interaction, an event leading to apoptosis and thereby a powerful mechanism aimed at inducing tolerance to self-antigens (Fig. 1.22).

- The armamentarium of T epitopes has intriguing repercussions for SIT, based on the *switching of immune responses* from Th2 to Th0 T cells. A prominent feature, however, is a feasible switch to Th1 and DTH (Chap. 13).
- Administration of peptides corresponding to T epitopes (at least 10–12 amino acids) elicits a *T cell tolerance* to a complete antigen in humans [404], hence immunodominant T cell epitopes of an allergen actually also function as high inducers of T-cell nonresponsiveness [619].

What was summarized above on sCD40 makes it feasible to consider an isotype switching inhibition after the second signal expressed by ILs, to stop T–B interactions via CD40 and CD154 thus far achievable in vitro [132]. In addition, CsA abrogates CD154 stimulation in mast cells [174]: such data explain why CD154 regulatory effects are inhibited on APCs and IL_{12} as regards the stimulation of adhesion molecules useful for T cells and macrophage production of inflammatory ILs [187].

- A not yet explored and totally different strategy has the objective of suppressing B_{IgE} cells in patients with IgE-mediated atopy using mABs activated by a procedure tested on B cells expressing membrane-bound IgE, more precisely including amino acid residues and Ig membrane-anchoring segments extending from the C-termini of H chains. One potential application could be a methodology that can modulate isotype-specific antibody production to suppress B cells undergoing switch recombination, but not circulating IgE or IgE bound to mast cells [108].
- A fusion protein of 2 major allergens bypasses IgE binding and mast cell/basophil IgE FcεRI cross-linking and protects from IgE development [282].
- A recent study that certainly has striking consequences on AD treatment has shown that FK-506 and CSs inhibit both NFAT and AP-1, and hence IL_5 transcription [394]: since TFs are alike for diverse ILs, it is probable that further studies will report the inhibition of additional ILs.
- As outlined in Chap. 22, atopic children, some with HIgES, have been successfully cured with intravenous IgGs (IVIgs), suggesting that *IVIgs inhibited IgE production* of PBMCs (T cells) cultured in vitro and stimulated by IL_4, without influencing IgA and IgM levels, and also of normal B cells stimulated by IL_4 + anti-CD40.
- The significance of CSMs results from their ability to block antibody production following in vivo administration of CD152-Ig and anti-CD86 [115]. CD152-Ig specifically abates IL_4 production and if administered early after the start of an infection *blocks its progression* [242, 685].
- A new strategy *to inhibit or,* depending on the case, to *cause apoptosis* (Tables 1.68, 1.69) [576] acts on NF-κB as well as TNF-α [599], or modulates bcl-2/ced-9, or alternatively ICE/ced-3 [267, 661], or ced-4: if a mammal homolog is identified the issue could encompass all changes deriving from apoptosis dysregulation.
- A recent approach could be aimed at *eliminating eosinophil influx* into airways, which has been achieved treating animals with anti-MIP-1α, anti-RANTES and anti-MCP-3. So the rationale to focus CCR3 antagonists is available, a potential mediator of CCL chemokine effects on eosinophils [9].
- Another strategy is *the blockade of chemokine receptors:* CCR3, CCR4 and CCR8 are the most obvious targets for therapeutic investigation.
- Attention is also turning to small molecular weight (SMW). SMW IL_4 inhibition seems to *decrease IgE synthesis,* lung eosinophilia and mucous production and could be used to treat asthma and other atopic disorders.
- The development of new therapies for atopic allergy is now focusing on local Th1-driving IL_{12}-promoting substances to target both the development of new Th2 cells and the persistent population of established allergen-specific Th2 cells to *revert established effector*

Table 1.69. Partial list of the agents inducing apoptosis

Inducers			
Physiological activators	**Damage-related inducers**	**Therapy-associated agents**	**Toxins**
Ca	Antimetabolites		
CSs			
GF withdrawal	Free radicals		
Loss of EC attachment	Nutrient deprivation -		
Neurotransmitters			
Glutamate	Tumor suppressors p53	Methotrexate	
Dopamine	CTL	γ Radiations	
N-m-*d*-aspartate	Oxidants	UV radiations	
TGF-β	Oncogenes myc, rel, E1A	Arabinoside, vincristine, nitrogen mustard	
TNF family	Heat shock	Chemotherapeutics:	Ethanol
FasL	Viral infections	Cisplatin, bleomycin	β-Amyloid peptide
TNF	Bacterial toxins	Doxorubicin, cytosine	

Modified from [576].

Th2 cells in humans into predominant Th1 phenotypes [530].

The newly described IFN-λ and $IL_{27–29}$ that mediate antiviral activity in cells in response to viral infection are the major initial weaponry against most viruses (Table 1.5).

The development of new therapies for atopic allergy is now focusing on local IL_{12}-promoting substances to target both the development of new Th1 cells and the persistent population of established allergen-specific Th1 cells:

- IL_{27}/WSX-1 plays an important role in the down-regulation of BHR and lung inflammation during the development of allergic asthma via its suppressive effect on IL production [357].
- Since IFN-γ induces T-bet expression, T-bet would be an attractive target to identify anti-asthmatic drugs [155].
- Anti-human IL_{13} antibody also looks promising for lowering tissue eosinophil levels and is in preclinical trials [44].
- Antagonists of proteinase-activated receptor or inhibitors of proteases that activate this receptor may be worthy therapies for asthma [502]. Tryptase is a therapeutic target in asthma and selective tryptase inhibitors can reduce allergic airway inflammation [392].
- CD300a was shown as a future potential target for the treatment of allergic and eosinophil-associated diseases. By suppressing the activity of human eosinophils, cross-linking of CD300a on the eosinophils inhibited the IL_5/GM-CSF antiapoptotic effects and blocked the release of TNF-α, $IL_1β$, IL_4, 3T3 fibroblast proliferation, IL_5-mediated JAK2 phosphorylation, eotaxin- and IL_5/GM-CSF-mediated ERK1/2 and p38 phosphorylation, but also blocked IFN-γ release [366].
- Selected defensins and/or chemokines may be good candidates for the development of vaccine adjuvants, since they are believed to enhance adaptive immunity markedly [660].
- The effect of dietary vitamin E on atopic disorders is beneficial since it blocks TF binding to two pivotal IL_4 promoter binding sites for NF-κB and AP-1 and interferes with promoter activity upon T cell activation [311]. Moreover, vitamin E suppresses CD95L (APO-1/Fas) mRNA expression and protects T cells of HIV-1-infected individuals from CD95-mediated apoptosis, evidence that vitamin E can affect T cell survival [312].

Allergens

Following the increased prevalence of atopic disease, it has become even more necessary to have at hand purified allergens to obtain more characterized diagnostic and therapeutic tools [94, 127, 433, 639, 675]. Allergens responsible for immune reactions are mostly proteins and are classified into major, intermediate and minor allergens based on the frequency with which an IgE-mediated allergy occurs. This characteristic can be analyzed with laboratory methods; CRIE (crossed radioimmunoelectrophoresis) is the most frequently used technique in two phases. In the first phase, all proteins of a given allergenic substance are separated electrophoretically and recognized as precipitates in specific rabbit antisera. In the second phase, a pool of RAST sera positive for the substance under scrutiny is applied to the procedure:

sIgE will only fix to allergenic proteins, subsequently read by autoradiography utilizing anti-IgE antisera marked by radioactive tracers. Thus *major allergens* are defined as components that bind IgE in 50% of the sera from a group of patients with the same allergy, *minor allergens* when components react with 10% of the sera, and *intermediate allergens* when components share halfway properties. Most commonly, an allergenic source may enclose allergens belonging to the three above types: for instance, egg white contains at least 20 different proteins, but only four or five of these proteins are allergenic. Actually only major and minor allergens are taken into account. However, not all patients recognize all major allergens and some patients only recognize allergens that are not recognized by the majority of allergic patient sera (Chap. 13). From a clinical point of view, allergen sources can be classified as inhalant allergens: pollens, mites, animal danders, molds, chemical/pollutant substances, drugs; ingested allergens: foods, chemical substances, drugs, etc.; insect allergens: insect venoms; and contact allergens: foods, chemical substances, topical drugs, cosmetics, etc. [94, 126, 433, 639, 675].

Table 1.70. Completely cloned allergens (recombinant DNA) with the pertinent amino acid sequence

Foods	Ara h 1, Mal d 1, Met e 1, Sin a 1
Animals	Bos d 1, Can f 3, Chi t 1, Fel d 1
Mites	Blo t 5, Der p 1, Der p 2, Der p 3, Der p 5, Der p 6, Der p 7, Der f 1, Der f 2, Der f 3, Der f 9, Der f ?, Eur m 1, Lep d 1
Molds	Alt a 6, Alt a 7, Alt a 10, Asp f 1, Asp o 2, Cla h 3, Cla h 4, Cla h 5, Cla h 6, Pen n 1
Pollens (plants and trees)	Aln g 1, Amb a 1, Amb a 2, Amb a 5, Amb p 5, Amb t 5, Bet v 1, Bet v 2, Bet v 3, Car b 1, Cor a 1, Cry j 1, Cry j 2, Cyn d 1, Dac g 2, Hol l 1, Hor v 1, Lol p 1, Lol p 2, Lol p 5, Ole e 1, Par j 1, Pha a 1, Pha a 5, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 11, Poa p 9, Tri s 11, Zea m 11
Insects	Api m 1, Api m 2, Bla g 2, Bla g 4, Dol m 1, Dol m 2, Dol m 5, Myr p 1, Ves v 5

Data from [112, 496, 604].

Allergen Standardization

Standardization regards commercial allergens, provided that they correspond to the main rules established with respect to their content of major and minor allergens, safety and potency or biological activity. It is highly necessary because crude allergen extracts incorporate all components of original materials, hence not only all potentially sensitizing allergens, but also irrelevant material with potential primary irritant activity. We define allergen extracts as complex mixtures of substances with a known composition, reflecting that of a relevant allergen source material that could include even 100 proteins, but with a known total allergenic potency that is constant between batches [126]. However, the extracts may vary according to the quality of the initial material and be insufficiently purified and also unstable. Therefore, allergen extracts contain major allergens in inadequate concentrations, and are contaminated by irrelevant components to which the patients are not sensitized, or important allergens are lost during extractive procedures due to proteases purified together with allergens [126]. In addition, measurement of allergen levels present in planned extracts is often difficult because of a mixture complexity, or because allergen components vary from one supplier to another, either for allergen potency or quality, or from one year to another or one production lot to another of a single supplier [112]. *It is not possible to compare the potency of allergen extracts produced by different manufacturers, even if quantified with the same techniques* and if done, it might lack the indication of major and minor allergens [112]. To ensure uniformity among future batches, units of measure have been introduced. As a consequence, manufacturers can employ appropriate, although of varying calibration, methods for allergen extracts, provided that such methods are clearly documented and constant. The WHO/IUIS has established International Units (IU), the Biologic Units (BU) are widely used in northern countries and in several European countries, and in the US are expressed as Bioequivalent Allergy Units (BAU). Such methods are expensive and demanding; therefore promising improvements in the procedure are currently *recombinant allergens* (RA) (Table 1.70) [112, 496, 604, and Internet data, August 2006].

Standardization Techniques

Allergen standardization consists in the adoption of methodologies ensuring both uniformity and reproducibility between different batches of the same allergen extract, that is, the procedures to select the raw materials, assemble and control allergen extracts, which allow suppliers to produce different batches with the same allergen provided with wholly matching characteristics. The system based on SQ units (standardized quality unit), crossed immunoelectrophoresis (CIE) and CRIE makes it possible to demonstrate the single epitopes of standard extracts. For each new extract, the existence and amount of all allergens is controlled by autoradiography; subsequently the batches presenting quantitative differences are equilibrated to standard concentrations using quantitative immunoelectrophoresis [126]. Reference internal extract (in-house standard) is prepared to guarantee the protein composition of allergen ex-

tracts via immunochemical analyses, such as CIE, CRIE, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), high-performance liquid chromatography (HPLC), isoelectrofocalization (IEF) and other molecular biology techniques. Currently, it is necessary to employ in vitro and in vivo standardization methods, both based on interaction between allergens and sIgE produced by sensitized patients [675].

In Vitro Standardization Methods

The most important in vitro standardization techniques are based on binding inhibition of pooled sera containing sIgE to allergens and on gel precipitation.

RAST inhibition quantifies single allergens or total allergenic potency of raw extracts: soluble antigens, identical or cross-reacting with antigens of solid phase, are combined with the serum pool. The resulting binding inhibition of anti-IgE antibodies labeled with I^{125} to solid phase antigens shows the identity of the soluble antigen with that bound to the solid phase. Consequently, varying amounts of soluble allergen extracts are added to the RAST first phase as inhibitors. Dose-response inhibition curves may determine activity relative to reference extracts: parallel inhibition curves indicate similar composition, while nonparallel curves do not. The necessary reagents require:

- *Allergens* derived from high-quality materials where all major and minor allergens are represented.
- *Pooled sera* containing IgE from a panel of patients characterized for their sensitivity to the allergen in question.
- *Reference extract* of high quality.

Similarly, ELISA inhibition, rocket electrophoresis, isoelectric focusing, gel electrophoresis, etc. are used [94].

Analysis of single allergens is accessible only when major allergens have been identified, thereby anti-allergen-specific antibodies allow subsequent allergen quantification with an immunological essay. For this purpose, several techniques are utilized, for example, an enzymatic essay to characterize the extracts as regards *Hymenoptera* venoms equipped with several enzymes [433].

Gel precipitation techniques include CIE, CRIE, immunoblotting and histamine release by basophils.

In Vivo Standardization Methods

The standardization method established in Scandinavia expresses the results as HEP (histamine equivalent potency), the allergenic activity of an extract is estimated measuring the skin response to the extract in a pool of subjects with known allergy to the tested allergen. SPTs test the quantity of the extract that produces a wheal the same size as the wheal size produced by histamine hydrochloride at the concentration of 1 mg/ml: this result corresponds to one HEP unit. However, the results are now usually expressed as BUs, which is defined as the wheal equivalent to histamine 1 mg/ml = 1,000 BU/ml. The BU gives valid indications on total biological activity, but not on the presence in the extract of single allergens; BUs are referred to as the in-house standards of each manufacturer, and therefore depend on patient selection, reactivity and compliance [112]. An analogous technique is skin activity reference allergen/histamine (SARAH) [433]. The approach established in the US based on AUs (allergy units) is valid for mites and other allergens: reference controls regarding the extracts are Der p-sensitive individuals intradermally tested with increasing doses, with subsequent evaluation of the flare rather than the wheal size as with HEP units. The reference standards for subsequent allergen dilution is the sum of erythema diameters. For other allergens, the AU provoking reactions of the same diameter in patients with multiple sensitizations is defined as BAUs. However, extracts standardized in BAUs are not comparable with corresponding BU extracts, since the techniques do not reduce the likelihood of detecting a different potency in lots of the same allergen extract produced by different manufacturers, nor the variability among lots of the same manufacturer, nor the problems of related extract potency between BUs and two different societies [112]. There is variability due to isoallergens, different isoforms of the same allergen, contained only in varying levels in the extracts [551]. Finally, besides the variations between the techniques used by laboratories, we must also take into account different subjects in patient pools [94].

Units for SPTs and SIT

Both the US FDA (Food and Drug Administration) and Nordic guidelines prevailing in Europe can be followed. In the first case, an allergen concentration is defined that can be recommended for SIT and is based on intradermal skin testing of the most sensitive patient: 100,000 AU/ml. In the second case, the unit is based on SPTs done on patients with average sensitivity and corresponds to the concentration eliciting a wheal that is the same size as the wheal produced by 10 mg/ml histamine and equals 10,000 BU/ml [126]. The ideal extract should comprise definite quantities of all major and minor allergens of each biologically potent allergen: such analyses obviously play a highly relevant role in assembling SIT extracts, which should include only allergens (or allergen epitopes) pertinent to the single patient [112]. According to Scheiner et al, SPTs have a high sensitivity (82.1 %) and specificity (66.3 %); to their detriment are the rapid denaturation of some natural allergens (such as the apple) during extraction processes. Furthermore, in the extracts the allergen levels can be naturally reduced (such as the cypress), or following destruction via enzymes present there [496].

Advantages of Standardization

The principal advantage is the testing of standardized extract biological effects, and the variability in relative titers of major allergens is much lower than extracts that have not been standardized (two- to threefold vs ten- to >100-fold). Another point in favor is clarified by the therapeutic effectiveness: consistent data show that when SIT maintenance doses correlated with major allergen levels are reached, evident improvements in patient symptom scores are noted [126].

Among the more recently achieved results in above-mentioned studies, we include the delineation of antigenic or allergenic epitopes, either major or minor and/or dominant in individual antibody repertoire, using techniques based on recombinant DNA. In addition, cloning single-helix DNA cDNA to an RNA chain, which was synthesized by inverse transcription, cDNA-coded protein amino acid sequences drawn from cDNA libraries were established, a complex of DNA cloned fragments representing the whole genome [443]. Hitherto, several allergen amino acid sequences have been determined, above all arthropods and pollens, employing cDNA-based techniques and it is auspicious that the list is so extensive that it includes all allergens [360] and Table 1.70. We stress that with knowledge of primary tools, it is reasonable to predict spatial conformation, and that computerized programs can help disclose both biochemical properties and biological functions. These results will bring out and make it possible to evaluate protein allergenicity [678]. Moreover, using synthetic peptides based on known sequences, it is practical to determine T and B cross-reactive epitopes, as well as the regions of molecules containing them [551]. The dominant epitopes referred to as Amb a 1, Bet v 1, Lol p 1, Poa p 1 and Sin a 1 were identified in parallel, even if studies done on mice are not always applicable to human beings [501, 511].

Several RAs are now available (Table 1.71) [319, 498, 604]. RAs should be preferred without exception: RAs can be precisely manipulated, targeted, engineered and formulated at defined concentration and potency. They may be produced in suitable purity and batch consistency and hence might offer a perfectly standardized diagnostic material. A WHO and IUIS international committee has fixed in IUs some standardized allergenic extracts, to which laboratories should refer, thus providing more quantitative and meaningful extracts than methods that are by now obsolete [675]. However, the majority of foods and molds have not been even partially characterized. In the US, the assortment is wider: the FDA (last updated: 2, 26, 2006) has also approved cat epithelium, Can d, Der p, Der f, several *Hymenoptera* venoms Api m, Dol a, Dol m, Pol a, Ves g, Ves p spp, pollens (Agr a, Ant o, Cyn d, Dac g, Fes e, Hev b, Lol p, Ole e), Phl p, Poa p, Amb a and Amb e (ragweed), although for Lol p 1, Lol p 3 and Ole e the complete amino acid sequence is available [94, 496, 501]. Standardized allergen extracts are commercially available. The FDA has established that all missing allergens should be standardized [94]. In several countries of the European Union, allergen extracts are subjected to registration and/or a strict quality control. For example, with RAST inhibition, it is controlled that the levels of biological potency of produced lots remain constant. Since ≈50 major allergens might cover up to 90% of all IgE specificities, commercialized but not standardized extracts will be excluded from such regulations regarding only small groups of patients residing in specific geographical areas [94].

Table 1.71. Recombinant allergens

Animals	Fel d 1, Mus m 1
Food	Api g 1, Api g 4, Dau c 1, Mal d 1
Grass pollen	Par j 1, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Phl p11, Phl p12
Insects	Bla g 1, Bla g 2, Bla g 4, Bla g 5
Mites	Der p 1, Der f 2, Eur m 1
Molds	Alt a 1, Alt a 2, Asp f 1 to Asp f 18
Tree pollen	Aln g 1, Bet v 1, Bet v 2, Cor a 1, Hev b 3, Hev b 7, Hev b 8, Hev b 9, Hev b 10, Hev b 11
Weeds	Art v 1

References [319, 498, 604] and Internet data, August 2006.

From this point of view, particular observations refer to *profilins,* vegetal panallergens present in many organisms (Table 1.72) [468, 594, 596–598, 602], prominent allergens in the *pollens of trees, grasses and weeds,* all involved in cross-reactivity observed in pollinosis patients between foods and pollens of only distant phylogenetically correlated plants, even in latex [597, 603].

Cross-reactions among allergens are outlined in Table 1.73 [17, 52, 128, 134, 406, 417, 434, 518, 598, 603, 604]. Above all, pollinosis patients suffer from cross-reactions and type I reactions between isoallergens of group I pollens with a MW of 26–32 kD, especially of Phl p and Lol p, with a marked degree of analogy in amino acid sequences. Furthermore, Phl p 1 has several T epitopes [497]. Mal d 1 has homology with *Fagales* group I [543]; Mal d 1, Api g 1 and Cor a 1 belong to the group of proteins related to pathogenesis, expressed by vegetables in stress conditions.

We now examine the allergens thus far identified and characterized, underlining the most recent updating, based on recent revision [639]. Allergens have the C or P letters depending on whether the related data on amino acid sequences are complete or partial [667]. In addition, we found Lol p 11 as the second allergen after Bet v 2 present in the pollens of vegetables and trees [134], and others [348, 543] (Table 1.74) [8, 15, 17, 60, 89, 98, 112, 121, 128, 134, 149, 199, 204, 265, 295, 310, 319, 348, 355, 365, 397, 384, 416, 405, 434, 471–473, 478,

Table 1.72. Profilins purified and characterized by allergenic raw materials

Provenance	MW (kD)
Pollens	
Ambrosia artemisiifolia	10–38
Artemisia vulgaris	14
Betula verrucosa (Bet v 2)	15
Betula verrucosa (Bet v 3)	30
Cynodon dactylon (Cyn d 12)	≈14
Helianthus annuus	≈16
Hevea brasiliensis	14
Lolium perenne	≈12
Mercurialis annua (Mer a)	14–15
Olive (Ole e 2)	15–18
Phleum pratense (Phl p 12)	44
Phoenix dactylifera	≈14
Zea mays	≈14
Foods	
Apple (Mal d 1)	18
Banana (Mus xp 1)	15
Carrot (Dau c 1)	16
Celery (Api g 4)	≈15
Cherry (Pru av 4)	15
Fennel	
Hazelnut (Cor a 1)	17
Kiwi (Act c 1)	30
Litchi	15
Muskmelon	13
Peach (Pru p 4)	14
Peanut (Ara h 5)	15
Pepper (Cap a 2)	14
Pineapple (Ana c 1)	15
Soybean (Gly m 3)	14
Sunflower (Hel a 2)	15.7
Tomato (Lyc e 1)	14
Watermelon	13
Zucchini	13

Data from [468, 594, 596–598, 602].

497–499, 511, 543, 549, 551, 577, 604, 639, 652, 674]: see Figs. 1.67–1.80 for examples. Table 1.74 is completed with the structural and antigenic homologies of Fel d 1 with other superior felines (jaguar, lion, leopard and tiger), of Can d 1 with other Canidae (wolf, jackal, etc.) and of Equ c with other Equidae (donkey, mule, zebra, etc.). CM allergens are shown in Table 1.75 [24, 121]: five different casein molecular species were identified in a purified form, synthesized by structural genes localized on the same chromosome. Regarding animal panallergens, *tropomyosin*, present in Pen a 1, Met and 1 and Der f 10, homologous to Mag44, is a band I muscular protein inhibiting contractions, unless its position is not blocked by troponin present, for example, in Bla g 5, etc. [107, 297, 649]. It is notable that tropomyosin is shared by Met e 1 and Pen a 1 with Tod p 1, Der f 10, Der p 10, Lep d 10 and Ani s 2 [8]. Several allergens are included in the lipid transfer protein (LTP) family: a 50-kD salt-unextractable protein not affected by heat treatment be-

Table 1.73. Main cross-reactions among allergens

Foods
Act c 1 (kiwi) with Phl p (Timothy) and Bet v (birch)
Gad c 1 (cod fish) with several other fishes
Mal d 1 (apple) with Bet v 1, Bet v 2, Api g 1 (celery) and Pru p l (peach)
Met e 1, Pen a 1, Pen i and Tod p 1 = squid (tropomyosins) along with other mites and insects (see below)
Egg and chicken (bird-egg syndrome, Chap. 9)
Limpet with Der p (Chap. 20)
Animals
Can f 3 (Dog) with albumin of Fel d 1 (Cat)
Fel d 1 with dander antigens and of other felines (partial) and pork meat (pork-cat syndrome)
Can f, Fel d, Equ c (horse) and sheep have interspecies cross-reacting epitopes
Rat n 1 (Rat) with Rat n 2
Insects
Chi t 1 (*Chironomus thummi*) with Hb of other chironomids
Mites
Der m 1 cross-reacts with other mites
Between Der p 1 (Mite) and Der f 1 and Hel a 1 (snail)
Der p 1 has 85% homology with Eur m 1
Between Der p 2 and Der f 2
Der f 7 has 86% cross-reactivity with Der p 7
Der p 10, Der f 10, Lep d 10, Anis 3 (nematode) and Per a 7 (American cockroach) (tropomyosins)
Per a 1 with Bla g 1
Plants and trees
Amb a 1 (ragweed) with Amb a 2 and vice versa, with Cry j 1 (Sugi), tomato and corn
Bet v 1 and Bet v 2 with Pru av 1, Pru av 4 and Api g 4
Bra j 1 (mustard) with Sin a 1 and vice versa
Car b 1 (hornbeam), Cor a 1 (hazel), Aln g 1 (alder) and Que a 1 (white oak) with Fagaceae
Hev b 5 (latex) with Act c 1
Mer a (Mercurialis) with Art v (Artemisia), Fra e (*Fraxinus*), Ole e (*Olea*), Par j (*Parietaria*), Ric c (*Ricinus*)
Between each Phl and its group and between Par j 1, and Par o 1
Stressed vegetables express some PR, Protein related to pathogenesis, including Mal d 1, Api g 1 and Cor a 1

See in Table 8.14 the latex cross-reactions and in Table 9.48 the cross-reactions between foods and vegetables. Can f can also be named Can d. Additional cross-reactions may occur between two profilins combined (Table 1.72), for example, Gly m 3 and Bet v 1, which may trigger severe clinical reactions. Data from [17, 134, 299, 406, 417, 434, 518, 598, 603, 604].

longing to corn has been reported [416]. This is a major allergen that has not come from the WHO IUIS Allergen Nomenclature Subcommittee[639].

Fig. 1.67. *Ambrosia tenuifolia* (short ragweed)

Fig. 1.68. *Cynodon dactylon* (Bermuda grass)

Fig. 1.69. *Festuca elatior*

Fig. 1.70. *Phleum pratense* (timothy)

Fig. 1.71. *Betula* (birch)

Fig. 1.72. *Olea europea* (olive).

Fig. 1.73. *Lolium perenne* (rye grass)

Fig. 1.74. *Artemisia* (mugwort)

Technically, the allergens are classified according to linnaean nomenclature, where any species is indicated using a binomial composed of two Latin names: the first three letters of the abbreviations indicate the genus and the first letter of the species name, followed by a number, progressive, referring to epitope historic or temporal identifications. Instead of Roman letters, Arabic numerals are used to show the identification order [639]. For some allergens not included in the above-mentioned revision, we kept the previous specifications [397] with the Roman numerals already attributed.

Table 1.76 [601] summarizes the T epitopes of many allergens and Table 1.77 [601] the association of single allergens with HLA molecules and IgE responses, with several factors of relative risk.

Fig. 1.75. *Parietaria officinalis*

We show the foods derived from genetically modified organisms (GMO) or crop plants in Tables 1.78 and 1.79 [110, 348]. In GMOs, insect protection is achieved by means of plants producing insecticide proteins not toxic for human beings, while herbicide tolerance is mediated by plants provided with enzymes disarming herbicides. A prerequisite is that such products be subjected to extensive assessments to ensure food safety and digestibility [348]: however, it is feasible that genes of a given plant are transferred to a nearby one and that other genes damage the so-called useful insects, also including fish deriving from monosexualization or maternal DNA doubling. Therefore, verification of potential allergenicity of transgenic food modified with genetic engineering now appears to be necessary, such as in the case of soy (Fig. 1.81) [42]. The challenges posed by GMOs and bovine spongiform encephalopathy (BSE) will be discussed in Chaps. 9 and 24.

Fig. 1.76. *Parietaria judaica*

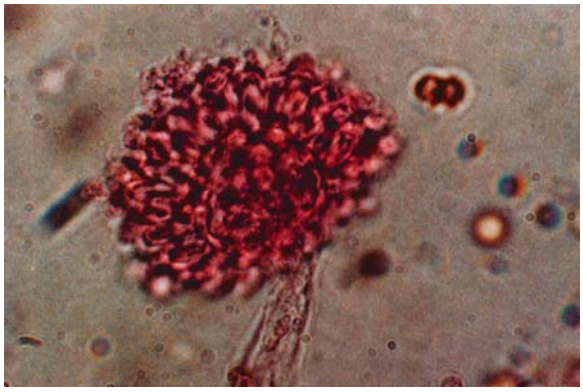


Fig. 1.77. Fungi. *Aspergillus fumigatus*

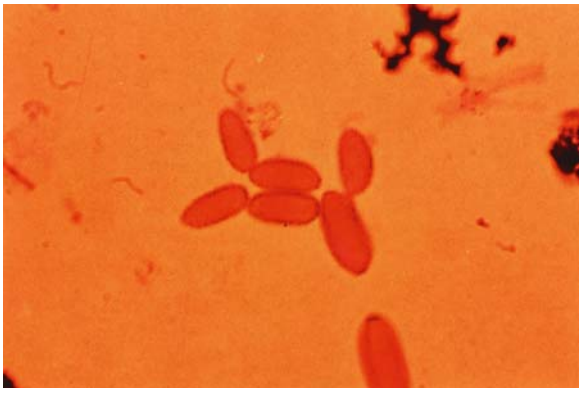


Fig. 1.78. Fungi. *Cladosporium*

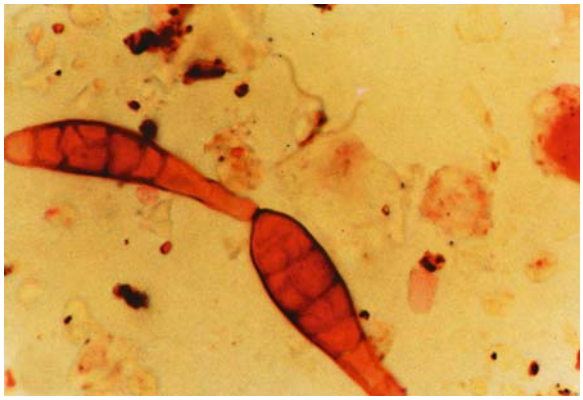


Fig. 1.79. Fungi. *Alternaria*

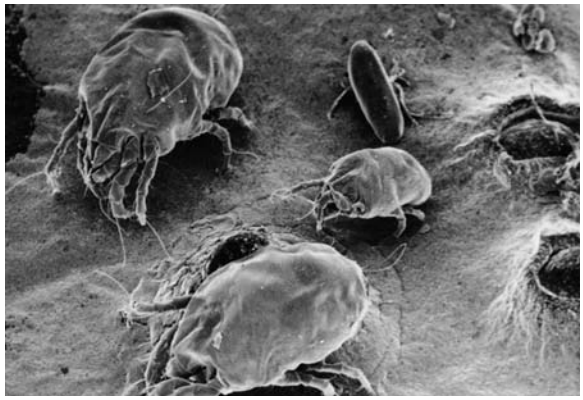


Fig. 1.80. EM view of mite family: egg, larva and adult

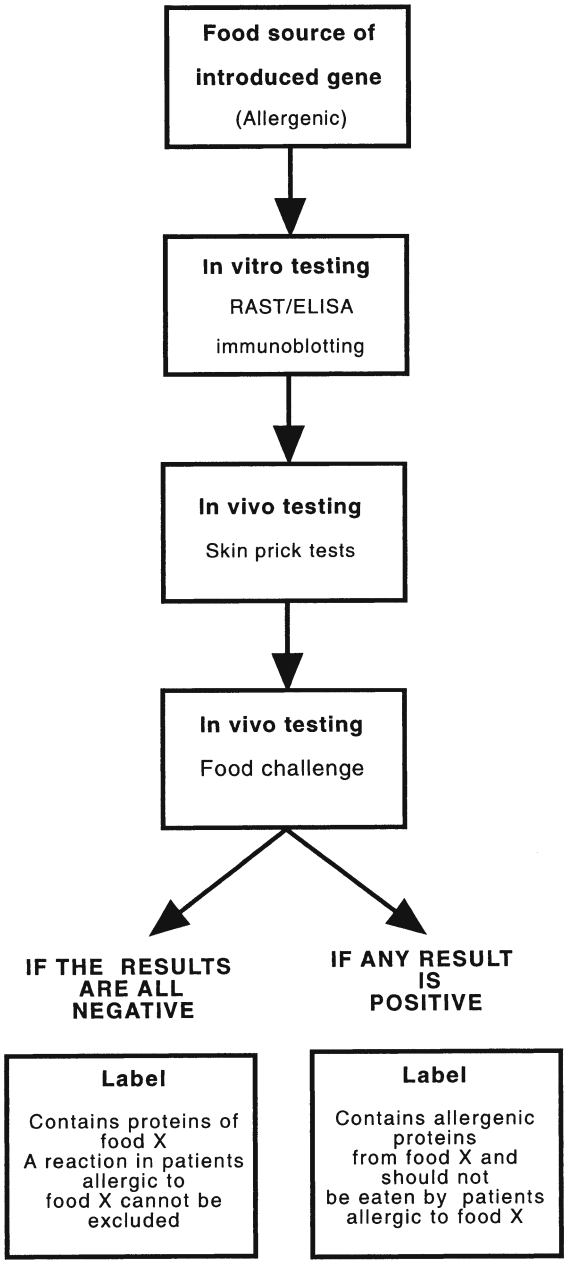


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Fig. 1.81. Allergy risks of transgenic foods. Flow chart for investigation of genetically modified foods for potential allergenicity before their release on the market, with suggestions on labeling of the pertinent foods. (Modified from [42])

Table 1.74. Allergens characteristics

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
1. *Foods* (listed independently of the family)					
Abalone (*Haliotis midae*)	Hal m 1	49			
Apple (*Malus domestica*)	Mal d 1	18	C		Profilin, hom: Bet v 1 [348, 604]
	Mal d 2		C		Hom: thaumatin
	Mal d 3	9	C		LTP
Apricot (*Prunus armeniaca*)	Pru ar 1		C		Hom: Bet v 1
	Pru ar 3	10	C		LTP
	Pru av 4				LTP [499]
Asparagus (*Asparagus officinalis*)	Aspa 01	9	P		LTP
Atlantic salmon (*Salmo salar*)	Sal s 1	12	C		Parvalbumin
Avocado (*Persea americana*)	Pers a 1	32	C		Endochitinase
Banana (*Musa paradisiaca*)	Mus xp 1	16	C		Profilin
Barley	Hor v 1	15	C	52	α-Amylase/trypsin inhibitor [348]
(*Hordeum vulgare*)	Hor v 9	30	C		
	Hor v 15	15	C		
	Hor v 16				α-Amylase
	Hor v 17				β-Amylase
	Hor v 21	34	C		Hordein
Black walnut (*Juglans nigra*)	Jug n 1	19	C		2S albumin
	Jug n 2	56	C		Vicilin-like protein
Brazil nut (*Bertholletia excelsa*)	Ber e 1 Bet e 2	9 29	C C		High-methionine protein, composed of two subunits
Carrot	Dau c 1	16	C		Hom: Bet v 1
	Dau c 4		C		Profilin
Celery (*Apium graveolens*)	Api g 1	16	C		Hom: Bet v 1, ribonuclease
	Api g 4				Profilin sharing IgE-binding epitopes with Bet v 2 [498]
	Api g 5	55/58	P		
Cherry (*Prunus avium*)	Pru av 1	18	C		Hom: Bet v 1, ribonuclease
	Pru av 2		C		Hom: thaumatin C
	Pru av 3	10	C		LTP
	Pru av 4	15	C		Profilin
Chicken (*Gallus domesticus*)	Gal d 1	28	C	34	Ovomucoid, protease inhibitor
	Gal d 2	44	C	32	Ovalbumin, hom: serine protease inhibitors
	Gal d 3	78	C	47	Ovotransferrin or conalbumin, iron transport protein
	Gal d 4	14	C	50	Lysozyme
	Gal d 5	69	C		Serum albumin
Cod fish (*Gadus callarius*)	Gad c 1	12	C		β-Parvalbumin, diffused cross-reactivity with other fish

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Corn (*Zea mays*)	Zea m 1	21	P		Lol p1 homolog [348]
	Zea m 11	14	C		The clone C13 is an Ole e 1 homolog [348]
	Zea m 14	9	C		LTP, a 50-kD corn protein that does not correspond to any known corn allergen, has been reported [416]
Cow (*Bos domesticus*)	Bos d 1	25			
	Bos d 2	22			
	Bos d 3	22			Ca-binding S100 hom
	Bos d 4	14.2	C		α-Lactalbumin
	Bos d 5	18.3	C		β-Lactoglobulin
	Bos d 6	67	C		Serum albumin
	Bos d 7	160			Immunoglobulin
	Bos d 8	20–30			Caseins
Cow's milk (Table 1.74)					
Cucumber (*Cucumis sativus*)		13			Profilin [468]
Grape (*Vitis vinifera*)	Vit v 1	9	P		LTP
Hazelnut (*Corylus avelana*)	Cor a 1	17	C	>90	Four variants of Cor a 1, 5, 6, 11, 16, all Bet v 1 hom [348] In 4 other variants IgE reactivity to Cor a 1.0401 was in 95%, to Cor a 1.0402 in 93%, to Cor a 1.0434 in 91%, to Cor a 1.0404. in 74% of sera [319]
	Cor a 2	14	C		Profilin
	Cor a 8	9	C		LTP
	Cor a 9	40	C		11S globulin-like protein
	Cor a 10	70	C		Luminal binding protein
	Cor a 11	48	C		Vicilin-like protein
Kiwi (*Actinidia chinensis*)	Act c 1	30	P		Recognized by IgE in 100% of cases, cross-reacts with Phl p and Bet v [417], cysteine protease
	Act c 2	24	P		Thaumatin-like protein
Lentil (*Lens culinaria*)	Len c 1	16	P		Vicilin
	Len c 2	66	P		Seed biotinylated protein
Lettuce (*Lactuca sativa*)	Lac s 1	9	LTP		
Muskmelon (*Cucumis melo*)	Cuc m 1	66	C		Serine protease
	Cuc m 2	14	C		Profilin
	Cuc m 3	16	P		PR-1 protein 13-kD components of melon, cucumber, watermelon, and zucchini were strongly recognized by the IgE antibodies of patients with melon allergy and were identified as profilins [468]
Mustard (*Sinapsis alba*)	Sin a 1	14	C		2S storage albumin
Mustard, oriental (*Brassica juncea*)	Bra j 1	15	C		Divided into IE-L, 2S albumin large chain, and IE-S2S albumin small chain

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Mustard, rapeseed (*Brassica napus*)	Bra n 1	15	P		2S albumin
Mustard, turnip (*Brassica rapa*)	Bra r 2	25	P		Hom: prohevein
Pea (*Pisum sativum*)	Pis s 1	44	C		Vicilin
	Pis s 2	63	C		Convicilin
Peach (*Prunus persica*)	Pru p 3	10	P		LTP, Pur p I contained in the peel [310]
	Pru p 4	14	C		Profilin
Peanuts (*Arachis hypogea*)	Ara h 1	63.5	C	>90	Vicilin seed storage protein
	Ara h 2	17	P	>90	Conglutin and others with ± concern [60]
	Ara h 3	60	C		Glycinin seed storage protein
	Ara h 4	37	C		Glycinin seed storage protein
	Ara h 5	15	C		Profilin
	Ara h 6	15	C		Hom: conglutin
	Ara h 7	15	C		Hom: conglutin
	Ara h 8	15	C		PR-10 protein
Pear (*Pyrus communis*)	Pyr c 1	18	C		Hom: Bet v 1
	Pyr c 4	14	C		Profilin
	Pyr c 5	33.5	C		Hom: isoflavone reductase
Pepper (*Capsicum annuum*)	Cap a 1w	23	C		Osmotin-like protein
	Cap a 2	14	C		Profilin
Pineapple (*Ananas comosus*)	Ana c 1	15	C		Bromelin, hom: papain and group 1 of mites
Pistachio nut					Four antigenic fractions of 34, 41, 52 and 60 kD; the first one seems to have the highest binding capacity to IgE [384]
Plum (*Prunus domestica*)	Pru d 3	9	P		LTP
Potato (*Solanum tuberosum*)	Sola t 1	43	P		Patatin
	Sola t 2	21	P		Cathepsin D inhibitor
	Sola t 3	21	P		Cysteine protease inhibitor
	Sola t 4	16+4	P		Aspartic protease inhibitor
Rana esculenta	Ran e 1	119	C		α-Parvalbumin
Rice (*Oryza sativa*)	Ory s 1		C		Allergens RAP and RAG 1, 2, 5, 14, 17 [348]
Rye (*Secale cereale*)	Sec c 20				Secalin
Saffron crocus (*Crocus sativus*)	Cro s 1		P		
Sesame (*Sesamum indicum*)	Ses i 1	10	P		2S protein
	Ses i 2	7	C		Albumin
	Ses i 3	45	C		Vicilin-like globulin
	Ses i 4	17	C		Olesin
	Ses i 5	15	C		Olesin

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Shrimp					
Metapenaeus ensis	Met e 1	34	C		Tropomyosin [295]
Penaeus aztecus	Pen a 1	36	P		Tropomyosin, major allergen [543]
Penaeus indicus	Pen i 1	34	C		Tropomyosin, or seralbumin, with diffused cross-reactivity [543]
Penaeus monodon	Pen m 2	40	C		Tropomyosin
Snail (*Helix aspersa*)	Hel a 1	36	P		Tropomyosin
Soybean (*Glycine max*)	Gly m 1	7	P	>90	Glycoprotein, MW of the monomeric form; Gly m is divided into 7 subunits: the best known are Gly m 1A and Gly m 1B [348], then the Kunitz tryptic inhibitor (3 subtypes)
	Gly m 2	8	P	>90	
	Gly m 3	14	C	>90	Profilin, β-conglycin 3 major subunits, α-1, α-2 and β with MW at 76, 72 and 53 kD, respectively, and lectin
	Gli m 4	17	C		SAM22, Pr-10 protein [265]
	Gly m Bd	30			Isolated from the crude 7S-globulin fraction, β-conglycinin, a trimer with MW at 150–200 kD
	Gly m Bd	60			Glycinin, a hexamer with MW at 300–400 kD, A5–B3 subunit
		30			Component from soybean constituted by two polypeptides (A5 and B3) that cross-react with CM caseins [478]
		25			Protein of GMO soybean reacting with IgE of some patients [674]
		50			Allergen pertaining to soy aeroallergen (asthma outbreaks during unloading of soybean from ships with significant hom with chlorophyll A-B binding protein precursors from tomato, spinach, and petunia [89])
Squid (*Todarodes pacificus*)	Tod p 1	38	P		Tropomyosin
Tomato (*Lycopersicon esculatum*)	Lyc e 1	14	C		Profilin, Ole e 1 homolog
	Lyc e 2	50	C		Isoallergen
	Lyc e 2	50	C		β-Fructofuranosidase
Walnut (*Juglans regia*)	Jug r 1	19	C		2S albumin
	Jug r 2	56	C		Vicilin
	Jug r 3	9	P		LTP
Watermelon (*Citrullus lanatus*)		13			Profilin [468]
Wheat					
Triticum aestivum	Tri a 18	17			Wheat germ agglutinins A and D [348]
	Tri a 19	65	P		Gliadin
Triticum durum					Wheat germ agglutinin [388]
Zucchini (*Cucurbita pepo*)		13			Profilin [468]
Other fruits					Strawberry, banana, tangerine, cherry and kiwi (Chap. 9)

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
2. Fungi (molds)					
Alternaria alternata	Alt a 1	28	C	<80	Similar to Alt a-29, Alt a bd 29 and Alt a 31-kD I_{1563}, 70-kD glycoprotein (GP70)
	Alt a 2	25	C	42	Aldehyde dehydrogenase [348]
	Alt a 3	70	C		Heat shock protein
	Alt a 4	57	C		Isomerase
	Alt a 5	45	C		Enolase
	Alt a 6	11	C	8	P_2 acid ribosomal protein [348]
	Alt a 7	22	C	7	
	Alt a 8	29	C		Mannitol dehydrogenase
	Alt a 10	53	C	51	Aldehyde dehydrogenase [543]
	Alt a 11	45	C		Enolase
	Alt a 12	11	C		Acid ribosomal protein
Aspergillus flavus	Asp fl 13				Alkaline serine protease
Aspergillus fumigatus	Asp f 1	18	C		Mitogillin toxin/ribonuclease [348]
	Asp f 2	37	C		
	Asp f 3	19	C		Peroxisomal protein
	Asp f 4	30	C		
	Asp f 5	40	C		Metalloprotease
	Asp f 6	26.5	C		Mn superoxide dismutase
	Asp f 7	12	C		
	Asp f 8	11	C		Ribosomal protein
	Asp f 9	34	C		
	Asp f 10	34	C		Aspartic protease
	Asp f 11	24			Peptidyl-prolyl isomerase
	Asp f 12.	90	C		Heat shock protein
	Asp f 13	34			Alkaline serine protease
	Asp f 15	16	C		
	Asp f 16	43	C		
	Asp f 17		C		
	Asp f 18	34			Vacuolar serine protease
	Asp f 22w	46	C		Enolase
	Asp f 23	44	C		1.3 ribosomal protein
Aspergillus niger	Asp n 14	105	C		β-Xylosidase
	Asp n 18	34	C		Vacuolar serine protease
	Asp N 25	66–100	C		3-Phytase
	Asp n ?	85	C		
Aspergillus oryzae	Asp o 2		C		
	Asp o 13	34	C		Alkaline serine protease
	Asp o 21	53	C		TAKA-amylase A

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Candida albicans	Cand a 1	40	C		Three more allergens at 37, 43, 48 kD [543]
	Cand a 3	20	C		Perixosomal protein
Candida bodinii	Cand b 2	20	C		
Cladosporium herbarum	Cla h 1	13		>60	
	Cla h 2	23	C	43	Enolase [348]
	Cla h 3	53	C	36	Aldehyde dehydrogenase [348]
	Cla h 4	11	C	22	Acid ribosomal protein P2 [348]
	Cla h 5	22	C	22	
	Cla h 6	46	C	20	Enolase [543]
	Cla h 8	28	C		Mannitol dehydrogenase
	Cla h 9	55	C		Vacuolar serine protease
	Cla h 12	11	C		Acid ribosomal protein P1
Coprinus comatus	Cop c 1	11	C		Leucine zipper protein
	Cop c 2				
	Cop c 3				
	Cop c 5				
	Cop c 7				
Fusarium culmorum	Fus c 1	11	C		Ribosomal protein
	Fus c 2	13	C		Thioroedoxin-like protein
Malassezia furfur	Mala f 1				
	Mala f 2	21	C		Peroxisomal membrane protein
	Mala f 3	20	C		Peroxisomal membrane protein
	Mala f 4	35	C		
	Mala f 5	18	C		
	Mala f 6	17	C		
	Mala f 7		C		
	Mala f 8	19	C		
	Mala s 9	37	C		
Malassezia sympodialis	Mala s 1	18	C		
	Mala s 5	17	C		
	Mala s 6	17	C		
	Mala s 7		C		
	Mala s 8	19	C		
	Mala s 9	37	C		
	Mala s 10	86	C		Heat shock protein
	Mala s 11	23	C		Mn superoxide simutase
Penicillum brevicompactum	Pen b 13	33			Alkaline serine protease
Penicillum chrysogenum	Pen ch 1	33		100	Two more allergens at 64 and 62 kD [543]
	Pen ch 13	34			Alkaline serine protease
	Pen ch 18	32			Vacuolar serine protease
	Pen ch 20	68			*N*-acetyl glucosamine

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Penicillum citrinum	Pen c 3	18			Peroxisomal membrane protein
	Pen c 13	33			Alkaline serine protease
	Pen c 19	70	C		Heat shock protein
	Pen c 22w	46	C		Enolase
	Pen c 24		C		Elongation factor 1β
Penicillum oxalicum	Pen o 18	34			Vacuolar serine protease
Psilocybe cubensis	Psi c 1				
	Psi c 2				Cyclophilin
Saccaromyces cerevisiae					Two allergens at 40 and 48 kD [543]
Trichophyton rubrum	Tri r 2		C		
	Tri r 4		C		Serine protease
Trichophyton tonsurans	Tri t 1	30	P		
	Tri t 4	83	C		Serine protease
3. Grass pollens					
Gramineae					Nearly all allergens show hom: groups 1–3 [543]
Agrostis alba (redtop)	Agr a 1	?	P		
Anthoxanthum odoratum (sweet vernal)	Ant o 1	34	P		
Cryptomerica japonica	Cry j 1	38	C		Cry j 1 is divided into 1A and 1B [348]
	Cry j 2	37	C		Of the same allergenicity [199]
Cynodon dactylon (Bermuda grass)	Cyn d 1	32	C	100	Has several isoforms
	Cyn d 7		C		
	Cyn d 12	14			Profilin
	Cyn d 14	9	C		
	Cyn d 15	9	C		
	Cyn d 22w				Enolase
	Cyn d 23	9	C		
	Cyn d 24	21	P		PR-protein
Dactylis glomerata (orchard grass)	Dac g 1	32	P	>95	
	Dac g 2	11	C	75	
	Dac g 3	C			
	Dac g 5	31	P	>90	
Festuca elator	Fes e 1	34	P		Moreover, Fes e 1A and Fes e 2B [348]
Festuca pratensis (meadow fescue)	Fes p 4w	60			
Helianthus annuu (sunflower)	Hel a 1	34			
	Hel a 2	15.7	C		Profilin
	Hel a 5				Expansin
Holcus lanatus (velvet grass)	Hol l 1	11	C		

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Lolium perenne (rye grass)	Lol p 1	27	C	>90	Group 1
	Lol p 2	11	C	60	Group II
	Lol p 3	11	C	70	Group III
	Lol p 4	57	C	74	
	Lol p 5	11	C	80	Lol p IX, Lol p Ib, ribonuclease (unknown)
	Lol p 9				90% of patients allergic to darnel recognize it together with Lol p 1 [43]
	Lol p 10	12			Cytochrome C, hom: group 10
	Lol p 11	18	65		Hom: trypsin inhibitor, 44% of homology with Ole 1 [189], further Lol p IV, 11 kD and 3 allergens at 30, 34 and 50 kD [347]
Phalaris aquatica (canary grass)	Pha a 1	34	P	77	Hom: group 1
	Pha a 5				Has 4 isoforms [476]
Phleum pratense (Timothy)	Phl p 1	27	C	80	Cross-reacts with group 1 allergens [497]
	Phl p 2	10, 12	C	62	
	Phl p 3	10, 12	C		
	Phl p 4	50, 60	P		Significant hom: Amb a 1/2
	Phl p 5	32	C	80	Ribonuclease (unknown) is divided into 5a and 5b [348]
	Phl p 6	11	C		Additional allergens Phl p of 32 kD and Phl p of 38 K [348]
	Phl p 7		C		
	Phl p 11		C		Trypsin inhibitor hom
	Phl p 12		C		Profilin
	Phl p 13	55–60	C		Polygalacturonase
Poa pratensis (blue grass)	Poa p 1	33	P		All with unknown sequence and group 10 homology
	Poa p 5	31/34	P	>95	
	Poa p 9	29, 35	C		In 3 forms: KBG31, KBG41, KBF60 [348]
	Poa p 10	29			Cytochrome C
Sorghum halepense (Johnson grass)	Sor h 1		C		
Zea mays (maize)	Zea m 1	21	P		
	Zea m 11	14	C		
Euphorbiaceae					
Hevea brasiliensis	Hev b 1	58	P	23–>80	Major allergen, rubber elongation factor
	Hev b 2	34/36	C	21	Major allergen, β-1,3-glucanase, microhelix component
	Hev b 3	24	P	36	Prenyltransferase
	Hev b 4	50–57			Component of microhelix complex
	Hev b 5	16	C	56–92	Major allergen (Table 1.73)
	Hev b 6.01	20	C		Hevein precursor
	Hev b 6.02	5	C		Hevein

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Hevea brasiliensis (continued)	Hev b 6.03	14	C		C-terminal fragment
	Hev b 7.01	42	C	83	Hom: patatin from B-serum, cross-reacting with avocado, potato and tomato
	Hev b 7.02	44	C	23	Hom: patatin from C-serum defense-related protein
	Hev b 7.03				Inhibitor of rubber biosynthesis
	Hev b 8	14	C	>90	Latex profilin structural protein
	Hev b 9	51	C		Latex enolase
	Hev b 10	26	C		Mn superoxide dismutase
	Hev b 11	33	C		Class I endochitinase defense-related protein
	Hev b 12	9.3	C		LTP
	Hev b 13	42	P		Esterase
Ricinus communis (Castor bean)	Ric c 1	11	C	96	Small chain, 4 kD, large chain, 7 kD, 2S storage albumin
	Ric c 2	47			Crystalloid protein [543]
4. Weeds compositae					
Ambrosia artemisifolia (short ragweed)	Amb a 1	38	C	>90	Pectate lyase; hom: Amb a 2, Cry j 1, tomato and maize
	Amb a 2	38	C	>90	Pectate lyase; hom: Amb a 1, Cry j 1, tomato and maize
	Amb a 3	11	C	51	Shows homology with electron transport proteins
	Amb a 4	23			
	Amb a 5	5	C	17	Ra 5
	Amb a 6	10	C	21	Ra 6, lipid transferase (?)
	Amb a 7	12	P	20	Ra 7, shows hom with electron transport proteins
	Amb a 10	12			Cytochrome C
Ambrosia psilostachya	Amb p 5				Hom: Amb a 5
Ambrosia trifida (giant ragweed)	Amb t 5	4.4	C		Hom: Amb a 5
Artemisia vulgaris (mugwort)	Art v 1	27–29	C	>70	
	Art v 2	35	P	33	
	Art v 3	12	P		LTP
	Art v 4	14	P		Profilin
Mercurialis annua	Mer a 1	14–15	C		Profilin
	Mer a 2				
Mercurialis perennis, etc.					
Parietaria judaica	Par j 1	12	C	100	[511] or 2 proteins at 8.8 and 9.8 kD, respectively, with allergens homologous to those of Par o 1 and di *P. mauritanica* and with great cross-reactivity [17]
	Par j 2		C		LTP
	Par j 3		C		Profilin

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Parietaria officinalis	Par o 1	15	P	100	[397] or 3 proteins at 8.8 and 2 9.4 kD, respectively, R 100, with several isoallergens with similar MW [98]
	Par o 2	11		82	Phospholipid transfer protein
Parthenium hysterophorus (feverfew)	Par h 1	31			Extensin
5. Tree pollens					
– Betulaceae					
Alnus glutinosa (alder)	Aln g 1	17	C	>90	Hom: Bet v 1 [348]
Betula verrucosa (birch)	Bet v 1	17	C	>95	PR, isoform Bet v 1 N
	Bet v 2	15	C	10	Profilin
	Bet v 3	20	C	<10	Profilin [348]
	Bet v 4	8	C		
	Bet v 6	33.5	C		Hom: isoflavone resuctase
	Bet v 7	18	P		Cyclophilin
Carica papaya (Papaya)	Car p 1	23	C		Papain
Carpinus betulus (Hornbeam)	Car b 1	17	C	>90	Bet v 1 hom [348]
Castanea sativa (Chestnut)	Cas s 1	22	P		Hom: group 1 of Fagales [543] and with Bet v 1 [348]
	Cas s 5				Chitinase
	Cas s 8	13	P		LTP
Cupressus arizonica	Cup a 1	43	C	81	[355]
Cupressus sempervirens	Cup s 1	43	C	81	
	Cup 3 3w	34	C		
Fraxinus excelsior (ash)	Fra e 1	20	P		
Juniperus ashei	Jun a 1	43	P		Pectate lyase
	Jun a 2		C		
	Jun a 3	30	P		Hom: thaumatin, osmotin, amylase/trypsin inhibitor
Juniperus oxycedrus (prickly juniper)	Jun o 4	29	C		Hom: calmomodulin
Juniperus rigida	50	100			[543]
Juniperus sabinoides (mountain cedar)	Jun s 1	50	C		
Juniperus virginiana	Jun v 1	43	C		
Ligustrum vulgare (privet)	Lig v 1	20	P		
Quercus alba (oak)	Que a 1	17	P		Hom: group 1 of Fagales [543] and with Bet v 1 [348]
– Oleaceae					
Olea europaea	Ole e 1	16	C	>90	Allergens present also in Fra e 1, Lig v 1, Syr v 1 [369] hom: soybean trypsin inhibitor and Lol p 11
	Ole e 2	15–18	C	25	Profilin
	Ole e 3	9.2			Ca^{++}-binding protein
	Ole e 4	32	P	80	Hom: Ole e 1
	Ole e 5	16	P	35	Superoxide dismutase

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Olea europaea (continued)	Ole e 6	10	C		Cysteine-rich protein
	Ole e 7		P	47	
	Ole e 8	21	C		$Ca2^{+}$-binding protein
	Ole e 9	46	C		β-1,3-glucanase
	Ole e 10	11	C		Hom: Glycosyl hydrolase
Phoenix dactylifera(date)	Pho d 2	14.3	C		Profilin
Syringa vulgaris	Syr v 1	20	P	16	
– Plantaginaceae					
Plantago lanceolata	Pla l 1	18	P		English plantain
Platanaceae					
Platanus acerifolia	Pla a 1	18	P		Major allergen [15]
	Pla a 2	43	P		Major allergen [15]
	Pla a 3	10	P		LTP
– Taxoidiaceae or Pinales					
Cryptomeria japonica	Cry j 1	41–45	C	>85	Pectate lyase, hom Amb a 1
	Cry j 2	57		76	Polymethylgalacturonase
6. Animals					
Cat (*Felis domesticus*)	Fel d 1	38	C	>80	Allergens in sebaceous glands and saliva; cross-reaction with pig meat [127]
	Fel d 2		C	23	Albumin
	Fel d 3	11	C		Cystatin
	Fel d 4	22	C		Lipocalin
	Fel d 5w	400			IgA
	Fel d 6w	800–1000			IgM
	Fel d 7w	150			IgG
Cavia porcellus	Cav p 1	20	P		Lipocalin hom
	Cav p 2	17	P		Allergens present in hairs, urine, saliva
Oryctolagus cuniculus	Ory c 1	17			Present in saliva
Dog (*Canis domesticus*)	Can f 1	25	C	>70	Allergens present in skin, saliva, parotid gland
	Can f 2	27	C	23	Parotid gland
	Can f 3	69	C	40	Albumin
	Can f 4	18	C		
Horse (*Equus caballus*	Equ c 1	25	C	100	Lipocalin, the allergen is in the horsehair
	Equ c 2	18.5	P		Lipocalin
	Equ c 3	67	C		Albumin
	Equ c 4	17	P		
	Equ c 5	17	C		Two more 14- and 39-kD proteins [149]

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
– Rodents					
Mouse (*Mus musculus*)	Mus m 1	19	C		Prealbumin; allergens present in urine, liver
	Mus m II (Ag3)	16			Reciprocal homology [543]
Rat (*Rattus norvegius*)	Rat n 1	21	C	60	Allergens in urine, saliva
	Rat n 2	16	C	90	
	Rat n III	>200			
7. Worms					
Anisakis simplex	Ani s 1	24	P		
	Ani s 2	97	C		Paramyosin
	Ani s 3	41	C		Tropomyosin
	Ani s 4	9	C		
Ascaris lumbricoides	Asc l 1				[348]
Ascaris suum (worm)	Asc s 1	10	P		
Thaumetopoea pityocampa	Tha p 1	15			Amino acid sequence with no homologies to any other protein described [365]
8. Insects					
American cockroach (*Periplaneta americana*)	Per a 1	20–25	C	50	Cr-PII, Per a 1 reacts with Bla g 1 [434]
	Per a 3	72–78	C	83	In addition, a protein of the allergenic fraction, Cr-PI, perhaps major allergens [616]
	Per a 7	37	C		Tropomyosin
Australian jumper ant (*Myrimecia pilosuls*)	Myr p 1		C		
	Myr p 2		C		
Black fire ant (*Solenopsis richteri*)	Sol r 1		P		
	Sol r 2		C		PL
	Sol r 3		C		
Bumble bee (*Bombus pennsylvanicus*)	Bom p 1	16	P		PL
	Bom p 4		P		Protease
Cat flea (*Ctenocephalides felis*)	Cte f 1				
	Cte f 2	27	C		
	Cte f 3	25	C		
Midge (*Chironomus thummi*)	Chi t 1–9	16	C		Hemoglobin
	Chi t 1.01	16	C		Component III
	Chi t 1.02	16	C		Component IV
	Chi t 2.0101	16	C		Component I
	Chi t 2.0102	16	C		Component IA
	Chi t 3	16	C		Component II-β
	Chi t 4	16	C		Component IIIA
	Chi t 5	16	C		Component VI
	Chi t 6.01	16	C		Component VIIA
	Chi t 6.02	16	C		Component IX

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Midge (*Chironomus thummi*) (continued)	Chi t 7	16	C		Component VIIB
	Chi t 8	16	C		Component VIII
	Chi t 9	16	C		Component X
European hornet (*Vespa crabo*)	Vesp c 1	34	P		PL
	Vesp c 5	23	C		Antigen 5
German cockroach (*Blattella germanica*)	Bla g 1	20–25	C	50	30%–50% prevalence of IgE antibody
	Bla g 2	36	C	58	Aspartic protease
	Bla g 4	21	C	40–60	Lipocalin
	Bla g 5	22	C		Glutathione transferase
	Bla g 6	27	C		Troponin
	Bla g Bd	90			77% of patients with IgE antibodies [204]
Honey bee (*Apis mellifera*)	Api m 1	16	C		PLA_2, in addition Api III, V and VI at 49, 23 and 105 kD, respectively
	Api m 2	41	C		Hyaluronidase
	Api m 4	3	C		Melittin
	Api m 6	7–8	P		
	Api m 7	39	C		Serine protease
Mosquito (*Aedes aegiptii*)	Aed a 1	68	C		Apyrase
	Aed a 2	37	C		
Paper wasp (*Polistes dominulus*)	Pol d 1				
	Pol d 4	32–34	C		Serine protease
	Pol d 5				
Red fire ant (*Solenopsis invicta*)	Sol i 1	37	P		PL
	Sol i 2	13	C		
	Sol i 3	24	C		Hom: vespid group 5 allergens
	Sol i 4	13	C		Hom: Sol i 2
Tropical fire ant (*Solenopsis geminata*)	Sol g 2				
	Sol g 4				
Solenopsis saevissima	Sol s 2				
Giant Asian (*Vespa mandarina*) hornet	Vesp m 1				
	Vesp m 5				
Vespula flavopilosa	Ves f 5	23	C		Antigen 5
– Wasp					
Polistes annularies	Pol a 1	35	P		PLA_1
	Pol a 2	44	P		Hyaluronidase
	Pol a 5	23	C		Antigen 5
Polistes exclamans	Pol e 1	34	P		
	Pol e 5	23	C		Antigen 5
Polistes fuscatus	Pol f 5	23	C		Antigen 5
Polistes metricus	Pol m 5	23	P		Antigen 5
Wasp (*Vespula vidua*)	Ves vi 5	23	C		Antigen 5

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
White face hornet (*Dolichovespula maculata*)	Dol m 1	35	C		PLA_1
	Dol m 2	44	C		Hyaluronidase
	Dol m 5	23	C		Antigen 5
Yellow hornet (*Dolichovespula arenaria*)	Dol a 5	23	C		Antigen 5
Yellow jacket (*Vespula flavopilosa*)	Ves v 1	35	C		PLA_1: has 67% of sequential identity with Dol m
Vespula germanica	Ves g 5	23	C		German yellow jacket, antigen 5
Vespula maculifrons	Ves m 1	35	C		Eastern yellow jacket, PLA_1
	Ves m 2	44	P		Hyaluronidase
	Ves m 5	23	C		Antigen 5
Vespula pennsylvanica	Ves p 5	23	C		Western yellow jacket, antigen 5
Vespula squamosa	Ves s 5	23	C		Southern yellow jacket, antigen 5
Vespula vulgaris	Ves v 1	35	C		PL
	Ves v 2	44	P		Hyaluronidase: has 92% of sequential identity with Dol m
	Ves v 5	23	C		Antigen 5: has 69% of sequential identity with Dol m and 60% with Pol a; it is possible to set an order of cross-reactivity hyaluronidase >antigen 5> PLA_1 [577]
9. Mites					
Acarus siro	Aca s 13	14	C		Fatty acid binding protein
Blomia tropicalis	Blo t 1	11–13		>47	Cysteine protease
	Blo t 3	24	C		
	Blo t 4	56	C		
	Blo t 5	14	C	70	Shows hom: other allergens
	Blo t 6	25	C		Chymotrypsin
	Blo t 10	33	C		Tropomyosin
	Blo t 11	110	C		Paramyosin
	Blo t 12	16	C		
	Blo t 13		C		Fatty acid binding protein
	Blo t 19	7.2	C		Anti-microbial pepsin homology
Dermatophagoides farinae	Der f 1	25	C	79	Cysteine protease, hom: Der p 1, Eur m 1, papain, cathepsins B and H
	Der f 2	14	C	83	Variants 2.1, 2.2 and 2.3
	Der f 3	34	P	42–70	Trypsin, hom: Der p 3, Der p 6, Der f 6, other trypsins and proteases
	Der f 6	30		31	Chymotrypsin, hom: Der p 3, Der p 6, Der f 3, other chymotrypsins and proteases
	Der f 7	22		46	86% homology and cross-reactivity with Der p 7 [469]
	Der f 9				[112]

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
Dermatophagoides farinae (continued)	Der f 10	39	C	81	Tropomyosin, hom: Mag44, highly reactive with IgE like Der f 1, 2 [8]
	Der f 11	98	C		Paramyosin
	Der f 14		C		Mag3, apolipophorin
	Der f 15	98	C		Chitinase
	Der f 16	53	C		Gelsolin/villin
	Der f 17	53	C		Ca binding protein
	Der f 18w	60	C		Chitinase
Dermatophagoides microceras	Der m 1	25	P		Cysteine protease
Dermatophagoides pteronyssinus	Der p 1	25	C	>90	Cysteine protease, hom: Der f 1, Eur m 1, papain, cathepsins B and H
	Der p 2	14	C	>90	Lysozyme?
	Der p 3	28/30	C	51 to >90	Trypsin, hom: Der p 6, Der f 3, Der f 6, other trypsins and proteases
	Der p 4	60	P	25–46	Amylase
	Der p 5	14	C	>55	
	Der p 6	25	P	39	Chymotrypsin, hom: Der p 3, Der f 3, Der f 6 and other chymotrypsins and proteases
	Der p 7	22–28	C	53	IgE and monoclonal antibody bind to Der p 7 [518]
	Der p 8	26	C		Glutathione transferase
	Der p 9	28		>90	Serine protease, hom: groups 3 and 6 of mites
	Der p 10	36	C		Tropomyosin
	Der p 14		C		Apolipophorin-like protein
Dermatophagoides siboney	Der s 1	25			
	Der s 2	14			Correlated with Der f
Euroglyphus maynei	Eur m 1	24	C		Cysteine protease, hom: Der p 1, Der f 1, papain, cathepsins B and H [543]
	Eur m 2		C		
	Eur m 14	177	C		Apolipophorin
Glycyphagus domesticus	Gly d 2		C		
Lepidoglyphus destructor	Lep d 1	14–16	P		Hom: group 2 of mites
	Lep d 2				
	Lep d 5		C		
	Lep d 7		C		
	Lep d 10		C		Tropomyosin
	Lep d 13		C		
Tyrophagus putrescentiae	Tyr p 2		C		

Table 1.74. (Continued)

Name (species)	Allergens	kD	C/P	R	Notes and reference (if related)
10. *Homo sapiens* human autoallergens [639]					
	Hom s 1	73	C		
	Hom s 2	10.3	C		
	Hom s 3	20.1	C		
	Hom s 4	36	C		
	Hom s 5	42.6	C		

Allergens are usually ordered according to their common name: those corresponding to foods are listed among foods.
B column shows the percentage of reactivity [543]. All the known homologies are included [543]. The Der (p, f, m) 1 are considered major allergens, similarly to Der (p, f, m) 2; the latter ones, contrary to the first group, are thermostable and pH resistant; 80%–90% of Der p I is contained in the stools and 10% in the body. All insect allergens correspond to the primary antigen 5 and have identical MW; Can d is employed parallel to Can f, *Canis fidelis* [639].
Updated from [639], other data from [8, 15, 17, 60, 89, 98, 112, 121, 128, 134, 149, 199, 204, 265, 295, 310, 319, 348, 355, 365, 397, 384, 416, 405, 434, 471–473, 478, 497–499, 511, 543, 549, 551, 577, 604, 639, 652, 674].
C/P complete or partial availability of data, *hom* homology, *LTP* lipid transfer protein, *PL*, *PLA*$_1$, *PLA*$_2$ phospholipase, phospholipase A$_1$, phospholipase A$_2$, *PR* pathogenesis related protein, *RAP* rice allergenic protein, *RAG* rice allergen, *R* risk.

Table 1.75. Cow milk allergens

Allergens	MW (kD)	g/l	%	Stability at 100°C	Allergenicity
Caseins (Bos d 8)		24–28	80	+++	++
αs1	23–27	15–19	42		
αs2	23				
β	24	9–11	25		
κ	19	3–4	9		
γ_{1-3}	12–21	1–2	4		
Whey proteins		5–7	20		
β-Lactoglobulin (Bos d 5)	36	2–4	9	++	+++
α-Lactalbumin (Bos d 4)	14.4	1–1.5	4	+	++
Serum albumin (Bos d 6)	69	0.1–1.4	1	±	+
Immunoglobulins (Bos d 7)	0.6–1	2	–	+	
IgG	150–170	0.5–0.8	1.7		
IgM	900–1,000	0.05–0.1	0.2		
IgA	300–500	0.02–0.05	0.1		

Casein is the major antigen and allergen [121].
β-Lactoglobulin has four genetic variants.
Data from [24, 121].

Table 1.76. T-cell epitopes of allergens

Allergen source	Allergen	Size[b]	T-cell epitopes	Individuals tested	T cells
Perennial allergens					
Acarids					
Dermatophogoides pteronyssinus	Der p 1	24 kD, 222 aa	45–67, 94–104, 117–143	2	TCC
	Der p 1		110–119, 110–131	1	TCL and TCC
	Der p 1		1–14, 1–56, 15-94, 57–130, 95–208		
			188–222, 209–222	18	PBMC
	Der p 2	15 kD, 129aa	1–15, 11–24, 20–33, 29–42, 38–51		
			47–60, 56–69, 92–105, 101–114, 116–129	5	TCL and TCC
	Der p 2		1–20, 11–35, 22–50, 36–60, 51–77, 61–86		
			78–104, 81–96, 91–105, 87–112, 105–129	18	PBMC and TCC
	Der p 2		11–25, 16–31, 21–35, 22–40, 71–86		
			81–96, 82–100, 111–129	1	TCL and TCC
	Der p 2		20-33	2	TCC
	Der p 2		1–15, 11–25, 21–35, 31–47, 41–55, 51–65		
			61–75, 71–86, 81–96, 91–105, 101–115		
			111–129	24[a]	PBMC
Mammals					
Felis domesticus	Fel d I	17 kD	39–52, 53–66 (chain 1)		
		70–92 aa	9–21, 22–35, 57–70 (chain 2)	4	TCL and TCC
	Fel d I	(dimer)	1–17, 9–25, 18–32, 29–42, 37–55, 44–60		
			56–70 (chain I)	53[a]	TCL
			1–22, 12–33, 23–48, 34–59, 49–68, 60–82		
			74-92 (chain 2)		
Seasonal allergens					
Trees					
Betula verrucosa	Bet v I	17 kD, 159aa	2–16, 11–22, 61–72, 77–88, 85–96		
			113–124, 145–156, 147–158	6	TCC
	Bet v I		1–16, 27–40, 35–48, 75–92, 77–92		
			93–110, 141–156	2	TCC
	Bet v I		1–16, 11–26, 61–76, 63–78, 65–80, 75–90		
			77–92, 95–110, 97–112, 111–126		
			113–128, 127–140, 141–156	9	TCC
	Bet v I		1–15, 8–23, 19–33, 29–43, 46–63, 58–73		
			65–79, 73–87, 82–96, 90–104, 117–131		
			99–113, 126–140	3	TCL and TCC
Cryptomeria Japonica	Cry j I	41–45 kD, 353 aa	327–346, 337–353	1	TCC

Table 1.76. (Continued)

Allergen source	Allergen	Size[b]	T-cell epitopes	Individuals tested	T cells
Grasses					
Lolium perenne	Lol p I	34 kD, 240 aa	191–210	1[a]	TCC
	Lol p I		Several[c]	6[a]	PBMC
	Lol p I		1–20, 11–30, 21–40, 31–50, 41–60, 50–70		
			71–90, 91–110, 101–120, 111–130		
			121–140, 131–150, 141–160, 151–170		
			171–190, 181–200, 191–210, 221–240	8[a]	TCL and TCC
	Lol p I		Several[c]	6[a]	PBMC
Phleum protense	Phi p I	34 kD, 240 aa	22–36, 25–39, 34–45, 70–84, 73–84		
			91–102, 97–111, 91–102, 100–114		
			109–123, 121–134, 127–138, 130–141		
			142–155, 157–168, 169–183, 211–225,		
			226–240	9	TCC
Poa protense	rKBG60	28 kD, 268 aa	peptide 5. 99–118, 109–128, 149–168		
			159–178, 169–188, 199–218, 219–238		
			229–248, 239–258, 249-268	13[a]	PBMC
Venom allergens					
Insects					
Apis mellifero (honey bee)	Api m I (PLA_2)	19 kD, 134 aa	50–69, 83–97	1	TCL
	Api m I (PLA_2)		45–62, 74–91, 76–93, 81–92, 81–98,		
			107–124, 111–128, 113–124, 114–131	40[a]	PBMC and TCC
Food allergens					
Birds					
Chicken	Ovalbumin	43 kD, 385 aa	1–33, 198–231, 201–213, 261–277	4	TCC

[a] Even though the presence of several T-cell epitopes was described, T-cell epitopes that were recognized by more than 50% of all individuals tested have been identified in these studies.
[b] Sizes are shown as SDS-PAGE mobility of the native protein (in kD) and as number of amino acids (based on the recombinant sequence).
[c] These papers describe reactivity with several peptide pools. Exact amino acid sequences are not clear.
aa, Amino acids, *PBMC* peripheral blood mononuclear cells, *PLA_2* phospholipase A_2, *TCC*, T-cell clone, *TCL* T-cell line.

Table 1.77. Allergen association with HLA molecules and IgE responses

Allergens	HLA-DR	IgE^+ (%)	IgE^- (%)	RR
Amb a 5	DR2/Dw2	100	24	65
Amb a 6	DR5	85	14	35
Amb a 6	DR5	40	6	23
Lol p 2	DR3	47	15	5.3
Lol p 3	DR3	43	18	3.5
Lol p 3	DR3	57	7	18
Alt a 1	DR4	26	16	1.9
Der p 1	DR3	16	17	>1
Der p 2	DR3	19	16	>1
Bet v 1	DRw52a/c	62	33	2.5
Bet v 1	DRB3*0101	51	30	2.5
Fel d 1	DR1	16	9	2.0
Lol p 1	DR3	36	7	7.3
Lol p 1	DR3	33	14	3.1

Some allergens are shown twice since they are reported in different studies.
Data from [601].
RR relative risk.

Table 1.78. Transgenic or genetically modified foods

Introduced proteins	Crop products and targets
ACC deaminase, antisense PG, antisense ACC synthase	Delays without impairing the tomato's natural ripening and softening to obtain a more concentrated juice
Phosphinothricin acetyltransferase	Renders corn tolerant to herbicides
Neomycin phosphotransferase II	Protects potato from insects and delays tomato's natural ripening and softening
Glyphosate oxidoreductase	Renders corn tolerant to herbicides
Btt-HD1 insecticidal protein	Protects corn and tomato from insects
Btt-HD 73 insecticidal protein	Protects potato from insects
CP4 EPSPS synthase	Renders corn, soy and sugar beet tolerant to herbicides
β-D-glucuronidase	Renders soy tolerant to herbicides

There were controversies regarding GMO. Prohibitions and/or restrictions are expressed almost everyday in several countries.
ACC 1-amino- 1-cyclopropane-carboxylic acid, *Btt Bacillus thuringiensis* subspecies *tenebrionis*, *Btk* = *Bacillus thuringiensis* subspecies *kurstaki*, proteins from strains HD-73 and HD-1, *CP4 EPSPS* 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* strain CP4, *PG* polygalacturonase.
Modified from [347].

Table 1.79. Additional GM foods

Apple	Carrot	Kiwi	Rapeseed oil	Rye
Apricot	Cauliflower	Lemon	Orange	Soybean
Asparagus	Celery	Lettuce	Papaya	Spinach
Barley	Chicory	Licorice	Pea	Strawberry
Bilberry	Colza	Lotus	Peach	Sugar beet
Black currant	Eggplant	Maize	Plum	Sweet potato
Broccoli	Fennel	Melon	Potato	Tomato
Buckwheat	Grape	Mustard	Raspberry	Walnut
Cabbage	Horseradish	Oats	Rice	Wheat

Modified from [110].

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Fetal and Neonatal Immunology and the Mucosal Immune System

Immunodeficiency and Immaturity

Immunodeficiency and immaturity are two distinct concepts, both characterizing the neonatal period: it is a transitory period for the immune system, opening exquisitely new problems [86]. Immunodeficiency depends on the immaturity at the molecular and functional level of immunocompetent cells, and to accomplish diverse and sophisticated roles such cells should complete their differentiation. The neonatal incapacity to respond to sudden exposures to antigens and microorganisms is not exclusively a consequence of immune immaturity, but also of a missed immune experience. Neonates should leave a situation of immunological dependence, dictated by the necessity of avoiding maternofetal reactions during pregnancy, to adapt to the new requirements of extrauterine life. During intrauterine life, the fetal immune system (FIS) is modulated in a suppressive direction so that the reactions directed against the maternal HLA antigens do not occur, in concert with mechanisms of maternal tolerance correlated with fetal genetic and antigenic heterogeneity, to avoid a graft-versus-host disease (GvHD). There is a wealth of evidence showing that both fetus and placenta are provided with a number of HLA antigens encoded by paternal genes, thus representing a paternal allograft to the mother. The lack of convincing evidence explaining why this foreign tissue is not rejected is a gap with deep origins, whereas the FIS, immunologically primed by the second trimester, could in turn prime GvHD [48, 221, 229]. A type of maternofetal balance is thus created that can be outlined as follows:

- The syncytiotrophoblast (the syncytial outer layer of the trophoblast) supplied with HLA class I- and II-negative antigens is interposed between maternal uterine cells and the conceptus, thus acting as a partial barrier to maternal immunogenic material [48].
- There is production of class II-dependent antibodies, but not of class I-restricted cytotoxic T lymphocytes (CTLs) [229].
- Specific antibodies prevent generation of paternal-antigen-directed cell-mediated immunity (CMI) at the syncytiotrophoblast level [229].
- Nonspecific serum factors limit the effects of CMI, either systemic or local [221].
- In the presence of adequate mitogens and antigens, fetal T lymphocytes are inclined to suppress adult lymphocyte proliferating responses and further differentiation, that is stimulated by Th2-like ILs of activated fetuses [169], thus avoiding that maternal T cells prime a GvHD in the conceptus [229].
- Th2 T lymphocytes in particular produce IL_4, IL_5, IL_{10} at the maternofetal interface during the entire pregnancy, thus inhibiting suppressor Th1 T cells and related Th1-like interleukins (ILs), insuring fetal survival until birth even if ILs depress immune responsiveness by impairing responses against maternal and fetal pathogens [221].
- Prevalence of Th1-like ILs, unless there is a shift to Th2 T cells, induces "immune" abortions or recurrent spontaneous abortions of unexplained etiology [85], as demonstrated by Th1-like IL production from an abortion-inclined placenta [221].
- Presently, deleterious Th1-like ILs can compromise pregnancy: IFN-ɣ activates NK (natural killer) cells able to damage trophoblast tissue and with a potential role in immune abortion, also inhibiting IL_4, IL_5, IL_6 involved in various phases of B-cell development and Th2 T-cell proliferation [219].
- IL_{10}, in synergy with IL_2, is believed to stimulate activity of LAK (lymphokine-activated killer) cells (Chap. 1), which discriminate between self and non-self, thereby contributing to fetal protection shutting off maternal immune responses biased toward humoral immunity [219].
- Trophoblast cells, although devoid of HLA-A or HLA-B molecules, when in contact with maternal cells directly express HLA-G molecules but are not able to protect from NK-cells killing specific targets not expressing HLA molecules [160].

Successful pregnancy is therefore associated with a bias toward Th2-skewed IL response [167] postulated to limit cellular damage in embryonic tissues [102]. Repeated contact with common environmental allergens during the postnatal period probably redirects the fetal Th2-skewed immunity toward a Th1-skewed immunity in nonatopic babies, whereas the Th2-dependent allergic sensitization is reinforced in atopic babies [166].

As a corollary, at the end of pregnancy there is a shift to humoral immunity and a gradual maturation of Th1-like T-cell ILs and IFN-ɣ, NK-cell and TNF production,

to mount a rational strategy of antimicrobial defense, whereas the active Th2 response results in protective immunity for neonates that have not developed an endogenous immunity [221, 229].

Fetal–Neonatal Immune System: Immunocompetence or Immune Depression?

The particular susceptibility of newborns having left the protective husk of the maternal uterus to food, inhalant and infectious antigens, who remain exposed to a relevant antigenic stimulation, objectively critical, has induced several investigators to provide insights into the functional and phenotypic characteristics of the immune system components during intrauterine life and the B- and T-cell ontogenesis [224]. The human fetus has a genetically programmed capacity to recognize and respond to a great number of antigens, while the neonatal immune system, not yet "educated" to the extrauterine world, is unprepared for the new functions allowing the fetus to elicit an effective specific immune response [225]. However, after the termination of passive immunity, babies' chances of survival will depend on their induction of adaptive defense mechanisms. As a consequence, T and B lymphocytes, immature at birth, should establish contact with numerous environmental antigens to organize an adequate immune defense [144].

Recent studies on experimental animals underline that *neonates are immunocompetent,* thus immune responses to antigens do not differ practically from those of adult subjects [59, 171, 183]. In this respect, the neonatal period should no longer be considered in ontogenesis as a passive immune window, essential to acquire the tolerance of subsequent years, but as an immunologically normal and active period [59]. The concept of a *window period* dates back to a 1945 report, after which the theory of tolerance was postulated, based on two cattle twins injected at birth with HSCs from a genetically nonidentical donor, later able to accept transplants from the same donor [156]. This report has long influenced the understanding of the neonatal period, and was followed by studies by Burnet and Medawar.

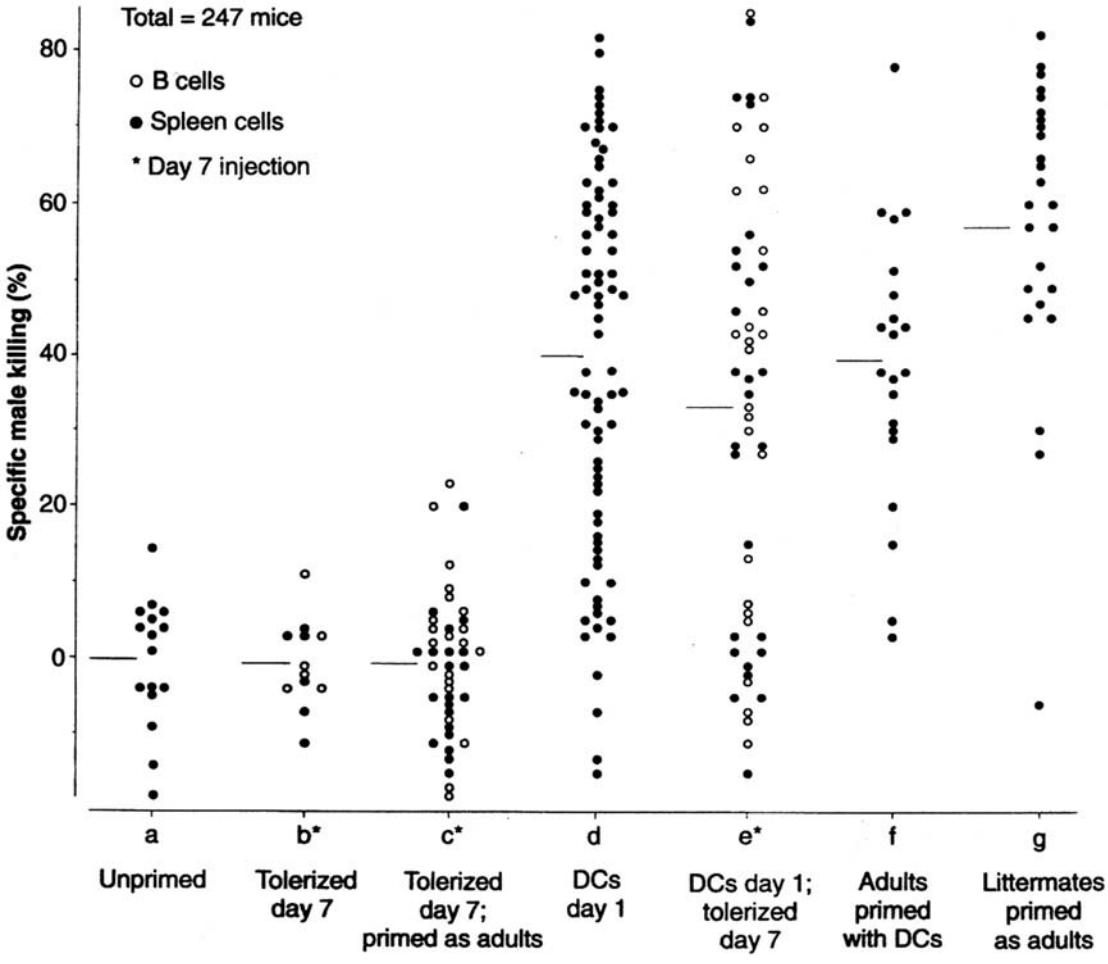

Fig. 2.1. Neonatal T cells first primed with dendritic cells (*DCs*) are resistant to tolerance induction

Table 2.1. Reduced or delayed activation of neonatal immune components

Deficit/delayed production	References
Complement components	[50, 53]
Neutrophil function	[225]
IFN-γ activation by macrophages	[137]
Macrophage phagocytosis and chemotaxis	[222]
T-cell IL secretion	[225]
B-cell immunoglobulin production	[202]
Gene expression of immunoglobulin V region	[225]
Isotype switching from IgM to IgG and IgA	[170]
Expression of CD154/CD40	[152]
sIgA generation	[25, 140]
Memory T-cell generation	[39, 83]
CD45RA/CD45RO	[126, 164]
NK cells	[40, 232]
Production of poor levels	
GM-CSF (50% of adult levels) [29]	IL_6 [168, 233, 234[b]]
IL_2 [71[a], 82, 226[a]]	IL_{10} [38, 168]
IL_3 (10%–25% of adult levels) [219]	IL_{13} [168, 223[a]]
IL_4 (0%–0.3% of adult levels) [71[a], 127, 158]	TNF-α (50% of adult levels) [54]
IFN-γ (0.8% of adult levels) [126]	CD21 [210[a]]
IFN-γ [71[a], 168, 172]	CD23 [210[a]]

IL interleukins.
[a] Not reduced according to [158] and [201].
[b] Not reduced according to [172].

Additional and interesting reports have cast greater light on these aspects of neonatal immunology, including research on development and behavior of HLA molecules, IgA antibodies and secretory component (SC) in premature infants and full-term neonates who were either stillborn or who died during the first 3 weeks of extrauterine life or in the postnatal period [174]. The enigma of nonmaternal antibodies in newborns of hypogammaglobulinemic mothers [73] has further documented the role of idiotype/anti-idiotype antibodies [74].

Since the immune system is still maturing in neonates during the first days of life, they may be suddenly confronted with a vast array of potentially dangerous microorganisms, which would take up residence and circulate to the intestinal mucosa [40]. The detrimental action mediated by certain bacteria may put into effect the adhesion of additional organisms that normally do not challenge the intestinal mucosa; however, the immune system response to virus primes a Th1 or Th2 response and is thus critical in the development of protective immunity [183]. Neonates are not immunologically naive, but can mount significant immune responses to environmental antigens, a possible result of prenatal sensitization. Neonatal immunocompetence has been demonstrated by newborn mice that were given dendritic cells (DCs) on day 1 and were fully able to activate neonatal immune T cells (Fig. 2.1, d) to the same extent as were the adult female controls (Fig. 2.1, e) [171]. In the spleen, the essential organ for defense against capsulated bacteria, the marginal zone and CD21 (C3d receptor) are absent in neonates, thus making its role difficult [162]. Instead, the marginal zone is active in infants and the CD21 levels are still low; however, CD35 (C3b receptor) primed to common antigens [134] is found in the B-cell zone [162]. However, the neonatal inadequacy to mount an effective antimicrobial response, *is amplified by IFN-γ deficiency* and upstream reduction *in memory T cells*, as well as by several functions, as detailed in Table 2.1 [25, 29, 38–40, 50, 53, 55, 71, 82, 83, 127, 130, 137, 140, 152, 158, 164, 168, 170, 172, 201, 210, 222, 223, 225, 232–234, 239]. Moreover, CMI induction is delayed, the CTL response is decreased (Table 2.2) [71, 101, 130, 219, 224, 225], and immunoglobulin (Ig) class switching is limited [201]. Fetal and neonatal IFN-γ and IL_4 levels are depressed (Table 2.3) [3, 71, 102, 127, 158], especially when family history of atopy (FHA) is positive [126], even for several years [3, 168]. The fetus (human amniotic fluid at 16–17 weeks of gestation) has 0.0 pg/ml of IFN-γ [102]. No differences in the very low or undetectable CB (cord blood) levels of IL_2-, IL_4- and IFN-γ-producing Th and T-suppressor/cytotoxic lymphocytes were found between neonates from atopic and nonatopic parents [71].

Table 2.2. Cellular immune response at birth

Function	Finding
Total lymphocyte count (10^9/l)	↑/↓ 4.8 (2.8–7.8)
Absolute and percentage number of T cells	=/↑
T:B cell ratio	3:1
Antigen recognition	=
Specific response to antigens	=
Cytotoxic capacity	=
Graft-versus-host reactivity	=
Delayed skin hypersensitivity	↓
G-CSF	N/↓
GM-CSF	N/↓
M-CSF	N
IFN-α	N/↓
IFN-β	N
IFN-γ	↓
TNF-α	↓
TNF-β	N
MIF and LIF	↓
PHA stimulation	=/↓
NK and K cells	↓
$CD1^+$	↓
$CD2^+$	↓
$CD3^+$ (10^9/l)	↓ 3.1 (1.4–4.9)
$CD4^+$ (10^6/l)	↑ 2489 (1004–3590)
$CD6^+$	–
$CD8^+$ (10^6/l)	↓ 565 (338–1308)
$CD9^+$	–
$CD10^+$	–
CD14	–
$CD25^+$	↓
$CD28^+$	N/↓
$CD38^+$	N
CD4 $CD45RA^+$ (10^6/l)	↑/↓ 1252 (432–2255)
CD4 $CD45RO^+$ (10^6/l)	↓ 150 (25–1073)
CD57+	↓
IL_1	N
IL_2	N
IL_3	↓
IL_4	↓
IL_5	↓
IL_6	↓
IL_6	N
MIP-1α	↓

Reference values are given in parentheses where available. The figures are median CB values and range related to high-risk neonates, but the data were similar for low-risk and high-risk neonates [71]. Additional data on neonatal cytokines are summarized in Tables 2.7–2.9. Appendix 1.3 shows the main lymphocyte subpopulations in neonates and infants, and Tables 1.34 to 1.39 show similar data also in cord blood.
Data from [101, 130, 219, 224, 225].
LIF leukocyte inhibiting factor, *MIF* migration inhibiting factor, *PHA* phytohemagglutinin.

Table 2.3. Fetal and neonatal mean values of IL_4 and IFN-γ compared to adult values

Mean values	IL_4	IFN-γ
Fetus (pg/ml) (range)	18.67 (0–69.70)	0 (9–218.74)
Neonate	<3.5, undetectable [126] or absent [158]	28.67±1.55 U/ml
% of Th-producing cells		
Low risk (range)	0.08 (0.01–0.49)	0.21 (0.04–0.60)
After PMA stimulation	0.28 (0.15–1.52)	4.05 (1.26–13.11)
High risk (range)	0.07 (0.0–0.87)	0.28 (0.0–2.58)
After PMA stimulation	0.57 (0.08–3.68)	4.11 (0.9–18.39)
Adult	30.5±11.44	3,564±1,297 U/ml

Measures: 118 pM, 149 pg/ml.
IL_4 reaches adult levels after 15 years of age, IFN-γ reaches adult levels at the age of 3 or later [3].
Data from [71] (low- and high-risk babies), [102] (fetus), [126, 158] (IL_4), [126] (IFN-γ).
PMA Phorbol myristate acetate.

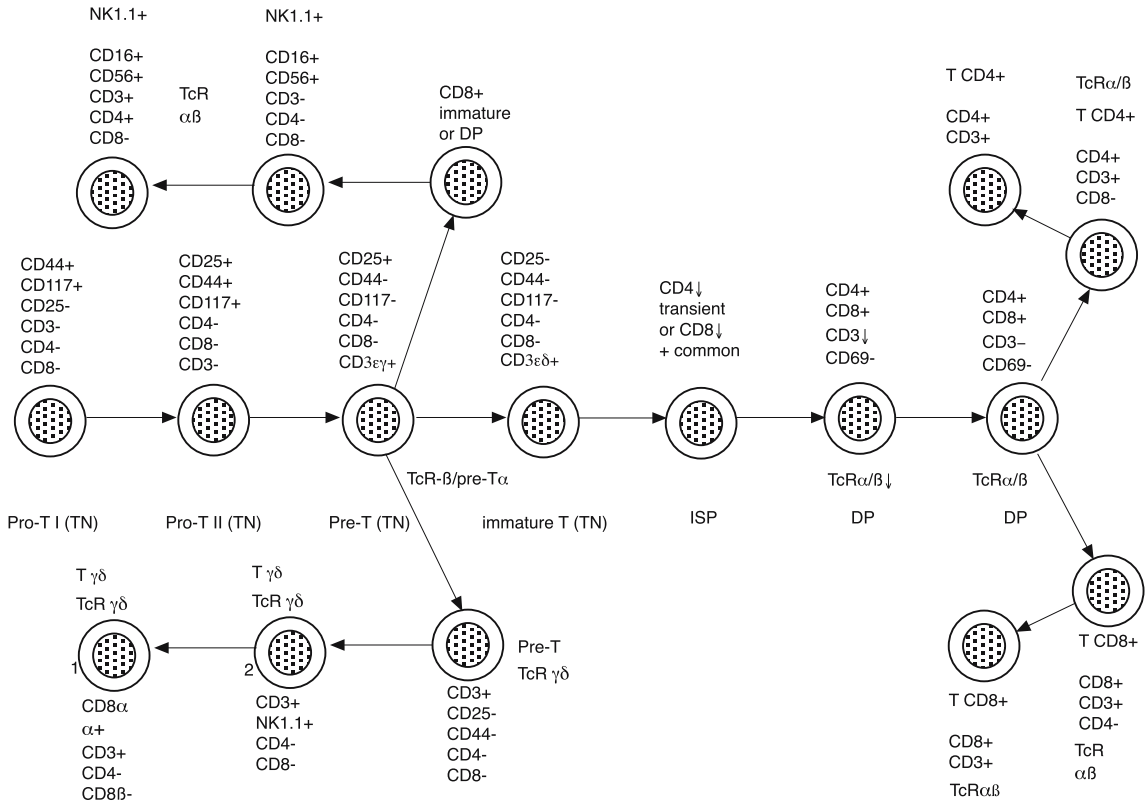

Fig. 2.2. Lymphocyte CD4, CD8, and NK cell development and receptor expression. The negative cells (–) are present but not expressed. TcR-γδ cells shown as *1* are found predominantly in intestinal epithelium. The cells shown as *2* are found in nonintestinal epithelial surfaces, e.g., skin. *TN* triple negative, *ISP* immature single positive, *DP* double positive *NK1.1* NK cells. (Modified from [1])

Ontogeny of the Immune System

In the fetal liver HSCs generate specialized cells, such as polymorphonuclear (PMN) cells and macrophages, DCs, NK cells, and mediators of inflammation, such as mast cells, basophils and eosinophils [123]. T and B lymphocytes, derived from pluripotential HSCs in the fetal liver [123], unlike other cells of the organism are characterized by a biphasic differentiation, according to whether they depend on antigen stimulation. The inductive phase occurs in the thymus for T cells and in the bone marrow for B cells, where both types of lymphocyte undergo an irreversible differentiation, consisting in genetic rearrangements leading to TcR (T-cell receptor) and BcR (B-cell receptor) expression [137]. By 18–20 weeks of gestation, the human thymus differentiation appears to be complete and T lymphocytes are provided with TcR. From the first trimester, T and B cells start to express clonal diversity, while the CTL production, one of the more outstanding effector functions of T cells, begins in the middle of fetal life. From this period onward, a specific antibody response may also be present, quantitatively and qualitatively different from adult responses: fetal and neonatal B cells possess a fully limited skill of synthesizing antibodies of IgG and IgA isotypes, even if precursor B cells expressing such isotypes are present. In the central phase, a selection process takes place since the lymphocyte's fate is not the same for all of them: during maturation the cells interact with HLA proteins localized in thymus positioned on the APCs (antigen presenting cells) surface associated with peptides for T cells and antigen epitopes interacting with BcR for B cells. The first selection concerns T lymphocytes with TcR that recognize both exogenous and autologous antigens, always in the context of HLA molecules: the lymphocytes with affinity for autologous antigen, that is self-reactive clones, are removed or anergized and die (apoptosis) and only T cells with specificity for exogenous antigens survive [159] (Fig. 1.22, Tables 1.16–1.19). During fetal life, the immune system undergoes an active proliferation of elements and functions (Table 2.4) [102, 120, 130, 144, 149, 159, 215, 219]. A still unclear crucial progress is the early expression of a pre-receptor on the cell surface, in both T and B cell development. The pre-TcR on thymocytes is formed by a TcR-β chain associated with a structure called pre-Tα. The equivalent pre-BcR contains an H IgM chain associated with two invariant surrogate L chains: λ_s and $V_{pre\text{-}B}$. Even if the ligands for these pre-receptors are not yet known, signaling from these molecules is necessary to continue molecular development [179, 225] (Fig. 2.2 [1], Tables 2.5, 2.6 [130]).

Table 2.4. Development of immune system in fetus

Gestation (weeks)	
5	First rudiments of spleen
5.5	Synthesis of complement detected (C3)
6	Start of myelopoiesis
6–7	Fusion of thymic lobes
7	Hemopoietic cells in liver
7–8	Lymphocytes appear in peripheral blood (1,000/mm^3), pro-B cells in liver
8	Synthesis of complement (C2, C5)
8–9	Lymphocytes in thymus
8.5–10	T-cell receptor (TcR) development in thymus, TcR-γδ before TcR-αβ
9.5–11	Maturation of pro-B cells in liver and spleen with IgA, IgD, IgM, IgG surface markers, serum IgM levels detectable
10	Corticomedullary differentiation appears
10–11	Lymphocytes in bone marrow and intestine
11	IgE antibodies in liver and airways CD40 abundantly expressed HLA class II expression
11–12	Intraepithelial lymphocytes in the gut
11–24	Gut expressing CD3, CD14, CD20, CD28, CD40, CD68, CD86, CD154, CTLA-4 HLA class II and APC (antigen presenting cells)
12	Hemopoietic cells in thymus and spleen Mononuclear cells in spleen, antigen recognition Stem cells in bone marrow
12–14	Pre-B cells in bone marrow, first identifiable Peyer's patches in the gut
12–15	Thymus becomes lymphoid
13	Graft-versus-host reactivity is present IgE antibodies in the amniotic liquid Transplacental HIV transmission
14	Response to passive hemagglutination by thymus lymphocytes First MALT and tonsil rudiments $CD3^+$ cells detectable in increasing numbers
14–16	Mature neutrophils, first rudiments of ethmoidal and maxillary sinuses
15	Complete differentiation of T and B cells, Langerhans' cells in tonsils
15–16	Lymphocytes in blood, first cytotoxic T cells
15–17	Serum NK cells and IgM levels detectable
16	Corticomedullary differentiation complete, primary follicles in tonsils First lymphoid follicles start forming $CD20^+$ cells abundant in the lymphoid follicles of rudimentary PPs CD83 immunoreactivity detectable (mature DC cells) $CD154^+$ and $CD152^+$ cells detectable in the lymphoid follicles
16–17	IL_{10}, TGF-β (full levels)
17	Serum IgG levels detectable First PBMC proliferative response to PHA. Some PBMCs may release IFN-γ
18	Lymphocytes migrate into lymph nodes, complement levels detectable

Table 2.4. (Continued)

Gestation (weeks)	
18–20	Complete differentiation of T lymphocytes with TcR γδ TcR T cells in the epithelium Lymphoid follicles and Peyer's patches with primary B follicles and T zones FIS is active (see Tables 2.5 and 2.6)
20	Maturity of thymus, memory lymphocytes the ability to synthesize immunoglobulins is complete
20–25	Lymphocytes in blood about 10,000/mm^3, T cells = Th1+Th2 Increased B-cell traffic
21	IgE antibodies in spleen
21–22	CD25$^+$ levels reach 50 % of neonatal levels The FIS may be primed by maternal allergens
22	Complement levels detectable in serum PBMC proliferative response to more antigens The fetus can react to food and inhalant allergens of maternal origin
22–24	Secretory component in duodenal mucosa and trachea
24	Peyer's patches are scattered throughout the gut
25	Differentiation of white and red pulp in spleen
30	IgA level detectable in serum
31	B cells with membrane-bound IgD detectable in serum
33	IgG levels reach maternal levels

Data from [102, 120, 130, 144, 149, 159, 181, 215, 219].
FIS fetal immune system, *PBMC* peripheral blood mononuclear cells, *PHA* phytohemagglutinin, *PP* Peyer's patches.

Table 2.5. Percentage (mean ± SD) of T-cell-related surface molecules in human fetuses aged 18–20 weeks and in cord blood (*CB*) of term neonates

Surface molecules (CD)	Fetus	CB
CD1	6± 5	1± 2
CD2	58± 8	64±12
CD3	53±19	52±12
CD5[a]	52±14	48±16
CD7	64± 7	66±13
CD4	41± 8	37±12
CD8	21± 6	21± 7
CD4/CD8	3± 2	1± 1

Modified from [130].
Significance (p=0.004) for CD1 and CD4/CD8.
[a] % Related to CD5*CD19$^-$ lymphocytes.

Table 2.6. Percentage of CD7$^+$ or CD3$^+$ expressing a second molecule (mean ± SD) in human fetuses aged 18–20 weeks and in *CB* of term neonates

Surface molecules (CD)	Fetus	CB
CD7+CD38	91±14	94±5
CD3+CD28	87±14	75±21
CD3+TcR-αβ	88± 9	94± 3*
CD3+TcR-γδ	4± 2	2± 1**
CD3+CD45RA	69±10	85± 9***
CD3+CD45RO	8± 6	7± 5
CD3+CD25	11± 4	4± 3
CD3+HLA-DR	1± 2	1± 2

Modified from [130].
* p=0.03, ** p=0.02, *** p=0.001.

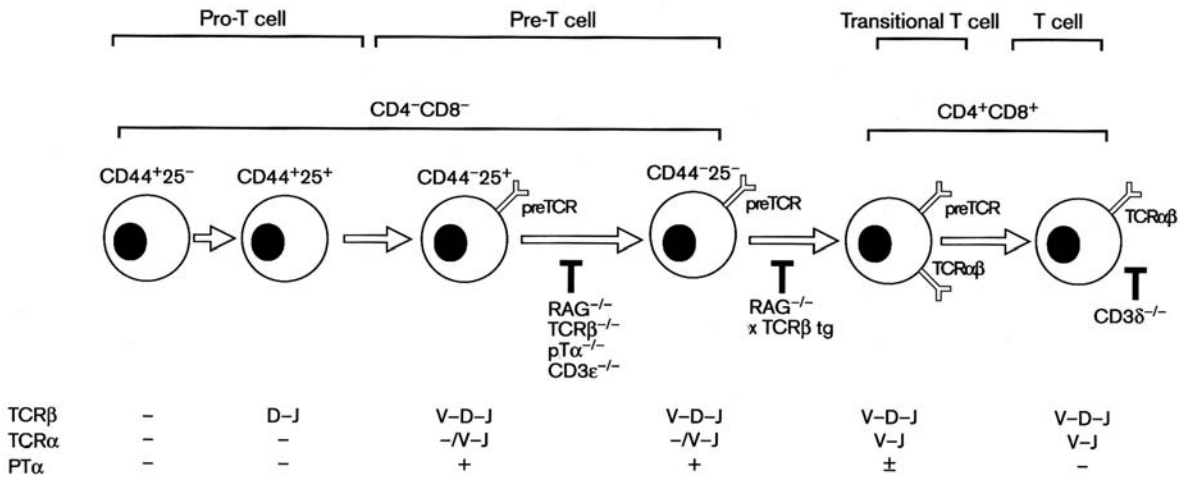

Fig. 2.3. T-lymphocyte differentiation schema evaluating expression and function of the pre-TcR

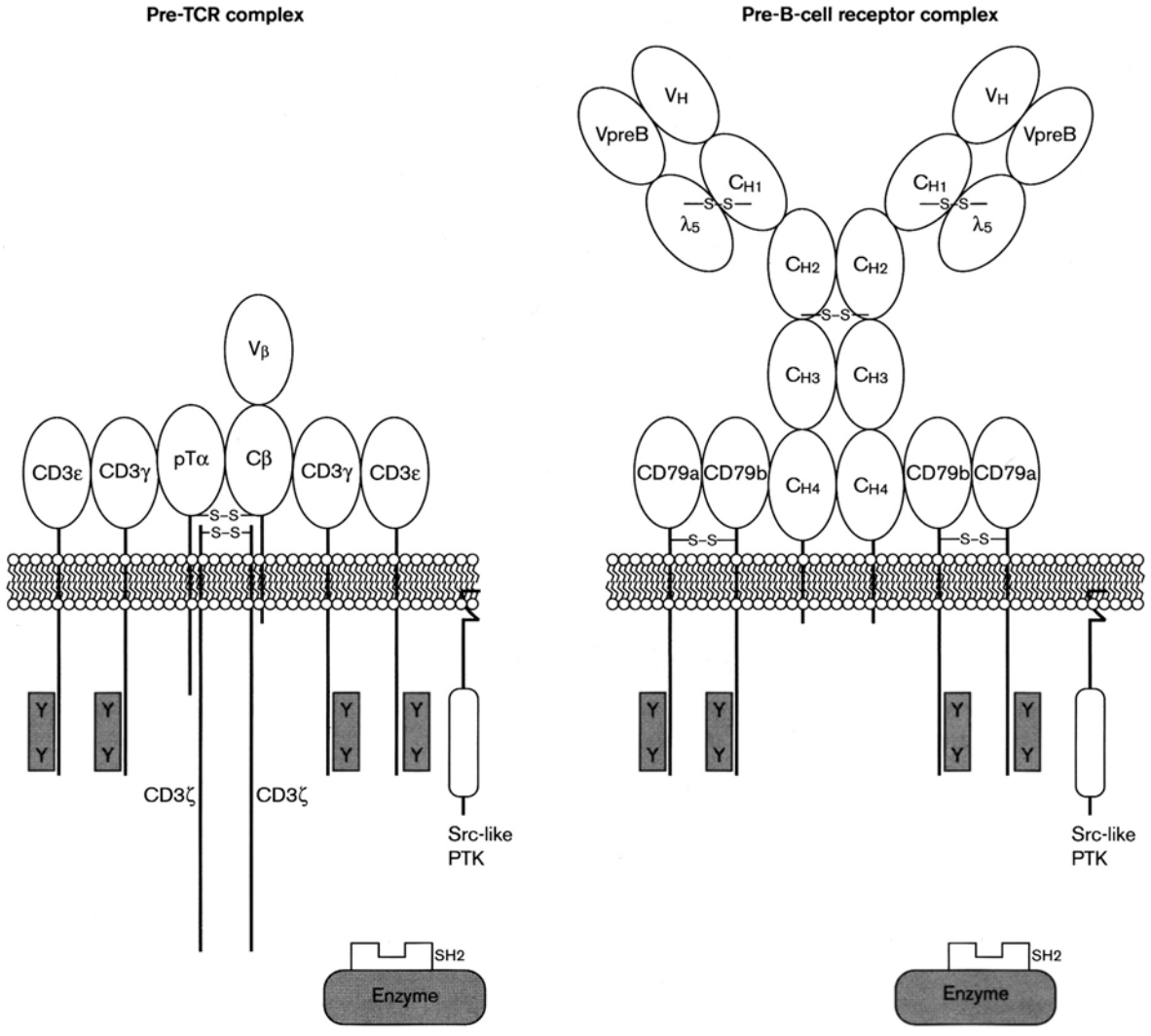

Fig. 2.4. Models of pre-TcR and pre-BcR complexes. *Ovals* indicate Ig-like domains, *boxed YY symbols* represent ITAMs that play a role in pre-TcR or pre-BcR signaling for development. *TAM* immunoreceptor tyrosine-based activation motif

T Cells

T cells arise from bone marrow hemopoietic precursors and at around the 8th week of intrauterine life colonize the fetal thymus, where the lymphoid cells or thymocytes undergo different degrees of differentiation, each characterized by changes in surface antigens. Immature thymocytes are identified in the cortical zone at 8–9 weeks and in the medulla at 10 weeks, where the thymocytes undergo further differentiation at 18 and 20 weeks of gestation [3]. This process regards 5% of the thymocytes escaping apoptosis; negative selection occurs next and is mediated by bone marrow-derived APCs, which eliminate autoreactive cells by clonal deletion or anergy (Chap. 1). Lymphoid cells (about 5%–10% of thymic lymphocytes) express antigens shared by bone marrow cells of the hemopoietic lineage. Figure 2.2 shows different stages of lymphocyte development, including CD4, CD8, NK cells and receptor expression: not shown is the common origin of cell lines from a common precursor, CD34, an early marker of hemopoietic precursor cells, which binds to bone marrow stromal cells [1]. At 7–8 weeks of gestation lymphoid cells with the CD7 phenotype migrate into the prethymic mesenchyme, membrane CD7 is the marker of mature cells, along with the ε chains of cytoplasmic CD3. By 10 weeks thymocytes acquire new antigens identified by means of monoclonal antibodies: CD1 (expressed only during the thymic residence), CD2 (which remains for the entire life-span) and CD3 [321, 123], as well as by 8 and 14 weeks, CD71, CD2, a receptor for sheep erythrocytes, identified also by anti-CD2 antibodies and expressed in all thymocytes and circulating T cells [6]. CD2, CD3, CD7 belong to IgSF (immunoglobulin superfamily) (Table 1.4).

By 15 weeks of gestation, CD7 and CD3 undergo varying stages of differentiation [32], are TdT-positive (terminal-deoxynucleotidyl-transferase), and then express CD4, CD8 and the TcR-β chain. The next stage is marked by membrane expression of both CD3 and TcR-αβ, whereas TdT cannot be detected at the passage from cortical to medullary regions [32]. More than 90% of CD3 and CD7 express CD38, a transducer of activation signals (Table 2.6), which along with CD71 are not specific markers of T cells, since they also belong to cells of other lineages [123]. By the stage of immature T cells, there are TN (triple-negative) cells expressing the IL_2 receptor (CD25), CD34 (mediates apoptosis) and CD117 (signal transducer) [1]. Only in the next stage do ISP (immature single positive) cells express CD4 or CD8, until cortical cells (70%–80%) express $CD4^+CD8^+$; however, in the medullary stage they divide (10%) into two subpopulations with TcR-αβ (Fig. 2.2). Although immunocompetence is already acquired in this stage, it is unlikely that qualitative changes take place while lymphocytes remain in the sheltered intrauterine environment [123].

From a molecular point of view, the first developmental stages of T cells imply the rearrangement and expression at the cell surface of TcR genes. At this level, selection can start, more precisely at the earlier stage fetal TN thymocytes (Figs. 2.3, 2.4). The γδ receptor of T cells (TcR-γδ) is the first receptor in human ontogenesis associated with CD3, soon followed in order by TcR-αβ expression (about 8.5–10 weeks of fetal life) [72]. At a later stage, thymocytes expressing TcR-γδ progressively decrease in number: as summarized in Table 2.6, γδ TcRs at 18–20 weeks of gestation are 4.5%–7.8% αβ TcRs [130]. At 12 weeks of gestation, antigen recognition is present, around 14 weeks T cells acquire surface markers, leave the thymic environment for further differentiation, expressing $CD45RA^-$ and $CD45RO^+$. At 18–20 weeks of intrauterine life, the distribution of diverse subpopulations is roughly comparable with that of a mature thymus [144] with CD4 T cells that are twice the number of CD8 T cells [130]: at 20–25 gestational weeks, 10,000 T cells/mm^3 are found in blood [144].

Compared to adults, there are evident quantitative differences, as demonstrated by the lower numbers of CD3 and a reversed CD4/CD8 ratio due to the scarce number of CD8 [130, 147] (Table 2.5). By 19 weeks of gestation, $CD3^+$, $CD4^+$ and $CD8^+$ T cells gradually increase in number, reaching a peak at about 6–7 months after birth [174], whereas CD45RO neonatal levels

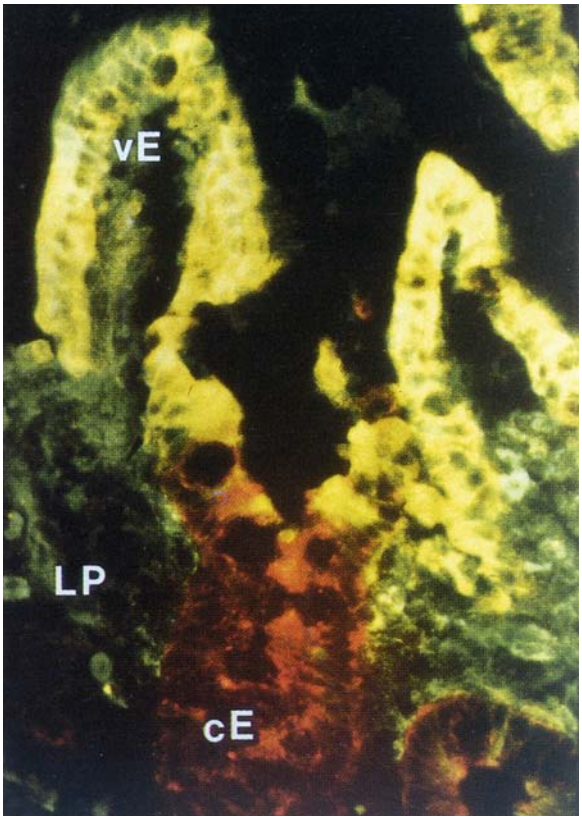

Fig. 2.5. Duodenal mucosa of a 1-week-old neonate. The cells are stained *green* for HLA-DR and *red* for keratin. The keratin-positive vE is also intensely positive for HLA-DR. *cE* crypt epithelium, *LP* lamina propria

Table 2.7. ILs promoting the proliferation and differentiation of thymocytes in vitro

IL(s)	Responding cells	Effects
IL_2	H DN	Proliferation, $IL_2R\alpha$
IL_4	H CD3 DN	Proliferation and maturation; TcR-γδ maturation
IL_7	MSP	Proliferation and differentiation
	H CD3 DN	Proliferation and maturation
IFN-γ	MDN day 15 fetal	IL_4-mediated inhibition, proliferation
TGF-β	M total thymocytes	Inhibition of proliferation

Only the effects induced by unstimulated ILs have been included.
Data from [34].
IL(s) interleukin(s), *H* human, *M* murine, *DN* double negative, *SP* single positive.

Table 2.8. Evident effects that IL(s) modulate the proliferation and differentiation of thymocytes in vivo

IL(s)	Effects
IL_1	The majority of day 13–17 M fetal Thy constitutively express IL_1R
	Fetal Thy epithelial and bone-marrow-derived stromal cells constitutively produce mRNA for $IL_1\alpha$ and $IL_1\beta$
IL_2	H fetal Thy express $IL_2R\alpha$, especially in pre-T cells (SP, SN, DN)
	Day 15 fetal Thy constitutively express IL2 and IL_2R genes
	Day 14–17 fetal Thy constitutively express high- and low-affinity IL_2R
	IL_2-IL_2R intervention seems to be necessary for M fetal Thy differentiation
	Anti-$IL_2R\alpha$ antibodies administered to pregnant mice block T-cell development in neonatal offspring
	Anti-$IL_2R\alpha$ antibodies inhibit the thymic regeneration in sublethally irradiated mice
	$IL_2R\beta$-human $IL_2R\alpha$ heterodimer induce the accumulation of T-cell precursors in the thymus and periphery
IL_3	Originates from T cells, mRNA expression is reduced, but mononuclear cells produce high levels of IL_3 after LPS stimulation
IL_4	Day 15 fetal Thy constitutively produce IL_4 mRNA and express IL_4R
	Populations of adult mice DN Thy express IL_4R
	The constitutive production of IL_4 in IL_4-transgenic mice inhibits DP Thy and mature peripheral T-cell development
IL_6	Promotes the differentiation of Thy -1^+ IL_2R^+ donor Thy after intrathymic transfer into irradiated hosts
IL_{10}	Promotes the growth of progenitors of the erythroblastic series and immature Thy in synergy with IL_2 and IL_4

Data from [3, 34, 115].
Thy thymocytes, *M* murine, *DN* double negative, *SN* single negative, *SP* single positive, *LPS* lipopolysaccharides.

Table 2.9. ILs promoting the growth, proliferation and differentiation of intrathymic T cells

IL(s)	Growth-promoting effect (proliferation, differentiation) of							Induction of expression of	
	pro-T	pre-T	TN	DN	DP	CD4 SP	CD8 SP	TcR-αβ	TcR-γδ
IL_1[a]	?	–	–	+	–	±	±	?	?
IL_2	++	++	++	+	–	+	+	++	+
IL_4	++	++	++	+	±	++	++	+	++
IL_6[a]	–	–	–	–	–	+	+	?	?
IL_7	+++	+++	+++	+++	–	+	++	–	+++

Data from [64].
DP double positive, *DN* double negative, *SP* single positive.

[a] Costimulatory activity.
? Not clear.

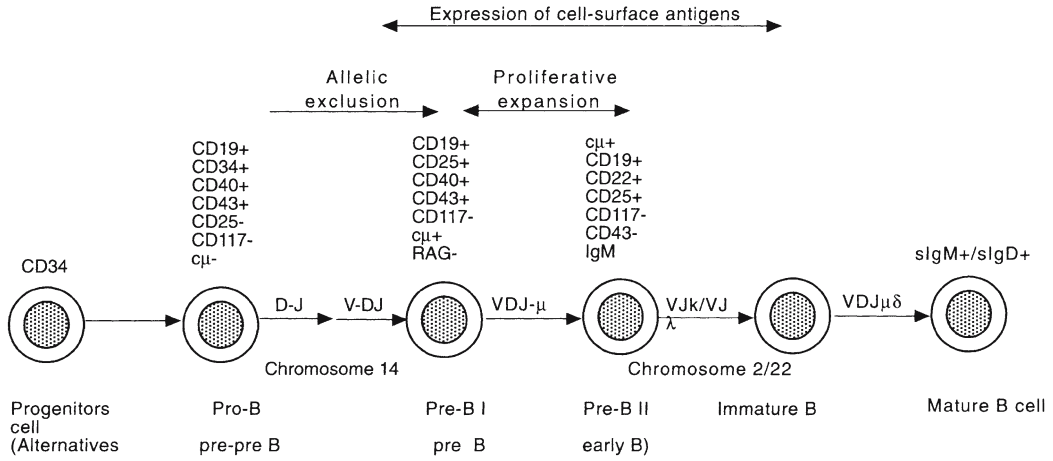

Fig. 2.6. Stages of B lymphocyte development and receptor expression. The CD-negative cells (–) are present but not expressed: *cμ* are H chains of cytoplasmic IgM antibodies. (Data from [1, 31, 122])

remain stable (Table 2.6). So fetal thymocytes, still expressing several functional capacities, at birth appear in a stage of substantial maturation. HLA class I antigens are ready in the fetal intestinal epithelium [174, 179], whereas HLA-DR expression, scarce in CB (cord blood) [147], is positive in the gut by 1 week of postnatal life [174] (Fig. 2.5). Between 11 and 16 weeks of gestation, both HLA class II and costimulatory molecules are present, CD40-CD154 and CD86-CD28/CD152 counter-ligands of B-cell CD80 and CD86 provide the costimulatory signals required for the initiation of antigen-specific reactivity in the human fetal gut as early as 16 weeks of gestation [102].

In distinct stages of T-cell ontogenesis, several ILs have positive effects (Tables 2.7–2.9) [3, 34, 64, 115]. Table 2.1 outlines their deficits or delayed activation: an intrinsic abnormality of CD4 T cells is associated with the pathogenesis of atopic disease.

NK cells derive from CD8 DP T cells and acquire CD16 and CD56 (Fig. 2.2), which are IgSF members.

B Cells

As the primary site of hemopoiesis shifts from the fetal liver to the bone marrow, by 12 weeks the latter becomes the site of B-cell generation during the fetal life; at birth this function relies almost exclusively on bone marrow and to a lesser degree on the spleen, immature in the neonate [162]. B cell functions, after leaving the bone marrow, where IL_7 and IL_{11} contribute to B-cell maturation, are always under the control of T cells, until the final differentiation in plasma cells [179, 225] (Fig. 2.6) [1, 31, 122]. B-cell maturation is characterized by differences in the expression of genes dictating H and L chains and the organization of different chains. In the earliest phase, lymphoid HSCs generate pro-B cells, which are identified in the liver from 7–8 gestational weeks and characterized by CD106 provision of CD45, TdT enzyme, IL_7 and SCF (stem cell factor), equal to CD117 receptors and DJ rearrangement on both pairs of chromosome 14, dictating two H chains (one for each parent) [123]. Pro-B are dividing cells, free of recombinations and expressing different L-chain genes that have begun the process of H-chain gene rearrangement, distinctively have no detectable Ig on cytoplasmic membranes, and express HLA class II molecules: CD19 and CD22 (Fig. 2.7). As early as 9 weeks of fetal life, the first pre-B I cells appear following a VDJ rearrangement and the production of Hμ chains, characterized by the expression of μ cytoplasmic chains, but not of L chains. Pre-B cells no longer present the TdT enzyme, but IgM or IgD, C3, CD20, which along with CD19 remain until the stage of blasts [106]; active partners are the adhesion molecules (CD49d/CD29 and CD44), SCF, IL_3 and IL_7 produced by the same cells. On chromosomes 2 or 22, B cells start the VJ rearrangement of λ or κ genes, the L chain synthesis and expression of a monomeric IgM receptor [123]. When pre-B I differentiate into pre-B II cells, L chains are also expressed and display IgM or IgMs (surface) [31]. The BcR function is present from the pro-B stage and active from the pre-B stage (Fig. 2.8).

From Fig.2.4 the similitude of the two pre-receptors is evident as concerns Ig-like domains (*ovals*) and ITAM (*boxes*). Any further gene rearrangement flops since RAG-1, RAG-2 or other recombinase components are lost (Chap. 1): IgMs (surface) antibody is regarded as essential for the closure signals. By 12 and 14 weeks of gestation, immature B cells differentiate into B cells co-expressing IgM and IgD, both with the same V region, and acquire the Fc receptors for IgG [144]. During ontogenesis, all B cell clones derive from progenitors expressing IgM or IgMs; many B cells express all three isotypes together [3]. Immature B cells expressing IgAs or IgGs also co-express IgMs and IgDs, whereas co-expres-

Fig. 2.7. Surface antigens of B cells. *T* T lymphocytes, *M* Monocytes, *Pla* plasma cells

sion of IgAs or IgGs is uncommon. All the maturation up to this point has taken place in the bone marrow and is antigen-independent. The presence of mitogens or an appropriate combination among antigens, T cells, T-cell-derived ILs, macrophages and accessory cells is necessary thereafter [122]. Mature B cells interacting with antigen lack membrane IgD (mIgD) and expose the other isotypes in sequence on their membrane. The end stage of B-cell development is the differentiation into plasma cells, when surface Igs, HLA-DR, CD19, CD20, CD21 and CD22 are lost; to complete Fig.2.6, CD19, CD20 and CD22 are expressed continuously from the pre-B-cell stage [106]. B-cell surface antigens are summarized in Fig.2.7. Pre-B cell production occurs in the bone marrow by 12 weeks; by 11–16 weeks of gestation, pre-B cells are as high as 1–4% of nucleate cells and outnumber B cells with mIgM with a ratio of 2:1. By 30 weeks of gestation, there is no detectable pre-B cell in the fetal liver [3]. The pre-B cell proportion in the spleen and bloodstream is lower than that of B cells. In the adult, pre-B cells are produced only in the bone marrow [144].

By 11 weeks of intrauterine life, the human fetus synthesizes IgE antibodies in the liver and lungs, which, similarly to IgA and IgM, do not cross the placenta [120]: elevated fetal levels of IgM and occasionally of IgA have been associated with intrauterine infections [169]. IgG are seen by 17 and IgA by 30 weeks of gestation (Table 2.4). The expression of SC is in the duodenal mucosa [174] and fetal trachea with increasing levels [204] at 24 weeks of fetal life (Fig. 2.9) [204], until SC reaches normal values at 1 week after birth, a production stimulated by both TNF-α and IFN-γ [19]. Therefore, a normal fetus can rely on IgG antibodies, as an exclusive result of active and gradual transplacental transport from the mother, until at 33 weeks of gestation fetal IgG achieve maternal levels [120]. IgE antibodies, practically absent *in utero,* are synthesized after birth, rising slowly at about 3 weeks [174, 204] (Fig. 2.10) [204], despite reports of CB IgE levels also in nonatopic babies, independent of maternal diet during pregnancy [82].

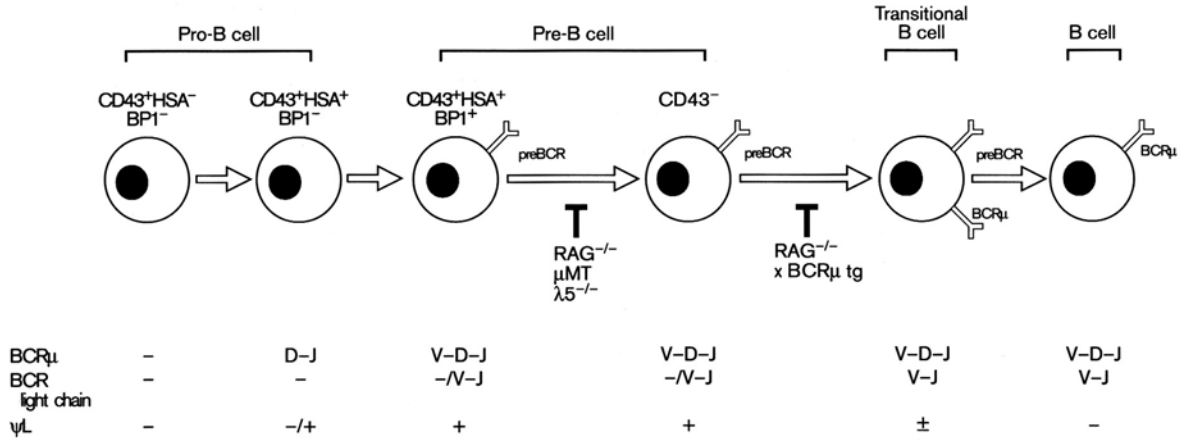

Fig. 2.8. B-lymphocyte differentiation schema evaluating expression and function of the pre-BcR

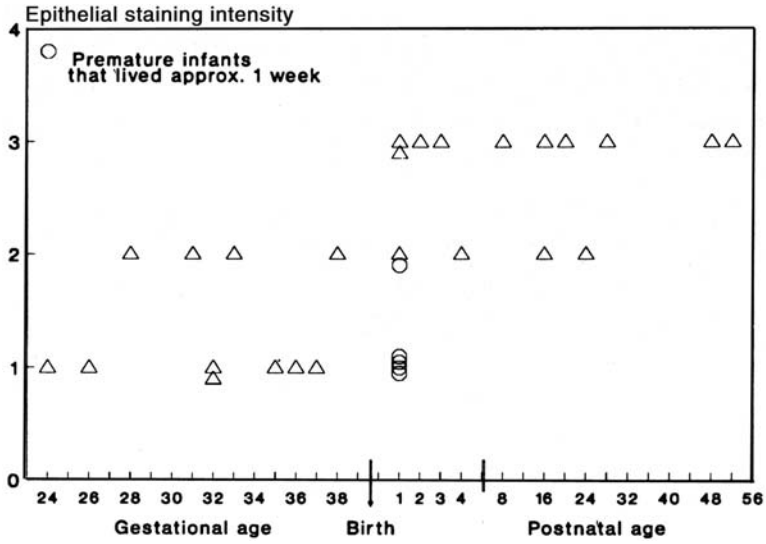

Fig. 2.9. Secretory component (SC) expression in tracheal wall according to gestational and postnatal age

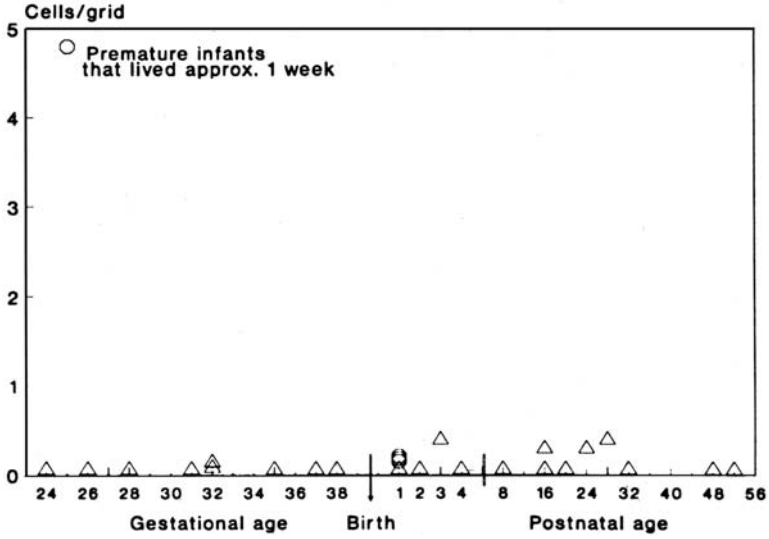

Fig. 2.10. IgE expression in tracheal wall according to gestational and postnatal age

Phagocyte Cells

Phagocyte cells appear beginning in the 2nd month of intrauterine life: the differentiation and subsequent maturation of progenitor cells is promoted by hematopoietic CSFs (colony-stimulating factors) stimulating colony proliferation and differentiation, distinct in GM-CSF (granulocyte macrophage-colony stimulating factor) and M-CSF (monocyte/macrophage-colony stimulating factor) and by IL_3. In the liver and peripheral blood of fetuses 8–21 weeks of gestation, several CFU-GM (colony forming unit – granulocytes and monocytes) have been identified. Mature monocytes are found in lymph nodes and spleen by 4–5 months of gestation. Macrophages mature gradually during the fetal life: before birth and in the neonatal period, they are scarce in number and immature in the lung alveoli; however, soon afterwards a massive influx of mature cells in the airways is noted [86]. Chemotaxis is relatively deficient in neonates [238] and is 79% of the mean adult value [54] (Table 1.65).

Complement Factors

Complement has been identified in fetal tissue by 6 weeks of gestation and serum levels are detected at 30 weeks (Table 2.4); however, C1q, C2, C3, C4, C5 concentrations are modest in neonates (C3 0.60%–0.79% and C4 0.41%–0.59% of the mean adult value) [50], and correlated with gestational age (GA) [53]. Significantly, the impact of GA in the deficit of components (and of opsonization) is more evident in neonates with a lower GA [50]. Hemolytic complement titers of normal full-term neonates are about 50% that of the mother [169]. Concentrations increase rapidly, reaching mean adult

values by 3 and 6 months [50], with the exception of both C1q and properdin levels, which, although approaching 90% and 71% of adult levels, respectively, in term neonates [235], they remain low until 12–18 months of age [53].

Neonatal Immunodeficiency

As pointed out previously (Table 2.4), the fetus is able to recognize and respond to a wide assortment of antigens because this capacity is genetically programmed. To reach this objective, an elaborate interrelation among several components of the immune system is necessary, dependent on their developmental cycle, which even though in unending evolution [40], is not free from severe deficits of B and T lymphocytes. If the intestinal mucosa is a frequent entrance for several infectious agents, it is because fortunately Mother Nature, always a provident protector of the human species, has selected a seat such as the intestinal mucosa, passively protected by the immune factors present in breast milk, for the first contact with potentially pathogenic substances [155]. Human milk supplies the vulnerable newborn not only with critical nutrients, but also with the powerful Ig defense against the assaults of antigenic macromolecules and of infectious agents [27]. The particular characteristics of the neonatal immune system are summarized in Tables 2.1, 2.2, 2.5, 2.6; see also Tables 1.34–1.39. Table 1.38 shows significant differences between CB and venous blood of the same children at 5 days of life.

Cellular Immunity

In CB and in term and preterm neonates aged 1 day, of adequate weight for GA, an absolute proportion of cells positive for CD4 and CD8 monoclonal antibodies is found, significantly more than the counts in low-birthweight (LBW) neonates for GA. A neonate of whatever GA has an absolute number of T lymphocytes greater than adult values, as well as quite a number of cells positive for CD6, CD9, CD10 [225] and CD1, *an index of normal lymphocyte development* [86]. These positive data are matched by the persistent CD4/CD8 ratio increase (Tables 1.38 and 2.5) and the clear activation deficit of both CD2 and CD3 [165] equal to about 64% and 52% in CB, respectively (Table 2.5), corresponding to the initial low density of the memory $CD45RO^-$ cells also in CB (Table 2.6) and virtually of the whole $CD4^+$ subset (10%), in parallel with the scarce proportion of $CD45RA^+$ compared to adult vales (15% vs 50%) [86, 164]. The high expression of CD38 is a marker of immaturity when associated with CD45RA cells and of immune activation when co-expressed by CD45RO cells; we can hypothesize that CD38 makes immature fetal and neonatal CD45RA cells more responsive to signals mediated by previously encountered antigens during ontogenesis or at early stages of their functional maturation [130]. The selective shortage or depressed expression of IL genes may be ascribed to poor CD2 and CD3 levels [6], whereas TcR and CD8 modulation is unchanged 165]. The contrast between the initial activation and subsequent decrease in T cells is confirmed by the apparent hyperreactivity to mitogens at 1–2 days and the reduced T-cell proliferation at 5 days of life [17], thus suggesting an inadequate maturation and/or a shorter T-cell life cycle, mainly of CD8 [225]. Neonatal T-cell immaturity demonstrated by a scarce proliferation via CD2 and CD3 stimulation [6] is stressed by the IL_2R restoration of neonatal PBMC (peripheral blood mononuclear cell) unresponsiveness to CD2 and CD3 [164]. IL_2 binding to its receptor, with normal neonatal levels [217], induces T-cell proliferation [164], therefore there are transitory supernumerary T cells, perhaps reflecting an excessive activation in response to the first exposure to environmental antigens of the immature immune system. A demonstration may be the transient peak of IL_2R serum levels, which remain above adult ranges over 2–3 years, independently of T-cell levels, thus suggesting that IL_2R activators are T cells isolated in the periphery, maybe in mucosal sites [46]. $CD45RA^+$ $CD45RO^-$ T cells predominate among neonatal circulating lymphocytes until an antigenic challenge occurs. A study has postulated that $CD45RA^+$ T cells may suppress or alternatively prime Ig production [181], thereby explaining, at least in part, the unusual susceptibility to infections of the neonatal age. The persistence of naive subsets of CD45RA during the first years of life, even following antigenic challenges, as shown by an *increased expression of $CD45RO^+$ cells soon after birth,* although with lower levels when compared with adult levels [236], might be responsible for the deficit of CD2 and CD3 [165] or of CD11a/CD18 [147]. The apparent immunodeficient status of T-cell subsets could persist until the age of 4–5 years [164]. Subsequently, CD45RO levels gradually increase (as the responses to CD2 and CD3) until they match adult levels at 10–15 years of age [86, 147], whereas CD45RA levels progressively decrease [164]. Phenotypic analysis indicates that about 75% of neonatal and 87% of fetal CD3 T cells co-express CD28, important for interactions with CD80, however poorly expressed by fetal and neonatal B cells [130]. Obviously the deficits of all lymphocytes are quantitative, as demonstrated by the number of T and B lymphocytes and of total T lymphocytes, which in the first 2 years of life are 2–3-fold more elevated than in normal adults [149] (Table 2.1).

The generation and activation of CTLs are only 60% of those in adults [215]: this is an additional factor favoring the neonatal susceptibility to infections. Phenotypic characterization of neonatal NK cells has demonstrated substantial differences compared to adults [40], in neonates the levels were only 50% of the adult values [232], thus suggesting a defect in the initial activation of the neonatal precursor cells [86]. Studies show that NK

cells reach adult levels at 1–5 months of age, while the activity induced in vitro by IL_2 (LAK activity) is normal or increased [232]. The number of cells with CD11b, CD16 and CD56 phenotype or NK cell surface markers shows that these cells are increased during infancy to early childhood and are significantly greater than in adults; the CD57 values reduced up to age 4 but only at 9–13 years does no difference exist between children and adults [232]. IFN-α at the single-cell level did not enhance the binding ability of NK-cells at any period, whereas IL_2 had augmentative effects on NK cells [232], present in normal concentrations [158, 201]. However, in neonates the lytic and CTL activity is poor, hence the defense against infections by HSV (*Herpes simplex* virus) and *Cytomegalovirus* may remain uncovered [222].

Interleukins, particularly the production of IL_6 after stimulation with IL_1, similarly active in the antimicrobial defense, have a low concentration in fetal and term and preterm neonatal cells, but the levels can be normal at birth if opportunely stimulated [234]. Fetal levels of IL_2, IL_{12} and IFN-γ are undetectable, levels of IL_{10} and TGF-β were detectable in all fetuses [102]. In neonates, the T-cell ability of secreting bioactive ILs such as IFN-γ and IL_4 (0.8 % and 0.3 % in adults, respectively) (Table 2.3), IL_3, GM-CSF, IL_6, IL_{10}, IL_{13}, TNF-α (50 %) [225], IFN-ɣ, and IL_2 [71] is markedly reduced (Table 2.1). T-bet, a member of the T-box family of transcription factors, is repidly induced in early developing Th1 cells and is absent in developing Th2 cells. IFN-γ requires the introduction of T-bet into T cells which preferentially induces the conversion of these T cells into IFN-ɣ producing Th1 cells [208]. T cells of infants who manifested allergic symptoms produced higher levels of IL_{13}, compared with those of nonatopic infants [154]. Babies at risk for atopic disease in infancy displayed defective IL_{13} production at birth when compared with babies with no FHA [223]. A correlation was found between $CD4^+IL_{13}$ from CBMC lymphocytes derived from atopic mothers and the occurrence of wheezing and/or asthma during the 1st year of life [200]. IL_6, a central differentiation factor for activated B cells and in the induction of IgAs B cells to become sIgA cells [138], is also deficient [167]. The amounts of IL_4 and IFN-ɣ as shown in Table 2.3 are <1 % of adult values, consistent with their antigenically naive status [127] or with a neonatal maturative defect, perhaps because the cells are unable to sustain the expansion of T-cell clones. Impaired IL_4 production may depend on an altered CD21 and CD2 on B-cell expression by CBMCs that may limit IgE production [211]. However, the majority of fetal PBMCs release IFN-ɣ beginning in the second trimester of pregnancy [219], while CB production is poor compared to the initial activation [17]. As discussed earlier, neonatal defects (CD2, CD3, CD45RO) appear to occasion the low production of IFN-ɣ, IL_3 and IL_4, which play a crucial function in modulating antigen-specific and not antigen-specific immune responses [164, 165]. This maturational immaturity may impair T-cell normal function, thus

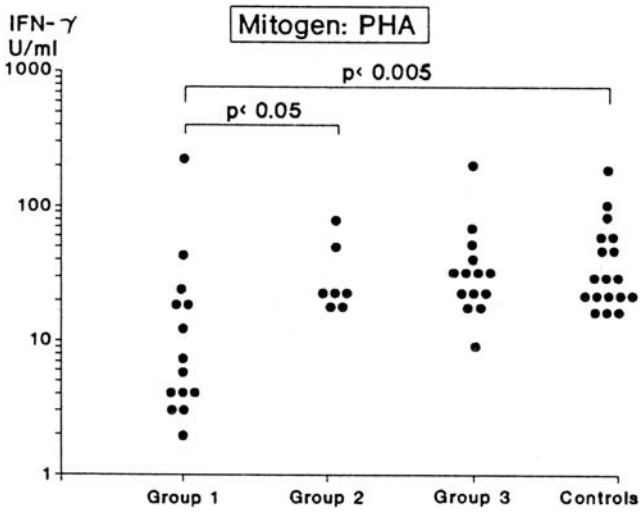

Fig. 2.11. IFN-γ production induced by PHA. Group 1 neonates with positive family history of atopy (FHA), group 2 with FHA and high cord blood IgE (CBIgE), group 3 only with high CBIgE. *Group 1* neonates showed a significantly decreased PHA-induced IFN-ɣ production compared to controls. *PHA* Phytohemagglutinin [172]

contributing to reducing both generation and activity of antigen-specific CTLs, and B cells through a lack of cognate help, or to suppressing Ig production, until it interferes in the modulation of the specific and unspecific immunity afferent phase [127], and to compromising the neonatal resistance to infections, including AIDS [222]. In this context, IL_{15} is important. It has an activity that regulates T-cell growth, T-cell cytotoxicity, and NK activity [3]. Animals that lack IFN-γ that binds to the IFN-γR, or the Jak-STAT signaling pathway to which IFN-γR is coupled, display disruption of both innate and adaptive immunity [208].

As regards IL_4 inhibition by IFN-γ in the neonatal age, IFN-γ appears to increase IgE synthesis by lymphocytes, instead of suppressing it [161]. IL_4 production seems to increase after birth by reaching greater levels in atopic babies than in nonatopic infants and by playing a critical role in IgE synthesis, while IFN-ɣ maturation (as well as of other ILs) is complete only at 3–5 years [127]. IFN-ɣ deficiency in the first months of life may stem from different causes such as an intrinsic inability of T cells to synthesize it, an increased sensitivity of T cells to PGE, or an ineffective activity of accessory cells such as macrophages [217], which neither produce nor activate IFN-ɣ [137]. *IFN-ɣ deficit* (Fig. 2.11) [172], independent of IL_4, but paralleled by CD45RO decrement [127], is based on the increased incidence of certain infections (Table 2.10) [28, 84, 144], and *facilitates the onset of atopy in neonates, especially in at-risk neonates*, when they are unable to react [26]. However, the APCs encountered with antigens may result in the production of IL_{12}, which on the one hand up-regulates IFN-ɣ expression and on the other hand, acting on

Table 2.10. Causes of the increased neonatal susceptibility to serious bacterial infections

Impaired opsonizing activity
Up-regulated opsonizing activity more evident vs Gram bacteria
Absence of opsonins specific for group B streptococci
IgM antibody levels = 0.05 % of normal adult level
Chemotactic defect
Relative defect in the generation of chemotactic activity
C3, C4, C5 levels = 50 % of maternal levels
Incomplete development of complement pathways
Functional defects of polymorphonuclear (PMN) leukocytes and monocytes
Decreased phagocytic and bactericidal activity
Reduced GM-CSF expression and production
Significant decrease in neutrophil chemotaxis
Decrease in Clq and C4 levels
Impaired cAMP and cGMP intracellular levels
Membrane defects[a]
Reduced actin polymerization
Prevalence of band PMNs with reduced chemotactic activity
Dysfunction of microtubule system
Defects in signal transduction
Defects in cell surface receptor up-regulation and motility
Cytoskeletal rigidity
Defects in deformability
Defects in microfilament contraction
Defects in O_2 metabolism
Defects in intracellular antioxidant mechanisms
Reduced candidocidia in preterm babies[a]
Defect in CD11b/CD18 and CD62L activity[a]

Data from [28, 84, 144].
[a] See the text.

CD45RA, induces the production of IFN-ɣ by neonatal human CD4 Th1 cells [230].

The analysis of the connections between malnutrition and immunodeficiency has shown deficits of CMI in neonates of mothers with vitamin B_6 deficiency (Chap. 21). Crucial is the function that EGF (epidermal growth factor) and TGF-α, present in the human fetal intestine in the second trimester [143], can have in facilitating the growth of mucosal epithelial cells, thus amplifying the anatomical integrity of the protective barrier and favoring early gut closure [143]. The passage of antigens that occurs during the first 3 months of life gradually decreases [142]. A specific danger may come from maternal smoking, which may reduce EGF levels in milk and colostrum (Chap. 24) and delay the process of intestinal maturation.

Humoral Immunity

Data on B lymphocytes are abundant, but often contradictory. The whole Ig production, including IgE antibodies, is deficient in neonates who are also atopic; this deficiency may be caused by an intrinsic immaturity of lymphocytes not favoring antibody synthesis or secretion [86]. CD4 T cells, even if present in neonates in great concentrations, have a reduced ability to regulate the antibody responses belonging to IgG and IgA isotypes [202], since the amounts of such antibodies expressed by stimulated neonatal B cells are markedly less than those produced by adult B cells [201, 220]. Supplementation of neonatal B cells with IL_2, IL_4 or IL_6 results in the correction of the above deficits and production of all major Ig isotypes, thus the consequent deficiency of these ILs results in an inconsistent antibody synthesis in neonatal B cells [202]. This depressed activity may also depend on a CD154/CD40L deficit, an important factor contributing to B cell immaturity [154], which could affect switch events, as seen in hyper-IgM and common variable primary immune deficiencies (PID). This deficit might be balanced by colostral CD3 T cells expressing in vivo CD154, with levels significantly higher than those in the circulation [13]. The majority of neonatal peripheral B cells express CD5 and activation markers such as CD21 and IL_2R, and in lesser amounts CD1c [9, 52]. Neonatal B-cell proliferation is endorsed by IL_2 or IL_4 without needing further signals such as anti-μ or mitogens compared to their adult counterpart [9, 220], reportedly due to a preactivation by exposure to transplacentally acquired antigens or, alternatively, by an intrinsic property of the CD5 subset [86]. A significant proportion of neonates is possibly equipped with B subsets expressing mIgD, mIgM, CD23 and CD11b, on which CD5 expression is variable [9]. CD5 could be associated with sIgA activity [149]. Some immature B cells with mIgM, even if stimulated with mitogens, secrete only the IgM isotype, thus denoting a further CD4 deficit. Moreover, B cells spontaneously secrete IgM autoantibodies against a range of autoantigens: a role in overall regulation of B-cell development has been postulated [9].

Maternal IgG transfer across the placenta to the fetus by 16 weeks of intrauterine life gradually increases during the last 3 months, mostly during the last 4 weeks, thus explaining why IgG concentrations in the CB of term neonates are 5 %–10 % higher than maternal levels, and preterm infants have IgG concentrations lower as their GA is short. They are therefore at a greater risk of

being susceptible to infections [62] and having a profound and prolonged physiological hypogammaglobulinemia (hgG) between 3 and 6 months of age [169] compared to full-term infants. This hgG is transient (Table 1.15) when maternal IgG levels gradually fall and the infant has not yet synthesized IgG in sufficient amounts, so that serum IgG levels in full-term infants drop up to 300–400 mg/dl [26]. Therefore, in preterm infants intravenous (IV) IgG substitution is indicated, which also significantly increases IgG subclasses [2]. Since IgG subclasses cross the placenta in different concentrations, a hierarchy ($IgG_1 \rightarrow IgG_3 \rightarrow IgG_4 \rightarrow IgG_2$ [62] or $IgG_1 \rightarrow IgG_3 \rightarrow IgG_2 \rightarrow IgG_4$ [50]) can be hypothesized, with the levels similar in all GA groups: 70%, 7%, 20% and 3%, respectively [50].

The importance of adequate IgG concentrations originates from phagocytes requiring specific antibodies to eliminate capsular polysaccharide antigens [137]. The close correlation between neonatal antibody status and susceptibility to bacterial infections is clearly demonstrated for group B streptococci, which are the predominant causative agents in neonatal infections from Gram-positive bacteria [144]. Unlike adults, during their convalescence neonates and infants with bacteremia synthesize high titers of specific antibodies of IgM class for the most part, which in adults mainly belong to IgG isotypes: this difference may depend on neonatal B-cell restriction of the IgG responses induced by suppressor T cells [169]. Antibody responses to somatic antigens of several Gram-negative bacteria (*Escherichia coli, Salmonella, Shigella*), belonging to the IgM isotype, do not cross the placenta [120]. Instead a small quantity of IgG antibodies to *Bordetella pertussis* pass, reflecting the very reduced level of maternal serum [36]. However, neonates and infants may be at risk following pertussis vaccination, a source of intriguing controversies, due to an overestimation of the risk of probable chronic neurological sequelae, actually of 1:310,000 DPT (diphtheria tetanus pertussis) doses = 0.000003% [90]. Large trials have provided a trend inversion favored by the introduction of acellular pertussis vaccines. The tetanic and diphtheria antitoxins are instead transferred across the placenta in adequate levels and confer an efficient passive protection during the first months of life [36].

Innate Immunity

Normal full-term neonates have a physiological deficit of complement functions compared to adults, particularly in preterm infants [63]. Measuring the values of both pathways of complement activation, compared to normal serum, the classic complement activity in the sera of term and preterm neonates was 52%±36% and 41%±29%, respectively, and the alternative complement activity was 59%±25% and 49%±12%, respectively [63]. A transient deficit of the bactericidal activity in term neonates, more enduring and marked in preterm neonates, can be responsible for the deficient opsonic activity [84], possibly corrected by the addition of IV IgG [45]. Although borderline, phagocytosis may also be deficient, owing to a diminished opsonic activity for staphylococci, *Streptococcus pneumoniae* and *E. coli*, possibly ascribed to a decrease in antibodies and complement levels [84, 121]. This deficit may be more evident in stressed newborns, especially preterm neonates [121]. Recent trials have shown a decrease in precursor HSCs, as well as a functional disorder of neonatal neutrophils (NN) or PMN cells, especially in times of stress or infections [28, 84], independently of GA and birth weight [169]. NN deficits include their differentiation, activation and functional activity, so they are less adherent and more rigid than those from adults, which results in impaired diapedesis and chemotaxis [84]. NN microbicidal and oxidative functions, such as phagocytosis, are delayed transiently in term neonates and more lengthy in preterm ones, likely due to the demonstrated MPO (myeloperoxidase) deficiency in the granulocyte cytoplasmic granules, implied in bacterial killing [28, 84]. In close relationship with this deficit is the poor IFN-α production induced by HSV-infected cells [37]. PBMC ability to normally migrate and eventually localize to sites of inflammation is mediated by interactions with gelatin and fibronectin to a degree comparable to that of adult cells [177]. GM-CSF expression and production by neonatal PBMCs are <50% compared to adult PBMCs [56]. Recombinant GM-CSF primes in vitro PMN activity [29]. As seen in Table 2.10, several functional deficits cause the unique neonatal susceptibility to bacterial infections [84]: in particular, PMN chemotaxis values, low at birth, increase by 11 and 32 days of life up to normal adult levels; the deficit persists longer in preterm babies [54]. There is evidence that the responsible mechanism [237] resides in the membrane deficiency leading both to greater cell rigidity, probably dependent on poor PMN locomotion, and a reduced membrane deformability, preventing the interactions between chemotactic factors and membrane receptors [144]. Studies show that a decreased actin polymerization could explain the observed abnormalities of PMN chemotaxis, possibly secondary to a negative regulation of profilin, coinciding with a decreased passage from monomeric G-actin to filamentous F-actin [81]. PMN mobility is nearly insignificant (the values at the age of 15 years are 50% of the mean adult value). However, the pathogenesis of NN impaired adhesion, diapedesis and migration, although provided with secretory vesicles and granules [110], depends on the deficit of both CD11b/CD18 [212], expressed by NN at only 60%–70% of the mean adult value, and CD62L [24, 212], whereas fetal neutrophils express normal levels of CD62L [196]. Adhesion molecules initiate a process that allows PMN extravasation into inflamed tissue with the goal of neutralizing the inciting stimulus [112]. Similar dysfunctions have been noted in eosinophils [194, 195]

and monocytes [212]. These findings may relate to the poor CB expression of NN receptors, or to a reduced gelatinase release or a poor ability to synthesize the peroxidase-negative proteins of granules [110]. However, delayed apoptosis and the subsequent survival of neonatal PMN lead to their prolonged persistence in tissues and to an enhanced up-regulation of CD11b, thus contributing to the pathogenesis of inflammatory disorders [112]. NNs express IL_1 [44], thus compensating for the missing IL production by neonatal macrophages, but fail to respond to both LPS (lipopolysaccharide), up-regulated by IL_{19} and TNF-α, which at low doses stimulates NN functions [20]. NN chemotaxis and adhesion deficits, the cause of LAD (leukocyte adhesion deficiency), might instead protect neonates from bronchiolitis that never develops in the first few months of life.

Neutropenia is defined as a neutrophil count of 100–500 cells/μl or an absolute count $<2.0\times10^9$/l based on the complete blood cell count, significantly correlated to a birth weight <2.5 kg [7]. During a 20-month study period, 87 neonates with neutropenia made up 6% of all admissions to a neonatal intensive care unit: 41% of episodes could be attributed to infections; the mean duration of neutropenia was 2 days and 70% of all episodes lasted for about 7 days [7]. We stress that differently from what occurs in granulocyte PIDs (Chédiak-Higashi syndrome, chronic granulomatous disease, etc.), hitherto no biochemical basis has been demonstrated that accounts for the myriad of functional deficits reported in newborns. Additionally, the clinical manifestations of patients with a primary disorder of chemotaxis are classically characterized by a selective localization of infections to the skin and subcutaneous (furuncles, abscesses), otitis media, pneumonia, and lymphadenitis [238], while newborns are predisposed to a high incidence of sepsis secondary to phagocyte defects [28, 84]. It appears that diverse dysfunctions of adhesion molecules, complement, opsonins, phagocytes, and aspecific factors may contribute to the high neonatal susceptibility to bacterial infections and a trend to become systemic (Table 2.10). O_2 consumption, H_2O_2 and superoxide production are normal, thus confirming a regular function of O_2-dependent bactericidal mechanisms [121, 197]. Macrophages have been studied very little: the circulating cells show quantitative values and phagocytic and bactericidal activity overlapping adult cells [121]. The recruitment of the above cells into inflammatory foci is delayed, a major factor explaining the usually low skin test reactivity of neonates [86], who also show an IL_6 deficiency [168, 233], TNF-α [55] enhances IL_{10} secretion [3], which is also reduced [168], and vital integrins [212]: this may contribute to the poor inflammatory activity. Macrophage function as APC is rare in neonates, due to a reduction in HLA antigens [174].

Mucosal Immune System

The lymphoid tissues of the mucosal surfaces of gastrointestinal (GI), respiratory tracts, etc., on the whole called MALT (Fig. 2.12) [11], in addition to the primary function of keeping the delicate epithelial surfaces sterile in contact with the external environment, should also be able to recognize the external antigens and to respond by sensitizing CD4 T cells. If there is a malfunction in normal functions, the host is at risk of enduring cell-mediated tissue damage: inappropriate responses of T cells underlie chronic allergic disease. CMI resembles a double-edged sword, since many aspects of this cascade imply the immanent risk of being detrimental to the structures to be protected. Thus it is clearly necessary to reduce the CMI impact on mucosal surfaces by limiting this impact to the minimum needed for the appointed goal, the elimination of pathogenic antigens [33].

Although manifold defense barriers, both natural and immune (such as sIgA), provide reliable defense lines for the epithelial external surfaces, the high solubility and low MW of many nonpathogenic antigens, either inhalant or dietary, make antigen penetration within epithelial tissues a frequent event. In the lungs, the contact between T cells and environmental antigens is potentially immediate, due also to the intervention of T cells

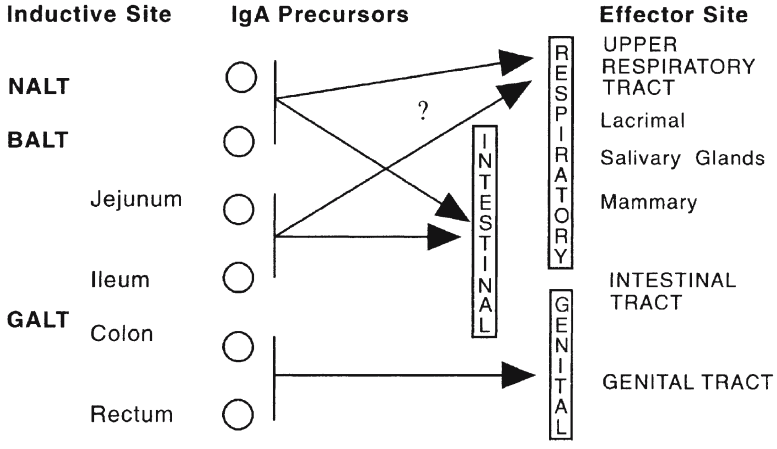

Fig. 2.12. The MALT: inductor and effector sites. *MALT* mucosa-associated lymphoid tissue, *BALT* bronchus-associated lymphoid tissue, *GALT* gut-associated lymphoid tissue, *NALT* nose-associated lymphoid tissue. (Modified from [11])

patrolling adjacent structures, above all beneath the alveolar epithelial surface. Similarly the GI tract is the seat of equally sound T-cell subsets [188]. There should be specific control mechanisms to restrict lymphocyte responses to antigens that are harmless to the organism. The concept of MALT is universally accepted: the cooperation with a variety of organs and systems has operated so that the mucosal immune system interacts with the peculiar properties of these organs, and has evolved exquisitely refined strategies to concert a balanced and differentiated regulation of T-cell activation, well suited for the specific requirements of the unique tissue microenvironments [79]. At birth MALT structures are absent: aggregates of lymphoid cells to be considered as MALT precursors have been found in the intestine [22] and the lungs [68] at about 14 weeks of gestation in the human fetus; lymphoid aggregates more identifiable with MALT appear about 7–10 days after birth and increase later on during childhood and adolescence [187]. Therefore, neonates should be prepared to deal with new problems such as the bacterial colonization of the gut, toxic by-products of bacteria and virus (enterotoxins and endotoxins) and intervention of exogenous factors. These substances, if allowed to penetrate the defense barrier, can cause either a local response (with a variable immune mediation), or potential dangers, both general or localized to distant shock-organs, with modality and severity linked to the type of immune response, not always univocal [19]. As a consequence, the first line of defense in order of time, including MALT, is against the absorption of food antigens: therefore the important neonatal adaptive changes to the extrauterine environment include the development of a mucosal barrier against the penetration of antigens and their fragments present chiefly in the intestinal lumen [187].

Immune Components of the Intestinal Mucosa

At 11 weeks of gestation, T cells migrate into the fetal gut; however, their numbers are low in the epithelium and lamina propria, where cells similar to macrophages and DCs are mostly present [133]. Between 11 and 24 weeks, APC markers and costimulatory molecules are seen within human fetal gut associated with expression of HLA class II (a), CD14 (b) and CD68 (c) (APC), CD20 (B cells) (d), CD83 (DCs) (e), CD40 (f), and CD86 (costimulatory molecules expressed by APCs) (g) and control (h) (Fig. 2.13a–h) [102]. Mature DCs are seen at 16 weeks, associated with immunoreactive CD86 [102]. By 14 weeks, isolated T cells and aggregates of B and T cells can be identified in the lamina propria and within 20 weeks distinct cellular zonation develops; plasma cells are absent [133]. Figure 2.14a–e [102] shows human fetal gut expression of CD3 (a), CD28 (b) CD40L (CD154) (c), and CTLA-4 (CD152) (d) and control (e). Surprising is the abundance of HLA class II and CD154 expression. Immunohistochemical studies of fetal small intestine have shown that lymphoid aggregates appear in the lamina propria at 14 weeks, and express $CD3^+$ and $CD4^+$ or $CD8^+$ antigens, with predominance of $CD4^+$ T cells, with a steady increase in the numbers after birth [199]. Intraepithelial lymphocytes (IEL) until 11 weeks of gestation are 0.03% of epithelial cells, at 11 weeks $CD3^+$ IEL can also be seen and by 17–19 weeks are 2%–5% of epithelial cells: only 50% of IEL express $CD8^+$, the remainders are $CD3^+$, $CD4^-$ and $CD8^-$ (about 10%), $CD3^+$, $CD4^+$ and $CD8^+$ (about 6%) and $CD3^-$, $CD7^+$ (about 12%) [133]. Heterodimer TcR consists of both αβ and γδ chains; the IEL γδ population mainly contains double-negative T cells; however, the number of γδ TcR is higher compared to adult levels (about 20%) [133, 199]. By 18–21 weeks, about 50% of IEL are γδ TcR [133]. CD3, CD4 and CD8 IEL are consequently very few in number in the fetal intestine [133]. We deem that low IEL numbers in infants and children denotes that IEL levels increase after birth and that antigen-driven stimulation is not the only factor leading to lymphocytes populating the intestinal epithelium [188]. *Peyer's patches* [163] (PP) in maturation (Table 2.4) do not comprise T or B lymphocytes, but HLA-DR aggregates and CD4 T cells: continuing the differentiation, by 19 weeks they contain distinct T and B zonations [199]. In the center, B cells are IgMs, IgDs and express CD5, surrounded by T-cell zones, mostly $CD4^+$; every cell is class II^+ [133]. At birth, PPs contain abundant lymphoid tissue, but there is no evidence of a germinal center (GC) and of secondary lymphoid follicle formation, also due to the low antigenic exposure of the intestinal mucosa (Fig. 1.43). This explains why B cells concentrate in primary follicles and are sparsely distributed in the lamina propria of the fetal intestine [199]. After antigenic challenge, both sensitized T cells and Ig-secreting precursor cells migrate from PPs to mesenteric lymph nodes, then they enter the systemic blood circulation via the thoracic duct. After further division and subsequent maturation, B cells migrate to the lamina propria where they differentiate into Ig-secreting plasma cells [179]. B cells with IgM are the first to appear and predominate during the early formative period, and later on B cells with IgA are prevalent; this difference is upheld, so that at age 2 years the ratio is 1:4 [114]: parenterally fed preterm neonates fail to develop Ig-secreting cells (IgSC), possibly due to their lacking specific stimulation [111]. B-cell definitive maturation probably involves regulatory T-cells, macrophages, and IL_{4-6}; several of these B cells apparently belong to clones of previous differentiating states, provided with J-chain (J = joining) (a polypeptide with MW = 15.6 kDa), synthesized by plasma cells, which binds to two H chains of IgA via covalent bonds independently of the Ig expressed [22]. After birth, GCs develop in PPs due to intestinal colonization from luminal bacteria: mucosal B cells are produced and during the first months of postnatal life, plasma cells originating from antigen-stimulated B lymphocytes

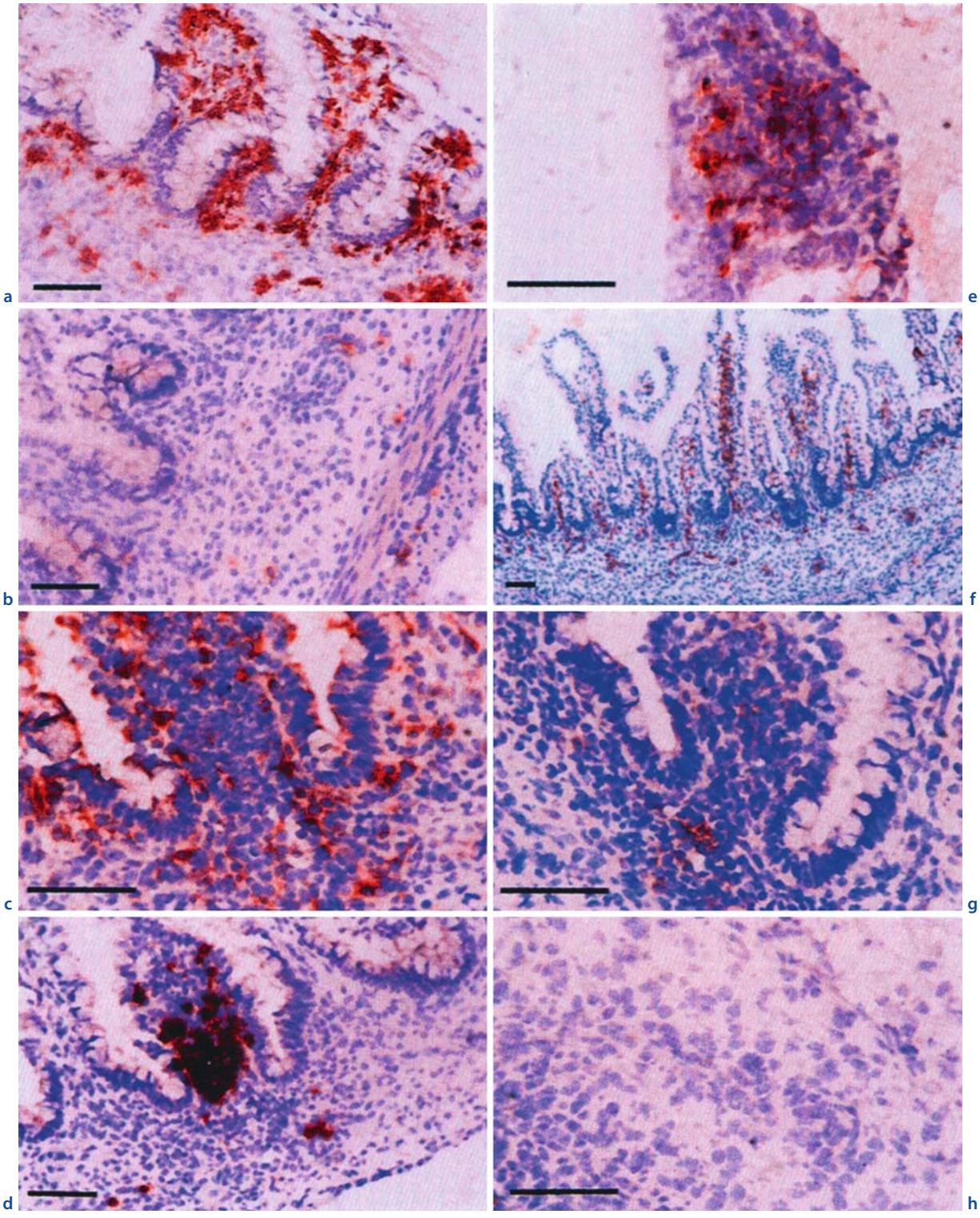

Fig. 2.13 a–h. Fetal gut samples at a range of gestational ages (11–24 weeks). APC markers and costimulatory molecules within human fetal gut. Immunohistochemistry was used to evaluate the expression of HLA class II (**a**), CD14 (**b**) and CD68 (**c**) (APC), CD20 (**d**) (B cells), CD83 (**e**) (DCs), CD40 (**f**), and CD86 (**g**) (costimulatory molecules expressed by APCs). An isotype-matched control was always included (**h**). Scale bar, 0.2 mm

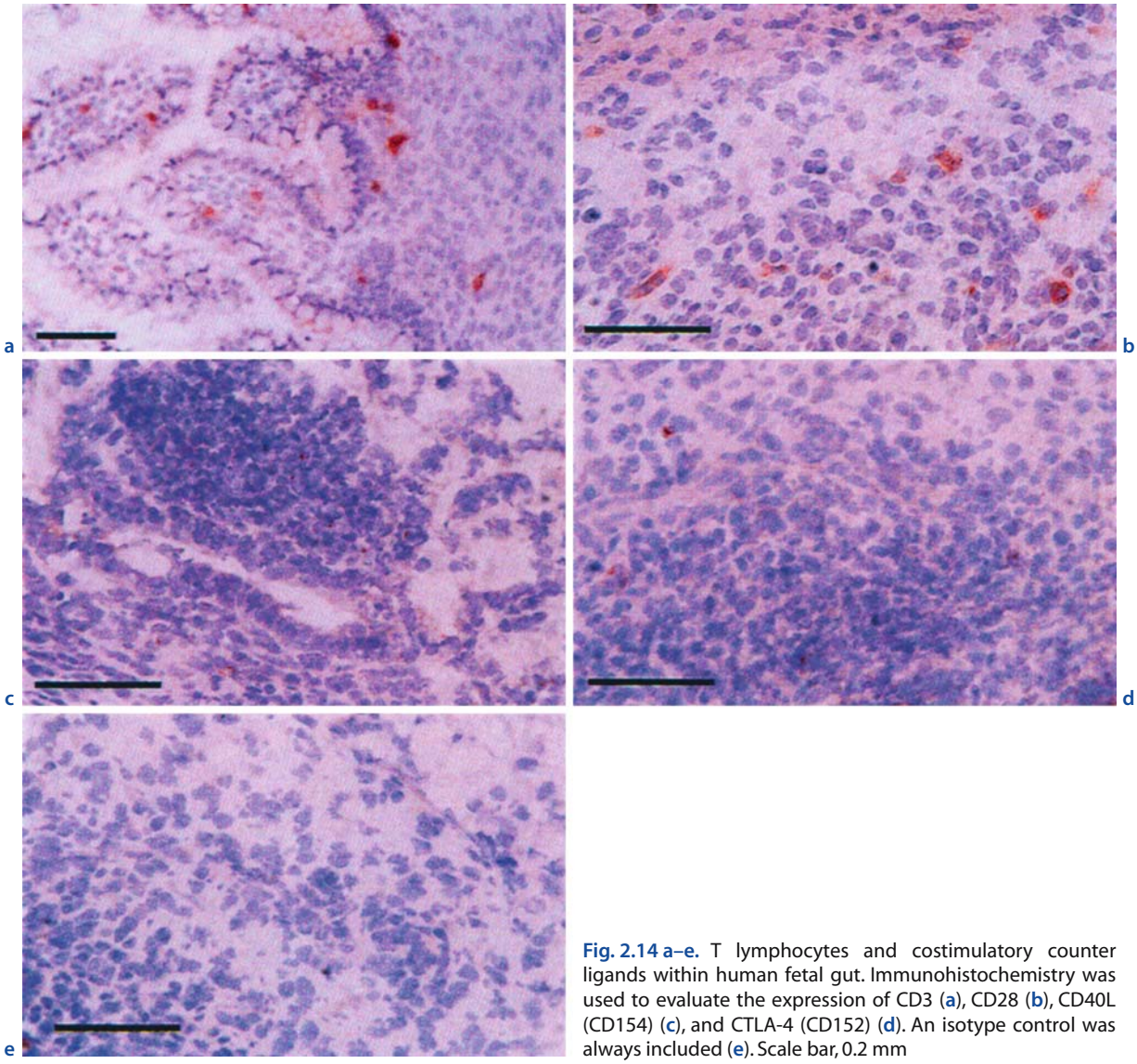

Fig. 2.14 a–e. T lymphocytes and costimulatory counter ligands within human fetal gut. Immunohistochemistry was used to evaluate the expression of CD3 (**a**), CD28 (**b**), CD40L (CD154) (**c**), and CTLA-4 (CD152) (**d**). An isotype control was always included (**e**). Scale bar, 0.2 mm

populate the lamina propria. GCs, along with lymphoid follicles, at about 12–24 months reach levels comparable to adult levels, but plasma cells only at about 2–10 years [199].

The report of IgG+ and IgA+ antibodies in fetal intestinal tissues suggests the *fetal origin of B-cell differentiation* independent of antigenic stimulation, a fetal immune response to an intrauterine infection, or an immune response induced by anti-idiotype antibodies from the mother [141]. The fetus responds to infections by the early production *in utero* of IgA and IgM antibodies; moreover, the striking finding of sIgA and sIgM (secretory) to *Escherichia coli* 0 and poliovirus type 1 in amniotic fluid and meconium, urine and saliva already at birth speaks in favor of the hypothesis of a *fetal isotype switch* [141] in response to the transplacental passage of IgG anti-idiotype antibodies [74]. Therefore, since it is unlikely that the fetus has been exposed to poliovirus antigen *in utero*, the intervention of idiotype/anti-idiotype anti-poliovirus antibodies of maternal origin is possible [141]. However, the report of aggregates of B_{IgA} lymphocytes confirms that isotype switch of B lymphocytes occurs *in utero*, as demonstrated by the finding of sIgA and sIgM in the saliva of neonates of hypogammaglobulinemic mothers [73]. These observations have moved the experimental studies on *maternofetal immunology* far forward, establishing that the amniotic fluid from an IgA-deficient mother contained IgA antibodies, which were also found in the neonate, thus suggesting a fetal origin: we emphasize that in Sweden where the study was conducted, an inactivated poliovirus vaccine has been used for many years [73]. The detection in CB of anti-idiotype anti-poliovirus antibodies suggests that once transferred via the placenta they can prime specific immune responses in the fetus [138]. However, anti-idiotype antibodies have

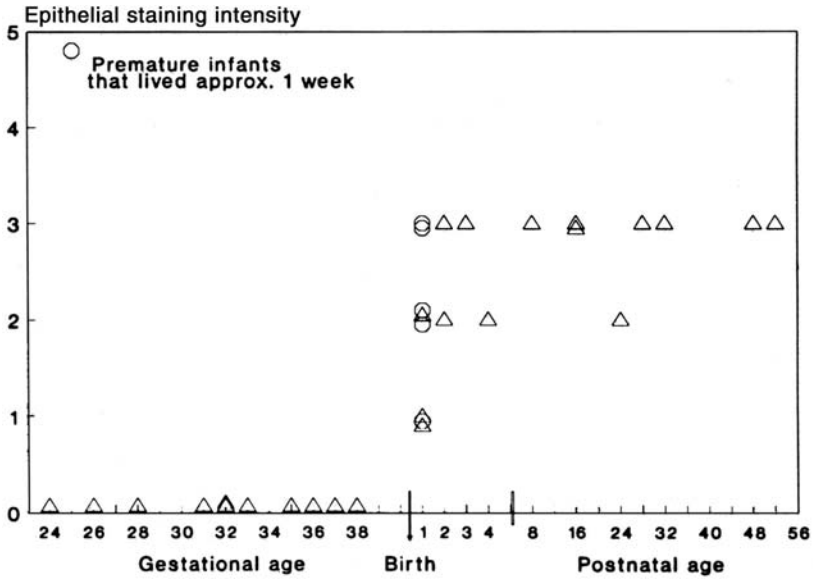

Fig. 2.15. HLA-DR expression in tracheal wall according to gestational and postnatal age

been found also in maternal serum and milk [74]: anti-idiotype anti-poliovirus and anti-*Escherichia coli* antibodies transferred from the mother can therefore actively induce specific immune responses in the fetus without previous exposure to transplacentally transferred antigens, or by being passively transferred to the newborn via breast milk [74]. Such a transmission can have two-way effects: it can either enhance the ability of the fetus and young infant to mount a protective immune response or increase the risk of developing allergy during early life [8, 74].

At birth, both T cells and accessory cells of the mucosa are comparatively better developed; in the lamina propria several HLA class II^+ macrophages and DCs are identified. Furthermore, $CD3^+$ T lymphocytes are identified both in the lamina propria and at the epithelium, crucial in the postnatal period when the intestinal epithelium permeability is greater [187]. Activation of lamina propria T lymphocytes elicits their proliferation and increases lamina propria density and IEL numbers [205]. The source of mucosal T cells during the postnatal period is unclear: studies in the animal model indicate that certain IELs develop from T cells activated by antigens within PP. Subsequently, T cells, through a pathway similar to that of B lymphocytes, recirculate back to the lamina propria [205]. When class II^+ macrophages are recruited in sufficient number, in the proximity of T $CD4^+$ cells, intraluminal antigens may trigger reactions of T cells [188]. Recent evidence has demonstrated that the fetus does not express immunologically regulated epitopes because no expression of HLA class II antigens is detected on the fetal intestinal epithelium or that of 1-day-old neonates [179], but HLA class II^+ cells were detectable from 11 weeks of gestation [102] and HLA class I expression is present [179]. From the 2nd postnatal week, HLA-DR molecules may appear in the intestinal epithelium to interact with CD4-antigens, apparently reflecting a response to environmental substances [174, 204] (Fig. 2.15), even if class II complexes are not yet uniformly distributed [174].

Immune Components of the Mucosal Barrier

An important adaptation of the neonatal GI tract is the development of a mucosal barrier opposing the transmucosal passage of antigens and antigenic fragments, which is an elaborate system of immunological and nonimmunological defense mechanisms, at the very critical moment when the local and systemic immunity is still immature, to combat potential dangers such as the bombardment of microorganisms and the invasion of antigenic substances. The immunological components are either humoral, sIgA sIgM, IgA, etc., or cellular, lymphocytes, mast cells, neutrophils, macrophages and eosinophils of the gut mucosa, cells of the mucosal coat and reticuloendothelial system (RES) and potential erythrocyte growth factors, among which EGF is in the first line [42] (Table 2.11) [19, 33, 42]. Therefore, the local immune system plays a crucial role in the local defense against infectious and food antigens. Above all, it has been postulated that sIgA may block the uptake of ingested antigens, and that IgA, IgG and IgM make it possible to eliminate the macromolecules penetrated into the gastroenteric tract.

There are *two IgA subclasses,* namely IgA_1 and IgA_2, accounting for 90% and 10%, respectively, of the daily serum IgA, but because of GALT B-cell expansion, there is a shift toward more IgA_2 production in postnatal salivary glands in the first weeks of life; during the first 3 months after birth the IgA_1/IgA_2 ratio approaches the normal adult value [22]. This may imply an ongoing postnatal influx of IgA precursors from PPs where the IgA_2 subclass predominates [22]. The sIgA system is

Table 2.11. Immunological and nonimmunological defense

Immunological defense
1. Humoral immune system
Secretory IgA and IgM antibodies
Serum IgA and IgG antibodies
2. Cellular immune system
Lymphocytes organized in follicles
Lymphocytes localized in the lamina propria
Intraepithelial lymphocytes
Additional cells of the intestinal mucosa
Mucus layer
Eosinophils
Macrophages
Mast cells
Neutrophils
Cells of the reticuloendothelial system
Potential growth factors for human intestinal cells
EGF
Insulin-like growth factor I
Insulin-like growth factor II
IL_2
Insulin
PDGF
TGF-α
TGF-β
Nonimmunological defense
1. Block of the entrance of ingested antigens
Ciliary activity
Glycocalyx
Intestinal flora
Microvillous membrane
Mucosal coat
Peristalsis
2. Breakdown of ingested antigens
Digestive enzymes
Gastric acids and biliary salts
Kupffer cells
Lysozyme of intestinal epithelial cells
Pancreatic enzymes
Saliva

Data from [19, 33, 42].
EGF Epidermal growth factor, *PDGF* Platelet derived growth factor, *TGF* Transforming growth factor.

expressed in higher proportions in secretions, especially in the large bowel (59%) and mammary (37%) and salivary (34%) glands. Additionally, IgA_2 of the GALT tends to produce the J chain with a greater frequency than sIgA [193], until both IgA immunocytes express elevated levels of J chain (94%–97%) [22]. Such increased results are extremely useful since IgA_2, at variance with IgA_1 immunocytes, by strong, noncovalent bonds with SC, are highly resistant to bacterial proteases, rich in the gut [187]. Because of its unique structure, sIgA is thus able to adversely affect proteolytic enzymes while carrying the immune functions of enteric mucosa. sIgA immunocytes have a dimeric or polymeric form, unlike the monomeric form of serum IgA antibodies: the J chain appears to facilitate IgA polymerization [22]. SC is a glycoprotein found on the basal and lateral aspects of local epithelial cells. SC has receptor characteristics, belongs to IgSF, its gene is located at the long arm of chromosome 1 and binds to dimeric IgA [188], generated by IgA-producing plasma cells [22]. J-chain-containing dimeric IgA is transported actively by transmembrane SC via a process of endocytosis, from the luminal face to the mucosal layer of intestinal epithelial cells, and is then transferred into secretions [22]. SC is an integral part of the latter cells' membranes: in particular it plays a crucial role in the bowel lumen, protecting IgA molecules from degradation by enzymes and toxins [188]. IgM and IgG plasma cells bind neither to SC nor to J chain, which is produced and degraded by these Igs more frequently than the cells of peripheral lymph nodes [193].

However, at birth the maturational immaturity of the GI tract is unmistakable: only IgG of maternal origin are seen and other IgG are received by breast-milk-fed neonates [33]. Serum IgGs are reduced in infants beginning in the 3rd month of life: in children aged 2–15 they represent only 5% of total Igs (vs 80%–90% of IgA immunocytes). IgG_1 is the preponderant subclass in normal ileal and colonic mucosa (as well as in the airways and in normal serum), while IgG_2 cells are usually more abundant (20%–35%) than IgG_3 cells (4%–6%) in the distal gut mucosa, at variance with the levels observed in the upper airways [186]; such disparities may be influenced by different local stimulatory agents [206]. IgA, appearing late in both phylogenesis and ontogenesis, are undetectable at birth compared to the other Ig classes and sIgA in the usually sterile intrauterine environment [170]. Three studies have examined Ig-producing cells in the trachea [204] and intestinal mucosa [174, 179] in neonates aged 1 day [179] and by 24 weeks of fetal life until the first postnatal months [174]; however, in 1- to 5-day-old infants no Ig immunocytes were detectable [179, 203]. A few IgM and IgG immunocytes were present during the entire period investigated: by 2 weeks of life some B_{IgM} cells were observed in the lamina propria of neonates [174] and the first B_{IgA} cells appeared [204]. IgSCs are scarce (mostly B_{IgM}) within the first 5 days of life, but by 1 month the levels are increased in 77% of infants, primarily B_{IgA} with levels at 0.8 IgSC/10^6 PBMCs (mean 1±1) [203]. Thus, IgA immunocytes are present by 2 weeks of extrauterine life (Fig. 2.16) [204] (Fig. 2.17) [174], increasing parallel to IgMSC [174], until the levels were similar at 6 weeks of age [174]; IgM antibodies slowly increase (Table 1.15) at about 5–6 months of life. However, within the 1st year of life, IgM antibodies predominate on the other isotypes, whereas in the adult intestine the ratio IgA: IgM: IgG is 20: 3: 1 [187].

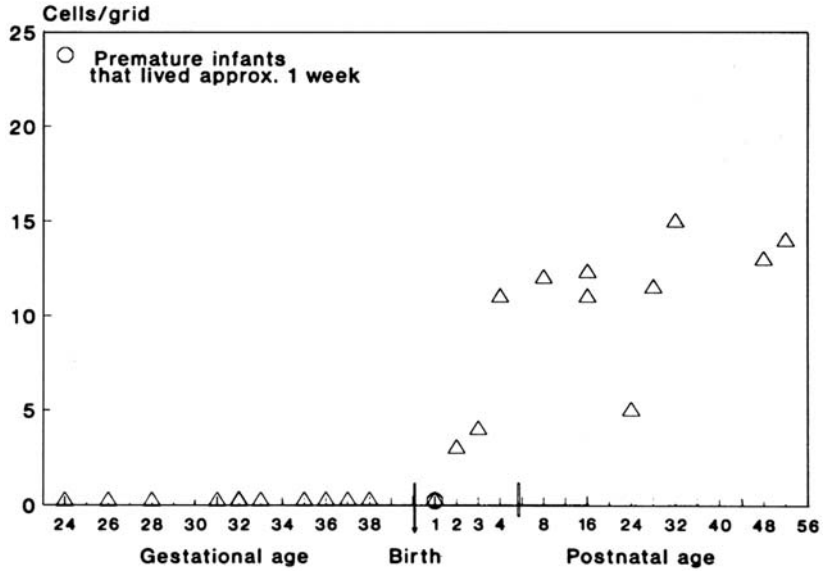

Fig. 2.16. IgA expression in tracheal wall according to gestational and postnatal age

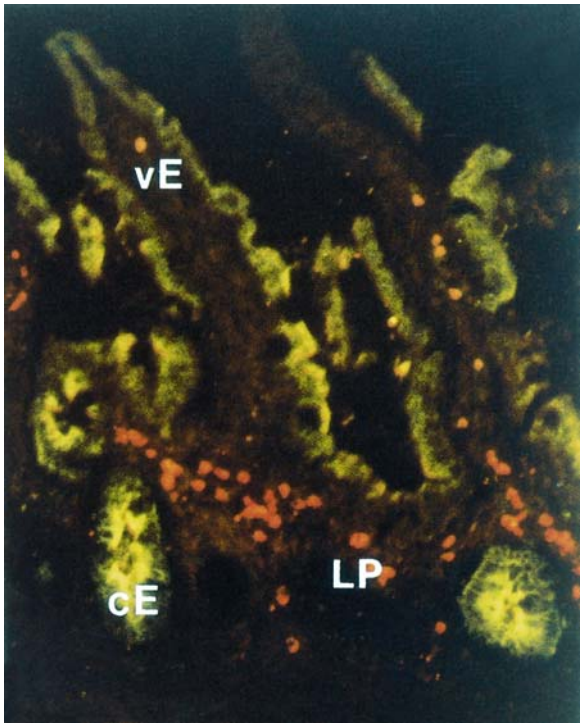

Fig. 2.17. Duodenal mucosa of a 12-week-old infant. The cells stained green for SC and red for IgA (colocalization). *cE* crypt epithelium, *LP* lamina propria, *vE* villous epithelium

For the first 6 months, there is an admixture of IgD-producing cells in the parotid glands, highlighting the dichotomy of MALT between NALT and GALT [22]. The levels of sIgA increase by 1–2 weeks of life [140, 190] and mean adult values are approached by 4 weeks in 92% of infants [190] or by 2 [25] to 12 [140] months, certainly after local exposure to antigens. Using more sensitive methods, IgA_2 antibodies in saliva are <15% 2 weeks after birth and by 6 months 24.4% of total sIgA, with 15.5% of maternal levels by 4 months [58]. CD_{154} expressed by colostral T cells could trigger isotype switching to IgA and sIgA [13]. Recently, salivary sIgA were found in 75% of neonates aged one day (190.2 mg/ml) and in 77.1% of neonates aged 2–10 days (216.4 mg/ml) [179]. In turn, IL_6 has a key role in the conversion of IgA into sIgA [138]. A study on salivary and fecal IgA antibodies has recently found measurable IgA levels during the 1st week of life. The levels peaked at the age of 30 days but decreased and remained stable from 3–8 months, but no differences were found between breast-fed and cow's-milk(CM)-fed infants. However, the ratio of fecal IgA was reversed [116]. At 2 weeks of age an increase in SC levels was observed, concluding that SC synthesis seems to be independent of B lymphocytes: its increased expression shortly after birth along with HLA-DR and $CD3^+$ T cells suggests that T-cell-derived ILs may be responsible for the induction of both SC and HLA-DR [204]. The SC-mediated selective epithelial transport of dimeric IgA and pentamer IgM antibodies ensures a first line of defense against environmental factors active in the first 3–4 weeks of life [22]. Moreover, *sIgA existing in colostrum and breast milk* exclude macromolecular absorption, facilitating their removal via the feces, thereby elaborating a unique immune system passively protecting the vulnerable neonate before the development of the mucosal barrier and the provision of secretory Igs [218]. The endogenous synthesis of sIgA antibodies starting before the appearance of both serum IgA and food antigens is likely antigen-driven, resulting from the colonization of the mucosal surfaces by commensal bacteria, and represents a sufficient stimulation for sIgA levels, which gradually increase in the first months of life [58]. Serum IgA antibodies develop more slowly, attaining adult levels only at about 10 years of life (Table 1.15), but breast milk may have a stimulatory effect on IgA production in neonates and/or may favor IgA concentration in the colon [116].

The more rapid maturation of IgA antibodies suggests that they play a positive role that is more important than serum sIgA antibodies, at least in the first periods of life. An indirect support of sIgA antibody effectiveness comes from the knowledge that in subjects with IgA-selective deficiency there is a steady increase in sIgM titers to food antigens [214]. However, it seems paradoxical that newborns are deprived of secretory Igs in the first days of life, when this immune and anti-infectious protection is of crucial importance [114]. It is of great significance that in the saliva of neonates IgM antibodies are also included, IgM with the SC glycoprotein, probably delivered to secretions over the pathways normally used by sIgA antibodies; thus when IgA are scarce or absent, the transport mechanism becomes available for IgM antibodies. In spite of their poor stability compared to sIgA, sIgM antibodies may play an active role in the mucosal defense until sIgA is up-regulated upon repeated antigenic exposure [170].

Furthermore, it has been demonstrated that *low sIgA levels may be associated with an increased risk of atopic disorders* [135], and that *colostral sIgA and sCD14* [184] *levels are significantly reduced in the mothers of neonates developing CM allergy (CMA)* compared to mothers of nonallergic children [185]. The sIgA increase from 15.5% to 52% of maternal levels was more rapid in exclusively breast-milk-fed infants compared to bottle-fed infants [58]. The prominent localization in both the stomach and the intestine acquires a particular significance, since activation of mucosal immunity should protect the underlying tissues without inducing inflammatory responses damaging the fragile epithelium [22]. At this level, particular emphasis is placed on the polyvalent sIgA and its striking avidity for antigens. sIgA are equipped with four paratopes (which are formed by dimers), thus making sIgA more effective than serum IgG and monomeric IgA antibodies in the agglutination mechanisms; also lacking opsonizing activity, they do not induce phagocytosis mediated by local and/or circulating macrophages, nor do they induce the classic complement activation devoid of the pertinent receptors [170].To perform this job, the mucosal immune cells acquire several properties *peculiar to mucosal sites* [57]. Therefore IgA molecules transported across the epithelial cells of the mucosa are able to prevent both invading organisms and potential macromolecular antigens from adhering to or penetrating the mucosal surface [186].

sIgA spread within the coating mucous film to constitute a protective *antiseptic paint,* so to speak, which neutralizes the invading virus, interferes with bacterial colonization, prevents the uptake of heterologous macromolecules and plays a role of immune exclusion since binding to sIgA blocks intraluminal antigens and prevents their transport across the epithelial barrier [19]. As a consequence, foreign molecules lose their ability to trigger immune reactions also in sensitized subjects, and since they preclude their absorption, are finally digested by intraluminal proteolytic enzymes [205]. The high concentrations of sIgA and IgA cells in the MALT milieu attest to their local significance [218]. As stated above, the absence or paucity of sIgA cells in babies with CMA is perhaps a consequence of lacking or poor colostrum and breast milk ingestion, which may provoke a statistically significant reduction in antibody-producing cells when compared to regularly breast-fed infants [193]. sIgA rise in number in children acquiring CM tolerance [92].

Much interest has focused on sIgA antibodies, synthesized in great part by MALT B_{IgA} lymphocytes, present in several mucosal tissues, including BALT, which supports the existence of a common mucosal immune system [19]. *A chief role is played by T cells:* it has been demonstrated in animal models that B_{IgA}-cell precursors are sensitized by antigens in the lymphoid tissues of gut mucosa (PPs), then migrate to mesenteric nodes for further maturation, enter the systemic circulation and are guided by homing receptors. Independently of the meeting point, they recognize the HEVs (high endothelial venules) (Fig. 1.3) along mucosal surfaces of the intestinal, respiratory, and urogenital tracts and mammary gland, where they produce IgA-secreting plasma cells, due to CD4 T-cell cooperation [188] (*enteromammary axis*). Figure 2.18 [198] depicts this immune system, a very prominent and unique means of delivering maternal IgA to protect the immature surfaces of infant gut [19, 198]. Lactogenic hormones may promote the circulation of B_{IgA} cells toward mammary glands, thus synthesizing sIgA cells produced against a large variety of antigens previously penetrated in the intestinal lumen [26]. By a reverse and complementary route, mammary gland plasma cells release sIgA against heterologous substances present in the maternal gut [198]. As a demonstration of the close relationship between GALT and mammary glands, it was found that the TcR-$\gamma\delta^+$ proportion was twofold to fourfold greater in the mammary gland than in the bloodstream [15]. Moreover, 85%–95% of colostral TcR-$\gamma\delta^+$ co-expresses CD103 (HML-1) [12]: thereby, such TcR-γδ, differently from the bloodstream where CD103 are absent, have a phenotype similar if not identical to that of intestinal TcR-γδ. This finding suggests that both types may come from common precursors of MALT or in turn that a lymphocyte homing process, including TcR-γδ directed there, may occur between the gut and the mammary gland over the last trimester of pregnancy and the whole breast-milk feeding period [15]. The concept of an enteromammary axis is also indirectly confirmed by a series of animal experiments that have noted that early milk leukocytes are able to resist digestion with trypsin, a characteristic that allows them to survive in the GI tract [16, 93], and that orally administered milk cells are measurable in the gastric and duodenal content of neonatal mice, and were shown to cross the gut mucosa and enter the circulation and the spleen of neonatal lambs [16, 93].

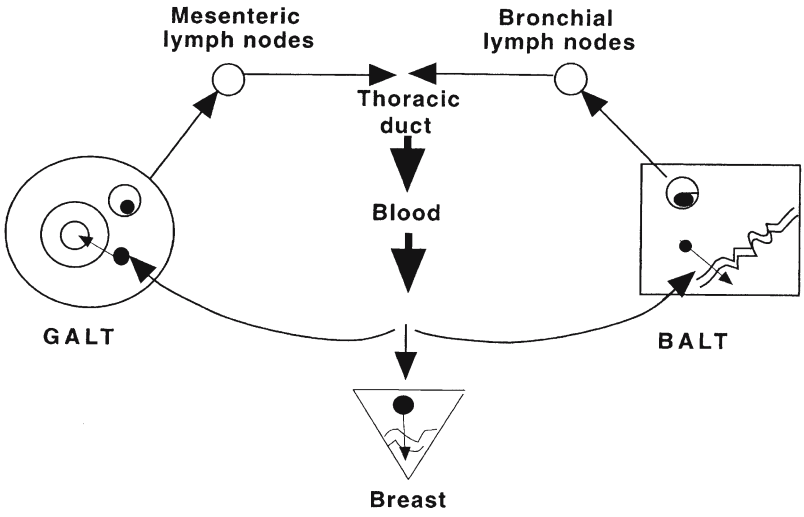

Fig. 2.18. A diagrammatic view of the enteromammary axis. *GALT* gut-associated lymphoid tissue, *BALT* bronchus-associated lymphoid tissue. (Modified from [198])

Maturation of gut mucosa B cells is stimulated by CD4 and depressed by CD8 T cells; both subpopulations are not static, but by complex circuits remain in a state of dynamic equilibrium: the CD4 T cells on the one hand stimulate B cells and on the other hand activate CD8, which inhibits antibody responses (feedback suppression) [26]. Several lines of evidence suggest that CD4 and CD8 specific for the diverse Ig classes acquire a particular finality between the various highly specific immune regulator mechanisms characterizing MALT [19, 206]. To enhance the selective production of IgA antibodies, T cells coming from GALT promote in B lymphocytes the isotype switching from IgG and IgM to IgA antibodies, with a double duty of assuring a lesser absorption of antigens and preventing systemic reactions due to complement activation [57]. A regulating role is also played by breast milk through proteins present there, with the capacity to both stimulate and suppress T-cell proliferation. Above all the suppressor effect may influence the depression of systemic immune responses, in synergy with the contemporaneous GALT activation [145]. Within oral tolerance, much interest is focused on the expression of suppressor activity of both CD8 cells and HLA class II molecules, in addition to surface epithelial cells possibly inducing anergy/apoptosis of Th2-like $CD4^+$ lamina propria T cells (Chap. 1). Moreover, *the circuit of T contrasuppressor cells,* moves from PPs [57], or the TcR-γδ IEL population [188], to prevent the CD4 T-mediated suppression, thus releasing IgA (and possibly IgM) immune responses. The outline may be completed by T cells with *suppressor effects on IgE synthesis* [188]. In conclusion, the intestinal barrier regulation against antigenic macromolecules involves an intricate network of cellular interactions that ultimately could favor secretory immunity, via the above IgA-associated contrasuppressor activity [57].

Food macromolecules can be taken up intracellularly – through epithelial or membranous cells – or intercellularly – via the tight junctions. Endothelial cells lead to absorption via the process of *endocytosis* (by which the greatest part of antigens are processed) from the *microvillous membranes* (MVM) [41]. Polypeptide macromolecules are therefore surrounded by MVM as microvesicles that then fuse originating, as stated earlier, phagosomes that fuse with lysosomes, creating phagolysosomes, where the macromolecular constituents of the ingested particles are degraded. However, some unmodified macromolecules escape the final digestion and via *exocytosis* are released into the intercellular space. Hence, the barrier can be crossed by food antigens via *microfold cells (M cells)* [181], membranous epithelial cells overlying PPs (<1% in humans) derived from Lieberkühn crypts. They have few microvilli (in the gut) or vibratile cilia (in the airways), a richly vacuolated cytoplasm, an only slightly developed glycocalyx and few lysosomes able to endocyte macromolecules that transport to PP lymphoid tissue [206]. The number of M cells is low in humans, but they perform important tasks, since they can contribute to the uptake and processing of polypeptide macromolecules and transfer of antigenic information to immunocompetent cells, thus playing the role of connection between the gut epithelium and subepithelial system [170]. M cells also transport HIV-1 [182]. However, the follicle-associated epithelium overlying PPs does not secrete IgA, so there is a gap in the barrier and, despite the ongoing sIgA response, a microbial invasion is likely [22]. The tight junctions are impermeable in healthy intestine, but if they suffer from pathological insults during the inflammatory processes, they loosen and become leaky, thus facilitating the passage of antigens and their fragments [187]. The macromolecular degree of absorption is correlated to the antigen concentration in the bowel lumen: so the early bottle-fed infants are subjected to a huge antigenic assault; consequently *pinocytosis* is extended to all mucosal epithelial cells, paralleling the massive penetration of undigested macromolecules [182]. If the first line of barrier defense is disrupted, both local IgG production and serum IgG antibody exudation are increased: if these critical defense mechanisms are insufficient, additional

defenses made up of the nonimmunological components are ready to take on these functions [33].

Nonimmunological Components of the Mucosal Barrier

The factors constituting the second line of mucosal defense, working independently and in concert with the local mucosal immune system, enter the circulation to control the proliferation of microorganisms passed into the gut, augment their adhesion to limit the absorption of antigens able to overcome the local immunological defense mechanisms and to dismantle the mucosal barrier (Table 2.11). In concert with the elaborate local immune system already developed, these factors make the gut impermeable to CM proteins, thus preventing the upregulation of immune reactions in the majority of cases, despite the presence of IgE. The defense factors, all of which help to provide maximum protection, include [33]:

- *Gastric barrier* and enzyme content of the GI tract. Neonates have a physiological deficit of such factors: the achlorhydria, facilitating the microbial proliferation and the enzyme deficiency, predisposes to a reduced breakdown of food macromolecules, thus favoring antigen absorption.
- *Gut microflora.* It is formed by two different populations: one autochthonous population proliferating in a stable way, and one allochthonous population proliferating only following the ingestion of particular foods. Along the enteric tract, true ecological niches are found, which, depending on the type of microorganism, are characterized by optimal conditions different for their development. Bacteria can attach to mucosal surfaces via their structures provided with antigenic power; the acidulous bacterial flora in particular plays a crucial role in preventing or limiting the gastroenteric infections that facilitate allergen access [76]. For example, lactobacilli, such as *Lactobacillus bifidus* predominant in breast-milk-fed neonates' microflora, are able to produce antibacterial substances and/or prevent microorganism growth competing with receptors or nutritive factors [84]. However, differences in the composition of the gut microflora might play a role in the allergy prevalence.
- *Peristaltic movements:* a physical barrier to prevent bacterial proliferation, likely to be present in case of intestinal content stasis. Moreover, conditioning the duration of the transit time of a food bolus plays a primary role in the function of intestinal absorption of nutrients [218].
- *Intestinal surface* secretions include:

1. The saliva, which contains lytic enzymes (lysozyme) and polymers, limits the bacterial attachment to epithelium.
2. The chlorhydropeptidic gastric secretion contributing to fragment and denature intraluminal antigens.
3. The pancreatic and enteric proteolytic enzymes.

- *Mucosal surface.* This surface, consisting of the MVM and its overlying mucus layer, forms with secretions a glycoprotein layer on epithelial cells, an important protective barrier acting as a mechanical obstacle to foreign antigens and bacteria, incorporating and removing them from intestinal walls. However, in the neonatal period specific phospholipid changes may contribute to a disturbance of the protein/phospholipid ratio of the MVM membrane [41], of the composition and thickness of the mucus protective layer [218], and might interfere with defense functions (Chap. 3). Likewise malnutrition may reduce secretions and bottle-feeding may reduce the intrinsic qualities of the mucus, thereby reducing the mucosal barrier defense function, a clinical disease state unknown to breast-milk-fed neonates receiving mucin with more fucose and glucosamine and fewer sulfates compared to bottle-fed neonates [213].
- *Liver filtration* of luminal antigens: the liver, with *Kupffer cells,* is the second line of mucosal defense, in order of importance, after sIgA and the anatomical, functional and enzymatic systems of intestinal mucosa. All foreign substances taken up by the bowel gain access to the portal circulation: the more active and ready the clearance capacity is, the more harmless the antigenic aggression is [218].

Several observations suggest that local immune defenses contribute widely to protecting the host against potential dangers of aggression across the mucosal barrier, especially when they operate in a mutual accord. To understand the complex atopy pathogenesis, we consider evaluating the host general immune reaction of prime importance [19, 33].

Additional Defense Factors

Some *aspecific factors of defense* protect the intestinal surface when the defense mechanisms of the complicated milieu of the intestine are incompletely developed to prevent antigen aggressions. They act by expanding specific immune reactions thanks to substances with aspecific inflammatory action, such as ILs generated by activated T lymphocytes, mast cell and basophil chemical mediators and proteins with enzymatic action belonging to the complement cascade. In case the expansion of immune responses is dramatically heightened, these aspecific factors can provide the basis for the onset of hypersensitivity reactions, normally inhibited by sIgA [218].

Nutrition and Absorption of Antigenic Macromolecules

In neonates, especially in preterm neonates, the sophisticated system of *oral tolerance* is not yet completely developed, and is linked to a network of unique and

elaborate immune mechanisms that should ensure simultaneously a local strong antibody barrier and a block of the dangerous systemic overflow of Igs and therefore of CICs [57]. The intestinal mucosa, particularly in the first weeks of life, is physiologically vulnerable to pathological penetration of potentially harmful macromolecules, and is consequently susceptible to hypersensitivity reactions [33], a significant result since both neonates and preterm infants absorb more food allergens than adults. Bottle-fed neonates have an increased permeability to β-lactoglobulin (βLG) [118] and preterm infants may develop a CM sensitization more frequently than term infants [129]. In neonates, the rapid substitution of breast milk with CM led to concentrations of anti-CM protein IgG significantly higher than those found in gradually weaned infants; the results further confirmed how important breast milk sIgAs are in developing a protective role within the intestinal mucosa, reducing the absorption of detrimental antigens [78]. Irreconcilable conclusions are suggested by a methodologically correct study using questionnaires after 11 years [186].

Immunology of Breast Milk

Even during recent years it was presumed that breast milk was devoid of immune factors [26]. The relations between immunological factors, including ILs, EFA, and eicosanoids in breast milk, may influence the delicate balance of the gut immune milieu of the infant and thus may have health effects on the breast-fed infant [119]. It is increasingly manifest that breast milk contains a wealth of immune factors, which are designed to nourish and protect the vulnerable newborn during the critical postpartum period. Thanks to the *mammary gland, a true immune organ* [16, 67], breast milk is an excellent immune protection for neonates during the critical period of intestinal vulnerability, it is a remarkable protection against the dangers of a deficient intestinal defense system, based on unique immunological, anti-infective, anti-inflammatory and immunomodulating factors functionally interacting among themselves (Tables 2.12, 2.13) [12, 16, 27, 61, 65, 67, 80, 94, 105, 107, 119, 142, 153, 178, 198, 228, 231].

- Together with colostrum and in addition to T and B lymphocytes and Ig, *breast milk* provides IFN-γ, complement components and other bioactive molecules to protect against bacterial and viral infections [169]; it *is rich in vital cells (up to 10^6/ml),* thus ensuring a continuing supply of factors with an important immune role in the defense of the vulnerable infant. IgA and sIgA antibodies inhibit or prevent the penetration of harmful luminal antigens [135]. IgA and sIgA have the potential to lessen or abrogate possible hypersensitivity reactions [16, 27], with levels up to 0.5–1 g/daily or 0.2–0.3 g/kg of sIgA [228], in addition to anti-infective factors to protect infants against pathogens during the critical period, such as anti-VRS sIgA [77]. Accordingly, breast milk stimulates IgA and sIgA secretion, thus actively facilitating the maturation of the neonatal immune system, unlike bottle-fed neonates [5]. It appears that sIgA are significantly more elevated in the saliva of breast-milk-fed babies [58], whereas the production is developmentally delayed in the recipient baby: 4 weeks to 12 months are needed for sIgA maturation, with 1–2 years for lysozyme and about 2 years for memory (CD45RO) cells [67] in children deprived of *both breast milk and colostrum.*
- *Idiotype/anti-idiotype antibodies* may have lasting effects on the offspring immune system, activating both B- and T-cell clones, and thus priming protective immune responses [78], also inducing tolerance to environmental antigens such as food antigens [78, 79]. In 4-month-old breast-milk-fed infants, such antibodies generate serum and secretory responses to vaccines (tetanus and diphtheria toxoids) statistically more significant than in bottle-fed babies [75], and can also explain the occasional CB findings of IgE antibodies to several antigens [73, 140].
- *EFA,* polyunsaturated (PUFA), long chain (LCPUFA) C20 and C22: arachidonic (AA, 20:4ω6), docosahexaenoic (22:6ω3), dihomo-γ-linolenic (DGLA 20:3ω6) necessary also for intellectual development [43], notably the ratio 18:2ω6/18:3ω3 allow the incorporation of 22:6ω3 into the neonatal neural tissue and the retina [69]. The levels shown in Table 2.14, higher than the levels in control babies and in infants fed supplemented formulas [5], regularly absorbed [89], are present in breast milk at least over 8 weeks with a ratio ω-6:ω-3=5:1 [49]. ω-6 EFA are twice the amount of ω-3 in the brain and 1.3 times in the retina [69], linoleic acid (ω-6 series) and its isomers form collectively the conjugated linoleic acid (CLA) with levels between 0.2% and 0.4%. CB studies suggest a preferential and selective maternofetal transfer of LCP [113]; therefore the breast-milk-fed premature infants receive a higher LCP supply [131] and overall a threefold higher dietary LCP phospholipid concentration than LCP-enriched formulas, apparently because the infants transform dietary LCP into structural lipids, sparing them from oxidation [113]. Supplemented formulas may improve visual acuity in infants fed so [43]. Table 2.15 [49, 113] lists the factors possibly influencing LCP metabolism. Although EFA levels are variable, taking into consideration the marked differences in methods and dietary composition of the population studied [113], most EFA levels are comparatively similar in several European studies [70, 113]. Generally, ω-6 PUFAs, as well as eicosanoids derived from them, are considered proinflammatory, whereas ω-3 PUFAs are considered anti-inflammatory [30]. However, evidence is accumulating to suggest that ω-6 fatty acids may actually be anti-inflammatory via the effects of Th3 or Tr-1 (T-regulatory 1) cells and thus via TGF-β_2 and IL_{10} production. Recent results suggest that in addition to ω-6 fatty acids, the proportion of total PUFA and of total saturated fatty acids may be an important regula-

Table 2.12. Breast milk components with immunological, anti-infective, anti-inflammatory and immunomodulating activities

Components	Properties
sIgA 240 µg/mg/protein/day until 3rd day, ≈20 µg/mg/protein/day from 15 days to 6 months	Does not activate complement, suppresses PMN chemotaxis, blocks adhesion of microbial pathogens, prevents infections, limits the allergenic penetration
IgM about 100 mg/day	Activates complement, forms antibodies against bacteria and virus, retains opsonic activity after traversing the intestinal canal
IgG about 70 mg/day	Activates complement, has heat-stable opsonic activity, blocks toxins and virus
IgG subclasses	Anti-infective activity
IgD	Forms antibodies against bacteria
α-2-Glycoprotein associated with pregnancy	Inhibits the lymphocytes, lymphocyte blastogenesis, ADCC, and immunoglobulin production
Antioxidants: ascorbic acid, cysteine, β-carotene, α-tocopherol	Contrast superoxide production
Arylsulfatase	Degrades leukotrienes
Catalase	Degrades H_2O_2
Cytokines [61, 80, 178, 231]:	
IFN-γ (0.5 UI/ml)	Increases chemotaxis and opsonization, enhances Th1 and antagonizes Th2 cells
IL_1 (1 ng/ml)	Activates T lymphocytes
IL_6 (50 pg/ml)	Enhances IgA production and favors oral tolerance
IL_{10} (3,000 pg/ml)	Carries out anti-inflammatory activity in the infant gut, has effects opposed to those of IFN-γ on Th1 and Th2 cells
M-CSF, GM-CSF	Induces macrophage differentiation
TGF-β (1,000 ng/ml)	Enhances isotype switching to B_{IgA}^+ cells and favors oral tolerance
TNF-α (500 pg/ml)	Induces proliferation of epithelial cells from G-CSF; activates macrophage motility and SC production
Glycoproteins, glycolipids	Antiviral activity, offer protection against bacterial colonization
Fibronectins	Membrane protein, mediates cell interactions and adhesion Plasma protein with opsonic functions
Histaminase	Catabolizes histamine
Inhibitors of proteases	Reduce inflammation
α-1-Antichymotrypsin α-1-Antitrypsin	Degrades the enzymes active in the inflammatory reactions
Inhibitors of viruses	
Lactoferrin	Inhibits complement and inflammation, is a bacteriostatic and iron-binding factor
Lipids	Inhibit superoxide production, disrupt enveloped virus
Lysozyme	Inhibits PMN chemotaxis and free radical formation, antibacterial activity
Macrophages	Have sIgA, produce lysozyme and PGE_2, which reduces intestinal permeability
Modulators of growth	
EGF	Enhances maturation of epithelial cells: levels in "mature" BM 20–111 ng/ml, vs pasteurized CM 155 ng/ml [142]
NGF	
Neuropeptides: GIP, bombesin, cholecystokinin, gastrin, etc.	
Taurine	
Oligosaccharides	Receptors for certain microbes, block their attachment to mucosal sites; are prebiotics that promote the growth of probiotic bacterial strains
Polyamines	Spermine and spermidine
Prostaglandins	Inhibit degranulation of neutrophils, activation of lymphocytes, are cytoprotective, promote intestinal and cellular integrity, release brush-border enzymes
Protein binding B_{12}	Antibacterial activity, is bacteriostatic
Receptor analogs	Anti-infective activity, protection of mucosal barrier

ADCC antibody dependent cell mediated cytotoxicity, *EGF* epidermal growth factor, *GIP* gastric inhibitory peptide, *NGF* nerve growth factor, *TGF*-β transforming growth factor-β, *TNF* tumor necrosis factor.

Table 2.13. Evaluation of breast-milk immune activity

Poor or absent cellular reactivity

- Absence of basophils, mast cells, eosinophils, platelets
- T lymphocytes respond weakly to allogenic cells
- Poor activity of NK cells and ADCC
- Slow motility of neutrophils

Active cellular reactivity

- Macrophages contain IgA, produce IFN and lysozyme, modulate phagocytosis, epithelial growth, immunoregulation
- Neutrophils contain IgA, regulate chemotaxis, phagocytosis
- B lymphocytes promote Ig synthesis
- T lymphocytes promote cellular immunity, produce IFN, modulate cytotoxicity, immunoregulation

Features denoting that breast-milk lymphocytes are activated [105]:
- T cells: IFN-γ, CD45RO, CD25, HLA-DR ↑
- Macrophages: motility ↑ CD11b ↑ CD62L
- Neutrophils: chemotactic response ↓ CD11b ↑ CD62L ↓

Number of lymphocytes and other cells/mm^3 (mean±SD) [16, 94, 107]
- Total lymphocytes = 1,196.7±358.7
- B lymphocytes = 376.2±138.1
- T lymphocytes = 222.3±251, CD4=504.2±155.3, CD8=318.4±98.6; CD4/CD8=1.6±0.15
- Macrophages = 2,325.5±660.5
- Neutrophils = 948.9±368.3

Determinants (% of positive cells±SD) [12, 65]
- CD3 (T lymphocytes) 25.6±14.9
- TcR-αβ 24.5±15.6
- TcR-γδ 4.0±3.6
- CD4 13.6±8.7
- CD8 12.2±7.0
- CD14 (macrophages) endotoxin receptor 64.0±18.2
- CD19 (B lymphocytes) 10.2±5.3
- CD103 4.7±1.6
- IL_2R 10.5±4.6

TGF-β2 was detected in all breast-milk samples, whereas TNF-α and IL_{10} were detected in 16% and 9% of the samples, respectively [119]

Breast milk EFA are shown in Table 2.17, and nucleotides in Tables 2.16 and 2.17.
Data from [12, 16, 27, 61, 65, 67, 80, 94, 105, 107, 119, 142, 153, 178, 198, 228, 231].

Table 2.14. Essential fatty acid (*EFA*) composition of breast milk (*BM*) and of formulas supplemented or not with EFA (mean values, range)

EFA	BM	S+F	Control subjects	AA+DHA	DHA
12:0 Lauric acid	1.4–6.5	17.5	12.9	11.1	14.1
14:0 Myristic acid	3.8–10.2	7.2	5.1	4.5	5.7
16:0 Palmitic acid	19.8–24.0	10.2	7.7	9.0	7.9
18:0 Stearic acid	7.1–9.0	4.2	3.1	4.0	3.2
18:1 Oleic acid	30.7–38.2	16.1	39.5	42.1	40.2
18:2ω6 Linoleic acid	5.7–17.2	34.2	21.9	21.7	20.7
20:4ω6 AA	0.2–1.2	0	0	0.43	0
Total ω6	5.7–17.7	34.2	21.9	22.1	20.7
18:3ω3 α-Linolenic acid	0.1–1.8	4.8	2.2	1.9	1.9
20:5ω3 EPA	0.0–0.6	0	0	0	0.07
22:6ω3 DHA	0.1–0.9	0	0	0.12	0.23
Total ω3	0.3–3.3	4.8	2.2	2.0	2.2
ω-6/ω3	3.1–43.8	7.1	10.0	11.1	9.4
20:4ω-6/22:6ω3	0.7–5.0	ND	ND	3.6	ND

Data from [5]. *AA* Arachidonic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *S+F* Similac+Fe.

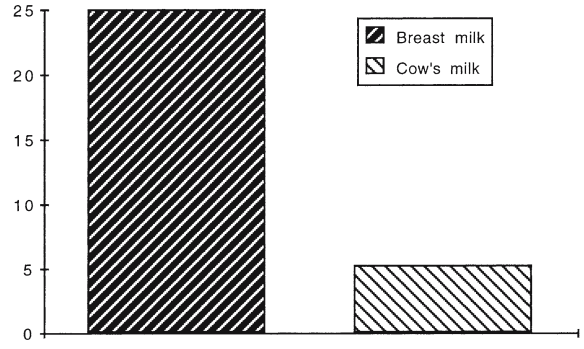

Fig. 2.19. Nonproteic N in breast and cow's milk. (Modified from [66])

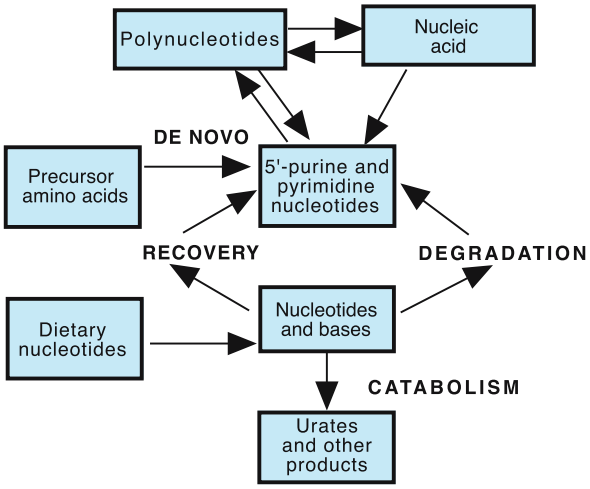

Fig. 2.20. Nucleotide metabolism. Modified from [66]

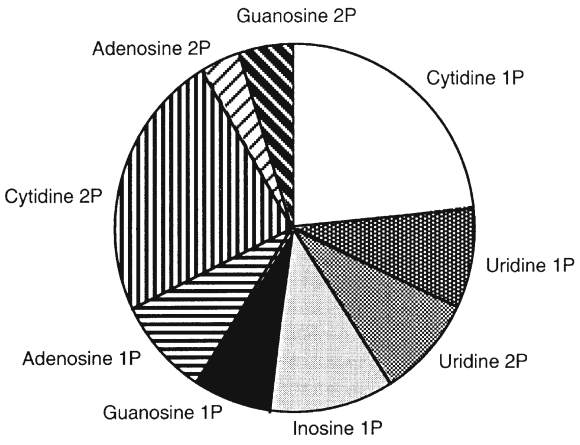

Fig. 2.21. Nucleotide content of breast milk. Mean of 2, 4, 8, and 12 weeks of breast feeding; values are expressed in µmol/l. *1P* monophosphate, *2P* diphosphate. (Data from [10])

tor of the breast milk immune milieu [119]. Recently, it has been reported that ω-6 LCPUFAs contained in breast-milk protect infants against atopy and respiratory disease by favoring an enhancement of the Th1 response [47] or Th2/Th1 switching [43].

Table 2.15. Factors potentially affecting neonatal LCP metabolism

Neonatal factors
Body stores at birth
Endocrine regulation (cortisone, insulin)
Energy balance
Energy expenditure
Enzyme and receptor activity
General health condition (stress, infection, etc.)
Growth rate
Rate of fat oxidation
Environmental and methodological factors
Composition of the maternal diet
Cultural and ethnic traditions
Dietary intake of energy: carbohydrates, protein, fats, etc.
Geographic background and socioeconomic status
Humidity
Methods used for measurements
Milk sampling conditions
Temperature

Data from [49, 113].
LCP long-chain PUFA.

- *Nucleotides*, monomeric units of polymeric DNA and RNA, present in breast milk (% of nitric products) five times more than in CM (Fig. 2.19) [66], are essential in energy metabolism, enzymatic reactions, and during rapid growth, have been reported to be especially important for the growth and maturation of the developing gut, in addition to enhancing immune system potentials in neonates [124]. The human body synthesizes nucleotides to cover its metabolic needs, and is also dependent on external supplies such as dietary nucleotides, whose metabolism is shown in Fig.2.20 [66], also denoting the contribution of dietary nucleotides. Breast-milk levels are delineated in Fig.2.21 [10], in Table 2.16 [124] and in comparison with supplemented formulas (Table 2.17) [210]. Breast milk is provided with the complete enzyme sequence to convert purine nucleotides to uric acid [210]. Nucleotides influence several indices of neonatal immune function [35]. They are essential nutrients for normal development, maturation and repair of the GI tract, since rapidly growing tissues such as the intestinal epithelium and lymphoid cells have limited *de novo* synthetic capacity and require exogenous sources of purine and pyrimidine bases [214]. Dietary purines are not significantly incorporated into

Table 2.16. Nucleotides as such and total potentially available nucleoside (*TPAN;* µmol/l) in a pool of 100 samples of breast milk (*BM*) distinct by stage of lactation

Type of BM	Uridine	Cytidine	Guanosine	Adenosine	TPAN
Colostrum Mean+(range)	26 (21–30)	71 (33–84)	21 (15–26)	21 (13–26)	139 (82–164)
Transitional BM Mean+(range)	32 (23–37)	86 (76–100)	30 (19–43)	29 (17–42)	177 (144–210)
Early mature BM Mean+(range)	48 (30–67)	102 (79–146)	45 (23–91)	46 (21–97) 240	(172–402)
Mature BM Mean+(range)	47 (36–58)	96 (73–124)	28 (22–40)	31 (24–49)	202 (156–259)
General mean±SD	38±13	88±24	31±18	32±20	189±70
Range	21–67	33–146	19–92	13–97	82–402

Colostrum, 1st and 2nd day; transitional BM, 3rd–10th day; early mature BM, 1 month after delivery; mature BM, 3 months after delivery.
Modified from [124].

Table 2.17. Levels (mean±SEM and limits) of nucleic acids and their metabolites found in breast milk at 3–24 weeks of breast-feeding, and in formulas supplemented or not with ribonucleotides

Compound	Breast milk	Not supplemented formula	Supplemented formula
Nucleic acid	23±19 (8.6–71)	<2	<2
Nucleic acid[a]	68±55 (25–209)	<6	<6
5′-CMP	66±19 (41–106)	–	60
5′-UMP	11±5.3 (4.8–21)	–	22
5′-GMP	1.5±1.6 (0–5.9)	–	8.4
5′-IMP	–	–	9.8
5′-AMP	5.7±4.9 (1.7–19)	–	14
Cytidine	5.4±1.6 (3.6–9.8)	6.2	8.5
Uridine	4.9±1.3 (2.8–7.8) 10	9.7	
Guanosine	–	1.4	1.2
Inosine	–	1.5	1.5
Adenosine	–	–	–
Guanine	0.76±1.3 (0–3.3)	–	–
Hypoxanthine	–	–	–
Xanthine	–	–	–
Uric acid	69±12 (47–86)	35	33
Orotic acid	–	240	188

All values are expressed in µmol/l. The first value of nucleic acid is expressed in mg/l.
– Not measurable. [a] Nucleic acid expressed as nucleotide equivalent. Modified from [210].

hepatic nucleic acids, but pyrimidines are: both are taken up by gut cells, which convert into uric acid the excess purines [214]. Carver [35] has documented that breast-milk-fed infants have higher NK cell and IL_2 production compared with a group of healthy babies fed a CM formula supplemented with nucleotides (41.7±4.7 vs 32.2±3.4), and levels significantly higher than infants fed a standard non-nucleotide-supplemented formula. At 4 months only breast-milk-fed babies maintained the statistical differences vs the standard CM-fed group [35]. Principally in preterm infants [166], dietary nucleotides may influence the conversion to LCPs of

linoleic (ω-6 series) and α-linolenic (ω-3 series) acids. High levels of NK cells and IL_2 may be a defense against pathogenic invasion [35].

- The *nucleic acids* contained in breast milk in a much higher quantity than CM are capable of inhibiting the Th2-mediated responses, thus in the breast-milk-fed infant they may redirect the original (fetal) immune responses toward the Th1 phenotype [207].
- Several factors ensure a nonspecific protection against potential pathogens, including the enteric colonization with nonpathogenic bacteria, lectins, additional carbohydrates different from lactose, providing a protective effect for the developing mucosa, lipids with antibacterial, antiviral and antiprotozoan activity, and growth factors stimulating gut closure, etc. [67].
- The leukocytes consist of neutrophils 55%–60%, macrophages 35%–40%, lymphocytes 5%, 80% of which are T lymphocytes [105], including memory T cells [226]. The total lymphocytes, B and T cells, CD4 and CD8 lymphocytes, macrophages and neutrophils are present in preterm babies in a significantly greater number [94].
- More *T cells* are present than those identified in peripheral blood such as TcR-γδ, CD8 and CD103 and an increased proportion of activated cells, which impart to breast milk cells a mucosal phenotype [65], and CD14, which inhibits IgE synthesis on monocytes (Table 1.2), whose reduced levels in amniotic fluid and breast milk and therefore in the neonatal GI tract are associated with the subsequent development of atopy, AD, or both [101]. Maturation of sCD14 plasma levels continued after birth, with adult levels achieved only by 4 months of age [101]. Probably for that reason, human breast milk contains high levels of the critical coreceptors *sCD14*. In addition, breast milk provides the neonate with a *TLR* (Toll-like receptors) signaling regulator, sTLR2. sTLR2 was found naturally expressed in plasma and breast milk. Milk sTLR2 levels mirrored those of the TLR coreceptor sCD14. Depletion of sTLR2 from serum resulted in an increased cellular response to bacterial lipopeptide [125]. Breast milk cells also expressed mRNA for TLRs (Toll-like receptors), TLR1, *TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR9, CD14,* and *LL-37*. In this way, an excessive local inflammation of the neonatal gut following bacterial colonization could be avoided [4].
- Regarding *defensin* mRNA expression in the breast milk cells, the concentrations were 33.0 μg/ml for human β-defensin 2 (HBD-2), 2.4 μg/ml for HDB-5, 1.7 μg/ml for HDB-5 and 3.1 μg/ml for human α-defensin 6 (HNP-6) [4]. Human *cathelicidin LL-37* mRNa was detected in human milk cells with an increase in relative expression levels at 30 and 60 days after parturition. The antimicrobial activity of LL-37 against *Staphylococcus aureus,* group A *Streptococcus,* and *Escherichia coli.* Thus cathelicidin secreted in mammary gland and human milk has antimicrobial activity against both Gram+ and Gram– bacteria, and can contribute to the anti-infectious properties of milk [148].
- *Macrophages* are functionally active by means of M-CSF present, with levels 10- to 100-fold above those found in human blood [80], an average of 30-fold [65], ready to bridge the early neonatal deficits, since they are provided with receptors for sIgA and may be activated via these receptors [170]. Breast-milk macrophages may increase sIgA levels to up to 5%–10% of breast milk content [228], yet they encompass intracellular sIgA, a valid means to progressively transport the antibodies directly into critical areas [173], in addition to phagocyting the CICs formed by sIgA to exclude potential pathogens invading breast milk. Macrophage concentration is greatest on the 6th day but can persist and act up to the 6th month [16]. They can also be primed to release large amounts of O_2 metabolites and could thus contribute to the protection of newborns against invading microorganisms [197]. In keeping with these findings, the ability of immunocompetent cells to survive sticking to the intestinal mucosal sites [93] and to secrete their soluble products is also a means to potentiate the local immune responses of neonatal gut as well as their systemic responses. A study on the cellular composition of breast milk has warned that the risk of the breast-fed infant developing CMA is moderately high if breast milk fulfills at least two of the following criteria: either <91% macrophages, or <52% macrophages, >5% neutrophils, and at least 1% eosinophils (odds ratio, OR 4.5, p=0.01) [98].
- In addition to *the ILs* listed in Tables 2.12, 2.13, almost all generated by macrophages, there are several others, including $IL_{1α}$, $IL_{2–5}$, IL_8 [191] and IL_2 produced by T cells [226]. Such ILs are therefore able to meet all requirements of breast-milk-fed neonates. Moreover, properly stimulated leukocytes produce TNF-α [175] and mononucleates GM-CSF as well [191]. In particular IL_{10}, present in placental lysates of second and third trimesters and in amniotic fluid [219], could be a bridge among the anti-inflammatory and immunomodulating factors forming the defense system of breast milk [61], also because it is necessary for the synthesis of IgA antibodies [123]. The undetectable expression of IL_{10} in preterm babies [100], as confirmed by the decreased secretion by neonatal monocytes and T cells [38], is related to severe respiratory manifestations and can predispose preterm neonates to chronic lung inflammation [100].
- Regarding the *chemokines* IL_8 and RANTES (regulated on activation normal T expressed and secreted), an intriguing hypothesis has been put forward that they facilitate the transfer of maternal cells into breast milk, their adhesion to intestinal walls and their migration into infantile immune tissues, leading to the modulation of neonatal immunity [231].
- Some *oligosaccharides* (Tables 2.12, 2.13), including lactadenin (glycoprotein associated with mucin), contribute to augmenting the potential defense of babies against infectious agents, acting as receptors for *E. coli* and *Vibrio cholerae*, preventing their adhesion to the intestinal mucosa, thus decreasing mucosal inflammatory reactions. Galacto-oligosaccharides are *prebiotics* that

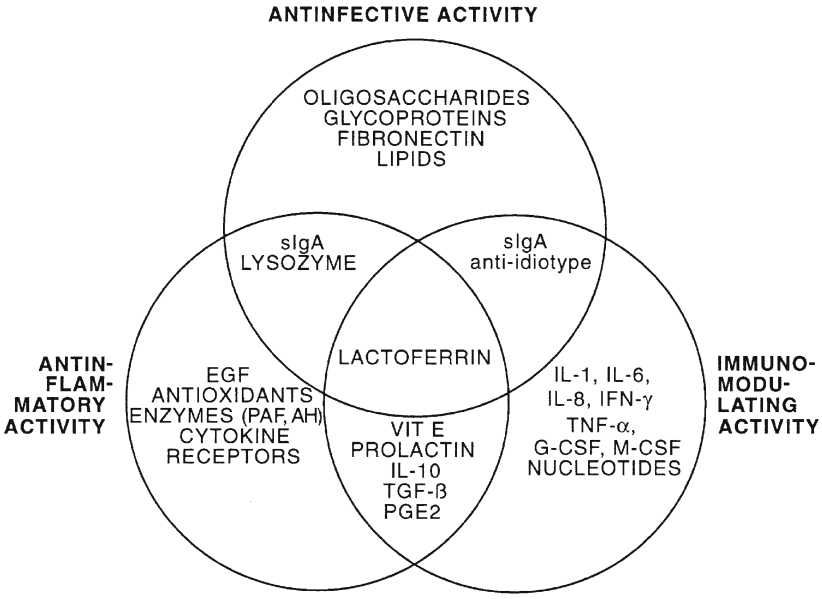

Fig. 2.22. Functional interactions of breast milk immune factors. *AH* acid hydrolases. (Modified from [60])

Table 2.18. Geometric mean levels (g/l) of IgG subclasses in colostrum, breast milk and saliva (mean levels, g/l; with relative rate of single IgG) and matched serum

	IgG_1	IgG_2	IgG_3	IgG_4	Total IgG
Colostrum	0.0372 (46.6)	0.0349 (43.9)	<0.0034 (<4.0)	0.0049 (6.2)	0.0804
Serum	6.209 (64.0)	2.585 (28.5)	0.577 (6.0)	0.194 (2.1)	9.986
C/S rate (%)	0.6	1.1	<0.4	1.4	0.8
BM	0.0251 (46.9)	0.0196 (43.1)	<0.0016 (<3.7)	0.0042 (5.7)	0.0469
Serum	7.546 (63.0)	3.204 (29.0)	0.786 (7.0)	0.201 (2.0)	12.280
BM/S rate (%)	0.3	0.6	<0.2	0.9	0.4
Saliva	0.0097 (27.9)	0.0187 (53.7)	<0.0016 (<4.6)	0.0013 (3.7)	
Serum	8.246 (60.7)	4.504 (33.2)	0.473 (3.5)	0.161 (0.8)	
Saliva/S rate (%)	0.1	0.4	<0.3	0.8	

Data from [108].
C colostrum, *BM* breast milk, *S* serum.

foster the growth of probiotic strains of bacteria, restore normal fecal flora, enhance nutrient bioavailability and reduce gut permeability, thus promoting neonatal gut endogenous host defense mechanisms [152] (see also Chap. 7).

- Anti-infective, anti-inflammatory and immunomodulating factors functionally interact among themselves (Fig. 2.22) [60].

Immunology of Colostrum

It took centuries for the alimentary value and the immunological value of nutritional sources to be acknowledged. Colostrum contains:

- IgA, IgM, IgG. As a compensation for poor quantity of IgG (3% of maternal IgGs), colostrum and breast milk contain significant concentrations of subclasses, ≈50% of maternal titers [55]. Table 2.18 also shows the salivary values [108].
- sIgA, macrophages and EGF with titers higher than those found in breast milk (Table 2.19) [14, 94, 98, 107, 108, 142, 182]. IgA antibodies [5–10 g/l) are transferred to newborns during the first 3 days after delivery: IgA can represent up to 80% of the total content of proteins [228, 231]. CD154 expressed by colostral T cells could trigger *isotype switching* to IgA and sIgA [13].
- Nucleotide levels not much lower than those of transitional breast milk (Table 2.16).
- Factors binding IgE and specifically *suppressing IgE synthesis* by B cells of atopic individuals [182] as well as IL_4 production [21].
- Factors eliciting a nonspecific protective function at the level of the mucosal barrier because the existent

Table 2.19. Immunological factors in human colostrum

Colostrum contains:

Lymphocytes (about 10%–15% of total cells) × ml ($\times 10^4$)
CD3 74.7±2.5
CD4 50.6±2.3
CD8 24.0±1.7
(mean±SEM)
T4/T8 cell ratio higher than that of circulation

(Mean and range)	Colostrum	Autologous blood
CD3+	69 (55–81)	75 (65–84)
CD3+/CD45RA+	12 (4–31)	49 (28–69)
CD3+/CD45RO+	78 (56–98)	54 (40–85)

Immunoglobulins (Igs)

IgA (mg/dl) days 1–2:	619.0±110.6	days 3–4	239.3±55.8
IgG (mg/dl) days 1–2:	31.4± 12.3	days 3–4	14.1± 5.0
IgM (mg/dl) days 1–2:	38.3± 7.8	days 3–4	5.3± 1.6

IgG subclasses (about 50% of maternal levels. see Table 2.18)
sIgA with levels higher than BM levels

IFN-γ (U/ml)

	Colostrum	Autologous blood
Anti-CD3	39.5±9.6	33.8±10.7
Anti-CD2	20.6±6.5	22.3±6.2

Macrophages 30%–47% of total cells (levels higher than BM levels)

Antioxidants (may serve to block neutrophil-generated toxic oxygen metabolites)

EGF
Precolostrum 130–180 ng/ml
Colostrum 35–438 ng/ml

Enzymes and proteins (levels higher than BM levels)

Lactate-dehydrogenase

Trace elements (levels higher than BM levels)

Human colostrum activities:

Hastens the development of an intact mucosal barrier

Enhances brush-border enzyme production (lactase, sucrase, and alkaline phosphatase)

Cytotoxic activity
ADCC
Lectin-dependent
NK cells

Decrease in antigen penetration in neonates

Presence of IgE suppressor factors

No. of lymphocytes and other cells/mm^3 (mean±SD)
Total lymphocytes = 1,532±520.2
B lymphocytes = 414.2±218.2; T = 1.095±347.6; T4=662.2±218.4, T8=425.5±143.9; T4/T8=1.6±0.35
Macrophages = 2,860±860.3
Neutrophils = 1,201.9±479

T cell data from [94, 107], Igs from [98, 108], $CD45RA^+$, CD45RO+, $CD3^+$ and IFN-γ from [14], EGF from [142], other cells from [94], IgE suppressor factor from [182].
ADCC antibody-dependent cell-mediated cytotoxicity, *EGF* epidermal growth factor, *SD* standard deviation, *SEM* standard error of the mean.

Table 2.20. Immunomodulating agents in breast milk

Colostrum
Levels of $CD45RO^+$, T ($CD3^+$) and IFN-γ cells have a considerable positive role in the context of the shortage of such cells in nursing infants. There is a double TcR-γδ supply compared to peripheral blood
Factors specifically suppressing IgE synthesis by B cells of atopic children
Human milk
Promotes the production of IgA and sIgA antibodies, thus actively favoring the maturation of the neonatal immune system
Idiotype/anti-idiotype antibodies prime both B and T cells to promote tolerogenic responses during exposure to environmental antigens, especially food allergens
Such idiotype/anti-idiotype antibodies generate better vaccine responses in breast-fed than in bottle-fed infants
IL_{10} represents a bridge between the anti-inflammatory and immunomodulatory factors in the breast milk defense system
Fibronectin modulates the infant immune response by binding to specific sequences of cellular DNA, thus inducing DNA transcription
There is a growing body of recent studies suggesting that breast-fed infants are at lower risk for developing atopic disease, including asthma, and type 1 diabetes mellitus, Crohn's disease, or secretory otitis media later in childhood

Modified from [67].

antibodies, not absorbed, stick to the intestinal wall, providing a function of *passive defense* [187]. In addition, they provide a *quantity of EFA equal to the recommended quantities* [176].

- Titers of $CD45RO^+$ and T-$CD3^+$ cells mostly expressing CD103 and of IFN-γ, which assume a highly positive significance in the context of the inadequacy of such neonatal cells alluded to above [14]. Moreover, there is a double volume of TcR-γδ cells compared with that in the peripheral blood [12] IL_5 and IL_{13} production is enhanced. However, this is offset by an increased IL_{10} production [21].
- HNP-1, HDB-2 and HD-6 were present in *significantly higher levels in colostrum* than in mature milk [4].
- Substances able to accelerate the development of an intact mucosal barrier, enhance the production of brush-border enzymes (lactase, sucrase, alkaline phosphatase), and decrease food antigen penetration through an anti-inflammatory activity within the intestinal mucosa (Table 2.19).
- Antioxidant substances that could inactivate O_2 toxic metabolites derived from excessive neutrophil secretion [23] (Chap. 1).
- Colostral B cells respond with Ig secretion to antigen stimulation [192]. Similar growth has been observed in culture; therefore, such results have given credit to the theory that B cells partially maintain their functional activities after having colonized the colostrum/breast milk [192]. This hypothesis was not confirmed since the IgE concentrations, similar in atopic and nonatopic mothers, are so low that they have no significant effect on IgE regulation in the neonatal age [51].
- In addition to breast milk, colostrum is rich in TGF-β, which may prevent the development of atopic disease during exclusive breast-feeding and promote specific IgA production in breast-fed babies [103].
- Together with TGF-β1 and β-2, colostral sCD14 is a co-receptor with TLR-4 for lipopolysaccharide (LPS) from Gram-bacteria needed for CD14-negative cells [184].

In conclusion, it seems *unscientific and against medical common sense* to deprive neonates of colostrum and transitional breast milk in the very first days of extrauterine life and thus add to the risk of potential infections [77].

All evidence yet gathered tends to prove that the maternal breast is an immune organ belonging to MALT [170]: breast milk not only has components protecting the vulnerable infant against the first infective and inflammatory episodes, but is also the vehicle for the transfer of immune regulation from the mother to her offspring, thus contributing to the maturation of the immune system of the newborn infant (Fig. 2.22). Several immunomodulating factors present in breast milk (Table 2.20) [67] that may actively modulate the immunological growth of the baby many of which are produced and are common to other mucosal sites, often share synergic features and provide a protective activity without inducing inflammation. Moreover, their production decreases with the duration of breast-milk feeding and in synchrony with the increased secretion of those factors from the neonate [60]. In addition, EGF has been shown to play a role in reducing macromolecular absorption and in promoting the functional maturation of the epithelial cells of the gut barrier (Tables 2.12, 2.13 and 2.19): indeed the proliferation of the intestinal epitheli-

Table 2.21. Mechanisms by which breast-feeding can delay atopy development in genetically at-risk neonates and infants

Protects from gastrointestinal and respiratory infections including bronchiolitis
Lessens exposure to antigens early in infancy
Stimulates intestinal mucosa maturation, thus limiting the penetration of free antigens
Transfers cell-mediated immunity
Transfers interleukins
Enhances secretory IgA production to exclude intestinal absorption of macromolecules
Has a positive effect on the gut microflora

Modified from [67].

Table 2.22. Mechanisms by which breast milk may promote gastrointestinal maturation

Amino sugars
Cortisol
EGF
Glutamine
Insulin
Insulin-like growth factors
Lactose
NGF
Somatomedin-C
Taurine
Thyroxine

Modified from [19, 231].
EGF Epidermal growth factor, *NGF* Nerve growth factor.

Table 2.23. Conditions potentially associated with an immature mucosal barrier

Disruptions of mucosal barrier function as caused in neonates and/or infants of pathogenic uptake of antigenic fragments
Absence of digestive enzymes
Antibiotic therapy
Avitaminosis A Gastrointestinal anoxia
Increased intestinal permeability
Inflammation
Malnutrition
No colostrum and/or breast-milk feeding
Presence of monomeric IgD and IgA antibodies
Transient selective IgA deficiency Reduced gastric acidity Immature gastrointestinal function
Neonate and/or infant clinical conditions possibly associated with immature mucosal barrier
Atopic dermatitis (AD)
Bacterial enteritis
Cow's milk allergy
Malabsorption
Necrotizing enterocolitis
Toxigenic diarrhea
SIDS
Viral infections in general and in particular rotavirus infection

Modified from [218].

um is more rapid in breast-fed animals compared to CM-fed ones [142]. However, pasteurized CM contains nearly similar EGF values, which likely do not resist subsequent handling [235].

sIgA is the major antibody of breast milk [16, 27, 170, 218]: primed B_{IgA} cells home to the mammary gland through the enteromammary axis and are transferred to the suckling newborn, where they act against noxious intraluminal antigens and also respiratory microorganisms [187]. In the neonatal gut, specific sites where maternal sIgA antibodies bind the glycocalyx of epithelial cells more firmly than the endogenous ones were detected. Furthermore, maternal IgA antibodies have been shown to block the antigen entrance into breast milk effectively [193]. Clearly, maternal MALT is in turn "educated" and, again through the enteromammary pathway, contributes to the *de novo* synthesis of sIgA. Studies on anti-idiotypic antibodies show that they are favored in infants by maternal antibodies [74, 169]. Maternal T cells are equally divided between CD4 and CD8 T cells, whereas in the bloodstream the ratio is 2:1, and they play a relevant role in that they promote the secretion of maternal IgA antibodies [169]. Even breast milk macrophages have a place in the local protection of the infantile gut [173]. Beginning from the first week of breast-milk feeding, the baby receives *spermine and spermidine*, with a virtual protective effect on food allergy (FA) [175]. Consequently, the immune defense provided to the offspring appears to be pivotal, first by colostrum and then by breast milk, in a particularly critical period concerning a possible sensitization [26], in which the maturation of the GI barrier and the antibody secretion are still inadequate [114]. Tables 2.21 [67] and 2.22 [19, 231] show how breast-milk feeding can prevent atopy development in genetically at-risk newborns and infants and promote the maturation of the GI tract with several mechanisms. Table 2.23 [217] lists the conditions potentially associated with an immature mucosal barri-

er, for instance the elimination of gut microflora following the increased use of antibiotics in infancy. It is not out of place to state that the *most common immunodeficiency* may be that of the *young infant deprived of breast milk,* with the ensuing deficiency of sIgA and other immune and defense factors [76]. This is demonstrated by the study of 24 variables, as presumed causes of neonatal septicemia: only the protection ensured by breast milk vs CM or formula reached statistical significance ($p<0.001$) [76].

As regards possible effects of *malnutrition* on breast-milk feeding, neither nutritional status nor ethnic origin influence the immunological components. Instead *Rotavirus* infections may cause a significant rise in intestinal permeability to antigenic macromolecules (βLG) [91, 216], in particular if associated with malnutrition [216], so that introducing CM can trigger an inflammatory reaction if the local microflora is depressed or absent [95]. A retrospective analysis confirms that the infection is associated with AD during the first 2 years of life, but excludes this association with FA [117].

Experimental Studies on Neonatal Tolerance and/or Immunocompetence

The neonatal period appears to have a major role in establishing tolerance to environmental antigens: in most species it is possible to induce a specific and long-lasting tolerance to orally administered antigens during this time span [146]. In neonatal mice, immune system immaturity favors priming to oral antigens instead of inducing tolerance; proteolysis is also immature and peptide fragments predisposing to intolerance cannot carry tolerogenic messages [79]. Such a delay may be due to T-cell clones and T-like IL immaturity (Table 2.1). Potential tolerance does not start before 4–7 days of life, and until 21 days is dose-dependent, since very low doses of antigen are able to prime young mice, which may develop an IgE response [146]. Young rats become tolerant to an antigen introduced in their weaning diet for 2 weeks, whereas adult rats fail to become tolerant, even if fed the same antigen for several weeks [227]. Pigs equally become tolerant when weaned onto an antigen, or alternatively if they receive a large dose of antigen at birth before gut closure [79]. Rodents are born much more immature than other species, therefore there is a *lower age limit for being able to develop oral tolerance* [206]. Oral tolerance to ovalbumin in mice can be prevented by agents that activate the RES (estrogens, muramyl-dipeptide, GvHD), while activation of APC, with a similar effect on tolerance, may interfere with the production of CD8 [146]. Recent evidence has shown that feeding one antigen to naive mice is associated with transient RES activation, and prevents induction of tolerance by feeding a second antigen [79], a finding of clinical relevance to the possible effects of the early introduction of solid foods (Chap. 4). Other studies emphasize that induction of tolerance is possible only in immunologically naive animals, because once stable memory is established in $CD4^+$ Th2 cells, persistent IgE responses develop. Further exposure to the relevant antigens only boosts the ongoing responses [139]. This form of tolerance is preferentially directed against IgE and the pertinent Th2-dependent IgE production, while preserving an ongoing Th1-dependent IgG response [131]. The crucial concepts of sensitizing low doses [97] and CD8-mediated immune deviation [139] will be analyzed in Chap. 4.

Time	Stage	
Gestation		
15ª–16ª week	Absence of blood lymphocytes (Table 2.4)	
17ª–18ª week	T lymphocytes	
	↓	↓
2°–3° trimester	Th1 + Th2 (normal)	Th1 + Th2 (Th2 excess) ← allergens
	IFN-γ	IL_4
	↓	↓
	Protective effects	Atopic phenotype
		↓
Birth		Biologic markers distinguish the atopic state ← allergens
		↓
Infancy		Atopic march

Fig. 2.23. Atopic march. (Based on a Symposium on Atopic Dermatitis in Children held in Brussels, October 11, 1995)

Recent experiments have demonstrated the clonal elimination of thymic autoreactive T cells: T cells expressing TcR Vβ able to deliver reactivity to endogenous superantigens (SA) are deleted in the thymus of mice expressing SA [104]. In transgenic mice, restricting the TcR specific for H-Y antigen to males, the TcR developed normally in female mice, but was eliminated in the thymus of males [109]. Comparable deletion of autoreactive thymocyte occurs, inducing tolerance in neonates [132]. Subsequent studies have partially defined the mechanism by which newborns fix their defense. As yet, two main categories of interpretation are convincing. Passive models suggest a negative selection of T-cell reactive clones after neonatal challenge with their own or foreign antigens. Active models suggest instead that following an early antigen contact, neonatal lymphocytes generate suppressive, anti-idiotypic, or deviated Th2 immune responses, thus paving the way for the atopic march (Fig. 2.23). However, these hypotheses do not explain why some viruses induce tolerance if given neonatally and others provoke an immune response with formation of related specific antibodies [171]. Three recent studies [59, 171, 183] re-examine the prevailing theories, putting the APC role in the top position. They conclude that the neonatal period is not an immunologically privileged window in ontogeny. Tolerance or sensitization

depends on APCs and the type of signals sent to T lymphocytes, which must decide which response is pertinent [171]. Neonatal cells are neither innately tolerizable nor able to make only Th2 responses: tolerance induction in adult animals was achieved by inoculating an increased dose of cells inducing tolerance. Therefore, these responses, relative to tolerance induction, are only quantitatively different [171] (Fig. 2.1). As a consequence, the susceptibility of neonates to virus-induced disease reflects not an "uneducated" immune system, but the type of response of lymphocytes, which in murine spleen are 2000-fold less than adult cells. Infection of neonatal mice with a dose of murine leukemia virus results in virus replication within 2 weeks in mice spleen and brain, but virus infection in 3-week-old mice leads to a vigorous protective CD8 CTL response that blocks viral replication [183]. Equalizing the dose to T-cell number resulted in an intensity of protective CTLs response that was inversely proportional to viral dose. Inoculation of neonates with high doses of virus leads to the induction of a nonprotective type Th2 response, thereby not caused by the immunological immaturity of the recipients [183]. Following the classic protocol, an antigen was subcutaneously injected into mice within 24 h of birth and the same antigen was reinjected at the adult age. Mice injected at birth displayed an impaired response in the lymph nodes but a marked proliferation of spleen cells [59]. T cells recall responses to antigens and so do not migrate into lymph nodes. Technical restrictions limited earlier studies to lymph nodes, so neonatal injections do not tolerize but induce memory cells [59]. Activation of the neonatal immune system was detected in CB immune cells (Tables 2.5 and 2.6), where we find [87] T cells, CD25 and CD45RO at different levels based on FHA [87, 130], moreover recording T responses to food and inhaled allergens [87] (Chap. 4). If memory cells are a prerequisite of tolerance, the related deficit can influence the subsequent exposure to allergens negatively. However, during fetal life and at birth poor T-B cooperation and lack of CD28-mediated signals may contribute to development of T-cell anergy and consequently to tolerance induction [130].

Local Immunity in the Respiratory Mucosa

The neonatal lung is a *secretory organ:* in animal fetuses, 2–3 ml/h/kg of fluid is secreted. This fluid flows to the oropharynx as a result of fetal respiratory movements and by contraction of smooth muscles, then is swallowed or added to the amniotic fluid. The protein content of such secretions is tenfold lower than plasma or lymph, thus suggesting that fetal epithelium is impermeable to macromolecules early in development. Given the importance of sufficient quantities of surfactant for normal neonatal airways, nodal points are the presence in fetal lungs of constitutive proteins including SP-A and the release of palmitic acid from alveolar capillaries, used by pneumocytes to synthesize surfactant [209].

Table 2.24. Anatomical and physiological systems involved in pulmonary defense

Anatomical defense mechanisms
Aerodynamic filtration of particles (2–3 µm for the upper airways, 0.5–3 µm for the lower airways
Bronchoalveolar fluid flow
Mucociliary transport
Lymphohematogenous drainage
Physiological defense mechanisms
B-cell-mediated antibody
Biological amplification systems (complement, coagulation, kinins, cytokines)
Non-phagocyte cells
Phagocyte cell-related events
Phagocyte cells
T-cell-mediated cellular response

Modified from [11].

BALT aggregates are uncommon in human fetuses: some follicles are seen in fetal lungs of 18-day-old mice transplanted into syngeneic adults [18]. BALT cluster cells appear at 7–10 postnatal days even though in infants aged <1 year, BALT is developed and organized when larger and more confluent aggregates appear [68]. BALT is a system with structures equivalent to PPs – with a scant division into distinct structural and functional areas – and also to GALT lymphoid tissues. BALT consists of numerous lymphocytes, with a predominance of B cells, organized into follicles and other lymphoid structures, while GCs are relatively rare. Lymphocytes are localized along the main bronchi of all lung lobes and around bifurcations into bronchioles, and also in the epithelial tissue found among the branches of the pulmonary trunk; the epithelium overlying BALT has no cilia and goblet cells [18]. BALT is provided with an elaborate blend of capillaries, arterioles, venules and efferent lymphatics: Holt therefore suggests that it *can control the antigenic traffic* both in the bronchial lumen and bloodstream [86]. M cells overlying the lymphatic follicles of BALT are similar to that of GALT [157]. BALT functions are also carried out by tonsils and adenoids, by 3–5 months of intrauterine life infiltrated by lymphoid tissue and favorably placed to mediate the airway and intestine immune protection (Chap. 15).

Table 2.24 [11] summarizes the number of anatomical and physiological components of pulmonary defense: the mucociliary apparatus develops in the first weeks of intrauterine life, at birth it is able to fulfill its function, and is very effective in removing >90% of particles >than 2–3 µm. Particles between 0.5 and 3 µm

penetrate to distal portions where 90% are deposited [11]. Expression of physiological and nonimmunological defense is the phagocytic system completed by B-cell antibodies, cell-mediated responses, and a set of biological amplification systems including complement [11]. Also of importance are the different cellular and extracellular nonspecific mechanisms such as surface fluids encompassing mucus, surfactant, fibronectin, etc. and epithelial defenses (cilia, goblet cells, mucus, serous glands and type I and type II cells) [11]. Table 2.25 [151] outlines several immune components involved in pulmonary defense. As the table shows, macrophages support the work of lymphocytes, Igs, etc. However, having previously alluded to such notable defenses, we underscore their immaturity in lung alveoli in the neonatal period (chemotaxis, microbicidal activity) in connection with respiratory distress, infections and O_2 toxic metabolites [11]. SCs, proteins (α_1-antitrypsin, α_2-macroglobulin, lysozyme) that protect pulmonary structures from the action of proteases (collagenase and elastases), destroying or inactivating them, are important. A further defense mechanism has been partially characterized: in the airways TcRs may not have the conventional αβ chains but the γδ chains. Hitherto their role and specificity do not appear to be convincingly defined in this field, but it is tempting to speculate that such receptors may have a wider specificity than the normal αβ TcR. In addition, γδ T cells could exhibit responses to SAs, particularly microbial and mycobacterial, with essentially polyclonal modalities. Thereby γδ TcR could play a crucial role as a first line of defense by killing infected epithelial cells before infection spreads across the basement membrane, that is before T-cell intervention, and the ensuing tissue damage [96]. From this, the increased γδ localization to certain mucosal surfaces, such as intestinal and respiratory surfaces, could protect the epithelial cells from inflammatory damage, selectively *suppressing IgE responses* [86]. A role in the defense of respiratory mucosa has also been credited to IgG_4, the only IgG not activating complement by the classic pathway and relatively present in secretions. HLA class II molecules are entirely absent, while after birth consistent aliquots of $CD3^+$ T cells appear and those of IgM and IgA produce cells [215]. However, even 1-day-old neonates have demonstrable levels of $IL_{1\alpha}$, $IL_{1\beta}$, IL_6, IL_8 and TNF-α in their airways [128].

This accumulation of varied observations points to neonatal immunodeficiency: as previously noted, the first 6 months of life are a period at high risk of sensitization to aeroallergens. Exposure to mites, animal epithelial, pollens, etc., in the first months of life increases the risk of respiratory allergy [86]. Neonates are exposed to a notable respiratory load: what neonates breathe is not made up only of normal components, but also by a large pool of pollutants (Table 2.26) [11]. Evidence that early and intense exposure to allergens and the result of several triggering factors in the first months of life can enhance T-cell recognition of aeroallergens and the consequent generation of immunological memory, thus leading to specific immune responsiveness. This might explain why over this period the immune system appears to be more susceptible to initial sensitizations caused by such allergens compared to subsequent sensitization, because children born outside the pollen season (when the immune system has more time to mature before its initial exposure to pollen allergens)

Table 2.25. Immune components involved in pulmonary defense

Lymphatic tissue
BALT
Lymph nodes
Lymphatic follicles
Cells
T and B lymphocytes
Macrophages
Secretion
α_1-Antitrypsin
α_2-Macroglobulin
C3, C4
Secretory component
IFN
Immunoglobulins (IgG, IgM, IgA, IgE)
Mucus
Opsonins
Surfactant
Transferrin

Data from [151].

Table 2.26. What the neonate breathes after birth

Acid pollutants: SO_2, aerosol, sulfates, etc.
Aromatic hydrocarbons: benzpyrene
Cigarette smoke
Exhaust gas Cars (also because of UV rays): NO, NO_2, O_3 Mopeds: benzene
Gas: CO
Heavy metals: Pb, Cd
Oxidants: NO_2, O_3, etc.
Total particulate

Modified from [11].
CO C oxide, *NO_2* N dioxide, *O_3* ozone, *SO_2* S dioxide.

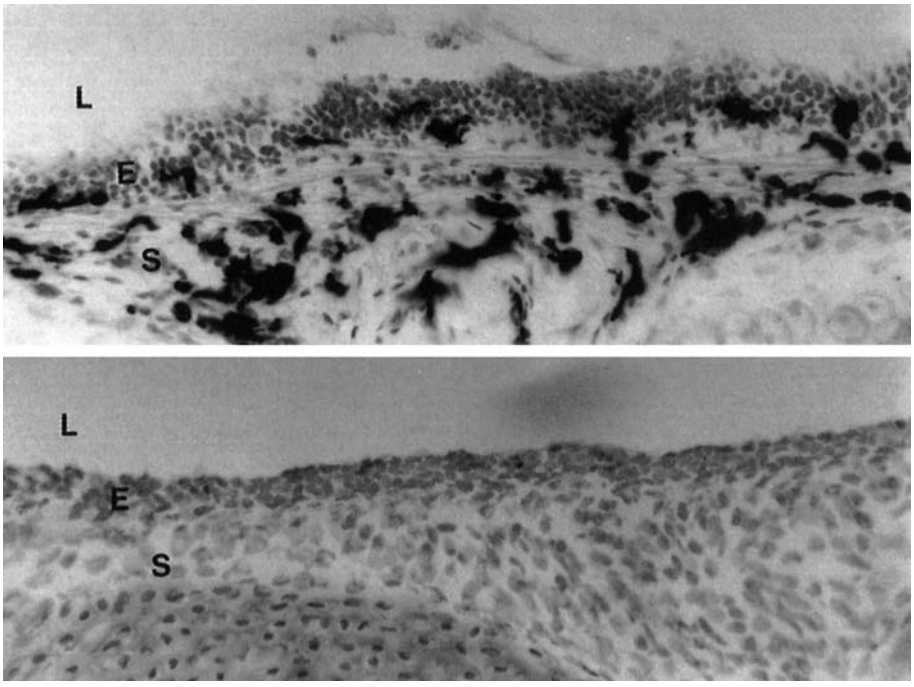

Fig. 2.24. Dendritic cells (DCs) in an adult and 8-day-old rat trachea. In the *upper panel* (adult) note DCs in and below airway epithelium, compared to preweanling (*lower panel*). *L* Airway lumen, *E* airway epithelium, *S* submucosa

are less likely to manifest pollen allergy during childhood [151].

Epidemiological studies have shown that atopic children infrequently show IgE antibodies to inhalant allergens before the second birthday, when they can instead produce IgE to foods during the first year of life. Since this transient defect is restricted to inhalant and not food allergens, it is possible that a BALT intervention, which in some way blocks T cells susceptible of sensitizing children to aeroallergens, thus preventing transfer of immunological memory, acquired during early infancy, into active IgE production. In experiments on animal models, it was suggested that failure to recognize inhaled allergens in the first months of life may depend on a selective maturative defect in local respiratory mucosa immune function [151]. In the rat model it seems to be due to the *absent or marked reduction in DCs at birth and their slow postweaning maturation* (Fig. 2.24) [88], demonstrated by HLA-negative precursors at the expression site of high HLA levels in adult rats [150]. Thus, the slower rate of increase in the peripheral lung DC population, in infant rats, the only cells expressing HLA [151], may determine the failure to recognize aeroallergens [151] and a sort of protection of the delicate tissues from potentially detrimental T-cell responses [150]. A parallel network of these intraepithelial cells is present in the lungs of human adults [68]. Concerning human neonates and nurslings, there is so far no definite information available, therefore the topic is open to speculation. In this context, it is likely that the key may be located in the BALT. We conclude with Table 2.27 [88], illustrating the factors that, in the nonimmunologically exposed host, might interfere with tolerance acquisition to inhalant allergens.

Table 2.27. Host and environmental factors associated with evasion of protective tolerance to aeroallergens

Risk factors	Possible negative effects
Diesel particulate	Promotes IgE responses
Estradiol	Affects macrophage and T cell function, increases TGF production
Histamine	Increases vascular and epithelial permeability
N dioxide	Induces respiratory epithelium damage
Pertussigen	Affects T cell function, increases vascular permeability
Virus	Induces respiratory epithelium damage
	Affects T cell function
	RSV promotes IgE responses

Modified from [88].
RSV respiratory syncytial virus.

Local Immunity in the Skin

In Chap. 7 we shall discuss the skin immune system (SIS), which makes up a complex system of immune surveillance at the skin level and is formed by lymphocytes, DCs, keratinocytes, etc. At 23–26 weeks of intrauterine life, the epidermis is still immature, formed by only 2 or 3 cell layers (the stratum corneum is poorly developed). At birth there is an accelerated maturational process and by 2 weeks of age, including in extremely LBW neo-

nates, the epidermis resembles that of term infants, almost similar to that of older ages [180]. LCs enter the fetal epidermis during the first trimester, and their number continues to increase over the entire length of gestation, forming Birbeck granules in the second trimester, when the relative distribution is different in skin layers, perhaps with reference to certain skin characteristics such as its structure and function [180]. Unlike airway DCs, HLA expression is very rapidly seen after birth [150].

Pediatricians and Neonates

Pediatricians welcome neonates that come into the world equipped to begin an immunologically normal and active period. They are without "windows," thus expanding the range of tolerance acquisition: therefore neonates grow well. It is the environment, as we shall see, that tends to spoil them, even with the air they first breathe. Food allergens exemplify the most frequent cause of sensitization in the first weeks of life, when the intestinal barrier is more permeable to harmful macromolecules. The mechanisms acting to control antigen exclusion and elimination are physiologically immature, thus allowing food allergens to penetrate across the intestinal barrier. The immune protection entrusted to neonates by breast milk intake is therefore significant. Several prospective studies confirm the protective value of breast-milk feeding vs atopic sensitization: in the neonatal age, frequent exposure to small doses of antigens are more harmful than high doses. Antigens can prime a critical IgE-specific response, according to Jarrett's stimulating studies. However, factors present in human colostrum suppress IgE synthesis. Hence pediatricians have an essential function, that of ensuring that neonates enjoy a healthy life, following the natural cadence of breast-milk feeding, which, during the critical period of intestinal vulnerability, provides substantial immune protection because of rich immunological, antimicrobial, antiviral, anti-inflammatory and immunomodulatory factors, functionally interacting among themselves. Clinical and scientific evidence suggests that the protection assured to neonates from breast-milk feeding is greater when it is exclusive and of suitable duration.

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Neonatal Immunology: The Neonate at Risk of Atopy

The Neonate at Risk: Predisposing Factors

Since it is not possible to act on the genotype, the scientists' research and the clinicians' experience are directed at eliminating or at least reducing exogenous predisposing factors. It is consequently necessary to combat the potential danger of such factors at birth and, if permitted, even before (Table 3.1) [122]. As a result of the incomplete maturation of the mucosal barrier, the risk of an allergic sensitization is higher [84, 117, 122, 127], because of the resulting immaturity of the local immune defense system [11], including Th1 cells, IFN-γ, B cells, sIgA and sIgM (secretory) antibodies, neutrophils, macrophages, and complement. Thus the neonate is vulnerable to penetration of harmful luminal macromolecules or their antigenic fragments and, if deprived of the unique immune factors of breast milk (Tables 2.12, 2.13, 2.20–2.22), the condition may become even more critical, and the impact of a retarded gut closure can be striking.

Several factors associated with the physiological immaturity, typical of the neonatal age, can interfere with the protective gastrointestinal barrier function [122] and thus result in an increased antigen entry in both normal and at-risk neonates (Table 2.23). Usually, the enterocytes provide absorption through a process of endocytosis beginning from the microvillous membrane (MVM), which complexes with polypeptide macromolecules to form microvesicles. Instead, during the neonatal age the membrane of such cells shows differences in composition and the MVM protein–phospholipid ratio is dramatically reduced, with a consequent increase in permeability of the intestinal barrier [22, 110]. Age-dependent structural alterations may quickly affect the composition and thickness of the protective mucous blanket, membrane fluidity, activity of membrane-bound enzymes, transport systems, receptor binding events, and protein adhesion to membranes [22], thus enhancing the antigen uptake and its intercellular transport [110].

Table 3.1. Maternal factors active during fetal life that can modulate atopy development

Cigarette smoke
Health state
Immaturity
Possible treatments
Transfers through the placental barrier

Modified from [122].

Factors that may account for mucosal barrier defects during the perinatal period are the following:

- The immaturity of the gastric barrier leading to increased uptake of intact proteins.
- The scarce production of protective mucus, whose glycoprotein molecules ensure that the local defense acts as competitor to the binding of antigens with the microvilli glycoproteins.
- The reduced gastric acidity that reaches levels comparable to those of adults about the 4th week of life.
- The decreased secretion of intestinal and pancreatic enzymes due to immaturity of calyciform cells of the intestinal mucosa and of pancreatic cells is shared with the deficit of natural antibacterial substances (lysozyme, biliary salts), thus explaining why small bowel proteolytic enzymes attain maturity only about the 2nd year of life.
- The reduced carbohydrate/protein ratio of mucin, possibly causing alterations of linking options for proteins, etc.
- The inadequate intestinal motility, following reduced peristaltic activity, delays food progression, which implies a longer contact of luminal macromolecules with gut mucosa, and consequently a higher antigen absorption [11].
- The absence of brush border surface area may allow macromolecules to reach the submucosa. The brush border will start to develop in the first weeks of life, contributing, together with the acquired maturity of the mucosal barrier, to gut closure, a process favored by breast-milk feeding [120].
- An adequate gut microflora (Table 2.11) has no role in preventing or outweighing gastroenteric infections, modulating immune responses to dietary antigens, and balancing the generation of proinflammatory and anti-inflammatory cytokines [99], but the acquisition of an appropriate gut flora may play a role in skewing toward Th1 lymphocytes. Since pregnancy is a Th2 phenomenon and allergy is marked by a Th2 dominance (Chap. 2), the development of a balanced Th1/Th2 immunity is critical [77].

Supplementary Feeding in Maternity Wards

In the complex process of supplementary feeding in the maternity ward, it is believed that the coexistence of physiological immaturity of the mucosal barrier function and the early ingestion of allergenic foods can favor the onset of food sensitization in at-risk babies, who are predisposed to potential IgE-mediated hypersensitivity reactions. In this picture, the role played by the early and occasional exposure to cow's milk (CM) or CM-derived formulas in the first 3 or 4 days of life should be highlighted, a time when the feeding of newborns with CM formulas increases their risk of sensitization. Jarrett's studies reviewed in Chap. 4 confirm that low doses can sensitize the predisposed individual; 49%–100% of babies sensitized to CM [8, 21, 53, 104, 112] were fed supplements of CM or of hydrolysate formulas (HFs) during the first 3 or 4 days of life in maternity wards. Among these babies, allergy to CM (CMA) was more frequent [37], up to 100% of babies, none of whom had symptoms at the first exposure to CM [53]. Our studies demonstrate that immediate reactions (anaphylactic shock, urticaria, etc.) at the subsequent feeding of CM, acting as a booster dose, should draw attention to a delayed effect of the so-called hidden bottle [21], since *in utero sensitization is very uncommon*. CM supplements (40–860 ml) were given to 39 neonates in amounts corresponding to 0.4–7.4 g of β-lactoglobulin (βLG) during the first 3 days of life spent in maternity wards [53]. The amount of βLG in 40 ml of CM formula is equal to the amount provided by ≈19 years of nursing one liter of breast milk daily [53], and according to subsequent measurements [108] 32 years. In a prospective study of 6,209 infants, who received supplementary feeding at the hospital, exposure to CM while in hospital (OR, 3.5; p=0 03) increased the risk of CMA. Of 247 infants who recovered while on an elimination diet, 118 (48%) reacted adversely to the CM challenge test performed at the hospital at a mean (± SD) age of 6.7±2.3 months [104]. However, others disagree. According to a retrospective analysis of record-charts completed by questionnaire data, feeding breast milk supplemented with CM or CM alone in the first 8 days of life seemed to reduce the emergence of atopic symptoms during the first 18 months [35]. In a group of 129 infants randomly assigned at birth to one of three feeding regimens: breast milk, CM, or a casein hydrolysate formula (CHF), during the first 3 days of life, IgE antibodies were equally distributed between the three groups. However, 2 out of 38 CHF-fed infants developed CMA [58]. Early, brief exposure to CM in breast-milk-fed children was not associated with atopic disease or allergic symptoms up to age 5. However, specific IgE (sIgE) to CM (2+ or more) was 5.8% (CM) vs 4.1% (placebo) at age 1 (RR (relative risk), 1.43), and 5.3% vs 3.0% at age 5 (RR, 1.77) [29]. After 7–14 years, some data may have gone unnoticed or neglected, such as the duration of breast-feeding, the timing of weaning, the diagnostic challenge tests and the dropout rates. It is possible that in the final follow-up, cases of CM-induced shock may be disregarded, as well as children who might achieve tolerance by 7 years of age and in an earlier examination would have modified the results. In a cohort of infants randomly assigned to receive at birth either a CM or a HF for 1–4 days, and then all breast milk, supplemented when necessary with HF until day 90 [106], the median titers of total serum IgE were statistically significant at day 5 in comparison with the volume and frequency of supplements received soon after birth and before breast feeding [106], maintaining the statistical significance up to 12 months of life, especially in at-risk babies [114]. In at-risk children, followed up prospectively from birth to 18 months of age and reexamined at age 4–6 years, according to the planned nursing schedule the cumulative prevalence of atopic disease was 18% in the group fed CM in the first days of life or 33% in the exclusively breast-fed group, while in subjects with biparental atopy the incidence was 11% and 61%, respectively [74]. The involved neonates were small for dates, with a gestational age (GA) of 27–42 weeks and 2 SD below the normal median weight, thereby similar to preterm babies who respond in a different manner to sensitization and the onset of atopic manifestations. In the follow-up, the prevalence of atopic disease was almost alike in both groups; however, only in CM-fed infants, there were *3 cases of skin prick tests (SPTs) positive for CM coinciding with RAST* (p=0.0223) [76]. In 118 children with CMA followed until age 8.6 years, IgE-mediated CMA was detected in 86 (73%) children. Among the risk ractors for persistent CMA at age 2.0 years were *CM exposure at the maternity hospital* (OR, 3.2; 95% CI, 1.4–7.8) and early sensitization to egg (OR, 2.8; 95% CI, 1.2–6.6) [105].

It is not surprising that the early administration of dietary antigens to newborn rat pups delays the process of gut closure [3], measured through the intestinal absorption of horseradish peroxidase (HRP), an enzyme employed in immunohistochemistry to disclose CM proteins, although the results of experimental studies cannot be extrapolated directly to human beings. The demonstration that feeding healthy newborns with a CM-protein HF during the first 3 days of life resulted in a delayed gut closure up to the 6th month, based on the observation that serum α-lactalbumin (ALA) absorption at 2 months of age was significantly higher in the HF-fed infants than in the breast- or CM-fed babies [56], suggests that administration of special formulas in neonatal age should be avoided.

Prenatal Sensitization

The likelihood of an *in utero* sensitization has been suggested by several reports of fetal IgE synthesis in infant cord blood (CB) (Table 3.2) [18, 27, 28, 32, 40, 47, 48, 58, 65, 69, 84, 127], including sIgE to penicillin [67] and microfilaria [125]. Such prenatal sensitization is a somewhat infrequent event occurring in 0.5% of newborn

infants, although with a frequency of 0.99%–2.2% for egg, CM and pollens in some babies (Table 3.2). The IgE production begins in the fetal liver and lungs by 11 weeks of intrauterine life [88], in human amniotic fluid by 13 [108] and in the spleen by 21 weeks [88] (Table 2.4). However, IgE-producing cells are rare until 9 months after birth [73]. Numerous IgE^+ cells were detected in the human placenta but had no evidence for local IgE production [115]. Antibodies against maternally ingested foods have been found in the amniotic fluid [82]. The fetus cannot therefore respond with IgE antibodies against food antigens ingested by the mother [82]. Such antigens have crossed the placental barrier, whose permeability is selective: only IgG can be transferred, because arterial CB consistently fails to contain maternal IgE, IgA and IgM [84]. As early as 1902, a placental transfer of egg-white was documented in 29% of rabbits [4]. Regarding the IgE determination in CB for predictive purposes, we should take into account the rather uncommon detection of specific antibodies against known allergens: actually such research in the CB of children who had developed atopy yielded negative results [31, 129]. More probably, high CB IgE levels in at-risk newborns would document the spontaneous synthesis of polyclonal IgE, secondary to the missed suppression of the synthesis of those antibodies [14]. However, recent studies have found memory cells in CB [78, 81] and allergens such as Der p, Lol p, ovalbumin (OVA), egg [52, 86], rye [52], and βLG [86], especially in at-risk neonates [86], thus documenting an evident *in utero* exposure to a critical amount of antigens. That priming of T cells can commence *in utero* is shown by low levels of allergen-induced proliferation in CB [86]. *Allergen exposure in the prenatal period can influence the fetal immune response,* since higher total IgE levels in HR children 3–5 days after birth are related to a larger prenatal exposure to Der p allergens [107]. In the first week of life a high total IgE was also found in 12.2% of boys and 6.2% of girls [59]. For immunological priming to occur, it is insufficient for an allergen to be merely present in the circulation: it must access tissue sites where it can be taken up by dendritic cells for processing and presentation to naive T cells. This does not occur in the circulation but in the secondary lymphoid organs (lymph nodes and spleen) where naive T cells normally reside [116, 117].

Reactions of immediate hypersensitivity have been documented at 5 [126] and 72 h of life [33], SPTs for foods were positive at the 3rd day of life in 1% of neonates [6], a very infrequent event, but possible. Studies done with anti-IgE antibodies have demonstrated that within the first 3 days of life, the skin contained IgE bound to mast cells in 14.7% of neonates and within 39 days in all infants [111]. Moreover, neonates aged 5 h to a few days can fix IgE to dermal mast cells [28]. Consequently, the functional placental barrier withstands the transfer of maternal IgE, thus protecting the fetus first and the neonate later against potential adverse effects of maternal antibodies, since CBIgE antibodies originate in a large portion of newborns, but this is not correlated with the IgE of maternal circulation. As IgE is unlikely to pass the placenta, the presence of elevated CBIgE must be the result of fetal immune response. Therefore an exclusively fetal origin is suggested if a neonate of a grass-sensitive mother has reacted to CM and wheat [126]. Given that IFN-ɣ and IL_4 levels are reduced in the first months of life [60, 102], this reaction cannot be ascribed to a functional immaturity of B lymphocytes, because CB B cells are ready for isotype switching in response to IL_4 stimulation [98] and at least to T-cell stimulation. The nonspecific production of IFN-ɣ by fetal PBMCs (peripheral blood mononuclear cells) from the second trimester of pregnancy onward

Table 3.2. Specific IgE antibodies (*sIgE*) in cord blood

Authors (reference)	Tested allergens	Incidence
Kjellman et al [65]	Pollens, house dust mite	0/100
Michel et al [84]	CM	3/136
Croner et al [27]	CM, fish, egg	0/165
Businco et al [18]	CM	1/101
Delespesse et al [28]	CM, grass	2/96
Hamburger et al [40]	CM, egg	0/55
Hattevig et al [47]	CM, egg	0/86
Zeiger et al [127]	CM, peanut, egg	0/24
Fälth-Magnusson [32]	CM, egg	0/212
Lilja [69]	CM, egg	0/170
Hattevig [48]	CM, egg	0/115
Juvonen et al [58]	CM	1/129
Total		7/1389=0.5%

In cord blood, sIgE to penicillin [55] and helminths [125] were also detected.
The order of the studies reflects the publication year.

Table 3.3. Factors promoting atopic sensitization during the first years of life

Genetic
Family history positive for atopic disease
Neonatal
Functional and anatomical immaturity of the gastrointestinal system with consequent immaturity of antigen exclusion and increased penetration of harmful luminal macromolecules through the intestinal barrier
Brush border not completely developed
Deficit of secretory IgA antibodies
Environmental
Early cow's milk introduction

Modified from [20].

could be viewed as an attempt to contrast placental IL_4 and IL_{10} production, and thereby the Th2 phenotype: failure of this machinery may favor the progression of the allergy march [123].

The factors favoring sensitizations in the first years of life are shown in Table 3.3 [20]. The predisposing factors important for the development of an atopic state, which will be discussed in Chap. 4, should stimulate the institution of effective interventions for allergen avoidance even before birth (Chap. 24).

Methods of Predicting the Development of Allergic Disease

The determination of serum IgE levels has been evaluated for several years to establish the diagnosis in the presence of symptoms of allergic disease, as well as to institute pivotal preventative measures. The value considered as normal is between 0.12 and 0.35 kU/l according to the geometric means of several studies, while levels between 0.35 and 1.28 kU/l are regarded as elevated [1]. Many studies have investigated the possible value of such examinations in CB to predict allergic risk early in the neonatal age (Table 3.4) [17, 25, 27, 29–31, 43, 44, 46, 48, 50, 64, 66, 72, 78, 103, 113, 120–122, 129, 130]. Croner et al [27] were the first to demonstrate that high CB IgE concentrations (CBIgE) could be predictive of subsequent atopic disease, observing that 52%–82% of neonates with high levels (and only 5%–30% of neonates with normal concentrations) developed atopic manifestations within the first 18 months of life. So far, we cannot affirm that CBIgE levels, even if specific, are an important predictive marker. Croner et al followed up 1,701–1,651 unselected children from birth to 11 years of age [25, 64], and 82% of subjects with CBIgE levels of 0.9 UI/ml developed an atopic disease, obvious in 68% and probable in 15% of cases. CBIgE sensitivity was 26%, specificity 94%, and effectiveness as a screening method 72%. Corresponding parameters of positive family history of atopy (FHA) were 45%, 74% and 64%, respectively (see Fig. 3.1) [25]. Significant differences among term and preterm neonates have not been noted, whereas newborn infants of atopic parents had significantly higher CBIgE levels. The risk of developing symptoms of allergic disease between 0 and 11 years of age was two- to sixfold higher (for asthma fivefold) in newborn infants with CBIgE levels of 0.9 UI/ml than in neonates with low CBIgE values [24]. In the follow-ups at 12–14 years, even taking into account the FHA positivity, asthma severity failed to have predictive effects [26]. Subsequently, the authors concluded that IgE titers at age 11 years correlated poorly with neonatal IgE concentrations [24]. In 1,123 children, increased CBIgE et the age of 2 was inversely associated with the manifestation and severity of early AD, whereas increased total IgE and sIgE levels showed a strong positive association with early AD, especially with the more severe form [54].

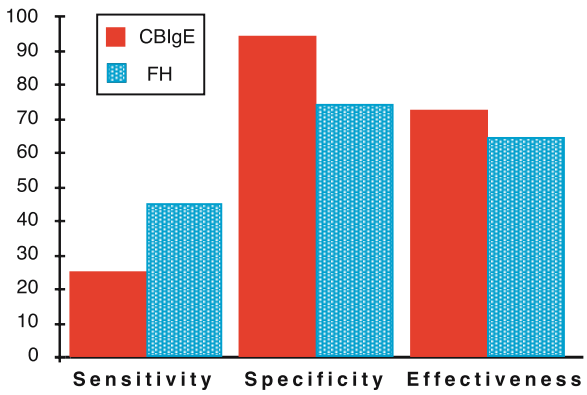

Fig. 3.1. Sensitivity, specificity and efficacy of the two screening methods. *CBIgE* cord blood IgE, *FH* family history. (Modified from [25])

Several studies have found that the CBIgE positive predictive value (PPV) varies between 21% and 42%–46% [50, 72, 120, 128], and others have found that sensitivity is 7%–22% [44, 47, 48, 50, 72, 103, 113, 120]. More precisely, mean sensitivity is 31% and PVV is 42.9%, while most recent works show that the values decrease to 23% and 32.6%, respectively. The mean values of specificity (85%), effectiveness (73%), and negative predictive value (NPV; 80.5%) are more often found to be higher (Table 3.4). A further controversy is that CBIgE is a predictor of neither atopic manifestations up to the age of 2 years [8] nor of atopic disease developed in 76% of infants with elevated CBIgE levels [7]. However, predictivity at age 6 years was significantly associated with aeroallergen sensitization [94].

As regards the *threshold level* (cut-off) to be applied, there is not yet an agreement among all investigators, but it is usually fixed between 0.5 and 0.9 kU/l (Table 3.4); employing a very sensitive PRIST (Paper radioimmunosorbent test), a cut-off value as low as 0.3 kU/l was most reliable [45]. Several investigators have considered cut-off values up to 1.3 kU/l, indicating that the higher the cut-off value, the greater the changes in the sensitivity, specificity and, in some cases, PPV values (Table 3.4).

Alternative Tests to CBIgE Determination

Given such controversial results, FHA positivity should be taken into account, which out of all the available tests has been proven as a much more sensitive marker than CBIgE levels for allergy screening purposes [50]. Furthermore, the positivity of double or single FH associated with CBIgE is sevenfold higher than $CBIgE^+$ alone, whereas FH together with $CBIgE^+$ levels reaches a value of 3.8% (Fig. 3.2) [7]. From Table 3.4 it is clear that the values, calculated by one-fourth of the authors, are of 44% sensitivity and 67% specificity; other authors have suggested that high CBIgE levels associated with FHA positivity, for the time being, are the best predictive methods available [77, 128].

Table 3.4. Predictivity of atopic disease based on CBIgE levels and/or family history

Studies	Author(s)				
	Croner et al [27]	Bousquet et al [17]	Kjellman and Croner [64]	Hattevig et al [46]	Fälth-Magnusson [31]
Year	1982	1983	1984	1987	1987
Selection	NS	Fam atopy	NS	NS	Fam atopy
Number	1,701	282	1,651	86	197
Follow-up (years)	1.5	2	7	7	1.5
Atopy diagnosis	Q (P)	Q (M)	Q (P)	M eval	Q + M eval
Cumul incid	8.3[b]	20[b]	18[a]	27[a]	48[b]
CBIgE					
Method	PRIST	PRIST	PRIST	PRIST	PRIST
C-O (IU/ml)	≥1.3	≥1.0	≥0.9	≥0.9	>0.9
High level (%)	5.3	20	13	7.0	23
Prediction: CBIgE					
Sensitivity (%)	44	58	40	14	24
Specificity (%)	98	85	94	95	79
Effectiveness (%)	94	77	84	73	
Pred val + (%)	70	58	51		
Pred val – (%)	95	88			53
Family history					
Sensitivity (%)	–	–	43	–	–
Specificity (%)	–	–	83	–	–
Effectiveness (%)	–	–	63	–	–

	Strimas and Chi [113]	Magnusson [78]		Zeiger et al [129]		Croner and Kjellman [25]	Hattevig et al [48]
Year	1987	1988		1989		1990	1990
Selection	NS	NS		P atopy		NS	Fam atopy
Number	83	190		237		1654	115
Follow-up (years)	1	2		2		11	1.5
Atopy diagnosis	M	Q (P)		M eval + P		Q (P)	M eval
Cumul incid	23[b]	20[b]		40[b]		33[a]	18[a]
CBIgE							
Method	PRIST	PACIA		PRIST		PRIST	PRIST
C-O (IU/ml)	≥ 0.5	≥0.9	≥1.2	>0.5		≥ 0.9	≥ 0.9
High level (%)	7	22	19	13		13	17
Prediction: CBIgE				Total[c]	AFA[c]		
Sensitivity (%)	22	74	68	67	22	26	10
Specificity (%)	96	91	93	66	94	94	82
Effectiveness (%)	78	87	88	–	–	72	–
Pred val + (%)	67	67	72	18	29	67	–
Pred val – (%)	82	93	92	94	91	72	–

Table 3.4. (Continued)

Studies	Author(s)					
	Strimas and Chi [113]	Magnusson [78]	Zeiger et al [129]	Croner and Kjellman [25]	Hattevig et al [48]	
Family history						
Sensitivity (%)	-	21	–	45	–	
Specificity (%)	-	61	–	74	–	
Effectiveness (%)	-	62	–	64	–	
	Lilja and Ohman [72]	**Hide et al [50]**	**Ruiz et al [103]**	**Twiselton et al [120]**	**Vronier et al [121]**	
Year	1991	1991	1991	1991	1991	
Selection	Atopy mat	NS	Fam atopy	NS	Q (P)	
Number	159	1,111	92	788	388	
Follow-up (years)	1.5	1	1	1	1.5	
Atopy diagnosis	M eval	M eval	M eval	M eval ?	Q (M)	
Cumul incid	22[b]	23*	49[b]	22[a]	35[a]	
CBIgE						
Method	PRIST	EIA ultra	ELISA	EIA ultra	PRIST	
C-O (IU/ml)	≥0.9	≥0.6	≥0.7	≥0.6	0.5	1.2
High level (%)	18	24	5.5	-	32	15
Prediction: CBIgE						
Sensitivity (%)	17	8.5	7	9	27	13
Specificity (%)	82	92	98	78	77	94
Effectiveness (%)	68	–	–	–	61	66
Pred val + (%)	21	21	60	26	41	88
Pred val – (%)	–	78	52	21	67	67
Family history						
Sensitivity (%)	–	72.5	–	–	–	
Specificity (%)	–	48	–	–	–	
Effectiveness (%)	–	–	–	–	–	
Pred val + (%)	–	29	–	–	–	
Pred val – (%)	–	85	–	–	–	
	Kondo et al [66]	**Hansen and Viborg [44]**	**Hansen and Odense [44]**	**Hansen and Viborg [43]**	**Hansen and Odense [43]**	
Year	1992	1992	1992	1992	1992	
Selection	Fam atopy	NS	NS	CBIgE 0.5		
Number	37	1,189[b]	1,625[b]	301	387	
Follow-up (years)	2	1.5	1.5	1.5	1.5	
Atopy diagnosis	M eval	M	M	M	M	
Cumul incid	19[a]	39	40			
CBIgE						
Method	ELISA	PRIST	PRIST	PRIST	PRIST	

Table 3.4. (Continued)

Studies	Author(s)								
	Kondo et al [66]	Hansen and Viborg [44]		Hansen and Odense [44]		Hansen and Viborg [43]		Hansen and Odense [43]	
C-O (IU/ml)	≥0.5	≥0.5	≥0.3	≥0.5	≥0.3	≥0.3	≥0.5	≥0.3	≥0.5
High level (%)	–	15	12						
Prediction: CBIgE									
Sensitivity (%)	43	17	32	15	24	67	34	46	28
Specificity (%)	77	86	70	89	60	72	87	78	89
Effectiveness (%)	70	59	56	59	56	72	82	75	82
Pred val + (%)	30	43	41	46	40	21	22	21	24
Pred val – (%)	85	62	62	61	60	95	92	92	91
Family history									
Sensitivity (%)	57	55		58		42		33	
Specificity (%)	77	62		63		54		62	
Effectiveness (%)	73	59		55		53		59	
Pred val + (%)	15	48		43		9		10	
Pred val – (%)	70	68		62		90		88	

	Eiríksson et al [30]	Vassella et al [122]			Edenharter et al [29]			
Year	1994	1994			1998			
Selection	M eval	M eval			M eval			
Number	180	148			499			
Follow-up (years)	2	1.5			5			
Atopy diagnosis	M eval	M			M			
Cumul incid	39[a]				38			
CBIgE								
Method	ELISA	PRIST			CAP RAST			
C-O (IU/ml)	≥0.23	0.6	1.0	1.5	0.35	0.70	1.25	3.00
High level (%)	–							
Prediction: CBIgE								
Sensitivity (%)	28	41	22	14	54	37	24	9.8
Specificity (%)	61	88	93	97	78	82	91	97.5
Effectiveness (%)	54	74	73	73				
Pred val + (%)	42				25	28	33	42
Pred val – (%)	62				89	87	86	86
Family history (ND)								

total = every cause, *AFA* associated with food allergy, *C-O* cut-off, *Diagn* diagnosis, *Fam* family, *Cumul incid* cumulative incidence (%), *Pred val* predictive value, *M* medical doctor, *mat* maternal, *ND* not done, *NS* not selected, *P* parents, *Q* questionnaire, *eval* evaluation.

[a] Only certain atopy.

[b] Certain + probable atopy.

[c] Study by Zeiger et al [129].

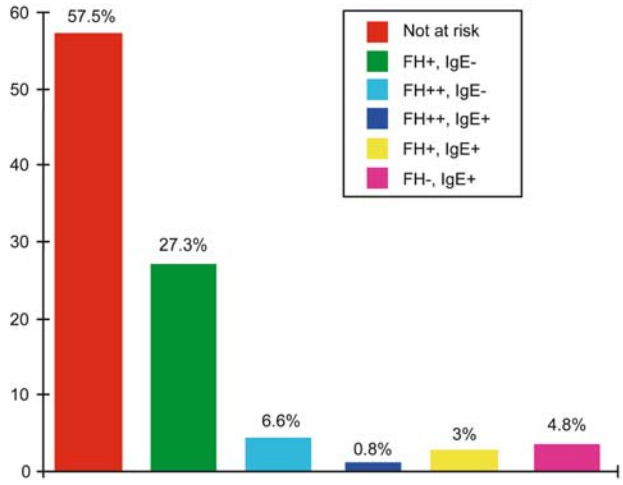

Fig. 3.2. Atopic risk of neonates. Percentage of positivity of FH and elevated IgE concentrations in CB. In this sample 22% of the mothers, 21% of the fathers and 10% of the siblings were atopic; 7.5% of the newborns had at least two atopic relatives. In total, 9% of the CBIgE levels were greater than 0.9 kU/l; therefore 57.5% of the newborn babies were not at risk of atopy. FH+, only one parent atopic, FH++, both parents. (Modified from [7])

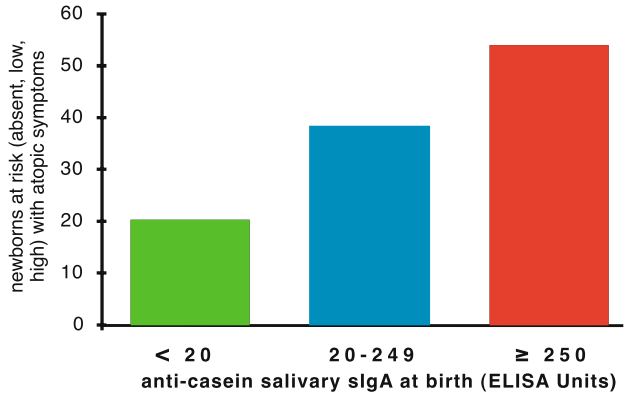

Fig. 3.3. Correlation between cumulative incidence of atopic disease during the 1st year of life and levels of salivary anti-casein sIgA at birth in newborns at risk (absent, low, high) with atopic symptoms ($p<0.05$). (Modified from [100])

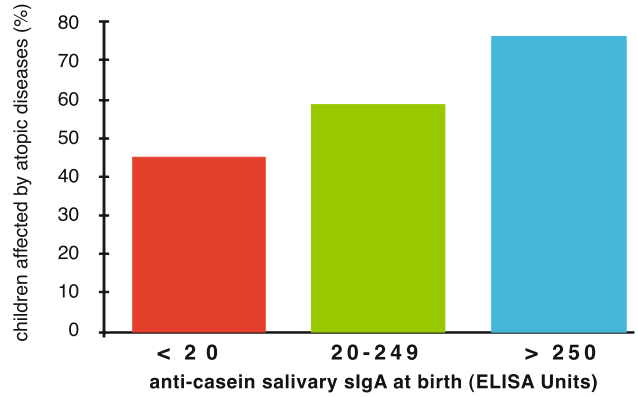

Fig. 3.4. Correlation between prevalence of atopic disease and anti-casein sIgA in high-risk (HR) children at the age of 3 years (p=NS). (Modified from [100])

Hansen et al [45] conclude that CBIgE levels cannot be used as a predictive factor regarding the risk of a baby developing atopic manifestations, assigning a PPV of 44%–48% and a sensitivity of 55%–58% to positive FH vs 15%–17% CBIgE [44]. They suggest evaluating CBIgE as a reliable predictor of atopic disease after the age of 18 months. High CBIgE concentrations are combined with total IgE, also high from 18 months to 5 years of age; however, high levels were also found in 14%–18% to 16%–17% of children aged 18 months with CBIgE under 0.5 kU/l, with and without atopic predisposition, respectively. At age 5 years the levels were 11.6% and 14.6% (with a cut-off value at 0.3 kU/l). Reconsidering their data, Hansen et al [42] highlight that if CBIgE values (with the same cut-off) are examined in parallel with the onset of atopic disease associated with elevated total IgE levels, in contrast with the low PPV of CBIgE alone, specificity is 87%–89%, sensitivity 46%–67%, but PPV remains as low as 22%–24%.

After a wide review of the contention, Croner [23] has concluded that an accurate FHA is adequate, and that routine use of laboratory methods may not be justified, also for financial reasons.

It is now firmly established that CB can be contaminated by maternal blood mixed with fetal blood [27, 39, 71, 103], in up to 4% [39], 7% [103], 14% [71] of cases, or in an insignificant volume, also due to intrapartum surgical procedures [30]. The measurement of IgA levels has less practical value, being of maternal and fetal origin [70, 71].

Many other predictors were proposed as an alternative to CBIgE (Table 3.5) [12, 63, 91]:

- Total IgE levels during the 1st week of life, avoiding possible IgA or sIgE presence [41, 70]. The use of such tests for general screening programs can also be challenging because of the difficulty of drawing enough CB: blood is collected on filter paper on the 4th or 5th day of life using the DELFIA method [34].
- Salivary anti-casein IgA levels, correlated with positive elevated FHA, CBIgE levels [101], and with the development of atopic disease during the first 3 years of life, as outlined by Figs. 3.3 and 3.4 [100], which show the relationship between anti-casein sIgA and the onset of atopic disease at 1 and, respectively, at 3 years. However, although the test has a sensitivity of 34% [100], much higher than the 7%–22% of CBIgE, it cannot be recommended as predictive, and not even associated with CBIgE levels, also due to the striking correlation between elevated sIgA and CBIgE concentrations [100].

Table 3.5. Predictor markers of atopy development assessed in neonates at risk of atopy

	Specificity	Sensitivity
Parents		
1. Family history (2 parents >1 parent >none)	Low	Low
2. Total and/or specific IgE	Low	Low
3. Skin prick tests	Low	Low
Mothers		
1. Serum: IgG to foods	Low	Low
2. Anti-IgE IgG	Unconfirmed	
3. Colostrum: IgG/IgA to defined allergens	Not known	
4. Amniotic fluid: IgE, IgD	Not known	
Neonate		
1. Total IgE at birth (CBIgE)	High	Low
2. Total IgE associated with salivary anti-casein IgA	Not known	
3. Salivary anti-casein IgA	Not known	
4. FcεRII (CD23)	Not known	
5. Lymphocyte subpopulations	Unconfirmed	
6. Proliferative lymphocyte response to food allergens	Not known	
7. Specific IgE	Low	Low
8. Eosinophilia associated with that of maternal serum	Not known	
9. Mast cells	Low	Low
10. Basophil releasability	Low	Low
11. Platelets	Low	Low
12. Polyunsaturated fatty acids	Low	Low
13. Phosphodiesterase mononuclear leukocytes	Low	Low
14. IFN-γ production	Unconfirmed	
Day 5		
Total IgE	Not known	
Infants		
1. Serum: IgE	Low	Low
2. Specific IgE by sensitive technique	High	High
3. Serum IgA, saliva IgA	Unconfirmed	
4. Nasal eosinophils	Unconfirmed	
5. Nasal basophils	Unconfirmed	
6. Suppressor CD8	Unconfirmed	
7. CD4/CD8, etc.	Unconfirmed	
8. Skin prick tests	High	Low

Data from [12, 63, 91].

The sIgA increase could suggest a basal lymphocyte hyperresponsiveness in atopic children, or depend on an antigenic stimulation, for example of casein fragments in maternal circulation that modify the baby's immune response.

- CB-soluble FcεRII (CD23) levels in infants who have developed atopic symptoms 5–13 months after birth were significantly higher than those in infants who were free of atopic symptoms [60]; others have found no dif-

ference in CB levels of sCD23 between controls and children with subsequent atopy and AD [97].

- CB eosinophilia has been recently re-examined [19]. However, a high CB total eosinophil count does not seem to be an inherited factor predictive of future development of atopy, nor was there a correlation with maternal counts [50].
- Nasal eosinophils and metachromatic cells at 3 months of life [15]; the peripheral eosinophilia may have a role in prediction, and is also significantly correlated with both CBIgE and total IgE levels [15], because they are associated with the development of atopic disease at 6 years [16]. However, such data have not proven ideal for screening purposes, since they may come too late for instituting primary preventive measures, particularly effective only if started at birth.
- Proliferative response of CB lymphocytes to maternal food antigens (OVA and bovine seralbumin [BSA]) [66], a test that in 37 neonates followed up for 2 years has shown to have very high specificity (93.3%) and sensitivity (85.7%) in predicting not only the onset of allergic diseases in general, but also the particular type of food allergy (FA) within the first 2 years of life. However, significant differences between responses of maternal and HR neonates' CB lymphocytes should not be a significant problem for the contamination of maternal blood. CB PBMC proliferative responses showed highly significant differences vs anti-CD3 in 34 HR infants with atopic dermatitis (AD), whether associated with FA or not, followed up for 1 year. Instead, IFN-γ production was found to be significantly lower than the above parameters. AD children had the highest responses to anti-CD3 and the lowest responses to IFN-γ, while in those with AD associated with FA, the responses after stimulation with βLG and OVA were similar [124].
- CB lymphocyte subsets [72], several deficits of which are reported in Tables 2.5 and 2.6, data not confirmed in HR neonates who had elevated CB total numbers of CD2, CD3 and a CD4/CD8 ratio significantly different among the babies classified as atopic or not at 18 months; however, with wide overlapping values and the ratio normalization at 2 and 6 months of age. Sensitivity, specificity and PPV values were too low for a possible screening [72], a conclusion not confirmed by others [87].
- Platelets with low CB levels [81]; however, subsequent reports have failed to find such levels useful as a test complementary to CBIgE and FHA [91].
- IFN-γ production by CB PBMCs of HR neonates, significantly reduced compared to healthy neonates [68, 102], independently of IgE concentrations [102]. Concordant figures in 21 HR infants at 12 months of age come from the analysis of the correlation between SPT positivity for foods and mRNA expression for IL_4 by CB lymphocytes prestimulated with βLG and OVA: there was a correspondence between mRNA expression for IL_4 and SPT positivity for OVA, but the correlation between IFN-γ expression and SPT positivity for βLG was negative [85]. In two trials, IFN-γ levels were not seen to be useful for the prediction of atopic disease [36, 92].
- Anti-IgE IgG antibodies, associated with a reduced allergy prevalence in infants at risk, a test with a rather poor sensitivity, even if higher levels appear to have a protective effect since they are synonymous with a lesser development of atopic disease at age 18 months: only 9% of infants with high anti-IgE IgG vs 29% with low levels [121].
- Phosphodiesterase (PDE) activity, whose CB increase is controversial since it lacks a significant predictive value. Nor does it seem to be relevant as a screening method without a correlation with CBIgE [90]; the PBMC PDE increase is sometimes not suitable [90], and therefore elevated PDE levels do not represent a primary deficit in atopic subjects [15].

The several tests done in CB, even at an early age (IgE and IgG, SPTs and lymphocyte subsets) have not yielded reliable results [72]. No predictive value has been related to the atopic symptoms recorded at varying ages in 500 babies prospectively followed up over 7 years [83] and in 180 infants evaluated at the age of 18–23 months [30]. Such tests had a 25%–79% sensitivity, a 40%–74% specificity, and 58% efficacy [91].

In the studies reporting CB antigen-specific T-cell responses, it is noteworthy that the study group was modest and the results appear to be near the detection limits employed by the conventional lymphoproliferative assays, but possible alternatives have been studied [66, 121]. In addition, the poor variability of the values of stimulation indices, oscillating within a range varying

Table 3.6. Factors or techniques which can influence the neonatal PRIST determination

1. The neonatal PRIST is influenced by blood sampling technique, depending on whether the blood from the umbilical vein or when the neonate is 4–5 days of age is aspirated or gravity-collected: contamination of maternal IgA should be investigated if the latter technique is employed.
2. IgA levels are significantly lower at days 4–5 of life (hence IgE and IgA samples should be collected at birth at the same time).
3. IgE levels show very good equivalence in cord blood and samples collected at 4–5 days and correlated with maternal levels, but only with that measured at delivery.
4. Consequently, besides an active pre- and postnatal synthesis of IgE antibodies, there is a tangible prenatal maternal influence.

Data from [71].

Table 3.7. Factors reported to be associated or not associated with CBIgE

Factors associated with increased CBIgE levels
Atopy in 1st degree relatives and/or high levels of serum IgE [18, 27, 53, 79]
Gestational age of <38 weeks [30]
Intrauterine exposure to alcohol and/or caffeine [12]
Intrauterine exposure to progesterone and metoprolol [13, 84]
Male sex [10, 12, 27, 38, 43, 61]
Maternal atopy [12, 79, 84, 119]
Maternal smoking during pregnancy [5, 10, 80]
Month of birth [12, 43, 61]
Related to cord blood [70, 71] Contamination by maternal blood Cord blood sampling techniques
Season of birth [12, 38, 43, 61, 119] Higher IgE levels in spring and winter [43]
Factors not influencing CBIgE levels
Intrauterine exposure to albuterol administration [84]
Diet during pregnancy [31, 48, 94]
Male sex [94, 107]
Maternal smoking during pregnancy [12, 31, 53, 93, 95, 107]
Prenatal oral contraceptives [84]
Racial differences [1, 2]
Season of birth [53, 107]

Table 3.8. Genetic/immunological characteristics of neonates and infants at high risk of atopy

Positivity of family history (2 parents >1 parent >none)
High levels of phosphodiesterase in monocytes
High levels of circulating eosinophils
High levels of IgE antibodies in cord blood
High levels of nasal eosinophils/basophils
High levels of specific IgE
High levels of total IgE
Dysregulation of IL_{13} production
Increase of soluble FcεRII (CD23) in cord blood
IgA antibodies deficiency (transient)
C2 deficiency
CD4 deficiency
Opsonization defect
Genetic localization (chromosome *11q*)
Positivity of skin tests for foods or pollens at birth
Presence of specific IgE antibodies to foods in cord blood
Presence of specific HLA alleles
Decreased production of INF-γ
Numerical reduction of T cells and of lymphocyte subsets (CD8)
In vitro lymphocyte proliferative response to food antigens
Thrombocytopenia in cord blood

Data from [63, 92, 127].

between 0.39 and 3.29 [66], is perplexing. As recently stressed (Table 3.6) [71], above all when the IgE levels exceed the normal threshold values, one must keep in mind that CB IgA values between 14.1 [43, 71] and 32 µg/ml [10], with an incidence of 0.7% [94], are indicative of contamination. The mean value of 28.2 µg/ml does not protect from possible contamination [96].

- A useful marker of high-risk neonates may be the increased production of IL_{13} by neonatal CD4 T cells [92, 109], and Il_4, which was detectable in ten of the atopic children (28.6%) and in only two (5.7%) of the control subjects [97].
- Although significant differences were observed in CB-soluble CD14 (sCD14) levels, where children with atopic mothers had the highest levels, the same pattern could not be observed in the same children at 2 years of age [51]. Babies will encounter very high sCD14 levels in breast milk (Table 2.10); however, breast-fed infants with AD were associated with lower sCD14 levels in the breast milk of their mothers [57].

CBIgE levels can also be influenced (mostly as an increase) by several factors summarized in Table 3.7 [1, 2, 5, 10, 12, 13, 18, 27, 30, 31, 38, 43, 48, 61, 70, 71, 79, 80, 84, 93–95, 119]. However, in 2,631 children, male sex was significantly associated with an increase in total IgE and CBIgE (p=0.001). Maternal smoking during pregnancy (even >15 cigarettes) failed to reach significance [12], whereas the maternal intake of caffeine (200–800 mg/day) and alcohol (at least three doses of 12 mg/week) has been included among the harmful factors [12].

It seems logical to attribute such controversial data either to a higher maternal awareness in the last few years of allergy risks, with a consequent reduction in fetal antigenic exposure, or the meaningful criterion of eliminating all sera with elevated IgA levels. Significantly, pollen exposure in atopic mothers confers intauterine stimulation of fetal IgE production [119].

Table 3.8 [63, 92, 127] outlines the immunological and clinical characteristics of neonates and infants at HR of developing atopy. Moreover, the utility of T-cell determination is recommended by some authors [87] and not by

others [72]. In conclusion, we deem it reliable to stress that:

- The CBIgE levels within normal limits cannot definitely exclude the risk of developing an atopic disease, as demonstrated by false-negative results, averaging 21%–37% [127, 128], and 4%–11% of false-positive results, with high titers not always correlated with the development of allergic diseases [7, 120]. Accordingly, 73%–65% of newborn babies with one and, respectively, two atopic parents had CBIgE levels <0.35 kU/l, whereas 13% of neonates not at high risk had CBIgE at 0.35–0.7 kU/l and 10% >0.7 kU/l [10]. More studies [7, 39, 70, 122] confirmed these data.
- Only 0.8% of babies with biparental FHA have elevated CBIgE at birth, which was observed in 4.8% of babies with negative FHA (Fig. 3.2), and according to others 18.2% (only one atopic parent) and 8.3% with two atopic parents [122].
- Only 7 out of 103 (6.8%) neonates with double FHA have levels above the 90th percentile [70].
- Only 28% of neonates of atopic mothers show levels of 0.5 kU/l compared with 26% of control babies [39].
- A low correlation between the IgE levels at birth and later in childhood suggests that different effector mechanisms may be operating at different ages [55].
- We have done a meta-analysis on CBIgE levels determined by various authors in neonates [43], either at risk (8 studies, 468 neonates) or not at risk (10 studies, 9,955 neonates), which showed that the mean level was 0.33 in the first group of studies, range, 0.11–1.1;0.30 in the second group, range, 0.09–0.80. Only 8 of 18 studies also specified the median range: <0.1–0.25. Furthermore, the value of results is not given in all reports by the selection of an adequately representative cohort, compared with an unselected control group.
- When the investigation is not carried out in the general population, but only in a highly selected group, defined, for example, based on FHA positivity, then CBIgE determination, even if by chance valid in 100% of all parameters, serves exclusively to further subdivide and circumscribe a category of HR neonates.

CBIgE concentrations fail to appropriately meet the basic prerequisites and at present cannot be used as a balanced and realistic marker of the risk of developing atopic disease or as a screening instrument for primary prevention [9]. Until definitive strategies become available, we encourage taking a relevant FH for preventive purposes (see Fig. 3.2), an approach recommended by Kjellman [63]. Therefore, even disregarding the economic factors, such drawbacks *fail to justify CBIgE use* as the only validated test within a screening program finalized to select candidates for taking part in studies for allergy prevention [62]. Unfortunately none of all the alternative procedures proposed so far has shown a satisfying level of sensitivity and specificity, in addition to being easily applicable.

We deem that at present the most suitable predictive test is the family atopy score (FAS) (Table 3.9) [63], which may help identify at-risk babies, hoping that in future a potential rational method becomes available that allows the early identification of families where an effective preventive allergen avoidance should be set forth. For pediatricians, the consideration that the association of FHA with skin dryness has a sensitivity of 80% and a specificity of 85% ($p<0.01$) [91] will be decisive for early identification of neonates to be enrolled in prevention programs.

IFN-ɣ reduction, if it could be standardized, could be a predictive marker of the atopic march not secondary to atopic manifestations [102, 117, 124], with reference to the deficit of Th1 lymphocytes observed in CB of at-risk neonates.

Table 3.9. Family atopy score (*FAS*) according to family history

Relatives	Score	
	Obvious	Probable
Father	2	1
Mother	2	1
Siblings	2	1

Paternal and maternal points are added to the highest score in any sibling, a FAS 3 means that there is a statistically increased risk that allergy will develop in that child.
Modified from [63].

Pediatricians and Neonates at Risk

Pediatricians should focus on future trends in prevention because most atopic diseases appear in the very young and sensitization is known to occur early in life. Early intervention strategies to block the atopic march are dependent on identification of neonates at HR for later development of atopic disease. The promotion of allergen tolerance, with resultant atopy prevention, will likely be achieved in the immature neonate's immune system more readily than in the mature immune system. We believe that the future health of the population depends on innovative strategies for preventing the atopic and immunological diseases of infancy and childhood. CBIgE as a predictor of atopic diseases in infants has been discussed in a large number of papers with contradictory results. Even when elevated CBIgE levels are identified as a strong risk factor for sensitization, their poor predictive value may make them useless as a basis for preventive measures. If atopy is indeed a major risk, the ability to measure some markers associated with the atopic trait would be beneficial, especially because atopy has its roots in neonates and toddlers, and interventions aimed at primary prevention can be made a reality. A positive FAS increases the risk of subsequent atopic disease in infancy and childhood.

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Genetic and Environmental Predisposing Factors

Genes and Atopy

The atopic state is an individual's augmented and persistent genetic predisposition to produce allergen-specific IgE antibodies after exposure to common allergens, with a significant correlation between IgE-producing B cells and relative total serum IgE levels in the systemic circulation, significantly more elevated than the levels of nonatopic children [201]. We best understand the pathogenesis of the atopic diathesis by analyzing the regulatory mechanisms of IgE synthesis, based on genetic and environmental factors that are not always well known [48]. Genetic control appears to be exercised not only on atopic disease as such and the type of sensitization, but mainly on a predisposition not specifically directed to elicit the onset of atopic disease. Fundamentally, a growing body of evidence emphasizes the role of environmental factors [409, 504] in determining the type of sensitization and form of atopic disease precipitated in genetically at-risk babies in relation to the allergens encountered and the conditions regulating this event [182]. These conditions are linked to genetic predisposition, and specific chromosomal regions appear to be important for linkage of atopic disease-related phenotypes. Heredity regulates the transmission from parent to offspring of the susceptibility to different phenotypes of atopic responsiveness. In this context, the development of atopic disease is dependent on environmental exposure to common aeroallergens, respiratory infections and even pollution. Accordingly, sensitization may occur in different ways: by inhalation, ingestion, contact or puncture. Figure 4.1 exemplifies the sequence of events leading to the development of atopic disease.

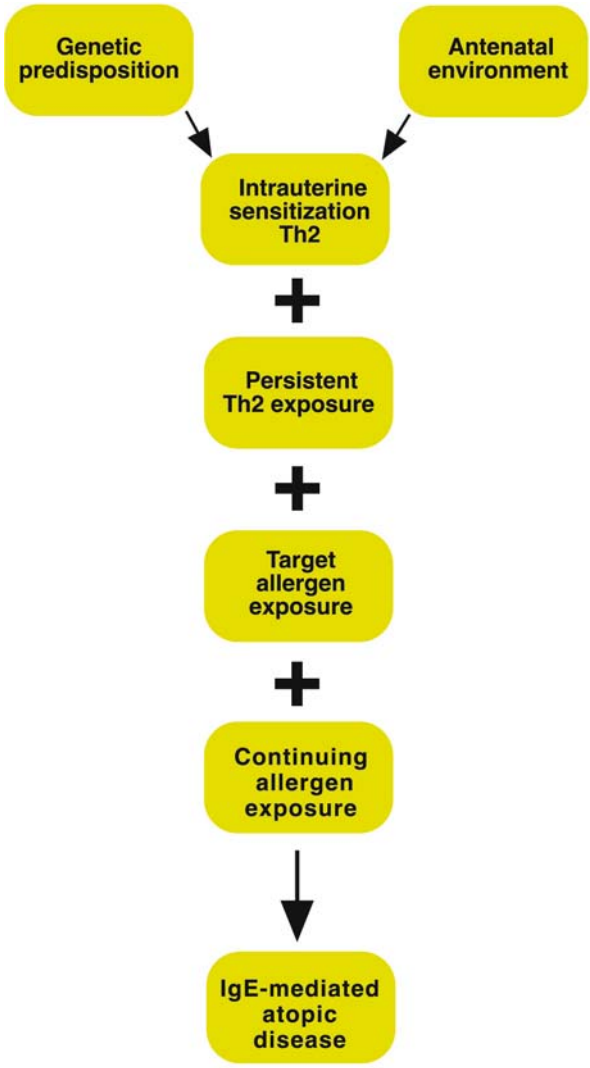

Fig. 4.1. Pathogenetic sequence leading to atopic sensitization

Genetics of Atopy

In 1916, Cooke and Van der Veer convincingly substantiated that atopy runs in families, that family history of atopy (FHA) is linked to genetic factors [76]: inheritance models are first monogenic with a mendelian autosomal dominant trait and then autosomal recessive traits have been proposed [173]. The challenging hypothesis that atopy is genetically determined has gained increasing credibility, based on the following points:

1. A genetic predisposition to atopic disease and an association of atopy with well-identified chromosomes.
2. A preferential maternal heredity of atopy.
3. A genetic HLA-mediated control of the capacity of synthesizing high total IgE and specific IgE levels.

4. Atopy is correlated to a T lymphocyte and pertinent interleukin (IL) dysregulation and to a hereditary maturative defect of immunocompetent cells.
5. Atopy could be conditioned not so much by IgE hyperproduction as by IgA delayed maturation.
6. Atopy could be programmed in the intrauterine life.

1. The existence of a genetic predisposition for atopic disease was first supported by the transfer of atopy to a nonatopic recipient by an asthmatic bone marrow donor [4, 439], conversely clearing of asthma in asthmatic recipients of normal lungs [86]. Transfer of IgE-mediated hypersensitivity can occur after liver transplantation and have serious consequences, such as an anaphylactic reaction to cashew nut that developed in a man with no history of nut allergy 25 days after receiving a liver allograft from a 15-year-old atopic boy who died of anaphylaxis after eating peanut [330].

In Chap. 3 we recorded CB (cord blood) lymphocyte proliferative responses to food allergens [213], thus suggesting an *in utero* sensitization. Moreover, CB $CD4^+$T cells proliferate in response to Der p (*Dermatophagoides pteronyssinus*) with a different IL pattern according to whether there is neonatal atopy: allergen-specific CD4 of neonates from nonatopic parents are Th1, whereas children with an atopic background show a prevalence of Th0 or Th2 clones [98]. As is emphasized by the IFN-ɣ deficit in the neonatal period, Th2 lymphocytes are able, in prospective, to recognize allergens and generate IL_4/IL_{13}, IL_5, IL_6, IL_9, and IL_{10} in virtually all newborn infants [344].

If an increased and persistent production of antibodies to common allergens, already evident at birth, is the main characteristic of atopic subjects, which genetic mechanisms regulate both IgE production and genesis of atopic disease? Immunologists have asserted the IgE hyperproduction as a central feature of atopy and consequently have looked for a possible deficit of immune homeostasis, dependent on T cells: this approach has led to an increasing amount of evidence helping understand the regulation of IgE synthesis [354]. Several investigations have demonstrated that high IgE levels are usually inherited with a recessive mechanism, but – as we observed in Chap. 1 – are not consistently associated with atopy. Studies on the animal model have confirmed the recessive mode of inheritance of high IgE levels via non-H2 genes [182]. According to an attractive hypothesis, the dominant allele of the IgE regulatory *locus* limits the number of clones stimulated in response to allergens with consequent production of low IgE levels: this *locus* is not included in HLA. In mice, the capacity of responding to a given allergen is modulated by immune response (Ir) genes, localized in humans mostly in HLA. In humans, it seems that any HLA-D genetic sequence can be found in subjects responding to particular allergens: certain alleles appear to be more frequent in atopic subjects. The greater frequency of some alleles in a population compared to others could be the expression

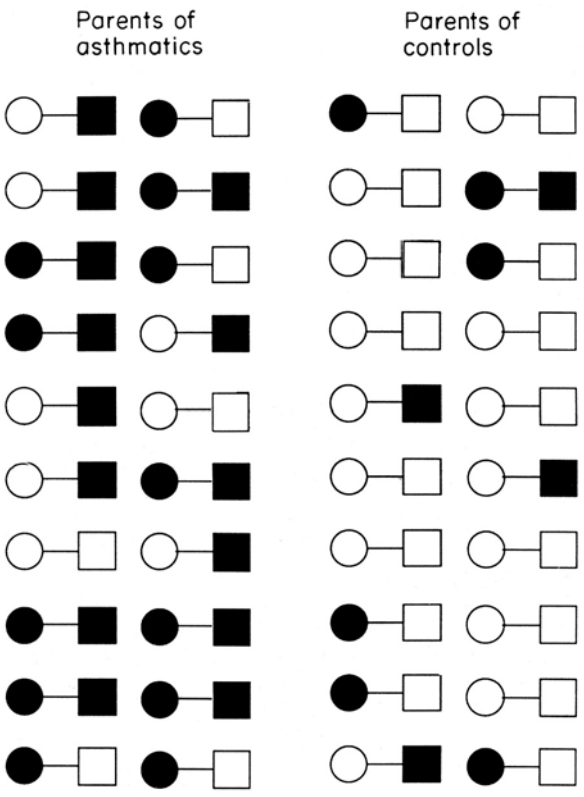

Fig. 4.2. Frequency of atopic IgE responsiveness (*shaded symbols*) in the parents of atopic asthmatics (*left*) and control individuals (*right*)

of a general genetic intervention owing to immune hyperreactivity or, alternatively, could originate from a particular Ir gene selection, while the atopy gene, being proximal to Ir, would be passively dragged along [182].

Family studies may involve twins, helpful in differentiating genetic and environmental factors. Studies on the genetic basis of atopy have found a decisive genetic effect on IgE levels in twins: the IgE variation is significantly lower in pollen-sensitive monozygotic (MZ) than in dizygotic (DZ) twins (0.15 vs 0.51) [37]. The concordance of atopic dermatitis (AD) and asthma was higher in genetically identical MZ twins [114]. MZ twins have a greater concordance ratio for atopy [37, 173], while in DZ twins BHR (bronchial hyperreactivity) and total and specific IgE prevail [37]. A recent twin family study supported a strong genetic component for asthma [227], while in MZ twins, correlation coefficients were significantly higher for IgE levels, since about 50% of variations could be attributed to genetic factors, with a great concordance (71%) for IgE responses to allergens [153]. In a population of unselected twins, a high IgE genetic dependency was shown on the order of 75%–85% [186]. These and other studies in twins [33] show that IgE inheritability (the part of variability attributed to genetic factors) is between 50% and 84%, and that the overrid-

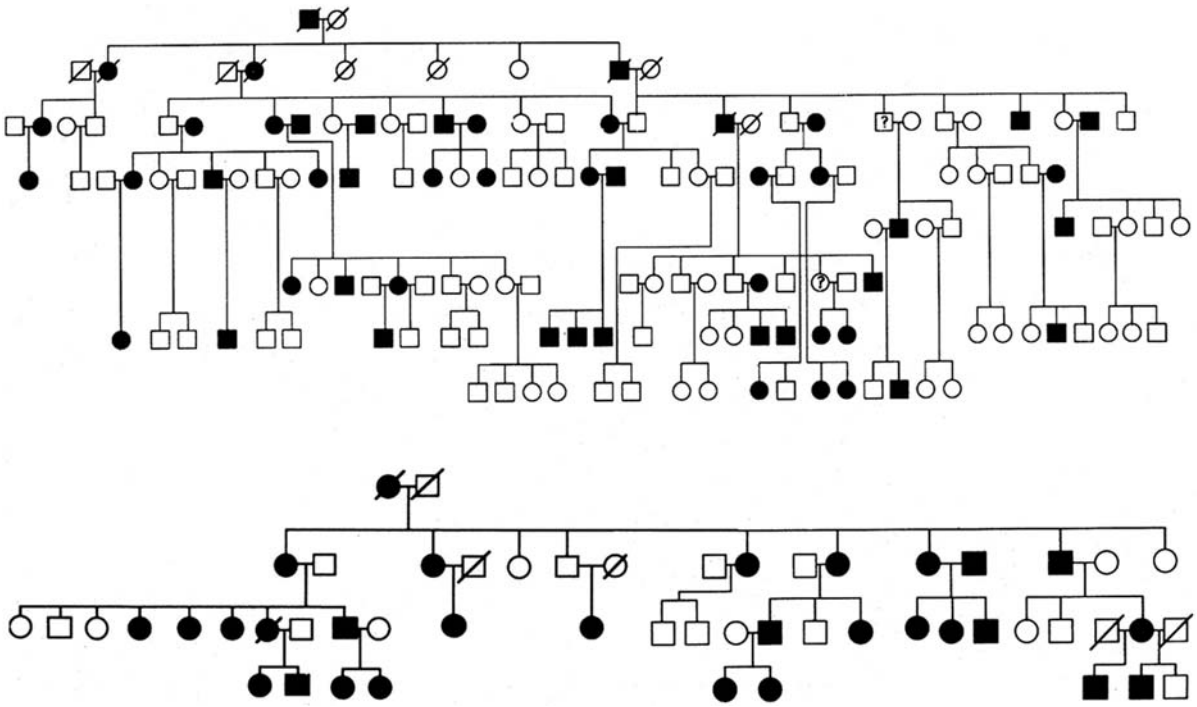

Fig. 4.3. Frequency of atopic IgE responsiveness in two extended families: (*shaded symbols*) atopic, (*open symbols*) normal, (*?*) uncertain phenotype, (*Ø*) dead

ing influence on IgE levels is genetic rather than environmental [153]. A common experience is that the higher the IgE levels the higher the risk of atopy [77]. However, no univocal decision was made on the model of heredity based on IgE levels. In 1916, a positive FHA (FHA^+) was found in 48% of the probands compared with only 14% of the control group, so almost 75% of children with a bilateral FHA were at risk of developing atopic disease vs 50% with a monolateral FHA. The authors concluded that atopy was inherited as a mendelian autosomal dominant trait [76]. By contrast, others conclude that the high IgE state is based on autosomal recessive inheritance [138, 265]. Subsequently, a study on 20 patients and their parents selected according to an FHA positive for IgE-mediated asthma and allergic rhinitis (AR) has demonstrated that atopy is vertically transmitted, since 90% of the 20 atopic subjects had at least one atopic parent compared with 25% of controls (p=0.005), so concluding for a mendelian model of inheritance [77] (Figs. 4.2, 4.3). In these probands and their families, the distribution of specific allergic responses among the members of the same familial nucleus was heterogeneous, thus suggesting the influence of other factors [77]. Several studies have established that atopic disease is multifactorial (Table 4.1) [34, 72, 77, 78, 138, 155, 265, 266, 269, 277, 278, 495, 496]. The terms "polygenic" and "multifactorial" mean that several genes along with a battery of environmental factors influence the clinical expression of atopy; "genetic heterogeneity" means that different genes or combinations of genes determine a disease phenotype, apparently the same or similar to a clinical perspective [262]. A multifactorial pathogenesis is well suited for atopy transmission [114, 205] and is confirmed by the following data: AD and atopy inheritance is not 50%, as required by an autosomal dominant transmission, and atopy incidence is much greater than that of typical monogenic disorders, in which external factors do not play as significant a role as in atopic disease [206].

The interactions between IgE, allergens, HLA-D genes and TcR represent the development of what is called *forward genetics* [173], focussing on the analysis and functional mapping of prospective candidate genes. A further approach of molecular biology is *reverse genetics,* usually termed positional cloning, primarily employing PCR (polymerase chain reaction) to amplify microsatellite regions of genomic DNA or family members [262], which tries to identify the nucleotide markers within the genome for a more effective localization of the genes correlated with the atopy phenotype [32, 182]. In genetic studies the term "linkage" is used, that is, a condition where two genes are both localized on a single chromosome and are usually inherited together, and the lod (logarithm of the odds) score. A lod score of ≥3.0 expresses a significant evidence of association, =1,000:1 [318], but it may be false positive in 1:1,000 cases [263].

Table 4.1. Models of genetic transmission of serum IgE levels in family studies

Authors (reference)	Year	No. and type of family	Type of heredity
Gerrard et al [138]	1978	173 Nuclear	Autosomal recessive + polygenic component
Marsh et al [265]	1981	28 Nuclear	Autosomal recessive
Blumenthal et al [34]	1981	3 Extended	Genetic heterogeneity
Hasstedt et al [155]	1981	5 Extended Mormon	Polygenic
Meyers et al [277]	1982	42 Nuclear	Mixed
Meyers et al [278]	1987	23 Nuclear Amish	Codominant
Cookson et al [77]	1988	40 Nuclear and 3 extended	Autosomal dominant
Coleman et al [72]	1993	95 Nuclear	Genetic heterogeneity
Marsh et al [266]	1994	11 Amish	Gene polymorphism
Martinez et al [269]	1994	798 Nuclear	Autosomal codominant gene
Xu et al [495]	1995	92 Families	Mixed recessive gene
Xu et al [496]	2000	200 Families	Two genes, a recessive and a dominant gene
Cookson et al [78]	2001	148 Nuclear	Genetic heterogeneity

Genome-Wide Screens

As yet, several genome-wide screens have successfully identified several chromosomal locations that are likely to contain asthma and atopy genes [18, 73, 92, 105, 144, 215, 228, 234, 272, 306, 307, 489, 494, 497, 498, 501]. In addition, several candidate genes for association with atopy in family studies are outlined in Table 4.2 [17, 18, 21, 26, 73, 74, 77–80, 82, 83, 92, 105, 123, 124, 126, 144, 154, 171, 176, 208, 215, 222, 228, 234, 261, 266, 272, 279, 287, 288, 305–307, 342, 360, 376, 380, 397, 398, 435, 447, 450–452, 457, 463, 487, 489, 496, 498, 501, 502, 503, 506, 511] and in case–control studies (Table 4.3) [26, 78, 109, 165, 166, 178, 201, 361]. Recently, 80 additional genes related to asthma development have been reported [257].

The genome-wide studies have mapped specific chromosomes with high lod scores for asthma or BHR [73, 92, 105, 228, 307, 487, 497], AD [222, 234], seasonal allergic rhinitis (SAR) (lod =1.52–2.01] [502], perennial allergic rhinitis (PAR) [144] (lod =1.04–2.83), IgE [92, 105, 228, 234, 489, 502], specific IgE [234, 489, 497], asthma-related phenotypes, including forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) [498], SPTs [92, 105, 215, 307], and eosinophils [92, 105, 489].

The CSGA genome-wide screen [73] was done in 266 families and used 323 polymorphic DNA markers that were distributed across the genome. All of the affected family members met the same criteria for asthma as the probands. Evidence for linkage was observed in African-American families for *1lq21*, and a lod score =2.0 (p=0.002). Evidence for linkage to *lp32* was found in Hispanic families, with a lod=2.92 (p=0.0002). Seven other chromosomal regions had lod scores >1.0 (*2q*, *5q*, *1lp*, *12q*, *14q*, *17q*, and *21q*). In Caucasian families, evidence for linkage was found at *6p21*, with a lod score of 1.91 (p=0.003). Lod scores >1.0 were found at *8p23* (lod=1.06), *l9ql3* (lod=1.02), and *20pl3* (lod=1.07). Across the entire population, there were no regions with lod scores >2.0; the highest lod score was found at *14q32* (lod=1.23; p=0.017). Two other regions (*5q31* and *12q22*) had lod scores >1.0, whereas seven other regions (*lq*, *4q*, *8p*, *11q*, *12p*, *13q*, *17q*) had lod scores >0.5 [451].

Candidate Genes for Asthma and Atopy

On *chromosome 2q*, CD28 was found [161], also considered an accessory molecule in the regulation of IgE antibodies.

Among the present multitude of genes that may play a role in elevated IgE levels and atopic disease, the genes on *chromosome 5q31–33* encode for the production of several ILs (IL cluster) (Fig. 4.4) [266] and other candidates, including β_2-adrenoreceptor (ADR) [342]. On chromosome 5q31-q33 specific *loci* contribute to susceptibility to atopy and are linked to the development of asthma in childhood [501]. The corticosteroid (CS) receptor is located in close vicinity in the *5q31-q32* region *6*. In a study of 11 Amish extended families, a significant linkage was found to IgE (sIgE) antibodies, with more significant statistical differences than with the IL_4 gene; however, no significant linkage was found to specific IgE antibody concentrations [266]. The analysis of 170 subjects has shown that polymorphism within the IL_4 promoter region may enhance IgE production and Th2 expression [262]. The most significant linkage of IgE is with the D5S436 marker [279]. Moreover, the genes regulating both BHR and elevated IgE antibodies are coinherited by sibling pairs concordant with asthma and

Table 4.2. Linkage between chromosomal region markers and atopy in family-based genetic studies

Chromosome	Locus (marker)	Linked trait/phenotype	References
1	*D1S518*	Total serum IgE levels	[498]
1p21	*D1S3728, D1S2846*	Total serum IgE levels	[496]
		Asthma	[105]
1p32	*D1S2134*	Asthma	[450]
1p36	*D1S239*	SPT + to cockroach	[307]
1p36.2	*D1S2667*	Seasonal allergic rhinitis	[502]
1q21	*D15252, D15498*	AD	[83]
	D175784, D175928		
2	*D2S1780*	BHR to methacholine challenge test	[498]
2q	CD28	T-cell proliferation	[176]
2pter	*D2S2298*	Asthma, specific or total IgE	[489]
2q12–q33	*D2S442*	Allergic rhinitis	[144]
2q33		Asthma	[73]
2q24–q32	*D2S1776, D2S1391*	Eosinophils, total serum IgE levels	[215]
3p		BHR	[376]
3p24.1	*D3S1266*	Total serum IgE levels	[502]
3p24.2–22	*D3S2432, D3S1768*	BHR, asthma symptoms	[306]
	D3S3564	Loose asthma	[307]
3q13	*D352460*	Allergic rhinitis	[144]
3q21.3	*D3S3606*	AD, total serum IgE levels	[234]
	D3S3606	AD	[222]
3q29	*D3S1311*	Total serum IgE levels	[496]
3q25–q26	*D3S3053*	Specific IgE to Der p 1	[215]
	D3S1311	Total serum IgE levels	[496]
4	*D10S1435*	SPT + to cockroach	[498]
	D4S1627	Total serum IgE levels	[272]
	D4S5243	Total serum IgE levels	[272]
4q13.3	*D4S392*	Seasonal allergic rhinitis	[502]
4q24–q27	*D4S2394*	Allergic rhinitis	[144]
4q35	*D4S426*	Total serum IgE[b], atopy[b], BHR	[92]
4q35	*D4S2417, D4S408*	Mite-sensitive childhood asthma	[501]
5p	*D5S1470*	BHR	[307]
5q	*D5S1462*	Loose asthma	[307]
	D5S2014	Asthma symptoms	[307]
5q13–q15	*D5S1719*	Allergic rhinitis	[144]
5q-15	*D5S1480*	Loose asthma	[306]
		Asthma	[73]
	D5S2849	SPT to HDM	[215]
5q23–q31		Asthma	[73]
	D5S1505, D5S5816	Total serum IgE levels	[496]

Table 4.2. (Continued)

Chromosome	Locus (marker)	Linked trait/phenotype	References
5q31–q33	Several loci	Several ILs[a] stimulate B-cell switching, MCs and eosinophil functions	[74, 154, 266, 279, 305, 342, 360, 447, 452, 511]
		BHR	[215, 279, 342, 447]
		IgE	[266, 279]
	D5S820, D5S393	Mite-sensitive childhood asthma	[501]
		Atopic dermatitis	[26]
5q31	*D5S404*	Atopic dermatitis	[126]
	IL4, IL9, D5S393	Childhood asthma/atopy	[305]
5q31.1	position 169	CD14, total serum IgE	[21]
5q31–q33	*IL12B locus*	Mite-sensitive childhood asthma	[501]
5q32	ADR	Bronchodilation	[305]
5q33.1	*D5S410*	Total serum IgE levels	[502]
6p21	*D6S2439, D6S2427*	Total serum IgE levels	[215]
	D6S2439, D6S2427	Total serum IgE levels	[496]
	D6S1281	Asthma	[450]
6p21.3	HLA	APCs, allergen-specific IgE Ab	[123, 261, 435, 506]
	D6S260–D6S276	Eosinophil counts	[92]
		Asthma	[73]
	D5S291	Asthma, specific or total IgE	[489]
6p21.3–23	TNF polymorphism	Mediator of inflammatory response	[78]
6p24–p22	*D6S2434, D6S1959*	Eosinophil counts	[215]
6p24–p23	*D6S277*	Allergic rhinitis	[144]
7p14–p15		Asthma, high serum IgE levels	[228]
7p15.2	*D7S2280*	BHR, IgE	[92]
7q11–q22	*D7S820, DS7S821*	Total serum IgE levels	[215]
8p	*S8S1136*	Strict asthma	[307]
8p23		SPT to HDM	[215]
	D8S1130	Asthma	[450]
9q32	*D9S1784*	Asthma, specific or total IgE	[489]
9q34.3	*D9S1826*	Seasonal allergic rhinitis	[502]
10	*D10S1435*	FEV1	[498]
10q		IgE	[215]
11p13	*D11S907*	IgE	[105]
11q	*D11S97*	IgE, atopy (asthma, rhinitis)	[397]
11q13	*FcεRI-β*	IgE, SPT, asthma	[92]
11q13	*D11S96, D11S901*	IgE	[79]
11q13	*FcεRI-β*	Atopy[b]	[77, 82, 287, 380, 398, 505]
		Allergic rhinitis	[398]
		BHR, total serum IgE levels	[452]
		Atopic dermatitis, atopy	[126]
	D11S903, FcεRI-β	Atopic dermatitis, atopy	[124]
	FcεRI	Asthma	[305]

Table 4.2. (Continued)

Chromosome	Locus (marker)	Linked trait/phenotype	References
11q21	*D11S1985*	Asthma	[450]
11q22	*D11S2017*	SPT to HDM	[215]
12p13	*D12S391*	SPT to HDM	[215]
	D12S374	Allergic rhinitis	[144]
12p13.1	*D12S364*	Total serum IgE levels	[502]
12q		IgE	[80]
12q13	*D12S351*	Asthma, specific or total IgE	[489]
12q13.12–q23.3	*D12S326*	Asthma	[18]
	D12S1052	Allergic rhinitis	[18]
12q14–24.2		Asthma	[73]
12q15–24.1	*D12S375*	Loose asthma	[306]
12q15–q24	IFN-γ Th2 T-cell inhibition	Asthma, total serum IgE levels	[17]
12q23	NOS, mast cell GF	Inflammatory mediator	[171]
12q23–q24	*D13S1493, D13S218*	Total serum IgE levels	[215]
	PH-D12S2070	Total serum IgE levels	[496]
12q24.2	*D12S86*	Total serum IgE levels	[502]
12q24.31	*D12S366*	Eosinophil	[105]
13q	*D13S787*	BHR	[306]
	D13S787	Asthma symptoms	[307]
13q	*D13S153*	Atopy	[92]
13q11	*D13S175*	Mite-sensitive childhood asthma	[501]
13q12–q13	*D13S1493, D13S218*	Total serum IgE levels	[496]
13q12–14		Atopic dermatitis	[26]
13q14	*D13S788*	SPT	[215]
13q21.3-qter		Asthma	[73]
14q	*gata193a07*	Loose asthma	[307]
14q11	*TcRα/δ*	Specific IgE Ab, allergen-specific IgE, T-cell activation	[288]
14q11.2–13		Asthma	[73]
15q11	*D15S822*	Eosinophils	[215]
16q22.1	*D16S289*	Asthma[b], atopy[b], IgE levels	[92]
	D16S539	SPT for molds	[307]
16q-tel	*D165520*	Total serum IgE levels	[83]
17q		Asthma	[73]
17q12–21	*D17S250, D17S787*	Asthma, SPT	[105]
17q21	*D17S1290*	SPT to HDM	[215]
17q23	*D17S2193, D17S1301*	Eosinophils	[215]
17q25	*D15252, D15498*	AD	[83]
	D175784, D175928		
19p11	*D18S178*	Specific IgE to Der p 1	[215]
	D19S178	Strict asthma	[306]

Table 4.2. (Continued)

Chromosome	Locus (marker)	Linked trait/phenotype	References
19q	*Q19S900*	BHR	[307]
19q13	*D19S198*	Asthma	[450]
19q13.3	*D19S178*	Asthma	[73]
19q13.3	*D19S601*	Atopy	[457]
20p	*D205115*	Asthma and AD	[83]
20p13	*ADAM33*	Asthma	[451]
20p13	*D20S473*	Asthma	[450]
21q21	*D21S1440*	Strict asthma	[306]
21q		Asthma	[73]
22	*D22S685*	FEV1	[498]
22q11	*D22S420*	Specific IgE to Der p 1, SPT to HDM	[208]
	D22S345	SPT	[208]
22q13	*D22S1150*	Allergic rhinitis	[144]
Xp21	*DXS9907*	Allergic rhinitis	[144]

Several studies report a genome-wide search [73, 92, 104, 144, 215, 227, 234, 306, 307, 487, 450, 501, 502], one study found a significant coincidence of AD findings on chromosomes *1q21*, *17q25*, and *20p* with previously observed linkages to psoriasis [83]. The 80 genes related to asthma can be found in the Ensembl Genome Browser – Ensembl v27 (http://www.ensembl.org/) [257]. Two major *loci* for atopy are on chromosomes 2p12 and 16q21 [222].

[a] IL_3-IL_5, IL_9, IL_{13}, GM-CSF.

[b] Association with maternal heredity.

Ab antibody, *ADR* β_2-adrenergic receptor, *ADAM33* (α disintegrin and α metalloproteinase), *APC* antigen presenting cells, *BHR* bronchial hyperreactivity, *GF* growth factor, *HDM* house dust mite, *IL* interleukins, *MC* metachromatic cells, *NOS* nitric oxide synthase, *SPT* skin prick tests.

Table 4.3. Association between alleles at specific *loci* and atopy in case-control genetic studies

Position	Locus/marker	Associated trait	References
5q31–33	Gene cluster ILs	Total serum IgE levels	[109]
	D5S436, D5S643	Atopic dermatitis	[26]
6p21.3	TNF-α polymorphism	Asthma	[78]
11q13	*FcεRI-β/L181*	Total serum IgE and RAST to grass, atopy	[361]
	FcεRI-β/L181/L183	SPT to Der p and grass, BHR	[166]
	FcεRI-β/G237	RAST to Der p and grass, BHR	[165]
	D11S534/253	Total serum IgE levels	[109]
	D11S527/68	BHR	[109]
	IL4 gene polymorphism	Atopic dermatitis	[201]
13q12–14	*D13S218*	Atopic dermatitis	[26]

All the studies cited here have high significant statistical differences.
BHR bronchial hyperreactivity, *ILs* interleukins, *SPT* skin prick tests.

BHR [257, 342], thus confirming that the region regulating IgE has a *locus* on chromosome *5* [266, 279]. A similar allele association of IgE antibodies is with IL_9 [109]. Clearly, the polymorphism on chromosome *5q* has possible functional consequences: for example, the gene polymorphism of the IL_4 promoter is found in 64% of asthmatic compared to 15% of control patients [41]. Moreover, multiple allelic forms of the IL_4 promoter do exist, associated with an increased transcriptional activity of T IL_4^+ cells [407]. This polymorphism is also

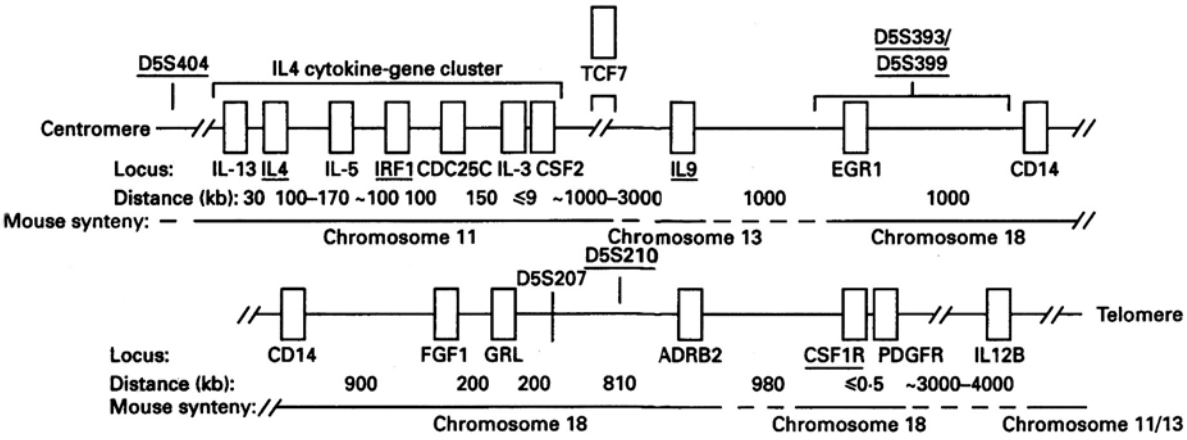

Fig. 4.4. Chart showing genes of known physical location and certain polymorphic markers in and around chromosome *5q31.1–q33*. Band *5q31.1* extends approximately from IL-13 to CD14. The IL-4-cytokine gene cluster includes several genes important in atopic diseases. IL-4 gene cluster, IL-4-cytokine gene cluster. *ADRβ2* β2-adrenergic receptor, *CSF2 GM-CSF* granulocyte macrophage-colony stimulating factor, *FGF* fibroblast growth factor, *IRF1* IFN regulatory factor-1, *PDGFR* platelet-derived growth factor receptor: CD140, *kb* kilobase

linked to the *Arg 16* allele mutations associated with nocturnal asthma and *Gln 27* allele with BHR. Functional studies indicate that a *Gly 16* substitution increases stimulus-related receptor desensitization, thus challenging patients with severe asthma or with a significant nocturnal component associated with an ADR depression [441]. Asthma may be induced by *ADR* gene polymorphism and more precisely by its *Gln 27* form, whereas asthma should be less probable when the *Glu 27* form is prevalent [149]. The candidate genes on chromosome *5* are numerous; thus a clear conclusion is perplexing [31]. A polymorphism in the 5′ flanking region of the gene for the leukocyte antigen CD14, which was mapped to chromosome *5q31–33*, encoding the high affinity receptor for endotoxin, was associated in allergen-sensitized children, with lower serum IgE levels and sensitization to fewer allergens [15]. Interestingly, on chromosome *5q23–35*, a region homologous to human chromosome *5q*, a mendelian trait encoded by T cell and airway phenotype regulator (Tapr) was identified. Tapr is genetically distinct from known IL genes and controls the development of BHR and T cell production of IL_4 and IL_{13} [275]. Positional cloning identified a gene family located at human chromosome *5q23–35* that encodes T cell membrane proteins (TIM, T-cell immunoglobulin and mucin-1). Major sequence variants of this gene family (TIM) were completely co-segregated with Tapr. The human homology of TIM-1 is the hepatitis A virus (HAV) receptor, which may explain the inverse relationship between HAV infection and the development of atopy [275].

The genes encoding the HLA complex are located on *chromosome 6p*, where a strong linkage exists to celiac disease (Chap. 9) and to inflammatory bowel disease (Chap. 18). Since the HLA class II *locus* on chromosome *6* is very polymorphous, especially in the exon corresponding to genes α and β hypervariable regions (HVRs) of each HLA subregion, it is a site of great relevance because it presents allergenic peptides via HLA to effector cells. These HLA genes are also important in allergen-specific IgE responses [123, 261, 503]. Moreover, total IgE shows evidence of linkage to chromosome *6p21* [215, 496]. Linkage has been found for eosinophil count in a genome screen of atopic families [92]. In Caucasian sib pairs, there was evidence for increased allele sharing for markers on *6p21.3.23* [73]. Another linkage is with TNF genes, which is important in mediating inflammatory responses, as is HLA in antigen presentation [78].

Evidence for linkage was found in a 20-cM region of *chromosome 7p14-p15* for three phenotypes: asthma, a high level of IgE and the combination of the phenotypes [228]. In region *q32* is the TcRβ chain (Fig. 1.14).

A gene on the long arm of *chromosome 11* in region *q13* appears to be a key player linked to a specific atopic phenotype (interpreted as a tendency to produce elevated IgE antibodies) and may be correlated to a predisposition to atopy [80]. Significantly, some primary immunodeficiencies, including deficits of IgA, opsonization, C2, IgG_2 and a wide series of functional defects of T lymphocytes, are connected with an increased risk of developing atopic disease (Chap. 22). In particular, the atopic status responsible for asthma and AR seems to be linked to chromosome *11* [80], although such data are questionable and less reproducible [80, 182]. The Oxford group has genotyped parents in several nuclear atopic families related to the transmission of chromosome *11q13* [506], continuing the studies on the mendelian autosomal dominant model and examining >300 sibling pairs [82, 166, 287, 380, 398, 399]. A parallel study on genetic inheritance [505] has demonstrated that in 21 atopic sibling pairs, the concentrations of Der p were

significantly higher in both mattresses and bed linen (but not on the bedroom floor) of a Der p-allergic than in the bed linen of his brother, sensitized to a different allergen [507]. In other 15 perfectly matched pairs (also for bed occupancy since birth) in whom a brother was nonatopic, there were no differences, not even in soil findings. Genetic segregation and related gene analyses, based on IgE antibodies as recruitment criteria to the atopic state, have clarified that heredity was of a dominant type and coded by the *locus 11q* [505]. It was similarly ascertained that sIgE responses to Der p were partly dependent on the allergen load, underlining the key role of the environment [504]. Although the Oxford group results found evidence linking at least one major gene of atopy to chromosome *11*, with a lod score between 10 and 3.38 [174, 287], there has been much dispute over the linking of atopy to this chromosome. Results in the Japanese population demonstrated that the *11q locus* governs IgE responsiveness (lod score =4.88) [397], especially if the atopy is severe [398]. Subsequent studies by Anglo-American and Dutch researchers [5, 72, 92, 167, 258, 251, 355] have found no association with chromosome *11q13*. The discrepancy may also be due to differences in populations, such as ethnicity and how the families studied were recruited, and/or to differences in definition of phenotypes, or different methods used [399], although no definite cause has been ascertained. Studying the patients with AD as probands, the association of atopy with the *11q13* gene disappeared, but the lod scores of these studies were too low (≤0) to direct criticism against the data referred to above [263]. Also localized on chromosome *11q13* is the subunit of the high-affinity IgE receptor FcεRI-β, which could be associated with elevated IgE of atopic patients [380]. Subsequent studies have confirmed the Oxford data [74, 154, 398, 452]. Two of these studies have found replicated linkage between atopy and chromosome *11* by sib-pair analysis [74] and using lod score methods in a set of extended families [398].

The gene encoding for FcεRI-β sequences on chromosome *11q13* is strongly linked to asthma and BHR, regardless of the atopic state: studying the whole population instead of a selected group strengthens the link between *11q13* and BHR [452]. The gene encoding for FcεRI-β, if maternally inherited, has been linked to a potential risk factor demonstrated by the high genetic association observed between polymorphism of *Leu181/Leu183* variants, atopy and BHR [166] (Table 4.3), shown in highly atopic Japanese [398] and English [399] patients. Other studies have not found the same linkage with FcεRI [46, 148, 211, 305]. Subsequent evidence has linked chromosome *11q13* and FcεRI-β to atopy [77, 81, 124, 126, 287, 380, 505], AR [398], BHR and IgE [452], AD [124, 398], and asthma [305]. The FcεRI-β *locus* showed significant allele sharing in affected sib pairs for BHR and for SPT positivity [257].In the region lying between *11q11* and *11q13*, Clara cell protein (CC16, previously CC10) was localized, a polymorphous gene released by nonciliated cells of the proximal airways and usually inhibiting airway inflammation, making this an area requiring further study on the role of Clara cells in asthma pathogenesis [158].

On *chromosome 12q*, the genes of asthma are significantly linked to IgE levels [17, 18, 489] and to AR [18, 144]. This chromosome contains the NO-synthase (NOS), a gene important in the inflammatory response [177]. On chromosomes *11q* and *12q*, either linkage or association of alleles from two markers separated by only a few centimorgans was observed with either log IgE or BHR [17, 109], a finding consistent with the clinical correlation observed between high IgE levels and BHR [176]. In a sibling-pair analysis conducted on 29 Afro-Caribbean families, *12q15–q24.1* with several candidate genes that influence IL regulation and IgE production was linked to asthma and IgE production [17]. Evidence for linkage for atopy was also detected in Hispanic sib pairs aged 11.63±4.95 years [92] and for asthma in Caucasians and Hispanics [73]. Recently, no evidence was found for linkage of the genomic region *12q13–24* to elevated IgE antibodies and related specific sensitization to common inhalant allergens or atopy in a study failing to focus on the asthmatic and inflammatory aspect of atopy [159].

Chromosome 13q maps atopy [92], AD [28], asthma [307], mite-sensitive childhood asthma [501], and SPTs [215].

On *chromosome 14q12*, the α chains of TcRαδ, associated with total and specific IgE [288], and NF-κB are mapped (Chap. 1); several other *loci* related to respiratory allergy have recently been localized [92] (Tables 4.2, 4.3).

Chromosome 20 maps AD [83] and above all asthma [83, 451, 497]. Van Eerdewegh et al conducted a genetic linkage analysis on 460 pairs of siblings from affected families in the US and UK and identified a *locus* on the short arm of chromosome *20* that was linked to asthma (lod, 2.94) and BHR (lod, 3.93). They assessed 135 polymorphisms of 23 genes in this region and identified by positional cloning the *ADAM33* gene, belonging to the ADAM12, ADAM13, and ADAM19 subfamily, which encodes a protein-processing enzyme known as a metalloprotease, significantly associated with asthma [451].

Other Regions of Interest

Novel regions of interest have been described, showing evidence for linkage to several chromosomes:

- *Chromosome 16q*, asthma, atopy, IgE [92], SPTs for molds [307], and IgE antibodies [83].
- *Chromosome 17q,* asthma [73, 105], SPTs [105, 215], AD [83].
- On *chromosome 19q13.3* a suggestive linkage of asthma [73], atopy and positive SPTs [457].
- *Chromosome 20*, asthma [83, 497] and AD [83].
- *Chromosome 21*, asthma [70] and strict asthma [306].
- *Chromosome 22*, specific IgE to Der p 1, SPT to HDM [229], and AR [144].

As regards the preferential *maternal pattern of inheritance,* Cookson et al [82] provided the first evidence for a maternal effect to atopy at the *11q13* marker for the FcεRI. The population under scrutiny [82] had in common an excess of alleles of maternal origin, whereas no excess in sharing paternal alleles was seen, a result different from the expected distribution; 62% of the sibling pairs affected by atopy shared their maternal allele, most likely pointing to a *genomic imprinting,* with activation of the maternal *locus* and parallel suppression of the paternal one [82]. These results show a significant sharing of maternally inherited alleles in region *11q13* in sib pairs with atopic IgE responsiveness. There lies the risk factor of the maternal inheritance of the polymorphism associated with atopy and BHR [166] and with FcεRI-β [398]. Atopic siblings share maternal but not paternal alleles in the chromosome *16p12* region, which increases the risk for enhanced IgE responsiveness. The most obvious candidate gene in this region is the gene for $IL_4RA\alpha$. Further evidence for inheritance through maternally transmitted alleles and atopy at different *loci* has been reported, including chromosomes *4q35.2*, *11q13*, *16q24.1* [96].

2. The genetic association of maternal atopy with chromosome *11* is undoubtedly interesting, although the origins remain for the most part unclear [165]. This is further proof that genomic imprinting [398], or the preferential expression of a paternal or maternal allele, imparts a decisive influence on mammalian development, in addition to the inheritance of polygenic human disease [150]. Several epidemiological studies highlight that inheritance of atopy and asthma is higher through mothers compared to fathers [2, 14, 151, 184, 220, 225, 255]. The evidence accumulated from the reported studies on the one hand points out that atopy is inherited at a given *locus,* and on the other hand provides an explanation for the frequent finding of a *greater risk for the children of atopic mothers.* Several observations suggest a maternal transmission to fetal and neonatal immune systems in evolution or a genomic imprinting by which the paternal gene expression could be abrogated, although it could be reactivated while being transmitted from the mother, thus paving the way for carriers. This hypothesis is in accord with both inheritance patterns, dominant and recessive [150]. Others explain this preference by the *Carter effect* [62]: atopy is a polygenic condition, thus if more predisposing genes are possessed by an individual, atopy expression is more probable. Since the threshold is higher in females, they inherit more genes for a disease expression and consequently their male offspring are more at risk [514].

In conclusion, the genetic mapping of atopy is far from simple, especially when taking into account the increasing prevalence of atopic disease in the past 3 decades (Chap. 5), and it is difficult to explain on the basis of genes alone. Furthermore, linkage of atopy with a gene on *11q13* could not be shown when patients with AD were taken as probands [72]. Thus more than one gene seems to be involved.

3. The interactions among numerous factors, genetic and nongenetic, lead to the disease phenotypic expression of manifold genetic traits that regulate general and specific responses to allergenic epitopes. These should be considered as determinants interacting in atopic disease, correlated with both profile and frequency of allergenic exposure and with the involvement of several HLA-D antigens and TcR as contributors to atopy pathogenesis, and thus of genetic and molecular bases of atopic disease [312]. A predisposing factor for allergic disease could be represented by the particular genetic set of HLA class II antigens, as based on the demonstrated correlation among these molecules and IgE responses to specific allergens. Following this pathogenetic hypothesis, recent studies have suggested that in atopic patients there are three types of *genetic alterations:*

- Increased response to antigens dependent on peculiar genes linked to the HLA haplotype.
- Significantly increased frequency related to some HLA haplotypes.
- Increased IgE production apparently independent of HLA.

Scientists have therefore concentrated their interest on two distinct and interdependent questions: whether a correlation exists between atopic disease, IgE levels, and HLA haplotypes and why there is IgE hyperproduction in atopics.

The possible correlations between atopic disease and/or HLA haplotype and IgE levels have basal mechanisms that are operative since fetal life, because the T-cell repertoire cannot be influenced by HLA haplotypes of a single subject [247]. Studies have speculated on a reciprocal genetic influence between HLA genes controlling specific immune responses and non-HLA genes controlling IgE synthesis. However, there is no IgE response in the absence of Ir genes; therefore the individual has inherited HLA alleles that induce synthesis of antibodies different from IgE in the presentation of allergenic peptides to TcR; yet subjects with an HLA–Ir association can also be sensitized in the absence of IgE responses. In addition, alterations of regulatory mechanisms in atopic subjects are multiple, they have a genetic basis [354] and different associations may lead to the development of an atopic phenotype with a Th2 prevalence [483] (Fig. 4.5).

The role played by HLA molecules in regulating immune response has been an incentive to undertake studies that may link the different disciplines of genetics and immunology; this new and fertile field of research has provided an impetus for the study of the association between particular HLA alleles and IgE responses to specific aeroallergens. In these studies, a product of the *HLA-DRAB3* gene (DRw52a) is a class II molecule assigned to the presentation of HDM allergens to T cells:

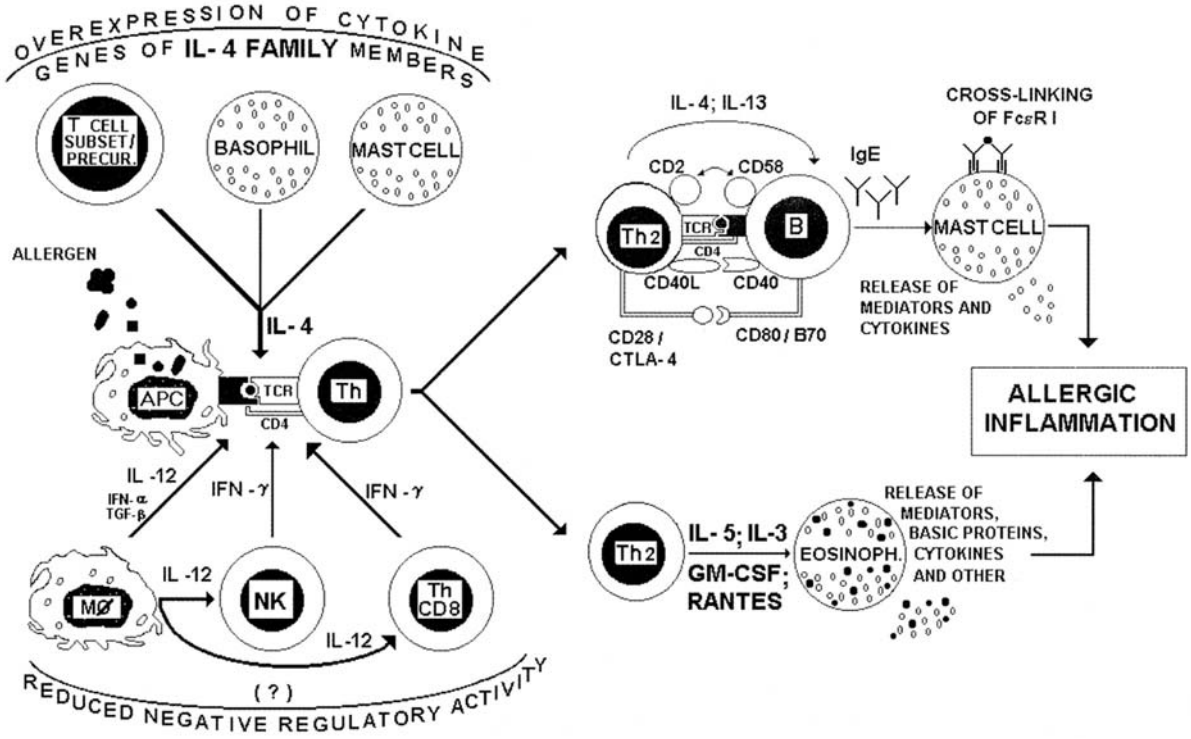

Fig. 4.5. Possible genetic, cellular, and molecular alterations contributing to the expansion of Th2 lymphocytes and to triggering the allergic inflammation in atopic subjects. *APC* antigen presenting cells, *IL* Interleukins, *MØ* Macrophage

following a peptide binding to DRw52b, there was no cell activation [312]. Moreover, an analogic peptide inhibited the proliferation of Der p-specific T-cell clones in vitro, and the HLA-DP recognition restricted T lymphocytes [163], thus opening the way to a possible functional T-cell inactivation by employing these peptides [312] (Chap. 13). Further progress in the research may provide pivotal insights for clinical studies: this approach offers unique opportunities to identify the Der p T-epitope repertoire, that is, the Der p 1 immunodominant regions containing several epitopes activating HLA class II-restricted T cells belonging to various regions [164, 510], or distributed along the primary sequence [510] (Tables 1.76, 1.77). The Der p 2 T-cell epitopes are present in all regions [311]. These epitopes are recognized by allergen-specific T lymphocytes isolated from atopic and nonatopic patients. Taking a more targeted approach to the HLA restriction to HDM allergens, T lymphocytes are directed toward several allergens, and even taking into account only Der p 1 and 2, there are several T-cell epitopes available (Table 1.76) (in addition to B cells). Efforts must focus on defining the Der p allergen presentation to T-cell clones by HLA class II molecules. Consequently, much emphasis has been placed on Der p 1, 2 and 3 allergenic epitope recognition by HLA-controlled T lymphocytes and the ensuing Th2 rather than Th1 T cell production, independently of HLA regulation [311]. One way forward may be the study of additional allergens, thus providing evidence for sIgE responses to Amb a 5, Amb t 5, Amb p 5, Amb a 6 pollen allergens associated with HLA-DR2 (*HLA-DRB1*0102*) alleles, Lol p 1–3 with DR3 (*HLA-DRB1*0103*) and DR5 (*HLA-DRB1*0105*) alleles [177], as well as the Der p family with DRB1 (*HLA-DRB1*0101*), DRB3 and DRB5 alleles [311] (Appendix 1.1). Additional studies have provided convincing evidence for sufficiently defined correlations with IgE antibody increase to other HLA alleles in atopic subjects compared to controls: *HLA-DRB1*0101*, *DPB1*0402* and *DPB1*0501* alleles to Der p 1, and the *HLA-DPB1*0401* allele to Fel d 1, Can f 1, Alt a 1, Phl p 5, Der p 1, and Der p 2 [163]. Significant linkage of HDM-specific IgE response to the *DPB1*0401* allele was found in sib pairs with IgE responsiveness to the HDM allergen [413]. Differences are evident in Japanese subjects, in whom *HLA-DRB1*1502* and *HLA-DRB1*0405* alleles to Der p 2 are common [313]; instead, *HLA-DRB1*1101* is frequent in Caucasians [458]. *HLA-DRB1*0101*, along with *HLA-DQA1*0101* and *HLA-DQB1* 0501* alleles, may be the gene predominant for immune response to the Chi t 1 allergen [435] or it may not [357]. However, several findings were not confirmed. In 83 UK atopic families, Young et al established a strong association with HLA alleles and IgE levels for Alt a 1 and Phl p 5 with the *HLA-DRB1*0104* allele, Der p 2 with the *HLA-DRB1*0103* allele and Fel d 1 with the *HLA-DRB1*0101* allele, which

showed the greatest statistical significance [506], along with that between anti-bet v 1 sIgE and HLA-DR3 in European patients [123]. The correlations of HLA class II and sIgE to Amb a 5 with *DR2*1500*, Amb a 6 with *DR5*, Ole e 1 with *DQ2*, Par o 1 with *DRB1*1101* and Lol 1–3 with *DR3* [32] have been confirmed, as well as associations with high IgE levels to Amb a 5, Amb t 5 and Amb p 5 with *HLA-DR2/Dw2* [520]. Since ragweed-allergic patients have *DR2* and *Dw2* sequences [519], it has been hypothesized that αβ1 heterodimers are the main HLA class II molecules mediating Amb a 5 presentation [520]. Previous data on Der p 1 and the associations with HLA-DR or DP alleles appear to be inadequate [506], while the allergen epitope recognized by HLA-DP and reactive in atopic subjects has been characterized [163]. Furthermore, particular Der p 1 epitopes are probably recognized by multiple HLA-DPB1 alleles provided with a greater heterogeneity in HLA class II-restricted alleles of the same region as the allergens [177]. In highly atopic patients, there is a great proliferative response of T lymphocytes to Der p allergens, HLA-DR-restricted by $CD4^+$ T cells, while in low atopic or nonatopic patients, the response is HLA-DQ-restricted by $CD8^+$ T cells [273].

Several studies show that HLA-D genes are critical for IgE responses [262]. Table 1.77 summarizes a recent investigation on the association of single allergens with HLA alleles and IgE levels, with a different relative risk factor: the HLA-IgE correlation is highest for allergens with a lower MW, which is probably caused by spatial limits; they thus contain only a restricted number of T epitopes compared to other allergens. However, exactly how the *locus* of HLA class II genes regulates the immune response of human beings is unknown [35]. This raises the issue of whether an interdependence between *IgE-regulating genes* and specific HLA molecules is achieved by inducing an immune IgE response [262]; hitherto class I gene products could be implied in the expression of diseases associated with class II antigens [207]. Ir genes, which condition sIgE production, could be localized either in the vicinity of HLA class II genes (in an association disequilibrium with certain alleles), or in the genome. However, their expression depends on specific HLA genes: it appears that positive associations between IgE and HLA class II molecules are correlated with strong $CD4^+$ (Th2) responses to the immunodominant regions of processed allergens, hence regulating IgE-mediated hypersensitivity responses [177].

Genetics of Pediatric Atopic Disease

In *pediatric atopic disease* the *DRB*0100/*0300/*1100* and *DPB*0201/*0401* haplotypes are significantly associated with asthma, AR and atopy (asthma and/or AR and/or AD), but more with Der p, establishing the role played by HLA antigens in atopy development [414]. A linkage between childhood asthma and atopy [305], and HDM-sensitive asthma, is on chromosomes *4p35 5q31–q33* and *13q11* [501], near the $IL_{12\beta}$ *locus,* and on chromosomes *11q22* and *12p13*, which have been found with the phenotype "HDM sensitization" [215] near the IL_{18} *locus*. Further data on the immunogenetic *respiratory allergy* regard significant associations with the HLA-B7 haplotype, DR2 almost exclusively in asthmatics, and with HLA-B8, DR3 more frequent in rhinitis patients, both ragweed-sensitive [35]. In patients with ragweed-induced asthma and rhinitis, IgE concentrations were higher in atopic patients compared to controls and in asthmatic compared to rhinitis patients [35]. A linkage between *HLA-Dw2* and skin reactivity to Ra5, a very purified component of ragweed pollen, cannot be hypothesized [264], because the sensitivity to Ra5 is linked to different HLA haplotypes in different families, in some of them without any link to HLA [33]. In pairs of asthmatic brothers, a recessive gene linked to HLA controlling IgE responses to HDM may confer susceptibility to asthma [59], and in Chinese schoolchildren Der p is associated with *HLA-DQw2* [180]. Instead the association with *DPB*0401* is absent in a mulatto population [58] and in Der p-sensitive patients, either nonatopic or with an SPT diameter <2 mm and with *HLA-DR*, *DP* and *DQ* [237]. In atopic asthmatics, there is a strong association with HLA class II alleles *DR4*, *DR7* [11, 12], *DQA1*0301* and *DQB1*0302* [11] and with atopic disease in general [12]. In Dac g allergic patients and controls, a positive association was found with *DRB1*0301* and *DRB1*0401* and with sIgE to Dac g 1 and Dac g 5, respectively, while TNF-α is encoded by a gene in the HLA class II region on chromosome *6p21.3*, thus playing a crucial role in asthma pathogenesis [78].

The growing literature previously discussed on *asthma genetics* [17, 18, 73, 82, 83, 105, 166, 176, 228, 305–307, 342, 452, 457, 489, 497–499, 501] and so-called loose asthma [306, 307] and on BHR [92, 109, 165, 166, 215, 279, 306, 307, 342, 493] (Tables 4.2, 4.3) has shown striking progress, correlating BHR either to FcεRI (chromosome *11q13*) [166, 452] or to IgE hyperproduction (chromosome *5q31.1*) [342] or not [109]. These trends of genetic research, following the suggestion of an autosomal dominant pattern of inheritance [246], are strengthened by the association with DNA markers on chromosome *5q31–q33* [31, 236] (Fig. 4.6) [236]. Recently, linkage of BHR or atopy was confirmed for markers on chromosomes *5q* [215, 278, 279, 305, 307, 493, 496], *6p* [261, 506], *7q* [92], *11q* [77, 82, 126, 287, 380, 398, 399, 452, 505], *14q* [288], and *17q*, which contain known candidate genes and five new regions (*2q*, *5p*, *11p*, *17p*, and *19q*) [73] (Table 4.2). Although the association of BHR and IgE levels with the same or different genes on this chromosome is still quite uncertain [318], the dependence on atopy may be strengthened by the absence of maternal asthma transmission in patients with BHR not linked to atopy [452]. The heredity factor is materialized in a wheezing risk of 1.7 if only one parent and of 17.9 if both parents suffer from BHR [116], in addition to an asthma risk prevalent in male offspring [514].

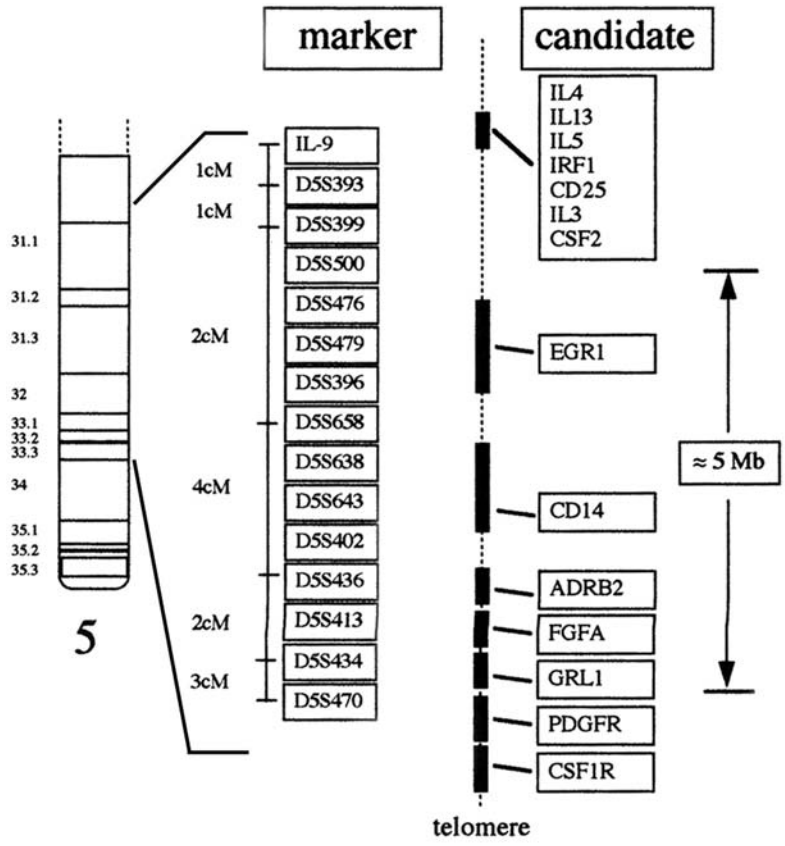

Fig. 4.6. Genetic map of the chromosome *5q31.1–q33* linked to genes providing susceptibility to BHR and atopy

The *AR genetics* has been known since 1916 [76], with positive patient associations with *HLA-DQ3* [136, 373] and negative with *HLA-A2*, as well as an *HLA-A3* and *B7* haplotype increase in those patients manifesting asthma later on [136]. *HLA-DR2* (*HLA-B7*, *SC31*, *DR2*] and *HLA-B8*, *SC01*, *DR3* haplotypes distinguish subjects with asthma from those with rhinitis if ragweed-allergic [35] and *HLA-A* and *B loci* PAR subjects [238]. A linkage between IgE responses underlying AR and chromosome *11q* was found in Japanese families [398]. In 123 children, genotyping data of 400 microsatellite markers suggested linkage of SAR to chromosomes *1p36.2*, *4q13.3*, *9q34.3* [502] and *12q13* [18]. A subsequent study in 79 children revealed one major candidate region on chromosome *4q24–q27* and eight minor candidate regions (Table 4.2) likely to contain susceptibility genes for PAR [144].

In *AD genetics,* positive associations with the HLA system have been thoroughly examined but not fully established [206, 387], and no definite consistent association has been found with HLA-A, -B, -C and -DR antigens [374], neither with a gene on *11q13* [72] nor in children with cow's milk (CM) allergy (CMA) and in their parents with *HLA-A*, *HLA-B* and *HLA-DR* alleles, nor with alleles of the *HLA-D* domain such as *DQ* and *DR52/53* [388]. A recent study in children with AD and CMA found a positive association with *HLA-DQ*, and that DQ^+ children had a prevalence for humoral rather than cellular responses [52]. Evidence for linkage and allelic associations for AD was found in 192 children for markers on chromosomes *13q12–14* and *5q31–33* [26]. A study of 12 families with AD supports the findings of the IgE gene FcεRI-β at *11q13* [124]. A linkage on chromosome *5q31*, a major *locus* that causes a general predisposition to atopy, was found in 50 families with offspring with AD that started at 3–10 months [126]. A significantly preferential transmission to AD offspring of the *T* allele of the –590C/T polymorphism of the IL_4 gene was reported [201] (Table 4.3). Because the *T* allele is associated with increased IL_4 gene promoter activity compared with the *C* allele, the data suggest that genetic differences in transcriptional activity of the IL_4 gene influence AD predisposition [201]. In severe AD, an allele for the $IL_4R\alpha$ subunit that segregates with atopy was found; the *R576* allele of *$IL_4R\alpha$* being strongly associated with AD may predispose persons to allergic diseases by enhancing IL_4R signaling function [162]. On the whole, the data suggest that IL_4 gene expression plays a crucial role in AD pathogenesis. A major susceptibility *locus* for AD was found to map to chromosome *3q21* [222, 234]: this *locus* provided significant evidence for linkage of allergic sensitization by paternal imprinting, further enhancing the presence of an atopy gene in this region [234]. This susceptibility which was not reported for asthma or atopy-related phenotypes, was identified by a genome-wide linkage scan [234]. Addi-

tional positive associations have been established with DPB, DRB [414], HLA-A1 and -B5 in patients with AD, but complicated by asthma and/or AR [444]. Kuwata et al [226] studied the transporter (TAP genes) polymorphism associated with allergen-processing genes in AD and reported a tendency toward an increased frequency of TAP-1 637Asp in subjects with AD, which may contribute to the pathogenesis of AD in combination with *DRB1*1302/DQB1*0604* [207]. An association between AD and genetic variants of mast-cell chymase (MCC) located on chromosome *14q11.2* has been found [259]. Since there is no association of MCC with atopic asthma, AR, or nonatopic asthma, these findings suggest that the genetic basis of AD may involve the interaction of MCC variants (which promote skin inflammation) [259] and FcεRI-β on chromosome *11q* (which promotes enhanced IgE responses and atopy) [399]. Further, AD patients produce IgE antibodies against environmental allergens regulated by HLA-class II-restricted $CD4^+$, although cytotoxic HLA-class I-restricted $CD8^+$ subpopulations may predominate in the infiltrates in AD lesions [343]. The role of atopic heredity in the development of AD is well established and is a significant risk factor (OR >1) [384]. A population-based twin study demonstrated pair wise concordance rates of 0.72 in MZ and 0.23 in DZ twins with AD [387].

Genetic studies on IgE antibodies, whether or not linked to markers localized on several chromosomes, to cytokines IL_3-IL_5, GM-CSF [342] and IL_9 [109], show more or less significant evidence for linkage of high IgE antibodies or atopy on chromosomes *1q* [496], *2pter* [489], *2q* [215], *3q* [234, 496], *4* [272], *4q35* [92], *5q* [31, 36. 109, 215, 266, 279, 280, 342, 496, 502], *6p21* [489, 496], *6p21* and *7q11* [215], *6q* [496], *7p13* [92], *7q* [215, 228], *9q32* [489], *10q* [215], *11p* [79, 92, 105], *11q* [80, 109, 452], *12q* [17, 80, 215, 489, 496, 498], *13q12* [215], *14q* [288], *15q*, *16q-ter* [83] 16q22.1 [92], *17q* [92], *22q* [329] and to two independent *loci* regulating IgE [109, 280, 495]. Each of these regions contains several important genes that are biologically relevant to IgE regulation (Tables 4.1, 4.2). Controversial results [36] may be explained by the heterogeneity of specific determinants of IgE antibodies due to a pleiotropism of pertinent genes [38] in different populations subjected to varying environmental factors, and analytical methodologies [496]. We recall the inheritance of IgE in 50%–84% of cases [33] and family studies suggesting a major *locus* contributing to IgE antibodies [23, 34, 38, 153, 274], apart from elements of complexity deriving from studies on recessive [104, 138, 269, 279] and dominant [269] inheritance of elevated IgE. The results of studies based only upon IgE antibodies are not concordant: parents with low IgE have offspring with elevated sIgE [262] and subjects with $SPTs^+$ and $sIgE^+$ have a low profile of IgE antibodies, so the concordance with SPTs and sIgE is to be found only in 36% of cases with elevated concentrations [211].

A characteristic trait of atopics might be that of inheriting *particular sequences of HLA class II molecules* (mainly in α-1 and β-1 HVRs) expressing preferential bonds to some allergenic peptides more than to others [365]. The incessant presentation of allergenic peptides on the ample surface of *dendritic cells* (DC) with both IgE receptors is unusually able to amplify the recognition by passing $CD4^+$ T cells with an allergen-specific TcR, thus favoring their proliferation, circulation and colonization in different lymphoid sites [354]. The recognition process is specific and genetically restricted [173], even at the clone level: T lymphocytes of atopic patients proliferate in vitro in the presence of very reduced levels of purified allergens [98]. Consequently, allergen-specific T lymphocytes selectively colonize the tissues of target organs where they orchestrate and maintain the aggression of the tissues invaded by allergic inflammation [98, 257, 361, 454].

In the last few years it has become evident that allergen-specific clones of activated $CD4^+$ cells are able to produce high levels of IL_4/IL_{13}, up-regulating IgE production in B cells from healthy donors in the absence of IL_4, thus revealing that the generation of *high levels of serum IgE* is the characteristic of atopic subjects [183, 363]. Progress in the knowledge of transplants gives rise to hypothesizing an epiphenomenon and attention has been focused on a *primitive defect of T cells,* which is strengthened by ascertaining that atopic individuals may have a greater number of circulating clones of $CD4^+$ T lymphocytes (Th2-like), which provide optimal help for antibody responses [354]. Therefore, the quantitative expansion of specific clones of activated T cells increases the probability of peptide recognition from allergen-specific B cells, which capture allergen via their surface Ig (immunoglobulin) molecules and present the peptide bound to class II HLA molecules. This view of the HLA-peptide association for the presentation to T cells offers the key to reconsidering the relation between IgE response and HLA: it appears that different molecules may possess distinct capacities of presentation either of allergens taken up in *minimal amounts* (ng/year) or of epitopes correlated with a given allergen [354]. The signals transmitted during HLA-TcR interactions influence the type of ongoing IgE-mediated reaction. In addition, the epitope immunogenicity, as well as their capacity to drive immune response toward preferential production of IgE rather than of other isotypes, largely depends on the APC recruitment of T lymphocytes [354]. On the one hand, such studies update the mechanisms critical for the allergen penetration into the mucosa and the subsequent atopic sensitization; on the other hand, they contribute to enlightening certain genetic aspects, indicating that atopic subjects undergo:

- An increased number of Th2 lymphocyte clones predisposed preferentially to produce IL_4/IL_{13} compared to normal subjects.
- A decreased number of Th1 lymphocytes producing IFN-ɣ and IFN-α aimed at inhibiting this process [328].

First Hypothesis. *The uncontrolled increased IgE levels in atopic patients might depend on a genetically*

determined defect of T cells: IgE regulation in the animal model and probably also in humans is strictly controlled by T lymphocytes, and especially down-regulation of IgE synthesis is modulated by cytotoxic T cells. In atopic patients, an effective or relative lowering of allergen-specific CD8 T cells has been demonstrated by a rise in the CD4/CD8 rate (Tables 1.35, 2.5) even in BALF (bronchoalveolar lavage fluid) (Tables 1.40, 1.41), therefore inducing a *selective highlighting of the CD4 Th2 phenotype, involved in the generation of* IL_4 *and* IL_5 [365].

Second Hypothesis. In atopic patients, a clue might be offered by *a defect in the regulation of cytokines as well as by an imbalance between* IL_4 *and its antagonists,* emphasizing that ILs, mainly IL_4 and IL_{13}, are major players in IgE synthesis. The reason now arises as to how specific clones of allergen-specific T cells produced by atopics promote a Th2-like profile (Table 1.10) secreting a different pattern of ILs [363]. Research has shown that the overflow of Th2 preferentially delivering IL_4 elicits several mechanisms, including an uncontrolled amplification of both IgE synthesis and eosinophilia characteristic of atopic disease. Coordinate production of IL_4 and IL_5 by Th2 cells results in the Ig gene switching and expressing germline transcripts in B cells and in selective accumulation of eosinophils in the tissues with the working equivalence of IL_{13} and IL_4. The first 6 months of life are a critical time window for the initiation of immunological changes resulting in the development of atopy: an environment favorable to Th2 responses is also orchestrated by IL_6 toward Der p immune responses [450] and IL_4-driven IgE synthesis, where it plays an obligate role (Chap. 1). The selective development of a Th2-like T-cell IL profile in HR (high risk) children who develop atopy is due to increased production of Th2-like T-cell ILs, caused by impaired allergen-induced IFN-γ production in the neonatal period. Furthermore, the decreased allergen-induced IL_{10} levels observed in 12-month-old atopic children may result in a lack of down-regulation of the inflammatory process [450]. Thus, the conclusion evoked *in atopics is the conversion from Th1-like T-cell ILs into Th2-like T-cell ILs.*

An important conclusion of the Melbourne Epidemiological Study of Childhood Asthma that started 40 years ago (Chap. 5) is that patients with persistent and severe atopic asthma have a reduced HDM-induced Th1 response, whereas those with resolved asthma do not. Reduced HDM-induced IFN-γ production was associated with increased severity of asthma, thus implying that a reduced IFN-γ production could be an important factor contributing to ongoing severe asthma. The finding that all subjects with a history of asthma displayed increased HDM-induced Th2 (IL_5 and IL_{13}) responses, irrespective of the presence or absence of asthma, suggests that increased Th2 responses reflect the presence of the atopic state *per se* rather than being specifically linked to asthma [403].

The second hypothesis consequently confirms that the *intrinsic genetic dysregulation of T lymphocytes in atopic subjects* could acquire the *phenotypic aspect of a postnatal delay in their functional maturation,* so driving the overexpression of IL_4 and other IL genes present on chromosome *5* (Table 1.5), of crucial importance for IgE switch recombination and differentiation of precursor T cells into dominant Th2 T cells at the expense of Th1 T cells, evidence stemming from several tissues affected with atopic disorders. Recent data may ultimately provide the immunologic explanation for the postnatal delay in T cell functional maturation. The Th3 cells, termed T regulatory (Tr1), are induced by semi-mature DCs under the influence of regulatory ILs, including IL_{10}, TGF-β and IL_4 Th3 cells, secrete IL_{10} and or TGF-β, but no IL_4 and scarce or no IFN-γ and *are capable of suppressing Th1 and Th2 responses* [283]. $CD4^+$Th2 T cell and IL_{3-5} hyperproduction and low IFN-γ levels [98, 170, 361, 450, 454], a product of IL_4/IL_{13} that results in the preferential differentiation of B cells into plasma cell IgE, thus explaining why IgE synthesis is found to prevail compared to other isotypes. The Th2/IgE predominance may induce the passage from mono- to polysensitization in infants with AD and food allergy (FA), with prevalent respiratory symptoms [143].

What genetic or immune mechanism, at least in atopics, appears to underlie the complex and presently unclear dysregulation affecting T-cell subsets, preferentially interacting with Th2 cells? It is clear that all subjects, regardless of FHA, respond to parasite infections evoking a Th2/IgE response whatever the genetic background, and mycobacteria promote Th1 T-cell generation [363]. Several studies have clarified the grounds for this substantial dichotomy, establishing that allergens preferentially evoke Th2 responses in the atopics, but Th1 responses in nonatopic patients [354]. This finding may reconcile different views on the facilitated and amplified Th2 production and of the specific and decisive role of heredity [262]. Such inheritance may depend on the clonal expansion and rearrangement of the TcR gene β chain, and the production by the T-cell clone of high levels of Th2-like T-cell ILs and of markedly reduced Th1-like T-cell ILs [98]. Additional factors such as the APCs perform variable functions; after the demonstration that in nonatopic donors they were able to skew Th0 T cells to express a Th2 phenotype. It is thus possible that TcR diversity greatly influences the differential compartmentalization into Th1 or Th2 in target organs [313]. IL_4 present in the paracortical zone of lymph nodes, and in the microenvironment where the antigen presentation to Th0 cells takes place, appears to be the most significant factor responsible for CD4 differentiation into Th2 T cells, thus representing the chief threat closing the pathogenetic circle of IgE synthesis [354]. A clue may be that atopic patients are also equipped with Th0 precursors able to interact with IL_4 in basal conditions and in the absence of adequate stimuli and with no difference between allergen-specific Th2 in both FA and respiratory allergy [363].

An additional hypothesis is that the PGE, elevated in atopics and with selectivity for certain APCs, may inhibit the Th2 IL prominence, also considering the blocking effect of IL_4-dependent IgE production by B cells [328]. Several immunoregulatory properties also include the relevant role played by PGE_2 in inhibiting the IL secretion by Th1 cells without affecting IL secretion by Th2 cells. Such action is associated with the rise of cyclic adenosine monophosphate (cAMP) intracellular levels, a relevant effect following the observation that various agents able to elevate cAMP concentrations show similar behavior toward the T-dependent IL production [331]. The PGE-mediated decrease in IL_2 and IFN-γ together with IL_1 production by macrophages inducing PGE synthesis and necessary to Th2 activation and IL_5-production by cAMP may tip the scales to the IgE recombination machinery [134, 331]. In humans, PGE_2 also interacts with B lymphocytes, stimulating their proliferation after IL-independent CD40 activation [134]. The crucial prerequisite needed to verify the outlined hypothesis is the PGE_2 hyperproduction in one or more lymphoid organs, which could underlie the Th2 hegemony in atopics [134]. The scene is complete; however, B and T cells have ample chances of interacting with PGE_2 and/or the cells secreting it, including macrophages, fibroblasts and follicular DCs (FDCs) in the spleen and lymph nodes [331]. For now, the scene of hyperactivity of the cascade of events triggered in atopics stems from indirect observations or extrapolated parallels; it is hypothesized that experimental results concur to explain the apparent contradictions.

Studies on allergen-induced lymphocyte proliferation in children with AD and FA have demonstrated during reactions in an active stage that Th1 T cells produced IL_2 and IFN-γ and expressed FcεRII on B cells in a greater amount compared to controls [213]. Thus in atopic children, the Th1-mediated mechanisms are active near the Th2-mediated mechanisms. Interestingly, CD4 T cells derived from CB showed a different IL profile depending on the newborn's parental atopic status. Der p 1-specific T-cell clones derived from CB of nonatopic parents' newborns showed a prevalent Th1 profile, whereas when both parents were atopic CD4 T-cell clones of at-risk newborns exhibited a Th0 or Th2 profile [333]. CB IL responses specific to the HDM allergen showed a Th2-skewed profile similar to that in atopic adults associated with a slightly detectable IFN-γ, irrespective of atopic risk or later atopic disease [345]. Taken together, these data demonstrate that in addition to the allergen structure and the type of APCs, the genetic abnormalities favor the preferential Th2 response in at-risk neonates. As stated in discussions in Chap. 2, in vivo the fetal immune environment is directed toward a Th2 response as a result of IL_4 and IL_{10} production by the amnion and placenta.

4. The hypothesis that a *genetically determined defect of T lymphocytes in atopic subjects and postnatal delay of functional maturation* appear to be associated in at-risk neonates [333]. In infants divided into at HR and low risk (LR), depending on the presence or absence of at least one atopic parent, symptomatic HR compared to LR infants had decreased levels of CD45RA and CD45RO and increased levels of activated T cells (CD25), with highly significant differences [282]. In CB mononuclear cells (CBMC), CD45ROs were significantly higher in children developing atopy at 12 months compared to those who did not [452]; however, CD45RO levels are scarce in both the fetus and the normal neonate (Table 2.6). So a postnatal maturational delay of T lymphocytes is reflected on the subsequent acquisition of immune memory, which may be delayed in HR children [172]. The CD45RO cell decrease appears to have a greater effect on T-cell activation [282]. A first hypothesis postulates a possible T-cell suppression, either innate or maternally influenced in the uterine environment. The FHA genetic influence was demonstrated by IFN-γ decreased levels [239, 358, 432, 431, 450]. Another hypothesis proposes that if CD45RO cells are a prerequisite of immune tolerance, a reduced presence of memory T cells might result in an altered postnatal exposure to allergens with consequent atopy development [282]. In accordance with a presently more reliable hypothesis, the immune system is more activated and stimulated at birth (Tables 2.5, 2.6, Fig. 2.1) owing to the *in utero* exposure to allergens. Thus the reduced CD45RO titers in HR children imply either a different exposure related to the maternal influence, or a hereditary maturative defect of immunocompetent cells [282], or above all the reduced secretion by CD45RA cells in imbalance with CD45RO cells, of Th1-like T-cell ILs such as IL_2 and IFN-γ [196]. These findings provide a plausible mechanism to the persistence of fetal Th2 responses during early childhood in atopic children [345]. The *first 6 months after birth* may be a pivotal time window for the beginning of immune changes resulting in the atopy expression, associated with skewing of immune responses away from a Th1 to a Th2 IL profile in HR children, caused by impaired IFN-γ production in the neonatal period [345]. These findings provide a plausible mechanism to explain the persistence of fetal Th2 responses during early childhood in atopic children [345]. Moreover, the decreased allergen-induced IL_{10} concentrations in 12-month-old atopic children may result in the lack of down-regulating inflammatory processes [452].

Recent reports on hereditary defects found in HR children who later develop atopic disease confirm that in normal subjects T cells are led to adopt a Th1-like profile depressing IL_4 function with significantly elevated secretion of IFN-γ compared to atopics; on the contrary, in HR children [239, 345, 358, 432] *elevated levels of IL_4 and impaired IFN-γ responsible for switching B cells to produce sIgE are positively demonstrated in atopics* [328]. In neonates, an evident decrease in both cytokines is seen in their levels (Tables 2.1, 2.3). IFN-γ was detectable in human amniotic fluid at 16–17 weeks

Table 4.4. IL_4 and IFN-γ levels in children at high (*HR*) and low (*LR*) risk of atopy compared with adults

Cytokines	LR	HR	Adults
IFN-γ (U/ml)	13.27 ±16.17	20.69 ±26.51	57.65 ±58.64
IL_4 (pg/ml)	2.667 ±4.948	6.328 ±7.379	9.730 ±13.420

Data from [170].
See in comparison the neonatal levels shown in Table 2.3.

Table 4.5. IL_4 and IFN-γ levels in atopic (*A*) and nonatopic (*NA*) children stimulated by $CD3^+$ $CD45RO^+$ and $CD3^+$ $CD45RO^-$

	IFN-γ (U/ml)	IL_4 (pg/ml)
A $CD3^+$ $CD45RO^+$	15.5 (9.4–26.6)	61.9 (<LM–353.2)
NA $CD3^+$ $CD45RO^+$	35.7 (16.8–59)	76.5 (30.2–1180)
A $CD3^+$ $CD45RO^-$	7.7 (2.2–17.4)	<LM
NA $CD3^+$ $CD45RO^-$	21.7 (6.3–36.8)	<LM

Data from [196].
LM limit of measuring.

Table 4.6. Additional IL_4 and IFN-γ levels in atopic children

	IFN-γ (U/ml)	IL_4 (pg/ml)
Atopic		50.8± 45.8
Nonatopic		211.8±105.3
Very atopic	47.2	38.5
Low atopic	147.6	24

Atopic/nonatopic from [203], very/low atopic from [432].

Table 4.7. Cytokine levels (ng/l) in healthy children aged 1–19 years compared to adults after stimulation with mitogens

Cytokine	Mitogen	≤10 years	≥10 years	Adults
IFN-γ	PHA	13×10^{3a}	31×10^3	48×10^3
	PWN	50×10^3	54×10^3	61×10^3
IL2	PHA	1,200[a]	1,300	1,800
	PWM	1300	1,400	1,500
IL-1α	PHA	80	100	90
	PWM	270	270	250
IL-1β	PHA	870	1,100	810
	PWM	2,900[a]	3,100[a]	2,300
TNF-α	PHA	340[a]	490[a]	920
	PWM	390	300[a]	510
Results after normalization of PBMC count				
IFN-γ	PHA	11×10^{3a}	25×10^{3a}	56×10^3
	PWN	35×10^{3a}	51×10^{3a}	74×10^3
IL2	PHA	940[a]	910[a]	2,300
	PWM	960[a]	1,100[a]	1,800
IL-1α	PHA	50[a]	90	100
	PWM	200[a]	300	290
IL-1β	PHA	610	820	960
	PWM	2,300[a]	3,000	2,700
TNF-α	PHA	230[a]	530[a]	1,200
	PWM	340[a]	390[a]	680

Data from [118].
[a] Significance compared to adults, which increases after normalization.
PHA Phytohemagglutinin, *PWM* Pokeweed mitogen, *PBMC* Peripheral blood mononuclear cells.

of gestation in only 11 (26%) of 42 samples [194]. How can we justify this deficit in the first few years of life? There is evidence of a post-transcriptional defect [431] resulting from notably deficient IFN-γ secretion compared to controls vs increased mRNA expression [429], or from the poor expression of CD2 and CD11a/CD18 by unstimulated CD45RO lymphocytes [291], or impaired IL_{12} or IL_{18} production (Table 1.5). Impaired IFN-γ production was only observed after allergen-specific stimulation of CBMCs from children developing atopy [452]. Also, neonatal IL_4 impairment appears to decrease as the child ages, so that IL_4 may play a leading role in IgE synthesis [203]. In atopic and nonatopic children, the differences in IL_4 levels are often contradictory, since they are also significantly reduced in nonatopic children (Tables 4.4–4.6) [170, 196, 203, 432], in healthy and HR children with very elevated IgE levels compared to LR children [170] and adults [170], with an additional reduction of IL_2 levels [196]. In healthy subjects aged 1–19 years, $IL_{1\alpha}$, $IL_{1\beta}$, IL_2 TNF-α and IFN-γ levels are reduced compared to adults, and IFN-γ increased only after 10 years of age (Table 4.7) [118]. This is the main difference: IFN-γ values are constantly low in neonates [430] (Table 2.3) and in HR infants [291, 358, 429, 473], to the point of resulting predictive of the subsequent atopy expression [423, 429, 473] and of sensitization to inhalants [270]. *Thus the reduced secretion of IFN-γ at birth is a primitive marker* [430, 473] *and a result of the atopic march* [430], in accordance with the postnatal immunodeficiency [170] rather than an effect of the disease process [430]. Recent studies in animal models demonstrate that selective decrease of IFN-γ gene expression in T cells *in utero* and in early postnatal life may be due to hypermethylation of CpG (cytosine-phosphate guanine) sites in the proximal promoter, which results in reduced capacity to transcribe IFN-γ-

specific mRNA [480], whereas an IFN-ɣ hyperproduction caused by CpG oligodeoxynucleotide (ODN) resulted in the exacerbation of dermatitis in 38.5% of mice [426]. Further mice experiments show that high mobility group A1 (HMGA1)-deficient mice exhibited an IFN-ɣ induction silencing, because IFN-ɣ gene expression requires HMGA1, the architectural transcription factor mediating IFN-ɣ enhanced formation [65]. The greater IL_4 concentrations in atopic polysensitized children compared to monosensitized children, and those 12-fold lower levels of IFN-ɣ compared to controls [429], and even lower levels of IL_2 in infants with double FHA [270], appear to establish a correlation with atopy severity [429]. At birth and *up to the age of 10 years,* the defective IFN-ɣ generation in children by PBMC (peripheral blood mononuclear cell) response to specific stimulation is a predisposing factor to the development of atopic disease, thus causing up-regulation of ILs associated with a Th2 response and the atopic phenotype [430, 431, 473]. Studying the ontogeny of IL production, IFN-ɣ release in atopic children was significantly reduced in the 0–2-year, 2–5-year, and 5–10-year age groups when compared with nonatopic children [402]. Therefore, it is confirmed that the delayed postnatal maturation of this important aspect of cellular immune function is a key determinant of genetic predisposition to atopic disease [170].

In HR atopic-prone babies compared to babies with nonatopic parents, IL_{13} secretion is defective at birth in PHA-stimulated CB MCs, contrasting with IFN-ɣ deficiency. Since IL_{13} is a prototype of Th2-type ILs, this finding may reflect an inherent immaturity or suppression of T-cell IL responses in such babies [486].

The significant reduction in IFN-ɣ and IL_4 levels in atopic children, either with asthma [432] or AD [429], confirms the inverse role of these ILs in the pathogenesis of atopic disease, and that the imbalance is not an epiphenomenon [358, 432], but the expression of a *primitive functional abnormality of T cells* [39] associated with a significant reduction in NK cells in HR compared to LR children [239]. The clinical result is the significant, persistent elevation of IL_4 titers in infants who developed atopic disease during 18 months of observation, compared to healthy controls, parallel to IFN-ɣ levels under detection limits, with *the highest levels of IL_4* at 18 months *in infants with FHA+* [39]. Even if Th2 T cells were not produced, as occurs in mice with the deleted IL_4 gene (or made not functional) [214], it is hypothesized that during early responses a small population of $CD4^+$, $NK1.1^+$ cells may come on the scene [499], or one prematurely migrating from the thymus with double-negative TcR-αβ (DN) [516], or metachromatic cells releasing IL_4 localized after FcεRI stimulation [45]. Mice significantly devoid of mast cells show normal Th2 levels [479].

Other studies indicate that soluble FcεRII (sFcεRII) levels are significantly higher both in infants compared to older children and in atopic infants (aged <1 year) compared to nonatopic infants [203]. These studies emphasize the strict correlation between sFcεRII levels, an increase likely due to IL_4, and IgE antibodies: since IgE-BFs (Chap. 1) have the capacity of modulating IgE synthesis, which suggests that sFcεRII in turn is an IgE-BF, they could indeed be able to increase IgE in atopic infants [203].

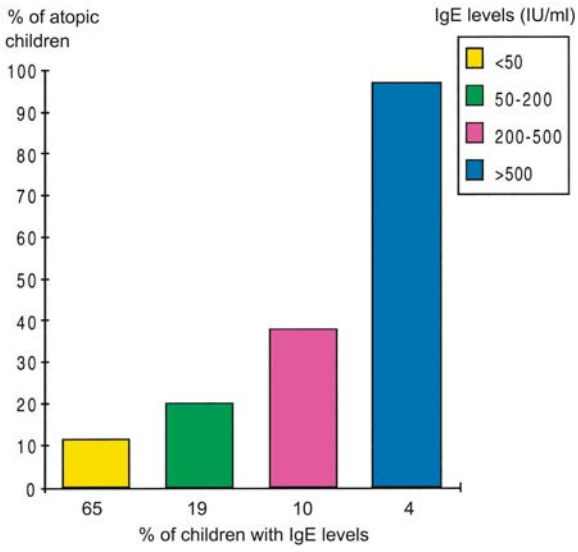

Fig. 4.7. IgE levels and risk of allergy. The higher the IgE concentrations, the greater the likelihood of atopy. (Data from [256])

Also defective is the kinetics of airway DC postnatal maturation, which sequestrates and transports aeroallergens to T lymphocytes from the regional lymph nodes: as seen in the animal model (Fig. 2.24), DCs only develop at the age of weaning. Also in humans, the responses to aeroallergens may develop after the age of 1 year [157].

Evaluating the heredity role in atopy, the role played by IgE molecules in the genesis of atopic disease is remarkable (Fig. 4.7). As outlined in Table 3.4, CBIgE levels in neonates of atopic parents are elevated, and significantly related to atopic inheritance [433], even if there is an evident heterogeneity [180]. Long-term prospective studies have contributed to delineate *the IgE natural history* [157]. Serum IgE antibodies develop in two phases (Table 4.8) [169]: to common food allergens before the 1st year of life in atopic and normal infants, but decline in most babies to undetectable levels by the age of 2 or 3, in healthy children first. IgE antibodies to inhalant allergens have a more markedly biphasic distribution: more often they develop at 1.5–2 years, a peak is attained at about 4 years and takes longer to switch off in normal babies, often not stopping until age 5–7, whereas *IgE responses increase more rapidly in atopic babies, who maintain their serum IgE reactivity to inhalant allergens into later childhood, and about 30% maintain elevated peaks to one or more allergens beyond the age of 12 years* [28, 156, 157, 169, 447, 513]. It was demonstrated that up to age 2 the infants were sensi-

Table 4.8. IgE response during early childhood

	Food allergens	Aeroallergens
Exposure levels	µg–g	ng
Initial appearance of serum IgE	3–6 months	1–2 years
Duration of transient IgE responses	1–2 years	3–5 years
SPT reactivity	+ (transient response)	– (atopic children +)
Clinical sequelae	+	?
Likely mechanism(s) for Th1/CD8 selection	Immune deviation, T-cell anergy	Immune deviation

Modified from [169].

tized to egg (16.4%), CM (13.1%), grass pollen (6.2%), and HDM (3.0%). At age 7, the prevalence of atopic sensitization was inverted: to grass pollen 23.3% (a 3.7% increase) HDM 15.7% (a 5.2% increase), CM 13.6%, and wheat 11.4% [184]. In a parent study, FA at age 2 was a strong predictor for the development of asthma; school age children sensitized to inhalant allergens at age 7, either without (OR, 3.44) or with (OR, 9.43) concurrent FA, had an increased risk of having asthma which was strongest for perennial inhalant allergens compared with seasonal inhalant allergens [184]. HR infants, though residing in allergen-free houses at 9 months of age, exhibited significantly greater antibody levels than FHA-negative infants not protected by environmental measures [156]. In the 817 infants with AD of the ETAC (Early Treatment of the Atopic Child) study from 13 different countries, there was a catalog of factors associated with a larger risk of developing high antibody titers: male sex (62%), exposure to egg (45%), cigarette smoke (41%), kitchen gas (59%) and proximity to plants (44%) [474]. In the first 24 months after birth, the number of children at risk of atopy with sIgE to foods was significantly higher than those with sIgE to Der p 1 (p=0.0006) [48]. In 931 healthy newborns, higher FHA scores and exposure at 18 months of age to egg and to Der p allergen led to a higher incidence of AD at 3 years of age [181]. Similar patterns of IgE responses yielded significant data: only minute doses of allergen are sufficient to drive a primary sensitization, µg for foods and ng for aeroallergens (Table 4.8).

In animal studies, mice exposed to an aerosol of allergens mount an IgE response and divide into two categories: the tolerant mice, with low tendency to produce IgE, which will never again respond to the same allergen with IgE, and those with a marked propensity to IgE production, in whom a repeated exposure to allergens amplifies the antibody response. In the first case, the leaders are Th1 T cells, in the second the pro-IgE Th2 [169]. Thus, feeding low doses (ng) to nonreacting mice produces tolerance, whereas feeding larger doses to highly reactive neonates genetically at risk (mg) does not, since small doses sensitize them [178]. The data we expose in Chap. 9 on the deluge of artificial formulas are a failure for this thesis: given that larger doses truly induce tolerance, millions of neonates and infants, whether or not at risk, have been fed CM and weaned early with solid foods, but on the contrary the atopy prevalence has risen in geometric progression. Early postnatal allergen exposure is a risk factor for development of long-term, primary allergic sensitization, and a recent study suggests that the basis for this risk may be the presence in the newborn of small populations of allergen-specific T cells that are primed during intrauterine life [344].

Holt has been studying the pathogenetic theory on the different genetically determined effects in both GALT and BALT for several years, and Table 4.8 summarizes IgE responses to allergens during early childhood. Holt argues that IgE down-regulation is more rapid and effective in GALT than in BALT: the reason is that the higher concentration of dietary allergen may drive the allergen-specific T-cell selection process much faster and more efficiently in the GALT than the parallel process in the BALT, which is stimulated by intermittent exposure to ng of aeroallergens [169]. More precisely, feeding high food allergen concentrations directly produces T-cell anergy that establishes a functional deletion, whereas continued exposure to low doses produces tolerance, a misnomer for the term "immune deviation" [169], and IgE down-regulation by allergen-specific T cells, with a major component of the suppressor CD8 phenotype [515] stimulated by TGF-β (Fig. 1.40). Studies based on the adoptive transfer show that this form of tolerance is CD8-mediated [515] and in this process prominent roles are played by IFN-γ and TGF-β [178, 515]. IFN-γ production by T cells from lymph nodes draining GALT in response to allergens [179] suggests that most T cells that evade anergy may acquire the Th1 phenotype [169]. Others [171] continue that Th1 cells are tolerated and not the Th2, Th1 hyporesponsiveness being IL_4-mediated. There seems to be a gradient of sensitivity to tolerance induction, with Th1>Th2>B lymphocytes, with a likely outcome in IgE hyperproduction at the systemic and mucosal level [169]. Jarrett stressed that the "very low doses" may stimulate other than suppress IgE responses [187]. We add that at-risk neonates are continually fed low sensitizing doses in the neonatal period: in their CB an effective reduction in CD8,

CD45RO and IFN-γ is reported [291] (Tables 2.5, 2.6), and the gut is physiologically immature. In early life, *the CD8 suppressor deficit* is markedly clear (Tables 1.35–1.39, 2.5), which is the basis of atopic disease. An early Th2 priming is capable of deviating subsequent immune responses toward the selection of potentially pathogenic Th2-polarized memory [344]. In infancy, IgE are synthesized and other factors are conditioned, including intensity and duration of allergen exposure (Table 4.8), diet, maternal smoking and possibly presence or absence of a protective factor that stimulates Th1 responses more than Th2 responses: this is the evidence that asthma has multifactorial causes [175]. A third line of Th (Th3) may be prominent in this context, considering that the induction of experimental autoimmune encephalomyelitis is inhibited by tolerant mice and that the injection of anti-TGF-β blocks the effect [66]. Th3 T cells can be beneficial to the host, since they can function in infection by limiting pathogen-induced immunopathology [286].

The regulation is at the genetic level also for the recognition of varying allergens by the immune system: specific responses of atopic individuals are activated by only certain allergens with which they make contact; the different reactivity makes it possible to distinguish mono- and polysensitized subjects. IgE hyperproduction continues and persists in all atopics, and rises strikingly following an allergen exposure; however, at a certain point it proceeds endlessly from the initial exposure (ongoing production).

5. The phenotypic expression of atopic disease depends not only on IgE hyperproduction and/or both CD8 and IFN-γ defects, but also on IgA hypoproduction. Having observed significantly lower sIgA concentrations in the milk of IgA-deficient mothers affected with atopy [351], we suggest that the maternal deficit of IgA antibodies may play a permissive role in the expression of atopic symptoms, thus appearing to be a more prominent factor than positive predisposition to atopy [253]. As previously outlined, the IgA deficit of a mucosal site is reflected diffusely in other sites, in the first place in the gut. These data have been supported: Fig.4.8 [249] shows that high or intermediate IgA levels combine with low IgE concentrations, and low IgA levels correspond to measurable IgE levels. The results show that low IgA values are correlated with the most severe clinical manifestations and with an increased cumulative incidence of atopic disease [249]. From the demonstration that serum or skin IgA levels are inversely correlated with the expression of clinical atopic disease, we might conclude that *atopy is conditioned by the delayed IgA maturation rather than by IgE hyperproduction.* A prospective study of the association between viral infections and the onset of allergic disease observed in atopic children that serum IgA levels were uniformly in the low range for age during the first 30 months after birth [128]. The sIgA levels are also reduced in the skin of subjects with AD (Chap. 7); low IgA levels may coincide with an increased susceptibility to respiratory infections (Chap. 22).

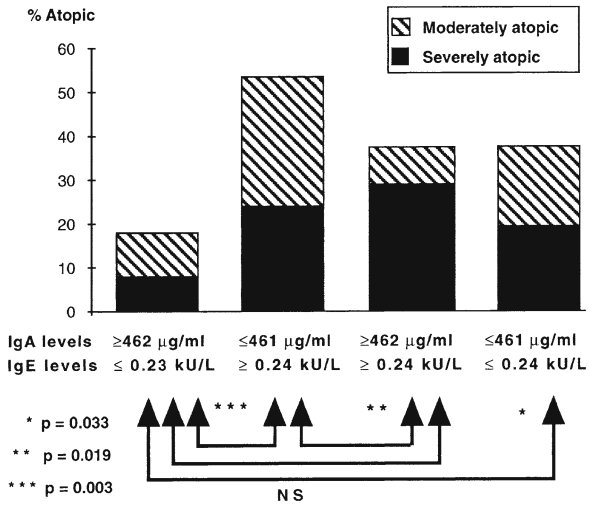

Fig. 4.8. IgE levels and risk of atopy. Percent of atopic subjects. Percent of children with IgE levels. Serum IgE levels (IU/ml). The higher the IgE levels the greater the risk of atopy. (Modified from [249])

In conclusion, the great protagonists of genetic and immunological progress are strictly concatenated: the HLA genes load the gun, so to speak, to *shift the balance of T-cell subsets toward a polarized Th2 dominance*, which along with IL_4/IL_{13} are the builders of IgE synthesis. The expression of atopic disease is the result of a complex interplay between genetic predisposition and environmental exposure. The impact of factors influencing the Th1–Th2 selection during infancy is somewhat modified by the inherent importance of bystander respiratory infections, which may *release strong IFN-α/ IL_{12} signals* in the lymph nodes seat of the Th1–Th2 selective process, *skewing the chair in favor of Th1-type protective immunity* [363], most likely by intervening DCs [252]. Thus, a reduced capacity to produce IL_{12} could lead to aberrant Th2-type responses in atopic children. During the formative period, several changes encourage Th2 allergic responses to allergens, which may otherwise yield Th1 responses in the same baby under dissimilar conditions. Hitherto, allergens are increasingly viewed as the targets of these responses, rather than the primary cause of atopic disease. Additional evidence comes from a study on the spread of pollutants, which found that IgE levels were three times higher in children of the former East Germany, with no correspondence with sIgE to seven different allergens, with the net conclusion that high serum IgE levels do not necessarily correspond to atopic sensitization [20]. However, IgE and atopy map to the same chromosome *4q* [92]. Investigations in atopic children in eastern and western Germany show a high prevalence of sIgE against inhalant and food allergens and atopic sensitiza-

tion [476] and that Th0 lymphocytes prevail in children who grew up in East Germany, regardless of whether they were atopic, whereas children of West Germany, particularly when they are atopic, show a Th2 polarization [352].

Prenatal Genetic Factors and the Fetal Immune System

6. A vast array of factors acting during intrauterine life have been indicated as promoting atopy development in children (Table 3.7). The research on *in utero* sensitization, recently amplified by substantial contributions, has given this factor a salient place among the prenatal factors. CB T lymphocytes proliferate in the presence of Der p 1 and Lol p 1 [333], while when testing CB PBMCs with α-lactalbumin (ALA) [320], β-lactoglobulin (βLG) [332] and bovine seroalbumin (BSA) [213], there was a meaningful proliferative response. Expanding the panel of allergens tested in CB (ALA, βLG, BSA, α, β and κ caseins), a proliferative response was recorded for a broad set of allergens [423]. Moreover, from as early as 22 weeks of gestation onward, the *fetal immune system* (FIS) is active and begins to respond to a similar variety of allergens, including βLG, ovalbumin (OVA), Der p, Fel d 1 and birch pollen [193], and normal-term fetuses showed positive lymphoproliferative responses to one or more common allergens, including whole HDM extract (46%), βLG (44%), OVA (42%), rye allergen (24%) and Fel d 1 (22%). Both an *intrauterine sensitization to foods* [193, 320, 332, 344, 423] *and aeroallergens* [193, 333, 344] was demonstrated, hence supporting the concept that fetal programming by the mother during the second and early third trimester of pregnancy results in FIS T-cell priming [102]. This shows that allergen exposure in the prenatal period can influence the fetal immune response, irrespective of maternal or paternal allergy or asthma [401]. Transplacental transfer has been demonstrated by numerous groups as the likely route of allergen transfer to the fetus, for example, the passage of OVA and bet v1 across the human placenta [424]. Over 11–24 weeks of gestation, HLA class II$^+$ cells, CD68$^+$ and CD40$^+$ cells were present throughout the lamina propria; few CD3-positive cells (T cells) were observed in fetal gut. With the emergence of lymphoid aggregates (14–16 weeks), CD83$^+$ (DCs) and CD20$^+$ cells (B cells) could also be detected (Figs. 2.13, 2.14) [194]. The recent observation of HDM and Fel d1 in amniotic fluid collected during amniocentesis at 16–17 weeks of gestation and in CB provides direct evidence that both transplacental and transamniotic routes of exposure can occur [168]. At 16–18 weeks, human amniotic fluid also contained intact IgE at levels that increased as maternal circulating levels increased, IgE that might bind to CD23$^+$ cells within the lymphoid follicles of the fetal gastrointestinal tract. However, circulating levels are very low, since there is no evidence of IgE production [436]. Prenatal exposure to HDM allergens was associated with total serum IgE at birth [401]. Using fractionation of CBMC CD45 isoforms, it was established that allergen-specific fetal Th cells can be sensitized *in utero* [102]. Expression of the CD45RO by T cells correlated with increased gestational age, indicating that fetal immune memory develops as gestation progresses, but supporting the idea of intrauterine antigen priming [196]. Consequently, the hypothesis that fetal T cells are exposed during gestation to maternally derived allergens is supported by a line of evidence [424]. Studies have demonstrated beyond controversy that CBMCs proliferative responses strongly correlated with an increased risk of subsequent childhood atopic disease development [282, 345, 473], especially when neonatal cells are stimulated with BSA [213]. Such occurrence should be taken into account when an anaphylactic reaction occurs at the first feeding of a given food. However, no sensitization to inhalant allergens *in utero* was found, but responses to tetanus toxoid in the absence of exposure, thus suggesting an additional mechanism of priming for these cells [405]. The anti-allergen lymphoproliferative responses of CB T cells are closely connected, with a peak response to OVA especially in infants aged 6–10 weeks, who in addition had 30% positive CB responses to HDM [171]. At present, these results do not differentiate between atopy-prone and non-atopy-prone children [423]. At 27 weeks gestation, the FIS is capable of IL_{13} secretion, and at birth in atopy-prone babies, but this production is defective [486]. Going back to the Th2/Th1 alternation during pregnancy (Chap. 2), this placental prevalence of Th2-like T-cell ILs may direct Th0-like FIS cells to the Th2 rather than to the Th1 phenotype; moreover, maternal exposure to allergens may prompt the development of fetal T-specific responses [473]. Recent findings suggest that the risk of AD was significantly elevated in children whose mothers had active asthma during pregnancy (RR 1.8) or AD during pregnancy (RR 1.70) [224]. Therefore, we can propose timing of these responses: as seen in Tables 2.4–2.6, the FIS is immunologically active before 16 weeks of gestation, as demonstrated by Jones et al [194]: CBIgE positive to foods (Table 3.2) appear to promote the outcome of an IgE-aspecific synthesis, perhaps due more to an absent suppression rather than to an allergenic stimulation. The above data show why the prospective and randomized studies on diet restrictions to mothers of children who are at risk for atopy during the last trimester of pregnancy had negative results. Several immunodeficiencies benefit from *in utero* transplantation and from *in utero* prenatal diagnosis (Chap. 22).

A study on a birth cohort of 24,690 children suggests that exposure to antibiotics *in utero* is associated with an increased risk of asthma in a dose-related manner (hazard ratio [HR] 1.68), and similar associations are present for AD (HR, 1.17), and AR (HR, 1.56) [276].

Postnatal Genetic Factors and Related Influence

A large body of evidence has accumulated suggesting that asthma and atopy run in families and that family factors play a crucial role (Fig. 4.9) [205]. Epidemiological studies and clinical observations have contributed to quantifying the risk, establishing that when FHA is *negative,* 5%–16% of children are affected by atopic disease, when FHA is *positive,* the percentage rises with the number of atopic first-degree relatives, increasing more when both parents have the same type of atopic disease (Table 4.9) [205]. With an atopic *father*, 33% of siblings may suffer from atopy, vs 45% if the *mother* is atopic. For asthma, the values are 25% and 40%, respectively [205]. The occurrence risk is 40%–45% when in a family two first-degree relatives are affected (one parent and one brother) [360]. FHA was the single most important predictor for atopy in 713 children, and a dual FHA doubled the risk for asthma and quadrupled the risk of rhinitis [433]. FHA is the most vigorous predictor of atopy, BHR and asthma, and this effect is extended until the age of 18 years [392]. A history of asthma in the mother poses a relative risk of 2.3, of 1.6 in the father [16]. In 1,123 MAS children with or without AD, FHA scores in ≥2 atopic family members and was present in 19.4%–36.2%, AD in 9.8%–27.5%, AR in 41.4%–53.7%, and asthma in 21.8%–31.2% of cases [183]. A classic study has demonstrated that 11 out of 13 (85%) children with double FHA were clinically allergic within 5 years vs 2 out of 28 (7%) of the "well-baby" controls [128]. Thus, siblings of asthmatic parents have a significant prevalence of atopic disease compared to those of nonasthmatic parents. The number of affected relatives is not negligible: differentiating CMA prevalence and based on genetic factors, depending on one or two or more first-degree relatives, values of 4.8%–24% are found in healthy, unselected infants and of 19%–54% in infants with CMA, with very significant differences between the two groups [386]. A further demonstration of the studies on genomic imprinting, particularly convincing, is maternal atopy: CB IgE levels are strictly correlated with maternal levels [243]. However, both maternal and paternal allergy increased the risk of an elevated total IgE at birth [202]. The sibling risk is modulated by total IgE positivity [505]. The children of atopic mothers have a higher risk [1, 392] of 26% if correlated with respiratory atopy vs 13% risk if the father is atopic [206], with an OR of 4.4 vs 1.5 (Table 4.10) [108]. Children having an atopic relative are more prone to having asthma, AR, or AD (OR, 2.54–3.81] [290]. The risk of a child being asthmatic was fourfold greater if either the mother or the father had asthma than if the child had been born to nonasthmatic parents [227]. Sibling atopy was a stronger predictor of clinical disease than maternal or paternal atopy [433]. Although some of these effects may be poorly defined, the risk of developing atopic disease is striking even if only one parent is affected, and increases when the disease is AR or AD (Table 4.11). The risk also varies in connection with the disease: children with parental AD have more than a double risk for AD

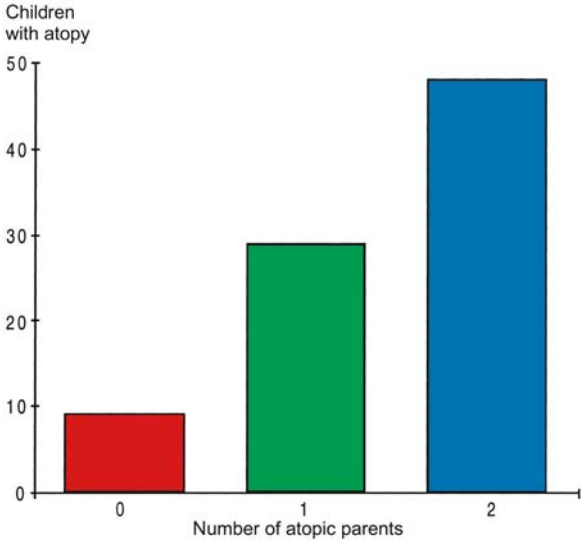

Fig. 4.9. Family history positive for atopy and risk of developing allergic disease. (Data from [205]). *0* No atopy, *1* No. of atopic fathers, *2* No. of atopic mothers

Table 4.9. Risk of developing allergic disease according to family history for atopy

Family history	Risk (%)
Both parents atopic	40–60
Both parents atopic with identical manifestations	50–80
One affected parent	20–40
One affected sibling	25–35
Negativity for atopy	5–16

Data from [205].

Table 4.10. Risk of developing allergic disease according to parental atopic diseases (odds ratio)

Child atopic disease	Asthma	Allergic rhinitis	Atopic dermatitis
Asthma	2.6, 1.5, 4.4	1	1
Allergic rhinitis	2.5	3.6, 4.1, 3	1.7
Atopic dermatitis	1.5	1.4	3.4, 3.9, 2.5

The presence of three figures indicates, in the order shown, the relationship with atopy of one parent (1), of the father (2), of the mother (3).
Data from [108].

Table 4.11. Risk of developing allergic disease according to parental atopic diseases (%)

Child atopic disease	Father (%)	Mother (%)
Asthma	15	13
	2.85 (1.8–4.49)*	2.67 (1.65–4.35)**
Atopic dermatitis	22	17
	2.42 (0.16–3.65)*	2.13 (1.35–3.35)**
Allergic rhinitis	33	49
	2.03 (0.16–2.74)*	2.17 (1.66–2.84)*

The first figures indicate prevalence, the second figures the relative risk and the confidence intervals.
Data from [1].
*$p<0.0001$, ** $p<0.02$.
According to our unpublished data, 289 atopic children had 163 (56.4%) atopic parents and/or brothers and sisters (in 90% of cases with respiratory allergy) vs 49 out of 300 (16.3%) nonasthmatic children ($p=0.0001$).

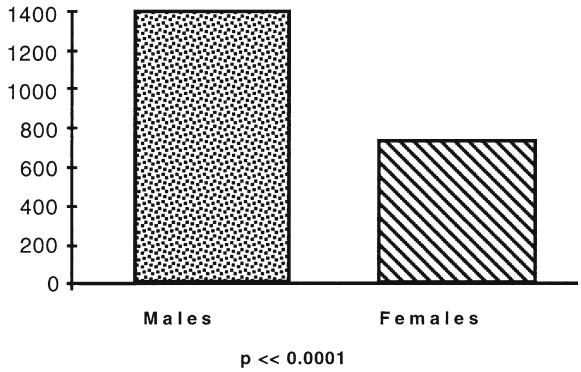

Fig. 4.10. Sex difference in 2,124 children studied

compared to children with parental asthma, while children with parental asthma have an even higher risk for asthma compared to children with parental AD [108]. To confirm the previous data, the statistical analysis reveals very significant differences, with the highest risk with FHA^+ in cases of biparental positivity ($p=0.0028$ vs uniparental, $p=0.0001$ vs double negativity), of atopy in the mother or in her family vs paternal atopy ($p=0.0001$) and of the same or another atopic disease in her son, but doubled if both parents are atopic ($p<0.001$) [3].

The data on 729 children and their parents (Table 4.11) [1] indicate a clear association between respiratory and cutaneous allergies in both parents and their children, again confirming the genetic and multifactorial origin of atopic disease. Two studies should be confirmed by subsequent literature, since they have underlined wholly original occurrences. In one study children have a higher prevalence of atopy, which is lower in children of the former East Germany [465]. In the other study, AD has a higher prevalence in firstborn offspring, unlike previous reports in asthmatic children [485]. In conclusion, on the one hand atopy predisposition is directly proportional to the degree and the number of affected relatives, on the other hand, 23% of children without any FHA also develop allergies [108], thus highlighting the role of environmental factors.

The role of IgE hyperproduction is another mainstay: asthma incidence is significantly more frequent at 12–54 months of age in the offspring of atopics with IgE ≈980–1500 UI/ml [200] and in 36% of 11-year-old children with IgE >1,000 UI/ml [390]. When comparing the total sum of sIgE to allergens, atopic children with current asthma had significantly higher sIgE levels than nonasthmatic atopic children ($p=0.0005$). Similarly, total IgE levels were significantly higher in atopic children with current asthma than in nonasthmatic atopic children ($p=0.018$) [184]. In clinical practice, the *high family concordance between type of disease and sensitization to specific allergens* is frequently observed: there are families in which all components are allergic to grass pollens and others in which all components are sensitized to *Parietaria* pollens; altogether there are families allergic to pets, confirming that immunocompetent cells are activated only by defined allergens [182].

The importance of *sex* has often been clarified: males are more at risk than females, with extremely significant statistical differences (Fig. 4.10) concordant with additional studies [16, 44, 146, 202, 206, 216, 221, 224, 290, 384, 390–392, 420, 432–434, 474, 487, 513, 514] and with total IgE and CBIgE levels (Table 3.7). This risk increases and in the respiratory allergy it may be occasioned by the reduced airway caliber in males, even though lung volume is greater (Chap. 11). This preference can also be explained by the genomic imprinting or the Carter effect [62]. Male children were significantly more likely to have doctor-diagnosed AD than females [224]. Boys with an allergic mother had a higher risk of sensitization at age 4 [202].

Similarly, *the age of symptom onset* (Tabe 5.5) *and the severity* of the clinical manifestations may be correlated with genetic controls: children with FHA^+ present with allergic symptoms more precociously than those with FHA^- [1, 3]. Moreover, DCs are very efficacious APCs [468] and very active in babies only a few months old affected with AD and FA, whereas they are activated in the lung later, as clinically confirmed by extensive experience and a study we have conducted [55], which evidence a lower age of onset for AD than for asthma. CMA onset may occur shortly after CM introduction with immediate reactions [267], and in infants aged <10 weeks T cells are able to respond with proliferative responses to Der p, *Lolium* and OVA [445]. As much as 29% of unselected infants followed from birth to the age of 18 months suffered from ≥2 episodes of asthma, in addition to responses to pet allergens [146]. The data agree with recent reports, stating that in the first few years of life, an active process occurs to recognize environmental allergens, even at levels low enough to elicit negative

SPTs, and the still immune system responds to the first encounter with an undifferentiated production of Th1 and Th2 without provoking sensitizations [28]. During subsequent encounters, the genetic predisposition induces either phenotype to prevail [169]. At 12 months in at-risk children, sIgE to aeroallergens are rare or barely detectable, as in children who later develop high sIgE concentrations to the same allergens [368]; negative SPTs and RAST to a group of aeroallergens at age 9 months turn positive at age 6 years [270].

As for BHR, the rate of Th2-like cells present in the respiratory mucosa of atopics with a specific pattern of responses to aeroallergens and an IL profile driving IgE synthesis is significantly greater than the rate found in nonatopics, hence an acceptable conclusion could likely be that specific Th2 cells appear in the mucosa soon after inhaling aeroallergens and that the Th2 IL-like cells start the allergic cascade of symptoms [98]. Regardless of the significant association between atopy and BPT (bronchial provocation test) to methacholine, BHR and asthma prevalence is greater in atopic children compared to controls [70]. Accordingly, adaption of homes for at-risk children should be planned even before birth, and in the first few months of life they should be fed following particular rules to prevent allergy or at least lessen its course.

Several authors comment that racial factors are strictly linked to genetic factors based on studies demonstrating that asthma prevalence is scarce in autochthonous populations (Chap. 5). However, it is known that African and Caucasian children show the same proclivity to express atopic disease [442].

Environmental Factors

Although molecular studies are in great fervor and may dispose of sophisticated equipment to analyze primary and tertiary structures, it is not yet possible to characterize the physicochemical properties of the molecules participating in allergen–antibody reactions, whereas atopic diseases have a clear distinctive character in children never to be found at subsequent ages. The immune system bewares of especially environmental allergens: the sensitization breakthrough and amplification and the commutation from a genetic predisposition to a true atopic state may be endorsed by a wide spectrum of factors. Association between FHA^+ and IL production at age 9 months and not in the CB suggests that to be loaded, genetic factors need exposure to environmental factors [270]. Indeed, several of them may be committed to the phenotypic expression of atopy, either emphasizing risk, or directly prompting symptom onset, respiratory if triggered by aeroallergens, cutaneous and/or digestive by food allergens. However, clinical manifestations of the respiratory system or of other systems are frequent, provoked by food ingestion or even inhalation when foods are diffused in the environment.

On this matter, the effects of the 1973 energy crisis cannot be underrated, with homes designed to minimize heating costs, fitted with insulating materials, and especially in Nordic countries with double-glazed windows and central heating, resulting in restricted air exchanges between outdoor and indoor environments. Air exchanges in these houses are therefore reduced compared to older houses, sometimes to minimal levels, with an increase in dampness and indoor mold growth because of the tightly sealed building, leading to warm humid habitats for HDMs and a consequent increase in prevalence in infantile sensitization. Appliances with a water reservoir such as humidifiers and vaporizers are a potential reservoir for mold growth as well. In 4,990 children living in homes with dampness problems, all with smoking parents, there was a detectable increase in AR and allergic asthma compared with children not exposed to these risk factors, but also in comparison with children exposed to only one factor. The risk was higher in children with a positive FHA, in whom 22 % had manifest asthma if exposed to both risk factors [7].

Month of Birth

Early infancy seems to be a period of particular susceptibility to sensitization, as indicated by epidemiological and experimental studies. For example, the season of birth may affect the future expression of allergy, particularly in children with a propensity to atopic manifestations. The physiological immaturity of the mucosal barrier has a pivotal influence on subsequent atopy development: several studies have agreed that the month of birth may condition the type of sensitization to pollens or HDM early. Table 4.12 [2, 49, 87, 199, 303, 369, 383, 392, 421, 475] includes 27,536 subjects in 29 different studies, with a convergence of sensitization to grasses of 83.5 % and to Der p of 98.4 %, with highly significant differences (p=0.0001). Our study [49] consisted of 2,124 children and of a control group including all the children born in the same years and the same district of the entrants, born throughout all seasons. We have demonstrated that children born in September and October and in spring express a significantly greater prevalence of allergy related to inhalant Der p and pollens, respectively (in both cases p<0.0001) (Figs. 4.11, 4.12) [49]. To explain the apparent discrepancy of certain studies, a Der p-negative result may depend on taking into account a great number of months (from May to December); others have found differences in winter months for pet danders [303]. The majority of pollen studies are positive, with wide differences, while a prevalence in winter months in English adults may depend on a diagnosis that was only clinical [49]. There is a concordance with CB T-cell proliferation: the neonates studied in the months between May and November show a response positive to Lol p 1, unlike those examined in the subsequent period [333]. A Finnish study

Table 4.12. Studies on the relation between month of birth and sensitization to grass pollen and/or house dust mite

Authors	Year	Country	Age (years)	Grass pollen	No.	House dust mite	No.
Reed	1949	US	15	No	150		
Smith	1970	Great Britain	Children	No	410		
Kleiner et al	1975	US	Adults	No	210		
Björkstén and Suoniemi	1976	Finland	0–29	Yes (Mar–May)	1,421		
Soothill et al	1976	Great Britain	0–2			Yes (Sep–Oct)	58
Pearson et al	1977	Great Britain	Adults	Yes (Dec–Feb)	476		
Warner and Price	1978	Great Britain			5–14	Yes (Jul–Dec)	87
Tan and Voothorst	1978	Holland	NS	Yes (Feb–Apr)	1,286		
Robert and Carron	1979	France	0–16	Yes (Mar–May)	667		
Smith and Springett	1979	Great Britain	5–15	No	1,715	Yes (May–Oct)	1,286
Kemp	1979	New Zealand	3–14	Yes (Jun–Sept)	86	No	86
Settipane and Hagy	1979	US	Students	Yes (May–Sept)	1,109		
Björkstén et al	1980	Finland	0–29	Yes (Feb–Apr)	2,171		
Suoniemi et al	1981	Finland	15–17	Yes (Dec–May)	605		
Björkstén and Suoniemi	1981	Finland	0–29	Yes (Mar–May)	7,095		
Korsgaard and Dahl	1983	Denmark	15–30	No	336	Yes (May–Sept)	240
Bonini et al	1984	Italy	Adults			Yes (Jul–Sept)	69
Carosso et al	1986	Italy	10–36	Yes (Mar–Jun)	207		
Beck and Hagdrup	1987	Denmark	0–41	Yes (May–Nov)	210		
Businco and Cantani	1988	Italy	1–14	Yes (Mar–May)	439	Yes (Jun–Sept)	1,685
Johnson	1989	US	1–18	No	631	Yes (Jun–Sept)	631
Åberg	1989	Sweden	3–16	Yes (Apr–Jun)	394		
Kozanoglu and Güneser	1991	Turkey	Adults			Yes (Aug–Sept)	208
Croner and Kjellman	1992	Sweden	12–14			Yes (Aug–Oct)	332
Karachaliou et al	1995	Greece	2.5–20	Yes (Mar–Aug)	324	Yes (May–Aug)	56
Rubio Sotés et al	1995	Spain	7–42	Yes (Mar-Jun)	1,827		
Sears et al	1996	Canada	9–15			Yes (fall–winter)	223
Nilsson et al	1997	Sweden	0–15	No (Mar–May)	209		
Saitoh et al	2001	Japan	12–13	Yes (Dec–Jan)	274	Yes (Nov–Dec)	325
Total					22,252		5,284

Data from [2, 49, 87, 199, 303, 369, 383, 392, 421, 475]. Additional references are in [49].
NS not shown.

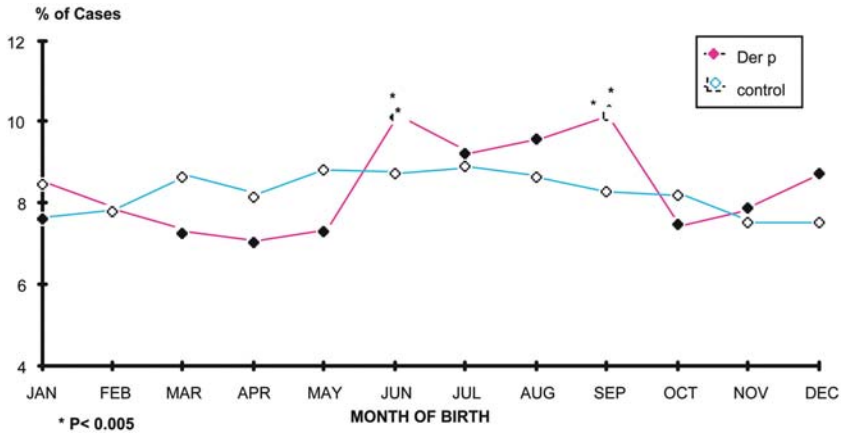

Fig. 4.11. Percentage of children sensitized to Der p (no. 1685) and of controls as a function of the month of birth. (Modified from [49])

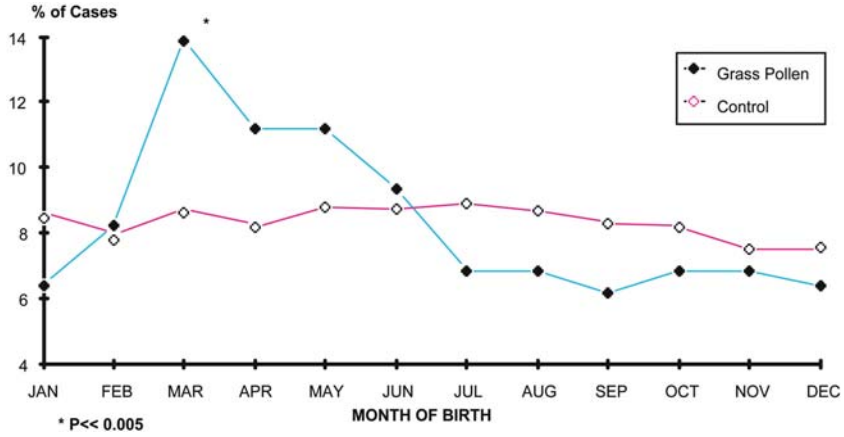

Fig. 4.12. Percentage of children sensitized to grass pollen (no. 439) and of controls as a function of the month of birth. (Modified from [49])

reported that if all neonate entrants were born in July and August, that is, within the season with a more reduced risk (25%) they had later development of allergic manifestations as children and young adults: the confirmation was after the 17-year follow-up [372]. It is therefore tempting to believe that there is of relationship between month of birth and specificity of the allergic sensitization, triggered by minimal doses of allergens.

Geographic and/or Racial Factors

Migration from industrializing to industrialized countries has been identified as an asthma risk factor in black children born in the UK of immigrants, more than in indigenous white children, whereas black children born in West Africa and the West Indies moving to the UK also had a low prevalence of associated atopic diseases, which was credited to a lower allergen bombardment in their home countries [442]. Similarly, Pacific islanders moving to New Zealand had a much higher prevalence than that of their countrymen who remained at home [442]. Another study has shown that the origin of such differences was not geographic, but depends on the age at which the family emigrated: if after the 1st year after birth, the prevalence is related to that of the original population, if before birth, it is related to that of the community where the family now resides [442]. Therefore, we conclude that asthma prevalence is more likely to be influenced by environmental than by racial and genetic factors. Allergy to Der p 1 is likely caused by the introduction of a Western lifestyle to rural peoples, by recent acquisition of mattresses instead of well-aired mats that are not infested by HDMs [442], which prefer the seldom washed cotton blankets encasing the person completely during sleep, and therefore harbor armies of HDMs [443]. This could explain the 70-fold increased asthma prevalence in the adult population over the last decades [442]. For example, a dramatic increase in bronchial asthma has been reported in Kuwait since the mid-1950s, which seems to be attributable to the importation of *Prosopis* trees [117]. The nearly absolute absence of Der p in dry areas such as Tucson, Arizona can be connected with geographic factors [491]. Overall, the data indicate that in "virgin" populations, as well in early in life, an increased exposure to Der p is followed by an increased asthma prevalence.

Residential Influences

Residence in an urban environment constitutes a greater risk of atopy than county life (p=0.000): an OR risk is related to pollens, cat danders, damp and unusu-

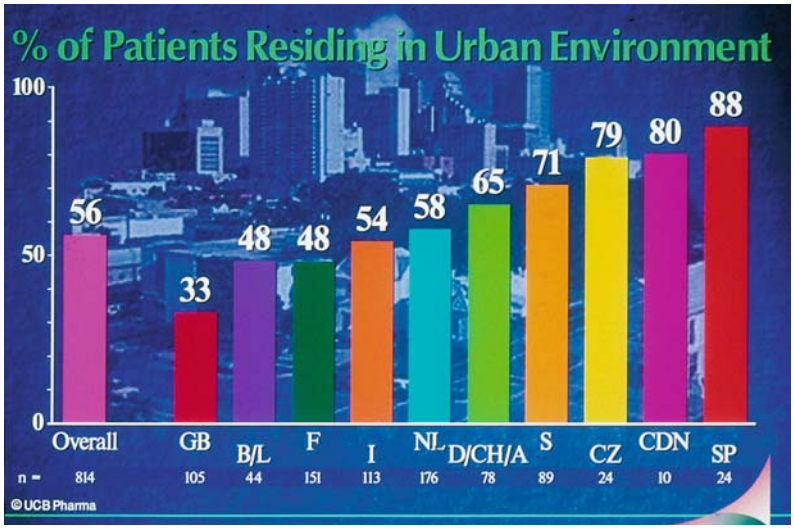

Fig. 4.13. ETAC Study: approximately 56% of children reside in an urban environment, the highest frequency is in Spain (88%), the lowest in Great Britain (33%)

al odors (p=0.03) [42]. A mean of 56% of the ETAC study children live in a city (Fig. 4.13). It is likely that this is the effect of a greater concentration of environmental pollutants in the city compared to the country [40, 42, 43], which could also combine with an increased risk of expressing AD [384]. In the population of Swedish children with asthma (3%–9.5%) 17.8%–71.5% have cats at home, and 36%–66.3% of asthmatic Polish children have dogs, in either country or urban homes [43]. In atopic children aged >2 years, additional, highly significant associations are found between condensation on window panes, either isolated or associated with smoke, and early exposure to dampness and an increase in allergic asthma and BHR [482], which along with favorable temperature (T) facilitate Der p propagation into houses. Other studies have reported no valuable differences on dogs, horses, Der p and tobacco smoke [42], but on the great frequency of furred pets (47%), caged birds (20%) and aquarium fish (23%) [7]. Children living in rural zones and in a damp house with parents who smoke show the greatest frequency of asthma (8.7%) and BHR (19%), both with a RR (relative risk) of 1.3; the damage due to dampness was significantly associated with increased occurrence of asthma, AR, prolonged cough after airway infections, and exercise, more severely in children with FHA^+, thus establishing a significant correlation between dampness and atopic disease [7]. Dampness and molds in the baby's bedroom amplify the risk of allergic asthma [68]. Therefore, it is unclear whether the increased asthma prevalence reflects a direct influence of indoor and outdoor pollutants on airways or an incremented allergic response to aeroallergens due to increasing pollution, of which an adjuvant effect has been supposed [28]. Asthma prevalence increased despite the low local rate of pollution, which was lower in a highly polluted city compared to other less polluted places [43].

Early Exposure to Environmental Hazards

Allergic manifestations develop more easily in children exposed in the first few years of life to an elevated allergenic load [171], thus demonstrating that the prevalent environment during the early formative period is fundamental in predisposing or not to sensitization and to persistent Th2 reactivity, taking into account the precocity and intensity of the exposure. To understand how "sterile" neonates can be sensitized to environmental allergens, foods or inhalants, profound modifications following the exposure to a myriad completely novel substances may overturn routine practices established previously [171]. As noted above, a different procedure regulating the sensitization to foods and inhalants, as well as inhalant sensitization, is carried out more precociously to the perennial allergens HDM and animal epithelia (danders, hairs, feathers) than seasonal allergens, and among these, firstly to allergens with extended or continuous diffusion (grasses, *Parietaria*) and then to allergens with short and circumscribed pollination (*Compositae*, trees). Der p asthma arises at a much earlier onset age than asthma to pollens (p<0.0001) (Fig. 4.14), as demonstrated by high levels of anti-Der p IgG_1 antibody at 3–12 months [370] and an age of ≈3.8 years in Der p-exposed children admitted to hospital with asthma exacerbations [411]. However, during early childhood, even a low-level HDM exposure stimulates T-cell responses [29], which are ultimately shaped by an allergen-driven T-cell selection process during infancy and early childhood [169]. In keeping with this, doctor-diagnosed AR is present during the 1st year after birth [491]. Therefore, a static equation is that not only food but also inhalant sensitization may occur in early life [405]. Der p, Fel d 1 and mold allergens are proteolytic enzymes able to clear a way directly through the bronchial epithelium, deceiving the normal defense barriers: the airway penetration is facilitated by concomi-

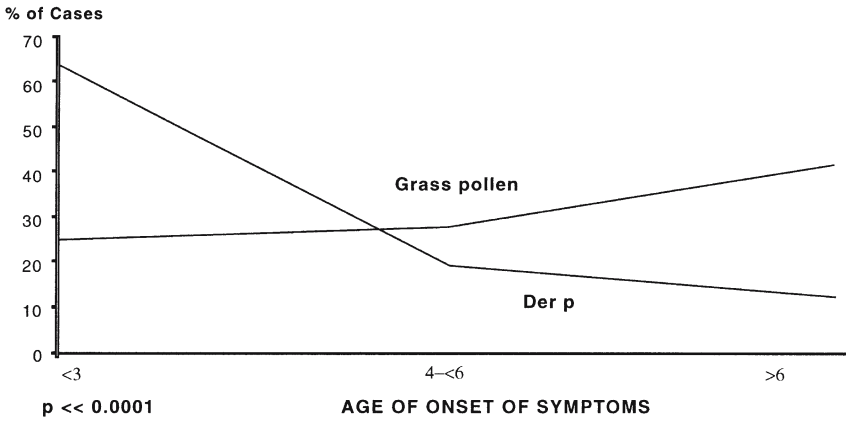

Fig. 4.14. Age of onset of symptoms and type of sensitization in 1,685 children sensitized to Der p and in 439 sensitized to grass pollen

tant viral respiratory infection (VRI), passive inhalation of environmental tobacco smoke (ETS) and pollution exposure. As previously alluded to, another area of increasing interest is the different compositions of the gut flora between infants who will and infants who will not develop allergy, which may be demonstrated before the development of any clinical manifestations of atopy. In comparison with healthy infants, babies who developed allergy had lower bacteria counts during their 1st month to 1st year of life. If the gut microflora drives the maturation of the immune system, negative changes affecting its composition might play a role in the higher prevalence of allergy [30].

Aeroallergens

The *allergen levels* of some inhalants are as follows (g/dust). Der p and Bla g 2, 2 µg; Fel d 1.8 µg. In 4-year-old entrants, the sensitivity to inhalants was significantly associated with FHA^+ [434]. A study on 1812 children living in Munich, 2242 in Dresden and 1222 in Leipzig has shown that the rates of atopic sensitization to inhalants were always *higher in the children aged 9–11* compared with those aged 5–7, with higher figures in children aged 9–11 from Dresden and Leipzig compared with those from Munich. There were no significant differences in the prevalence of SPT reactivity to at least one allergen, and increased sIgE against inhalant allergens between Dresden and Munich. Yet, despite the higher sensitization against cat (see below), tree pollen and HDMs, the prevalence of asthma and BHR in 9–11-year-olds was lower in Dresden than in Munich [476]. As noted by previous investigators, infantile asthma is evoked by aeroallergen allergy, with differences in the clinical manifestations depending on the causal agent: in Der p 1-sensitized children, either asthma or PAR symptoms exacerbate in autumn and often also in spring, whereas in children with pollen sensitization, the symptoms appear typically in the seasonal period of pollination. However, due to the intervention of other triggering factors, the pattern may extend clinical manifestations. In addition, inhalant allergens produce skin lesions by contact and by blood penetration after having been absorbed by the respiratory mucosa and thus through the skin mast cell cycle (Chap. 7). The pollens cross-reacting with foods (Chap. 9) induce symptom worsening in children allergic to the related food during pollination; cases of anaphylaxis after consuming a food containing an inhalant have been recorded (Chap. 24) (see Table 5.18 with the related prevalences).

Mites

Reportedly, Aristotle was the first to observe mites (Fig. 1.80) (350 BC) in some cheeses, but only in the seventeenth century did the first microscopic study appear, credited to van Leeuwenhoeck (1632–1723), followed by Ramazzini in 1713, who also described "farmer lung disease." The first report that mites may be the cause of respiratory problems in humans dates back to Ancona [6], who described cases of asthma driven by *Pediculoides* (now *Pyemotes*) *ventricosus*. We have therefore known the decisive role played by aeroallergens in the sensitization process for 80 years. Although in the 1920s, arguments were put forward about house dust, relating pillows, mats, chairs and bed quilts to AR and asthma onset [416] and anti-mite environmental measures were recommended [97], several decades passed before the relationship between *Dermatophagoides* and what, for lack of a better term, was defined as "dust allergy" was recognized. The scientific basis of allergy received a major boost when Voorhorst documented the source of *Dermatophagoides* allergens with clinical and histological observations reported in a classic paper [466]. His discovery, which was revolutionary at the time, was later confirmed by several reports, the number of which had grown exponentially.

The genus *Dermatophagoides*, species *pteronyssinus* (Der p) (11 allergens), *farinae* (Der f, 13 allergens) (Table 1.74) belongs to the Pyroglyphidae family, such as mites (*Acarus* genus, *Arachnida* class). Cross-reactions are between Der p 1 and Der f 1 and between

Der p 2 and Der f 2; Der p 1 has 85% homology with Eur m 1. Der p 1 and Der f 1 have a sequence homology of 80%. As detailed in Chap. 1, tropomyosin is shared by Der f 10, Der p 10 with Met e 1 and Pen a 1 and with Tod p 1, Lep d 10 and Ani s 2. Mites (Fig. 1.80) have four pairs of articulated legs (and are distinct from insects, which have three pairs), easily discernible buccal appendices and a striated cuticle, the average length of the life cycle is 122.8±14.5 days at 16 °C. The females depend on environmental conditions: within their life span they may produce 2–3 eggs/day during ≈26- and 34-day reproductive periods [336], from which active six-legged larvae appear within the first 5 days, they are then transformed into eight-legged protonymphs for a short time of about 2 days. After about 2 more days, they change into tritonymphs. After about 8 more days, quite mature mites emerge. Male Der p measures about 250 µm and females about 350 µm, with a mean weight of 3.11 µg [336]. Coprophagic HDM *feed* principally *on products of skin desquamation,* so *their ecological niche is concentrated inside, around and under the bed,* because humans enwrapped in bedding lose a host of dead skin cells, a mean of 0.5–1 g of dust/day, which attracts large armies of mite allergens, and may feed several thousands of them for 3 months. Der p levels increase during sleep 10-fold more than in everyday life and 1,000-fold while making the bed [375]. Because indoor humidity is the key factor for Der p growth and development, indoor T is about 20 °C and human body heat brings this T to 25±3 °C in mattresses. Relative humidity exuded from the human body (transpiration) may favor mite development [322]. Recent studies have observed that mites survive at relative humidity of 45%–55% at 15 °C and of 55%–65% at 25 °C, and thrive better at 25 °C and 75%–80% relative humidity, correlated with the maximal number of Der p 1 in mattress dust and on the floor under the bed. The preferred T is 25 °C, but HDMs survive at higher T: at a T of 45 °C HDMs survive 24 h, at 50 °C 4 h and at 60 °C only 1 h; at low T mites become inactive, but can survive at –1 °C for 3 weeks, and at –25 °C are dead after 6 h. Der p 2 are more thermoresistant [322]. Consequently, in temperate dwellings, mites fail to proliferate when indoor relative humidity is <50% and T of 20–22 °C. However, mites also resist sharp variations of such parameters via the regulation of water exchanges made by transpiration through their cuticle; if local humidity falls, HDMs take shelter in the niches offering the perfect habitat, that is 2 mm over the floor [337].

During its life cycle, Der p excretes fecal pellets ≈300-fold its weight, so ≈1,000 each containing 100 pg of Der p 1, particles with a chitinous peritrophic membrane ranging from 10 to 40 µm in diameter, reduced in young mites. In the form of fecal pellets, mites become airborne but fall within 10 min in still air, thus accumulating in house dust with an estimated number of 100,000/g [337]. The particles are surrounded by a viscous substance by which pellets adhere to textile fibers, which are unlikely to be removed by using common cleansing procedures. They are instead split by mechanical action such as walking on carpets and are decomposed when the viscous particle is attacked by molds. When the flat begins to be warmed up, the pellets are dried and pulverized, thus acquiring a diameter equal to that of pollen grains. Unwaxed chitin is not waterproof and allergens elute from fecal particles very rapidly [260]. Mite feces containing Der p allergens have the ideal measures to be airborne and inhaled whenever their support is lifted and shaken. Thus allergens increase in the air during activities that disturb dust, using a vacuum cleaner not provided with the proper microfilter, for example. The particles seen through a light ray contain dead mites and their feces, the most frequent cause of respiratory symptoms. A relatively small number of particles are inhaled per day; however, children who become allergic to HDM in their homes have an increased risk of asthma and continued exposure contributes to morbidity [336].

Much has been learned about Der p 1 noxiousness as resulted from immunohistochemical studies leading in atopic subjects to the isolation of cell lines or Der p 1-specific T-cell clones, demonstrating an in vitro IgE allergen-dependent production driven by IL_4. The association of responses between T cells and HLA-DRB3 (DRw52), and to a lesser degree DRB1, points out that since *DRw52* is in a position of linkage disequilibrium with other HLA alleles, such specific restrictions are expressed in about 40% of the general population. Mite-sensitive childhood asthma is on chromosomes 4q35, 5q31-q33 and 13q11 [501]. Being on an IL cluster (Fig. 4.6) enhances its allergenicity [10]. This impairs proliferation and IFN-ɣ secretion of Th1 T cells, favoring Th2 development, and finally enhancing total IgE production [139], thus clarifying why the prevalence of mite sensitization in humans is so high. While initial studies were focussed on Der p 1, a new chapter was opened up after finding that anti-Der p 2 IgE are present in 40%–88% of patients, with peaks of 92%, leaving in the background both Der p 1 (78%) and Der p 3 (16%); 89% of mite reactivity was to Der p 2 in 9-year-old children (mean) [308]. In HR infants a larger prenatal exposure to HDM allergens was associated with the presence of elevated total serum IgE at day 3–5 after birth [401]. Platts-Mills and his co-workers [336, 337] have convincingly proven *that Der p 1 is one of the principal and more diffused etiologic agents of infantile bronchial asthma.* Therefore, some problems of mite-sensitive asthmatic children can be brilliantly downgraded by providing a substantially dust-free bedroom with a proper degree of humidity [375]. The weight of interventions for allergen avoidance is highlighted by infant conditions: they spend 80% of their time at home (adults spend a mean of 33% of their time) [27], and Der p 1 levels are higher at night [375]. Mites and their feces are preferentially recovered in overcrowded residences and whenever dust is collected and relative humidity rises, in addition to bedding where there is the greatest mite sampling:

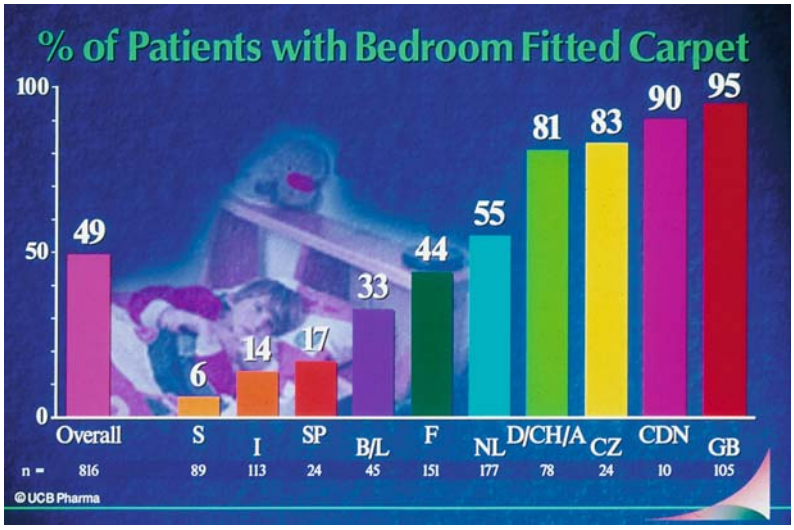

Fig. 4.15. ETAC Study: on average 49% of children have fitted carpets in their bedroom, the highest frequency is in the Czech Republic (95%), the lowest in Sweden (6%)

mattresses, pillows, curtains, carpets, upholstery fabrics, draperies, etc. [222], but also in upholstered seats of public places, in 9% of patients in homes with MDM levels >10 µg/g [88]. Rugs and wall-to-wall carpeting are found in 49% of infant bedrooms (ETAC study) (Fig. 4.15) (see Chap. 24).

When mites die, they disintegrate into a thin dust, which is inhaled by neonates and infants more easily than intact mites [293], a finding related to the greater prevalence of Der p 1 allergy in children born in autumn [49] (Table 4.12). Der p 1 proliferates in summer with a peak in July, declining gradually until October and dying in winter; the Der f 1 peak is in August, with two minor peaks in April and October [244]. The Der p 1 year cycle, as demonstrated graphically by an interesting study, is marked by asthma recrudescence, maximal from the end of summer and the beginning of autumn and minimal in spring, from March to May [449], in parallel with the greater medication requirement in September and October [204], the number of peripheral eosinophils, the response threshold to histamine and the connection between mite levels, humidity rate and BHR variations [449]. In evaluating the impact of Der p 1 and 2 sensitization, SPTs were positive in 33.3% of subjects with no allergic symptoms and/or asthma [460], correlating SPTs to dust samples collected from mattresses. The patients with no treatment had the lowest mite value at home, whereas patients with frequent asthma attacks and daily treatment had the highest values [460]. However, the mite number cannot be related to asthma exacerbations [221], even when exposure is greatest [204].

The values of 10 µg for maximal level equivalent to 500 mites and of 2 µg for the lowest level equivalent to 100 mites can be considered as the threshold [230, 325, 336] for sensitization of genetically predisposed school-aged children and consequent allergic symptoms [221]. The level of 2 µg corresponds to an increased risk of sensitization and BHR, a critical limit for subjects with other sensitizations [221]. In 92 atopic children with sIgE to HDM, mite concentrations above 2 µg/g dust were found on their bedroom floors [357]. In a prospective study on 68 children, exposure to high levels of allergen at age 1 increased the risk of asthma at age 11 and was related to an early asthma onset [409]. Studies on children admitted to hospital showed that as many as 80% were both sensitized and exposed to high levels of allergen at home [336]. A level of 10 µg increases the risk of symptomatic or acute asthma, which increases when mite concentrations run to 1,500 mites/g of dust; in borderline cases concentrations rise to 5,000–6,000 mites/g of dust [336]. High levels of Der p 1 are 10 µg (maximal level); 2 µg (lowest level/g of dust) and expression of clinical manifestations are strongly correlated [337]. In atopic children, the sensitization grows in parallel with exposure to Der p 1 [230, 232]. Worldwide and in the US, the highest mite prevalences in homes are of Der p and Der f. Eur m may also be prevalent in some temperate geographic areas, and at times, their density may even exceed that of Der p and Der f. Blo t is a cardinal source of allergen in homes in the subtropics and tropics, thus confirming the wide Der p diffusion. More or less similar are the concentrations in Great Britain [409], Sweden [293], the US [51, 337] and in schools [115]. Confirmation comes from other pediatric studies done in Brazil [13], France [460], Germany [230, 232], Japan [376], Great Britain [411] and New Zealand [390] on the association of asthma with exposure to >2 mg of Der p 1/g of dust. Very enlightening is the study on 68 children whose parents were affected with asthma or AR. The children were followed up for 11 years: at this date, 52% were atopic and 94% with active asthma had SPTs positive to Der p 1. No child exposed to <2 µg/g of dust was sensitized in the 1st years of life, whereas the children exposed to >10 µg/g of dust had a fourfold risk of asthma. Over the 11 years, 3 of 68 children were admitted to hospital and each of them was allergic to mites and had been exposed to >30 µg Der p 1/g of dust at age 1 year

[409]. It was also found that Der p 1 levels of >5 µg/g of dust in infancy predicted positive SPT results and high IgG levels to Der p by the 5th year of life [368]. The *growing exposure to* mites and other home allergens may critically contribute to the general increase in both morbidity and mortality associated with asthma. Thus, the age of the first wheezing was inversely correlated to the level of exposure at age 1 year [409], demonstrating that 65% of children hospitalized for asthma had been exposed to Der p 1 at a level >10 µg/g of dust [411]. The sensitization to Der p 1, cats and molds is a significant risk factor for asthma expression, with a cumulative risk between 2 and 14 [389]. Such sensitizations arise more frequently in the 1st 18 months [230], even *in infants aged 11 months* [156]. Recently, levels of household HDM allergen at age 6 months were associated with HDM sIgE at age 7 years, but failed to find a relationship with asthma. In our opinion the levels were too low (0.2 µg/g) [232], and justify the failure to find the expected association.

Der p 1 allergen predominates in houses throughout the year [113, 337], with no differences between houses of asthmatics, neighbors and controls [323], and is often associated with cockroach exposure [337], with the lowest levels (<5 ng/m^3) in school [113]. As yet, the number of mites thriving within domestic walls is *much greater than what is commonly reported* in 86% of dwellings and in 25% of those *with neonates* [24]. Der p levels of ≥2 µg/g of dust were found in 55% of cases and levels of 10 µg/g of dust were more frequent in floor dust than in carpets [94]. However, employing more sensitive tests to measure mite numbers, a Der p/g of dust value >1 µg should be considered at risk [337]. At a cut-off of 1–0.5 µg Der p/g of dust, significant differences between atopic children sensitized and not sensitized to Der p 1 were found [346], but the enigma remained unsolved by measuring concentrations per m^2, which was not analogous to results expressed in g/dust [455]. In Tokyo, Sao Paulo and Sydney, mite levels were found of *>10 µg,* reaching even 35.1 [376], *38.4 to 51 µg/g* of dust [260], respectively. It is conceivable that current paradigms have been revolutionized: it is clear above all that only in 22.8% of dwellings of asthmatic children were mite levels ≥2 µg/g; in 51.4% they were between 2 and 10 µg/g, in the remainder 25.7% they were *between 10 and 100 µg/g* and in about the half *between 50 and 100 µg/g* [51]. Der p 1 air levels were between 18 and 89.6 pg/m^2 and Der p 2 from 5.1 to 24.5 pg/m^2 [376]. New aspects of severity come from the discovery that Der f 10 is a tropomyosin highly correlated with sIgE, thus a relevant allergen of the Der family, and that Eur m has 85% homology with Der p 1. We emphasize that mites do not thrive at <1,000–1,500 m only, as was previously believed [461], but flourish even at about 6,000 m, independently of T and of relative humidity, with levels ≫2 µg .

Recently, HD endotoxin (HDE) has been investigated. Exposure to HDE is associated with wheezing in the 1st year of life among infants with a familial predisposition to asthma and allergies [319]. A recent report showed that homes of allergen-sensitized infants had significantly lower levels of HDE compared with homes of nonsensitized infants. Moreover, HDE concentrations correlated with increased proportions of IFN-ɣ-producing CD4 T cells [137] (see also Chap. 24).

Pollens

Pollens (Figs. 1.68–1.73), another well-known cause of atopy, are the male gametophytes of seed plants: a truly impressive number of pollens via aerial transport reach a recipient flower (reproductive destination) to affect fertilization when an ovary is encountered (*anemophilous* pollination). In the *entomophilous* mechanism, the pollination is mediated by specific vectors, most often insects, and pollen allergy in this case occurs only by direct contact with the pollen source (in farmers, florists, etc.) [406]. Flowering plants with anemophilous, wind-borne pollination discharge large numbers of light-weight, buoyant pollens which, due to their small diameter (15–60 µm), can be transported and remain in the air over long periods. One m^3 of grasses produces 1.5×10^9 pollens/year (=1.5 mg of Phl p 5), which during the blooming period pour into the air a maximal amount of 219–250 m^3/day and are dispersed over a distance of several km by wind currents, with the goal of spreading as widely as possible, including on the airway mucosa of the persons nearby, thus accomplishing an outstanding allergenic activity [408]. Few plants with entomophilous pollination and some types of flowers have proven allergenicity: the pollens of most trees, for example, conifers (pine, cedars, etc.), rarely acquire a cardinal role from the allergenic point of view. Several factors foster both pollen diffusion and concentration: season, climate, local flora, 25–30°C T, 60%–90% relative humidity, 5- to 15-km/h wind speed, and rain. The amount of pollen in the air rises on windy and/or sunny days compared to cool and rainy days, is greater in plains, valleys and in the country compared to mountain areas, on the coast and in urban areas [406]. However, starch granules are detected in the atmosphere even on sunny days without rainfall [209]. The pollen effect is manifest in the following cases:

- Allergenicity able to sensitize predisposed or atopic subjects.
- Anemophilous pollination.
- Emission of large amounts of pollen.
- Wind-transport over wide areas.
- Wide territorial distribution of the producing plant.

Respiratory symptoms are provoked by starch micron particles constituting pollen granules. These have a breathable size (<3 µm in diameter) and may therefore penetrate into the lower airways of the respiratory tree, triggering asthmatic symptoms in sensitized patients [209]. The starch granules, copious in air samples taken

during the pollen season, have a highly significant 50-fold increase/m^3 of air on days following significant rainfall [209]. Rain accompanied by atmospheric T inversions tends to concentrate particles close to the ground, thus rupturing the granules by osmotic shock, each of which releases into the environment about ≈700 1- to 2-µm starch granules with allergens ready to enter the damp mucosal environment and to elicit IgE-mediated responses in the lower airways [422]. Consequently, asthmatic patients, generally city dwellers, crowd the emergency departments during thunderstorms inducing air turbulence, or shortly afterwards [209]. When rain is followed by sunny days, the alert decreases due to a rapid water evaporation; thus both spore production and vitality subdue. However, active atmospheric mixing carries pollens aloft, hence diluting levels to which patients are exposed near the ground. During flowering, 10–100 ng of pollen are inhaled daily, so 1 µg is inhaled yearly [110].

Several peculiarities of pollens are well known, but the dose-response rate remains open to conjecture. The priming effect and the minimal amount necessary to trigger asthma attacks are typical (Chap. 12). The allergen load has a greater effect on sIgE development rather than on IgE, skin reactivity and clinical manifestations. Grasses, *P. officinalis and P. judaica, Compositae (Par j),* etc., cause respiratory allergy, so that pollen exposure in the first 4–6 months of life [53], or shortly before the season [372], promotes the sensitization. Single species of anemophilous trees, among which birch and horse chestnut (*Aesculus hippocastanum*), usually shed pollen briefly in given localities, producing symptoms in 5.1% of town children [341]. Finally, pollen grains under humid conditions secrete significant amounts of eicosanoid-like substances able to recruit neutrophils and activate eosinophils. This process of *allergy initiation* precedes the allergen–APC interaction and may be the first step in the process of atopic sensitization [359]. Recent findings suggest that grass pollen sensitivity in childhood is accompanied by a progressive accumulation of allergen-primed T cells and progressive deviation of the allergen-induced IL response toward a Th2 response in atopic subjects throughout childhood [404].

Pollen growth is *accelerated everywhere by ozone depletion*. The resulting increased pollution leads to hyperproduction of carbon dioxide (CO_2), which is associated with pollen overgrowth. For example, ragweed sensitization has actually doubled its pollen production over the last 10 years, not due to an incidence upsurge, but due to the pollen overgrowth [341].

Fungi

Fungi (Figs. 1.77–1.79), also called molds, have long known associations with human disease. Their diffusion into homes is facilitated by the previously alluded to energy crisis, resulting in poorly ventilated interiors: air, T and humidity reduction is optimal for fungal spore proliferation [406].

Two major types of fungi are observed [53]:

- *Fungi outdoors* become more prevalent in summer in temperate areas, but also in the months of May and June as weather becomes warmer (T about 20–30°C). Rain and relative humidity (75%–95%) increase the recovery of anemophilous spores. Air currents, as in the case of pollens, may transport the spores even hundreds of km from their source; they spread copiously during dry weather subsequent to rainy days especially when humidity rises. A high atmospheric load of fungi often takes place once snow covers the ground or the ground is frozen, because devitalized vegetables represent a favorable culture medium for saprophyte mold growth.
- *Fungi indoors* dominate samples taken throughout the year in ordinarily ventilated interiors. We emphasize that indoor, humid places can be frequent sources of allergy, from 33% to 86% of cases [54].

Fungi may also behave as *parasites:* various yeasts are employed in the preparation of wine, milk, baked foods and some cheeses. Parasites are veritably facultative when they grow on living plants or plant foods, which are used after the decay of parasite organisms, thus closing the circle because they turn again to saprophytes [406]. However, yeast-sensitive children should follow a more or less restricted diet (Chap. 24).

The best-known fungi belong to the *Deuteromyces* group (fungi imperfecti), and their recovery is dominated by spores of *Alternaria alternata* (*Alt a*), 9 allergens (cigar-shaped, 18 µm long, 5 µm in diameter) and *Cladosporium* (*Cla h*) species, seven allergens, both belonging to *Hyphomycete* class and the *Hyphomycetal* order and grow on leaf surfaces. *Asp f* (*Aspergillus fumigatus*): 19 allergens are implicated in children with allergic bronchopulmonary aspergillosis or chronic granulomatous disease (Chaps. 11 and 22). Alt a 1 allergen is correlated with the onset of symptoms in sensitive patients [207]. Allergic sensitization with SPT reactivity and clinical signs of sensitivity to inhaled fungal spores is generally not correlated with the seasonal patterns of presence of the spore molds in the atmosphere [91]. Both *Alt a 1* and *Cla h* frequently trigger asthma, with 100–3,000 spores/m^3 of air risk levels, respectively. *Alt a* is the *third most common cause of sensitization* after Der p and grass pollens at 4 years of age [434]. Fungi sensitization may be low, although in the atmosphere and in confined settings they are in concentrations remarkably higher than that of pollens (1,000:1 for *Cla h*): evaluation of mold allergy is difficult because of their poor propensity to release allergens when close to the respiratory mucosa [91]. The spores achieve their highest levels mostly in very moist and slightly windy weather and are recovered on the skin and airway mucosa. Measuring less than pollens (*Penicillium* <5 µm), this exposure to fungal spores can adversely affect the daily respiratory status of some asthmatics [91]. Such exposure to the

spores can be aggravated because *Alt a* can release mycelia more rapidly than other molds, potent allergens that worsen respiratory symptoms in allergic patients more than the spores [54]. The spores also provoke AR, but are encountered in the lowest airways where the ciliary function does not operate, so that spore clearance from respiratory mucosa requires a rather long period [91]. Eating mold-containing foods may provoke allergic (Chap. 24) or toxic reactions, especially if aflatoxins are involved [362].

SPT positivity to molds in 5.5% of children with asthma and in 19.5% of those with AR points to molds as critical indoor allergens [85]. *Alt a* spores are diffused according to the geographic position, the season, the prevalent atmospheric conditions, the time of the day and in all seasons of the year, but above all in summer [326]. The spores are more prominent in internal regions with a dry climate than in humid and warm sea resorts (15.2% vs 4%, $p<0.001$), while sensitizations depend on the exposure duration in addition to place of residence, thus representing a very potent allergen [326], also liberating spores and mycelia. A dry climate favors spore detachment and the consequent potential to trigger sensitization [53] *Alt a* may be associated with *Cla h* in 14.3% of dwellings [434]. Clinically, multiple sensitizations to *Alt a* are harmful, as highlighted by 6.4%–17.7% of children presenting with SPT positive for *Alt a* (mean, 13%) [85, 346], which we have found in 15.5% of 6,040 children [53]. Further, in the three studies, only 0.2%–2% of children were monosensitized (mean, 1.3%), a discrepancy of 4.8% (2.1% to *Cla h*) [434], and the significantly reduced monopositivity we observed was also related to the extracts in use (1.3%) [53]. The children aged 9–11 had SPTs + for *A. tenuis* in 0.3%–1.4% of cases [476]. With respect to the diagnosis and concordance between SPT and RAST, SPT positivity included all cases, RAST positivity only 63% [53]; no correlation between the two methods was noted [55]. In subjects suffering from asthma and perennial AR, often very severe and difficult to treat, a sensitization to molds should always be suspected and readily identified in the indoor setting, owing to a substantial association with asthma severity, hospital admissions, AD and FA [53]. In young adults, mold sensitization (and sensitization to Der p 1) implies an elevated BHR risk, reduced by half in patients sensitized to pollen and pet allergens [81].

Pets

The relative allergens are much more diffused compared to Der p 1 allergens: Fel d 1 and Can f 1 air levels are 160- and 110-fold higher than Der p 1, on the ground they are 59- and tenfold higher, respectively, in the homes of at-risk children [293]. The progression of cat allergy is proved by the high proportion (≈20%) of babies aged <2 years with positive RAST to Fel d 1 [111] and the association of the early Fel d 1 exposure with high antibody titers (RAST ≥1) in ETAC babies followed up for 36 months, 36% of whom had a pet at home [474]. Both families and children try to minimize and even resist recognizing pet-related symptoms, fearing that the beloved animal will be banished from the home. It was not a surprise to find that 70 French subjects who were advised to remove the cat became asthmatic before age 20: only 11% of them accepted, 39% preferred to take the pet, but 50% who first accepted cat avoidance *bought another cat* before the age of 20 [100]. Cat hair is much more sensitizing than dog hair. However, the soluble allergens induce symptoms in atopic children and more easily than mite and pollen allergens. The easily airborne particles bearing cat allergens are smaller, thus allowing penetration into the airways in higher numbers and consequently diffusing everywhere. Thus symptoms are experienced soon after the contact [65, 293].

There are three *cat* allergens: Fel d 1–3. Both dander and hair allergenic content is similar; all the three allergens can bind to human IgE antibodies. The primary allergen (Fel d 1) originates from lacrimal, sublingual and anal glands [453], urine, epithelial and squamous cells, but mainly from cat sebaceous glands, with a gradient from 10 to 1 from the hair base to the point [65] and the apex in the areas rich in these glands, such as the tail. From the tail, Fel d 1 is scattered in the dander; either with its saliva, or as a result of habitual hair licking or tail wagging, the cat spreads Fel d 1 to the whole environment [65], so allergens also are found in male urine, environmental air, furniture, mattresses, sofas and the ground, especially the carpets, unlike the Der p 1 allergen [94].

Thirty minutes after a cat enters a 30-m^3 room are sufficient to increase Fel d 1 concentrations up to 30–90 ng/m^3, or 127 ng/m^3 [94], rising up to 3,120 ng/g of carpet dust [293], 16,840 ng/m^2 in school carpets, with a tenfold increase in dwellings [113] and up to 100 ng/m^3 in homes with cats [376]. In cat-allergic children, Fel d 1 allergen inhaled through the nose and absorbed may exacerbate asthma. The threshold level is 8 µg/g, but within 20 min Fel d 1 levels rise up to 20 ng/m^2. Characteristically, 15% of Fel d 1 (vs <0.3%–1.0% of Der p 1 and 2, respectively) remains easily airborne after turbulence of environmental air; by adhering to particles <2.5 µm in diameter it is able to penetrate the lungs deeply, and 6 min are enough to inhale 8 ng of allergen [94]. Therefore, a sensitized subject often develops acute symptoms within minutes of entering a home with a cat and a highly sensitive subject could inhale the quantity used in BPTs within 20 min, even when the cat is absent [337]. In an apartment, both cat and dog allergens are significantly more concentrated in living rooms than in bedrooms or in mattresses [292], in dust, where concentrations vary from 0.8 and 1.6 µg/g of dust (homes without cats) up to maximal levels of 642–948 µg/g of dust [376] and 100 µg/g of dust in carpets (homes with cats) [94], and are very low in

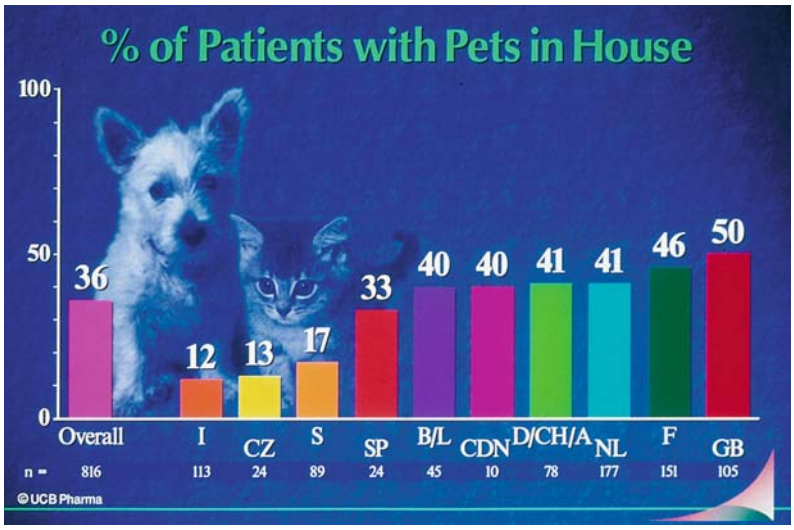

Fig. 4.16. ETAC Study: on average 36% of children have pets in the home, the highest frequency is in Great Britain (50%), the lowest in Italy (12%)

kitchens and bathrooms [339]. The children aged 5–7 had SPT+ for cat in 2.7%–3.3% of cases, and children aged 9–11 had SPT+ for cat in 8.6%–9.1% of cases [476]. Airborne levels are between 1,100 and 23,000 pg/m^2 [376]. Following repeated reports of elevated Fel d 1 levels in homes constantly without cats and in normally cat-free private or public buildings such as subways, department stores, schools, offices, hospitals [119, 293, 294], upholstered seats of cinemas and other public places, where measurable airborne concentrations were present [88], it was hypothesized that the cause was Fel d 1 ubiquity. As a consequence, exposure to cat allergens may occur in a variety of settings, transported by clothes and shoes of cat owners [10], and highly dependent on the activity taking place, including cotton operatives [88, 119], and ventilation systems [448]. Fel d 1 allergen levels were found in the mattress dust of *families without cats:* second-hand exposure to cat allergen is detrimental in terms of allergy development in infants [160]. The proof of passive transfer is likely in the significantly high levels of Fel d 1 and Can f 1 on school benches compared to floors [294]. There, the levels of cat allergen are high enough to induce sensitization and cause perennial symptoms in cat-sensitized asthmatic children [294]. In the end, children living in cat-free houses may be exposed to Fel d 1 at school, in houses of cat-owning schoolmates and in their own home due to passive transfer: levels as high as 8 µg of Fel d 1/g of dust in cat-free houses shows that unlike outdoor allergens, sensitization to indoor allergens is more often associated with asthma [10]. Occult sources of animal dander allergen are found in homes without these animals [10], but animal dander sensitization commonly occurs in children born in settings with nearly undetectable allergen exposure and who have never had contact with pets [375].

Can f 1 allergens have been purified; about 5%–30% of nonasthmatic atopic subjects and 30%–50% of those with asthma produce SPTs positive for Can f 1. Sensitizing levels are >10 µg/g of dust [376]. Can f 1 allergen may constitute a marker for studying dog allergy, since it reacts with sIgE antibodies of about 70% of dog-allergic patients. Cat allergens are found in dander, urine and mainly in dog hair and saliva; however, in assessing Can f diffusion in homes, we find that studies on the dimensions of particles to which Can f 1 binds are rare. A marked cross-reactivity between Can f 3 with albumin of Fel d 1 has been found (Table 1.73). Cat allergen levels can vary from 60 ng and 866 µg/g of dust and airborne up to 10,500 pg/m^2 [376]; they are higher in living rooms compared to bedrooms and other rooms [10], especially with rugs or wall-to-wall carpeting and, similarly to Fel d 1, stuck to the seats of public places more often than inside private homes [88]. Significant levels have been detected in dog-free houses [10, 456], a finding explained by frequent contact with the animal in the houses of grandparents (22%), friends and neighbors (14%) [456], with subsequent transfer to the child's own house. The earlier the exposure in life, the higher the chances of sensitization: at birth domestic pets are found in 31% [24] and in 25%–27% of homes with babies aged <12 months [100]. In infants of the ETAC study aged 17 months (mean), a pet was present in 36% of cases (Fig. 4.16) and a cat in 16% of cases (Fig. 4.17). In our study on pet allergy, we have found that 54 cats and 30 dogs were in the homes of 289 HR children since birth, with an incidence of allergy of 18.7% for cats and 10.4% for dogs, but 16 cats and six dogs were in the home of 300 healthy controls with a total incidence of 7.6% (p=0.0001) [54]. The negative effects of early exposure to pet allergens is demonstrated by an increased cat presence during the first 3 months [421] or of a dog during the 1st year of life [456] and is associated with BPT positivity. As much as 51.5% of English asthmatic children with cat allergy had housed a cat since birth, vs 11.4% of nonallergic children (p=0.0003), and the association between SPTs positive to Fel d 1 and cat presence in the environment

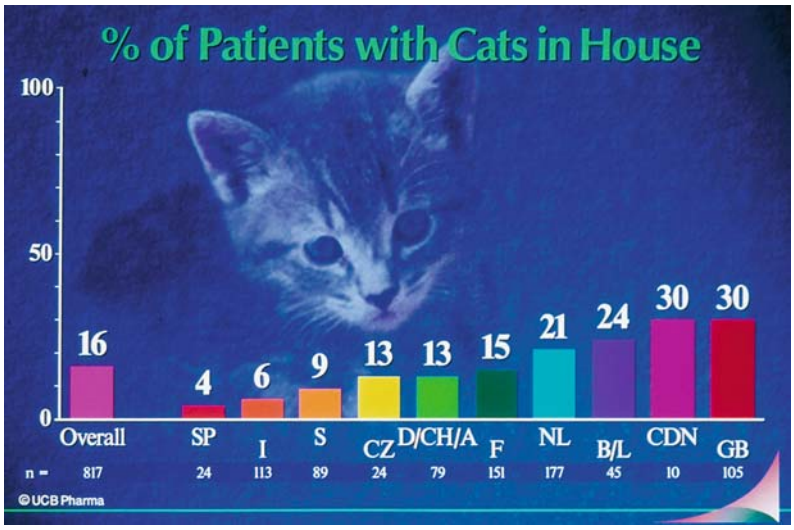

Fig. 4.17. ETAC Study: on average 16% of children have a cat in the home, the highest frequency is in Great Britain and in Canada (30%), the lowest in Spain (4%)

was more significant at the age of 2–7 years ($p<0.007$) [472]. After 1 year, more BPTs are positive in children housing a dog since birth than in children not housing a dog [456]. In atopic children with or without Der p 1 allergy, the direct exposure to domestic pets for at least 1 month in the first few years of life was statistically significant compared to controls, and high in children <2 years [482]. With continuing cat exposure in the adolescent period, clinical symptoms worsen and positive SPT prevalence increases compared to controls [421]. Such differences might go back to the different intimacy with which both animals are handled because most families of atopic children keep cats compared to controls [471], many dogs are often kept outside or at least out of bedrooms, and the proposed thresholds of exposure that have been associated in children <18 years with an increased risk of sensitization are >2 µg/g for Can f 1 and >1 µg/g for Fel d 1, and with asthma symptoms in allergic children are >10 µg/g for Can f 1 and >8 µg/g for Fel d 1 [10]. Houses completely without cats or dogs have consistent Fel d 1 or Can f 1 levels [10, 472], equal to >8 µg/g of Fel d [337], or <16 pg/m^2 or <0.02 pg inhaled/min by a child, and in homes with these animals, levels are 1–100 ng/m^2 [376], the sensitizing dose for an infant being of 3–300 ng/daily or 1–100 µg/year [110]. Importantly, for the Can f 1 and for the Fel d 1 house index, 55.7% (SE=2.2) and 66.0% (SE=1.6) of US homes, respectively, exceeded the sensitization threshold and 34.9% (SE=2.1) and 34.7% (SE=1.5) of US homes, respectively, exceeded the asthma symptom threshold [10]. Domestic pet sensitization is active even if the exposure started and continued after babyhood [100], indicating a prolonged sensitization. Appropriate investigations are necessary to re-evaluate pet allergy compared to the Der p 1 lower prevalence [376].

Epidemiological studies show that Fel d 1 sensitization attains highly significant levels in children followed up to the age of 11 years [410]; SPT positivity to Fel d 1 and/or Der p 1 increases 5-fold the risk of developing asthma, even 10-fold if associated with grass sensitivity. Over 50% of children with SPTs of >8 mm in diameter were asthmatic at age 13 years [391]; 12 children of 12–45 months with severe asthma had a more frequently ongoing contact with domestic pets than the 12 non-allergic children [382]. Contact was daily in 61% of 276 asthmatic infants [146]. In 174 neonates aged 3–5 days the median levels of exposure were 484 ng/g dust for cat, and 270 ng/g dust for dog [401]. In young asthmatic children, early exposure to cat increased the risk of allergic sensitization and further development of more severe asthma later in childhood. Such children should not live in homes with pets during their first few years, above all if the pet is a cat and a parent a smoker [281]. For pet-allergic children allergen avoidance may not be possible: although a dog or cat had lived in only 49.1% of US homes in the previous 6 month, Can f 1 and Fel d 1 were detected in 100% and 99.9% of these homes, respectively [10].

A common characteristic of dogs, cats, horses, sheep, etc. is having interspecies cross-reacting epitopes (Table 1.73).

Birds and Feathers

Avian allergens are connected with allergic alveolitis (Chap. 11) and in forms possibly complicated by FA (Chap. 9). Aged feathers, including those contained in down pillows and comforters, often offer excellent refuge for dust mites (Chap. 6), thus fostering mite sensitization.

Horse

Horse allergy is in a phase of recrudescence: hair, dander, serum and urine are immunogen, the allergens are detected also in certain houses, there transported by

subjects in contact with horses (79% of cases) [339]. Riding a horse is widespread in Sweden (21.3% in cities and 31.4% in country) and in Poland [43].

Rabbits and Rodents

Ory c 1 is a saliva constituent contaminating rabbit fur and is recovered in the environmental dust due to progressive dehydration. In guinea pigs two allergens have been found, mainly in urine, less in saliva, but also in the hair and pelt, and is diffused in elevated concentrations as recovered in the environmental dust. Mice have two major allergens found on hair follicles: Mus m 1 resides, also found in urine, Mus m II, also on the skin surface. Rat allergens are cross-reacting; Rat n 2 is mostly a male-derived allergen.

Cockroaches

In house dust, cockroaches (*Blatta orientalis*, *Periplaneta americana*, *Blattella germanica*) are a fundamental source of indoor allergens, especially in multi-unit housing and in urban areas [51] and are key allergens for infants and children [99, 133]. Bla g 1 and 2 levels in kitchens and bathrooms are 50-fold higher than those detected in other household places, unlike Der p/f 1 and Fel d 1 levels [339]. Cr-PI, perhaps a major allergen, is so hidden but so potent as to induce lymphocyte proliferation with production of IL_4 [190]. sIgE are in 69% of children positive to Der p 1 and/or to cockroach and/or to cat, vs 27% of control children [339]; 60% (21/35) of children have a double sensitization vs 13.6% [3/22] of control children [51]. Children with allergies had positive SPTs to cockroach in 12.7% of cases, including those with AD, asthma, rhinitis and urticaria [329]. Atopic adolescents sensitized to Bla g have a more severe asthma than the general population, with sIgE more elevated and high steroid-dependence [174]. Cockroaches are found in places and houses with prevalent humidity and poor air exchanges, but rarely in cool places with dry winters [292]. In 20% of homes with no visual evidence of cockroach infestation, significant levels of Bla g 2 were found in at least one dust sample, but uniformly distributed in all the rooms, unlike Der p 1 and Fel d 1 [339]. Bla g 2 levels >2 U/g have been detected in 65% of schools and in the air of other rooms [88]. The association of sensitization and exposure to cockroach allergens (Table 5.20) is a major risk for children with asthma exposed to high levels of cockroach allergen in their bedrooms, resulting in more clinical symptoms and a greater degree of morbidity [367]. Studies have raised the possibility that tropomyosin may be the basis for cross-reactivity among cockroaches, mites, shrimp, and squid (Table 1.73) , thus again linking respiratory allergy to FA. Asthmatic children may have sIgE to not yet classified insects, which are also detected in apparently free houses [242].

Insects

Several genera of insects, including butterflies, moths, midges, and mosquitoes, contain species that may induce inhalant allergy, as cockroaches do in asthmatic children [367], thus causing worldwide allergy. The sensitization may be more frequent because of widespread home aquariums [7, 492]. Pollen-sensitive patients are more exposed. Allergic symptoms are provoked by fish fodder containing Hb of *Chironomus thummi thummi* larva (13 allergens), or of *Tubifex*, muddy red worms. Relevant information comes from a report on 2,119 Chi t 1-sensitized patients with 33% of children: in patients polysensitized with asthma, conjunctivitis and urticaria, an expression inducible after HLA-DR antigen-specific stimulation on $CD3^+$, CD23 and CD25 was shown, while that on TcR α/β was decreased [241]. Association of HLA class II sequences encoding *DR1* and *DQ5* specificities with hypersensitivity to *Chironomus* allergen Chi t I has been reported [435].

Cage Baits

Allergy to baits of larvae and worms, with SPT and sIgE positivity of subjects with asthma and rhinoconjunctivitis is similar [492].

Environmental Pollutants

The worldwide rising public concern in the last years concerns the noxious effects of air pollution on children's respiratory health: children the world over are the greatest victims of environmental degradation, despite the great strides made over the past 10 years in improving both children's well-being and the environment (Tables 4.13, 4.14) [21, 481]. The pervasive problem of automotive emissions is the chief source. Experimental data suggest a correlation between the modifications of the surrounding ecosystem and the increased risk factors for the respiratory system, which is naturally exposed to

Table 4.13. Main outdoor air pollutants

Gaseous agents	CO NO/NO_2 O_3 SO_2
VOC	Benzene Methylene chloride Toluene Xylene
Particulates	PM_{10} TSP
Metals	As, Cd, Cr, Cu, Pb, Hg, Ni

Data from [21, 481].

Table 4.14. Main indoor pollutants

Type and source	Concentration	Indoor/outdoor ratio
Outdoor pollutants		
O_3	0–10 ppb	<<1
SO_2	0–15 µg/m^3	<1
Outdoor/indoor pollutants		
CO	5–50 ppm	>1
CO_2	2,000–3,000 ppm	>1
NO	10–700 µg/m^3	>1
TSP	10–1,000 µg/m^3	1
Indoor pollutants		
Formaldehyde	0.01–0.5 ppm	>1
Radon	0.01–4 pCi/l	>1
Synthetic fibers	0–1 fiber/ml	1
Aerosols		>1
Allergens		>1
Hg		>1
Microorganisms		>1
Organic substances		>1
Polycyclic hydrocarbons		>1

Modified from [21, 481].
PM_{10} particulate material <10 µm, *TSP* total suspended matter, *VOC* volatile organic compounds, *ppb* parts per billion, *ppm* parts per million, *pCi* picoCurie.

the consequences of air pollution owing to its particular structure and direct contacts with the outside; several air-polluting agents facilitate both sensitization and IgE production by enhancing or aggravating asthma in subjects with BHR [19, 20, 23, 121, 205, 216, 217, 471, 488]: cigarette smoke, gas exhaust (GE) particles, atmospheric dust, smog (smoke + fog), combustion products, building materials, volatile substances with a strong and pungent smell, including perfumes, toilet products, deodorant and insecticide sprays, cooking smells, damp, thermal stimuli, chemical agents derived from combustion of industrial residues such as CO, CO_2, SO_2 (sulfur dioxide), or due to an ultraviolet (UV) light-induced photochemical change on GE such as NO (nitric oxide), NO_2 (nitric dioxide), O_3 (ozone), formaldehyde, detergents, solvents, paints, etc, and in children with AD the T variations. Among unforeseeable events, we include the asthma outbreaks that occurred in Barcelona and Naples caused by the unloading of soybeans from ships in the harbor: installing filters on silos in Barcelona prompted a significant decrease in asthmatic symptoms [9]. Air pollutants bound to pollen may enhance allergic sensitization [20].

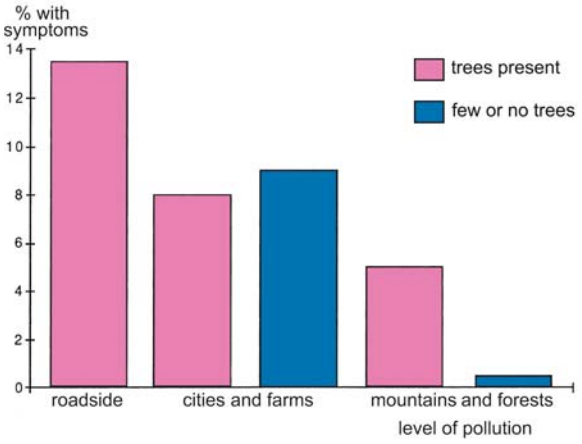

Fig. 4.18. Prevalence of rhinitis due to cedar pollen. *Pink bars* indicate trees present, *blue bars* indicate few or no trees. (Data from [185])

A Japanese study in mice immunized with cedar pollen-bound diesel exhaust particles (DEPs) (20- to 100-fold more numerous than in gasoline motors) showed that DEPs have a more adjuvant effect on IgE immune responses in mice than in control animals, thus facilitating the onset of atopic disease. As shown in Fig. 4.18 [185], sensitization to red cedar pollen is related to air pollution from cars, as it was found that the closer the people lived to a motorway, the more they suffered from AR [185]. In the least or most polluted quarters of Tokyo, the AR prevalence has attained rates of 1%–2% in children aged 0–14 years [285]. Additional evidence comes from DEPs, 0.01–10 µm in width, with <10 µm mean diameter (particulate matters <10 µm, PM_{10}), made of quartz, $CaSO_4$ and rock salt, which can bind to pollen grains, thus increasing their allergenicity [135]. Animal experiments have shown that mice were sensitized only when they inhaled pollen allergen mixed with DEPs, and that sIgE concentrations were correlated with the amount of inhaled DEPs [285]. Further, DEPs exert a direct effect on in vivo B-cell IgE production by acting on the ε germline [427]. In Australia and New Zealand there was a 6.0% increase in asthma admissions in 5–14 year-olds in relation to a 5.1-ppb (parts per billion) increase in 24-h NO_2 [19] and in Brazil there was a 31.4% increase in asthma or bronchiolitis admissions also due to NO_2 [121]. In Oslo, Norway, the asthma admission rate over 10 years has more than doubled; however, the higher admission rates were located along the main motor roads through Oslo [60]. In industrialized areas, the increased pollinosis prevalence is correlated to the duration of exposure [476]. There is also evidence of the different NO_2 and SO_2 diffusion in polluted townships and in the rural environment in two countries: NO_2 was equally prevalent in both Polish and Swedish cities and reduced by half in a rural zone; SO_2 was 7.5-fold more frequent in Polish than in Swedish

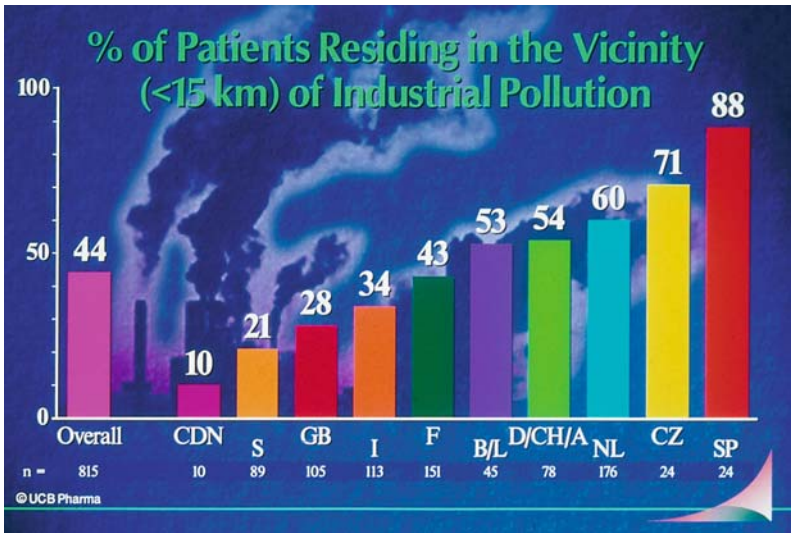

Fig. 4.19. ETAC Study: on average 44% of children reside in the vicinity (<15 km) of industrial pollution, the highest frequency is in Spain (88%), the lowest in Canada (10%)

localities [43]. In two industrial towns located 30 km apart (Upper Silesia, Poland), the children exposed to pollution (PM_{10}, NO_2, SO_2) suffered from wheezing and asthma, although with differences between the two towns [514].

The data above confirm that allergic sensitization is more probable in an urban environment, in parallel with industrialized areas, compared to areas with less traffic and other country areas [40]. Thereby, the atmospheric pollution increase is an emerging environmental plague related to the interaction between chemical substances foreign to the body (xenobiotics) and the immune system [22], which may lead to increased medical examinations and hospital admissions for asthmatic crises in younger children [361] and additional respiratory manifestations [106]. Significant is [304] the clear coincidence between a >100% increase in allergy prevalence that occurred 25 years apart and the increased fuel consumption, thus of traffic fumes that have replaced coal fire smoke as the major atmospheric pollutant. The greater prevalence of respiratory allergy in children living in Munich is probably related to the NO_2 [464] and GE [487] predominance. A significant correlation between GE and PEF (peak expiratory flow) and of MEF_{50-75} (maximal expiratory flow at 25–75% VC) reduction, in addition to recurrent dyspnea, correlated with the volume of car traffic in children aged 9–11 years [487]. Residing on a road with frequent motor traffic is a risk factor for AD [384], asthma and rhinitis [290], and if in one lives on a road open to heavy traffic, the risk of bronchiolitis in the first 2 years of life increases up to 41% [68]. The ETAC study children with AD in 44% of cases live <15 km away from sources of industrial pollution (Fig. 4.19). An unusual prevalence of BHR in schoolchildren living in the vicinity of power stations [161] was congruent with increased IgE levels significantly correlated to the degree of air pollution [23]. Atopic stigmata (OR 3.7) and AD (OR 1.4) rose depending on the lesser or greater pollution load [21]. Consequently, hospital admissions for asthma in an area with particulate air pollution mainly affect children aged 1–4 years [438] or <3 years, increasing 2.5-fold in 10 years [60]. Up to 35% of children residing in areas with high pollution reacted to BPT vs only one of the control children [469].

Another area of increasing interest is that the effects on humans depend on age, airway size, pre-existing illness, and pollutant characteristics: hydrosoluble gases such as ammonia, chlorine, formaldehyde, H fluoride, H_2SO_4 (sulfuric acid) and SO_2 dissolve in the upper airways, and can reach the lower airways only when their concentration is high. However, phosgene, NO_2, O_3 are less hydrosoluble and dissolve slowly, thus reaching the lower airways where they increase airway inflammation [60]. NO_2 and O_3 exert their noxious effects upon the airways, as a source of free radicals (FR) of O_2 mediators of inflammation [60]. The total impact of pollution on the asthmatic manifestations in children may be of 10%; no correlations with asthma have been subsequently ascertained [63]. The study in children living in three German cities has shown that the concentrations of SO_2 in ambient air were mostly due to emissions from power plants, heavy industry, and private heating, which were increased by a higher sulfur content of the coal burned in winter. Moreover, annual mean NO_2 concentrations certainly grew due to increased automobile traffic. In conclusion, the children had current asthma in 5.8%–10.3%, wheeze in 6.9%–9%, waking with cough in 16.4%–20.4%, morning cough in 10%–12.4%, runny nose with itchy eyes in 4.4%–15%, and AD in 13.5%–17.5% of cases [467]. Both figures of alert and warning states are in Appendix 4.1 and the equivalents of the parts per million (ppm) of atmospheric pollutants:

- *O_3* is very polluting from a clinical point of view (>180–>361 µg/m^3, mean per hour), a reactive allotrope of O_2 originating in the stratosphere by photochemical processes and UV rays, which convert diatomic molecules into triatomic molecules [189]. The wide damag-

ing effects of these rays, the UV-B rays, are only partly absorbed by the O_3 layer [245]. Ambient UV-B rays have the potential to decrease host resistance and immunity to some infectious diseases, thus leading to their increased incidence, severity, and duration [189]. The thick O_3 layer in the lower stratosphere between 15 and 25 km in altitude absorbs UV rays, thus forming a protective shield (ozonosphere) [245]. Yet the primary consequence of O_3 loss observed over the past 2 decades (ozone depletion or ozone hole) generated by human activity, particularly NO_2 release and photodegradation products of anthropogenic CFCs (chlorofluorocarbons) [245], is a global warming of the atmosphere, which advances pollination and promotes pollinosis. Its extension is equal to threefold the US surface. The parallel result is the thermal expansion of the oceans and the melting of the Svalbard Islands and the Greenland ice complex over the last century equivalent to –0.6 mm/year of sea-level rise [284], and the palm trees possibly growing on the Alps (Reuters). CO_2 is increasing in the atmosphere: its concentration, estimated at 280 ppm before the industrial revolution, has risen from 317 ppm in 1960 to 368 ppm in 1999. O_3 levels tend to be highest in warm and sunny weather and peak in the mid-afternoon hours, when children playing outside are more at risk [350]. The primary effects of O_3 and acidic sulfate exposure are exacerbations of asthmatic symptoms requiring hospital admissions and reducing pulmonary function tests (PFT) [63]. Studies indicate that symptoms are always more severe in the asthmatic group, with a significant correlation between admissions and increased O_3 levels [327].

The results are not less toxic when O_3 is formed by sun ray action on SO_2 and reactive hydrocarbons, both released by motor vehicles and industrial wastes. The ensuing chemical reactions usually lead to mixtures of pollutant gases in the lower layers, processes by which oxidative pollution is integrated and strengthened by photochemical pollution [245]. The ambient air standard for O_3 is ≤0.12 ppm over 1 h of the year [327] (1 ppm=2,000 µg/m^2). At a concentration of 0.08 ppm of O_3, this agent provokes epithelial damage, increased centroacinar inflammation in the airways, and structural alterations of the lower airways, as indicated by leakage of lactate dehydrogenase, albumin and total protein, and an increase in neutrophils, eosinophils, mononucleated cells, IL_6, IL_8, GM-CSF and PGE_2 is observed in BALF [101]; at 0.15 ppm, there is cilia shortening and necrosis in monkeys [350]. In vitro exposure of human epithelial cells to 400–800 ppb O_3 induced significant release of GM-CSF, TNF-α and CD54, which was blocked by cell treatment with 10^{-5} M nedocromil sodium [101]. UV-B exposure alters the patterns of IL secretion from keratinocytes in culture, and alters the morphology, decreases the number, and modifies the activity of Langerhans' cells [189]. Asthmatic adults exposed to 0.12 ppm O_3 at rest for 1 h and subsequent allergen challenge experienced a bronchial response and nonrespiratory manifestations, including nausea, malaise, headache and decreased ability to carry out sustained exercise; the patients required significantly less inhaled allergen to induce a 15% decrease in FEV_1 after an O_3 exposure than after a clean air exposure [289], hence O_3 interacting with common allergens could potentiate the host capacity of producing bronchoconstriction. In children exposed to 0.02 ppm O_3, a consistent drop of PEF values persistent up to 1 week has been found, suggesting damage in the respiratory tract such as a reduced ciliary clearance and an increased epithelial permeability; repeated exposures may result in persistent BHR [518]. Prolonged exposure is irritant and toxic for pulmonary cells and magnifies the bronchospastic response to allergens, thus impairing exercise performance and PFT in elite cyclists [210]. The PFT changes suggest that the effect of O_3 exposure on allergen-induced bronchoconstriction may be more significant than the effect of O_3 alone in asthma [327]. In children exposed to O_3, a deficit of CD4 and an increase in CD8 T cells with their ratio inversion have been observed compared to control children [518]. In another pediatric cohort [130], increases in environmental O_3 exposure were related to an increased nasal inflammatory response, similarly to Der p-sensitive asthmatics exposed to 0.4 ppm O_3 for 2 h, thus concluding that eosinophil inflammation is also transmitted to the airways [327]. Pollution catalyzes O_3 depletion, thus more UV-B rays, no longer absorbed by O_3 layer, reach the surface, potentially decreasing host resistance and immunity to infections [189], especially in atopic children [60]. The results relating both allergic sensitization to pollens and growing AR prevalence to the increased traffic rates and the coincident interaction with photo-oxidant agents are by no means insignificant [135]. Possibly, the long CFC atmospheric lifetime (up to two centuries) foreshadows that human beings will still have to deal with the repercussions of O_3 depletion for several generations, until the Cl level approaches pre-1970s levels [245]. The O_3 hole continues to widen, despite CFC decreases; possibly CO_2 increases CFC levels.

- *CO_2* is a polluting and toxic compound, with concentrations increased from 250 ppm at the beginning of the industrial era to 350–400 ppm currently (+66.6%), as noted previously, but in 2020, 92% of its emissions will be due to the car gases [481]. Consequently, the CO_2 ability to absorb the heat of the earth's surface has proportionally increased, and is a third cause of O_3 depletion. Another reason is Amazonian deforestation, which reduces CO_2 absorption by trees (–60%). The Rio Summit in 1992 has not stopped this devastation: today an area as wide as a soccer field disappears every 12 s (Johannesburg Summit 2002).
- *SO_2* emitted from diesel engines, refineries, coal-fired power plants, etc. is a source of important problems [381] (<125–>250 µg/m^3, mean per day): at 8–12 ppm, throat soreness appears, at 20 ppm eye irritation and immediate cough, corresponding to the highest tolerated dose in prolonged exposures; 50–100 ppm SO_2

(1 ppm=2,860 μg/m^2) are tolerated even for 30 min, but after a short exposure to 400–500 ppm, PFTs may deteriorate, and at 1,000 ppm lung resistance may increase >100% [101]. SO_2 may act as an aspecific bronchoconstrictor agent, similar to inhaled histamine or methacholine: the rapid onset of bronchoconstriction after exposure suggests a neural mechanism of action for SO_2 in asthma [327], but airway inflammation may result from short-term SO_2 exposure [381]. SO_2 enhances acute bronchoconstriction and lowers the threshold of aspecific BHR [334], because it causes airway inflammation of remarkable severity [381], effects that are exaggerated by cold air [334]. SO_2 decreases mucociliary clearance in both normal and atopic subjects at 0.02 ppm (urban level often common over long periods of time), especially with exercise-induced bronchospasm and/or mouth breathing, and BHR at 0.5 ppm [437]. In the BALF of healthy nonsmoking adults 4 h after exposure to 8 ppm SO_2, a significant increase was found in the number of lymphocytes, macrophages and mast cells, their number increased to peak values 24 h after exposure, and after 72 h returned to normal [381]. The SO_2 effect has been demonstrated in an epidemiological survey by a significant increase in BHR prevalence in children living near a paper pulp plant discharging 0.5 tons of SO_2 and 0.08 tons of hydrogen sulfide (H_2S)/day, as compared to children living in a less heavily polluted area [7]. Children living in a community where a steel mill was located also had increased respiratory symptoms [340]. Drawing conclusions from correlations between two pollutants, namely SO_2 and NO_2, may be justified since the association significantly emphasizes the bronchoconstrictor response to sensitizing allergens [440]. However, the hazard of similar associations is illustrated by an inverse correlation between SO_2 levels and *hospitalizations for severe asthma in 0–14-year-old children* [437]. Moreover, in children aged 6–15 years, asthma prevalence was highest and most significant in children living in the zone with the highest O_3 levels compared to those living in the vicinity of a highway with high SO_2 and NO_2 levels or in a zone with intermediate characteristics [385].

- *NO_2* (1 ppm=1,880 μg/m^2) is a precursor to photochemical smog derived from reactive hydrocarbons and is generated by the burning of fossil fuels in outdoor air in urban and industrial regions [90] and the use of unvented gas cooking stoves [210]. Outdoors, the most significant NO_2 source is GE fumes, although power plants and other industrial sources that burn fossil fuels also release NO_2 into the environment [90, 381]. The increased indoor exposure to combustion products of gas cooking stoves, fireplaces and space heaters, such as NO_2, acrolein, particulate material, formaldehyde and hydrocarbons, is significantly correlated with asthma exacerbations [315] and BHR worsening, also in nonasthmatic subjects [379]. The previous results have not been confirmed in a cohort of 1,205 healthy infants during the first 18 months of life [379], but such pollution may increase IgE levels in 6–12-year-old children [216, 488]. NO_2 reduces the efficacy of lung defense mechanisms, and exposure to 400–800 ppb caused *dysfunction of ciliary beat frequency of bronchial epithelial cells*. This may reduce either the threshold for allergen exposure [101] or the permeability of bronchial mucosa to allergens, but allergens remaining longer on the cells increase the chances of contact with immunoreactive cells, thus producing sensitization and increasing asthma prevalence [101]. Prolonged exposure induces the generation of mediators such as LTC_4 and influx of proinflammatory ILs (GM-CSF, TNF-α and IL_8) in the respiratory tract, with significant differences between cells produced by atopic and nonatopic subjects [101]. In vivo exposure to 0.01–0.06 ppm of NO_2 (>200–>400 μg/m^3, mean per hour) provokes BHR in exposed subjects, notably the asthmatic, with differences between exposure and symptoms of 24 h for NO and of 7 days for NO_2 and increase in lymphocytes, macrophages and mast cells of BALF [101]. The reduced mucociliary clearance and the increased production of inflammatory ILs by epithelial cells exposed to NO_2 in vitro [334] uncover the NO_2 capability to alter airway function, to the point of playing a significant role in wheezing development in children, and particularly in girls [371].
- *NO* (>15–>30 mg/m^3, mean per hour) is a reactive gas found in nerve, endothelial, epithelial and vascular smooth cells, macrophages, neutrophils, mast cells and lymphocytes, induced by $IL_1\beta$, IFN-γ and TNF-α. NO has a short half-life (<5 s) and may act as an intracellular messenger or a paracrine substance; leading to vasodilation and bronchodilation, NO production contributes to self-defense but is a *double-edged sword* in that it induces inflammatory and auto-oxidative damage [517]. NOS, a class of enzymes collectively known by this name, appears to fulfill a powerful homeostatic regulation of microcirculation permeability, to modulate the local perfusion and host defense mechanisms. NOS types are the enzyme catalyzing NO formation (endothelial, neuronal) known as cNOS (constitutive) and iNOS (inducible) formed in activated immune cells and vascular smooth cells with a Ca^{++} independent activity [302]. However, the NO hyperproduction up-regulated by iNOS expression, initiated by ILs and/or endotoxin, may suppress the enzyme activity with a decrease in cGMP and an increase in Ca^{++}, thus leading to bronchoconstriction [302]. Both IL_4 and IL_{10} inhibit NO production by macrophages, but not the production by endothelial cells, thereby expanding Th2 activation [219]. Inhaled CSs suppress iNOS [302]. Interestingly, NO has been identified as the main neurotransmitter of the nonadrenergic noncholinergic (NANC) pathway [208] and as such may contribute to a constant vasodilator tone [394], but it may play a role in the *early onset of asthma in children* [517].
- *PM_{10}* daily levels of the standard limits (Appendix 4.1) are correlated with a 3%–6% PEF decrease, increased reports of respiratory symptoms, and increased use of

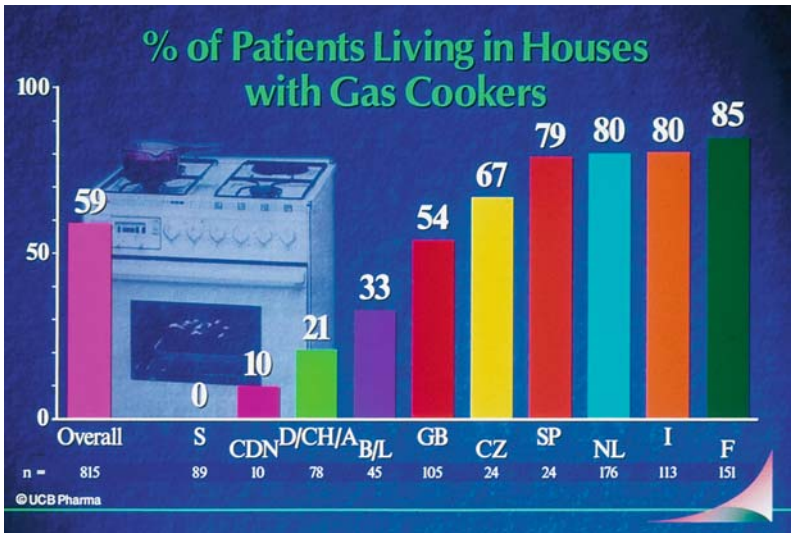

Fig. 4.20. ETAC Study: on average 59% of children live in houses with gas cookers, the highest frequency is in France (85%), the lowest (0%) in Sweden

medications in American asthmatic children [340]. Significantly increased were hospital admissions in asthmatic children associated with PM_{10} [19]. The Six Cities study found that chronic cough and chest illness in children were especially associated with total suspended particles (TSP) <15 µm in diameter, but not with flow measurements (FVC, FEV_1, MMEF) [106]. In Italy air pollutants appear to aggravate symptoms in already sensitized subjects [84]. TSP of 0.02–10 µm (>90–>180 µg/m^3, mean per day) have been correlated in children and adolescents with PFT decreases and symptom exacerbations, since these particles have been shown to be readily inhaled and deposited into the lungs where some remain indefinitely [210]. In a cohort of 4,400 children aged 1–5 who were surveyed in 1998 and in 2001, exposure to primary PM_{10} from fossil-fuel combustion as it is found in most cities was associated with an increased prevalence of cough with ORs of 1.21 and 1.56, respectively, and of current wheeze with ORs of 0.99 and 1.28 respectively. The ORs for incident symptoms were 1.62 for cough, and 1.42 for wheeze. These data confirm the association between locally generated primary PM_{10} and new-onset symtpoms with a consistent association with primary PM_{10} and incidence of wheeze [334].

- *VOCs* such as ammonia derivatives (NH_4), also found in household products such as paints and hair sprays, are of concern to public health because of their diameter, between 0.1 and 1.0 µm. They are readily inhaled and deposited into the alveoli, with a latency time of 5 days. Signs and symptoms may include eye and upper respiratory irritation, dyspnea, rash, itching, headache, nausea, and vomiting [40].
- *Indoor pollutants* (Tables 4.13, 4.14) include kerosene, gas, coal, wood cookers or stoves and fireplaces, which emit NO_2, CO, formaldehyde, aromatic polycyclic hydrocarbons and particulate substances <2.5 mm in diameter: unvented gas cooking stoves add 25 ppb NO_2 to the background level in a home, which increases NO_2 to 200–400 ppb during cooking, a major cause of unsuspected pollution [210]. A meta-analysis of eight studies investigating 40,269 children aged 0–15 years found a significant FVC and FEV_1 reduction in three studies involving a total of 85.7% of these children [378]: this is a significant risk factor for children with AD if the devices are unvented [384]. In Polish homes, gas cooking stoves and heating are very widespread, with a resulting production of smoke up to levels of 32.3 µg/m^3 (mean), with a threefold increase in respiratory symptoms [43]. In the ETAC study (Fig. 4.20), 59% of homes have gas cooking stoves.
- At least 2.7 kg of *formaldehyde* is produced during the combustion of 450 kg of gasoline: US car and airplane discharge is >300×10^6 kg/year of this relevant environmental pollutant found in foam insulation, chipboard, and carbonless copy paper. The minimal dose needed to provoke a reaction is 0.3 ppm. However, no effects are reported in 50%–70% of cases at an exposure of 0.05–1.5 ppm; 0.05–1 ppm is the odor threshold, 0.01–2 ppm provokes eye irritation, 0.1–25 ppm upper airway irritation, 5–30 ppm lower airway and pulmonary effects, 50–100 ppm pulmonary edema, inflammation, pneumonia, and >100 ppm leads to death [412]. Formaldehyde volatilizing from particle board and pressed wood products commonly used in the construction of new homes may also be found in carpet adhesives [412]. Its high content in the chipboard used in children's rooms may be responsible for IgE development [488]. In a particle board-paneled school, >0.075 ppm have been detected, able to cause *IgE-mediated sensitization* in 40% of schoolchildren [471], that is, the upper limits of values considered as inoffensive by WHO (0.050–0.080 ppm) [481]. Children are more susceptible than adults to toxic substances leading to IgE-mediated sensitizations [471], besides *hidden sources* such as cosmetics and cigarettes: a smoker of five cigarettes in a poorly ventilated room is exposed to concentrations three times the upper WHO limit of 1 Bq.

Table 4.15. Effects of gas present in traces in the atmosphere

	Asthmogenic effects	Smog	Acid deposition	Stratospheric O_3 depletion	Ozone depletion
CO_2				±	+
NO, NO_2	+	+	+	±	
N_2O				±	+
SO_2	+		+		–
O_3	+	+			
CFC				±	+

CFC chlorofluorocarbons.
Internet data.

- The use of *asbestos* has recently been drastically curtailed: when buildings become cracked and broken, they may liberate microscopic fibers that are pathogenetic when inhaled, such as fiberglass, used for acoustical and thermal insulation [229].
- *Radon* is a gas coming from radioactive decay products of ubiquitous uranium deposits in the soil. It enters houses through cracks in the foundations, porous cinder blocks, and granite walls, but easily volatilizes when water is in contact with air, at concentrations of 10×10^6 Bq/m^3 [229].

Table 4.15 summarizes the effects of atmospheric trace gases. Great attention has focused not only on the increase in CFCs chiefly used as propellants in pressurized metered-dose inhalers (pMDI), refrigerators, air conditioners, lighters, and Styrofoam insulation [245], but also on CO_2 and N_2O. The transition from CFC to non-CFC pMDIs has taken much longer than expected and has been far more costly than anticipated. However, alternatives based on hydrofluoroalkane (HFA) are currently being studied, also taking into account patient compliance [112].

Consequently, the convincing evidence that pollution increases atopy prevalence is clearer and clearer, as verified in Chap. 5. The results may be controversial: the children of highly polluted Leipzig and those of much less polluted Munich suffered from asthma and BHR in an insignificantly different way [464]. The Six Cities study in the US has found no connection between asthma, SO_2 and NO_2 [106].

In a positive light, there are the sources of renewable energy, including wind, sun rays, geothermal energy, hydroelectric energy, wave-motion, and tides.

Immunotoxicology

Immunotoxicology is the discipline concerned with the study of the immunotoxic potential of environmental xenobiotics on the human immune system components [22]. *Xenobiotics* (from Greek "ξενος," foreign) may be defined as the grouping of harmful components of the external environment, from the simplest chemical compound to the most sophisticated microbial agent. Most external agents confronting and eventually activating the host's immune system may evoke, in a variable way depending on the nature of the agent and the host's genetic disposition, antigen elimination or persistence with consequent perturbation of immune homeostasis or immunomodulating effects: both Fig. 4.21 and Table 4.16 summarize some of these effects [22]. Although immunotoxicology has become a prominent and respected discipline with global recognition since its inception nearly 30 years ago and has significantly contributed to the advancement of biomedical sciences, only relatively recently has the participation of investigators, clinicians, immunologists and allergists (but only occasionally and partly that of law-makers) been emphasized. Several of these approaches have been borrowed and utilized by clinical ecology, but they can have serious effects on the immune system of children, thus rendering them susceptible to infections or other disease conditions.

The immunotoxicity effects at the immune level stem from immune modulation and may be depicted as follows:

- Immune potentiating, which corresponds to an up-regulated or exaggerated immune response, to be expressed potentially in hypersensitivity and/or autoimmunity.
- Immunosuppression (or immune depression), underlying the generalized down-regulation of immune responses and may be materialized by an increased susceptibility to infections or sometimes irreversible forms of carcinogenicity [57].

Among the immunotoxic substances, some are ingested at low doses for a long time, such as those included in foods, either deliberately (additives), or inadvertently (contaminators), with potential risks for the immune system [57]. The salient characteristics of xenobiotics are otherwise unimaginable: immunotoxic substances exert their effects at remarkably lower doses than those normally expected. In particular, Ni and other metals may act as haptens, thus binding to host

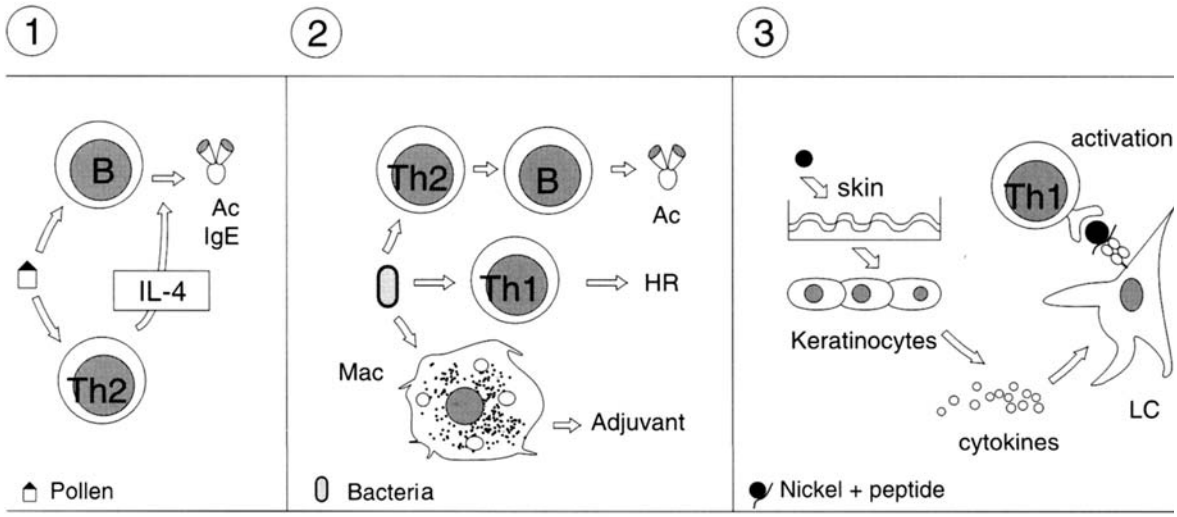

Fig. 4.21. Double function of xenobiotics: antigenic and immunomodulators. *Ac* antibody, *LC* Langerhans' cell, *Mac* macrophage

Table 4.16. Adverse effects of environmental agents on host target cells

Host target cells	Example of effects	Results
Circulatory system		
Endothelial cells	Increased intracellular pore size	Edema
Gastrointestinal tract		
Mucosal cells	Destruction	Hemorrhage
Smooth muscles	Increased contractility	Diarrhea, vomiting
Glandular cells	Increased secretion	Increased mucus production
Respiratory tract		
Smooth muscles	Increased contraction	Bronchospasm
Glandular cells	Increased secretion	Increased mucus production
Formed elements	Red cell destruction	Anemia
Skin	Disruption of epidermal cells	Dermatitis

Data from [22].

proteins, inducing symptoms and signs suggestive of either immediate or delayed immunoreactivity. Metabisulfites excite *an irritant action on the bronchial epithelium,* thus activating a vagal reflex. Na nitrite inhibits monoamine oxidase, *eliciting cellular anoxia,* and erythrosine by *altering the neuron membrane permeability* could provide support for behavioral modifications. Also in this context, there is a *toxic action at the epithelial level* of substance P (SP) and the risk of neuropeptides, but the involved mechanisms are poorly understood, in addition to certain mycotoxins, which putatively suppress both humoral and cell-mediated immune (CMI) responses [250]. It was not a surprise to find studies that have reiterated the old adagio *post hoc ergo propter hoc* (after the fact, therefore a corollary). There is the case of children with recurrent respiratory infections (RRI) ascribed to drinking water contaminated by industrial solvents, accompanied by numerous immune alterations also in their relatives: the factors should be understandably reversed to make the diagnosis [377]. According to Bellanti, who proposed a stimulating working hypothesis, the study of the immune modulation induced by pollutant chemical compounds allows a proper assessment of immune system alterations clinically induced by those agents, thus providing sound knowledge of the mechanisms altering the immune surveillance [22].

The following list delineates a few relationships between immunotoxicology and epidemiology of atopic disease:

- The *aerodispersed pollutants* of chemical origin may either directly sensitize the organism by targeting

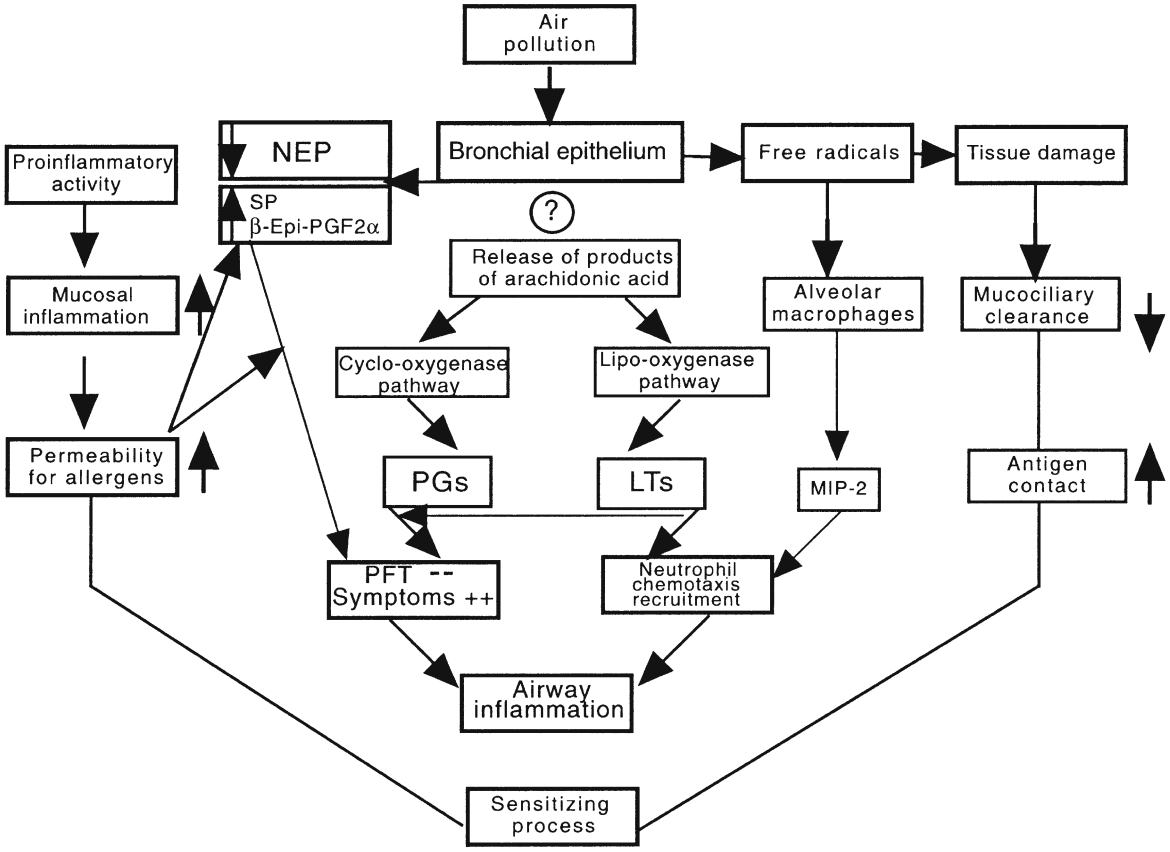

Fig. 4.22. Pathogenic hypothesis of sensitization enhancement by environmental pollution. The exposure to air pollutants can influence the development of the atopic march and clinical manifestations. Pollutants, in particular ozone, cause epithelial injury and diminish NEP activity, with a resultant increase in the neuropeptide concentration, such as substance P (SP). SP induces bronchoconstriction, increases airway permeability and activates neutrophils, while β-epi-PGF2α, marker of oxidative stress, induces pulmonary vasoconstriction. Alveolar macrophages through MIP-2 contribute to further stimulate neutrophils. *MIP* macrophage inflammatory protein, *NEP* neutral endopeptidase, *PFT* pulmonary function tests. (Modified from [219, 356])

immune reactions, or indirectly influence the host immune responses (by a potentiating or suppressing effect).

- Some *particulate pollutants* may act as adjuvants by potentiating IgE response to common allergens [22].
- *Atmospheric pollution may act as a precipitant factor of clinical manifestations* in subjects sensitized though still asymptomatic, or facilitating sensitization in nonatopic subjects [219, 356]. Figure 4.22 [219, 356] synthesizes the effects of pollution, in particular those supported by NO and O_3.

A wealth of chemical agents may play a central role in causing *immunodepression* in humans, such as *DEP-emitted SO_2*, which provokes an increased frequency of acute bronchoconstriction in asthmatic subjects, in addition to lowering the aspecific bronchial reactivity threshold. C powder (emitted by diesel engines) in contact with SO_2 plays a crucial role in the production of activated O_2, which by combining with unsaturated fatty acids, sets off a series of reactions damaging the bronchial mucosa: neoformed lipid peroxides prejudice the vessels, decreasing their functionality [377]. It is to be hoped that this further health offense may help establish safety levels with biodiesel, a product of natural origin deriving from vegetable oils (of rape, soy and sunflower). Above all it is S-free and consequently does not occasion SO_2 formation. Tables 4.17 and 4.18 [250] list a number of xenobiotic immunotoxicants, including gases, vapors, and particulates, and the results of several studies exemplifying the complexity of this issue. Table 4.19 [57] shows the experimental effects exerted by toxins potentially present in foods such as the aflatoxins and Table 4.20 [377] the environmental agents that compromise the immune system in the animal model. Moreover, bacterial exotoxins may target immune organs such as GALT by an immunostimulant effect, with a virtual rise in FA sensitization [57]. The intestinal mucosa may also establish long-term contact with elevated concentrations of immunotoxins during absorptive processes: such substances are able to

Table 4.17. Classification of immunotoxic xenobiotics associated with immunological changes

Class	Examples
Abused drugs	Ethanol cannabinoids, cocaine, opioids
Aromatic amines	Acetyl aminofluorene, benzidine
Aromatic hydrocarbons (solvents)	Benzene, hexachlorobenzene, toluene, xylene
Drugs	Diphenylhydantoin, lithium
Metals	As, Cd, Cr, Hg, methylmercury, Ni, Pb
Natural products	Selected antibiotics, estrogenic and fungal products, plant alkaloids, Vinca alkaloids, vitamins
Organic tin	TBTO
Others	Butylated hydroxyanisole, nitrosamine
Oxidant gases	CO, NO/NO_2, O_3, SO_2
Particulates	Asbestos, beryllium, cadmium, coal dust, silica
Particulate substances	PM_{10}, PM_{25}, TSP
Pesticides	Carbofuran, chlordane, trimethylphosphorothioate
Polyhalogenated aromatic hydrocarbons	Polybromurate polyphenyls, polychlorinated diphenyl compounds (PCB), polychlorinated dibenzofurans, tetrachlorodibenzo-*p*-dioxin (TCDD)
Polycyclic aromatic hydrocarbons	Benzopyrene, dimethylbenzanthracene, methylcholanthrene
Vapors (in addition to solvents)	Carbon tetrachloride, styrene, sulfuric acid, trichloroethylene

Data from [250].
PM_{10}, *PM_{25}* particulate material <10/25 µm, *TBTO* bis(tri-*n*-butyltin)oxides, *TSP* total suspended particles.

Table 4.18. Potential immunological changes of immunotoxic xenobiotics

Xenobiotics	Potential effects
Halogenated aromatic hydrocarbons	Thymic epithelium B-cell differentiation T-cell regulation
Polycyclic aromatic hydrocarbons	
Benzopyrene	T helper cells
Dimethylbenzanthracene	Macrophage function and proliferation
Benzene	Microtubule assembly
Dimethylnitrosamine	B-cell function
Mycotoxin	Protein synthesis
Organic metals	Immunotoxicity, immunosuppression, cell depletion, inhibition of glucose metabolism
Trimethylphosphorothioate	Macrophage function

Data from [250].

alter the local immune response, thus promoting the appearance of allergic responses or a greater susceptibility to infections [57].

The effects provoked by *dioxin* (tetrachlorodibenzo-*p*-dioxin, TCDD) stem from studies on the animal model, which appears to be very sensitive, while statistical increases in IgG levels, absolute and percent number of CD8, $CD4^+CD45RO^+CD45RA^-$, have been demonstrated in volunteers [300] as well as $IL_1\beta$ expression in response to epidermal keratinocytes with a potential role in the modulation of immune and inflammatory responses [500]. The effect on ILs is caused by the TCDD properties of mimicking the ligand of the Ah (aromatic hydrocarbon) receptor, the transcription factor inducing a gene battery including the ILs [131]. The explosion of a plant that manufactured the herbicide trichloro-

Table 4.19. Experimental immunotoxic effects induced by toxins potentially present in foods

Aflatoxins	Decrease in antibody responses and lymphocyte proliferation, suppression of phagocytic function, increase in mortality from infections
Citrinin[a]	Activation of B-cell- and T-cell-dependent immune responses
Ochratoxin A	Suppression/decrease in phagocytic function
Rubratoxin B	Decrease in antibody proliferation
Staphylococcal enterotoxin[b]	Suppression in vitro of immune responses
T2 Toxin from *Fusarium*	Decrease in antibody responses and lymphocyte proliferation, depression of B-cell- and T-cell-dependent immune responses, increase in viral reactivation and susceptibility to infections

Modified from [57].
[a] Isolated from *Penicillium citrinum*; note the opposing effects of citrinin and T2 toxin.
[b] See Chap. 7.

Table 4.20. Environmental pollution (chemicals and related substances) reported to affect the immune system of experimental animals

Substance and source	Immune system alterations
Benzopyrene Incomplete combustion of coal and other fossil fuels, cigarette smoke	Decreased antibody production by pulmonary lymph node cells
Beryllium (salts) Alloy production, aerospace, electronic and nuclear industries	Delayed cell-mediated hypersensitivity, lymphocyte transformation and cytokine production
Cadmium Paint and battery manufacturing, electroplating, smelting	Altered T- and B-cell function, antinuclear antibody formation immunosuppression, antiglomerular basement membrane antibody, amyloidosis
Carbofuran, Dizainon Pesticides	Alteration of serum immunoglobulin levels
Chlordane Pesticides, insecticides	Depression of contact hypersensitivity
Cigarette smoke	Decreased mitogen response, increase in either serum IgE levels (see text) or IgE production, enhancement of bronchial hyperreactivity; anaphylaxis after albumin aerosol
Diesel exhausts	Alteration of alveolar macrophages and leukocytes, reduced IFN-γ production, increased serum IgE levels
Demethylbenzanthracene Incomplete combustion of fossil fuels	Decreased splenic antibody production, decreased resistance to bacterial infection and tumor challenge
Fly ash Coal combustion	Decreased splenic antibody production
Formaldehyde Auto exhaust, smog, incinerators, biomedical research, insulation, industrial processes, cigarette smoke	Enhanced resistance to bacterial challenge
Lead Paints, gasoline combustion, battery production, welding, smelting	Decreased serum immunoglobulin and complement levels, depressed salivary IgA levels and ADCC
Mercury Organic and inorganic products, such as natural and industrial contaminants, pesticides, fungicides, jewelry, paints, foods, drugs	Autoimmune disease, hypergammaglobulinemia, decreased neutrophil chemotaxis

Table 4.20. (Continued)

Substance and source	Immune system alterations
Nitrogen dioxide Oxidant air pollutant, cigarette smoke	Alteration of antibody production in lung-associated lymph node cells, negative effects on mucociliary clearance and macrophages
Ozone Oxidant air pollutant	Increased susceptibility to infections, increased production of IgA antibodies in secretions and in lymphoid tissue
Pentachlorophenol Pesticide	Increased tumor susceptibility, reduced susceptibility to viral challenge suppression, increased macrophage phagocytosis
Silica Sand blasting, mining, glass industry	Altered T- and B-cell function, macrophage toxic effects, autoantibody formation and autoimmune-like disease
Sulfur dioxide Oxidant air pollutant	Depressed resistance to bacterial infections, increased immune response, enhanced synergism among pollutants, inflammation and alteration of fibrous septa
Tetrachloroazoxybenzene Herbicide manufacture and degradation	Decreased thymic weight, decreased splenic antibody production, inhibition of macrophage function, depression of bone marrow cellularity
Tin Canning processing of foods	Decreased splenic antibody production
Titanium dioxide Paints, cigars	Macrophage morphology and enzyme production abnormalities
Toxaphene Insecticide in agriculture	Reduced antibody production, suppressed delayed hypersensitivity responses, decreased macrophage phagocytosis
Trichloroethylene Industrial solvent	Decreased antibody and cell-mediated immune responses, inhibition of bone marrow stem cell colonization

Data from [377].
ADCC Antibody-dependent cell-mediated cytotoxicity, *IFN* interferon.

phenol in Seveso, Italy, on July 19, 1976, probably released >30 kg of TCDD into the environment. The net result is that human exposure to TCDD is linked to a lowered male/female sex ratio in their offspring, which may persist for years after exposure [286]. WHO experts (Press Release WHO/45, 3 June 1998) lowered the TCDD tolerable daily intake to 1–4 pg toxic equivalents/kg bw, and recommended that every effort be made to reduce exposure to the lowest possible level. Subtle effects may occur in the general population in developed countries at current background levels of 2 to 6 pg/kg bw. A preventive action limit for TCDD in feed for chickens and pigs was set to 2 pg toxic equivalents/g feed. This limit was effective in the detection of feed contamination and in the prevention of food contamination according to WHO tolerable daily intake. The adoption of the WHO level would have detected and prevented the 1999 crisis in Belgium, which followed the TCDD contamination of foodstuffs, and TCDD was subsequently discovered in the animal mash used to feed cattle instead of the usual forage, and finally to be a cause of *mad cow disease* (MCD) or *bovine spongiform encephalopathy* (BSE). The main cause was probably by feeding cattle with feed originally made from the carcass of an antelope infected with a similar form of BSE. The antelope was rendered for use as protein-rich animal feed after it died. The infected batch was then given to about 1,000 local cows between 1975 and 1977, passing the prions on to the cattle [Times 12. 3. 1990]. Cattle carcasses and carcass wastes were recycled through the rendering plants, increasing the levels of the now cattle-adapted pathogen in the protein supplement and eventually causing a full-scale BSE epidemic. The press reports that thousands of tons of BSE-infected cattle feed were exported from Britain to other nations over the past decade have set off the largest food scare in history. About 100 Europeans have thus far officially died since 1996 from the new variant of Creutzfeldt-Jakob disease (CJD), the human equivalent of BSE. There are many parallels with scrapie in sheep, also included in the recent definition of prion disease (Chap. 24). Unfortunately, the long chain of infected cattle continues: on May 20, 2003 the FDA learned that a cow has tested positive for BSE in Canada. On December 23, 2003, the US Department of Agriculture (USDA) made a preliminary diagnosis of BSE in a dairy cow in Washington state. On December 25, this diagnosis was confirmed. A preventive sampling strategy and regulations ensuring non-stop traceability of contamination to the raw materials utilized to produce cattle feed and human food are therefore mandatory.

Moreover, dietary antioxidants, polyunsaturated fats, and modern contaminants including phthalates, polychlorinated biphenyls (PCB), and organochlorine pesticides [349] are ubiquitous and persistent in the environment and may alter the intrinsic propensity for Th2 responses. The effects of these and other environmental

Table 4.21. Main pesticides

Class	Examples
Insecticides	
Carbamates	Aldicarb, carbaril
Organochlorines	DDT[a], lindane, aldrin[a], dieldrin[a]
Organophosphates	Parathion, malathion, diazinon
Vegetables	Pyrethrin, nicotine, permethrin
Anticryptogamics	Dithiocarbamates, sulfur, Cu salts
Acaricides	Acetyl bromide, ethylene oxide, paradichlorobenzene
Nematocides	Dichlorodibromopropane, trichloronitromethane
Herbicides	Chlorates, atrazine, carbamates
Molluscacidas	Metaldehyde, mercaptodimethur
Rodenticides	Phosphorus and derivatives, warfarin
Other pesticides	Chlordane[a], endrin[a], heptachlor[a], mirex[a], toxaphene[a]

Data from [141, 353].

[a] With β-hexachlorobenzene(β-HCB), polychlorinated biphenyl, dioxin and furans, belongs to the 12 POPs (persistent organic pollutants).

DDT dichlorodiphenyltrichloroethane.

changes are poorly understood, but are the focus of recent research. Undoubtedly, in addition to the introduction of many new chemicals into the environment, there have been many changes in the alimentation in industrialized countries over the past decades [101]. A recent study has, however, found that *in utero* exposure to background PCB levels is associated with *poorer cognitive functioning in preschool children* [321].

Pesticides (Table 4.21) [141, 353], a real plethora, are widely used also as herbicides, insecticides, fungicides, etc. The first of them, DDT, was banned in the US in 1972, but continues to be used in developing countries, so hundreds of millions of people are significantly exposed to pesticides each year. A number of insects develop a specific resistance to pesticides and their elevated stability provokes an environmental capillary diffusion, since ≈80%–90% of pesticides disperse through the air, soil and water. Vegetable and animal organisms gather DDT and other pesticides in their tissues up to 20 parts/10^9, more often in lipids, thus causing a progressive bioaccumulation along the food chain. Seventy-eight percent of countries report pesticide contamination as a threat to health [446]. Pesticides may pose serious risks to birds and human beings eating fish exposed to organochlorine pesticides in the paddles for only 6 months [353]. Pesticides depress immune system functions: for example, CD4/CD8 T-cell ratios and cell counts have been found significantly altered and lymphocyte proliferative responses suppressed, indicating severe dysfunctions in CMI. Clearly shown are the effects on B-cell function, macrophage phagocytosis, NK cell numbers and host resistance. Negative repercussions particularly affect *infants and children,* whose immune systems have not yet fully matured. In the more exposed areas, children are threefold more likely to have infectious diseases of the gastrointestinal tract and two- to threefold more likely to have infections of the respiratory tract than children living in areas of lower pesticide use [353]. WHO experts have grouped 12 pesticides not broken down naturally by light or other chemical substances, called POPs (persistent organic pollutants), which inevitably accumulate in the environment and because of their liposolubility target the tissues of living organisms. In children, exposure to pesticides may be magnified if they crawl or play on freshly sprayed surfaces. An infant's breathing is close to the ground where pesticides are at very high concentrations. Exposure may also result from dermal absorption. Most of these exposures do not cause poisoning, but severe symptoms may occur by overexposure [350]. However, pesticides added to crops enter the food chain and can have further damaging effects on metabolic functions. Data from a sample of US emergency rooms show that children <5 years of age were involved in 55% of 20,480 cases of *pesticide poisoning*, and 10% of children were later hospitalized. Insecticides and rodenticides accounted for 87% of these cases, and ingestion occurred in 76% of the cases [141].

The chemical substances outlined in Tables 4.20, 4.21, present in environmental air and other pollutants, in various ways carry on noxious actions on the immune system of human beings following involuntary exposures, thus calling attention to a much-needed examination of the mechanisms involved in immune surveillance alterations subsequent to such exposures, to prevent the severe complications of environmental immunotoxic plethora. These substances could be eliminated with a concerted action of international bodies and replaced with less toxic products.

Free Radicals

In the last few years, growing evidence states that *free radicals* (FRs), so called in that they carry a supplementary electron, can be of importance in immunology [338]. Approximately 1%–3% of inspired O_2 is used for the synthesis of $O_2^{\bullet -}$ (superoxide anion), the most reactive FR, which has a role in diverse fields, particularly in various lung diseases. The superoxide dismutase (SOD) then dismutates superoxide to H_2O_2. We should consider that FR oxidative changes deliver a large amount of O_2, and that we produce >2 kg/year on average. O_2 is not toxic as such, but due to products of reactions with H_2O,

which involve a series of extremely reactive substances, including H_2O_2 and hydroxyl radicals (OH). The production of these highly toxic molecules starts within 30 s of the target binding to the cell surface. When metabolic reactions dismantle an O_2 atom of an electron, FR tries to replace the missing electron by removing one from a molecule of cell membrane: thus a new FR is formed and a chain reaction begins [338]. FRs have a short half-life, measured in milliseconds, and react with other molecules found in the immediate environment. Usually, phagocytic cells use FR produced in small quantities in the mitochondria to kill microbes, and both neutrophils and macrophages protect themselves from FRs escaping from the phagosome, while FRs that have left the cell exert a toxic activity on nearby cells. On the other hand, FR release from phagocytes activates membrane phospholipase A_2, generating arachidonic acid (AA) and other lipid mediators, resulting in an amplified production of proinflammatory factors of lipid origin. FRs concur indirectly to neutrophil stimulation: IgG antibodies in FR presence show a characteristic fluorescence and form aggregates able to stimulate FR release.

An imbalance in superoxide production may activate a vicious cycle marked by contributions to the pathogenesis of many diseases. FRs can be involved in lymphocyte accumulation in inflammatory sites: in this situation the human phagocyte response to LTB_4 is inhibited by SOD and/or catalase, which reduces H_2O_2 to H_2O and O_2. Similar results have been obtained employing other chemotactic factors. SOD and/or catalase activation in varying types of inflammation shows that FRs play a crucial role in inflammatory processes, even if elaborate interactions surely occur and concur to the final cascade. Human beings in physiological conditions have the ability to cope with FR toxicity, by a series of defense mechanisms of various types, including the enzymes so far analyzed, in addition to glutathione peroxidase, but we can be exposed to an increased risk of cellular injury when the production is increased. Stress proteins have a potential defensive effect against oxidative stress, by playing an important role in protecting cells and tissues from injury. However, tissue sufferance oxidative stress may either contribute to or be the background of the tissular damage caused by the disease; moreover, FRs may worsen tissue damage in a more or less significant way. Mast cells and basophils have the ability to generate superoxide. In turn, reactive FRs activate histamine release from basophils, and activated neutrophils evoke histamine release from mast cells, thus creating a further amplification loop between immediate reaction and asthmatic inflammation [338]. Tachykinins (SP in particular) provide one more proof of the connection between FRs and asthma: SP is normally degraded by neutral endopeptidases, highly sensitive to oxidative damage and in turn can be degraded, for example, by ETS. In this occurrence, laboratory animals develop BHR to SP. BHR observed in cigarette smokers or in subjects exposed to O_3 may recognize a similar mechanism. A further class of FRs is elicited, in addition to normal metabolic challenges, by external causes, such as, for example, atmospheric agents, drugs, smoke, pesticides, radiation, cigarettes, etc. *Several antioxidant factors* such as natural vitamins, flavonoids, glutathione, uric acid in the epithelial lining fluid, and foods confer protection to neutralize excess FRs so that the bronchial epithelium escapes tissue injury (Table 4.22). Antioxidants normally bestow protection, but if the balance with oxidants is disturbed, the epithelium may undergo an oxidative stress, thus up-regulating eicosanoid metabolism, which contributes to early symptom development and PFT decrement and to arachidonate oxidative products chemotactic for neutrophils [219] (Chap. 22). Stress proteins could represent a self-protective mechanism that could promising-

Table 4.22. Natural and synthetic antioxidants present in foods

Enzymatic
Catalase
Glutathione-peroxidase
Superoxide dismutase
Nonenzymatic
Proteins and peptides chelating peroxiding metals
Uric acid
Albumin
Ceruloplasmin
Desferrioxamin
Glutathione
Lactoferrin
Selenium
Transferrin
Molecules binding O_2
β-Carotene
Retinoids
Natural vitamins A, C, E
Natural
Chocolate
Coffee
Flavonoids
Fruits
Green and black tea
Olive
Onion
Plant phenolis
Rosemary
Sage
Soy beans
Tomato seeds
Whey
Synthetic
Ascorbates (E 300–304)
Lecithins (E 322)
Phenols
Tocopherols (E 307–309)

Data from [338].
The complete analysis of synthetics (additives) is in Chap. 10.

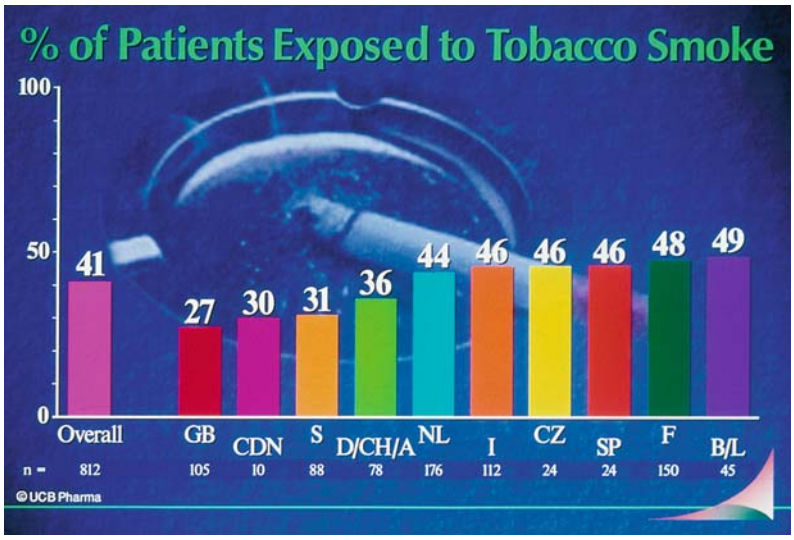

Fig. 4.23. ETAC Study: on average 41% of children are exposed to tobacco smoke, the highest frequency is in Benelux (49%), the lowest in Great Britain (27%)

ly be manipulated so as to provide a more effective shield against oxidative stress [338].

O_2 FRs and antioxidant defenses were evaluated in 210 asthmatic children aged 5–18 who had a 3-fold higher FH of asthma and a 4-fold IgE increase than in the healthy controls. Excessive production of superoxide, OHs and antioxidant enzymes was noted in the blood cells in asthmatic children, whereas SOD and FR scavengers in blood cells were significantly lower in asthma, thus stressing the correlation between asthma severity and O_2 FR production in asthmatic children [394].

Environmental Tobacco Smoke

ETS passively inhaled is the commonest respiratory irritant, especially in the home, where infants spend most time and older children more [27], considering that modern homes allow air exchanges in only 50% of cases vs 100% of older houses [28]. The role played by ETS is incontrovertible in exacerbating respiratory allergy and increasing its prevalence: in all likelihood the age of the child is a significant factor, thus younger babies are particularly at risk, also taking into account that 75% of smoke released in the environment is sidestream smoke, with greater T making it more toxic [132]. Hence ETS is the greatest factor in causing early asthma in children, correlated with the number of smokers [68], especially in children with a genetic predisposition for allergy [232]. It has been demonstrated that offsprings of smokers show an increased prevalence either of SPT positive to inhalant allergens or of BHR and suffer earlier and more frequently from atopic disease than the offsprings of nonsmoking parents [366, 425], as well as from AD and asthma (p=0.0001) [348].

The bulk of studies that have examined the relationship of parental smoking to wheezing and asthma in children have found a positive association [298, 425, 477]. The OR can be 2.1 between maternal smoking and childhood asthma [477], and an increased severity of the disease was seen in asthmatic children with smoking mothers compared to asthmatic children of nonsmoking mothers [298]. A clear connection with atopic sensitization has been established by other studies [7, 42, 146, 366, 482] and in particular with increased total IgE levels [488]. In children of atopic parents, total IgE levels are significantly higher and respiratory infections are more frequent compared to children of nonsmokers; consequently ETS occasions or worsens asthma in a high rate of children [484]. A synergy between pollutants, ETS and allergy has also been reported [7, 371, 385]. In 74% of infants aged <2 with bronchiolitis or asthma, the determination of salivary cotinine was positive, the main metabolite of nicotine, with a long half-life in body fluids with levels of 10 ng/ml, pointing to a high exposure to ETS vs 14% of children aged >2 [111]. An aspect to be evaluated is the detectable cotinine levels found even more often in children unexposed to any ETS source [75], thus implying an ETS community exposure. Dosing urinary cotinine has shown increments of passive ETS inhalation parallel to increased exacerbations of infantile asthma and decreases in the FEV_1/FVC rate and FEF_{25-75} (forced expiratory flow) levels [67]. Epidemiological studies document that ETS *passively inhaled* by children favors the onset and persistence of asthma; the association between parental smoke and symptoms such as cough, wheezing and airway inflammation is well demonstrated in children [147]. In children allergic or not to Der p 1, early exposure to ETS is a risk factor (41.5% of children) correlated with dampness of windows and/or of the home [7, 42]. Allergic infants with severe asthma are exposed to ETS more than nonallergic infants [382] and even FHA^+ is a hindrance for ETS [216]: it is significant to note that 65% of 210 asthmatic children had a smoking parent while in the control group it was ≈25% (p=0.0001) [394]. In the ETAC study, 41% of children were exposed to daily smoking indoors (Fig. 4.23); in

Poland, 59% of fathers and 51% of mothers smoke, and in Sweden the mothers are more often smokers (37%) than the fathers (30%) [43]. However, paternal smoke could not be associated with respiratory symptoms [44], perhaps because fathers are less present at home. Another threat is that of the older brothers possibly smoking at home: the current smokers aged <16 were 22.5%, the atopics 27.6%, a fivefold increase [396]. In a cross-sectional study on 770 children aged 7, which collected data with salivary cotinine determinations, *72.5% of children from nonsmoking households had detectable cotinine levels,* 10.1% of which was in the upper levels [419]. In an epidemiological investigation on 3,040 children aged 10–14, also based on salivary cotinine determinations, both parents smoked in 51% of cases, only mothers in 19%, only fathers in 17%, another household member or regular visitors in 3% of cases. However, 7%–11% of children had positive levels although not exposed to ETS [75]. The rising problem of finding *critical levels of cotinine in unexposed children,* logically *reached by ETS coming from anywhere, still awaits basic answers.* In children aged 7 with a HR of atopy, the levels were 24.3±20.7 and 6.5±6.4 ng/mg of creatinine depending on whether there were smokers in the house [512]. In a cohort of 199 asthmatics of 8 months to 13 years, mean urine cotinine was 5.6 ng/ml in those not exposed to smoke, 13.1 ng/ml in those exposed to maternal smoke or smoke in other households and 55.8 ng/ml considering all active smokers [67]. Often such results depend on smoking in parents of both asthmatic and control children, with no testing for the exposed offspring [317]. According to the parents of 19 asthmatic children, only 31% were exposed to ETS; when measuring salivary cotinine with high selectivity liquid chromatography, the rate rose to 69% [69]. The definitive proof was obtained using a method similar to that of Talbot (see Chap. 9): if the mother smoked immediately before breast feeding, a tenfold increased *nicotine concentration was dosed in the baby* [89].

The main toxic substances are summarized in Table 4.23 [132]: the levels of such substances are broadly dependent on the release of *sidestream smoke produced by the slow combustion of cigarettes* (without aspiration), often released in greater amount per cigarette than those of mainstream smoke exhaled back into the air by smokers [132]. The numerous and not completely known mechanisms by which ETS could act on the immune system are outlined in Table 4.24 [20, 47, 56, 67, 75, 107, 129, 132, 140, 145, 146, 191, 206, 268, 298, 366, 396, 425, 462, 490] and Table 4.25 [103], which quantifies the positive risk as related to pertinent parameters. Children exposed to ETS undergo a greater number of airway infections and suffer from changes in respiratory epithelium [145], with consequent facilitated allergen access to immunoreactive cells. These children experience a number of infections 16-fold higher than unexposed children, and the risk of respiratory infections increases 3.5-fold [15]. The ensuing restoration of infection-induced inflammatory alterations may be incomplete due to unending exposure to ETS [147]. Since these infections are asthmogenic, a vicious circle is started, especially in winter months when a greater virus transmission is facilitated: parents' smoking → VRI → asthma in the child. As a result, only a drastic inhibition of smoking can interrupt this circle. Consequently, children develop asthma (Chap. 11), infections of the lower airways (Chaps. 9 and 22) and more easily and prolonged secretory otitis media (Chap. 15). The effects produced on the offspring by ETS of 20 cigarettes consist in significant mean reductions of PEF (5%–6%), MEF_{75} (5.7%), MEF_{50} (4.9%) [107], FEV_1 (0.0008 l) and FEF_{25-75} (0.003 l/s) per cigarette with greater reductions and more significant statistical differences in children with active asthma [145]. More precisely, in asthmatic children aged 10 of average weight and height, FEF_{25-75} will be reduced by 6.8% if >1 cigarette/day is smoked in the home, plus a further reduction of 3.3% for a current respiratory infection, and 2.4% if >20 cigarettes/day are smoked in the house [145]. From the clinical point of view, ETS is associated with ongoing cough, effort-induced, nocturnal and recurrent wheezing, with highly significant differences based on the number of cigarettes smoked in the house daily [107]. ETS not only causes respiratory allergy in children at risk of atopy, but especially *an additional genetic factor,* because asthma is most likely occasioned when one atopic parent smokes and chiefly when both parents smoke [56].

The substances contained in ETS may variously influence Ig synthesis and stimulate the IgE system *in utero,* thus predisposing infants to atopic sensitization, since maternal smoke causes a significant increase in CB IgE

Table 4.23. Main toxic and/or noxious substances present in central tobacco smoke (quantity/filter-tipped cigarette)

Toxic substances	Amount
Nicotine	1–2.5 mg
Ammonia	50–130 µg
Carbon monoxide	10–23 mg
Carbonyl sulfide	18–42 µg
Cyanhydric acid	400–500 µg
Nitrogen oxide	100–600 µg
Toxic substances for the alveolar and/or ciliary epithelium	**Amount**
Acetone	100–250 µg
Acrolein	60–100 µg
Cyanhydric acid	400–500 µg
Formaldehyde	70–100 µg
Formic acid	210–490 µg

Data from [132].

Table 4.24. Mechanisms by which tobacco smoke could act on the immune system

General effects
Acts by irritating the respiratory mucosa
Predisposes to an increased susceptibility to respiratory viral infections
Adult smokers contract influenza more frequently
The incidence of influenza and of recurrent respiratory infections is higher in the offsprings of smoking parents
Potential mechanisms effective on the immune system
Direct action on mast cells, independently of IgE-mediated mechanisms, inducing mast cell degranulation
Increase in $CD4^+$ cells and changes in $CD4^+/CD8^+$ ratio
Dose-dependent increase in IL_4-producing Th2 cells
Increase in IgE antibody concentrations
Presence of specific IgE antibodies
Capability to impair both bronchial mucosa and epithelium, thus promoting the penetration of antigens, which in turn can sensitize the child, and stimulating IgE antibody synthesis
Hyperplasia of calyciform cells
Mucus hypersecretion
Alteration of ciliogenesis, so provoking the disappearance of cilia, which need months or even years to be re-formed
Damage of intercellular junctions integrity and of epithelium permeability:
The increase in bronchial epithelium permeability facilitates the allergen penetration into the sub-epithelial lymphoid tissue
The epithelial damage, especially the loss of cilia, could favor the persistence on mucosal surface of antigens and infectious agents and hence their penetration
The effect on alveolar macrophages inducing their increase in respiratory spaces, also critically altering their functions and metabolism, in particular as regards the arachidonic acid derivatives
Fulfills a potential up-regulation on T lymphocytes
Relationships with IgE levels and atopy
Neonates born to smoking mothers have increased CBIgE levels, independently of the family history of allergy
IgE concentrations are higher in smoking adults in the offspring of smoking atopic parents
In adult smokers the levels of serum and specific IgE antibodies are higher than in nonsmokers and in adults with airway infections are also higher compared to healthy individuals
A similar relationship is found in atopic smokers compared to nonatopic individuals
Even ex-smokers have high levels of serum IgE antibodies
There is a significant correlation between the number of smoked cigarettes and IgE levels
Infants and children of smokers affected with atopic disease present levels of serum IgE antibodies significantly higher than controls
Cigarette smoke favors the development of atopic manifestations in at-risk babies
The onset of atopic manifestations is earlier in children of smoking parents
Clinical data
Asthma and its exacerbations are more frequent in the offspring of smoking atopic parents
Passive smoking is responsible for 8% of all episodes of otitis media, prolonging the total duration of the affection
Wheezing is more frequent and has an earlier onset in the babies of smoking mothers
Asthma due to Der p is more frequent in children with a mother smoking during and after pregnancy
Children with a mother smoking during pregnancy develop bronchial hyperreactivity during the very 1st month of life
Maternal smoking during pregnancy may impair in utero airway development and/or alter lung elastic properties
Daily exposure to parental tobacco smoke is associated with an earlier and more frequent development of atopic disease compared to infants and toddlers of nonsmoking parents
Adult smokers manifest a higher frequency of infectious episodes of upper airways, therefore their babies have a higher risk of developing viral infections
Male children are more at risk than female children
Passive smoking in infants and toddlers is directly correlated to
Exacerbations of asthma attacks in children previously asymptomatic
Increase in number and severity of asthma exacerbations
Alterations of pulmonary function
Increase in number and severity of upper and lower airway infections

Data from [20, 47, 56, 67, 75, 107, 129, 132, 140, 145, 146, 191, 206, 268, 298, 366, 396, 425, 462, 490].

Table 4.25. Pediatric morbidity associated with household smoking

Condition	Pooled risk	At-risk children (%)
Asthma	1.43–1.46	8–13
Respiratory hospitalization	1.55–2.41	15–23
Lower respiratory tract infections	1.46–2.50	12–20
Cough	1.36	10–16
Middle-ear disease	1.19–1.58	2–13
Tympanostomy	1.60	1–26
Adenoidectomy tonsillectomy	1.20–2.06	16–24

Pooled risk is a relative risk for cohort studies (first figure) and an odds ratio for case–control studies (second figure when present); a value >1 shows a high risk of disease.
All the results show a statistical significance between 0.01 and 0.0001.
Data from [103].

and IgD levels, with a higher prevalence of atopic disease in the first 18 months after birth (Fig. 4.24) [256]. These data have not been confirmed by other studies of CB IgE [314, 316] and IgD [316]. Recently, late gestational smoke exposure was found to be associated with an increased risk for wheezing but not with asthma, yet data showed a tendency towards a protective effect of smoking on AR, and AD [254], or no significant relation to AD [183, 224]. Passive smoking was also protective at 12 months in children of allergic mothers, but not at age 4.

Several studies have certified a strong association of maternal smoke with the onset of the atopic march: *maternal smoke increases in utero IgE levels, provokes hypoplasia of fetal lungs and reduces the number of alveoli and/or alters passive respiratory mechanics* [14, 47, 152, 415, 425, 477], resulting after birth in PFT reduction, even more if linked to a hereditary basis for asthma [415]. It is well established that substances derived from the maternal environment can be found in the gestation-associated environment. Cotinine has been found in placental tissue, celomic and amniotic fluid and fetal serum from the first trimester when mothers have been exposed to cigarette smoke [188]. Prenatal maternal smoking was associated with reduced FEF rates in infants tested shortly after birth, thus smoking during pregnancy might *impair in utero airway development and/or alter lung elastic properties* [152], also suggesting an obstruction of small airways in dysmature lungs, which contain few alveoli and are smaller at birth, more marked than in the lungs of unexposed infants [47]. A precise conclusion is that *the fetus* is *the first victim of ETS,* although only one study [152] has clearly separated prenatal from postnatal maternal cigarette smoking, as completed by the PFT study at birth [415]. Further objective data underscore ETS effects in the first few years of life: 27% of neonates had cotinine CB levels higher than 5 ng/ml [24]; already in the 1st month of life, at-risk babies of mothers smoking during pregnancy had BHR [508] and symptom-free infants had PFT alterations in 8% of cases [507], and significant FEF reductions may actively predispose infants to the later occurrence of wheezing illness [152]. A strong association links maternal smoking with the development of asthma during the 1st year of life [14, 268, 477], placing the estimated risk at about twofold [268, 477], or fourfold [14] of nonsmoking mothers. Therefore ETS represents the main environmental factor hitherto identified in the etiology of early infantile asthma,

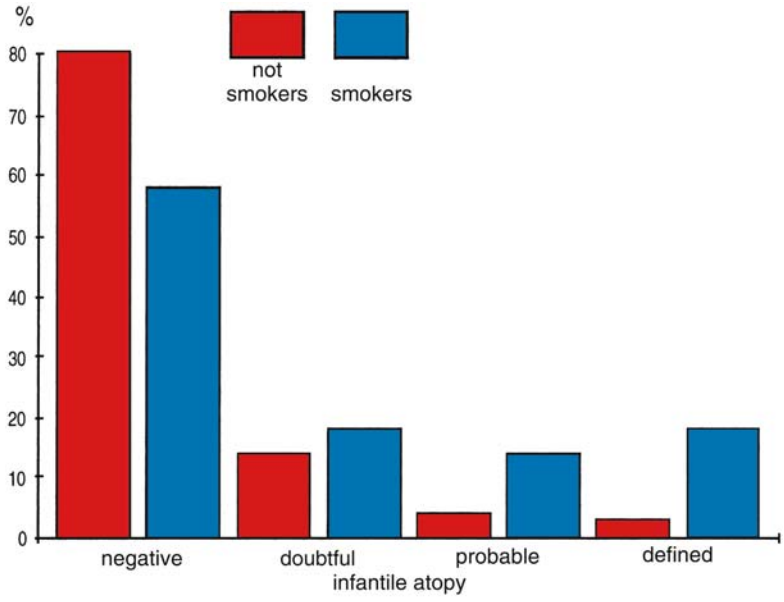

Fig. 4.24. Incidence of atopic disease among the children of smoking mothers. (Data from [256])

Table 4.26. Smoking mothers of 48 atopic children during and after pregnancy

	Smoking mothers 25/48 (52%)	
Smoking during pregnancy	>10 Cigarettes	<10 Cigarettes
16/25 (64%)	3	13
Smoking after pregnancy		
22/25 (88%)	9	13

$p = 0.0029$.

Table 4.27. Smoking mothers of 48 nonatopic children during and after pregnancy

	Smoking mothers 12/48 (25%)	
Smoking during pregnancy	>10 Cigarettes	<10 Cigarettes
5/12 (42%) (p=0.0065)[a]	2	3 (p=0.0066)*
Smoking after pregnancy		
9/12 (75%)	5	4 (p=0.0045)*

Data from [56].

[a] Compared with the mothers of atopic children.

to the point that about 40% of cases are related to chiefly maternal ETS [147].

Maternal smoke, even of only 10 cigarettes/day, is responsible for the earlier wheezing onset [490] even in the 1st year of life [129, 146], and is significantly predictive of asthma development in children [268], at school age [184], or after age 16 [420]. The mothers of atopic children with asthma and SPT and RAST positive to Der p 1 have smoked during and after pregnancy more than the mothers of nonatopic children (Tables 4.26, 4.27) [56]. We can therefore conclude that the principal source of ETS is maternal smoke during and after pregnancy and that the effect of maternal smoke of >20 cigarettes daily is significantly associated with asthma onset in children and that males are more significantly at risk than females [56, 268]. Smoking only during pregnancy or after delivery fails to modify the results [116]: the risk of developing BHR is positively associated with maternal smoke during pregnancy and/or in the 1st year of life [129]. In 276 unselected infants [146], the risk was extended at least up to 18 months of life. The association with ETS is statistically significant in Der p 1-sensitive children and of maternal smoke with the prevalence of atopic sensitization [465]. In 98.8% of 1461 asthmatic children of smoking mothers, the free running test increased heart rate to >170 beats/min, an effect sufficient to elicit bronchoconstriction in sensitized subjects [129]. The risk factor was quantified in 744 subjects aged <5: *children with mothers smoking at least 10 cigarettes daily were 2.55-fold more likely to develop asthma* and 15.7% lower MEF than children of nonsmoking mothers or those smoking <10 cigarettes daily [268]. *An OR of 2.1 was reported for asthma in children with mothers smoking even only 10 cigarettes daily compared with children of nonsmokers;* also highlighted were use of asthma medications (OR 4.6), asthma developing in the 1st year of life (OR 2.6) and increased numbers of hospitalizations [477], possibly leading to a greater risk of asthma in children with PFT anomalies [296] and to permanent PFT changes in children exposed *in utero* [470]. These studies suggest that maternal smoking during pregnancy may influence mechanical factors that are important throughout the whole life of the child. A consequence is that maternal smoke may implicate bronchial reactivity: symptoms were 47% more frequent in children of smoking mothers and a fourfold greater BHR to the histamine test than in children of nonsmoking mothers was reported [298]. After a longer exposure to maternal smoke, PFT abnormalities were more consistent [296]. Both obstruction and malformation of the lower airways, resulting from the exposure of the unborn child may predispose to a precocity of longer and more severe obstructive episodes, potentially worsened by poor effects of medications.

The true smokers were 52% of males and 49% of females: the mother smokes 20 cigarettes daily, but girls seem to be more susceptible considering that they spend more time at home and are more exposed [125]. Negative effects should be more pronounced in males for anatomical reasons; instead the picture has been reversed at the expense of females, with an elevated OR for smoking of either parent [125, 425]. This finding may be explained by the increased levels of dehydroepiandrosterone, an androgen of the metabolic pathway of testosterone and cortisol that has been demonstrated in the amniotic fluid of pregnant smoking mothers [240], *leading to a possible masculinization of the female fetal lung,* with effects similar to the male lung. A large body of epidemiological evidence has stressed the risks connected with ETS and suggested the urgency of firmly advising against maternal smoke in pregnancy and after [56]. This crusade is made more crucial by the observation that women who stop smoking in pregnancy often restart in the immediate postnatal period [56, 116] and that prolonged breast feeding in infants of heavy smokers ensures a protective effect against wheezing development [116]. However, ETS reduces EGF (epidermal growth factor) levels also in breast milk. The widespread nature of ETS is documented in Table 4.28 [43, 44, 75, 84, 125, 146, 198, 233, 335, 514]. Smoking as a serious problem for young girls is emerging in many countries. Figure 4.25 [140] shows the difference between active smokers aged 10–14 and 15–18 years in the US: the younger ones totaled 20% of smoked cigarettes and the only difference was the higher rate of females of

15–18 years smoking 1/2–4 cigarettes daily; in the younger group the prevalence was 17% for males and 19% for females, and at their age mild airway obstruction and slowed growth of lung function are already present [140]. A decline of FEV_1 and FEF_{25-75} was evident in the girls of 17–18 years who smoked. Perhaps youngsters are influenced by 67%–69% of smoking mothers [116, 140].

Table 4.28. Diffusion of passive smoke in exposed infants and children

Country	Percentage	References
Latium, Italy (polluted areas)	67	[84]
Latium, Italy (nonpolluted areas)	69	[84]
Denmark	66	[146]
France	35.5	[233]
Great Britain	53	[75]
Poland	50	[514]
Poland	55	[43]
Sweden	34	[43]
Turkey	74	[198]
US	43	[335]
Mean	54.7	
Parental smoke (reference)	**Fathers (%)**	**Mothers (%)**
Poland [514]	56.9	43.8
Poland [43]	59.1	51.1
Sweden [43]	30	37
Estonia [44]	46.2	23.7
Italy [125]	52	49
Mean	48.8	40.9

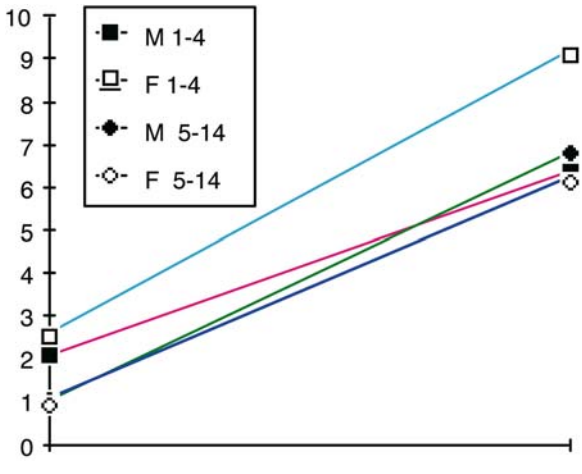

Fig. 4.25. Proportion of smoking children and adolescents (see text). *F* females, *M* males. (Data from [140])

The postulated links between the increase in atopic disease prevalence and smoking are proved by the significantly increased number of smoking girls aged 16–24 (9%) [467] paralleled over the same time span by the increased asthma frequency (6%) [304]. In an epidemiological study, the asthma prevalence is increased sixfold in those aged 16–33, especially in a period highly associated with active cigarette smoking [420]. It is needless to comment that in 1988 US cigarette manufacturers spent (or invested?) the equivalent of $100/s for cigarette advertising and promotion, and it appears that magazines that depend on advertising revenues are less likely than others to publish articles dealing with the hazards of smoking for fear of losing these revenues. The evidence on the dangers of smoking (Table 4.25) suggests that adopting adequate measures to warn smoking boys and girls, as well as women in their reproductive years, and mothers against smoking at all, particularly during and after pregnancy, can help ensure a normal development of infant airways in the generations to come. The parents of asthmatic children studied by Murray et al have reduced their children's exposure to smoke, so that the prevalence of asthma in their children has reached the levels of the normal population [297]. Although several countries have strengthened smoking regulations in public places, they are often hard to enforce [446].

Infections

Further insights into respiratory infections show strong epidemiological and pathophysiological evidence to link viral infections to exacerbations in childhood wheezing and asthma and to susceptibility to atopic sensitization: in 11 of 13 children, an upper respiratory infection occurred 1–2 months prior to allergic sensitization [128]. Based mostly on animal experiments, pups previously virus-infected, for example, by the respiratory syncytial virus (RSV), have supplied evidence that viral infections can promote IgE sensitization. Infection-induced release of potent mediator peptides, made by stimulated immunocompetent macrophages and monocytes, may result in the activation of immune responses. Host metabolism is altered in a way that may be debilitating and lead to malnutrition if continued for too long. Matching the changes produced in the intestinal mucosa, VRI may increase aeroallergen absorption via the respiratory mucosa, and epithelial permeability to a greater extent because of inflammation [127, 235]. Table 4.29 [50] recapitulates the numerous hypotheses increasingly proposed to elucidate the intriguing relationship between viral infections and their interference on IgE immunoregulation, thereby influencing development of atopic sensitization in experimental models [50].

Table 4.29. Possible mechanisms by which viral infections promote atopic sensitization in experimental models

Increase in total IgE levels by inducing clones of virus-specific IgE
Promotion of a direct epithelial damage
Epithelial damage + alteration of mucociliary clearance favor greater penetration of inspired viral allergens
Alteration of the allergen process at the mucosal level
Preferential reduction of IgE-specific T-suppressor cells
Increased release of histamine induced by IFN-γ and consequent increase of mucosal permeability
Increase in mucosal permeability may favor allergen absorption

Data from [50].

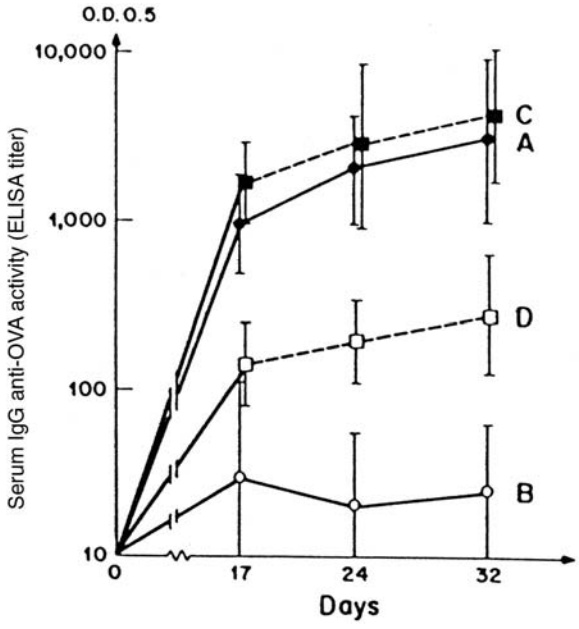

Fig. 4.26. Levels of serum IgG anti-OVA antibody in mice infected with RSV (*A* and *C*) or sham infected (*B* and *D*) on day 0 and given five intranasal doses of OVA without (*A* and *B*) or with alum adjuvants (*C* and *D*) on days 4–8. *OVA* ovalbumin, *RSV* respiratory syncytial virus. Reprinted with the permission of the Society for Experimental Biology and Medicine [127]

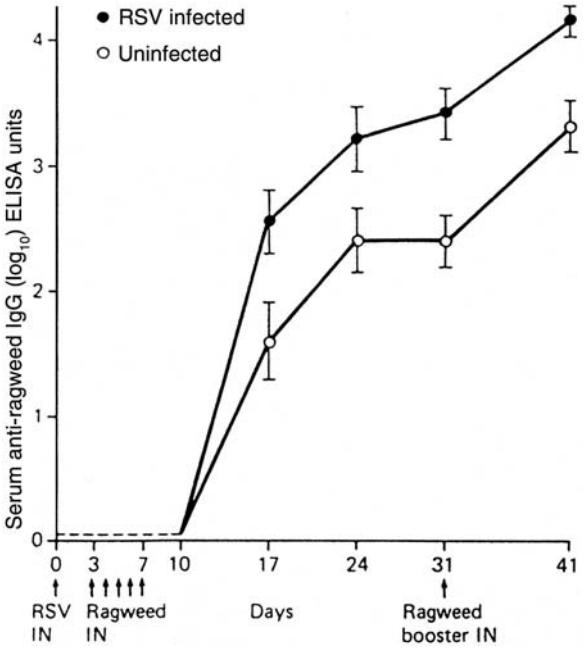

Fig. 4.27. Effect of RSV infection on serum anti-ragweed IgG production after 5 consecutive days of short ragweed pollen intranasal (*IN*) priming and a booster on day 31. Anti-ragweed IgE antibodies measured by ELISA. Reprinted with the permission of S. Karger AG, Basel

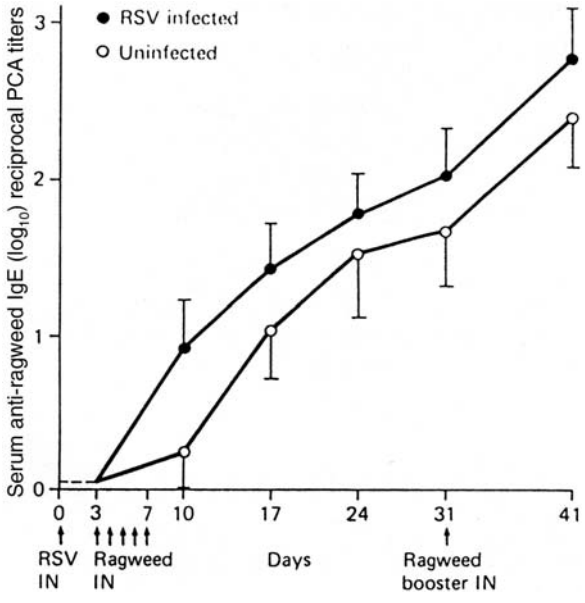

Fig. 4.28. Effect of RSV infection on serum anti-ragweed IgE production after 5 consecutive days of short ragweed pollen intranasal (*IN*) priming and a booster on day 31. Anti-ragweed IgE antibodies measured by PCA. Reprinted with the permission of S. Karger AG, Basel

Intranasally RSV-inoculated mice and exposed to OVA with or without adjuvant developed higher OVA-specific serum titers of IgG and sIgA antibodies. OVA serum concentrations were significantly higher in RSV-infected animals compared to uninfected animals or those exposed to OVA with the adjuvant (Fig. 4.26) [127]. The same team of investigators has demonstrated that RSV infection in mice may also significantly enhance IgG and IgE antibodies to ragweed administered by aerosol (Figs. 4.27, 4.28) [235]. These observations may explain why mucosa-restricted VRI may enhance the development of sensitization in children with viral-induced bronchopulmonary disease [127, 235]. RSV

may induce a consistent IgE response in the respiratory mucosa of infants with bronchiolitis, in whom cell-bound IgE persist longer compared to infants with other forms of respiratory illness due to RSV infection [478]. RSV is also capable of activating and stimulating eosinophils to release cationic proteins, thus amplifying its action in the pathogenesis of bronchiolitis and provoking persistent BHR after its infection.

Rhinoviruses have the ability to stimulate mast cells to release histamine, with foreseeable effects on older children, while RSV is the causative agent for wheezing in younger children. In an epidemiological investigation on schoolchildren, asthmatic episodes were accompanied by viral infections in 80% of cases and in 65% of cases *Rhinovirus* was detected [192]. *Rhinovirus* infections are prevalent mainly in April and September–December, in correspondence with the peak of asthmatic exacerbations [192]. In this outlook, the effect of viral infections on adhesion molecules regulating eosinophil and neutrophil migration is particularly interesting [50]. CD54-stimulating eosinophils migration is the main *Rhinovirus* receptor; this suggests that these viruses play a pivotal role in determining BHR. Recently, *Chlamydia pneumoniae* has been included in the asthma pathogenesis (Chap. 11). Interesting studies in infants and children have reported that airway viral infections are associated with respiratory allergy in children suffering from their first episodes of wheezing after 2 years of age, chiefly if they are at risk for atopy, while others continue to wheeze for years [509]. Moreover, a greater number of infections were found in babies in their first 2 years of life than in controls [50].

As both children and their airways grow, atopy exerts an ever greater impact on recurrent airway obstructive infections [61], so that while its influence is scarce in the first 2–3 years of life, as noted previously, it has a crucial role in provoking recurrent wheezing in schoolchildren [410]. In this context, studies done in Germany have revolutionized traditional concepts, leading to a critical re-examination of the role played by viral infections in facilitating allergic sensitization. The attendance in daycare facilities has increased the risk of early infections and of sensitization; however, *early infections may protect from atopy,* since 62.5% more babies in the former East Germany than in the former West Germany had access to daycare settings [464], and they are less atopic than the children attending daycare settings in the former West Germany [464, 465]. However, it appears that more children of former East Europe suffer from respiratory infections, and fewer SPTs are positive to inhalants [43]. The less ventilated modern homes with a wide use of rugs and wall-to-wall carpeting, and a scarce use of daycare facilities, might have protected children from exposure to infections, which when acquired in the first few years of life, could have protected from atopy onset [28]. The mechanism is far from being understood but revisiting other studies, the role played by infectious agents could be the inverse of current theories, that is, measles infection may protect children by significantly lowering the prevalence of allergy [393]. Evidence in support of the Th1/Th2 paradigm (a postulate of the "Hygiene Hypothesis") came from a large cross-sectional study of Japanese schoolchildren first immunized with BCG (Bacillus Calmette-Guérin) and then tested with tuberculin, thus showing *an inverse relationship between tuberculin response* (a Th1-mediated delayed hypersensitivity reaction) *and both allergic disease and a Th2-like T-cell IL profile* [400].

First, the initial observations on the protective effect of measles [393] and mycobacterial infections [400] have not been replicated in larger studies in Western communities. Second, the results of a recent study do not support the hypothesis that BCG vaccination in early infancy is associated with a subsequently decreased risk of atopic sensitization or of clinical manifestations of atopic disease in general [142]. Third, a study on 20,690 atopic children who had had measles has shown a positive association between measles and atopy, which was evident at all ages, in both urban and rural dwellers, and among subjects with many or few contacts at home or in daycare facilities [324]. An alliance between virus and mycobacteria may downregulate Th2 by inhibiting their ability to respond with IgE production due to a decline in Th2-like T-cell ILs: it is likely that PBMCs in response to a microbial challenge produce IL_{12}, IL_{18} and TNF-α by supplying a valid signal to inhibit Th2 proliferation directly or priming NK cells to generate IFN-γ, with ensuing Th1 preferential activation [363]. This hypothesis is substantiated by the observation that infections may indeed result as protective, especially if in concomitance with low socioeconomic status and domestic crowding [44], thus inducing frequent infections (Fig. 4.29) [79]. Thus, a declining number of infections in the first years after birth may occasion respiratory allergy [79]. However, the exposure to a range of infections *in utero* was associated with a small increased risk of developing allergic disease [276].

These data are in line with the revisitation of those theories binding atopy to viral infections [50, 356], which may be able to tip the scales toward Th2 [79]. From this point of view, the pertussis toxin is an adjuvant for IgE generation vs previously encountered antigens in a dose-dependent way; in humans pertussis is associated with BHR, which extends for several months, and in atopic children there is correlation between IgE and IgG response to pertussis toxins after booster immunization [309]. Viral infections cannot be underestimated: mainly in the first few years of life, they may damage the special mechanisms of the mucosal barrier, thus facilitating penetration of allergenic molecules and consequently B lymphocyte activation, isotype switching to IgE antibodies and the pertinent inflammatory cascade [50, 356]. At the same time, ETS fosters the increase in IgE concentrations (Table 4.24): *the pairing of virus and smoke modulates atopy genesis.* As we report in Chap. 22, children with RRI and with the tran-

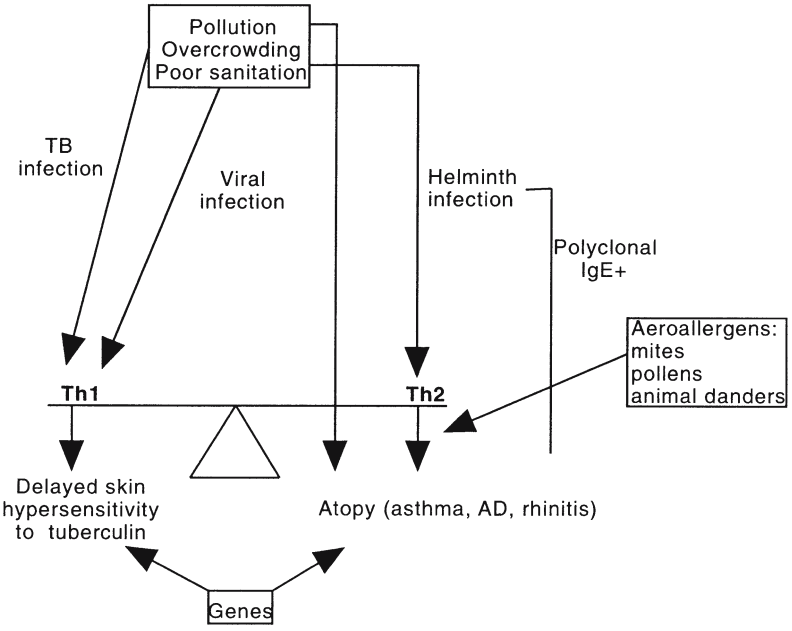

Fig. 4.29. Effect of infections. Atopy is reciprocally related to immunity to tuberculosis (*TB*). If a subject has predominantly Th2 cells, the Th2 phenotype interacts with environmental allergens to produce atopic dermatitis (*AD*). Infections may alter the Th1/Th2 balance, shifting it toward the Th1 phenotype. The clean living conditions of Western society, by reducing the incidence of infection, may tip the balance toward the Th2 phenotype and predispose individuals to the atopic march. (Modified from [79])

sient deficit in CMI are susceptible to undergoing the inactivation of normal mechanisms of antiviral defense.

Considering that several patients without a genetic predisposition suffer from atopic disease, it is obvious that the underlying source of asthma and atopy is not yet fully known. Viral infections often precede asthma onset, but more convincing is the frequent occurrence of unidentified viral agents, in line with certain epidemiological characteristics of asthma; therefore, the identification of causative agents may be the first step to define preventive strategies. The intervention is feasible when viewing an early vaccination as highly efficient in directing immune defenses toward host protection [169]. The original sin is that while the type of virus and the timing of exposure may play a critical role in both the initiation and induction of atopy and asthma, the immune system is no longer engaged in reacting to infections, tuberculosis, etc. Rarefied by vaccinations and effective treatments, it fails to produce Th1 and IFN-γ and becomes hyperactive to allergens, thus skewing to Th2 and IL_4 and contributing to the *unprecedented rising severity and prevalence of allergic disease*. Linked to Gram- infections is the polymorphism of the CD14 gene, encoding the high affinity receptor for endotoxin [15].

Dietetic Factors

Food allergens are the most frequent cause of atopic sensitization in the first few years of life, when the intestinal barrier is more permeable to protein macromolecules. The first 4 weeks of life appear to be particularly at risk because sIgA antibodies are absent at birth, since sIgA are able to limit the absorption of food macromolecules and play a protective role against viral infections. This absence is overcome by the very important immune protection ensured to neonates by receiving *colostrum and breast milk* rich in sIgA and other protective factors (Tables 2.14, 2.17). The first unequivocal demonstration of the role played by foods in FA dates back to Talbot, who reported AD development in a 3-week-old girl breast-fed from birth after the mother had eaten a pound (454 g) of *milk chocolate*. The symptoms cleared when the mother avoided chocolate and recurred when she ate it again [428]. A look at studies on atopy prevention suggests that dietetic restrictions in mothers breast-feeding children genetically predisposed to atopy development have yielded convincing results. Associating exclusive and prolonged breast-feeding with detailed dietetic measures and allergen avoidance, the prevalence of atopic disease and the severity of symptoms were reduced even in children already suffering from atopic disease (Chap. 24).

There is little doubt that *early weaning* inversely affects atopy development. A prospective study demonstrated a direct linear relationship between the number of solid foods introduced into the diet by 4 months of age and the subsequent development of AD, with a threefold increase in chronic or recurrent eczema at 10 years of age in infants who had received four or more solid foods before 4 months of age compared to those who received no solid foods by the same age (Table 4.30) [122], thus confirming the results of a classic study [197]. Introduction of other milk before age 4 months increases the risk of both asthma and wheezing [310].

Recent studies support the theory indicating that *anomalies of essential fatty acids* (EFAs), especially long-chain polyunsaturated EFA LC-PUFA, may negatively influence immunoregulation: according to the type and pathway of introduction, both T and B immune responses could be either amplified or suppressed. Breast milk LC-PUFAs suppress OX40 levels and de-

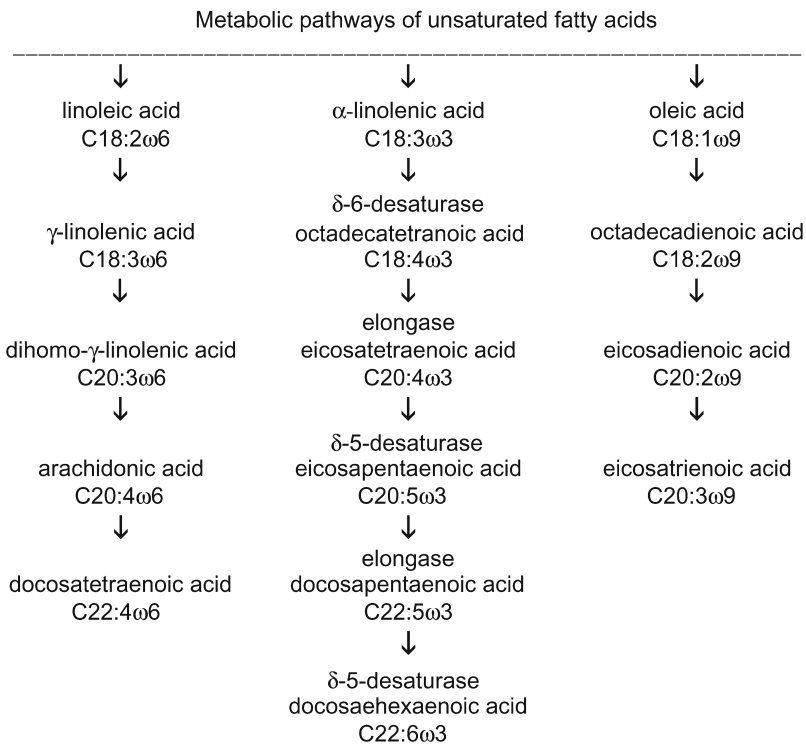

Fig. 4.30. Outline of fatty acid biochemistry

Table 4.30. Risk of recurrent/chronic eczema by early introduction of solid foods into the diet

No. of solid foods given (0–4 months)	Risk of eczema (%)	
0	13.7	13.2
1–3	17.0	16.4
4+	21.5	20.2
p	<0.05	<0.05

The first column shows the rates of eczema before adjustment for confounding variables; the second column shows the same data adjusted for confounding variables.
Data from [122].

crease Bcl-L and Bcl-2 expression, thus protecting against atopy, asthma, autoimmune diseases and diabetes mellitus [94]. EFAs consist of monocarboxyl side chains; their classification is based on the number of C atoms and double bonds, which reflect the main determinants of their specific biochemical and functional characteristics. Figure 4.30 outlines EFA, long-chain fatty acids (LCFA): the first two lines show polyunsaturated EFAs, the third line monounsaturated EFAs. EFAs derive from linoleic (ω6 series) and α-linolenic (ω-3 series) acids: ω-3 series acids are found in high concentrations in diverse fish varieties and, along with ω6 series acids, are the main constituents of the cell membranes of all tissues. From the linoleic acid supplied with food, via δ5- and δ6-desaturase and elongase enzymes, humans form a family of derivatives including, in order, γ-linolenic, dihomo-γ-linolenic and arachidonic acids. However, mammalian cells are devoid of desaturase enzymes that introduce double bonds into the ω-6 and ω-3 positions, so an insufficient energy intake may cause deficiency symptoms. The deficit could be the effect of an as-yet undemonstrated deficit of δ6-desaturase, due to deficiency of prostaglandin and leukotriene precursors. EFAs are acquired via placental transfer: the fetal daily requirement is 400 and 50 mg/kg of ω-6 and ω-3 series, respectively, but the immaturity of both desaturase and elongase enzymes determining their synthesis is destined to persist if infants are not breast-fed or do not receive opportune integrations (Chaps. 2 and 7). During the last trimester of pregnancy, the fetal brain accretes the necessary EFA rapidly, in contrast with solely small amounts of the precursors linoleic and α-linolenic acids [212]. We believe that only a few topics have aroused as much interest and debates in immunology as EFA levels. Koletzko et al [212] have produced a worthy review on breast milk variations of EFA levels in several countries. In particular, their meta-analysis of 14 European and 10 African studies [212] provides mean values that are well suited as a point of reference to evaluate the indications of these integrative treatment in atopic children. In persistent Epstein-Barr virus and human ID virus (HIV) infections, as well as in AD (in AIDS) there is a high incidence. As a consequence, atopic and nonatopic children may differ with respect to the proportion of various LC-PUFA received through breast-feeding or perinatal supplementation which promotes the Th1 phenotype and thereby *protects infants against atopic respiratory disease* [93].

Table 4.31. Number of fields where transgenic cultivations are being experimented

Country	No.
France	443
Italy	233
United Kingdom	179
Spain	140
Holland	109
Germany	105
Belgium	99
Sweden	53
Denmark	40
Finland	22
Greece	19
Portugal	12
Ireland	4
Austria	3

Source, NIH data.

In food, not only additives, pesticides (Table 4.21) and different pollutants are found, but also *genetically modified organisms* (GMO) are now part of food intake, and we are not aware of the extent of the impact they may have on the development of infantile allergy, not to mention the use of unspecified chemicals and the notable prolongation of preservation time [57]. Such a concern is intensified by observing that, either for the introduction of new compounds and additives or techniques devised for the genetic manipulation, even fresh food items differ in many respects from those available only a few decades ago [28]. The type of foods eaten has changed dramatically with the introduction of the GMOs (Tables 1.78–1.79), available everywhere (Table 4.31). Virtually nothing is known regarding the possible influences on childhood allergy of such changes in their diet [28]. The original sin of GMOs is fully debated in Chapter 9.

Figure 4.31 [71] illustrates the factors influencing atopic disease development from the fetus to the adolescent [71].

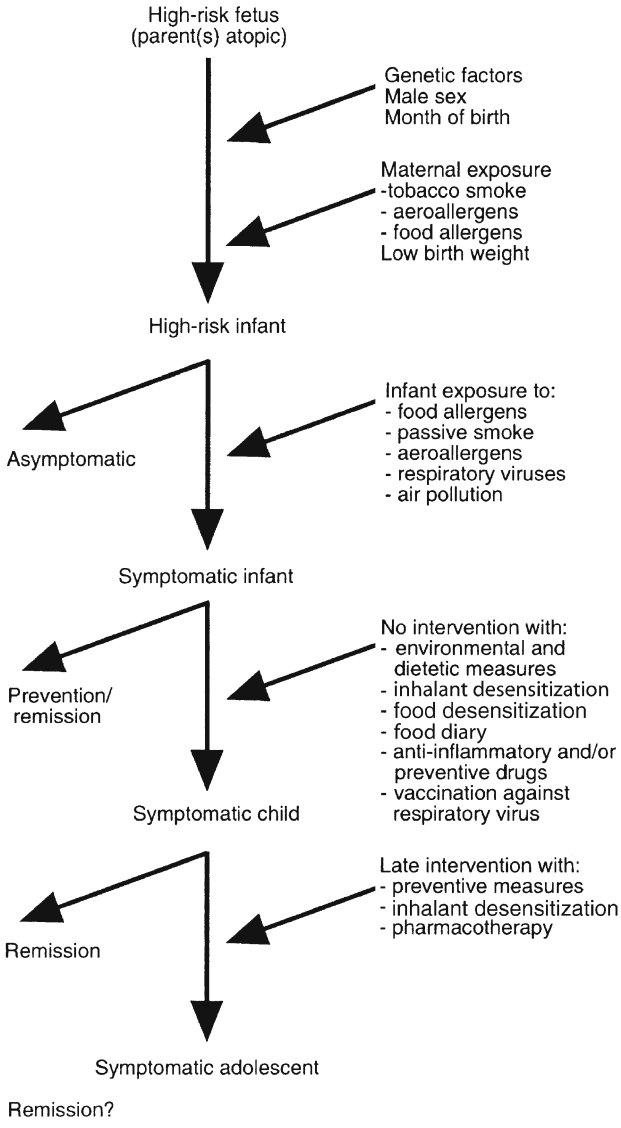

Fig. 4.31. Factors known to influence the development of atopy, from the fetus to the adolescent (see also Table 4.16). (Modified from [71])

Additional Factors of Importance for Atopy Development

Besides the factors incrementing IgE concentrations (Table 3.8), those arising more or less frequently as favoring the appearance of allergic disease are the following:

- Low birth weight (LBW) was not associated with asthma at 6 years of age [346] or at 13 years of age, while greater birth length was associated with an increased prevalence of reported asthma [233]: low (≤38.5 wk) or normal gestational age was an important determinant of asthma at 6 years of age in a birth cohort with FHA, significantly greater among boys (OR: 8.15) than among girls (OR: 1.90) [346].
- In premature babies, the incidence of asthma or wheezing is higher in those subjected to ventilatory assistance compared to unassisted babies [248]; specific risk factors for preterm babies are parental history of asthma, maternal smoking, siblings at home and neonatal O_2 supplements [116].
- In 8,088 children, infections in pregnancy and contraceptive use before pregnancy increased the risk of allergic disorders. Infections in the first term increased significantly the risk of AR and AD in the offspring [493].

- Complications and/or surgery either of the mother or neonate, including prematurity, delivery-related stress, delivery by Caesarean section, especially when complicated for medical reasons, prolonged O_2 treatment, anesthesia and surgery in the neonatal period, trisomy 21, infectious gastroenteritis, Hirschsprung's disease, prior intra-abdominal surgery in the clinical history of infants with CMA [459] and the number of hospital admissions in the first months after birth [116].
- Asthmatic children with recurrent early infections and antibiotic courses were at a lower risk of being symptomatic at school age, since the number of fever episodes and antibiotic courses were strongly inversely related to the prevalence of atopic sensitization [463]. A subsequent study in children from Belarus did not support the hypothesis that infection protects from recurrent wheezing or AD in the first year of life [216].

An analysis on 4,065 children has shown that early or threatened labor and malposition or malpresentation of the fetus were significantly related to a higher risk of developing asthma later in life (10.1%) [8], as well as neonatal illness in the 1st week of life [299]. Caesarean section had a strong effect on current doctor-diagnosed asthma in adulthood, with an adjusted OR of 3.23 (95% CI, 1.53, 6.80). However, no substantial effects were observed for atopy, AR, and AD [494].

Concerning latex and plants, see Chap. 8. In 16-year-olds a study has reported 167 cases of inhalant allergy caused by quilts due to raw silk employed in the fabrication; diagnosis was done by prick + prick test and RAST [120]. Jute, hemp, kapok and cotton widely used for carpets, underwear, etc. are irritants through contact, inhalation of the dust diffused, or as a possible substrate for growth of mites and molds.

Hygiene Hypothesis

The Th1/Th2 paradigm has been proposed as an explanation for the hygiene hypothesis; namely, the risk of allergic diseases is reduced by infection in early childhood [395]. An immunity skewed toward the Th2-type cytokine pattern at the cost of Th1-like ILs is a characteristic of being atopic [142]. Moreover, the results of a national cross-sectional survey (NHANES III) show a strong positive association between a history of Th2-mediated allergic disorders and Th1-mediated autoimmune disorders, and no epidemiological evidence to support the Th1/Th2 paradigm as conventionally understood [395]. The findings in a group of Estonian children do not support the hypothesis of an immune deviation with decreased Th1 and increased Th2 responses leading to atopic disease [195]. Therefore a mechanism of immune modulation throughout life, whereby both Th1 and Th2 responses are enhanced or suppressed in concert, provides a better way in which to view the effect of microbial agents on the development of atopic disease [395].

Originally, Strachan [417, 418] demonstrated an inverse relationship between birth order in families and the prevalence of wheezing, AR and AD [417], thus implying a different lifestyle. He suggested that the risk of allergic diseases is reduced by infection early in life. Moreover, several factors such as household size, infant dietary habits, vaccination status, antibiotic use, and migration history may alter immunoregulation. In a further comparison between East and West German schoolchildren [20], parasitic infections were significantly higher and atopic sensitization lower in the former than in the latter. Recent epidemiological studies have shown that children with siblings have a lower prevalence of allergies and asthma than do children without siblings. This association has been attributed to a preventive effect of cross-infections from older siblings in large families (the socalled *sibling effect*) and start daycare attendance at a younger age. This hypothesis is supported by a plausible biological mechanism, according to which early-life infections stimulate Th1 lymphocytes that may inhibit the expansion of allergen-specific Th2 lymphocytes, thus limiting the development of allergic diseases [359]. According to the hygiene hypothesis, widespread vaccination practices, and *increased use of antibiotics* in childhood, have led to a lower cumulative exposure to microbial pathogens, and as a consequence, to a prolonged propensity toward Th2-skewed immune responses, which prevail in atopic children who grow up in West Germany [352]. These results demonstrate differences in the Th1/Th2 response in two regions of Germany [352]. The atopic children from western Germany showed Th2 polarization; in eastern Germany, as in Estonian children [195], atopy was associated with Th0 responsiveness and production of IFN-γ in >60% of cases [352]. At birth there is a shift to Th2 T cells, which then prevail in FHA^+ neonates, whereas FHA^- neonates evolve toward a Th1-dominated pattern, as we stressed in Chap. 2. Subsequent studies have continued to explore the arguments for and against this hypothesis [395]. According to the postulates of the hygiene hypothesis:

- The number of siblings: the decreased prevalence of atopy is related to an increased number of older siblings [16, 465] or the presence of siblings did not have a significant association with sensitization at 12 month and 4 years [202].
- Daycare attendance started before the age of 1 year assured a lower prevalence of asthma, AR, and atopy compared to the children who started after the age of 1 year in a cross-sectional German study of schoolchildren [218]; at 2 years there was a lower RR of developing asthma [16].
- The type of dwelling (poorly ventilated homes, domestic crowding, furnishing, dampness, etc.) is often associated with atopy [28], which contrasts with increased prevalence in advantaged children [485] and the reduced prevalence [473] or not [146, 392] in connection with economic factors [464].

• In particular, domestic crowding is controversial [44, 488], because strikingly high IgE concentrations are dependent on the number of people living in one apartment [20], or because of a highly significant inverse relationship with atopic sensitization [44].

Recently, in a study of 2,531 children followed from birth to 4 years of age, early respiratory infections increased rather than decreased the risk of developing bronchial obstruction during the first 2 years of life and of having asthma at 4 years of age [299]. However, if a reduced microbial turnover at mucosal surfaces may be responsible for the association between hygiene and atopic disease among populations adopting a Western lifestyle, the sites involved may be those where APCs take up, process and present microbial and nonmicrobial antigens to specific T cells. The quality and intensity of bacterial stimulation would dictate the state of activation and the kind of accessory signals transmitted by APCs to surrounding cells of the innate and acquired immune system [271]. If a high microbial turnover educates our immune system, mimicking this education could safely revert the epidemic trend of atopy in a kind of early life immuno-education [271].

We conclude that the hygiene hypothesis is no explanation for the increasing prevalence of atopic diseases in the developed world (Chap. 5).

Interactions Between Genetic and Environmental Factors

Several factors that when isolated fail to influence atopy have the potential ability of reciprocal stimulation and provoke atopic disease in the first years of life, such as CD3, CD4, CD8 CB levels and parental smoking, becoming significant factors when associated [71]. In two-thirds of children with CMA (hospitalized at the mean age of 13.6±1.2 weeks of life), at least one of the following conditions was noted: parental atopy, no breastfeeding and one of the conditions pointed out above, with a significantly greater prevalence than in the average population [459]. Several genetic and environmental factors, alone or associated, condition raised IgE and CBIgE concentrations: male sex, cigarette smoke [513], parental asthma, SPT positive to egg at 4 months, and to CM, egg, peanuts at 1 year after birth, as well as nasal eosinophilia and basophilia [512]. We summarize in Table 4.32 [71] the most important genetic–environmental factors in HR infants and children.

Pediatricians, Genotype, Phenotype and Early Predisposing Factors

I would like to take a further step in my comments, by stressing the unique function of pediatricians, traditional advocates of children's health and above all a front-line source of information and guidance for parents, who live atopic disease with understandable anxiety and worry. Since intervention on the genotype is not achievable, it is more realistic to aim at modifying deleterious environmental factors but, apart from allergen prevention, a particular factor to be taken into consideration is the *quality of life of young patients* who, as previously seen, are the victims of pollutant aggression from the very day of their birth (Table 2.26). Even if there exists a substantial gap between what is likely and what we can put into practice, limiting the pollution impact is certainly achievable. Pediatricians should be a champion of no smoking, especially in homes with children, also suggesting that children stay away from homes with smokers. The introduction of CO_2 and other gases in the atmosphere cannot be influenced, yet one should stay away from places and moments with increased concentration of motor exhaust fumes. The ozone depletion leads to a T rise in thereby pollens begin damage earlier. Transgenic vegetables are another aspect of so-called progress that will be dealt with in Chap. 24. We stress that *children* are more at risk because they breathe more rapidly given that their airways are narrower than those of adults, so they have markedly increased needs for O_2 relative to their size, and *inhale more pollutant per kg/bw* than do adults and spend more time in outdoor activity [229]. Children are especially exposed to environmental toxins because kg for kg/bw they drink more water and eat more food. Moreover, their hand-to-mouth behavior increases the ingestion of any toxin that toddlers may find in dust or soil, and playing close to the ground may increase the exposure to toxins in dust, soil, and carpets. Much is still unknown about the long-term effects of exposure to pesticides, and children may be more vulnerable than adults. However, pediatricians should suggest that parents encourage children to eat a variety of fruits and vegetables: the health benefits outweigh the risks of pesticide residue consumption [229, 350]. According to the legend of Romulus and Remus, the twins were abandoned by their mother on the Tiber rivers and were suckled by a wolf. The twins survived in such a hostile environment as the Tiber river although they were fed with milk as different as wolf's milk. However, they grew so strong as to enable them to build Rome. This fascinating legend teaches us that *human newborns are able to overcome great difficulties*. However, there is no doubt that the twins would have failed to react in this way if pollution had been as widespread as in our times. Children in the New Millennium: Environmental Impact on Health, a new study released by three United Nations agencies, shows that every day 5,500 children die from diseases caused by consuming water and food polluted with bacteria [481].

Table 4.32. Main genetic–environmental factors responsible for or contributing to the development of atopic disease in high-risk infants and children

Genetic/immunological/perinatal factors
Positive family history of atopy
Altered IL_4/IFN-γ ratio
Altered CD4/CD8 ratio
Intake of alcohol and/or caffeine during pregnancy
Intrauterine exposure to drugs
Male sex
Maternal smoking during pregnancy
Month of birth
Prematurity, perinatal stress, surgery in the neonatal period
RFLP atopy in general
Markers with a probable genetic basis contributing to the atopy phenotypic expression
Number of mast cells/basophils and their releasability
Number of IgE receptors and affinity for IgE antibodies
Relationships with the autonomic nervous system (α-adrenergic hyperresponsiveness, β-adrenergic hyporesponsiveness, cholinergic hyperresponsiveness)
Target organ sensitivity
Clinical and environmental markers
Alimentation during the first days/months of life Absent or reduced breast-feeding Cow's milk allergens Egg allergens Additional food allergens Early introduction of solid foods Weaning period and procedure Food additives
Positivity of skin tests for foods and inhalant allergens
Presence of IgE antibodies specific for foods and inhalant allergens
Exposure to allergens during the first months of life, especially at birth Foods Inhalants
Early exposure to specific or nonspecific factors Antibiotic therapy ETS at home Home crowding Home dampness Presence of pets at home Urban air pollution (as adjuvant) Viral and/or bacterial infections
Range of allergen concentration
High prevalence of atopic disease
Severity of clinical manifestations
Multiple atopic disease

The hygiene hypothesis considers crowded living conditions, early exposure to pets and to group daycare as factors decreasing the risk of atopy: the truth may be also the opposite.
Data from [71].
RFLP restriction fragment length polymorphism.

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Epidemiology and Natural History of Atopic Disease

Epidemiology

In this chapter we analyze the unending worldwide increase of pediatric allergic diseases and their natural history. Of concern is the early age at which atopy starts its march in these defenseless children.

Definitions. Epidemiology, literally a study on the population, is the correlation existing between diseases and certain factors regulating the appearance, diffusion, and outcome of these diseases in a human community. During the last 3 years, the study of both frequency and distribution of atopic disease has been particularly intensified to better identify the factors triggering atopy onset.

The *natural history* of atopic disease usually establishes a sound scientific basis to arrange measures able to modify the phenotype, thus improving both the course and prognosis of these diseases.

We hereby add a number of definitions to calculate the pertinent titers [303]:

- *Incidence* of a disease is the frequency, that is, the number of new cases of a particular disease in a defined population identified during a given time (usually 1 year, or the last year), also called 12-month prevalence.
- *Prevalence* of a disease is the diffusion, that is, the number of cases of a disease in a given population at a specific period of time.
- *Point prevalence* is used at a particular point of enquiry, expressed as a proportion of the total population.
- *Period prevalence* expresses, over a specific period of time, the relation between the number of cases at a defined observation period and the total population.
- *Cumulative prevalence*, that is, the number of cases in a given population who have had a given disease [290].

The terms *lifetime prevalence*, the existence of a disease at any time in an individual's life, and the *current prevalence*, the existence of a disease at the present time of the individual's life, also-called *12-month prevalence*, have been recently added.

In the many tables of this chapter, we have tried to use a unifying method to link different data characterized by their non-homogeneity. The result is a high, unexpected prevalence/incidence of atopic diseases.

Lack of Uniformity of Diagnostic Parameters

Several parameters can invalidate the indispensable prerequisites of epidemiological data (Table 5.1) [20, 31, 33, 100, 112, 232, 294, 341] such as the lack of uniformity of terminology in establishing definitions and of criteria employed for the diagnosis [232]. Apart from most prospective studies, some are retrospective and bedeviled by problems of subject memory, such as the frequent reports affected by parents' recall bias, and liable to variations ranging from 25% [69, 127, 193] to 44% [142]. In a prospective study conducted on 480 newborns followed in a general pediatric practice through their third birthday, 28% were thought to have had an adverse food reaction [33]. Of the 133 children identified, only 38 (8%) had their symptoms confirmed by an oral food challenge (OFC). In a retrospective study, up to 25% of the conditions diagnosed in a child from birth to the age of 1 year were forgotten or not reported by the parents of 11-year-old children [69]. Only 37% of parents knew that their children suffered from asthma, and even fewer (32%) knew that their children had rhinitis [289]. Use of information obtained from both parents induces important bias. Such bias is reduced when the cases, for example of atopic dermatitis (AD) and asthma are diagnosed by doctors [268, 322]. Parents are less worried about respiratory problems, forget the wheezing episodes of their grown-up children, which were instead reported regularly in early infancy [193, 222], whereas only 68% of fathers and 72% of mothers recollected the feeding procedures [346]. Parents reported recurrent wheeze in 19.4%–25.9% of children, the doctor diagnosed even asthma in 6.9% of their offspring [332]. Over the years, 40% of the children failed to confirm the data originally elicited by their parents [142] and more than one-third of young adults did not remember that they had wheezing episodes during their infancy [303]. Parents with many children may not recall a relatively mild disease such as AR (allergic rhinitis) as accurately as parents with one or two children [330]. Some trials are based on objective measurements, others on responses to questionnaires, which when not standardized can be a catalogue of different definitions of disease and significant variations on prevalence data [232, 330]. Differences in diagnostic criteria may be due to relative-

Table 5.1. Parameters accounting for the discrepancy of data

1. Variable diagnostic criteria
2. Terminology used to establish a precise definition of disease
3. Lack of supportive allergic/immunological data
4. Heterogeneity in terms of age, sex and socioeconomic status
5. Criteria of selection (atopy of children, of parents)
6. Evaluation of family history
7. Evaluation of exogenous factors
8. Small number of children, hence more random fluctuation
9. Randomization, control groups, so the development of new diagnostic criteria, or the differing definitions of clinical cases
10. Representative criteria (general population or selected) of the samples studied
11. Diagnosis of atopic disease (doctor, clinical, laboratory, pediatricians, allergists, etc.)
12. Diagnosis of atopic disease (skin tests, sIgE study, provocation tests, blinded evaluation)
13. Statistical analysis of data
14. Prospective vs retrospective design
15. Possibly confounding factors
15. Drop-out rate
16. Compliance
17. Duration of follow-up: monthly, trimester, yearly controls, or start-end
Additional guidelines for studies on atopy prevention:
18. Environmental controls (smoking, mites, pets, chemicals)
19. Duration of breast-feeding or with special formulas
20. Dietary restrictions of the nursing mothers (diet duration, foods eliminated)
21. Time solid foods introduced
22. Type of foods introduced
23. Qualification and experience of study clinicians, definition of study population

Data from [20, 31, 33, 80, 112, 232, 294, 341].
sIgE specific IgE.

Table 5.2. Factors underlying the increased incidence and prevalence of atopic disease

Increased exposure to allergens
Environmental pollutants
Improvements in personal sanitation
Lower cumulative exposure to infections
Increased use of antibiotics in childhood
Increase in mobility in general and in social relationships
Decreasing numbers of siblings

Data from [171, 176, 330].

ly few studies involving clinical examinations [317]. In a trial on patients affected with AR, two-thirds were rejected at the military check-up because they did not respond to fixed parameters [334], while an observer found in children a 1.4-fold increased prevalence [3]. However, groups from medical practices tend to represent the more severe cases [155]. The sample variability has selection bias, but by selecting >400 patients, a type II error is avoided [290]. In the field of statistics, more and more frequently papers are committed only to χ^2; however, odds ratio (OR) confidence intervals and other specific tests should be employed [290]. A related crucial parameter is whether the trial has been carried out by using the double-blind placebo-controlled food challenge (DBPCFC). A DBPCFC study in subjects declaring a 7% allergy to food additives indicated a prevalence of only 0.023% [356]. In the field of food allergy (FA), the comparison between clinical response to elimination diet or food challenge test (FCT) and SPT or sIgE (specific IgE) results may invalidate the conclusion: SPTs may individuate as offending only the food that has provoked a type I immune reaction, but they do not identify the frequent late reactions (Chap. 6). The ISAAC (International Study of Asthma and Allergy in Children) has proposed a standard written questionnaire. Recently, the written questionnaires have been developed as video questionnaires obtaining a 78% concordance [231] with an ISAAC protocol. In Turkish children aged 8–11 the ISAAC found a prevalence of current wheeze of *11.5%* and of physician diagnosed asthma in *6.9%* [266].

In the tables showing epidemiological results, although we have preferred doctor-diagnosed or questionnaire data (such as ISAAC "has your child ever had asthma, eczema, rhinitis?"), methodological differences in several studies make it difficult to compare the magnitude of the difference in prevalence/incidence between countries, and among populations in countries of widely differing lifestyle and ethnic groups. Even when differences in methodology are allowed for, atopic diseases are alarmingly frequent and their prevalence has substantially and progressively increased in industrialized countries over the past 30 years. Undoubtedly, a wide spectrum of factors, both genetic and environmental, are mainly responsible for this; however, several recently appreciated features emphasize the multifactorial mechanisms behind any such disease (Chap. 4). Table 5.2 summarizes the factors that explain such an increase [171, 176, 330].

Table 5.3. Prevalence of food allergy (excluding CMA) according to several studies[a]

Author(s)	Year	Country	No. and type of cases	Frequency (%)	References
Retrospective studies					
Kajosaari	1982	Finland	866 NS healthy children	18[b]	[149]
Prospective studies					
Croner et al	1982	Sweden	1,701 NS neonates	1.5	[72]
Hattevig et al	1987	Sweden	59 NS healthy children	8	[130]
Bock	1987	USA	480 NS healthy children	7.7	[33]
Zeiger et al	1992	USA	288 at-risk infants	5	[359]
Bruno et al	1993	Italy	174 at-risk infants	1	[40]
Saval et al	1993	Denmark	1,052 atopic children	1.8[c]	[268]
Bergmann et al	1994	Germany	4,636 at-risk infants	3	[29]
Eggesbo et al	1999	Norway	533 NS	19.3	[87]
Kristjansson	1999	Sweden, Iceland	324 healthy	2.0	[167]
Lee et al	2001	Korea	38,955 ISAAC	6.5	[177]
Julge et al	2001	Estonia	273 healthy	1.5	[148]
Heinrich et al	2002	East Germany	2,002 students	3.5	[132]
Jazwiec- Kanyion	2003	Poland	3,079 NS children	4.2	[143]
Kurukulaaratchy	2003	Great Britain	169 atopic	13.6	[172]
Cantani	2004	Italy	141 NS children	13.6	Unpublished data

NS not selected.
[a] Mean 5.8%.
[b] Nineteen percent at 1 year, 27% at 3 years and 8% at 6 years.
[c] In total, 2.6% at 5–7 years, 0.9% at 8–10 years, 1.7% at 11–13 years and 2.5% at 14–16 years.

Age at Onset [51]

Table 5.3 [29, 33, 40, 72, 87, 130, 143. 148, 149, 167, 172, 177, 268, 359] shows the mean prevalence of FA, Table 5.4 [33, 68, 72, 86 , 88, 103, 108, 124, 133, 136, 142, 146, 192, 239, 249, 269, 270, 273, 299, 320, 326] of cow's milk (CM) allergy (CMA) and Table 5.5 [22, 29, 32, 33, 38, 48, 53, 68, 69, 71, 93, 96, 107, 118, 120, 140, 144, 148, 153, 163, 179, 187, 198, 208, 211, 241, 242, 273, 281, 291, 355, 358] the onset age of atopic disease; the onset of peanut allergy is as high as 91.5% within the 7th year [93]. In some studies, we find a quite accurate pattern of the onset age of AD [51], asthma and AR: Table 5.5 shows that 90%–95% of cases begin within 1 and 5 years. As shown by several investigators in different countries, asthma, AR, AD and FA are among the commonest childhood illnesses worldwide, and their prevalence may be progressively increasing in the pediatric population [63, 138, 343]. The Aberdeen colleagues report high figures: in over 25 years the asthma prevalence has increased from 4.1% to 10.2% and has more than doubled in 1992–1996 [215, 221], but above all the onset age has lowered (Fig. 5.1) [291]. Recently, an increased incidence has been

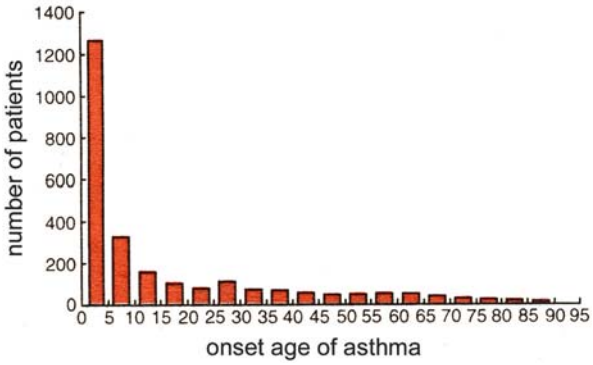

Fig. 5.1. Age of asthma onset vs number of patients

reported in the first 18 months, which was as high as 8% [39, 123], 15% [126] and 18% [29] for AD, 10% to 15% [29] for asthma, and a cumulative incidence of atopic disease of 19% in the first 18 months [127], and 34% during the 2nd year of life [29]. At variance, FA reaches a frequency of 2%–3% in the first 2 years [29, 127]. Moreover, several studies report infants with recurrent wheezing [30, 78, 122, 193, 257, 267], also with early symptom

Table 5.4. CMA incidence in different countries

Author(s)	Year	Country	No. and type of cases	No. of cases with CMA	Frequency (%)	References
Retrospective studies						
Vendel	1948	Sweden	Not known	1:7,500	0.013	[326]
Loveless	1950	USA	350,719 in general	3,691	1.5	[269]
Stintzing and Zetterström	1979	Sweden	4,311 infants	25	0.6	[299]
Prospective studies in unselected healthy infants						
Bachman and Dees	1957	USA	304 healthy infants	4	1.3	[269]
Johnstone and Dutton	1966	USA	240 healthy infants	2	0.8	[146]
Halpern et al	1973	USA	1,084 healthy infants[a]	20	1.8	[124]
			669 healthy infants[b]	6	0.9	[124]
Gerrard et al	1973	Canada	787 healthy infants	59	7.5	[108]
Jakobsson and Lindberg	1979	Sweden	1,079 healthy infants	20	1.9	[142]
Hide and Guyer	1983	Great Britain	609 healthy infants	15	2.5	[133]
Bock	1987	USA	480 healthy children	25[c]	5.2	[33]
				11[d]	2.2	[33]
Høst et al	1988	Denmark	1,749 healthy infants	39	2.2	[136]
Lucas et al	1990	Great Britain	777 preterm babies	34	4.4	[192]
				6[c]	0.8	[192]
Savilahti et al	1991	Finland	198 healthy infants	7	3.5	[270]
Schrander et al	1993	Holland	1,158 healthy infants	26	2.2	[273]
Eigenmann	2000	Switzerland	74 atopic	6	8.1	[88]
Sanz Ortega	2001	Spain	1,663 newborns	6	0.36	[249]
Eggesbo	2001	Norway	2,721	30	1.1	[86]
Garcia-Ara	2003	Spain	5,367 newborns	101	1.9	[103]
Studies in infants with one or two atopic parents and/or elevated CBIgE/sIgE levels						
Croner et al	1982	Sweden	64	6	9.4	[72]
Van Asperen et al	1984	Australia	118	8	10.25	[320]
Crespo et al	1995	Spain	355	87	24.5	[68]
Eigenmann	1998	USA	63	19	15.8	[89]
Pourpak et al	2004	Iran	119	37	45.4	[239]

Mean of 21 studies in preterm healthy infants, 2.32%, 5 studies in at-risk infants, 21.7%.
sIgE specific IgE.
[a] Free diet.
[b] CM-free diet.
[c] Diagnosis by oral provocation test.
[d] With double-blind, placebo-controlled food challenge.

onset in numerous cases [32, 48, 163, 193, 257, 355], 57% within 5 months [257] and 53%–59% within 12 months [163, 355] (Table 5.5). This is critical for males: 69% [193] or 66% of cases [145] with severe forms and bronchial hyperreactivity (BHR) [267] requiring hospital admission [257]. The highest figures in the asthma and AR onset were found in boys aged 0–5 [97]. In many babies, AR also begins in the 1st year of life [349].

Atopic March

What is called the *atopic march* is more evident in the earliest years of life (Fig. 5.2, Table 5.5): it is estimated that in the first 10 years of life, 10% of children suffer from AD, 20% from asthma, and 15% from AR [43, 69, 340, 341], while CMA prevalence (Table 5.4) is very different from that of FA. In adolescents, the cumulative

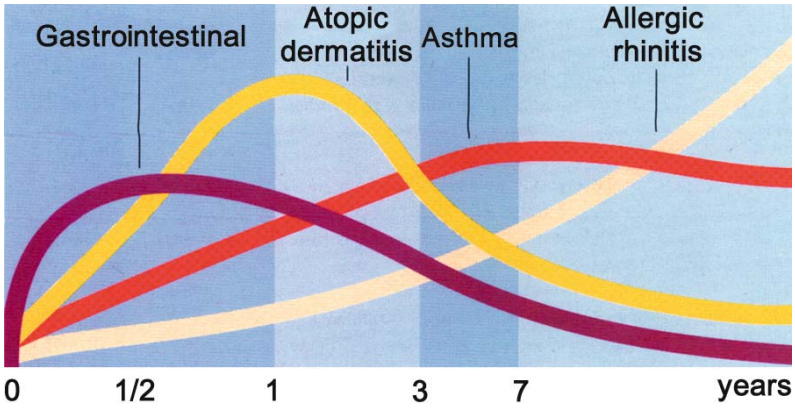

Fig. 5.2. Allergy prevalence according to age. Several children manifest more symptoms at the same time

Table 5.5. Onset age of atopic disease (%) in different countries

Country Age (years)	References	%	Subjects	Criteria and methods
Atopic dermatitis				
England	[32]			
<1		44	59 Males	A 1970–1973 study of 9,145 patients, all personally interviewed and SPT investigated
		31	42 Females	
1–2		7	10 Males	
		7	10 Females	
England	[153]			
0–0.5		47.5	2,354 Children aged 0–16 years	1989 interview by 3 family physicians, age, sex, age at onset and symptoms and signs of eczema recorded on a survey form, statistical analysis
0.5–1		12.7	50.5 Boys and 49.5 girls	
1–1.6		8.8		
1.7–3		8.3		
3–4.9		11.8		
England	[208]			
<0.5		72.2	99 Patients aged 16–17	Symptoms reported by patients, physical examination
England	[286]			
<2		8.0	3,000 Children aged 6–7 years	ISAAC questionnaire
Estonia	[148]			
0.5		4	273 Children aged 0.5–5 years	Population-based prospective study questionnaires, clinical examination, SPTs, total IgE levels, specific IgE only 2.9% of children had SPT ≥3 mm at 5 years
1		10.5		
2		15		
5		10		
Finland	[179]	47	300 Children aged 0–5 years	Retrospective cohort study
>3				
Finland[a]	[163]			
0–1		80	269 Children aged 0–8 years	Data from 2 questionnaires to 373+156 health nurses as part of a 1971 investigation and reviewed by the author
2–3		10	Out of a total of 271 aged 0–14 years	
4–5		3		
6–8		5		

Table 5.5. (Continued)

Country Age (years)	Refer- ences	%	Subjects	Criteria and methods
France	[118]	100	29 With severe clinical scores, eczema	SPTs, specific IgE, food challenge
<0.4	[241]	38.3	500 Children aged 5.7±3.2 years	Start in 1978, questionnaire to parents, physical examination, follow-up on a regular basis, prospective computerized study
<0.6		26.6	57.9% Boys, 42.1% girls	
<1		16.6		
<2		9.6		
<3		5.6		
Germany	[29]		1,314 Infants followed up from birth	CBIgE, specific IgE, started in 1990, questionnaires, clinical study
0.1–0.3		4.8		
0.3–0-6		5.5		
0.6–0-9		7.1		
1–2		8.8		
Italy	[52]			
0–2			220 Children aged 0.5->6 years	Prospective study, doctor-based clinical evaluation, SPT, total IgE levels, specific IgE
2–4		23	142 Males	
4–6		16	and 118 females	
>6		13		
Kuwait 0.1	[211]	3	Study of 10,000 consecutive new cases	Clinical study
Sweden 0.6	[120]	90	94 Atopic children, outpatient	History, SPTs
Food allergy				
Spain	[68]			
<1		48.8	353 Children with a mean age of 5.4 ±4.1 years, and a 2.2:1 male prevalence	Study done in 1989–1990, personal history, physical examination, SPTs, specific IgE (RAST), and open food challenges
1–2		20.4		
2–3		7.8		
3–4		5.7		
4–5		3.7		
USA	[33]			
1		80	480 Children followed from birth to age 3	Study in 1980–1982, 133 had reactions to foods, SPTs, open or blind food challenges
2		16		
3		4		
Estonia	[148]			
0.5		2	273 Children aged 0.5–5 years	Population-based prospective study questionnaires, clinical examination, SPTs, total IgE levels, specific IgE; only 2.9% of children had SPT ≥3 mm at 5 years
1		10		
2		1.5		
5		0		
Foods: allergy onset before 12 months (%)[b–f]				
	[242]	[198]		
CM	70	100	46 [22]	
Egg	68	64.1	81 [22]	
Cereals	62			
Fish	52	50.6		
Peanut	18.3 [93]	80[f] [358]		
Fruits	8	1.4		
Nuts	3			
Legumes	0	12.2		
Chocolate	0			
Allergic contact dermatitis				
Italy	[281]		3 Infants aged 6–12 months	Case reports

Table 5.5. (Continued)

Country Age (years)	Refer-ences	%	Subjects	Criteria and methods
USA	[96]		2 Infants aged 1 week and 7 months	Case reports
	[38]		2 Infants aged 6 and 9 months	Case reports
Urticaria				
Estonia	[148]			
0.5		2	273 Children	Population-based prospective study questionnaires, clinical examination SPT, total IgE levels, specific IgE Only 2.9% of children had SPTs ≥3 mm at 5 years
1		3	aged 5–5 years	
2		0		
5		1		
Asthma				
England	[32]			
<1		23	73 Patients	1970–1973 study of 9,145 patients personally investigated SPTs
2–5		32	98 Patients	
6–10		14	45 Patients	
Sweden	[69, 71]			
<1.5		31	80 Children with sex not stated	Questionnaires, clinical examination, medical records
<3		53		
Italy	[48]			
<1		29	3,599 Children	Epidemiological study done 1991 by 19 university and hospital centers, questionnaires done by 20 pediatricians
1–3		37	Aged <1 and <6 years	
3–6		25		
>6		4		
Finland[a]	[163]			
0–1		33	291 Males out of 299 and 163 females out of 173, all aged 0–8 years out of a total of 473 children aged 0–14	Data from 2 questionnaires to 373+156 health nurses as part of a 1971 investigation and reviewed by the author; boys had onset of asthma statistically more frequently at 0–3 years than the girls; ratio boys:girls, 1.8:1
		20		
2–3		43		
		36		
		36		
4–5		13		
		19		
6–8		7		
		20		
Italy	[53]			
0–2		19	220 Children aged 0.5–>6 years, 142 Males and 118 females	Prospective study, doctor-based clinical evaluation, SPTs, total IgE levels, specific IgE
2–4		20		
4–6		25		
>6		35		
Estonia	[148]			
0.5		0	273 Children aged 5–5 years	Population-based prospective study questionnaires, clinical examination, SPTs, total IgE levels, specific IgE; only 2.9% of children had SPT ≥3 mm at 5 years
1		0.50		
2		2		
5		6.5		

Mean age at onset	Ref	Country	%	Criteria and methods
Asthma				
4 years, 3 M, 8 F	[355]	USA		Epidemiological study
Within the 1st year	[163]	Finland	52.8	Epidemiological study
Within the 1st year	[278]	USA	59.3	Epidemiological study
Within the 2nd year	[107]	USA	45.2	Survey of a national sample, SPTs, spirometry
At ≥3 year	[60]	China	82.3	Epidemiological study
Within the 4th year	[291]	USA	80.2	Survey of a national sample, SPTs, spirometry

Table 5.5. (Continued)

Mean age at onset	Ref	Country	%	Criteria and methods
Within the 5th year	[29]	Germany	85	Multicenter study, cord blood IgE, questionnaire
Within the 5th year	[163]	Finland	82	Multicenter study
Before 6 years	[71]	Sweden	80	Multicenter study
Within the 7th year	[187]	Finland	82.2	Data from a national cohort
Within the 8th year	[107]	USA	90	Data from a national cohort
Within the 8th year	[163]	Finland	97 Males, 94 females	Data from a national cohort
Before 9 years	[71]	Sweden	95	Asthmatic children
Before 20 years	[144]	Australia	91.5–92.3	Parent-reported asthma

Country Age (years)	References	%	Subjects	Criteria and methods
Allergic rhinitis				
England	[32]			
4–5		12.5 6.5	112 Males (56%) and 88 females (44%);	A 1970–1973 study of 9,145 patients, 2,800 of whom aged 0–20, and 1,384 aged 5–15
6–10		19.5 7.5	65% of children were sensitive to	All patients were personally investigated and examined, SPT
11–20		17.5 17.5	grass pollens	
Estonia	[148]			
0.5		0	273 Children	Population-based prospective study questionnaires,
1	0		aged 0.5–5 years	clinical examination, SPTs, total IgE levels, specific IgE;
2	1			only 2.9% of children had SPT ≥3 mm at 5 years
5	5.5			
Finland	[163]			
0–1	35		200 Children	Data from 2 questionnaires to 373+156 health nurses
2–3		41	aged 0–8 years out of	as previously, rhinitis began later than asthma in 32.1%
4–5		18	a total of 205	of cases
6–8		12.7	aged 0–14	
Italy	[53]			
0–2		1	220 Children aged 0.5–>6 years	Prospective study, doctor-based clinical evaluation, SPTs, total IgE levels, specific IgE
2–4		5	142 Males	
4–6		8	and 78 females	
>6	17			
Switzerland	[350]			
1–4		13	182 Males and	Onset of allergic rhinitis was in 2/3 children
5–9		25	241 females as part	aged 1–14 years, no difference between urban
10–14		26	of a sample	and rural populations
15–19		18	of 2,524 persons	
South Africa	[201]			
Birth		29.7	771 Children aged 3 months to 15 years	Prospective study, single observer, SPTs, total and specific IgE levels

M males, *F* females, *SPTs* skin prick tests.

[a] The first figure indicates the males and the second the females.

[b] In Spain [242] in a study with 189 babies with food allergy, 93 had an onset before 12 months of age.

[c] In Spain [65] a study of 416 babies aged <1 year showed 617 immediate reactions to the pertinent foods.

[d] In Italy [22] 92 babies were studied, 49 males and 43 females, with a mean age of onset of 2.8±2.1 months, SPTs and specific IgE (RAST).

[e] An English study [93] on 60 children established the prevalence.

[f] In a preventative study done in the USA [358], 20% of 103 prophylactic-treated and 60% of 185 control infants were exposed to peanuts within their 1st year of life.

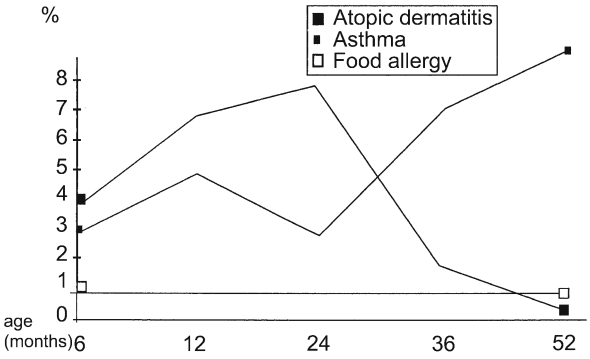

Fig. 5.3. Prevalence of atopic disease in 174 children at high risk of atopy according to their age. (Data from [39])

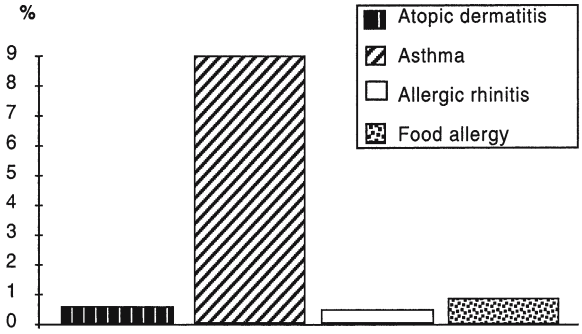

Fig. 5.4. Percentage of the 174 children with atopic disease at the 52-month follow-up. (Data from [40])

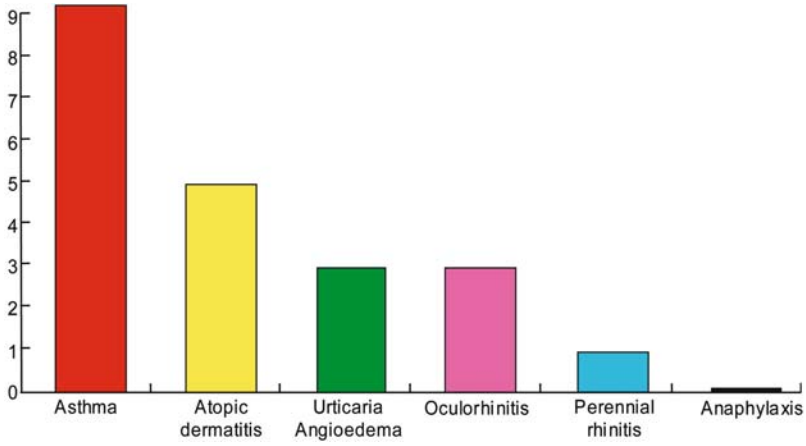

Fig. 5.5. Prevalence of atopic disease in children (for details, see text). (Data from [48])

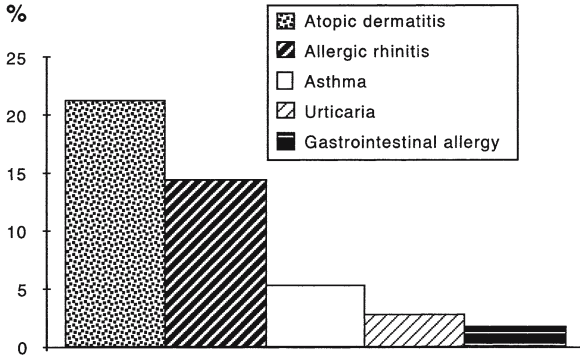

Fig. 5.6. Cumulative incidence of atopic disease in 1,654 children between 0 and 11 years of age. (Modified from [69])

incidence of atopic disease has a sensitivity frequency, between 25% and 31% and the prevalence is 21% [70, 321]. However, in different countries lower values are found, so that a dichotomy is observed: in Finnish children and adolescents the prevalences are 1.7% for AD, 6% for asthma and 4.3% for AR [240], evidently due to favorable environmental conditions. Therefore no univocal values are detected, except an increased prevalence in several countries or categories such as in preterm babies [192]. In 174 at-risk children followed-up for 52 months (Figs. 5.3, 5.4) [39, 40], the cumulative incidence of atopy was 18.5% [39], and 22.1% in the multicenter study (Fig. 5.5) [48]. These results should not be considered as timelessly invariable: for example, a considerable increase in asthma prevalence has been found in Australia [252] compared to 1969 [340] and between 1982 and 1992 [233]. Moreover, in Swedish children followed up for 11 years, the cumulative incidence of atopy was as high as 18.2% at 7 years and 32.5% at age 11 with a 23.7% prevalence [69]: Fig. 5.6 [69] shows the cumulative incidence of atopy between 0 and 11 years and Fig. 5.7 [70] the prevalence at age 11. Figures 5.8 and 5.9 depict the trend of atopic disease prevalence at distinct ages [53, 268]; in Fig. 5.9 [268], urticaria angioedema and oral allergy syndrome (OAS) are added. In the former East and West Germany [27, 329–331], AD prevalence is greater in the former East, the other atopic diseases in the former West. There is a substantial worldwide increase in the incidence of symptoms of asthma, AR, and AD in children aged 12–14 from 56 countries [311], including Arctic countries [166, 306].

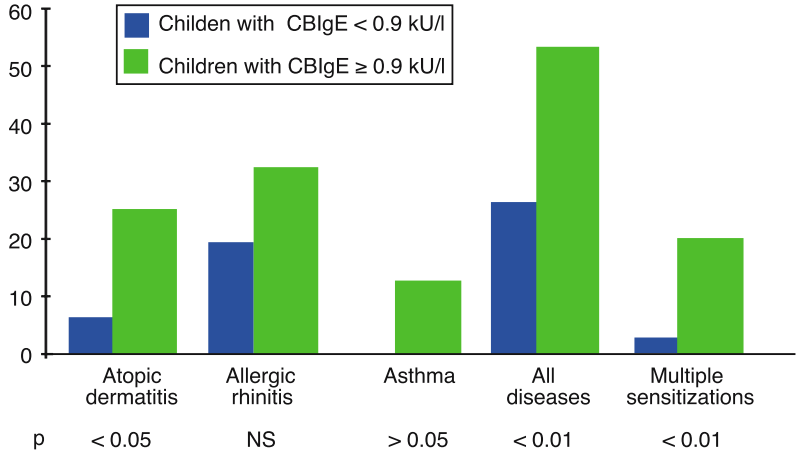

Fig. 5.7. Prevalence of atopic disease at the age of 11 years according to the cord blood IgE (*CBIgE*) levels. (Data from [70])

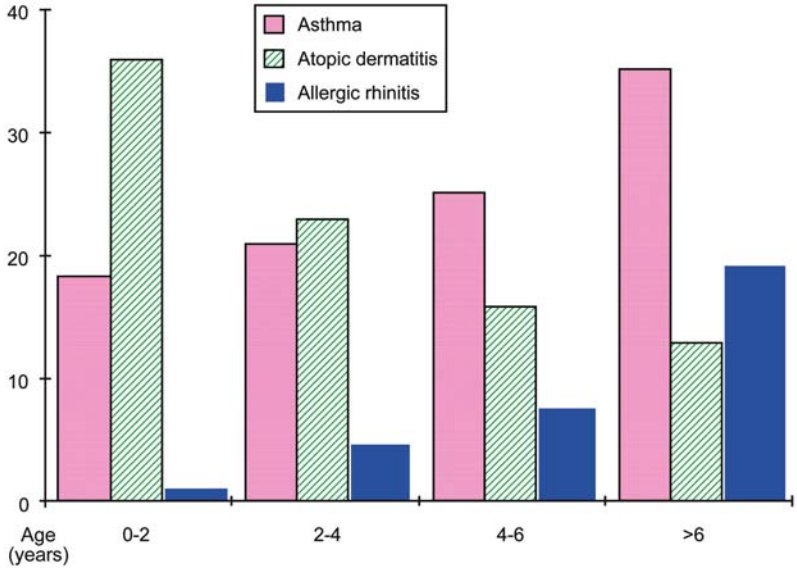

Fig. 5.8. Prevalence of the atopic march among 220 children divided into four groups according to their age. The slow decrease of AD prevalence, asthma progression and to a lesser degree AR should be noted. (Data from [53])

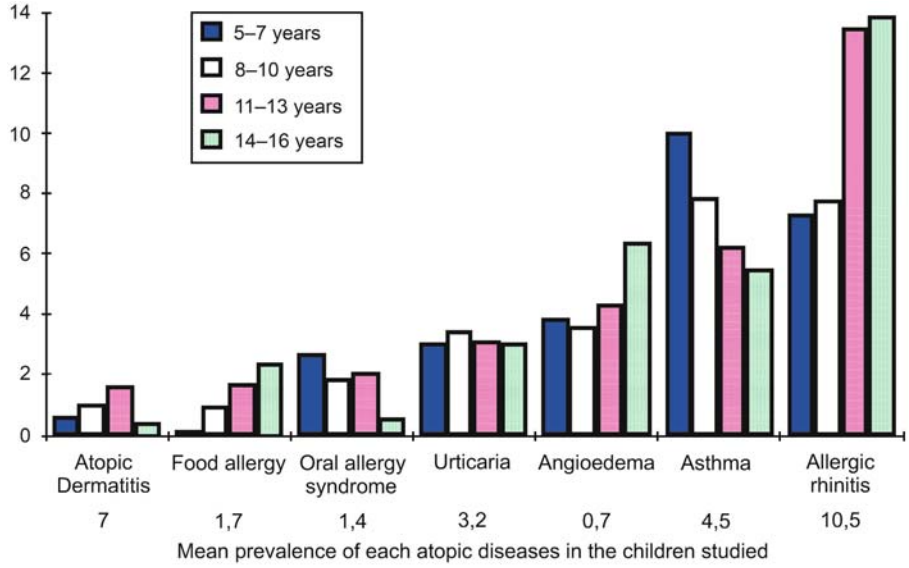

Fig. 5.9. Mean incidence of atopic disease in 1,052 children divided into four age groups; mean for males and females. (Data from [268])

Atopic Dermatitis

AD is very frequent in the offspring of atopic parents [271]. FHA (family history of atopy) rose up to 72.8 [128] – 83% of cases [239] and is an important risk factor for AD [81]. If only one parent is atopic the incidence is 9.5% at the age of 12–18 months; if both are atopic it rises to 12.8% (Fig. 5.10) [29]. In 0–4-year-old children with no FHA, 27.1% developed AD; of those with single or double FH, 37.9% and 50.0% developed AD, respectively [34]. AD rarely appears before 6 weeks of life (Fig. 5.11) and males are more involved than females (rate, 1.3:1) (Table 5.5). It is more frequent in children of high social classes and/or with a high education level [271, 306], in preterm babies [192], in twins [192, 276] and living in a detached house [306]. A greater incidence in females is found in Sweden in the first 2 years (1:1.8) [2], in Denmark from 5 to 16 years (1:1.9) [268] and at 8.1 years (1:1.2) [85], in Germany at 5–7 years in two out of four cities (1:2.5) [271] and at 8–33 years (1:1.5) [173], and in Hungary at 6–14 years (1:1.12) [128]. Additional risk factors are documented in Chap. 4 [271].

Table 5.6 [3, 6, 7, 15, 17–19, 23, 27, 29, 44, 45, 48, 52, 69, 72, 76, 79, 81, 83, 85, 94, 95, 113, 114, 116, 128, 129, 148, 153, 155, 158, 172, 177, 179, 183, 184, 187, 192, 193, 207, 209, 214, 232, 240, 241, 244, 254, 268, 271, 279, 286, 298, 307, 310, 315, 317, 323, 324, 329, 336, 337, 342, 351, 354, 362] outlines the values of prevalence and incidence of AD.

Data by Taylor et al [309] have shown that AD prevalence increased between 1945 and 1975 from 5.1% to 12.2% in England and between 3.1% in Bristol in 1945 and 17% in New Zealand in 1982 [95]. In Danish twins the prevalence significantly increased from 3.2% to 11.5% in 20 years or from 2.9% to 10.9% between 1939 and 1991 [276]. In 777 preterm infants with birthweight under 1,850 g, followed up to the age 18 months, AD incidence was 19.4% in general and 30% in twins [192]; in another 80 preterm infants, it was 7.5% compared to 5% of controls [76]. In a 3-year follow-up study [39], the prevalence rose to 8% at 24 months, decreasing to 2% at 36 and then fading (Fig. 5.3): the incidence was 4% at 6 months, 2% at 24 and 0% thereafter, and the cumulative incidence was 7.5% [39]. Likewise Sporik et al observed an analogous decline when children grow [296]. In 143 Greenland children followed up from birth to age 2, 16.78% of children developed AD. The incidence rate of AD was 177 cases per 1,000 child-years at risk, corresponding to a cumulative risk of 16% per year [306]. A recent worldwide ISAAC study on AD has found a 14.7% prevalence in 64,460 children aged 6–7, and a 14.6% prevalence in 135,559 children aged 13–14. In this group of children, the prevalence rates were as high as 46.9%–49.3% in Sweden, whereas the highest rate was 57.2% in Japanese children aged 6–7. Higher incidence values (≥15%) were found in 458,623 children aged 13–14 in northern Europe (Great Britain, Sweden) and especially in Japan and New Zealand in the younger group [339].

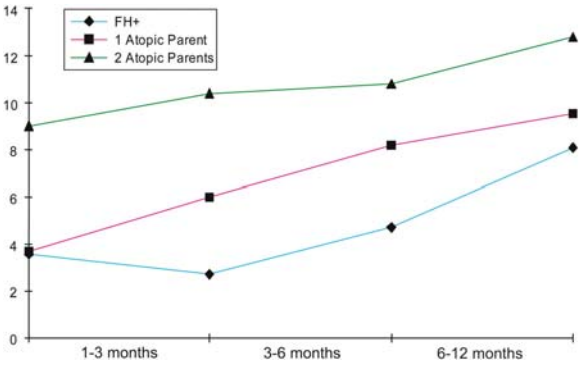

Fig. 5.10. AD in offspring of atopic parents: the more the parents are affected, the more AD incidence increases. For details see text. *FH* family history. (Data from [29])

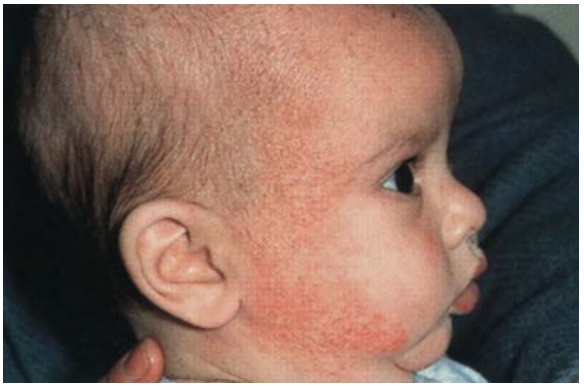

Fig. 5.11. A baby with atopic dermatitis

The *natural history of AD* in children is poorly known due to the few studies that have been conducted; however, it can be in part re-established on the available data indicating a significant increase in prevalence in recent years (Table 5.7) [3, 5, 10, 12, 43, 55, 69, 72, 92, 137, 174, 178, 215, 221, 233, 253, 282, 294, 309, 323], for example, up to 12.6-fold in England between 1946 and 1994. In a study of 1,654 children, it was 7%–25% at 11 years depending on low or high CBIgE [69]. Prevalence was the same among subjects irrespective of whether they had symptoms at examination as adults [109]. In twins it was 2.3% before 1960, 5.6% in 1960–1970, 10.7% in 1970–1986 and 11.5% in 1991 [276].

The rates of remission observed by various investigators are widely variable: between 17% and 90% of cases at 6 years of life. Only a study conducted on 2,000 children followed up in some cases for 20 years was more optimistic, over 84%, but the results are controversial because most patients suffered from seborrheic dermatitis [327]. Rudzki et al [261] reported that 41.8%–50.8% of cases of respiratory allergy in children with AD was associated with one or both atopic parents, re-

Table 5.6. Prevalence and incidence values of AD (%) in different countries

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References
Italy	0–>6	220	1993	9.3		[53][d]
	1.3	160	1989	6.2		[76]
	5[c]	634	1993	5		[48]
	6–7	20,715	1999	13.3		[339]
	9–10	1,760	2003	15.1	9.5	[79]
	9–15	930	1988	17.5		[10]
	13–14	26,447	1999	8.5		[339]
Albania	6–7	2,981	1999	1.5		[339]
	13–14	2,957	1999	1.3		[339]
Austria	6–7	5,787	1999	10.8		[339]
	13–14	4,866	1999	4.9		[339]
Belgium	6–7	6,533	1999			[339]
	13–14	1,515	1999			[339]
Denmark	5–7	1,052	1993		9.9	[268]
	8–10	1,052	1993		7.7	[268]
	12–16	1,501	2001	21.3	6.7	[209]
	11–13	1,052	1993		6.2	[268]
	14–16	1,052	1993		6.3	[268]
Estonia	1–5	273	2001	21.5		[148]
	6–7	3,070	1999	12.4		[339]
	11–12	979	2001	25.1	20.4	[15]
	13–14	4,984	1999	11.7		[339]
Finland	0–5	320	2003	57		[179]
	3–18	3,649	1980	4.3		[240]
	13–14	11,895	1999	24.1		[339]
	15–16	1,712	1990	9.7		[324]
France	4–14	505	1984	10.3		[187]
	6–7	3,202	1999	22.1		[339]
	13–14	18,544	1999	22.9		[339]
Georgia	6–7	6,770	1999	8.2		[339]
	13–14	6,748	1999	3.0		[339]
Germany	2	1,066	1992	18		[29]
	5–6	581	1989	8.6		[155]
	6	320	1992	12.3		[45]
	9–11	3,984	1990	19.5		[81]
	9–11	8,204	1990	20.2		[332]
Germany (ex East)	5–7	287	1991	17.6		[271]
	6	1,358	1992	15.7		[27]
	9–11	1,051	1992	13		[329]
Germany (ex West)	5–7	987	1991	10.7		[271]
	6	638	1992	12.9		[27]
	9–11	5,030	1992	13.9		[329]
Greece	6–7	1,654	1999	5.0		[339]
	13–14	2,561	1999	3.9		[339]
Great Britain	1.5	777	1989	19.4		[192][a]
	3–5	406	1993	20	14.5	[153]
	5	12,555	1975	5		[114]
	6–7	3,000	1999	27.8		[286]
	6–7	1,864	1999	27.6		[339]
	6–8	358	1993	19.5	9.9	[153]
	8–13	1,650	1990	12		[193]
	10	548	2003	33.9		[172]
	9–11	313	1993	25 M 18 F	13 M 8 F	[153]
	12	965	1993	15.9		[44]
	12–14	27,057	1999	22.5	16.4	[19]
	13–14	35,475	1999	22.3		[339]

Table 5.6. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References
Hungary	6–14	1,454	2003	15.1		[128]
Ireland	6–7	1,899	2003	11.2		[129]
	13–14	3,147	1999	9.4		[339]
Latvia	6–7	3,003	1999	8.0		[339]
	13–14	6,149	1999	6.0		[339]
Malta	5–8	3,506	2002	5.6 M 5.8 F		[207]
	13–14	4,184	1998	6.5 M 8.8 F		[206]
Norway	6–7	2,325	2004	10.7 M 9.5 F		[94]
	7–12	575	1994	19.6 M 27.6 F		[83]
	7–13	4,666	1995	31.7 M 27.4 F		[298]
Poland	6–7	4,974	1999	19.7		[339]
	13–14	10,167	1999	14.9		[339]
Portugal	6–7	5,129	1999	11.1		[339]
	13–14	10,751	1999	11.4		[339]
Romania	13–14	3,396	1999	3.7		[339]
Russia	13–14	3,411	1999	4.7		[339]
Spain	4–14	141	1998	25		[254]
	6–7	16,844	1999	16.2		[339]
	13–14	25,021	1999	10.2		[339]
Sweden	1.5	1,701	1977	4		[72][e]
	6–7	3,029	1999	35.7		[339]
	10–11	911 L	2001	32.0	24.6	[15]
	10–11	1,197 Ö	2001	26.6	32.1	[15]
	7	1,651	1980	7		[69][f]
	7–9	4,281	1991	29.1		[3]
	10–11	1,654	1989	11.9		[69][f]
	12	1,411	1993	22		[44]
	13–14	6,452	1999	48.0		[339]
Switzerland	4–6	3,270	1981	2.8		[323]
	6.8 (M)	1,105	2003	25.9		[337]
	6–15	2,879	2001	13.0		[351]
	15	3,500	1981	1.5		[323]
India	6–7	31,697	1999	3.1		[339]
	13–14	37,252	1999	4.8		[339]
Indonesia	13–14	2,249	1999	4.0		[339]
Iran	6–7	5,469	1999	6.4		[339]
	13–14	5,873	1999	8.6		[339]
Israel	13–14	10,057	2004	7.8	5.9	[116]
Korea	6–12	38,955	2001	7.3		[177]
Kuwait	12–15	38,955	2001	3.9		[177]
	13–14	1,056	1999	13.0		[339]
Lebanon	13–14	NS	1999	11		[244]
Oman	6–7	3,891	1999	7.4		[339]
	6–7	3,893	2003	7.5		[7]
	13–14	3,174	1999	14.4		[339]
	13–14	3,174	2003	14.4		[7]
Pakistan	13–14	1,829	1999	16.2		[339]
UAE	6–13	3,200	2000	11		[6]
Uzbekistan	13–14	4,662	1999	3.5		[339]
USA	1–5	NS	NS	2		[317]
	13–14	2,330	1999	8.2		[339]

Table 5.6. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References
Canada	6–7	5,758	1999	21.3		[339]
	13–14	4,929	1999	15.6		[339]
Argentina	6–7	3,005	1999	10.7		[339]
	13–14	6,004	1999	11.2		[339]
Brazil	6–7	7,261	1999	14.8		[339]
	6–7	3,005	2002	12.6 M, 13.8 F		[354]
	13–14	15,454	1999	10.3		[339]
	13–14	3,008	2002	11.7 M, 12.4 F		[354]
Chile	6–7	10,838	1999	9.7		[339]
	13–14	12,708	1999	7.7		[339]
Costa Rica	6–7	2,942	1999	8.2		[339]
	13–14	3,200	1999	4.5		[339]
Mexico	6–7	3,097	1999	3.5		[339]
	13–14	3,102	1999	3.9		[339]
Panama	6–7	3,043	1999	6.3		[339]
	13–14	2,885	1999	7.2		[339]
Paraguay	13–14	2,996	1999	16.3		[339]
Peru	13–14	3,158	1999	16.1		[339]
Uruguay	6–7	3,071	1999	9.6		[339]
	13–14	3,072	1999	7.4		[339]
Venezuela	4–21	NS	NS	3.3		[317]
China	6–7	7,754	2000		2.8–2	[362]
	13–14	19,007	1999	10.5		[339]
	14–18	737	1992	10.4		[184][b]
Hong Kong	3–10	535	1989	6.8		[158]
	6–7	3,618	1999	27.2		[339]
	6–7	3,618	2000		4.2	[362]
	13–14	4,668	1999	2.7		
	13–14	4,667	1995	5.5		[183]
	13–14	4,666	1999	5.4		[339]
Korea	6–7	8,109	1999	20.1		[336]
	6–12	38,955	2001		7.3	[177]
	13–14	9,983	1999	7.4		[336]
	13–15	38,955	2001		3.9	[177]
Thailand	6–7	3,828	1999		12.9	[315]
	6–7	2,658	2000	18.0		[310]
	6–7	7,457	1999	21.5		[339]
	<13	2,361	2000	41.2		[342]
	13–14	3,410	2000	9.9		[311]
	13–14	7,640	1999	26.3		[339]
	13–14	3,927	1999			[315]
Taiwan	6–7	4,806	1999	23.9		[339]
	7–14	8,345	1988	6		[307]
	13–14	11,400	1999	11.8		[339]
Vietnam	5–11	1,480	2003	3.3		[214]
Malaysia	6–7	15,285	1999	2.3		[339]
	7–12	747	2000	8.5		[218]
	13–14	18,636	1999	3.7		[339]
Philippines	6–7	3,558	1999	4.3		[339]
	13–14	3,207	1999	7.1		[339]

Table 5.6. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References
Singapore	6–7	656	1994	3		[113]
	6–7	2,353	1999	1.3		[339]
	12–15	4,147	1994	4.1		[113]
	13–14	4,206	1999	4.0		[339]
Algeria	13–14	1,173	1999	8.3		[339]
Ethiopia	13–14	5,978	1999	15.9		[339]
Kenya	13–14	6,287	1999	11.8		[339]
Morocco	13–14	9,359	1999	38.4		[339]
Nigeria	13–14	1,704	1999	10.1, 10.7 M, 9.5 F		[339]
South Africa	12	1,239	1993	11.1		[44]
	13–14	5,173	1999	9.6		[339]
Australia	6–7	10,899	1999	22.8		[339]
	8	2,360	1989	6.3 M 7.5 F		[85]
	8–10	380	1989	13.9		[232]
	8–11	1,016	1997	11.2		[84]
	12	965	1991	16 M 15.7 F		[23]
	13–14	12,280	1999	15.8		[339]
New Zealand	4	1,265	1981	16.7		[95]
	6–7	18,589	1999	26.7		[339]
	6–7	3,000	2001	15.0		[17]
	12	873	1991	14.3 M. 17.7 F		[23]
	13	662	1991	33.7		[279]
	13–14	19,023	1999	25.8		[339]
	13–14	3,000	2001	13.0		[17]

L Linköping, *M* males, *F* females, *NS* not specified, *O* Östersund.

[a] Study on preterm babies.

[b] With peaks of 25%–40% in children with severe AD.

[c] Median age.

[d] See Fig. 5.6.

[e] See Fig. 5.4.

[f] In some Swedish studies the cumulative incidence was calculated: 12% [1], 4% in children aged 1.5 years and 13.1% in those aged 7 years [69]. In the Chinese children, those of Beijing reported more asthma than the children from Urumqi; we report the Hong Kong children separately [362].

spectively, and in 25.2% of children with no FHA. Alarmingly, a high proportion of children with AD develop respiratory allergy: 46.9%–53.3%, with 48.7% in remission (Table 5.8) [2, 22, 49, 100, 104, 118, 137, 140, 156, 157, 160, 188, 208, 220, 241, 229, 258, 263, 264, 322, 327, 328, 332, 352, 353], but with peaks of 79% for asthma [118] and of 79% for AR [118]. The association with asthma occurs in 14% of children before 2 years of age and in 40% before 5–6 years, with AR in 25% of cases at 5–8 years [173, 353]. In comparison with the results of a recent analysis, in eight studies conducted in 1953–1966 all results were decidedly more favorable [275], either due to parental data or due to a lesser consideration of respiratory symptoms. The progression to respiratory allergy was evident in 18 atopic children followed up from the age of 6 months to 5 years: at 6 months all suffered only from AD, at 18 months 23.5% also suffered from asthma, at age 5, 38.9% still had AD, 50% asthma, and 44.4% allergic rhinoconjunctivitis (more diagnoses in the same child) [213]. Moreover, children may have pollinosis in 27% and urticaria in 40% of cases [14]. The more severe forms of asthma are a feature of children with unremitting AD [31]: infantile AD is therefore characterized by long-lasting morbidity [156, 157].

Rystedt [263] has suggested some unfavorable prognostic factors in order of importance:

- Severe AD or with atypical or widespread distribution of skin lesions in infancy
- FH positive for AD
- Association with AR and/or asthma
- Female sex and early onset

Table 5.7. Changes in prevalence and incidence of AD (%) in children reported from studies conducted in different countries

Country	Age (years)	Time span	Prevalence	References
Italy	6–7	1995–2002	4.4–5.2	[101]
	13–14	1995–2002	2.1–3	[101]
	8–11	1992–2002	24.4–24.8	[314]
Denmark	10	1986–1995	14–11	[137]
Germany	9–11	1991–1996	12.1–14.2	[332]
	5–14	1992–1999	10.3–11.5	[132]
	8–11	1992–1997	6.7–11.2	[84]
Great Britain	6–7	1946–1958	5.1–7.3	[309][a]
	5–7	1958–1970	7.3–12.2	[309]
	12	1973–1988	4.8–15.9	[43]
	8–13	1964–1989	5.3–12	[215]
	7.5–8.5	1978–1991	21.7–39.2	[6]
	8–14	1989–1994	12–17.7	[221]
	12–14	1995–2002	21.1–24.3	[12]
	8–9	1991–1999	18.1–31	[213]
Norway	7–12	1981–1993	8.6–9.6	[294]
	7–13	1985–1995	13.2–19.7	[282]
Sweden	1.5–7	1976–1977,1981–1982	4–7	[69, 72][b]
	7–11	1981–1982,1985–1986	7–11.9	[69]
	7–9	1979–1991	7–18.3	[3]
Switzerland	4–6	1968–1981	2.2–2.8	[323]
	15	1968–1981	2.3–1.5	[323]
Saudi Arabia	8–16	1986–1995	12–13	[5]
China	6–7	1995–2001	28.1–30.7	[178]
Hong Kong	13–14	1989–1994	18–32	[174]
Kenya	13–14	1995–2001	13.9–28.5	[92]
Brazil	6–7	1996–1999	13.2–11.4	[55]
	13–14	1996–1999	14.0–15.0	[55]
Australia	6–7	1993–2002	22.6–32.3	[253]
	8–10	1982–1992	20.3–24.4	[233][c]
			22.5–31.9	[233]

[a] The first study in the last 50 years documenting this rise.
[b] See Fig. 5.6.
[c] Study conducted in regions with two different climates (see Tables 5.12 and 5.22).

AD persisted in adulthood in 80% or 15% of subjects according to the persistence or not of these factors [263].

In 56 children followed up for 5 years, we reported that the following factors were significantly associated with persisting skin lesions [34]:

- AD onset after the 6th month of life
- Atypical or widespread distribution of skin lesions
- Persistence of food intolerance
- Elevated levels of serum total IgE and of sIgE for foods
- Positivity of SPT and/or sIgE for inhalant allergens at the first visit, with a predictive value for the development of respiratory allergy [49]

The remission of symptoms may be significantly lower in females, 29% vs 42% of males [2]. Furthermore:

- In Swedish children, 41%–47% were affected with AD before the onset of asthma [2, 71] and at follow-up 38%–41% suffered from both diseases [2, 69]. Early onset and positive FHA predisposed to respiratory allergy [2] and the risk was doubled if CBIgE were elevated [69].
- Among 1,052 Danish schoolchildren, 10% had asthma, 15% rhinitis, 26% angioedema and 20% OAS [268].
- In 55 asthmatic children prospectively followed up to 24 years of age [160], the cumulative incidence of AD was 54%; the persistence was 30% in infancy, 19% in adolescence and 28% in adulthood. Onset in adult age was only 7.45%.
- Wüthrich et al [156, 157, 352, 353] (Table 5.8) showed that patients followed from infancy and controlled at the age of 23 had a rate of persistence as high as 63.1%: in

Table 5.8. Prospective studies in children on AD natural history

Author(s) and reference	Year	No. of cases	Follow-up (years)	Remission (%)	Respiratory allergy (%)
Musgrove et al [208]	1976	99	15–17	42	21–32[g]
Vickers [327][a]	1980	2,000	5–20	90[c]	NS
Van Hecke and Leys [322]	1981	50	20	38	28
Wüthrich and Schudel [353]	1983	121	12	41	28
Wüthrich and Oehme [352]	1984	269	15	66	22–17
Sampson et al [264][a]	1985	113	1–2	40	NS
Queille-Roussel et al [241]	1985	200	7	26	34.5
Rystedt [263]	1985	955	24	38–60[d]	NS
Businco et al [49]	1989	56	5	57	50
Åberg and Engström [2]	1990	1,335	14	34–38[e]	28
Foucard [100]	1991	115	10	48[f]	25–64
Bardare et al [22]	1992	92	5	11	58–48
Guillet et al [118]	1992	29	3	17	79
Linna et al [188]	1992	40	11–13	18	53–78
Kissling et al [157][b]	1993–1994	106	23	40	80
Kissling et al [156][b]	1993–1994	47	23	28	NS
Kjellman et al [160]	1994	56	15	72	52–80
Oldak et al [220]	2000	52	6	83	53
Patrizi et al [229]	2000	118	4	88	38
Høst et al [137]	2002	39	15	97	62–52
Garcia-Aaet et al [104]	2004	66	3	68	NS
Rottem et al [258]	2004	46	8	34.7	10.8
Vinas Domingo et al [328]	2004	100	4	NS	55.2
Illi et al [140]	2004	1,314	7	43.2	52.4
Total and mean		**7,370**		**48.7**	**46.9–53-3**

NS not shown.
[a] Study including adults.
[b] Adults suffering from AD in their childhood.
[c] High prevalence of seborrheic dermatitis.
[d] The first figure refers to in-patients, the second to out-patients.
[e] Thirty-eight percent if AD is isolated.
[f] Thirty-four percent up to 6 years and 48% from 6 to 15 years.
[g] The first figure refers to asthma and the second to AR.

11.3% of cases there was a remission during infancy, 26.4% of patients were free of symptoms for a mean of 9 years, followed by a relapse in 19.1% of cases. In conclusion, 32.1% suffered from persistent AD from their infancy, >50% suffered from concurrent respiratory allergy, 71% were sensible to a season effect and 59.5% to stress. Respiratory allergy relapsed during infancy in 19.8% of patients.

- A greater rate of remission was present in 1,050 medical students followed during a 10-year period [101]: only 3% of them were affected with AD at the age of 24–>40.

In 46 children, full recovery was seen by the age of 8 years in 16 (34.8%), 8 of whom recovered within 3.3 years from the date of presentation (17 months) [258].

However, the main association of AD is with FA (Table 5.8), as has long been known, even if the role of IgE-mediated FA in AD is quite controversial, because of the scarcity of studies conducted using DBPCFCs, and not always based on Hanifin and Raika diagnostic criteria. FA could be responsible for AD in 67%–93% of cases, with an elevated risk of developing respiratory allergy [118]. When both diseases are associated, tolerance is acquired later and with unfavorable complications. Data on natural resolution of the condition are variable:

- In a classic study on 25 children with AD and hypersensitivity to egg demonstrated by DBFCs, at the follow-up at 2.5 years only 11 of 25 children (44%) had a spontaneous clearance of symptoms [99].

- In the 56 children of our study, a significant relationship was found between the outcome of AD and the development of food tolerance: 82% of the children who recovered achieved a complete or partial tolerance to the offending food. The offending foods were CM and egg in 69% of cases; tolerance to such foods was achieved at the age of 4; however, 50% of children had bronchial asthma and/or AR during the follow-up [49].
- After 8 years of follow-up, only 22 of 88 children (28%) with AD that developed in the first year of life, associated with high IgE levels and early sensitization, tolerated the sensitizing food, and 33 of 88 (38%) developed respiratory allergic disease [52]. Of the Der p-sensitized children with AD, 55.26% developed respiratory symptoms compared with 22.58% of the nonsensitized children ($p<0.001$). In these children, the OR of developing respiratory allergy was 4.23 [328].
- In 19 of 75 (25%) patients with AD and FA diagnosed by DBPCFC, tolerance was acquired after 1 year of elimination, in 4 of 44 (9%) after 2 years, and 20 patients after 3 years were not yet tolerant. The sensitizing foods in these 75 patients were CM in 81%, peanuts in 80%, egg in 76%, and wheat in 67% of cases. In the DBPCFC in 44 subjects the involved foods after 2 and 3 years were CM in 100% and egg in 90% of cases, other sensitizations were between 30% and 50% of cases. The high median age (7.9 years) and total IgE levels over 2 SD are, as previously discussed, unfavorable prognostic factors [265].
- In 250 children aged <2 years to 16 years, after 3 years of follow-up food sensitization was present in 93% of subjects, persisting in 73% of those aged <7 years and in 67% of those aged <16 years. The incidence of respiratory allergy was 19% at <2 years, 40% at 2–7 years and 86% at 7–18 years: 79% of children with severe AD suffered from respiratory symptoms within 3 years [118].
- In Italian children at the follow-up at 5 years of age, 31% of intolerant subjects were sensitive to CM, 25% to egg, 100% to fish and dried fruits [22].
- In 66 Spanish children the mean follow-up for 21 (32%) nontolerant infants was 58 months. The sIgE levels grew as the age of the infants increased, and *sIgE levels to case-in predicted clinical reactivity* [104].
- In the 1,749 children with AD, sensitization to food allergens was most prevalent in early infancy, replaced by sensitization to inhalant allergens later in childhood [137].

Oral Allergic Syndrome

A 1.4% incidence has been reported, associated with angioedema in 16%, with AD in 4%, with asthma in 11%, and with AR in 10% of cases (Fig. 5.9) . Moreover, 36% of asthmatics and 80% of pollinosis children with AR had OAS [268].

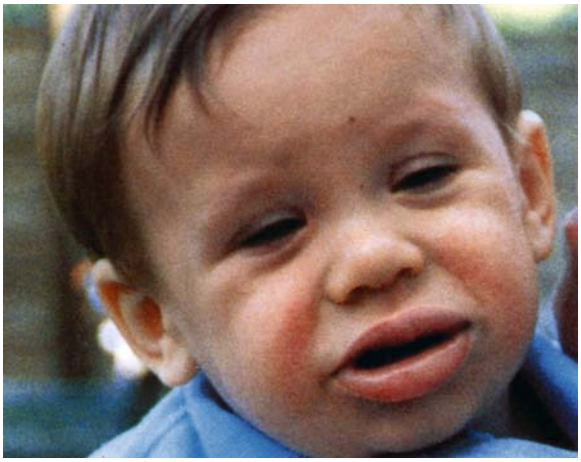

Fig. 5.12. A child showig a marked angioedema of the eyes, lips, and cheeks, following cow's milk ingestion to which the baby was allergic

Urticaria and Angioedema

Angioedema is more frequently associated with urticaria: in 10% of cases it can occur alone. In childhood, a self-limited form of this syndrome may have a 2%–5% prevalence [360]. Up to 7 years of age, the incidence is 1%–2.5% [124, 268], 2.2% in children aged <16 years [342]. Saval et al [268] found a prevalence of 3.2% for urticaria and 0.7% for angioedema (Fig. 5.9). Moreover, 23% of cases of urticaria are associated with angioedema [268], but in selected children aged 7–12, peaks may be as high as 8.8% in males and 17.7% in females [83]. When angioedema is provoked by foods (Fig. 5.12), higher levels have been described. Prevalence of urticaria is 3.2% in the first 3 years of life [33] (Fig. 5.5) and 1% at 5 years [148]. In Sweden it decreased from 5% to 4.5% between 1981 and 1993 and is significantly more frequent in females [294]. In Swiss children aged 4–6, it increased from 0.4% to 0.9% between 1968 and 1981, decreasing in adolescents (0.7% vs 0.5%) [323]. The coexistence of urticaria and AD has been studied [160] (18.9% in 10-year-old children with AD [172]), and in particular the positivity of FHA: the associated prevalence was 30% when only one parent was atopic and 34.8% when both were atopic (32%), while in nonatopic children with no FH, it reduced only to 21.3. After 15 years of follow-up, urticaria persisted in 8.2% of subjects who suffered from AD in infancy [352].

Allergic Contact Dermatitis

Childhood allergic contact dermatitis (ACD) is a much more widespread condition than commonly estimated: both sexes are equally involved at all ages [14, 243].

Table 5.9. Prevalence of ACD in children with AD (%) reported from studies conducted in different countries

Country	Age (years)	No. of cases	Year	Prevalence	References
Italy	1–16	282	1987	16.7	[14]
		431	2003	11.8	[111]
Denmark	12–16	1,501	2001	15.2	[209]
France	0.3–16	137	1999	43.0	[110]
	1– 3	68	1999	88.2	[259]
	3– 5	81		70.3	[259]
	6–10	110		54.5	[259]
	11–15	118		58.9	[259]
Germany	2–14	214	1992	32.2	[223]
	2– 4			5.1	[223]
	5– 7			12.6	[223]
	8–10			8.4	[223]
	11–14			7.0	[223]
Great Britain	5–12	125	1989	48.0	[243]
Greece	0–16	232	1996	43.5	[152]
Norway	7–12	424	1995	23.3	[82]
Portugal	0–12	329	1992	51.7	[115]
	0– 5			10.6	[115]
	6–10			24.1	[115]
	11–14			65.3	[115]
Poland	5–15	100	1996	60.0	[338]
Spain	4–14	141	1998	45	[254]
USA	2.6 (mean)	95	2000	24.5	[38]

Even if children are in contact from birth with potentially sensitizing objects (identification bracelets, necklaces, etc.), in children it is an uncommon finding in the 1st year of life, less frequent between 1 and 3 years of age [14, 115, 223], after which ACD becomes less rare, until the prevalence reaches values comparable to those of adults. Data on the prevalence of reactions summarized in Table 5.9 [14, 38, 110, 111, 115, 124, 152, 209, 223, 243, 254, 260, 338] modify appreciably this panorama. In two large samples, 2.1% [243] or 3.7% of children were tested and 57.8% of them were positive [14]. In those admitted to a pediatric clinic, levels of about 8%–10% are found. However in children with at least one positive patch-test, the prevalence was as high as 70% [64], or 88.2% in children aged 1–3 [260]. Several ACD infants aged between 1 week and 12 months have been reported. The 1-week-old infant developed ACD to the epoxy resin in his *vinyl identification band,* and a 7-month-old infant nickel dermatitis to the *snaps in his sleepwear* [96]. Two 6- and 12-month-old infants were allergic to nickel and a 12-month-old child to *para-phenylenediamine* [281]. Two more infants aged 6–9 months were positive to nickel [38]. Children aged 6.5±2.4 years may be positive to disperse dyes in their clothing [111].

Food Allergy

Adverse reactions to foods are commonplace in childhood allergy. Prevalence is subject to variations depending on certain parameters:

1. No unique marker of FA polymorphous image
2. Scarcity of reliable diagnostic tests that are easy to administer
3. Lack of studies on sufficiently wide samples
4. Patient selection only based on SPTs, RAST, or FCTs results

Additional shortcomings hold true for the often improperly used terms (Chap. 10) and the axiom of three FCTs (Chap. 9).

FA prevalence is generally identified with that of CMA, the more studied form of FA. FA prevalence is between 1% and 18%, with a mean of 5.8% (Table 5.3), that of CMA is between 0.00013% and 7.5%, with a mean of 2.25% in healthy babies (Table 5.4). In a group of children at high risk not receiving any prevention, FA prevalence increased up to 20%, and in selected young patients, as those affected with AD, the values may be higher [213]. In 777 preterm babies, the incidence was 10%–11% up to 19% in the sons of multi-

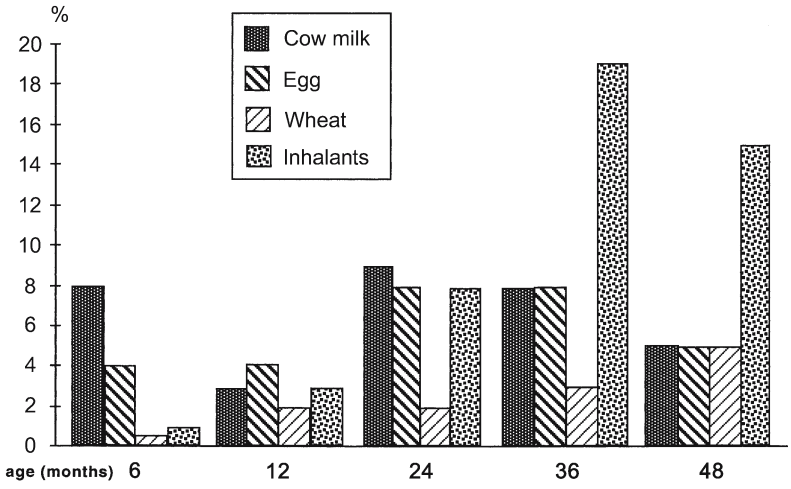

Fig. 5.13. Prevalence of IgE specific to foods and inhalant allergens in 174 children, which increases with age. Cow's milk, p=0.335; egg, p=0.362; inhalants, p=0.0001. (Data from [39])

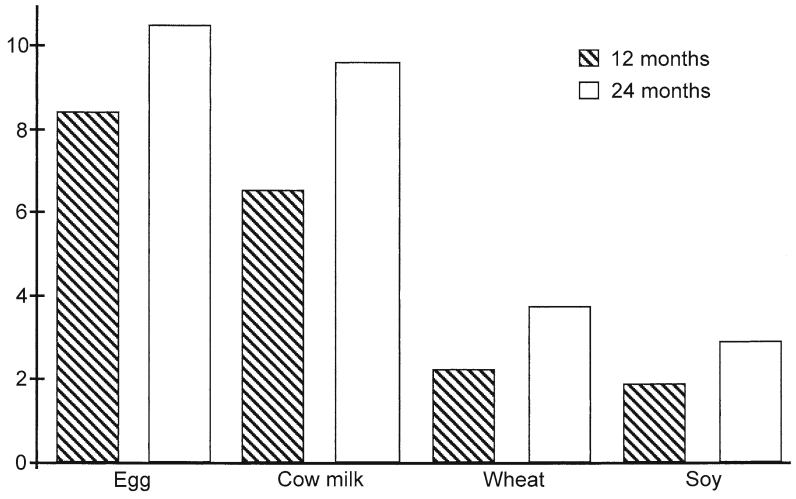

Fig. 5.14. Prevalence of food sensitization. (Modified from [29])

parous mothers [192], and in other cases it was 16.2% vs 13.7% of controls [76]. In a study on at-risk children, the prevalence was continuously 1% (Figs. 5.3, 5.4) and the incidence 1% at the age of 6 months [39] or 0% at 5 years [148]. In a multicentric study it was 6% (Fig. 5.5). CMA incidence is followed, in order of frequency, by allergies to egg and wheat, the foods most frequently provoking allergic reactions in children [39, 40, 264] (Fig. 5.13) [39] showing an upsurge of inhalant allergy. In the German multicenter study (Fig. 5.14), prevalence increased from 12 to 24 months [29].

The onset age of symptoms in IgE-mediated FA is a very significant factor: both allergies to CM and egg develop within the 1st year of life [29, 130], without any subsequent sensitization [131], or the symptoms appear in the 1st year in 80% of cases, and in 20% between 2 and 3 years [33] (Table 5.5). The onset may be biphasic: 69% of cases in the first 2 years, 8% in the 3rd year, then decreasing gradually until 7 years, rising to 10.5% in the subsequent period [68] (Table 5.5). CMA is alone in the first 3 months of life and provokes 30% of cases occurring in the 2nd month, and egg allergy follows (Fig. 5.15) [68]. The total impact in the first 4 years is for CM 97.5%, for egg 65%, for fish 35%, for other unspecified foods 8%. Such types of FA may also develop subsequently [68]. In the children of the ETAC study (mean age 17 months), CMA incidence was 53% (Fig. 5.16) and egg allergy was as high as 49% (Fig. 5.17). In particular, children with cod allergy had a high prevalence of asthma (60%), greater than in other atopic children studied, inhalant allergy in general (55%) and allergy to other foods (20%) (Chap. 9). In a study investigating children aged ≈3 years, the parents reported a prevalence of ≈25% during the first 4 years of life, but only 8% of such diagnoses have been confirmed by OFC [33]. These results confirm the suspicion that more elevated rates of incidence reported elsewhere referred in certain cases to highly selected children, and in other cases to very high rates, reduced by FCTs to the prevalences shown above.

The *natural history of FA* is not yet fully understood because most authors quote 40%–70% improvement at 3 years of age, whereas according to other authors, above all ourselves, a complete clearance of symptoms in this

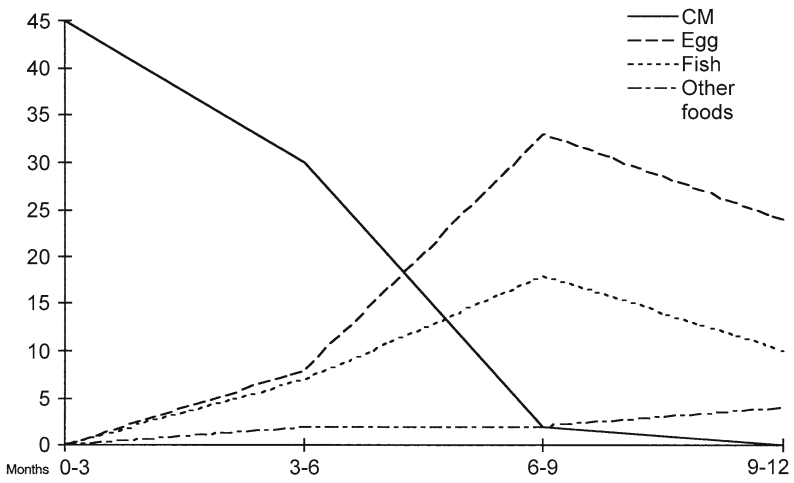

Fig. 5.15. Percentage in the 1st year of age of children with food allergy caused by foods correlated to the onset of symptoms. (Data from [68])

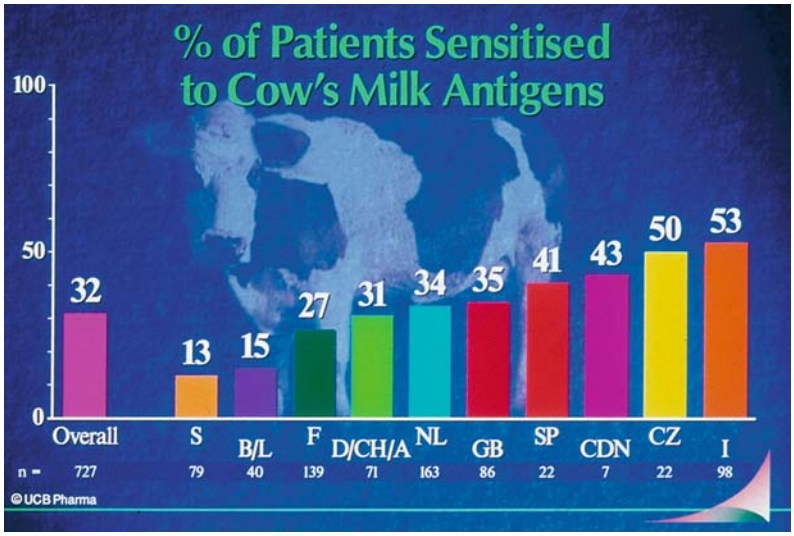

Fig. 5.16. ETAC Study. On average 32% of children are sensitized to cow's milk antigens, the highest frequency is in Italy (53%), the lowest in Sweden (13%)

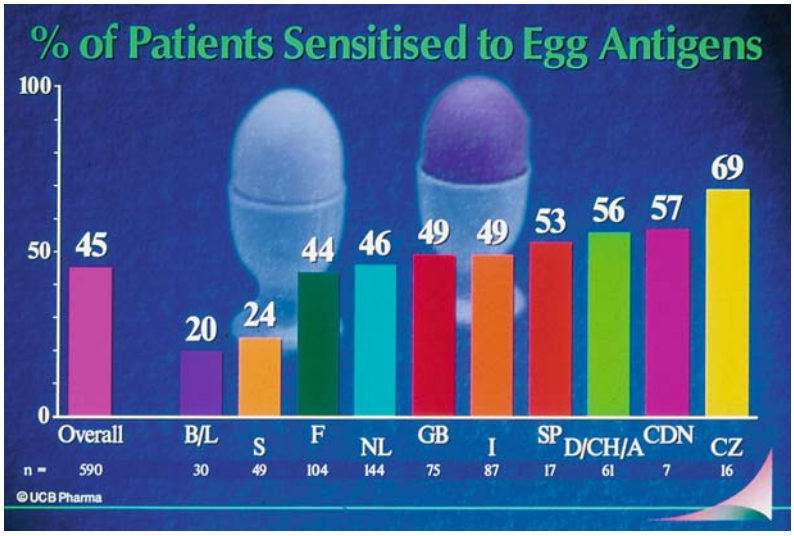

Fig. 5.17. ETAC Study. On average 45% of children are sensitized to egg antigens, the highest frequency is in the Czech Republic (69%), the lowest in Benelux (20%)

time span is uncommon, because of the frequent development of additional allergies. In particular, sIgE elevated levels for egg in infants are predictive of the subsequent appearance of respiratory allergy [130]. There is concordance with a computerized study showing a mean persistence after the initial diagnosis: in 71% of cases, CM intolerance persists after 3 years, in 50% after 6 years and in 28% after 9 years [199]. According to the initial level of serum IgE (<or >10 kU/l) at the age of 4 years, 90% or 47% of children, respectively, became tolerant to CM [272].

Few studies have investigated this aspect of FA:

- High rates of children are tolerant within a wide span of years: 67.6% [46] or 77% [136] at the age of 2 and 87% at 3 [136], but 32.4% were intolerant at the median age of 6–7 [46] and 72% at the age of 9 [52].
- Many children show additional sensitizations during follow-up: 73% [46], 67% of children with CMA and 27% of children with egg allergy, 54% had adverse reactions to other foods and 28% to inhalant allergens [52].

Remission therefore depends very much on the foods responsible for FA. When fish, shrimps and mollusks, peanuts and nuts are the offending foods, the symptoms will require a longer time to clear up: the children should undergo differentiated FCTs to check whether FA persists (Chap. 9). Probably, an early sensitization to inhalants is established in children with CMA including Der p, grasses and cat epithelium, even <2 years of age [134]; tolerance may be reached beyond childhood, and in several cases these diseases may persist into adulthood.

Asthma

Asthma, the most common chronic disease of childhood, now affects >15 million individuals in the USA and involves direct treatment costs exceeding US $6 billion for medications, hospitalizations and work absences. In children, the disproportionate increase in asthma prevalence, as measured by ambulatory visits, hospitalizations [262], and mortality was for those *younger than 5 years, for whom accurate diagnosis may be more difficult* [4]. Asthma accounts for 1.01 million lost school days, 1.21 million ambulatory care visits, 200,000 admissions with 1.9 million days in the hospital annually more than nonasthmatic children, 5% of whom complain of activity restrictions vs 30% of asthmatics [324]. In 1985 the prevalence of asthma in eight South Fore villages of the Eastern Highlands of Papua New Guinea was reported to have risen dramatically over the past decade and was 6‰ in those aged <20 [84], but the international ISAAC study has shown very contrasting and dramatic data [311]. Also, in many published studies different results are found, as shown in Table 5.10 [3, 6, 7, 11, 13, 15–18, 27, 35, 44, 48, 50, 53, 54, 56, 59, 61–63, 65, 66, 69, 79–81, 83, 84, 98, 105, 113, 151, 157, 169–172, 175, 182, 183, 184, 192, 193, 195, 196, 203, 204, 207, 209, 210, 212, 214, 217, 219, 225, 231–234, 240, 250, 251, 258, 261, 266, 268, 271, 277, 279, 283, 286, 289, 295, 297, 298, 301, 302, 307, 310, 315–318, 323–325, 329, 331, 344, 345, 351, 361, 363] showing prevalence, incidence, and wheezing rates. To these data we add personal findings. During the period October 2001–December 2003, we consecutively enrolled 592 children aged 4–11 years attending our division because they were affected with severe asthma (SPTs, sIgE and spirometry). There were 135 children during 2001 and 215 during 2002, with a 62.8% increase. During 2003, there were 242 children, *with an 88.8% increase compared to 2001.* We found 18.9 asthmatic children each month. The prevalence of childhood asthma has been described in high-risk cohorts, but not in randomized children. In a worldwide ISAAC study, 463,801 13- to 14-year-olds were studied in 56 countries and 257,800 6- to 7-year-olds in 38 countries. The variations in the prevalence of asthma symptoms were marked with a global total of 10.2% in the younger ones and 13.0% in the older ones. The prevalence of wheeze in the last 12 months was 13.8% in the older age group and 11.8% in the younger age group [16]. Table 5.11 [10, 119, 163, 210, 214, 218, 230, 251, 252, 286, 295, 298, 302, 361] details the prevalence or incidence of precipitating factors and asthmatic symptoms. The influence of environmental factors is confirmed, asthma being scarcely known in autochthonous populations residing in rural areas [318, 325], whereas in mixed populations the asthma incidence is more elevated compared with European countries [287]. In Canada the incidence is minimal [196], most likely because of a scarcity of allergens and/or pollutants, as demonstrated by studies in Gambia, New Guinea and among Eskimo children [65, 318, 344]. Asthma is 1.9-fold more frequent in European compared with Asian children, contrary to previous reports [225]; in black children it is instead 2.2-fold more frequent, a statistically significant risk of asthma [277]. Several studies have reported *an earlier age of onset, up to 59.3% of cases in infants aged <1 year* [357] *and 76% within 2–3 years* [163] (Table 5.5, Fig. 5.18) [302]. The figures are confirmed by a greater prevalence of asthmatic attacks in 5- to 10- vs 11- to 17-year-old boys [221]. More data confirm this trend.

In a Swedish study, the cumulative incidence was 5.3% and prevalence 3% between 3 and 11 years of age.

In a study on the month of birth in a selected cohort of 4,030 children aged 1–17 years with symptomatic allergy by inhalant allergens, asthma incidence was as high as 54% and AR as 30.7%. The prevalence of monosensitization to Der p was 79.3%, to grasses 20.6%, and to Parietaria 3.5% [47].

Sporik et al extend these data and conclude that wheezing and BHR prevalence does not decline until 11 years of age in 65% of children who wheezed after their second birthday [296].

The prevalence of atopic disorders differed significantly between children with asthma (60–81%: AR 20%–60%, allergic conjunctivitis 10%–45%, AD 20%–21%, FA 13% [119, 245], any allergic disorder 33%),

Table 5.10. Prevalence and incidence rates of asthma and wheezing in children reported from studies conducted in different countries

Country	Age (years)	No. of cases	Year	Prevalence (%)	Incidence (%)	Wheezing (%)	References
Italy	0–>6	220	1993	12.5			[53][b]
	5[a]	634	1993	9			[48]
	6–7	20,815	1998		8.6	7.3	[16]
	9–10	1,760	2003	12.4	7.0		[79]
	9–15	930	1988	4.4			[18]
	13	21,410	1997	9–10.4		3.4–7.8	[261]
	13–14	26,47	1998		9.9	8.9	[16]
Albania	6–7	2,981	1998		3.1	7.6	[16]
Austria	6–7	5,787	1998		3.9	8.9	[16]
	13–14	3,371	1998		6.0	11.6	[16]
Belgium	6–7	4,886	1998		4.2	7.3	[16]
	13–14	6,553	1998		8.1	12.0	[16]
Czech Republic	7–15	1,852	1993	2.3		27	[344]
Denmark	5–7	1,052	1993		3.8		[268]
	8–10	1,052	1993		3.1		[268]
	11–13	1,052	1993		4.3		[268]
	12–16	1,501	2001	6.9			[209]
	14–16	1,052	1993		6.3		[268]
Estonia	6–7	3,070	1998		1.4	9.7	[16]
	10–11	979	2001	2.5	20.4	8.8	[15]
	10–12	1,519	1993	2.9			[247]
	10–11	979	2001			8.4 I	[15]
	13–14	1,424	1998		3.0	10.8	[16]
Finland	3–18	3,649	1980		1.6		[240]
	13–14	11,985	1998		6.6	16.0	[16]
	15–16	1,712	1990	2.5			[324]
France	4–14	505	1984	3.8			[187]
	6–7	3,202	1998		9.3	8.1	[16]
	13–14	18,544	1998		12.6	13.5	[16]
	15.9	64,309	1976–1982	6.4			[234]
Georgia	6–7	6,770	1998		3.1	7.6	[16]
	13–14	6,740	1998		3.1	3.6	[16]
Germany	5–6	581	1989	7.7			[171]
	6–7	6,952	1998		3.6	8.5	[16]
	9–11	8,204	1990	4.7			[299]
	9–11	3,984	1990	7.6			[81]
	13–14	7,172	1998		5.7	13.8	[16]
	Children	704	1986	4.9			[169]
	10	6,083	1990	10.6			[345]
	10.8	481	1987–1990	5.8			[170]
	12–15	1,928	1991	6		33	[231]
Germany (ex East)	5–7	287	1991	1.4			[271]
	6	1,358	1992	1.2			[27]
	9–11[h]	1,051	1992	7.3			[329]
Germany (ex West)	5–7	987	1991	1.3			[271]
	6	638	1992	0.9			[27]
	9–11[h]	5,030	1992	9.3			[329]
Great Britain	<1	163	1990	9.1 M 5.4 F			[193]
	1.5	777	1989	22.5			[192][c]
	2–3	265	1990	13.4 M 7.2 F			[193]
	>4	383	1990	19.4 M 12.3 F			[193]

Table 5.10. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence (%)	Incidence (%)	Wheezing (%)	References
Great Britain (Continued)	4	160	2003	22.5			[172]
	5–7	5,472	1992	12.9		16.7 I, 24.4 P	[302]
	6–7	3,000	1999	27.7 M, 18.3 F	21.5 M, 4 F		[286]
	6–7	1,864	1998		22.9	18.4	[16]
	7	1,894	1965	18.2	8.1		[11]
	7–8	3,187	1988			14.8 I	[63]
	7–11	2,053	1989	12			[62]
	7–12	350	1990	12 E, 6.2 A		19.6 E, 11.9 A	[225]
	7.5–8.5	3,070	1991	12.8			[301]
	8–10	5,472	1992	12.6		15.9 I, 23.4 P	[302]
	11	1,198	1969	12	4.6		[11]
	11–13	5,472	1992	13.6		14.2 I, 21.7 P	[302]
	12	965	1993	12	9.1	15.2 I, 22.3 P	[44]
	12–15	2,091	1991	7		48	[231]
	13–14	35,485	1998		20.7	32.2	[16]
	14–17	5,472	1992	13.5		13 I, 22.3 P	[302]
	16	1,267	1974	11.5	3.3		[11]
Greece	6–7	1,654	1998		5.4	7.6	[16]
	9–12	290	2004		6.1	5.4	[289]
	13–14	2,561	1998		4.5	5.4	[16]
Ireland	6–7	1,899	2003		17.4		[129]
Latvia	6–7	3,003	1998		1.6	7.3	[16]
	13–14	6,219	1998		4.3	7.4	[16]
Malta	5–8	3,506	2002	20.1 M, 17.9 F	8.9 M, 8.7 F		[207]
	6–7	3,493	1998		7.5	8.8	[16]
	13–14	4,184	1998	29.3 M 26.8 F	15.8 M, 16.1 F		[206]
	13–15	4,184	1998		11.1	16.0	[16]
Norway	7–12	575	1994	11.2 M 13.2 F			[83]
	7–13	4,666	1993	11.3 M 6.6 F			[298]
Poland	6–7	4,974	1998		2.5	10.9	[16]
	10–12	410	1992	2.9		10.4	[35]
	13–14	10,167	1998		2.4	8.1	[16]
Romania	13–14	3,396	1998		3.7	3.6	[16]
Russia	13–14	3,411	1998		2.4	4.4	[16]
Sweden	0.5–16	4,990	1985	5.3			[13]
	6–7	3,029	1998		8.0	10.4	[16]
	7–9	4,281	1991	7.7–12.7			[3][c]
	10–11	1,654	1989	3			[69]
	10–11	2,108	2001			7.9–10.2 I	[15][d]
	10–12	665	1992	6.9		10.5	[35]
	12	1,411	1993	4	2.8	9.2 I, 21.7 P	[44]
	13–14	6,452	1998		10.4	12.9	[16]
	14	1,112	1987	9.4 M 12.9 F	4.1 M 9.6 F		[217]
Switzerland	4–6	3,270	1981	2			[323]
	6–15	2,879	2001	9			[351]
	7	1,495	1992	4.3		7.4 I, 18.4 P	[251]
	7	1,489	1990	6 M 2.5 F		10 M 4.6 F	[283]
	12	1,395	1990	8.2 M 3.5 F		7 M 4.7 F	[283]
	12	1,393	1992	5.9		6.0 I, 13.4 P	[251]
	15	1,461	1992	4.3		4.5 I, 11.8 P	[251]
	15	1,454	1990	5.5 M, 3.5 F		5 M, 4.1 F	[283]
	15	3,500	1981	2.8			[323]
Iran	6–7	5,469	1998		3.0	5.4	[16]
	13–14	5,873	1998		2.7	10.9	[16]

Table 5.10. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence (%)	Incidence (%)	Wheezing (%)	References
Lebanon	13–14	2,993	1998		11.6	14.4	[16]
Oman	6–7	3,891	1998		10.5	7.1	[16]
	6–7	3,893	2003		10.6		[7]
	13–14	3,174	1998		20.7	8.9	[16]
	13–14	3,174	2003		20.7		[7]
Pakistan	13–14	1,829	1998		7.3	8.6	[16]
Turkey	6–13	1,163	1969	2.1			[46]
	6–12	1,226	1994	17.4	8.3	23.3	[344]
	8–11	2,774	2003	6.9		11.5	[258]
	8–13	347	2003	6.9		11.5	[266]
UAE	13–14	3,200	2000	13		15.6	[6]
Uzbekistan	13–14	4,662	1998		1.7	9.2	[16]
Canada	0–14	28,778	1989	0.04 M, 0.03 F			[196]
	5–8	14,948	1994	4.7		13	[344]
	6–7	5,755	1998		14.7	17.6	[16]
	13–14	4,952	1998		16.5	28.1	[16]
United States	0–4	449	1991	11.3	8		[66]
	0–15	3,761	1974	5.8–10.4	0.3–1.2		[212]
	0.5–3	2,466	1989	4.6		8.8	[277]
	4–7	2,076	1989	5.9		7.7	[277]
	5–11	482	1991	16.4	8.9		[66]
	6–8	149	2001	28			[161]
	6–11	7,417	1963–65	7.6			[105]
	6–12	770	2004	59.1			[80]
	8–11	1,130	1989	4.8		5.4	[277]
	12–17	7,518	1966–1970	6.5			[105]
	12–17	354	1991	15.3	9		[66]
	13–14	7,508	1998		16.5	21.7	[16]
	13–17	497	2004	47.9			[80]
	15–20	1,836	1969	5.3			[65]
Costa Rica	5–17	2,534	1992	23.4			[295]
	6–7	36,264	2000	26.9		32.1	[210]
	13–14	52,549	2000	18.5		23.7	[210]
Cuba	0–11	291	1993	8.8	5.2		[344]
Mexico	0–11	2,761	1993	4.5	2.7		[344]
	6–7	3,097	2000	5.1		8.6	[210]
	13–14	3,102	2000	5.5		6.6	[210]
Panama	6–7	3,043	2000	19.3		23.5	[210]
	13–14	2,885	2000	16.9		17.6	[210]
Puerto Rico	0–11	914	1993	20.1	11.2		[344]
Uruguay	6–7	3,071	1998		12.0	18.0	[16]
Argentina	6–7	3,007	2000	6.5		17.3	[210]
	6–7	6,012	1998		5.3	16.4	[16]
	6–12	977	1980	2.9			[56]
	13–14	3,008	2000	7.9		11.8	[210]
	13–14	6,004	1998		7.3	10.9	[16]
Brazil	6–7	7,261	1998		13.1	23.3	[16]
	6–7	3,005	2000	6.1		21.3	[210]
	13–14	3,040	2004	35.1–29.9			[195]***
	13–14	3,007	2000	10.0		23.3	[210]
	13–14	15,454	1998		14.9	22.7	[16]

Table 5.10. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence (%)	Incidence (%)	Wheezing (%)	References
Chile	6–7	1,458	2000	9.7		9.7	[210]
	6–7	10,837	1998		12.1	27.1	[16]
	6–14	2,759	1979	2.7			[56]
	7	1,730	1992	9.4		26.2 I, 50.8 P	[251][f]
	12	1,820	1992	8	20.6 I, 45.9 P		[251]
	13–14	12,708	1998		10.7	10.2	[16]
	13–14	2,944	2000	12.4		11.7	[210]
	15	1,851	1992	7.4	17.2 I, 41.8 P		[251]
Colombia	0–4	680	1991	20.5			[54]
	5–9	519	1991	12.3			[54]
	10–14	551	1991	8.3			[54]
	15–19	421	1991	4.9			[54]
Paraguay	13–14	2,966	2000	12.2		19.4	[210]
Peru	13–14	3,158	2000	28.0		26.0	[210]
Salvador	13–14	3,162	2000	12.5		27.0	[210]
Uruguay	6–7	3,071	2000	12.0		18.0	[210]
	11–16	4,296	1981	7.5			[56]
	13–14	3,072	2000	15.3		19.0	[210]
Venezuela	3–15	1,968	1961	3.5			[56]
China	0–14	10,065	2003	1.97			[61]
	13–14	7,077	2003	12.4		7.2	[361]
	13–14	19,008	1998		6.7	4.2	[16]
	11–17	3,067	1990	2.4	1.9	6.3	[363]
	14–18	737	1994	1.6		1.1 I, 1.9 P	[184]
Hong Kong	3–10	535	1989	6			[175]
	6–7	3,618	1998		7.7	9.1	[16]
	7	519	1992	12 M, 7 F		7 I, 15 P	[181]
	12	623	1992	11 M 5 F		5 I, 10 P	[181]
	12–16	1,062	1992	6.6		3.7 I, 7.8 P	[182]
	13–14	4,667	1995	12.6		11.3	[183]
	13–14	4,666	1998		11.2	12.4	[16]
	15	547	1992	9 M, 5 F		4 I, 10 P	[181]
India	0–9	4,413	1966	0.16 M, 0.19 F			[344]
Indonesia	1–5	269	1993	6.5		10.7	[151]
	6–7	1,390	1998		6.6	4.1	[16]
	7–15	317	1982	3.8		1.3	[65]
	13–14	2,249	1998		1.6	2.1	[16]
Japan	NS	45,674	1992	4.6	5.2		[216]
	6–7	2,900	1998		18.2	17.3	[16]
	13–14	2,831	1998		18.9	13.4	[16]
Korea	6.7	8,158	1998		8.5	13.3	[16]
	6–12	36,955	2001			8.7	[177]
	12–15	36,955	2001	16.8	25.9	8.2	[177]
Kuwait	13–15	3,110	2000		5.5	16.1	[25]
Malaysia	6–7	15,285	1998		10.4	6.1	[16]
	7–12	747	2000	10.3			[218]
	13–14	18,636	1998	3.3		4.2	[16]
	13–14	409	1992	18.5	18.5	4.9 I, 7.7 P	[182]
Singapore	6–7	1,918	1994			16.5 I, 28.6 P	[113]
	6–7	2,353	1998	20.7	20.9	15.7	[16]
	12–15	4,102	1994		18.6	9.9 I, 18.6 P	[113]
	13–14	4,206	1998		12.7	9.7	[16]

Table 5.10. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence (%)	Incidence (%)	Wheezing (%)	References
Taiwan	6–7	4,806	1998			9.6	[16]
	7–14	8,345	1988	5.3 M 44.3 F	10.8		[307]
	7–15	NS	1994	19.7	9.0		[157]
	13–14	11,400	1998		18.6	5.2	[16]
Thailand	3–15	120	2004	18			[297]
	6–7	2,658	2000			10.2	[310]
	6–7	3,828	1999		6.7	5.5	[315]
	6–7	7,457	1998			8.2	[16]
	6–12	3,754	1987	4.3	20.5		[316]
	13–14	3,410	2000			11	[311]
	13–14	3,927	1999		1.6	12.6	[315]
	13–14	7,640	1998			13.0	[16]
Vietnam	5–11	1,460	2003	13.9	12.9		[214]
Algeria	6–14	5,200	1986	3.5			[59]
	0–25	4,677	1992	3.4	3.1		[344]
	13–14	1,173	1998		6.4	7.8	[16]
Ethiopia	0–9	2,939	1997	1.7 U, 1.1 R	2.4 U, 1.1 R		[355]
	13–14	5,978	1998		2.5	6.2	[16]
	10–19	3,957	1997	1.5 U, 1.4 R	1.8 U, 1.4 R		[355]
Gambia	8–14	231	1975	0			[344]
Kenya	13–14	6,267	1998		11.2	13.9	[16]
Morocco	6–14	1,804	1986	3.1			[59]
Nigeria	13–14	3,057	1998		18.4	13.7	[16]
South Africa	6–9	694	1979	3.2			[65]
	12	1,239	1993	11.5	8.7	17.8 I, 25.9 P	[44]
	13–14	5,173	1998		13.1	16.1	[16]
Tanzania	12.5	242	1977	7.9			[65]
Zimbabwe	5–19	4,293	1980	0.1			[65]
Australia	6–7	10,899	1998		27.1	24.6	[16]
	7	3,325	1992	24.1		23.1 I, 41 P	[251]
	7–10	30,000	1969	19.1			[65]
	8–10	380	1989	8.9		15	[232]
	8–10	1,668	1992	31.8		25.4 I	[233][g]
	12	2,899	1992	23.2		20.9 I, 36.5 P	[251]
	12	965	1991	14.1 M 9.8 F	9.1	15.2 I, 22.3 P	[23]
	12–15	1,519	1991	13		30 I, 45 P	[231]
	13–14	12,278	1998		28.2	29.4	[16]
	15	2,968	1992	22		18.6 I, 35.5 P	[251]
Australia (native Aborigine)	7–12	215	1991	0	0.1	1.4	[325]
New Zealand	6–7	3,000	2001	25			[17]
	6–7	18,568	1998		26.5	24.5	[16]
	7–10			16.3		18.7 I, 30.1 P	[204]
	11–13	857	1982	13.5			[203]
	12	873	1991	19.3 M 14.1 F	11.1	17.9 I, 26.6 P	[23]
	12–15	1,863	1991	11		28 I, 44 P	[231]
	13	662	1991	18.6	10.6	27.3 I	[279]
	13–14	3,000	2001	30.0			[17]
	13–14	19,023	1998		24.4	30.2	[16]
Papua New Guinea	6–20	257	1985	0	0.1	1.7	[318]
	8–11	1,016	1997	12.4		9.0	[84]

Table 5.10. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence (%)	Incidence (%)	Wheezing (%)	References
Philippines	6–7	3,358	1998		16.4	11.3	[16]
	13–14	3,207	1998		17.6	12.3	[16]
	<15	433	1991	3.9		20.6	[250]
	>15	727	1991	9.3		12.4	[250]
Maldives Islands	<15	2,903	1979			20.7	[65]
West Carolina	15	123	1978	49			[65]
Tahiti	14–18	64,309	1976–1982	11.5			[234]
Fiji Islands	8–10	781	1992	9.7		20.6 I, 25.1 P	[98]
Fiji Islands, Indian origin	8–10	843	1992	7.3		20.6 I, 23.6 P	[98]

Studies are arranged according to the age of the children studied. In the study of asthma symptoms in Latin America, if several cities of the same country were studied, we report the data of the related capital or most important city.

M Males, *F* females, *E* Europeans, *A* Asian (see text), *I* incidence of wheezing, *P* prevalence of wheezing (a single figure shows the incidence), *U* urban, *R* rural, *NS* not specified.

[a] Median age.
[b] See Fig. 5.6.
[c] Preterm.
[d] Two cities (Linköping and Östersund).
[e] The first figure shows the more polluted city, the second the less polluted city.
[f] In a Chilean city defined as pollution-free, the incidence of wheezing in children aged 7 and 15 years was 18.5% and 27.5%, respectively [251].
[g] Mean of two cities.
[h] The highest figures usually belong to children living in Munich, a city certainly more polluted than Leipzig and Halle [331] or to a more polluted area of Rio de Janeiro [195]. In an Italian study [261], the first figure shows the parental reports, the second that of their siblings; in different studies the cumulative incidence has been calculated: 18.2% in 7-year-olds, 21.8% in 11-year-olds and 24.5% in 16-year-olds [11], 5.3% in 11-year-old children [69] and 14.3% (11.3% at 0–4 years, 16.4% at 5–11 years, 15.3% at 12–17 years) [66].

Fig. 5.18. Early asthma onset: a young child using a peak flow meter

and children with other symptoms from lower airways (62%), thus concluding that atopic diseases have a notable prevalence independent of diagnosis [245].

The prevalence increases over 20 years (Table 5.12) [1, 3, 4, 5, 12, 37, 42, 43, 84, 90, 92, 105, 121, 137, 141, 165, 166, 168, 174, 190, 196, 203, 215, 216, 221, 224, 233, 234, 251, 252, 255, 219, 282, 287, 294, 295, 305, 312, 323, 333, 335], is modest in Canada with an increase of 0.01% in 0- to 14-year-old children [196], and in Finland with an incidence of new cases of 0.0015% [240]. In the USA, an increased incidence of asthma (definite + probable) from 183×10^5 in 1964 to 264×10^5 in 1983, in males more than in females, has been documented in the entire pediatric population of Rochester, USA [357]. An Australian study demonstrated a reduction in the 12-month period prevalence of reported wheeze, from 27.2% in 1993 to 20.0% in 2002, whereas the current wheeze *increased from 63.3% to 66.2% in the 1- to 3-year-olds* [253]. An ISAAC study has found in Australian 13- to 14-year-olds, a 28.2% prevalence of ever having asthma and a 29.4% prevalence of wheeze [16]. In northern Sweden, the prevalence of ever having asthma increased in only 1 year from 6.4% to 7.7% in 3,525 children aged 7–8 years [255]. In London in 13 years (1973–1986), there has been a remarkable increase in asthma, higher in females [42], with a parallel increase in school absences and hospital admissions [135], similarly in Canada from 1980 to 1985 [141]. In 1989, Anderson suggested that the prevalence of wheezing illness might have reached a plateau [8], or even a decline in admission rates [139], associated with a significant fall in school absences [10, 301] with no interference in normal

Table 5.11. Prevalence and incidence rates of precipitating factors and asthmatic symptoms (%) in children reported from studies conducted in different countries

Age	Sleep disturbances	Exercise	Night cough	Causes of wheezing				References
				Mites	Smoke	Foods	Pets	
0–14		63	57	36	65	50	61	[163][a]
3–15		47.5			36.7	6.1	2.9	[119]
5–11	1.9	6.7						[214]
5–17		10.4	12.5					[302]
5–17		8.6	7.6					[295]
6–7	5.8 (Maori) 5.7 (Pacific) 2.6 (European)							[230]
6–7		13	27.7					[286]
6–7	13.9	9.2	30.6					[210]
7	5.2–16.6	4.1–15	16.5–37.3					[251][b]
7		3.4	14.5					[252]
7.5–8.5		52.5		18.6	37.8	5.5	32.2	[10]
	8.5–13.9	6.2	31.9					[221]
7–12		5.9						[218]
7–13					13		9.5	[298]
9–11		23.5–25.4					14.7–20.7	[329]
12	3.2–12.3	7.2–20.4	10.6–34.5					[251]
12		4.5	9.5					[252]
13–14		25.6	19.3					[361]
13–14	9.5	19.1	28.7					[210]
15	1.6–9.8	6.9–23.1	8–28.2					[251]
15		10.1	9.2					[252]
	Pollens	**Emotional factors**	**Weather**	**Dampness**		**Pollution**		
0–14	59	37.6						[163]
3–15				12.9		25.6		[119]
7.5–8.5		35.4	56.4					[10]
9–11	17.7–31	8.8–22.2	35.7–50				14.7–24.6	[168]

[a] Parental reports.
[b] The lower numbers were reported in Switzerland. In 13- to 15-year-old Maltese children, there is 20.6% of effort asthma and 31.8% of nocturnal cough [206].

activities [10]. Instead, a 27% increase in asthma prevalence was found in Australia compared to a 1964 survey [252], and although a 7.3% decrease in the asthma prevalence was noted in 2002, a 31% prevalence is quite alarming [314]. Recently reductions were reported between 1993 and 2002 in the proportion of children attending an emergency department for asthma in the previous year (3.6%–2.3%), in children admitted to hospital (1.7%–1.1%) and in children taking asthma medication (18.5%–13.4%) [253]. The high proportion of children aged 1–3 with current wheeze increased from 63.3% to 66.2% [253]. Even if there was an 18% drop over 5 years in hospital emergency visits for asthma in children aged 2–14 years, a decline in the number of annual episodes per child and in the number of children needing further hospital treatment for the same episode, *the hospital admission rates rose by 35.9%* (28). In two Norwegian studies in 3,538 children admitted to an Oslo hospital for acute asthma from 1980 through 1995, the admittance rate *increased significantly among children aged 0-3 years* [145], *3.3-fold in children aged 1–2 years* [91] *or in the 1- to 47-month age group* [194],

Table 5.12. Changes in asthma prevalence and incidence in children reported from studies conducted in different countries

Country	Age (years)	Time span	Prevalence (%)	Incidence (%)	Wheezing (%)		Reference
					Incidence	Prevalence	
Italy	6–14	1995–2002				0.8–1.6	[101]
Austria	Children	1988–1997	24–34				[165]
Denmark	7–17	1986–2001		5.3–11.7			[312]
	10	1985–1995	2–9				[137]
Finland	19	1961–1989	0.1–1.8				[121]
France	21	1968–1982	3.3–5.4				[234]
	8–11	1992–2002	38.3–31		28.6–23.7		[314]
Germany	3–15	1992–1999	2.6–4.8				[132]
Great Britain	4–12	1973–1986	2–4.1				[42]
	<5	1978–1991	8.3–10.1				[252]
	5	1978–1991	2.5–2.6				[252]
	8–9	1991–1999	0.9–1.5				[60]
	8–13	1964–1989	4.1–10.2	10.4–19.8			[215]
	8–14	1989–1994	10.2–19.6				[221]
	12	1973–1988	5.5–12	4.2–9.1	9.8–15.2	17–22.3	[43]
	12–14	1995–2000	20.6–25.9		33.9–27.5		[12]
Hungary	Children	1995–1999	2.7–3.2				[90]
Norway	6–16	1981–1994	3.4–9.3				[219]
	7–8	1996–1997	6.4–7.7	5.3–5.7	11.7–10.7		[255]
	7–12	1981–1994	3.4–8	2–4.2			[294]
	7–13	1985–1995	5.1–8.6				[282]
Poland	6–7	1995–2002	4.0–5.8			25.5–28.6	[190]
			1.3–5.9			17.3–27.6	[190]
	13–14	1995–2002	2.3–6.8			14.6–21.8	[190]
			2.0–5.2			17.1–22.2	[190]
Sweden	17–20	1971–1981	1.9–2.8				[1]
	19	1966–1989	0.29–1.79				[121]
	7–9	1979–1991	2.5–5.7				[3]
	0–16	1973–1993	1.8–6.7				[306]
Switzerland	4–6	1968–1981	1.7–2				[323]
	13–14	1995–2000	10.1–12.3			17.6–20.4	[37]
	15	1968–1981	1.9–2.8				[323]
USA	0–17	1981–1988	3.1–4.3				[310]
	0–17	1980–1996	3.6–6.2				[4]
	6–11	1971–1974, 1976–1980	4.8–7.6				[105]
	12–17	1971–1974, 1976–1980,	6.0–6.5				[105]
Canada	0–14	1980–1990	0.02–0.04 M				[196]
	0–14	1980–1990	0.01–0.03 F				[196]
	3–7	1980–1983	3.8–6.4				[141]
Costa Rica	1–17	1989–1998	23.4–27.7				[295]
Japan	Children	1982–1992	3.3–4.6				[216]
Taiwan	7–15	1974–1991	1.3–5-8				[174]
China	0–14	1990–2000	0.9–1.5				[60]
Hong Kong	13–14	1989–1994	4.8–7.2				[174]
India	<18	1979–1999	9–29.5				[224]
Saudi Arabia	8–16	1986–1995	8–23				[5]
Singapore	4–17	1967–1994	4.0–19.5				[174]
	6–7	1994–2001	16.6–10.2		21.2–23.6		[333]
	12–15	1994–2001	9.9–11.2				[333]

Table 5.12. (Continued)

Country	Age (years)	Time span	Prevalence (%)	Incidence (%)	Wheezing (%)		Reference
					Incidence	Prevalence	
Kenya	13–14	1995–2001	12.6–28.5				[92]
Australia	8–11	1982–1992	9.1–37.7	4.5–6.6	10.4–27.6		[233][a]
			12.9–29.7	12–9.4	15.5–23.1		[233]
New Zealand	7–15	1964–1990	19.1–40.3				[251]
	11–13	1969–1983	7.1–13.5				[203]
	15	1975–1989	7.9–13.3		24.3–27.4		[287][b]
Papua New Guinea	<20	1973–1984	0.0–0.6				[84]
Tahiti	13–16	1979–1984	11.5–14.3				[234]
Arctic	15–19	1987–1998	4.3–20				[166]

The time span refers to data of the first and second follow-ups, usually collected on the same cohort and using the same methods.

[a] The two figures refer to data collected in two study towns, the first coastal and humid and the second inland and dry.

[b] In Maori children the wheezing increase is significantly higher, from 27.1% to 36.2%. In Polish children [190], we first report the children from Krakow and then the children from Poznan, a more industrialized city.

and wheezing illness continues to increase in Aberdeen [221]. In other English children the admission for wheeze increased from 6% to 10% during 9 years, in parallel with the treatment for wheeze (15% to 26%). However, from 1977 to 1986 hospital admissions increased more in children aged 0–4 than in children aged 5–14 [8]. In Honolulu these rates more than doubled from 1986 to 1989, and the *0–2 age group* was the largest [257]. In asthmatic children aged <14, there was a 98% rise in 10 years (vs 15% of all other illness) in accordance with a doubling of hospital admissions over 13 years [301]. In a French study, school absences reached a rate of 21% and the number of urgent hospital admissions 20.4% [176], clear proof of the increased prevalence and severity of asthma [252] . The data are at variance with the decrease observed in Sweden, up to 4.9-fold between 1973 and 1993 [305] and in the USA, which was caused by lower hospital admission rates among children aged 5–17. Younger babies had a fourfold risk of a significant number of readmissions [313], and in infants, especially of low birth weight (LBW), the hospitalizations were affected by increasing exposure to urban pollutants [202]. Recently, the Nordic country incidence of first hospital admission in 1999 was 2.17 per 1,000 children, readmission was noted in 16% of children, mean length of hospital stay was 2.64 days. *Hospitalization rate and BHR* were significantly higher in children <5 years old compared with 6- to 14-year-olds [162]. In Israel from 1990 to 1999 there were 1,584 admissions, 76.3% were first-time admissions, significantly increased over time, and 23.7% were re-admissions, significantly decreased, a finding was more significant in children aged <8. These data reflect the increase in the prevalence of asthma and changes in its treatment [259]. The overall asthma hospitalization rate remained relatively stable from 1991 (1.90) to 1995 (2.31), per 1,000 child-years [262], or rose from 38.0 to 59.7 $\times 10^5$ in 0- to 4-year-olds between 1980 and 1996 (decreasing, so to say, to 51.4 $\times 10^5$ in 1998–1999 [4]), but it was important that *the hospitalization rates for severe asthma rose 270% – from 0.57 to 1.55 – during this period*, while the hospitalization rates for mild asthma decreased from 0.26 to 0.12 per 1,000 child-years [262]. However, the rise in asthma hospitalizations was *24-fold higher among black children* from 1960 to 1997 [67], and in 1998–1999 they were more than threefold as likely to be hospitalized compared with white children [4]. The frequency of asthmatic symptoms seems to be related to unexpected causes (Table 5.11). Between 1985 and 1995 the severity of symptoms was unchanged, despite a large community-based asthma campaign and a *tenfold increase in the number of children receiving inhaled steroids* [36]. Data from Table 5.10 show that lower values are found in studies based on scientifically controlled parameters and definitions of disease (Table 5.1), as well as in countries with less pollution, and that higher levels may be directly related to atmospheric pollution [35, 119, 195]. Pollution may add to the very high prevalence of asthma and related respiratory symptoms [202].

The *natural history of asthma* includes an abundance of studies conducted in several countries, which cover the adult age, sometimes with contrasting results. A study on children living in two different Australian towns, the first coastal and humid and the second inland and dry [233], after 10 years observed an increase in wheezing incidence in both groups. Peat et al evaluated not only the questionnaire results, but also that BHR to histamine (not synonymous of asthma) increased above all in atopic children, as did Der p 1 levels [233]. This rise may match the rise in symptoms. The only positive study seems to be one done in Sweden on a wide and representative group of children, in 55% of whom asth-

ma stopped <14 years, in particular when the onset was in the first 2 years of life (74% of cases). We can presume that this group contained cases of wheezing with a more favorable outcome [2]: an early onset is a negative prognostic factor [53, 340]. In contrast, 11.5- to 14.5-year-old children with severe chronic asthma had an earlier onset of asthma symptoms, a higher incidence of other allergies, and SPTs especially positive for Der p and domestic pets, more frequent thorax deformities, elevated serum and sIgE and BHR in response to both the treadmill test and bronchial provocation test (BPT) with histamine [71]. Also taking into account heredity, 66% [119] to 87% of asthmatics have a positive FH [203] (see Tables 4.10, 4.11). A study from Singapore has found an asthma prevalence decrease in 6- to 7-year-olds, but it increased 2% in 12- to 15-year-olds [333], and from 8.7% to 9.6% in Swiss 13- to 14-year-olds [37] (Table 5.12).

We separately examined the prospective *Melbourne study* with the aim of determining the prevalence and natural history of asthma and wheezing bronchitis in children for as long as 42 years. The study started in 1963 [340] on 295 randomly selected 7-year-old asthmatic children, 74 with <5 episodes of wheezing with VRIs (viral respiratory infections) and 104 with five episodes of wheezing with VRIs, 113 with recurrent wheezing unassociated with VRI, and 105 second graders who had never wheezed to serve as controls. They had a medical examination at school that included a short questionnaire and interview and were followed-up with repeated evaluations until the age of 42.

- At the second follow-up at the age of 10, it was significant that only five children (1.7%) manifested severe wheezing, thus an additional 83 children from the same population were enrolled, who had a history of onset before age 3 with persistent symptoms at age 10, to make the group more representative [197].
- At the follow-up at the age of 14, ≈50% of subjects were still wheezing who, in the more severe cases, started before the age of 2. Bronchial obstruction was often present in the intervals between the attacks associated with PEF and FEV_1 anomalies and frequent thorax deformities. AR prevalence was significantly higher than in the controls and asthma was often associated with severe forms of AD [341].
- At the age of 21 years, the association with AR was frequent, not correlated to wheezing severity. The patients with more severe disease also suffered from urticaria and AD had started before their second birthday; compared with the previous follow-up, the cases of AD and AR had increased. In this review, 50% of subjects had minor wheeze, 25% had frequent symptoms, and only 5% of patients with persistent asthma in childhood were wheeze-free [197].
- In the subjects restudied at 28 years, a significant correlation between asthma severity, IgE levels and atopic disease was seen, with RAST significantly correlated with the classes of subjects at 21 and 28 years (p=0.0001). AR increased to 67% in subjects with more severe asthma and AD cases were significantly fewer. Compared with the follow-up at 14 years, only 32% of subjects were wheeze-free, 41% had less frequent wheezing and in 27% asthma persisted at 28 years: in 21% of subjects with symptoms that were less severe at age of 14, asthma was more persistent, and 39% of asthmatics were smoking [110]; IgE levels were very high in the severe forms (436.1±491.9 IU/ml) and high also in the controls (213.2±246.6) [154].
- In the follow-up at 35 years, in comparison with the previous review, *60% of subjects with persistent asthma at 28 years were unchanged*. Compared with the original diagnosis, 35% of subjects with mild wheezy bronchitis, 70% of asthmatics and 90% of those with severe asthma had persistent asthma, with a mounting progression. Current smokers amounted to 24%–34% of these subjects and AR occurred in 85% of subjects with severe asthma and in 43% of controls. The cause of the high rate of severe asthma was always that the group was "enriched" with children with more severe asthma at the age of 10 so that they had the greatest rate of persistence (63%) at 35 years [222].
- In the last follow-up at 42 years, most who had continued symptoms were little troubled by them, whereas most of those with asthma as children continued with significant wheezing into adult life, *and the more troubled they were in childhood, the more likely it was that symptoms continued*. The risk for AD or AR in later life increased with the severity of asthma in childhood. Subjects with mild disease *who had not taken inhaled steroids had no evidence of pulmonary function testing (PFT) deficit as adults* [236].
- In the people born on 3–9 March 1958 followed-up to age 33, the incidence of asthma or wheezing (42.9%) was associated with atopy, AD and AR. The high risk factor of active smoking is demonstrated by the great consistence of symptomatic groups at 16 years (33.3% were atopic and current smokers) and the relapses after prolonged remissions of childhood wheezing (13% before 33 years and 15% after), *which was more common among current smokers and atopic subjects* [303].
- Blair reported a 20-year follow-up of 267 children treated for asthma before the age of 12. In this study, 52% of patients were wholly or in a great part asthma-free, 21% were never asymptomatic, and 27% went into remission and then asthma caused a 3-year relapse. Blair concluded that *most children with asthma did not outgrow their disease* [31].
- In wheezing children reviewed after 13 years, AD incidence increased by 80% (from 21.7% to 39.2%) [10].

The study by Jenkins et al [144] is interesting because of the debate on clinical history reliability: in a follow-up after >25 years of a cohort of 8,700 schoolchildren recruited at the age of 7, only 59.9% confirmed the data given by parents in their infancy, with substantial underestimation based on the onset age: in 34.3% the onset was before and 20.9% after 7 years. Moreover,

16.9% of children defined as not asthmatic at age 7 suffered from asthma at the last follow-up. At this follow-up including a cohort of 1,494 subjects, 25.6% of those who were asthmatic at 7 years and 10.8% of those qualified as not asthmatic at the first check-up had current asthma (the prevalence of atopic asthma was 21.1% and 8.2%, respectively).

Moreover, 43% of 101 children reinvestigated after 16 years were still affected with asthma. It is striking that the prevalence of SPTs positive to pets increased by 26%, to molds by 16% and to pollens by 46% compared with the first visit at age 6–14, with no differences between current smokers and nonsmokers [109].

In 76% of 406 asthmatic children aged 8–12, 62% with positive FH suffered from asthma at 21–29 years, with an increase in FEV_1 mean percentile predicted values and in inspiratory vital capacity [256].

In a cohort of asthmatic children re-examined after 25 years, the proportion of subjects with BHR was statistically higher in the asthmatics studied, either symptomatic (88%) or not (42%), than in the group of controls (12.8%) [112].

In children followed-up to adulthood, *only 16% were symptom-free* in the year prior to the last follow-up, even if asthma severity was significantly decreased (compared to controls at 13 years), with FEV% and FEV_1 normalization and reduction in the sensitivity to aeroallergens [160].

To these data, which extend asthma natural history to the 3rd or 4th decade and contribute to clarifying that a long-term prognosis can be made for a substantial spectrum of patients, the study by questionnaires of 117 adults hospitalized for asthma at the age of 5–15 years provided a valid and original contribution: 47% were still affected while for the other 53%, who claimed to be free from specific symptoms, the results of spirometry and BPT showed BHR in 23% and rhinoconjunctivitis in 61% of cases [147].

In subjects followed from birth to age 22, the annual prevalence of both wheeze and atopy increased with age: 25% of adults showed both wheeze and BHR (asthma). The prevalence of BHR was *29% at 11 and 40% at 22 years.* Remission of wheeze was common in subjects <5 years of age and likely if wheezing occurred on < two occasions, but *wheeze at 11 years was likely to persist.* Sixty percent of the adult subjects with asthma developed sensitivity to common allergens *by the age of 2 years* and were showing BHR by mid-childhood. There was a steady increase in the *annual prevalence of atopy* over the 22 years, reaching 70% for the whole cohort by adult life [246].

In a cohort of 18,873 subjects involved in a large, nationally representative, cross-sectional study carried out in Italy from 1998 through 2000, asthmatic patients in remission had an earlier age at onset (7.8 vs 15.9 years, $p<001$) and a shorter duration of the disease (5.6 vs 16.1 years, $p<001$) than patients with current asthma. The probability of remission was strongly ($p<001$) and inversely related to the age at onset (62.8% in the <10 years age-at-onset group [74]). In a subsequent paper on 18,156 subjects, the *FH of asthma and allergy* was associated with a higher risk of developing asthma throughout life and worsened the prognosis of both childhood and adult asthma, and *early respiratory infections* were a strong risk factor for asthma starting at any time during a person's life. However, asthma onset at 0–10 years was associated in adulthood with a remission rate of 44% in males and 42% in females, which was reduced to 31% and 27%, respectively, when the onset was at 10–20 years [75].

In an unselected birth cohort of >1000 children, 14.5% had wheezing that persisted from childhood to 26 years of age. Moreover, 27.4% had remission, but 76 (12.4%) subsequently relapsed by the age of 26, together with persistently low PFT results. It was found that *the earlier the age at onset, the greater the risk of relapse* (OR, 0.89 per year of increase in the age at onset). The factors predicting persistence or relapse were *sensitization to Der p, BHR, female sex, smoking, and early age at onset.* Those with persistent or relapsing asthma had substantially impaired lung function at each assessment during childhood, adolescence, and adulthood. Most importantly, the findings suggested that outcomes in adult asthma may be determined *primarily in early childhood* [280].

It is not a surprise that the atopy score during infancy was high in 15 children who had severe atopic asthma at seven, and low in 15 who developed mild disease: 9.2 vs 5.9 [319].

Probably, some previous studies with discordant results have included subjects with a more severe form of asthma, without performing BPT and/or spirometry for diagnosis. Moreover, comparisons between children and adults do not take into account different indoor environments or lifestyles.

Examining the studies on pediatric asthma, three important points should be addressed:

1. Prevalence comparisons after decades, on the same terms, place and age distribution of the patients.
2. Evaluation by certain studies of patients of different ages, usually 15–16 or 21–25 years, in whom the prevalence might also decrease, but other asthmatic forms may arise, including daily shortness of breath or exercise-induced asthma. Moreover, the association with another atopic disorder is frequent, often respiratory such as rhinoconjunctivitis.
3. As Blair [31] has underlined, several studies reported a high proportion of remissions at puberty, but the restricted time span considered made it impossible to uncover the numerous cases of relapses after 3 years. In studies that have documented these relapses with long-term follow-ups of patients undergoing remissions of at least 3 years, a relapse in 27%–38% of subjects was noted.

We deem it necessary that prospective longitudinal studies be extended more often to 18 years and over, even better to 42 years such as the Melbourne study

[222], to better delineate either possible grow-outs or relapses, in order to provide an image that is not false-positive of the natural history.

The variability of prevalence may interact with genetic or environmental factors, but most often depends on the population selected for the study and the diverse prerequisites and parameters related to the diagnosis of childhood asthma. For example, in the first 2 years of life a substantial recurrence of terms such as "asthmatic bronchitis" and "recurrent wheezing" is recorded [141], plausibly caused by the lack of a precise coding of the disease labeled "asthma" [43]. The prevalence increase cannot be explained simply by nomenclature differences [8], since the prevalence of "wheezing" diagnosed separately has remained constant [43]. Some studies based on the differences between asthma and wheezing are interesting: wheezing has an incidence/prevalence at all ages that is almost always higher (and even much higher) than that of persistent asthma (Table 9.10). Moreover, it causes the same number of school absences in the first 5 days, while for longer periods up to >20 days, the total is 18% vs 35% for asthma [135]. The separation between the two conditions is challenging because the diagnostic method varies in several studies. In addition, many parents may forget minor episodes of wheezing in their babies with the passing years, thus underestimating their percentage: this may explain why 49% of such children elude diagnosis [158]. There are children with a transient early wheeze aged <3, likely in connection with a reduced airway development at this time and with VRIs, persistently wheezing children aged <3 and between 3 and 6, and others with a late wheezing aged between 3 and 6, associated with atopy and development of BHR heralding asthma onset [200, 296, 321, 347] and aeroallergen sensitization [308] over age 11 (Table 5.11). Thus the difference is between transient vs persistent wheezers, while we underline that "asthmatic bronchitis" and "asthma" are different labels for the same disease [222, 340]. The term "asthmatic bronchitis" included in the International Classification of Diseases (ICD) has only contributed to underdiagnosis and undertreatment of childhood asthma and to an increase in antibiotic usage [278] in >95% of children [60]. To confirm previous indications [97], the significant decrease in the diagnosis of "bronchitis" (34%–63%) and of "asthmatic bronchitis" (3–31%) [42] leads to concluding that "asthmatic bronchitis" and "asthma" are really two terms applied to the same disease. Increasing knowledge and progress in the diagnostic field have certainly led to an earlier diagnosis of infantile asthma, even if its underdiagnosis continues to be confirmed [283].

When considering the age of onset of asthma (Table 5.5), in the cohort of Anderson et al [10], all cases occurred within the first 3 years of life; in the Rochester study asthma developed in 93.5% of cases in children aged <1 to 14 years and in 59.3% of those aged <1 year [357]. Asthma caused by Der p 1 has an age of onset at <3 years, with a frequency of 3:1 compared to that of pollens ($p<0.0001$) and definitely at ≤3 years ($p=0.0001$) (Fig. 4.14) and rises to an even higher prevalence in several countries, also because serious environmental controls are not always adopted. As regards sex, as discussed in Chap. 4, males are always more affected than females [25, 48, 61, 66, 105, 113, 119, 129, 158, 161, 193, 196, 213,

Table 5.13. Prevalence of atopic disease based on sex

	Males (%)	Females (%)	Total (%)
Food allergy	75	25	1
Asthma	65	35	9
Atopic dermatitis	60	40	5
Urticaria and angioedema	56	44	3
Allergic rhinitis	59	41	4
Anaphylactic shock	100	0	0.1

Data from [48].
p (males vs females)=0.0000.

Table 5.14. Factors predicting the onset of asthma or wheezing in children

Prenatal factors
FH positive for atopy and/or asthma
Male sex of the child[a]
Type of feeding
Smoking in pregnancy
Mother's age <20 years at birth of the child[a]
Postnatal factors
Atopic dermatitis (AD) and allergic rhinitis (AR) (present or past)[a]
Especially early exposure to unfavorable environmental factors
Cigarette smoke
History of pertussis or pneumonia[a]
Infections of upper airways
Adenoidectomy or tonsillectomy[a]
Periodic vomiting or abdominal pain[a]
Factors not associated with statistical significance
Birth order
Low birthweight or premature birth
Social class
Number of children in household
Crowding in household
Smoking of parents
Separation from mother
Previous measles

Modified from [9].
[a] Factors more specifically predicting the subsequent onset of asthma. The highest relative risk is for AD in the 1st year, after the 1st year and in the past year and AR ever and in the past year, both at age 0–7 and later.

Table 5.15. Prospective studies on the natural history of asthma in different countries

Author(s) and reference	Year	No. of patients	Follow-up (years)	Remissions (%)	Notes
Williams et al [340]	1969	295	3	68	Schoolchildren aged 7 years
Williams et al [341]	1977	295	11	50	
Blair [31]	1977	417	22–28	52	
Martin et al [197]	1980	378	11–14	50 25 5	Subjects with mild forms Subjects with frequent symptoms Patients with persistent asthma
Linna [187]	1985	512	5	26	
Park et al [226]	1986	2,702	10	50	Asthmatic subjects
Kelly et al [155]	1987	378	18–21	31–41	Subjects in treatment 59% AR, 29% AD, 12% urticaria
Gerritsen et al [109]	1989	119	16	57	
Kelly et al [154]	1990	378	18–21		67% AR (severe asthma), less AD
Sporik et al [296]	1991	67	11	76	Onset of wheezing <2 years of life
Croner and Kjellman [69, 71]	1991–1992	89	11–14	42	56% AR, 36% AD
Kokkonen and Linna [164]	1993	131	20–24	28	
De Gooijer et al [73]	1993	60	27	47	
Oswald et al [222]	1994	378	25–28	65 30 10	Subjects with mild forms Subjects with frequent symptoms Patients with persistent asthma
Roorda et al [256]	1994	406	11–19	24	
Godden et al [112]	1994	121	25	39	
Jenkins et al [144]	1994	1,494	25	75	See text for details
Kjellman and Hesselmar [160]	1994	56	48	14	89% AR, 28% AD
Strachan et al [303]	1996	5,801	26	35	See text for details
Kjellman et al [159]	2000	55	21	16	Cohort followed up from age 9
Rönmark et al [255]	2001	3,339	2	10	Asthmatic children

Mean remission varies between 41.3% and 47.5% according to the more or less favorable values considered in the Australian study.
AD atopic dermatitis, *AR* allergic rhinitis.

225, 257, 283, 286, 295, 297, 310, 323, 332, 351], beginning at age <12 months [158] and decreasing later until the difference practically disappears. There are exceptions at 0–2 years [2], 4–8 [141, 323], at 14 years [92, 217] in Norwegian children [83, 298] and less so in teenagers [312, 323], until a reversal of prevalence is found at 15 [196] and 23 years [11]. In the USA, male prevalence is at least threefold greater in the 12–17-year-olds [66]. In our cohort of 4,030 children, the male:female ratio was 2:1 (p=0.0000) (Fig. 4.10); in the Rochester series, the ratio was 1.8:1 for the ages of <1 year to 14 years and in the 1st year of life 1.9:1 [3571], in Papua New Guinea 0.9:0.2 [84], until the same rate is found in all atopic diseases (Table 5.13) [48] (p=0.0000). Additional factors predictive of asthma development are summarized in Table 5.14 [9] and widely confirmed [303]. Further details concern the negative significance of bottle-feeding compared to breast-feeding [175], of LBW babies and smoking in pregnancy, even of 1–4 cigarettes, and at 5 years [185]. A crucial factor that should be investigated is the mother's age (<20 years at) childbirth [9, 185], while social factors may attain a statistical significance according to some authors [185, 323], but not others [9]. As regards birth order, firstborns are at risk in 36.3%–39.7% of cases [141]; additional studies have shown no relationship with the number of children in households [185].

The natural history of childhood asthma appears to be favorable: 22 prospective studies (Table 5.15) [31, 69, 71, 73, 109, 112, 144, 154, 155, 159, 160, 164, 187, 197, 222, 226, 255, 256, 296, 303, 340, 341] confirm that 41%–47.5% of children undergo a remission of symptoms at the start of their adult life [252], considering that unlike in adults, specific immunotherapy is able to positively

Table 5.16. Unfavorable prognostic factors of childhood asthma

1.	FH of asthma
2.	Male sex
3.	No breast-feeding, or breast feeding ≤3 months
4.	Mother smoker
5.	Chronic and persistent asthma and severe asthma arising during or before the 2nd or 3rd year of life
6.	Repeated wheezing in the 1st year of life
7.	Presence or persistence of AD, especially severe AD
8.	Coexisting AR
9.	Periodic vomiting or recurrent abdominal pain
10.	SPT positivity
11.	House dampness
12.	Pneumonia, respiratory infections or pertussis
13.	Adenoidectomy and/or tonsillectomy
14.	Delay in the diagnosis and/or inefficient treatment and/or prevention

Having or having had pets at home was associated with an increased risk for incidence of wheezing (OR: 2.9) [255].
Data from [5, 31, 255].
AD atopic dermatitis, *AR* allergic rhinitis, *SPT* skin prick test.

influence the natural history (Chap. 13). However, in children with severe asthma the remission rate is as low as 5% [197]. Yet some studies report a consistent association with other atopic diseases, with a firm persistence of both AR (56%–89%) and AD (29%–54%). In subjects studied over 14–21 years, the less troublesome their asthma was the more likely they were to grow out of it by the age of 21 [197]. Examining the influence of FH on asthma persistence in adulthood, some studies confirm the association [9, 73, 154, 155, 160, 232, 233, 303] and others do not [109, 112, 197, 222, 256, 280].

The unfavorable prognostic factors of childhood asthma are summed up in Table 5.16 [9, 31, 255], to which we add early-onset atopy, an important predictive factor of BHR [232]. Asthma onset before age 3 implies the occurrence of moderate or severe forms in 52% of adults vs 16% if the onset is afterward [160]. Additional factors include wheezing starting after 3 years [296, 321], Der p 1 [280, 321] and/or pollen [141] sensitization, air pollution [232] and BHR [280] at an early age at onset. Several unfavorable predictive factors mark missed remissions in adults: either childhood FEV_1 [256] or a wide panel of factors [144] such as female sex, AD, FEF_{50} decrease, positivity of an intercritical spirometry, positive FH for asthma and asthma in infancy, onset at an age <2 years or having suffered from <10 asthma attacks, symptom duration or severity, passive smoke, pets, soft furnishings, and living next door to busy roads [207, 256].

Although childhood asthma deaths are rare, mortality varied according to international comparisons. In pediatric patients in England, the rates are lessening in children 0–4 to 5–14 years of age [3]. In the USA, 100

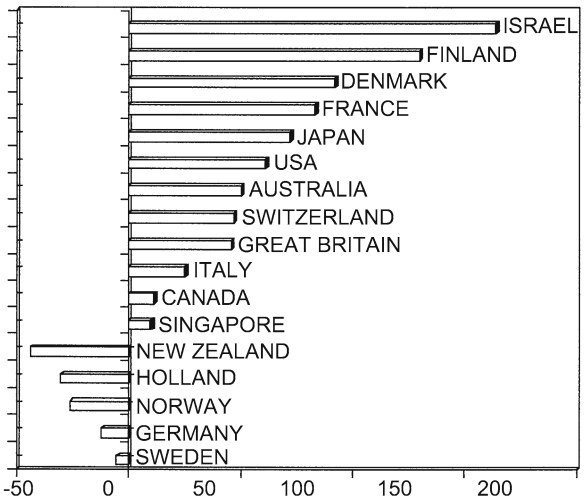

Fig. 5.19. Asthma mortality in 17 countries in 1987, calculated as rate of increase compared to 1980 rate among subjects aged 5–34 years. (Data from [218])

deaths per year are recorded in children, especially in the last decade there has been an annual increase in fatal cases, more likely in children 5–14 years than in the 15- to 34-year-old age-groups [357], with a time-trend of increase involving mostly the younger part of the population. US children aged 0–4 had the largest increase in prevalence, but adolescents had the highest mortality, and black children compared with white children were > fourfold as likely to die from asthma in 1997–1998 [4]. In 1988 (data $\times 10^5$), the year prevalence was 0.1×10^5 in 0–4 years, 0.3×10^5 in 5–14 years,

Table 5.17. Asthma mortality rate in 1990–1993 in persons aged 5–34 years

Country	Rate × 10^5 residents
Australia	1.13
Singapore	1.05
New Zealand	0.79
England	0.74
Japan	0.69
West Germany	0.56
Wales	0.52
Switzerland	0.50
USA	0.43
Israel	0.40
Canada	0.37
France	0.37
Italy	0.23
Austria	0.32
Finland	0.21
Sweden	0.12

Data from [24, 191, 278].

and 0.4 × 10^5 in 15- to 24-year-old individuals but could rise yearly to 1–2 cases × 10^5 [357]. In a survey of children aged 12–19, death rates per year were highest in New Zealand (1.75 × 10^5), intermediate in England and Wales (0.66 × 10^5) and South African Caucasians (0.67 × 10^5), and lowest in Sweden (0.38 × 10^5) [44]. Data from the USA show a rate 0 until 10 years, 0.04 % for 10- to 15-year-olds and 0.1 % for 15- to 20-year-old patients: if the total rate is 4 %, with a general total of 2.4 cases × 10^5, this increase is more pronounced for older subjects [291]. Asthma mortality in Israel is higher in the 5- to 34-year-old subjects, especially males [191], but remained stable between 1980 and 1997, with a mean rate of 0.226 × 10^5 [237]. However, it is not known whether these deaths attributed to asthma [291, 357] are also due to associated factors [348]. Figure 5.19 [278] outlines the increased rates between 1980 and 1987 relating to subjects aged 5–34; the 1990–1993 data are shown in Table 5.17 [24, 191, 278].

Allergy to Inhalants

The increased prevalence of specific allergens, especially that of pollens, due to an increase in environmental pollution and ozone depletion has been documented in Chap. 4; however, the reported different rates appear to depend on many factors [26, 42]. The results of various studies are summarized in Tables 5.18 [30, 35, 78, 84, 106, 120, 148, 150, 163, 235, 245–247, 289, 308, 331, 361] and 5.19 [271], showing that a very high proportion of infants develop multiple sensitizations [78, 292], a rate that is higher in small groups, but diffused through the entire pediatric age range, with rates significantly greater than in monosensitized children (Fig. 5.20) [292]. The ETAC study has established that 20 % of babies are sensitized to Fel d 1 and 17 % to Der p 1 (Figs. 5.21, 5.22). In Sweden, allergy to pets and pollen is prevalent [35], and is increased by 2.4 % in 12 years [294], the rates in Finland are higher [245]. Der p sensitization is more frequent in heavily polluted places [35, 247], with meaningful rates in Munich [331] and in other German cities [271]. It is

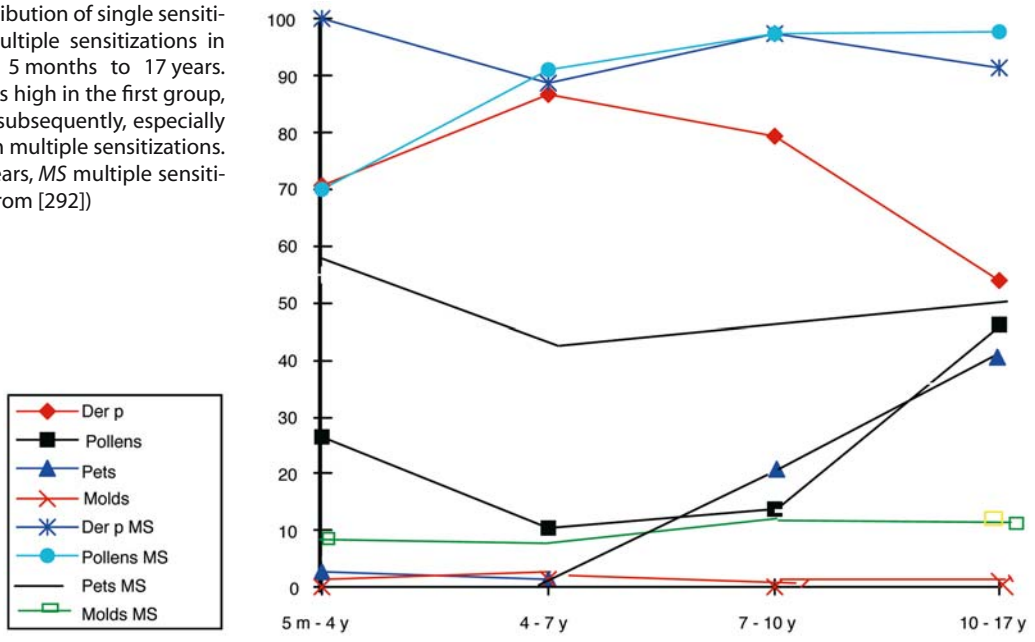

Fig. 5.20. Distribution of single sensitizations and multiple sensitizations in children aged 5 months to 17 years. The incidence is high in the first group, and increases subsequently, especially in children with multiple sensitizations. *m* months, *y* years, *MS* multiple sensitizations. (Data from [292])

Table 5.18. Prevalence of allergy to inhalants (%) in different countries

References	[78]	[18]	[308][a]	[106]	[150]	[245][b]	[235]	[331]		[35]			[247]		[163]	[351]	[361]
Age (years)	1	2–10	4	6–24	7	7–12	8.5	9–11		10–12			10–12		16	1–15	13–14[c]
								Leipzig	Munich	Sweden		Poland	Industrial	International			
										Urban	Rural						
No. of cases	49	53	61	4,295	1,755	244	170	2,335	4,451	351	289	358	589	631	737	2,879	2,126
Allergens																	
Mites																	
Der p	42	34	42.6	14	3.2	4.9	56	4.2	10.3	3.4	1.7	3.4	6.1	2.9	42.8	12.4	16.9–2.7
Der f		49				2	56										20.4–3.7
Pollens			31.1				65	7.9	21.3	20.2	13.8	9.8	4.8	3.5			
Ole e					10.9										5.6		
Grasses					18.5											22.4	
Ambrosia	14			9													
Artemisia						4.9											
Birch						28.7		3.3	12.7	12	10.4	2.2	2.6	2.2		11.4	
Cynodon				9.5		23.7											
Dac g		19															
Lolium	30			9		24.6											
Par o							40										
Phleum pratense						25.4											
Quercus			12														
Molds															2		
Alternaria	24	2	77	21													
Aspergillus	14																
Cladosporium	26	2	34.4			4.6											
Penicillium	18																
Pets																	
Can d 1	65		14.7	14		15.6	6	2.7	2.5	14.5	11.8	2	2	1.4			
Fel d 1	70	17	27.9	9		34.5	25	2.9	7.3	21.1	12.5	2.5	6.1	3.8		6.4	10.8–2.7

[a] Evaluations done on 61 out of 981 children.
[b] The percentage was calculated on the whole sample since the controls had more SPT+ than the asthmatics, extended polysensitizations were present.
[c] The first figure is related to urban, the second to suburban children.

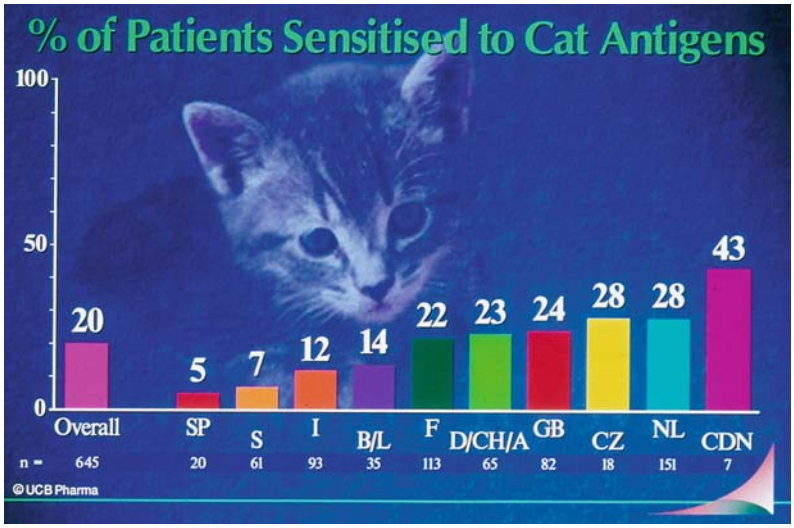

Fig. 5.21. ETAC Study. On average 20% of children are sensitized to cat antigen (Fel d 1), the highest frequency is in Canada (43%), the lowest in Spain (5%)

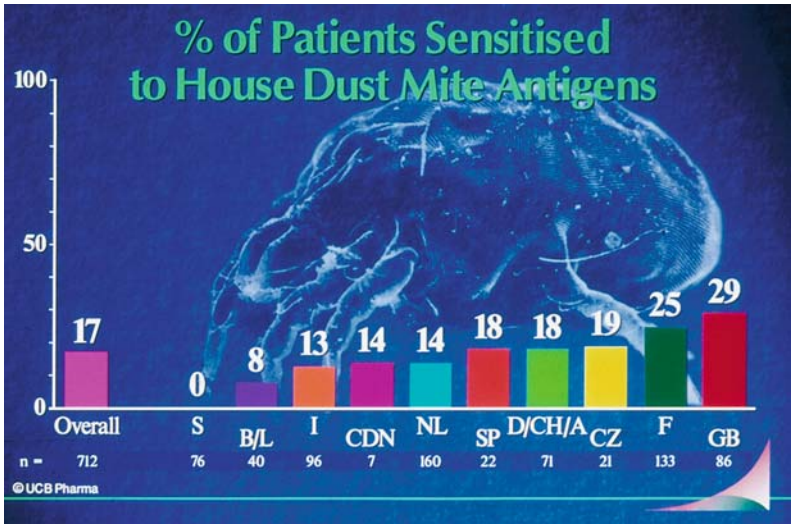

Fig. 5.22. ETAC Study. On average 17% of children are sensitized to dust mite antigen (Der p), the highest frequency is in Great Britain (29%), the lowest is 0% in Sweden

higher in France [205], in New Zealand [280], in Italy [235] and Wight Island [308], four- to fivefold higher after 10 years of age in Australia [233], reaches elevated levels in Hong Kong even in nonasthmatic children [182] and is often associated with mold allergy [245, 295]. Not only asthmatic children are Der p-sensitized (11.1%), but also those with AR (8.8%) and AD (6%) [307]. In Australia the prevalence of pet allergy reaches unexpected rates in the same time span with a 4.5- to 5.5-fold increase [233], contrary to Wales [296], and is lower (1.2%) in Sweden [294]. *Cat allergy* reaches high levels, which at 1 year of age are responsible for 70% of positive SPTs [78]. In a school, positive RAST and CAP increased from 8.5% in 1983 to 9.5% in 1992 [41]. Among 4- to 8-year-old children, prevalence is as high as 28%–34.5% [20, 169, 176, 225], in 9- to 12-year-old children it is 2.5%–7.3%, with no notable differences between polluted and unpolluted areas [35, 247, 331], with peaks from 21.1% [35] to 53% in Sweden [71], 28% in Wales [297], 63% in New Zealand [14], decreasing to 5% in former students [101]. This picture was remarkably complicated by the incredibly ubiquitous Fel d 1. The prevalence of *dog allergy* in Mexican infants is as high as 65% of SPT+ [78]; in children 4–8 years of age, it is lower, with 15%–16% titers [235, 245, 308]. In Sweden there is a peak as high as 49% [71], in Wales 35% [297] and in former students there is a 10% rate of allergy, documented with SPT positivity [101]. In New Zealand the figure is 48%, with high titers even for other mammals, from 11% to 23%, and birds from 11% to 17% [23], highest in South Africa with 76% (dog), 42% (cat) and 26% (birds) [44], the lowest apparently in Sweden, 17%, 23% and 21%, respectively [44], with the lowest rates in Poland [35] and Estonia [247]. Estonian children aged 5 had the highest SPT positivity to cat and dog [148] (Tables 5.18, 5.19). *In 13 studies comprising 16,638 allergic children, the incidence of pet allergy was 18.4% for cat and 12.7% for dog.* In France, 46.2% of asthmatic children live with at least one pet [176], as do 1.9% of students in China [184].

Table 5.19. Comparison between more or less polluted German cities (%) (SPT)

	Apparently more polluted	Apparently less polluted	Former East Germany
Betula	12	2.6	1.4
Grasses	15.5	9.2	12.9
Mites	9.4	8	5
Cat	16	5.6	6.8

Data from [271].

Table 5.20. Prevalence of cockroach allergy according to age

Age (years)	Prevalence (%)	Country	References
0.1–10	15	USA	[77]
1	16.6	Italy	[235]
1	42	Mexico	[78]
2–8	16.9	France	[30]
<3	14.5	USA	[102]
>3	15.6	Italy	[235]
3–6	35	USA	[102]
3–7	34	USA	[125]
3–15	25.7	USA	[50]
7–12	50	USA	[102]
13–14	11	China	[361]
14–18	36	China	[184]
Children	50	USA	[238]

Horse Allergy

Horse allergy is present in 2% of unselected children at age 5, 5% at age 7–16, 7.8% at age 7–12 [180, 245], in 16% of atopic children of equal age and in 21%–35% of asthmatic children aged 7–15 [245]. Increased prevalence in the later years is due chiefly to extrafamilial contacts (79% of cases) [180]. It causes conjunctivitis in 64%, asthma in 54%, rhinitis in 43% and skin symptoms in 18% of cases, and is associated with allergy to mites (79%), grasses (68%) and cat (64%) [180].

Cockroach Allergy

Cockroach allergy is increasing: 38 US children, 22 asthmatic and 16 controls, had sIgE for cockroach and in 71% of their houses the dust contained Bla g 1, especially in the kitchen, surprisingly also in 20% of unaffected houses [238]. In China, the prevalence is high in students [184]. In Israel cockroaches are present in the majority of houses, independently of the socioeconomic level of residents [158]. The prevalence of asthma to *Blattidae* is still higher, rising from 16.9% to 42%, with onset in the 1st year of life (Table 5.20) [30, 50, 77, 78, 102, 125, 184, 235, 238, 361]; thus it is necessary to look for multiple sensitizations [297]. In 50 asthmatic and not asthmatic children, several other insects have been reported such as ants, crickets, flies, grasshoppers, moths, some cross-reacting between themselves at the RAST inhibition, moths also cross-reacting with Der p 1, Bla g, Fel d and CM [186]. The prevalence of allergy to rodents and domestic birds is not very common.

Allergic Rhinitis

AR is very frequent: some authors state that AR onset occurs by the age of 5 years in 94% of cases [163] (Fig. 5.23) and in 21%–35% in the 1st year [163, 349], or in 36%–46% of cases from 4 to 10 years and in 35%–54% from 10 to 20 years (Table 5.5). The male:female ratio is 2.3:1 up to 10 years, then equal [32], or it is reversed from 5 to 14 years [350]. The prevalence variation in different countries is noteworthy (Table 5.21) [2, 3, 6,

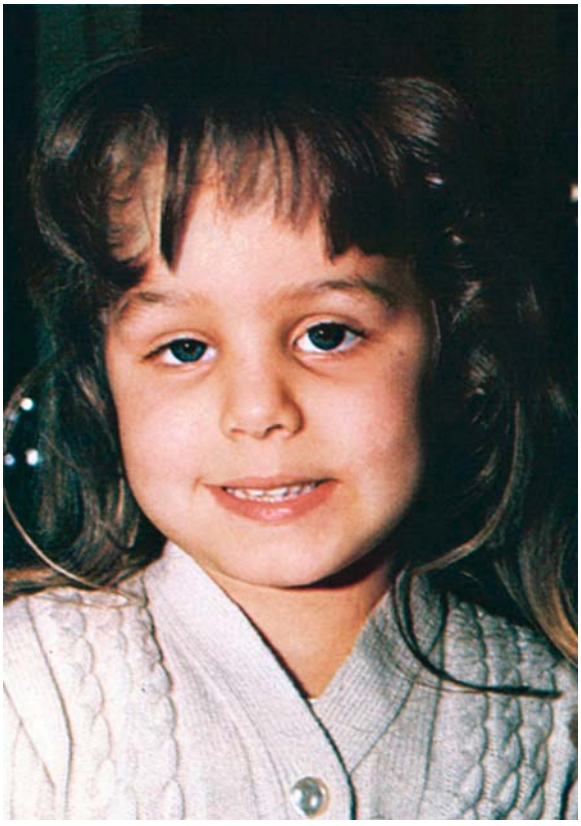

Fig. 5.23. Early onset of AR. Note the marked suborbital edema, the prominent suborbital folds (Dennie-Morgan folds) and other symptoms of multiple allergies

Table 5.21. Prevalence and incidence of AR (%) in children reported from studies conducted in different countries

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References[g]
Italy	0–>6	220	1993	3.5		[53]
	5[a]	634	1993	3 S		[48]
	5[a]	634	1993	1 P		[48]
	9–10	1,760	2003	13.2	9.1	[79]
	9–15	930	1988	13.1		[18]
Albania	6–7	2,981	1997	16.4	13.1	[304]
	13–14	2,957	1997	18.6	12.7	[304]
Austria	6–7	2,893	1997	13.2 (11.4–15)	11.7 (10.3–14.7)	[304]
	13–14	2,443	1997	26.9	21.6	[304]
Belgium	6–7	6,533	1997	19	14.7	[304]
	13–14	1,515	1997	44.8	36.4	[304]
Denmark	5–7	1,052	1993		7.4	[268]
	8–10	1,052	1993		7.8	[268]
	11–13	1,052	1993		14	[268]
	12–16	1,501	2001		15.7	[209]
	14–16	1,052	1993		17.3	[268]
Estonia	6–7	3,070	1997	15	11.6	[304]
	10–11	962	2001	21.6	6.2	[15]
	10–12	1,519	1993	7.4		[247]
	13–14	2,462	1997	30.9 (28.7–33.1)	21.9 (20.8–22.9)	[304]
Finland	3–18	3,649	1980		6	[240]
	13–14	2,974	1997	47.3 (43.6–55.1)	37.1 (33.3–45.5)	[304]
	15–16	1,712	1990	14		[324]
France	4–14	505	1984	3		[205]
	6–7	3,202	1997	25.8	22.2	[304]
	13–14	3,709	1997	54.7 (47.9–63.3)	46.5 (40.5–58)	[304]
Germany	5–6	581	1989	7.2		[171]
	6–7	3,296	1997	13.4 (11.8–14.9)	11.5 (10.4–12.5)	[304]
	9–11	3,984	1990	9.5		[81]
	9–11	8,204	1990	18.5		[332]
	13–14	3,586	1997	37.3 (36.5–38)	29 (28.7–29.3)	[304]
	10–18	8,771	1987	16.7		[350]
Germany (ex East)	5–7	287	1991	1.4		[271]
	6	1,358	1992	1.5		[27]
	9–11	1,051	1992	2.7		[329][c]
Germany (ex West)	5–7	987	1991	2		[271]
	6	638	1992	2		[27]
	9–11	5,030	1992	8.6		[329][c]
Great Britain	4	161	2003	10.6		[172]
	6–7	1,864	1997	23.7	21.2	[304]
	6–7	3,000	1999	25.7 M, 24.0 F		[286]
	12	965	1993	16		[44]
	12–14	27,507	1999	34.9	18.2 d	[19]
	13–14	2,366	1997	45.8 (34.4–50.2)	36.9 (30–40.8)	[304]
Greece	6–7	1,654	1997	13.1		[304]
	9–12	290	2004	12.6		[289]
	13–14	2,561	1997	17.2	14.4	[304]
Ireland	6–7	1,899	2003	20.2		[129]
	13–14	3,147	1997	48.6	41.8	[304]
Latvia	6–7	3,003	1997	16.7	13.4	[304]
	13–14	3,075	1997	31 (29–32.9)	22.3 (21.4–23.2)	[304]
Malta	5–8	3,506	2002	24.3 M, 22.5 F	47.4	[207]
	6–7	3,493	1997	23.5	20.8	[304]
	13–14	4,184	1998	49.2 M, 55.2 F	47.4	[206]

Table 5.21. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References[g]
Norway	6–7	2,325	2004	11.4 M, 11.2 F		[94]
	7–12	575	1994	21.8 M, 18.9 F		[83]
	7–13	4,666	1993	21.3 M 14.2 F		[298]
Poland	6–7	2,264	1997	21 (13–29}	18.1 (11.3–25}	[304]
	13–14	3,389	1997	24.7 (19–28.8)	18.3 (13.7–21.2)	[304
Portugal	6–7	1,710	1997	22.9 (18–26.9)	19.6 (14.7–23.4)	[304]
	13–14	2,688	1997	29.9 (28.5–31.4)	21 (19.7–22.7)	[304]
Romania	13–14	3,396	1997	15	11.5	[304]
Russia	13–14	3,411	1997	12.6	9.8	[304]
Spain	6–7	3,377	1997	19.4 (14.5–26.9)	13.5 (9.9–18.8)	[304]
	13–14	3,128	1997	44.3 (35.4–49.7)	32 (26.1–37.6)	[304]
Sweden	0.5–16	4,990	1985	7		[13]
	6–7	3,029	1997	17.1	14.1	[304]
	7–9	4,281	1991	6.5–19.1		[3][f]
	10–11	1,654	1989	13.6		[69]
	10–11	2,062	2001	12.3–14.1		[15]f
	12	1,411	1993	14		[44]
	13–14	3,226	1997	30.3 (25.4–35.1)	22.9 (19.2–26.7)	[304]
	14	1,335	1989	10.8		[2][d]
	14	1,112	1987	21	14.8	[217][d]
Switzerland	4–6 S	3,270	1981	1.1		[323]
	4–6 P	3,270	1981	0.2		[323]
	13–15	2,879	2001	17		[351]
	15 S	3,500	1981	6.1		[323]
	15 P	3,500	1981	0.6		[323]
Canada	6–7	2,878	1997	28 (25.6–30.5)	25.6 (22.6–28.6)	[304]
	13–14	2,476	1997	45.5 (39.5–51.5)	39.8 (33.8–45.8)	[304]
USA	6	747	1993	42		[349][a]
	13–14	2,503	1997	41.8 (38.9–47.2)	34.6 (29.5–40.6)	[304]
Argentina	6–7	3,006	1997	49.1 (47.8–50.4)	40 (38.3–41.8)	[304]
	13–14	3,001	1997	72.2 (69.2–75.1)	62.4 (59.6–65.2)	[304]
Brazil	6–7	2,420	1997	35.7 (33–40)	28.2 (22.8–33.8)	[304]
	13–14	3,091	1997	48.5 (35–68.2)	36.7 (24.1–55)	[304]
Chile	6–7	2,710	1997	25 (20.3–29.7)	21.3 (17.4–27)	[304]
	13–14	3,177	1997	27.5 (20–34.5)	21 (15.5–27.9)	[304]
Costa Rica	6–7	2,942	1997	32.7	26.6	[304]
	13–14	3,200	1997	39.2	30.9	[304]
Mexico	6–7	3,097	1997	64.8	23.2	[304]
	11–14	3,102	1977	59	22.2	[304]
Panama	6–7	3,043	1997	28.1	20.5	[304]
	13–14	2,885	1997	33.8	24.1	[304]
Paraguay	13–14	2,966	1997	80.5	66.6	[304]
Peru	13–14	3,158	1977	41.9	34.5	[304]
Uruguay	6–7	3,071	1997	33.8	25.1	[304]
	13–14	3,072	1997	51.5	34.5	[304]
Venezuela	4–21	240	1983	3.2		[56]
Algeria	13–14	1,173	1997	45.5	37.9	[304]
Ethiopia	13–14	2,989	1997	27.1 (4.2–50)	16.4 (3.2–29.5)	[304]
Kenya	13–14	3,134	1997	36.2 (32.4–44)	25.8 (20.5–31)	[304]
Morocco	13–14	3,120	1997	33.1 (29.7–36.1)	23.5 (19.4–26.6)	[304]

Table 5.21. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References[g]
Nigeria	13–14	3,057	1997	55.2	45.3	[304]
South Africa	12	1,239	1993	30.6		[44]
	13–14	5,173	1997	37.7	30.3	[304]
China	13–14	3,802	1997	36.3 (23.7–46.3)	30.5 (20.5–39.5)	[304]
	14–18	737	1992	1.6 S		[184]
	14–18	737	1992	2.7 P		[184]
South Korea	6–7	4,055	1997	36.1 (34.6–37.7)	31.4 (29.3–33.5)	[304]
	13–14	4,992	1997	37 (35.3–38.8)	30 (28.8–32.2)	[304]
Philippines	6–7	3,558	1997	21.6	18.4	[304]
	13–14	3,207	1997	35.9	27	[304]
Japan	6–7	2,900	1997	30.8	25.6	[304]
	6–12	Entire country	1984	15		[317]
	13–14	2,831	1997	52.6	41	[304]
Hong Kong	6–7	3,618	1997	36.5	32.9	[304]
	13–14	4,667	1995	53.7		[304]
	13–14	4,666	1997	52.1	44.5	[304]
Korea	6–12	38,955	2001	10.5		[177]
	12–15	38,955	2001	10.0		[177]
Vietnam	5–11	1,480	2003	34.9	27.6	[214]
India	6–7	2,264	1997	12.7 (2–28.6)	10 (1.5–23.8)	[304]
	13–14	2,655	1997	18.8 (4.4–47.9)	13.6 (3.4–37.7)	[304]
Indonesia	6–7	1,390	1997	21.2	20.1	[304]
	13–14	2,249	1997	61.7	32.6	[304]
Israel	13–14	10,057	2004	41.6	9.4	[116]
Iran	6–7	2,735	1997	8.8 (7.8–9.8)	6.4 (5.2–7.6)	[304]
	13–14	2,716	1997	24.5 (20.7–28.2)	17.6 (13.9–21.2)	[304]
Kuwait	13–14	1,056	1997	42.1	31	[304]
Lebanon	13–14	2,993	1997	33.4	25.4	[304]
	13–14	NS	1999	25.5		[244]
Malaysia	6–7	3,057	1997	15.8 (12.7–18.8)	12.6 (10–15.1)	[304]
	13–14	3,727	1997	44.6 (38.2–50.3)	33.5 (25.5–40)	[304]
Oman	6–7	3,891	1997	22	15	[304]
	6–7	3,893	2003	7.5		[7]
	13–14	3,174	1997	34.6	23.8	[304]
Pakistan	13–14	1,829	1997	34.8	29.9	[304]
Singapore	6–7	1,936	1994	6.3		[113]
	6–7	2,353	1997	29.3	26.3	[304]
	12–15	4,150	1994	3.7		[113]
	13–14	4,206	1997	50	41.2	[304]
Thailand	3–15	120	2004	53		[297]
	6–7	3,828	1999		18.5	[315]
	6–7	3,728	1977	28.6 (20.8–36.3)	25.6 (18.5–32.6)	[304]
	6–7	2,658	2000	38.4	33.5	[310]
	13–14	3,820	1997	48.7 (47–50.4)	40.8 (38.3–43.2)	[304]
	13–15	3,927	1999		38.3	[315]
Taiwan	6–7	4,806	1997	36.5	30.8	[304]
	7–14	8,345	1988	11.6 M 7.3 F		[307]
	13–14	11,400	1977	35.1	28.8	[304]
	13–14	3,410	2000	59.4	42.5	[311]
UAE	6–13	3,200	2000	14.9		[6]
Uzbekistan	13–14	2,331	1997	19.5 (14.8–24.1)	13.1 (8.8–17.3)	[304]

Table 5.21. (Continued)

Country	Age (years)	No. of cases	Year	Prevalence	Incidence	References[g]
Australia	6–7	2,725	1977	29.4 (25.7–32.5)	26.4 (23–29.9)	[304]
	8	2,360	1989	23.7 M 22.3 F		[85]
	8–10	380	1989	12.1		[232]
	12	2,189	1989	27.2 M 26.5 F		[85]
	12	965	1991	19 M 12.8 F		[23]
	13–23	3,070	1991	43.9 (40.2–48.5)	37.5 (33–41.5)	[304]
New Zealand	5–7	3,095	1997	26.6 (18.4–29.3)	23.6 (16.5–25.7)	[304]
	6–7	3,000	2001	10.0		[17]
	12	873	1991	23.1 M 18.7 F		[23]
	13	662	1991	27.3		[279]
	13–14	3,172	1997	45.9 (42.5–49.3)	38.8 (36–41.2)	[304]
	13–14	3,000	2001	19.0		[17]

M males, *F* females, *S* seasonal, *P* perennial, *RC* rhinoconjunctivitis.
[a] Median age.
[b] See Fig. 5.6.
[c] The highest levels were for children living in Munich, a city certainly more polluted than Leipzig and Halle [329, 331].
[d] Rhinoconjunctivitis.
[e] Fifty percent of cases were doctor-diagnosed within the 1st year of life [349].
[f] Survey in two different cities [3, 15].
[g] In the ISAAC study [311], when the mean data of each country are given, the minimal and maximal values of the prevalences are shown between parentheses.

Table 5.22. Prevalence and incidence of provoking factors and rhinitis symptoms (%)

Symptoms	Atopic subjects	Nonatopic subjects	In 1,058 subjects
References	[288]	[288]	[334]
Principal and severe symptoms			
Sneezing	32	16[a]	
Running	24	19	
Blocking	35	38	
Other symptoms			
Itchy eyes	80	60[a]	
Sinusitis	39	57[b]	
Catarrh	61	61	
Wheezing	39	18[a]	
Incidence of symptoms			
Seasonal	65	41[a]	
Perennial	76	85	
Diurnal	66	62	
Provoking factors			
Dust	76	63[a]	30
Fumes and/or perfumes	50	37[a]	
Pollen	63	25[a]	80
Pets	26	8[a]	15
Colds and/or infections	69	81[b]	10
Tobacco			20
Stress			10
Coloring material			5
Foodstuffs (milk + egg)			1

[a] Statistical significance for atopic subjects.
[b] Statistical significance for nonatopic subjects.

Table 5.23. Changes in prevalence and incidence of AR (%) in children reported from studies performed in different countries

Country	Age (years)	Time span	Prevalence	References
Italy	6–7	1995–2002	5.2–6.4	[101]
	13–14	1995–2002	4.1–6.3	[101]
Denmark	10	1985–1995	1–9	[137]
Germany	5–14	1992–1999	4.4–6.1	[132]
Great Britain	8–13	1964–1989	3.2–11.9	[215]
	12	1973–1988	9.4–14.9	[43]
	7.5–8.5	1978–1991	39–40.4	[10]
	8–14	1989–1994	11.9–12.7	[221]
	8–9	1991–1999	12.3–16.4	[12]
	12–14	1995–2002	38.4–37.4	[12]
Norway	7–12	1981–1993	4–3.8	[294]
	7–13	1985–1995	16.4–22.1	[282]
Sweden	17–20	1971–1981	4.4–8.4	[1]
	7–9	1979–1991	5.5–8.1	[3]
Switzerland	4–6 S	1968–1981	0.5–1.1	[323]
	4–6 P	1968–1981	0.6–0.2	[323]
	13–14	1992–2000	31.0–32.0	[37]
	15 S	1968–1981	4.4–6.1	[323]
	15 P	1968–1981	1–0.6	[323]
Saudi Arabia	8–16	1986–1995	20–25	[4]
China	6–7	1995–2001	38.9–42.4	[178]
Hong Kong	13–14	1989–1994	24–38	[174]
Kenya	13–14	1995–2001	14.9–38.6	[92]
Australia	6–7	1993–2002	14.9–19.8	[253]
	8–11	1982–1992	20.3–24.4	[233]
	8–11	1982–1992	22.5–31.9	[233][a]
	8–11	1992–2002	34.1–38.4	[314]
New Zealand	12–18	1982–1992	8.8–21.3	[14, 279]

S seasonal, *P* perennial.

[a] Study conducted in two cities (see Tables 5.7 and 5.12).

7, 13, 15, 17–19, 23, 27, 44, 48, 53, 56, 69, 79, 81, 83, 85, 94, 113, 116, 129, 171, 172, 177, 183, 184, 205, 207, 209, 214, 217, 232, 240, 244, 268, 271, 279, 286, 289, 298, 304, 310, 315, 317, 323, 324, 329, 331, 332, 349, 350, 351] as well as the significant increase with age change [268]. The top rate was attained by a US prospective study where in 747 children followed up from birth to 6 years of age, the prevalence was 42%, which in Sweden has fallen in the last 12 years [294]. AR is often underdiagnosed, mainly in asthmatic children, probably because rhinitis symptoms are in proportion less apparent, or because they are of minor importance compared with a more severe allergy [334]. These data are strengthened by the suggestion that up to 75% of asthmatic children and 53% of those with AD risk are affected with AR in the future (Table 5.8); other studies report that AR is a precursor of asthma in 3%–24% of cases [189]. The rate of SPTs positive to aeroallergens is high in 6- to 24-year-olds. Only for dog allergens is it <20%; for other allergens it ranges from 20% to 30% [106]. Two studies disclose no variations between urban and rural populations [300, 350], but one study has found AR more frequent in villages (10.5%) than in cities (8%) [350]. The low incidence of AR and allergic sensitization, for example, the highly polluted Leipzig [329] indicates that the decreasing prevalence of atopic diseases is indirectly caused by the increased exposure to environmental pollutants [132]; however, the greater incidence in firstborns is confirmed [300]. In parallel with Table 5.10, Table 5.22 sums up the prevalence/incidence of provoking factors [288].

We have analyzed the most significant studies in addition to those outlined in Table 5.21. In one study (Fig. 5.3), at the last follow-up the prevalence and incidence were 0%. Sporik et al [296] recorded a gradual increase with age.

The data for *natural history* are summarized in Table 5.23 [1, 3, 4, 10, 12, 14, 37, 43, 92, 137, 174, 178, 215, 221, 233, 253, 279, 282, 294, 323]:

- AR usually persists up to adulthood, complicating with asthma in 50% of cases. Prognosis is more favor-

Table 5.24. Risk factors related to AR

Parental atopy*
First child*
Onset during the 1st year of life
Mother smoking one pack of cigarettes per day during the 1st year of life
Maternal asthma*
Elevated serum IgE levels*
Early introduction of CM and/or solid foods
Households with dogs as pets*

* Statistically significant.
Data from [349].

Table 5.25. Allergens to which atopic children with doctor-diagnosed AR reacted

Allergen	Incidence (%)
Bermuda	69
Alternaria	49
Careless weed	39
Olive	38
Mesquite	31
Mulberry	29

Data from [349].

able in the monosensitized subjects who follow reliable preventative measures [101].

- In Sweden, AR incidence is fairly constant without remissions [2]. Prevalence increased from 4% to 14.5%, that of asthma from 3.8% to 5.3% (Fig. 5.6).
- In Japan, the prevalence (cedar pollinosis) increased from 8% to 12% from 1950 to 1987 [350].
- In studies done in Finland, 38% of adolescents also manifested AD [237]. In children aged 3–17 years checked after 8–11 years, only 9.7% were symptom-free at the last control; 23% of those with seasonal AR manifested a perennial AR and 19% had asthma, 34% of them with perennial AR vs 12.7% with seasonal AR [189].
- There were more children SPT positive for pollens after a mean of 26 years and 19 out of 36 (52.7%) adults with positive history were still affected [109].
- In a group followed-up from birth in 1953, the prevalence was 16.5% at 23 years [300].

The risk factors included in the US study are summarized in Tables 5.24 and 5.25 [349].

Allergic Conjunctivitis

Allergic conjunctivitis is frequent in children, although less than in adults. However, its prevalence is unknown because of the lack of epidemiological studies and the frequent association with several cases of AR, especially when the sensitizing lung allergen is a pollen. In the study on the month of birth, 12.7% of children were-affected [47], corresponding to 10% of another cohort [245]. In 154 children, eye symptoms disappeared or were effectively controlled by topical treatment [189], whereas in subjects with other allergies, the prevalence is over 60% [180, 245], and in patients controlled at 17 and 24 years of age, it persists in 80% of cases [160]. In 7632 children aged 5–14 between 1992 and 1999 the impact varied between 6% and 7.5% [132]. Between 1995 and 2001, the impact of itchy eyes/conjunctivitis increased from 11.8% to 24.1% [92] and from 13.6% to 17.2% [178].

Insect Allergy

Insect allergy syndrome, whose diagnosis and treatment were established >10 years ago, after recognition of the venom as the responsible agent, is a problem not so much for the number of involved patients as for the extent and severity of reactions, potentially fatal. Some 15%–24% of the general population appears to be sensitized to Hymenoptera venom following a medical diagnosis and SPT or RAST [57, 58], and every year thousands of reactions are reported. It is prevalent among the children of beekeepers (about 65%–70% are involved), with a small number of other children residing in the country [228]. At pediatric ages, this allergy has a prevalence of 10%–20% [117, 228, 274, 284] up to 32%–54% [21]; otherwise there are minimal levels, ranging from 1×10^6 to 0.15% [227, 285, 293]. Clinical evolution in children is more favorable than in adults. Mortality is also lower [228], which in adults varies from 0.45×10^5 in Switzerland to 0.09×10^5 in England (Chap. 17).

Prevalence of Atopic Disease at Various Ages

Figures 5.3, 5.4 and 5.13 show the differentiated prevalence according to age. In particular, Fig. 5.13 shows sIgE prevalence to foods and inhalants as a function of age. We have commented on the sIgE prevalence to foods; the increasing prevalence of inhalant allergy from 14 to 36 months can be seen in Fig. 5.20. Children

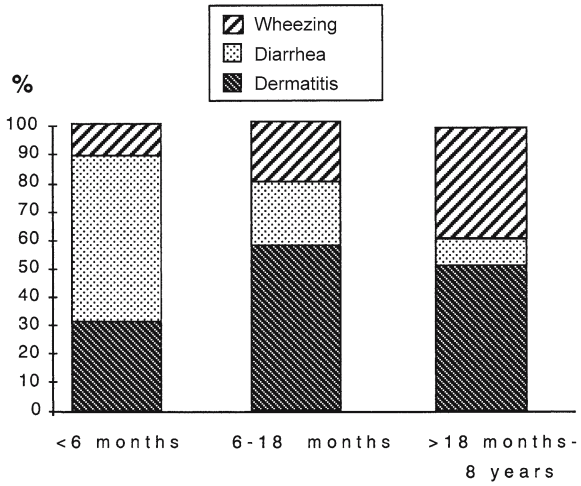

Fig. 5.24. Reasons for medical consultations according to age group

sensitized only to food allergens have an elevated incidence of AD. If the allergy to inhalants is exclusive the incidence of respiratory allergy clearly prevails over skin allergy [317]. The Hattevig et al study [131] confirms that sIgE to foods and the subsequent tolerance are evident from an early stage, while sIgE to aeroallergens appear later but do not disappear with age. Figure 5.8 from a study on 220 children [53] shows the ascending prevalence of atopic disease: a progressive increase of asthma and the fall of AD cases with age (whereas in the Hattevig et al study they increased >30 % at the last follow-up) [131]. Similar data in Fig. 5.9 show the prevalence of almost all the atopic disease in four age groups of 1,052 schoolchildren. Interestingly also urticaria angioedema and OAS prevalences are shown. The rate of 11- to 16-year-olds affected with AR is noteworthy [268]. Figure 5.24 [53] shows the most common reasons for consultations for children in the first years of life (>6 months–8 years): in babies diarrhea is by far the most frequent (Fisher = 0.008), in older children it is bronchopathy (Fisher = 0.013).

Prevalence of Immunodeficiencies

Very high is the relative incidence of primary immunodeficiencies (Chap. 22). Figure 5.25 is a world map reporting the estimated number of children (<15 years) newly infected with HIV in 2003 (total, 590,000–810,000) (Chap. 23).

Pediatricians and Epidemiology

Epidemiology is the study of the relationships existing between disease and certain factors intervening in the onset, distribution and evolution of these diseases, whether such diseases depend on the individual or on the surrounding environment. Allergic reactions and the diagnostic process are somewhat similar to a loaded gun. A great deal of knowledge is being obtained about a great variety of triggers that fire the charge: but why is the gun loaded? And what constitutes the load? We continue to focus on the triggers, and we have learned a lot about the load, but we do not know why children are hyperreactive to so many varied stimuli. While we are discussing the hygiene hypothesis, the prevalence of atopic disease such as AD, FA, AR and asthma is on the upsurge. These disorders have become a major health problem in industrialized countries, especially in infants and young children, because their prevalence has increased worldwide in the past several decades. Consequently, if the factors responsible for the increasing prevalence can be identified (Chap. 4), then there will be an opportunity to develop strategies to reverse these trends. The pediatrician is challenged to provide therapeutical and preventative interventions that achieve optimal health from infancy through adolescence. Regarding the natural history of atopic disease, it would also be helpful to identify infants who are at risk of developing allergy, so that these preventative measures could be started and used most effectively. An immense information campaign, which should benefit young children, adolescents, and their parents, is needed to make these

Fig. 5.25. Estimated number of children (<15 years) newly infected with HIV during 2003. UNAIDS. AIDS epidemic update. December 2003

measures more effective. Future progress will decisively depend on a better understating of the rising epidemiology of atopic disease.

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Diagnosis of Pediatric Allergy

From History to Clinical–Functional Examination

The diagnosis of atopic disease has particular characteristics, since *pediatric allergy is significantly different from adult allergy*. It is essentially based on a *detailed family and personal history*. The purpose of the history is not only to confirm or exclude a clinical pattern of suspected allergic disease, but also to identify and demonstrate the pertinent etiological factors. Diagnostic tests can help the physician if they are considered in connection to symptoms, but not if evaluated individually: infants and boys are too often labeled as atopic only because skin prick tests (SPTs) or RAST (radioallergosorbent tests) are positive. We cannot continue without underlining the special prudence that must be reserved for the possible inappropriateness of parent declarations, mainly when symptoms are not reported in a simple and objective way. However, to facilitate information exchange, it is necessary to take advantage of what parents report, listen to them, encouraging them to talk: a keen and accurate patiently gathered history concretely helps the pediatrician to individuate the direct causal relationship. Adler et al [2] have shown that if on the one hand SPTs, RAST and MAST-CLA tests were positive in 6.7% of asthmatic children but their parents declared they were affected with food allergy (FA), on the other *at least one test was positive in 23.3% of children declared healthy* [2]. In several cases we shall find a notable discrepancy between data referred to by families and data ascertained during the medical visit (Table 6.1).

Although in the last few decades the very great and multiform technological progress has in various ways changed the approach to children with atopic disease, diagnosis is very important, since both prophylaxis and the early institution of specific treatments are able to positively modify the natural history of atopic disease (Fig. 6.1) [179]. Therefore, diagnosis covers several points:

1. History
2. Medical examination
3. Diagnostic testing (in vivo and in vitro)
4. Specific provocation tests
5. Pulmonary function testing (PFT)

Table 6.1. Factors provoking asthma according to parents

Factors	%
Infections	70
Weather changes	49
Passive smoke	22
Physical exercise	17
Strong odors	7
Emotions	4
Food allergy	2

Data from personal results of a polycentric epidemiological study in Italian allergic children (unpublished data).

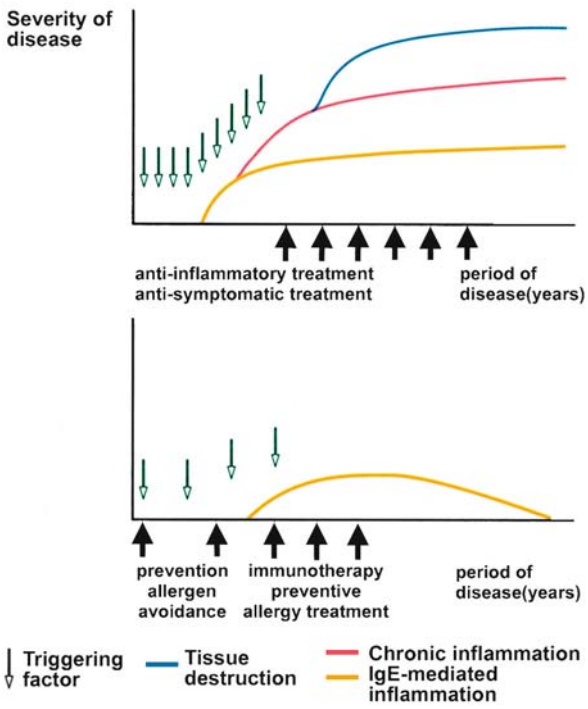


Fig. 6.1. The value of early diagnosis. The specific diagnosis of IgE-mediated allergic disease is very important in the early phase of the disease. By environmental prophylaxis and early treatment, it is conceivable to modify the natural history of allergy. Nevertheless, when the disease is already well-established, an antisymptomatic and/or anti-inflammatory treatment will become necessary. (Modified from [179])

The diagnosis of atopic disease in children, and above all the identification of pathogenic factors, needs the cooperation of a specialist center.

Allergy History

An accurate history is of *paramount* importance, because it is the key to gathering relevant information. After collecting a complete and accurate record, the nature, characteristics, frequency, as well as the possible severity of allergic symptoms will be defined [175]. As the interview continues, it is possible to exclude different conditions that present similar patterns. Subsequently, the clinical manifestations are explored: from the combined examination, specifically medical and with the possible help of diagnostic testing, the diagnosis is established [127]. To facilitate information exchange, listen patiently to the parent describing the history of the present illness as he (or she) deems appropriate, making the parent(s), who are generally unprepared and anxious, comfortable. If the baby weeps or screams or is naughty, the doctor may suggest entrusting the child to a parent until the moment of the visit, also to assure a greater concentration for both physician and parent(s).

Experience shows that:

1. History, although accurate, can be *silent,* thus complicating the diagnosis.
2. To ensure that the history is careful and detailed, the doctor should be friendly and unhurried, repeating if necessary the same question, even if only in a different form.
3. Nothing should be neglected, yet all data should be well investigated and properly arranged.
4. It is better to ask more questions or note more data, rather than to overlook them in the belief that the issues have been focused.
5. To continue methodically it is convenient to have a mental checklist at hand.
6. A record-chart or a computerized program with prearranged questions will make the task easier, but no issue should be neglected: with time, the opportunity of adding new questions or better detailing the already present ones can be evaluated.

The utility of *well-organized and drafted record-charts* is also underlined by the likelihood (or necessity) of updating them, considering second thoughts and unceasingly updating the written records with further data, which may often be recalled or provided in subsequent visits. Therefore, depending on his/her mood, the interviewer should decide whether or not to return to the history at a later time. A meaningful history therefore represents a diagnostic moment of great significance, even if it can be stressful, because history taking requires patience, listening and synthesis and abstraction abilities, as well as a great availability.

Family Allergy History

When the family history is positive, the chance that the patient is predisposed or sensitized and that some or all of his/her symptoms have an atopic origin increases: in this case it is helpful to recall the risk rates indicated in Tables 4.9–4.11. The immediate history of each family member is of the utmost relevance and should include both the mother and father of the child, as well as any information regarding other relatives, based on the family atopy score (FAS), as shown in Table 3.9. Moreover, because of the growing exposure to different cultures, medico-therapeutical trends not necessarily a part of our training, and our increasingly frequent encounters with citizens of other countries or continents, it is timely to investigate probable adhesions to homeopathic therapy and/or clinical ecology, as well as the family's cultural or religious eating habits.

Starting an *immunological diagnosis,* several questions must be answered, including unexplained miscarriages, cases of early death, morbilliform rashes during the neonatal period, early candidiasis or subsequent severe and recurrent infections, etc., leading to a PID (primary immunodeficiency), or whether addict parents have a child with ID, of as yet unexplained causes, as shown by infections from opportunistic germs, etc., suggesting a possible HIV infection.

Personal Allergy History

Following the protocol established by our division, we first investigate the mother's previous pregnancies, the related pregnancy and delivery, the neonatal period, alimentation, growth and subsequent development. If the mother smokes, ask whether she continued during or after the pregnancy and how many cigarettes she smoked or smokes daily. Regarding the type of delivery and the neonatal period, inquire whether there was any complication and/or the newborn was hospitalized for any disorder. Then accurately examine the type of feeding during the very first days, including the administration of colostrum and/or formulas different from breast milk in the maternity ward as supplements (Chap. 3). Also determine the month of birth, because there are potential implications for respiratory allergy (Figs. 4.10, 4.11). Table 5.5 shows, according to the child's age, which atopic disease will most probably affect him (her).

Inquire into the type of feeding (based on the record-chart or mental checklist) with more or less detailed questions (depending on the child's age). When feeding difficulties play a crucial role, it is timely to determine the onset of the problem with all the essential episodes, evaluating the child's growth in terms of weight and height.

Past and Present Allergy History

The *past allergy history* can give information directly related to the presenting symptoms such as episodes of anaphylactic shock, dermatitis, urticaria, vomiting, rhinitis, otitis, conjunctivitis, bronchitis, food allergy (FA), apparent multiple sensitizations, etc. In young children, episodes of vomiting and/or diarrhea, colic, and itching can be significant complaints. Itching is a common finding in AD (atopic dermatitis) and could be the first sign of an underlying disease. The purpose is now to define allergic symptoms, their onset, seat, intensity, duration, remissions or recurrence, etc. There may be a potential association with related allergy problems, viral or bacterial infections of the respiratory or gastrointestinal tracts. It is essential to collect information on temporal relationships and precipitating events, frequency of bettering or worsening, possible complications and/or relapses, treatments given and their responses. Then go on with the *present allergy history* to gather data on the age of onset and chronological sequence of symptoms: perennial or seasonal, or a daily, weekly, monthly, yearly occurrence. At this level, the decision is directed to what is most appropriate: selecting a diversified allergy history based on the prominent symptom's group, either AD–FA or asthma rhinoconjunctivitis.

Present Allergy History for Atopic Dermatitis and Food Allergy

When FA is suspected, the related history should be centered above all on the alimentation in the very first days of life and the type of feeding: exclusively maternal or supplemented and for how long, the diet prescribed to the nursing mother or, in case of bottle feeding, whether cow's milk (CM) or special formulas were used (Tables 6.2, 6.3) [32, 175].

Questions now explore at what age solid foods were introduced and which ones, verifying if necessary the data in Table 4.30, the age of onset and nature of symptoms, whether *general symptoms* were or are present such as shock, urticaria, skin rashes, wheezing, swellings, anemia, etc., and *intestinal symptoms* such as vomiting, diarrhea, colic, intercurrent infections, etc.

Starting with skin manifestations, note their morphology, time of onset, localization and timing of the lesions, also in view of the possible differential diagnosis with the seborrheic dermatitis or other skin affections. Note the relatively common contact urticaria associated with food antigens (Chap. 8).

Begin with a detailed description of symptoms, including the food suspected of provoking reactions, the time elapsed between food ingestion and symptom onset, the type of suspected food, whether it was fresh, raw, or cooked, conserved or lyophilized, the quantity of food that was necessary to produce the development of symptoms and their extent, whether similar symptoms occurred on other occasions when the food was consumed and the relative management, the response to diets and/or treatment, the chronology of occasional relapses or multiple sensitizations and the symptom outcome [34]. A "virgin" case is not always seen: diets of varying types have often been arranged, recommended by several specialists, or prepared directly at home even before consultation (Chap. 9).

Regarding medications, include vitamin and mineral supplements.

When the history indicates immediate symptoms, a *cause–effect relation* is easily suspected. Diagnosis will be more demanding in the following cases:

1. Late reactions (even up to 15 days).

Table 6.2. History pitfalls to be evaluated with attention when food allergy is suspected

Family history
Growth and development progression
Parents giving misleading indications
Dietetic, cultural or religious practices (vegetarians, Hebrews, Muslims, etc.)
Suspected food Freshness and/or cooking method Ingested amount Interval between ingestion and reaction Digestion and absorption Possible contaminations Possible cumulative effects
Relapses after ingestion of the same food
Probable dietetic or therapeutic measures

Data from [32].

Table 6.3. Frequent causes of confusion due to parents' misleading interpretations

Inclination to attribute all the clinical manifestations of the child to the last ingested food and to describe clinical findings similarly to one's own food allergy or that of a family member
Belief that a previously eliminated food is the responsible one, even if its exclusion was not productive
Probable confusion between aversion for a food and a true allergy
Tendency to interpret as an allergy the simple aversion for a food
Propensity to introduce arbitrary modifications to the diet or treatment
Propensity to set up inappropriate diets with consequent nutritive deficits

Data from [175].

2. Interferences of additional foods.
3. Multiple sensitizations.
4. A presumed sensitization is related to breast milk priming, for example AD in exclusively breast-fed children.
5. Severe manifestations (anaphylaxis) occurring at the first introduction of a food, apparently without a previous direct sensitization (supplements in the neonatal nursery), especially when *the history is silent*.

When a clear correlation cannot be established, particular attention should be focused on possible temporal or environmental relationships, including infant's age, the year, season, residence at home or in other people's home, households, relatives, baby sitters, etc.

Two different occurrences can be delineated in this approach: FA in its strict sense, characterized by hypersensitivity of the IgE-mediated type, documented by specific positive tests, or food intolerance, not sustained by IgE-mediated mechanisms (Chaps. 9 and 10).

Present Allergy History for Urticaria and Angioedema and Additional Skin Allergies

Clarify whether urticaria and angioedema are isolated or associated, or whether there are hereditary forms (hereditary angioedema) or acquired forms (apparently primary, associated with other affections, or physical forms). Although it is important to consider the main pathogenic causes, it is also critical to select them based on a single clinical history, especially when urticaria is induced by medications, food and/or contact. However, if a physical form is prevalent, Chap. 8 should be consulted for the diagnosis, as for the allergic contact dermatitis (ACD) allergic vasculitis, and urticaria caused by additives.

Present Allergy History for Asthma Rhinoconjunctivitis

The key question is whether symptoms mostly of a respiratory type refer to past episodes of wheezing, bronchiolitis, relapsing cough or bronchitis, recurrent respiratory infections, pertussis, bronchopneumonia, with special emphasis on the time or seasonal vs perennial appearance, course, intensity and therapeutic interventions. In older babies, also investigate a possible exposure of the upper airway: rhinitis (rhinorrhea, cough, sneezing, etc.), otitis (serous, recurrent, chronic), maxillary sinusitis, feasible intervention of (adeno)tonsillectomy, and the related causes and outcome. The chance that pneumonia and/or pertussis and/or persisting cough may underlie an unidentified asthma case should not be neglected, also because the trend of interchangeably employing the terms of asthma and bronchitis in children aged <5 years seems to be widespread: several young asthmatics were admitted to hospital with diagnosis of pneumonia or bronchitis [144].

If the child has suffered from asthmatic attacks, additional specific questions aim at determining what age the first episode took place, if respiratory symptoms are seasonal or perennial, then the yearly number of episodes, their severity and duration (dyspnea, shortness of breath), onset modalities, chronological sequence of breakthroughs, visits to emergency wards, hospitalizations, time course of symptoms and selective triggering factors (Table 5.11). Inquiry about seasons, climate, barometer and/or altimeter variations, places, indoors or outdoors, food, day or night, exercise-induced asthma (EIA), pets, pollens, passive smoke, odors, emotions, drugs, etc. is necessary. One may then be able to ask questions of the child beginning at ≈7 years of age such as night/day worsening, upon waking, during cleaning, in meadows or in the country, improvements, outdoors or in dry areas, yearly periods highlighted by more evident or attenuated symptoms.

As far as medication is concerned, the following must be recorded: intermittent or continuing treatment, routes of administration, daily or alternate-day dosage, whether or not asthma has provoked a reduction of physical activity, sleep, and school attendance, participation in social events, limitation in the use of public means of transportation and the capacity of going up or down stairs, etc. [29].

When rhinoconjunctivitis is suspected, one should check whether or not there are watery or purulent rhinorrhea, sneezing even upon waking, nasal obstruction and itching, oral respiration, postnasal drip, and mucus; eye findings such as tearing, itching and/or burning, eye redness, photophobia, edema, secretions; or allergic symptoms often associated with and related to otitis, including itching, otalgia, frequent infections, or general manifestations such as migraine, fatigue and possible secondary complications, including chronic nasal obstruction (CNO), sinusitis, disturbances of both smell and hearing, or sleeping, nasal hemorrhage, superinfections, etc. Similarly, we suggest thoroughly investigating whether parents report recurrent colds and bronchitis, chronic rhinitis and the like. All possible differential diagnoses should likewise be taken into account.

Environmental History

The allergy history should be completed by collecting information on the child's environment and living habits.

This includes the subject's home, the parents' or relatives' homes, as well as a possible vacation home:

- Location: urban, rural, in the mountains, at the sea. If urban, evaluate the degree of environmental pollution (severe, moderate, slight).
- Plants: type and location (indoor, outdoor); trees, weeds, grasses in the vicinity.

- Dampness, localized or generalized, air conditioning, humidifiers or fans, type of heating, air-heating, type of filters and frequency of cleaning and changes, fuel oil/gas/coal/wood heating, existence of fireplace, stoves, etc.

Bedrooms

- Location: basement or which floor
- Mattresses and pillows, type of filling (wool/down/hair/foam rubber or box-springs), encasing and purchase date
- Carpets and rugs, wall-to-wall carpeting, bedside rugs; wallpaper, curtains: if present, which material (cotton, nylon, etc.), whether washable and frequency of washing
- Furniture (upholstered furniture, etc.). If there is a closet containing household linen/clothes in the room where the child sleeps or studies, ask whether closet doors are open or closed, or rarely opened (at the change of season, etc.);

Child's Bedroom

- Other occupants, bed characteristics: when the beds have the same characteristics, return to the previous point
- Stuffed, plush toys, and whether kept in bed
- Pets (past, present and future, how long in household), which species, and whether kept in bed
- Indoor smoking: parents, relatives, regular visitors, locations allowed
- Stress: inquire whether or not there are causes for it
- Day-care facilities, baby-sitters: inquire about the location and environmental characteristics of daycare facility or child's schools, whether child consumes meals, snacks, etc., prepared locally there

Taking into consideration the pros and cons of the history, we suggest that the pros such as maximal convenience, absolutely low cost, direct contact with the child and his/her parents, and 100% noninvasiveness are immediately appreciated. If accurately done it may reduce the costs of further testing; the contras are represented by an evident subjectivity, the time required and the necessity of ensuring the child's (and parents') compliance [179].

Medical Examination

Some characteristic aspects can suggest the diagnosis such as allergic salute, infraorbital edema, nasal congestion, cough, Hertoghe's sign, etc.

In a child with *suspected dermatitis with FA* and skin manifestations, we observe the nutritional state, the type, localization and extension of lesions, usually corresponding to the child's age and the lesion severity. Body diagrams can be used (Figs. 6.2, 6.3), which arbitrarily divide the body surface into 20 areas. For each area the eczema is assessed in terms of:

- Redness (R)
- Vesicles, oozing/crusting (V)
- Excoriations (E)
- Lichenification (L)

Each type of lesion is given a score on a scale of 0 (none) to 3 (severe). The use of a scoring system quantifies possible improvement (or worsening) of skin lesions at subsequent visits. Note the data on the diagram to compare this with the next one.

Another method is based on different scores [20]:

0: Healthy child, no lesions

1: Mild, with scarce erythematous areas, with or without occasional scratching

2: Moderate, with areas of erythema, macule and scaly lesions, with or without obvious scratching, lasting >2 min each time

3: Severe, with marked and diffused erythema (>50%), scaly lesions also diffused (>25%), vesicles and/or pilary erection and incessant and conscientious scratching

The European Task Force on Atopic Dermatitis [161] has devised a composite index, applied in our division, the SCORAD (scoring of AD), which also takes into account lesion severity and subjective symptoms (Figs. 6.4–6.18) [161]. It is divided into three scores:

A Extent, according to the indications shown in Fig. 6.19 [161], which proposes a different evaluation of lesions for children <2

B Intensity, regarding the different symptoms, equally scored 0–3

C Pruritus and sleep loss (mean of the last 3 days or nights), each graded 0–10

Once A, B and C are calculated, the formula (A:5) + (B×3.5) + C is applied; the highest score in an infant <2 is (103:5)+(18×3.5)+20, thus A 20 + B 63 + C 20=103. In children aged >2, the calculation is equal because the total of A is 102. This way great emphasis is given to the symptom intensity: A (extent) and C (symptoms) are each equal to 19.4% and extent to 61.2%. Definitions of commonly used skin descriptive terms are summarized in Table 6.4.

In all children, the *medical examination* should include the auscultation of the chest, the examination of the abdomen and, depending on the circumstances, of ears, nose, and eyes. Also necessary is the accurate determination of heart (HR) and respiratory rates (RR), as well as the anthropometric evaluation with percentiles (Appendices 6.1–6.4), also considering growth and nutritional state. In asthmatic children, the medical examination should include spirometry and the peak expiratory flow rate (PEF or PEFR) (see "Additional Tests").

Table 6.5 [175] outlines the clinical symptoms that can be helpful in *differential diagnosis* [175] and Table 6.6 [104] concludes with *risk factors* for wheezing <3 and childhood asthma in children <3 years [104].

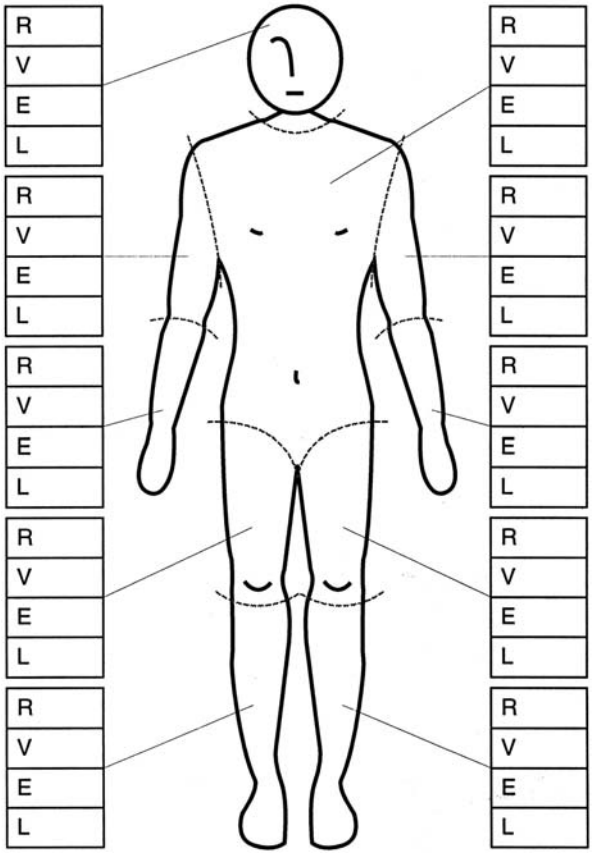


Fig. 6.2. Body diagram from our division for the evaluation of skin lesions: front view

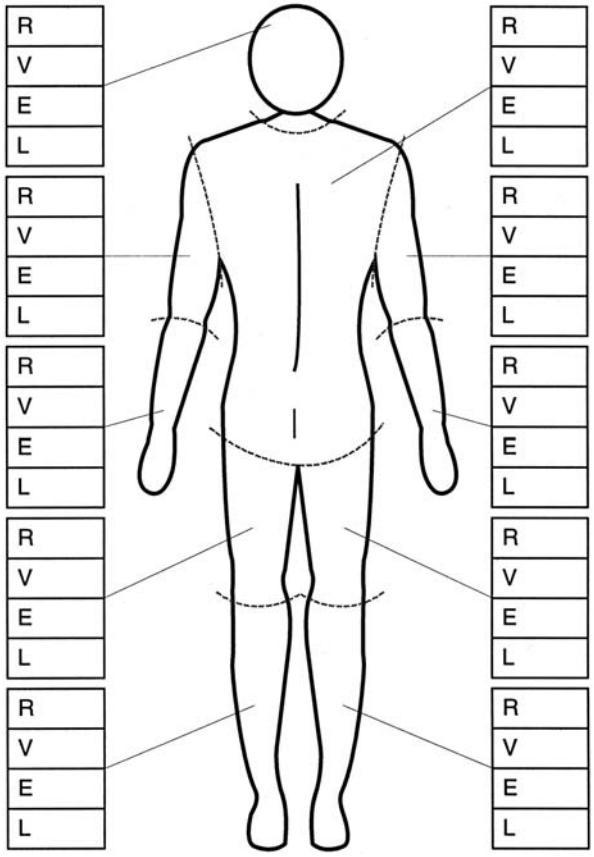


Fig. 6.3. Body diagram from our division for the evaluation of skin lesions: rear view

Photographic Atlas to Illustrate the Scoring System

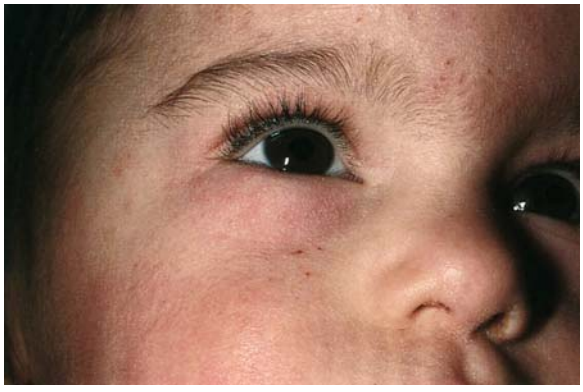


Fig. 6.4. Erythema, grade 1

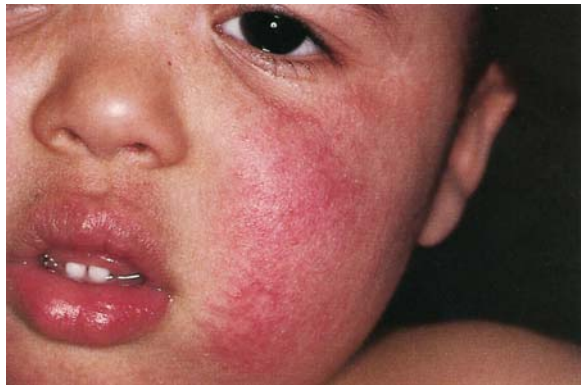


Fig. 6.5. Erythema, grade 2

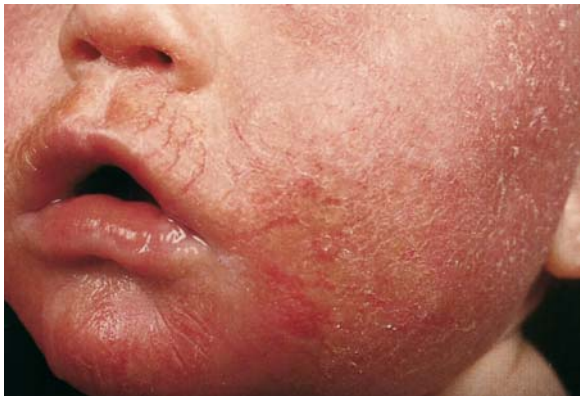


Fig. 6.6. Erythema, grade 3

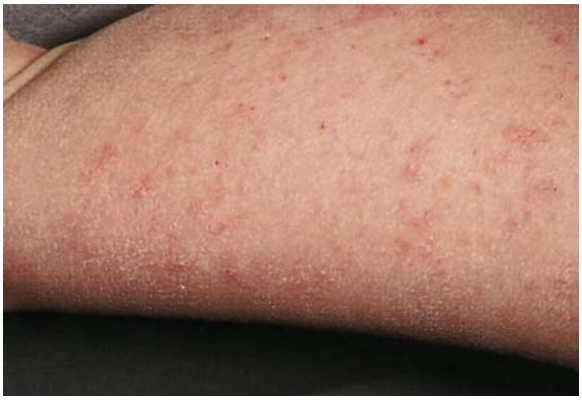


Fig. 6.7. Edema, papulation, grade 1

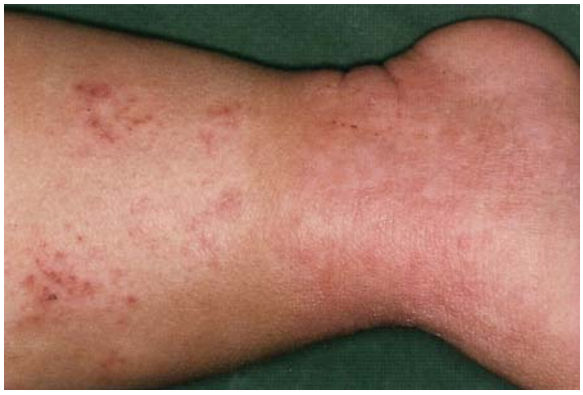


Fig. 6.8. Edema, papulation, grade 2

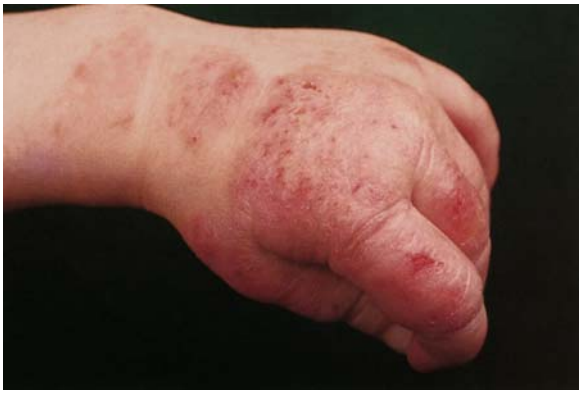


Fig. 6.9. Edema, papulation, grade 3

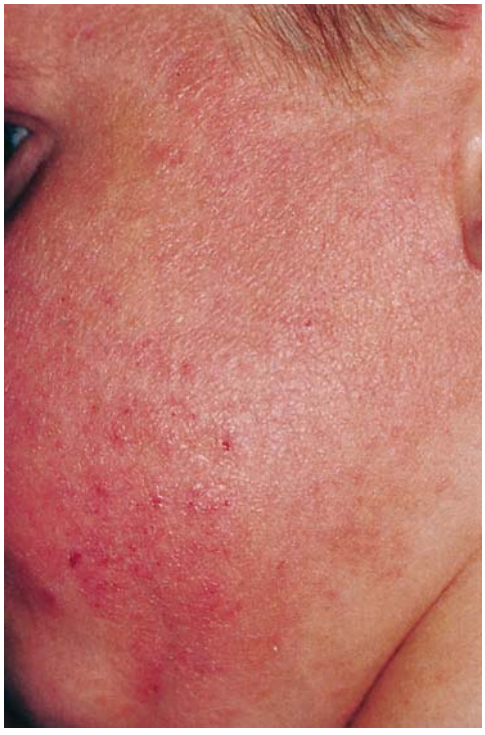


Fig. 6.10. Oozing, crusts, grade 1

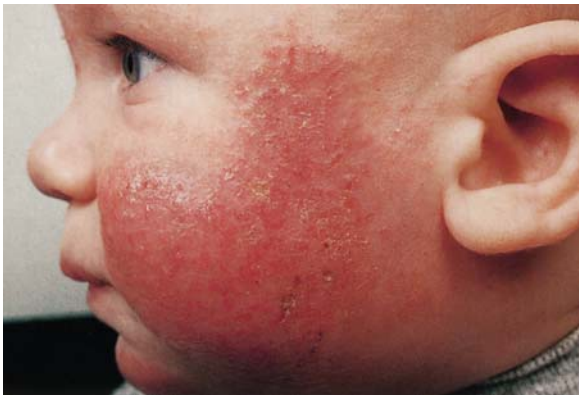


Fig. 6.11. Oozing, crusts, grade 2

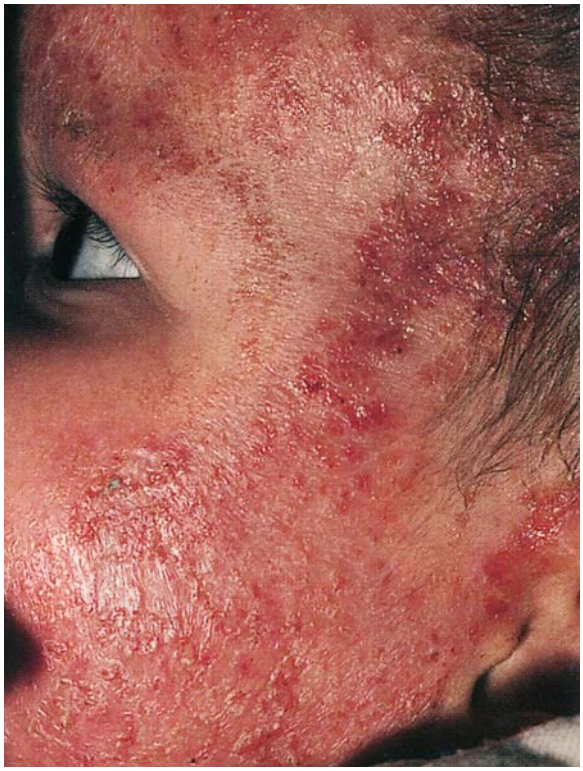


Fig. 6.12. Oozing, crusts, grade 3

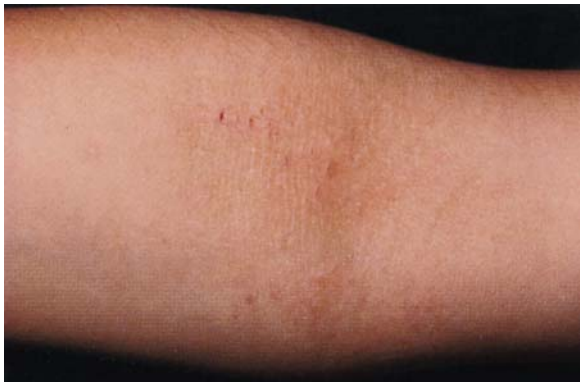


Fig. 6.13. Excoriations, grade 1

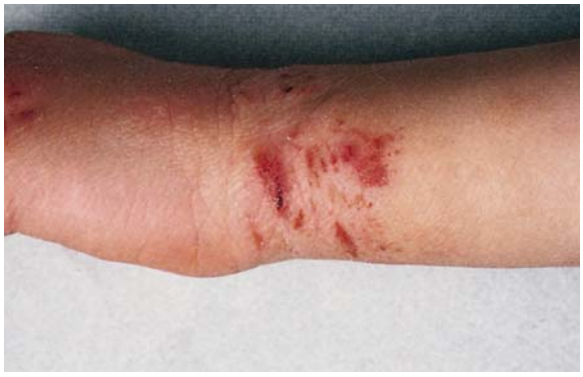


Fig. 6.14. Excoriations, grade 2

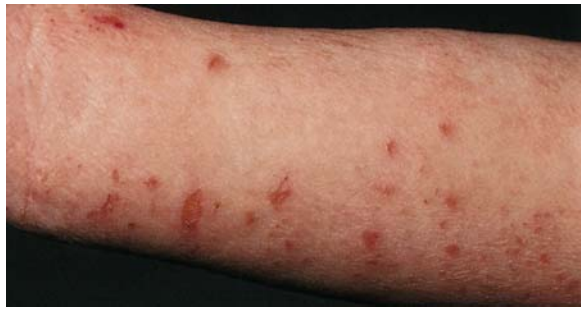


Fig. 6.15. Excoriations, grade 3

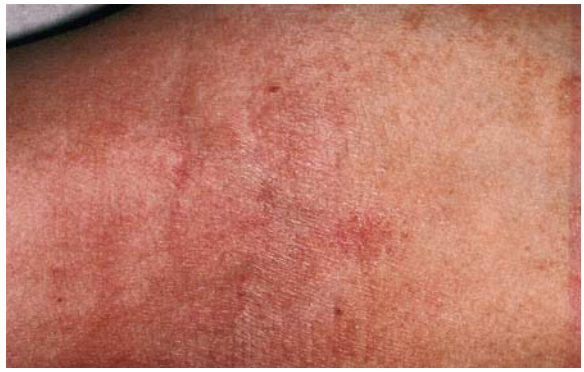


Fig. 6.16. Lichenification, grade 1

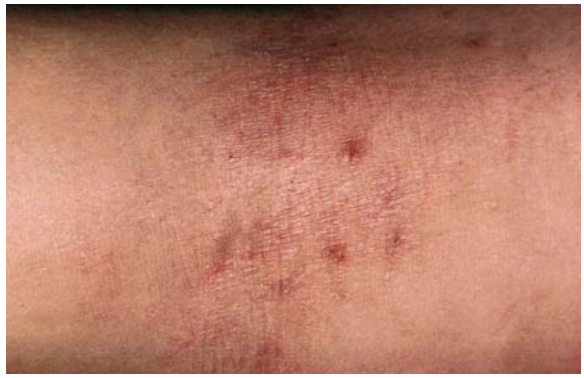


Fig. 6.17. Lichenification, grade 2

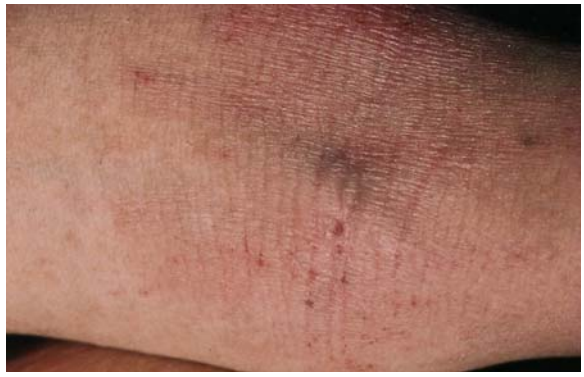


Fig. 6.18. Lichenification, grade 3

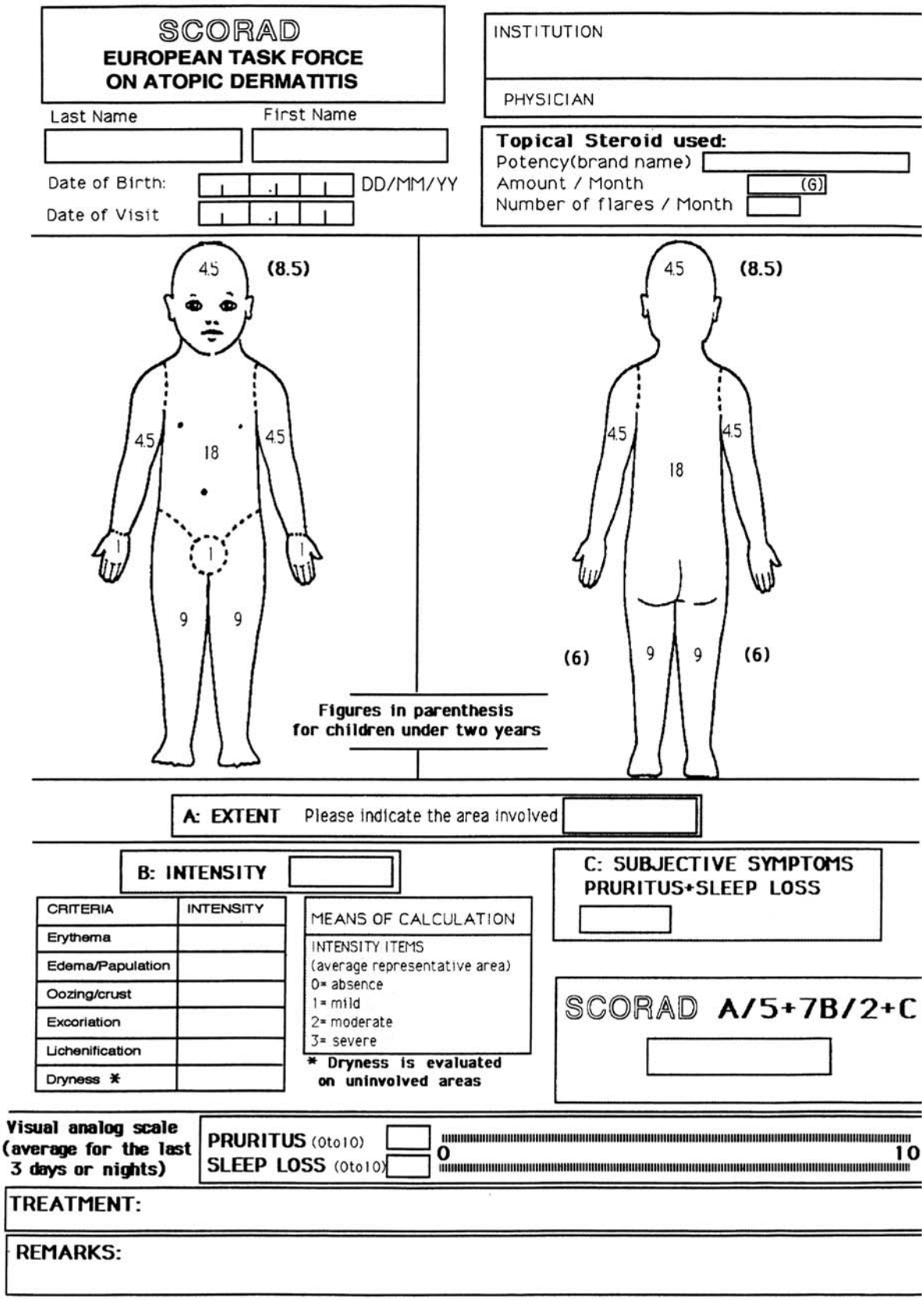


Fig. 6.19. Evaluation sheet of SCORAD severity index of AD lesions

Table 6.4. Descriptive terms useful for reporting skin lesions

Type of lesions	
Erythema: diffuse or localized redness (due to active or passive capillary hyperemia), disappearing with vitropression	
Macule: circumscribed color change, not palpable, of different form, color and dimensions, not disappearing with vitropression	
Patch: macule larger than 1 cm in diameter	
Papule: firm skin elevation (following hyperemia or phlogosis), 1 cm or less, colored or not, localized and persistent	
Wheal: evanescent elevation (due to edema), localized, consistent, pink-red or porcelaneous-white, surrounded by an erythematous flare; it is characteristic of urticaria	
Vesicle: small, clear fluid-filled elevation within the epiderm or between epiderm and derma	
Bulla: localized, clear fluid-filled elevation of varying dimensions, subepidermic or between epiderm and derma, elevated on the skin plain	
Scale: small agglomeration of horny cells, mono- or polystratified, easily detachable assuming the aspect of a thin lamella	
Characteristics of the lesions	
Number: one, some, several, many	Surface: smooth or rough
Location	Limits: net or faded
Distribution: disseminated, confluent, linear, figured	Consistency: soft, stiff
Extension: localized, regional, disseminated	Pain: to the touch
Shape: regular or irregular, roundish, annular, etc.	Aspect: mono- or polymorphous
Color: normal or abnormal pigmentation	

Table 6.5. Differential diagnosis according to clinical manifestations

Symptoms	Diagnosis to be evaluated
Airways	Asthma from inhalant allergens, foreign bodies, otitis media with effusion (OME), cystic fibrosis, IgA deficit, primary ciliary dyskinesia, dysgammaglobulinemia, anatomical defects (tracheoesophageal fistula, etc.)
Skin	Several diagnoses are possible (see Chaps. 7, 8)
Gastroenteric	
(diarrhea, failure to thrive, malabsorption)	
0–30 days	Food allergy, food errors, viral disease, galactosemia, disaccharidase secondary deficit, congenital lactase deficit, short bowel syndrome, intestinal lymphangiectasia, Wiskott-Aldrich syndrome
30 days–2 years	All the above-mentioned disorders, plus intestinal parasitosis, celiac disease, abetalipoproteinemia
>2 years	All the above-mentioned disorders, plus cystic fibrosis, ulcerative colitis, Crohn's disease
(Vomiting, diarrhea, abdominal distension/pain, melena)	
	Surgical causes (congenital megacolon, hiatal herniae, pyloric stenosis, tracheoesophageal fistula), infections, metabolic diseases (galactosemia, phenylketonuria, disaccharidase deficit), organ-specific diseases
Blood	Sideropenic anemia: several causes are possible
Central nervous system	Headache, hyperactivity, irritability, tiredness, behavioral and sleeping troubles

Data from [175].

Diagnosis Type

Before utilizing SPTs and other diagnostic tests, it is necessary to define disease [121]. In our division we use the following operational definitions of disease:

- AD diagnosis follows the appearance of erythematous or vesicular itching skin eruptions, involving typical seats (cheeks, extensor aspects of the extremities in infants, retroauricular sulcus), and a severity score as described in the previous section.
- For the diagnosis of urticaria defined as allergic, if appearing on at least two occasions within 1 h after exposure to a particular allergen and later to additives.
- For the diagnosis of asthma, we define the wheezing without asthma as a wheezing in the past 12 months without lifetime asthma; the wheezing with asthma as a

Table 6.6. Differentiation between wheezing and asthma in babies aged <3 years

	Wheezing <3 years	Asthma
Exogenous causes		
Allergen exposure	–	+++
Bottle-feeding	++	–
Passive smoke exposure	++	+++
Airway exposure to viral infections	++++	++
Endogenous causes		
Family history of asthma	–	++++
Atopy	+	++++
Male sex	++	+++
Immune response to infections	+++	?
Initial airway reactivity	–	++
Initial lung function	++++	?

Data from [104].

lifetime asthma with wheezing in the past 12 months; the severe wheezing as having >4 attacks of wheezing, or >1 nocturnal awakening/week due to wheezing, or speech limiting wheeze, in the past 12 months, the severe asthma as lifetime asthma with severe wheezing in the past 12 months. The wheezing is nearly always associated with upper respiratory tract infection (URTI).

- For the diagnosis of rhinitis, nasal discharge and/or blockage occurring continuously for at least 4 weeks plus the typical pale aspect of allergic mucosa on rhinoscopy, without any sign of infective rhinitis in other relatives, is required.
- For the diagnosis of gastrointestinal symptoms, recurrent colic, vomiting, and/or diarrhea after excluding ordinary eating problems, coincidental infections, and lactose intolerance.
- For the diagnosis of FA, skin, gastrointestinal, and respiratory symptoms occurring after the definite disappearance of symptoms after each of two dietary eliminations of the food in question and recurrence of identical symptoms after DBPCFC (double-blind, placebo-controlled food challenge) with the offending food, or after intake of a specific food on at least two occasions.

Results of both history and medical examination are helpful to determine which SPT is the most appropriate [127].

Immunoallergic Diagnosis

Among immunoallergic tests, in vivo tests, SPTs and provocation tests and in vitro tests are included as well as serum total and food-specific IgE (sIgE), eosinophil count and other tests. In this chapter we do not discuss elimination diets and FCTs (food challenge tests) and tests with drugs, which are to be found in Chaps. 9 and 19. The diagnosis of autoimmune disorders, PIDs and pediatric AIDS is covered in Chaps. 18, 22 and 23, respectively.

In vivo Immunoallergic Tests

The diagnosis of allergic disease in children requires a careful and critical evaluation of laboratory test results. Serum total IgE levels and sIgE are not always correlated with history, and all different tests employed may lack specificity and/or sensitivity.

Skin Prick Tests

SPTs were devised by Blackley [18], who was allergic, by putting grass pollen on a skin scratch done on the forearm volar face, where an itching wheal surrounded by a flare developed. This method was substituted in 1912 by intradermal testing, followed by SPTs in 1924 and by prick + prick (P+P) tests in 1978. In Chap. 1 we underlined the necessity of standardization, hence the allergen concentration in the extract should be dosed in BU (biologic units) [49]. We follow the indications of the European Academy of Allergy and Clinical Immunology (EAACI) [49].

Choice of Method

The best-known method that we prefer is SPTs, although intradermal skin testing, except for specific cases (Hymenoptera, drugs), is not recommended in children, because it has an increased risk of inducing a systemic reaction and a great number of false-positive results [41]. It has been revived by the AAP (American Association of Pediatrics) as an integral part of the ritual preceding anti-measles vaccination (Chap. 9); the so-called scratch test has been largely discontinued because of its lack of precision.

Indications

SPTs consist in allergen percutaneous administration. If correctly done and with standardized extracts the advantages are: rapidity, certainty, low-cost results, ease of performance, high sensitivity and is thus reliable and reproducible [176], because it has been widely experimented. As a consequence, SPTs are the most frequently used methods for a diagnosis of IgE-mediated allergic disease where skin-sensitizing antibodies are present. When skin mast cells have surface sIgE for intruding allergens, mast cells degranulate, releasing their media-

tors, thus producing a cutaneous wheal-and-flare reaction, a type I immune reaction [49].

SPTs are more sensitive and specific in respiratory allergies, thereby representing the first-choice investigation when an IgE-mediated allergic disease elicited by aeroallergens is suspected and in asthma epidemiological studies [45]. In FA diagnosis, SPTs are less reliable, because of different factors, including the instability of some allergens, cross-reactivity between allergens belonging to different plant species or to the same species, antigenicity loss during extraction and inadequacy of allergenic extracts (see "Prick + Prick Testing").

Age

SPTs can be done at any age, although infants can manifest less evident skin reactions than older children, since skin mast cells exhibit a lower number and express fewer IgE receptors and less mediator release, and total or sIgE values are reduced; this also applies to histamine tests. SPTs can yield reliable results even in neonates and in infants, aged even 1 [99] or 3 months [163], as we habitually practice SPTs. However, if these disturbances persist regardless of negative results, SPTs are repeated in a subsequent visit. We generally do not advise doing a large number of SPTs [37].

Seasonal Variations in SPT Reactions

No seasonal variations were disclosed other than the report of monthly influences (February and October +, July and August –) [118]. Selection of a season to perform SPTs may be important for pollen-sensitive children, who are more reactive during the pollen season since enhanced allergens by grass pollens increase wheal reactions to such allergens.

Allergens to Be Tested

In the commercial diagnostic collections, a large variety of allergens are found; nonetheless, the majority of them have no clinical significance, because either some allergens rarely cause sensitization or the child is rarely in contact with such allergens. As discussed above, we find it convenient to use mostly those allergens commonly responsible for atopic disease, taking into account the results of both history and clinical manifestations of each child. For the diagnosis of respiratory allergy, in most cases a limited group of aeroallergens are sufficient [19] (Table 1.74):

- *Mites: Dermatophagoides pteronyssinus* (Der p) (Fig. 1.80), *Dermatophagoides farinae* (Der f). It is not necessary to asses the value of *Euroglyphus maynei* (Eur m 1), since it cross-reacts with Der p.
- *Pet* allergens such as cats (Fel d 1) and dogs (Can f 1); bird feathers, horse hairs, etc., when requested.
- *Hymenoptera* venoms: when suspecting a related allergy, perform the tests in hospital employing all venoms of the most widely spread insects: *Apis mellifera* (honeybee), *Vespula* spp (yellow jacket), *Polistes* spp (wasp).
- *Pollens:* grasses (Figs. 1.69–1.73) including *Lolium perenne* (Lol p), *Cynodon dactylon* (Cyn d), *Parietaria* (P) (Fig. 1.75–1.77), *Compositae* (Fig. 1.67, 1.74), and possibly also Mercurialis annua [7], *Platanus acerifolia*, *Betulaceae*, *Cupressaceae* [193], two or three trees that are more frequent in the related geographic area, in addition to emergent pollens (Chap. 12). In children positive to Lol p and Cyn d, we also test *Phleum pratense* (Phl p), *Poa pratensis* (Poa p), *Dactylis glomerata* (Dac g) and *Festuca elatior* (Fes e). When a crossed sensitization between different types of a single pollen species exists, it is often sufficient to test only one for each species. We stress that the so-called ozone hole has caused an anticipation of pollination, favoring pollen spread in geographic areas where temperature (T) is increased. Therefore, certain species, for instance P, can be detected in regions that have become climatically more favorable.
- *Molds: Alternaria alternata* (Alt a) (Fig. 1.79), *Cladosporium* (Fig. 1.78).

In the great majority of children, the likelihood of respiratory allergy can be established utilizing extracts of grasses, Der p, P, Alt a, Fel d 1, Can f 1 [189]. Employing a panel as shown above, independently of history data, provides a complete skin testing, with possible disclosure of nonapparent sensitizations, which could be subsequently highlighted. Indeed skin positivity to different allergens can be ascertained about 2–3 years before the specific symptoms. The inclusion of Fel d 1 is imperative because it is found throughout the world, even when no contact is manifest (Chap. 5). The clinically important pollens vary according to location, so it is best to refer to the pollens prevalent in the region or country: some patients have been classified as affected with an intrinsic asthma, whereas they could be sensitive to unknown allergens and/or those not included in the common battery of allergens [7]. Another poorly defined aspect is the frequent cross-reaction among pollens, fruits and vegetables, namely, grasses, mugwort and birch with certain antigens in common with apple, wheat, spices, celery, carrot, etc. [111] (Table 1.73), partly explained by profilins and various related plant proteins that may function as cross-reacting allergenic components and therefore could explain that certain allergic patients display IgE-mediated reactions to other pollens as well as distantly related plants. (Table 1.72). A discrepancy may be provoked by certain Fel d 1 extracts that, due to cross-reactivity with Der p, give false-positive tests in cat-allergic patients [8]. In another study, Der p contamination of dog dander

extracts was shown to be the cause of false-positive SPT responses in patients sensitized only to Der p [171]. Such drawbacks can be prevented by using recombinant allergens (RAs) [117, 126, 151] (Tables 1.70, 1.71). Bird allergy is uncommon, often SPTs are positive to Der p contained in the feather bedding material [70], therefore extracts obtained from old feathers may be used.

The ISAAC (International Study of Asthma and Allergy in Children) protocol phase 2 module 3.2 includes the following aeroallergens: Der p and Der f, cat hair, mixed grasses [*Dac g*, *Lol p*, *Festuca pratensis (Fes p)*, *Poa p*, *Phl p*, and *Avena elatior*], *Alt a*, mixed trees (*Alnus glutinosa*, *Betula verrucosa* and *Corylus avellana*). This protocol is specifically intended to allow comparisons between centers [143].

For the diagnosis of FA, one should proceed on the basis of (1) epidemiological data (foods more regularly consumed in a typical Mediterranean diet for infants are CM, egg, wheat); (2) age: in an infant aged ≤6 months on an exclusive CM diet, the related proteins should be tested (Table 1.75), subsequently egg (two allergens), cereals, wheat, cod, peanuts, nuts, etc., adding bovine seroalbumin (BSA) if meat allergy is suspected; (3) history, recalling that alimentary customs influence the frequency of allergic sensitizations. In addition, children who are monosensitive to grass pollens may show an unusual prevalence of positive SPTs to food allergens (Chap. 9). We always perform SPTs to foods and inhalants in the same session; for fruits and vegetables see "Prick + Prick Testing."

Extracts to Be Employed

The principal obstacle is the inadequacy of extract characterization, purification and standardization, from which both qualitative and quantitative differences often originate, since the extracts produced vary considerably as extraction techniques are generally different, thus affecting SPT optimal potency and reproducibility (Chap. 1). A basic prerequisite of SPT reliability is biological extract standardization [128]. Although the number of RAs continues to increase (Table 1.70), at present several RAs related to food allergens are available. In addition, the digestion role in modifying food antigenic properties should be better defined. Gad c 1 is the most purified food allergen and hence the most reliable (Table 1.74), followed by peanuts, CM and egg. SPT sensitivity for codfish is 100%, for egg 70%, and for peanut it ranges from 45% to 100% [121]. Cereals yield highly specific responses from a biochemical point of view. However, there is no clinical correspondence, as we have demonstrated for chocolate, thus explaining why when employing commercial extracts from different producers to investigate the same group of patients, results can be very divergent and yield unexpected (false) negative results. Several of the above problems will be obviously unraveled using RAs: use of a single RA can play an effective role as a marker of severe atopy, delivering a useful means to individuate child sensitivity to a given allergen [117, 126]. The concentration usually utilized in conventional extracts (1,500–10,000 BU/ml) was shown as the most fitting to elicit a specific response without aspecific irritating reactions, being effective even when falling to 3,000 BU/ml [107].

Choice of Lancets

Presently, utilizing single-use lancets has gained popularity, especially if allergenic extracts coat the point (Prilotest): they are easy to use, prevent excessive traumas and provide homogeneous and reproducible results, in addition to assuring a more accurate standardization, but they are expensive. Table 6.7 [47, 105] summarizes the features of different devices in use: the Morrow-Brown needle has a new version [47] and the Duo Tip Test has a bifurcated needle [105]. Commonly used lancets such as the ALK lancet (module 3.2) are manufactured in such a way as to allow a small point to penetrate 1 mm into the skin with a collar or flare on the

Table 6.7. Characteristics of the different lancets available for skin prick tests

Name	Pricker	Stallerpoint	Allerprick	M-B	Duo Tip Test
Firm	D-H-S	Stallergènes	HAL		Lincoln
Technical data					
Needle tip (mm)	1	1	2	1	2.5
Total length (cm)	3	3	10	3	4
Diameter (cm)	5	2.8	3	3	
Material	Metal	Plastic	Plastic	Plastic	Plastic

Pricker: D-H-S, Dome-Hollister-Stier, Spokane, WA; Stallerpoint: Stallergènes Laboratoires, Fresnes, France; Allerprick: HAL, Hall Allergenen Laboratories, Haarlem, Holland; M-B: Morrow-Brown needle, Lincoln Diagnostics, Decatur, IL. ALK, Allergologisk Laboratorium A/S (Hørsholm, Denmark).
Data from [47, 105].

instrument to prevent further penetration of the point, also producing more consistent results. Shorter points are problematic in that they produce false-positive tests, while longer points are discouraged in infancy because they induce visible bleeding. In practice, either insulin needles or single-use 25- to 26-gauge hypodermic needles can also be used, both to be discarded after each test [120].

The present version of the MultiTest (MultiTest II) is a multipuncture applicator with eight heads loaded with seven antigens and glycerin as negative control. The heads, 1.9 mm in length, are grouped in two rows of four. It is also suggested for use in infants with different preparations [131]. In 72 Estonian 4- to 6-year-olds [71] and in older children [15], it appears to be exact and reproducible, except for drawbacks due to an excessive test proximity (each head is separated by 2 cm, and the two rows are separated by 3 cm). It seems to be suitable as a screening test with several major allergens [15], similarly to the Quintest, a five-headed device [105].

Testing Methods

Before Execution

- Check whether the child has previously shown severe and/or anaphylactic reactions to the allergen to be tested; in particularly sensitive children and when AD lesions are extended, it is wise to revert directly to an in vitro test.
- Investigate whether the children referred have withheld medications that could influence their response to SPTs [30]. The following withholding periods apply: for hydroxyzine and clemastine, 2–4 days; cyproheptadine, diphenhydramine, chlorpheniramine, promethazine and all short-acting antihistamines (acrivastine, azelastine, cetirizine, fexofenadine, levocabastine, levocetizizine, loratadine, oxatomide) and ketotifen, 7 days; theophylline 24–48 h. There is no need to discontinue inhaled short-acting bronchodilators and cromolyn or nedocromil sodium and inhaled corticosteroids (CSs). Oral CSs may interfere with late-phase hypersensitivity reactions only if administered for more than 1 week, whereas application of high-potency topical CSs should be discouraged [95].

Execution Technique

- Adequate skin cleaning can be accomplished by an ether-soaked cotton-wool wad to remove the grease from the skin, so facilitating the permanence of allergenic extracts at the site of application.
- Mark the skin with a ball-point pen for the allergens to be tested.
- Apply a small drop of each test extract to the skin of the volar forearm.
- Drops are placed about 5 cm from the wrist and about 3 cm distal to the elbow crease (reduced measures in younger children).
- Direct the sterile lancet through the allergen droplet, at an angle of 60° to the skin surface to reduce the variable pressure exerted by the investigator's hand.
- The puncture should be moderate to avoid unnecessary bleeding as well as false-positive tests, and lancets preloaded with allergen should remain in the skin for 1 s.
- When testing a battery of allergens, perform the tests in parallel lines placing the drops about 2–3 cm apart, occasionally using the left forearm, always discarding the lancet on completion of each test to avoid mixing allergens [95].
- Perform a positive control with histamine hydrochloride, 1 mg/ml [49] always approximately 4 cm away from other tests, otherwise histamine can infiltrate the area of other tests, giving questionable results [80] and then a negative control (normal saline, 0.9%). For children aged 9 years, 10 mg/ml histamine has been suggested [143]. Both controls must be applied in the same fashion and at the same distance as all the other tests [165]. Alternatively one can place one at the beginning and one at the end of the test battery.
- Gently wipe away the excess solution with a paper tissue (different for each test) approximately 1 min later, avoiding smearing of test solutions to adjacent test sites: this operation does not interfere with the intensity of skin response.
- Extracts should be kept refrigerated at 40 °C.

Especially in infancy, a laser technique is advisable instead of puncture testing, insuring a greater reliability and reproducibility, but with a disadvantage that cannot be overlooked: its high cost [75].

SPTs for allergy diagnosis should be performed in duplicate and quadruplicate for research purposes [59].

Scoring System

SPTs are read within 15–20 min in a standard manner. The size of skin reactions is related to the size of the histamine wheal resulting from the positive control (diameter, at least 3 mm) considered.

We no longer use the scoring system of Aas and Belin [1] and consider positive only children with a ≥3-mm wheal (equal or twice the size of the histamine wheal) with an area of ≈7 mm^2 (cut-off), to record the highest percentage of positivities [128]; if smaller (2 mm) it renders any evaluation difficult and does not warrant the trauma caused by testing [49]. The difference between 2 and 3 mm in mean diameter is equivalent to a tenfold difference in skin sensitivity, an important factor in epidemiological studies [59].

Cut-off values have been recently evaluated as follows (mean diameter/surface of wheal): CM 5 mm/29 mm^2, egg 4 mm/16 mm^2, wheat 3 mm/7 mm^2, peanut 6 mm/40 mm^2, soy 3 mm/9 mm^2, with significant differences in wheal size between children allergic or tolerant to the above foods, except for soy (NS) [52].

The method of comparing SPT responses with controls ensures the greatest reliability and reproducibility, also balancing the operator bias [98]. Studies that do not conform to this universal grading system (Table 5.1) make any evaluation and reproducibility of data difficult [98]. SPT dimensions were analyzed, for example, by Sears et al, who noted that the highest prevalence of asthma corresponded to a diameter >8 mm (Chap. 11), and Hill et al suggest that a response of 4+ is equivalent to a positive FCT [66]. More precisely, this equivalence is seen when skin wheal diameter is greater than a given size, that is CM, 8 mm; egg, 7 mm; and peanut, 8 mm; smaller in children aged <2 years: 6, 5, and 4 mm, respectively [160]. Especially with food and inhalant allergens and in very sensitive children, we see wheal diameters *>1–2 cm,* more with the erythema with food allergens and with inhalants, also 2–5-fold greater than the histamine wheal.

Evaluations are carried out and made more precise in three ways:

1. Calculating (in parallel with controls) the mean wheal diameter, by adding or multiplying the largest diameters and calculating their mean; however, since the reactions can be oval or irregular in shape, the largest and smallest wheal diameters are measured, added and divided by 2, to obtain the mean wheal diameter.
2. With a transparent millimeter ruler or one equipped with holes coinciding with increasing diameters (1, 2, 3, 4, 5 mm, etc.) to determine the wheal diameter and erythema diameter.
3. Using a computerized program with a tablet and a digitizer pen to very precisely outline the wheal on transparent paper; subsequently the SPT scanner calculates SPT areas [133].

Positive and negative controls should always be included:

- A positive control is extremely helpful to compare various responses, check the technique's reliability and exclude the inhibition of skin reactions possibly caused by a concurrent treatment.
- A negative control can reveal a likely aspecific skin hyperreactivity, as a marked red dermographism occurs in some children following the puncture microtrauma.
- A doubtful reaction due to wheal dimensions can be considered as negative in the presence of a great histamine reaction.
- Generally a smaller histamine dilution would be preferable, thus ensuring better reproducibility, inducing wheals which facilitate both comparison and differentiation between negative and positive responses [185].

Table 6.8. Mean value (cm) of the wheal induced by histamine according to age

Age (months)	Histamine	
	1 mg/ml	10 mg/ml
0–3	0.77±0.75	1.90±0.92
3–6	1.07±0.58	3.07±0.88
6–12	1.67±0.69	3.40±1.18
12–24	2.23±1.14	3.29±0.86
Adult	2.48±1.0	4.75±1.11

Data from [99].

Failure to respond to a positive control suggests that there is some interference with SPTs, such as parents failing to discontinue antihistamine drugs before testing [120]; one should consider such an occurrence and repeat the tests at the subsequent control visit.

Several investigators have considered the *end-point titers* as the extract progressive dilution giving a wheal size of 3 mm or a wheal size similar to that of the histamine-positive control [37].

IgE-mediated reactions appear within 1–2 h occasionally followed after 4–6 h by a delayed-type hypersensitivity (DTH) resolving in the subsequent 24–48 h. Possible systemic reactions, however exceptional, should not be underrated: thus it is recommended that SPTs are done directly by pediatricians or under their personal supervision [37].

- After SPTs are done, children should be supervised by pediatricians in the office/hospital for at least 20 min, the reading interval, as for SIT (specific immunotherapy), or longer for high-risk children [49].
- In children aged a few months to 2 years, positive control response (1 mg/ml) until 12 months is always <3 mm, as Table 6.8 [99] shows. If doubt arises with an 1-mg/ml dilution, it is advisable to repeat the test with a 10-mg/ml dilution [99]. Even in children aged 5–25 months, the control diameter is not significantly related to age: the 1.8±1 mm mean diameter (histamine 1 mg/ml) is <2 mm in children aged <12 months [45]. The popular notion that infants cannot be tested stems from the idea that they rarely produce detectable sIgE levels; however, we have seen infants with positive test results, including a 6-month-old girl with a 1 × 1-cm response to egg white.

Evaluations

One point should be clear: SPTs measure reactions to antigen-specific IgE; however, *SPTs are not diagnostic,* since a positive SPT alone does not indicate a definite clinical sensitivity to a given allergen, so it is necessary to analyze any response thoughtfully, case by case, con-

Table 6.9. Sensitivity, specificity and positive predictive value of SPTs, total IgE and PP in infants aged 6–18 months

Parameters (%)	SPTs		Total IgE		PP	
Months	6	18	6	18	6	18
Sensitivity	22	40	44	63	22	40
Specificity	100	100	70	55	100	95
Predictive value +	100	100	38	43	100	80

Data from [91].
PP Phadiatop Paediatric, *SPTs* Skin prick tests.

sidering history data. A puzzling test should be repeated and/or confirmed before making a decision, recurring to FCT or BPT (bronchial provocation test) if foods or inhalant allergens, respectively, are concerned.

During recent years, *SPT-negative results* have been the subject of intense discussion: it has been recently confirmed that if children with suspected FA present properly performed negative SPTs for one or more incriminated foods it would make an IgE-mediated FA very unlikely. These children might be better diagnosed by searching the allergens to which they may be sensitive. We have demonstrated that FCTs were positive in 7 out of 35 children with AD and negative SPTs to CM; therefore a method with a 20% ineffectiveness cannot be considered as fully reliable. In a recent study, 74% of diagnoses made with SPTs were not confirmed by FCTs [25]. In cases of rhinitis or conjunctivitis with a history highly indicative of allergic disease, SPTs can be negative since sensitizations are localized to the target organ; thus we suggest a nasal provocation test (NPT) and/or a conjunctival provocation test (CPT) (see "Provocation Tests").

Recently the SPT characteristics were evaluated at the cutoff level of 3 mm: CM hat 0.72 sensitivity and 0.62 specificity, and α-lactoglobulin (ALA) 0.66 and 0.64, β-LG 0.84 and 0.53, casein 0.55 and 0.87, respectively; PPV (positive predictive value) was highest for casein (0.78) and NPV (negative predictive value) highest for β-LG (0.81) [56]; see also Table 6.9 [91]. In general, SPTs (and RAST/CAP) for more standardized allergens (peanut, fish and egg white) are the most reliable, especially with immediate reactions to foods [149].

Prick + Prick Testing

P+P is a technique first employed in 1978 (Table 6.10) [33, 48], because fresh fruits and vegetables have extremely labile allergens that cannot be preserved. Therefore, for diagnostic purposes, it is preferable to employ fresh foods instead of commercial allergenic extracts, as using fresh material without preservatives such as glycerol is particularly suitable [48]. Some fruits contain vasoactive substances able to produce false-positive results. Foods should be fresh and not frozen since storing them at –16 °C causes some food proteins to lose their allergenic qualities; furthermore, several substances lose their allergenicity within 48 h [63]. The P+P technique is also very worthwhile when commercial extracts are not at hand, for example, exotic fruits, or when allergens are denatured to produce extracts and for a direct diagnosis of latex allergy.

Table 6.10. Studies done with the P+P method

Authors	Year	Fresh foods tested
Hannuksela and Lahti	1978	Apple, carrot, potato
Andersen et al	1978	Apple, potato
Dreborg and Foucard	1983	Apple, carrot, potato
Pastorello	1985	Apple, peach, plum, pear, cherry, apricot, banana
Ispano et al	1985	Apple, onion, tomato
Sacerdoti et al	1987	Wheat, egg, CM, chocolate, fish, tomato, etc.
D'Urso	1987	CM, egg, fish
De Martino et al	1988	Apple, peach, tomato, pear, egg, CM
Ortolani et al	1988–1989	Apple, peach, apricot, tomato, melon
Cantani and Mastrantoni	1990	Banana, apple, carrot, potato, tomato, wheat, egg
Sampson et al	1991	Hydrolysate formula
Ragno et al	1993	3 hydrolysate formulas
deWaard-van der Spek	1998	CM, egg, peanut

Data from [33, 48]
CM cow's milk.

Table 6.11. Reliability of P+P technique and RAST in the diagnosis of 3 HFs in 20 children with CMA

	NIDINA HA		ALIMENTUM		PROFYLAC	
	P+P	RAST	P+P	RAST	P+P	RAST
Specificity	0.69	0.22	0.89	0.89	0.80	0.63
Sensitivity	0.63	0.50	1	1	0	0
Predictive value +	0.62	0.34	0.62	0.50	0	0
Predictive value –	0.70	0.35	1	1	0.82	0.72

Data from [136].
P+P prick + prick.

Indications

We have successfully employed P+P with several foods, including CM, egg, wheat, the most common foods of children's diet and frequently suggested as inducing FA even by the 1st year of life, and several fresh fruits and vegetables, demonstrating P+P's greater reliability compared to commercial extracts disk. In addition to being easily arranged, P+P could be a safe and useful diagnostic method (Table 6.10). Applying the analytical reliability criteria for CM, egg and wheat, we have found a lower sensitivity for CM and wheat, and a higher effectiveness compared to SPTs. The P+P technique applied to hydrolysate formulas (HFs) has shown good diagnostic reliability (Table 6.11) [136]. The high P+P PPV and NPV supports the use of this simple and noninvasive method before prescribing HFs to babies with CM allergy (CMA) [136]. P+Ps gave unexpected results in a 9-month-old girl with severe CMA seen by us who had negative SPTs for CM. By performing P+P tests with CM diluted 1:1 with saline there was a wheal 1-cm-large-plus a 2-cm-large erythema (see also in Chap. 9). Similarly, this technique is specifically applicable to pollen-allergic babies with OAS [48].

Techniques

The technique is very simple. One pricks a normal, sterile lancet into a fresh food, assuming that it is solid, and immediately thereafter into the skin, as when doing an SPT [48]. When testing liquid foods such as CM and egg, one dips the lancet directly into the food. With other foods, including wheat, a mixture of flour and boiled water can be prepared [33] (Figs. 6.20, 6.21); similarly, HF formulas were diluted fresh according to the instructions on the can, as for a normal preparation [136]. As regards latex, one can perform puncture skin testing with latex gloves, obtaining positive results in 70 % of cases [114].

Fig. 6.20. Prick-by-prick method (for details see text)

Fig. 6.21. Prick-by-prick method (for details see text)

Patch Test or Epicutaneous Test

The PT (patch test) is the diagnostic tool of choice for ACD and selected AD cases. The significant correlation with total and specific IgE suggests a prominent IgE role in the reaction mechanisms, mirroring the sensitization process [84].

Indications

PTs confirm an ACD suspected case, recognize a sensitizing substance among many suspected ones, bring out the relevant but unsuspected substances, mainly in doubtful cases, and establish which materials can be tolerated by patients without risk, above all when very sensitive [16]. Because PT can aggravate an acute and disseminated dermatitis, or elicit an unspecific response in correspondence with the application site, it is wise to postpone testing to a later date until the lesions improve [153]. However, the risk of applying a standard panel is insignificant compared to the wealth of the information obtained [53].

Techniques

PT consists in placing the test material, usually commercial substances or extemporaneously prepared by specialists, in a proper vehicle and an adequate concentration is applied under occlusion on the child's upper back for 48 h. The ICDRG (International Contact Dermatitis Research Group) [16] and the NACDG (North American Contact Dermatitis Group) [95] have published lists of allergens for PTs, establishing pertinent concentrations and indicating application methods:

- Prefer the upper back skin, examining the area and excluding zones with minimal evidence of lesions [84], putting off testing if lesions are acute or disseminated. Applications on healthy skin yield positive results in 87 % of cases [153].
- Mark the outline of test sites with a spotlight fluorescent pen. Children should remain still during testing to avoid skin folds or streaking.
- Commonly available standardized test material varies according to the occlusive patches employed, requiring dilution to be nonirritating under patch conditions. The test substance is classically applied to 1 cm^2 of soft fabric under occlusion by an impermeable substance and subsequently applied to the skin with nonallergenic Blenderm tape.
- Test material is mounted on cellulose acetate and coated on a water-impermeable sheet of polyester covered with a protective sheet of polyethylene, and packed into a pouch of laminated foil made of Al, polyethylene, and polyester.
- Draw map of PT location on PT form.
- Inform patients that test sites should remain dry until removed. However, they should be immediately removed if severe itching, a burning sensation, or irritation occurs.
- Remove test 48–72 h [53] or 24 and 48 h [84], or 24 h for foods, after application [119]. Reading is done after at least 30 min (mostly after 1 h), so that a possible irritation produced by adhesive tape is reduced, making a delayed reading at 24 h [16, 53].

In children, the employment of *Finn chambers* is more practical and less invasive. These chambers are formed by small Al cups with an 8- to 12-mm diameter, a 50-mm^2 surface and a 20-ml volume, applied to the skin on nonallergenic tape (Scampor). The test substances, when incorporated in semisolid supports, can be directly applied to the small cups, when liquid a small disk of filter paper placed in the chamber is saturated by the test substance [37]. Another method, the TRUE test, consists of a mixture of 20 allergens incorporated in hydrophilic gels, coated on a water-impermeable sheet of polyester and dried to a thin film; the film once applied to the skin is hydrated by skin perspiration and releases the allergen; the high accuracy of the whole system and allergen distribution on a 9×9-cm polyethylene support provide a homogeneous allergen bioavailability [37, 53, 95]. The allergen material at hand is widely available, but you do not find it everywhere, thus test utility is limited to routine investigations [16].

The *Rapid Patch Test* is similar and includes three panels with a total of 30 allergens. *Epiquick* consists of 20 allergens on Finn chambers mounted on Scanpor tape. In children aged <4 years with AD and ACD, it is suggested to do a SAFT (skin application food test) to study food and contact allergens applied on uninvolved skin in a 0.5-cm^2 small cup [119]. Since children with AD are probably more likely to have irritant or false-positive reactions to metals, a shortened standard PT series for pediatric patients has been proposed [145].

To patch test latex, test latex hapten by means of specific extracts or apply a latex glove fragment on the upper back, to be read after 48–72 h [114].

The latex exposure test is required for a convincing diagnosis. In the test used, the child's wet hands are exposed to a wet rubber latex glove finger for 15 min, and if there is no reaction the whole hand is exposed to the whole glove for 30 min; vinyl or Tactylon gloves are used on the other hand as control [114].

Recently, the PT with foods (PTF), such as *the atopy patch test* (APT), has been introduced as a procedure enabling the identification of AD children reacting with AD worsening to the administration of the offending food [110], as well as reducing the need for OFC in these children [142]. In the case of *peanuts,* for the preparation of the PT material, peanuts were whipped and a

mixture was made with one part of petrolatum and two parts of peanuts: 20 mg of this material was applied to the uninvolved skin of the back using Finn chambers and Scanpor tape as above. The suggested occlusion time is 72 h and the results are read 30–60 min after removal. SPTs were positive in 16 patients (12%), whereas PTs for foods proved positive in 25 (19%) and a positive challenge was observed in 12 subjects (9%) [152]. For CM, egg, wheat, and rye, APT sensitivity was 60%–93% and specificity was 71%–97%. The SPT corresponding figures were 13%–41% and 97%–99% [162]. In infants with CMA the APT had a sensitivity of 79% and a specifity of 91% [44]. The *recent ready-to-use APT* had 79% sensitivity vs 44% of the APT, a similar specificity (93.8%) and a test accuracy of 82.9% vs 63.4% [74].

Evaluation of Patch Test Reactions

Evaluation of PT reactions can be based on ICDRG guidelines:

0 No skin change compared to controls
1 Weak positive, nonconfluent erythema and discrete papules
2 Strong positive, confluent erythema, papules, vesicles
3 Severe positive, redness plus blistering [53]
or based on the following scale [152]:
1 Erythema and edema
2 Erythema, edema, and few papules
3 Erythema, edema, and papules covering most of the patch test area
4 Erythema, edema, and papules spreading outside the patch test area or vesicles

Frequency of positivities greatly decreases from infancy to adulthood, as seen in a Der p study: 1–5 years, 60%; 6–10 years, 76%; 11–15 years, 50%; 16–20 years, 21%; 21–25 years, 29% [153]. Especially in AD children it is suggested that the cells infiltrating the PT site be studied with suitable techniques [153].

Photopatch Testing

A PT variant used for the diagnosis of photodermatitis is done by applying the suspected allergen on the skin in duplicate; after 24 h one of the tested skin sites is exposed to sunlight (10 min in summer, 30 min in winter). After 48 h the diagnosis is made with the same PT modalities [16]. Alternatively, artificial light sources can be adequate and provide enough of the long-wave ultraviolet light A (UVA) [95].

In vitro Immunoallergic Tests

Total Serum IgE

In allergy diagnosis, assay of total serum IgE levels (PRIST, paper radioimmunosorbent test) can play a significant role, taking into account the potential lack of correlation with the child's clinical state.

Indications

PRIST measurement has some definite indications:

- At birth, in cord blood as a predictive method of allergy (see Chap. 3)
- At every age as a mass screening
- To correctly classify allergic and nonallergic children
- In wheezing children
- To confirm an AD or FA diagnosis [27]

Total IgE Level Determination

The most commonly employed assays to measure total serum IgE levels (or other biological specimens) are:

- *Radioimmunologic assay* (RIA): the most classic is the Pharmacia PRIST, which measures anti-IgE antibodies covalently attached to a solid phase such as a paper disk, or a plastic microtiter well is incubated with an appropriate dilution of the serum or other biological liquid. A complex of anti-IgE-IgE antibodies is formed and by adding anti-IgE marked with I^{125} a new complex of anti-IgE antibodies–anti-IgE-marked antibodies is formed and directly measured.
- *Immunoenzymatic* assay (EIA, enzyme immunoassay, ELISA, enzyme-linked immunosorbent assay, and Instant ELISA), employing enzymes such as alkaline phosphatase and peroxidase as a detector system, instead of a radioisotope. The unit of measure used is the kilo-unit × l (ku/l) or the International Unit × ml (IU/ml).

An IgE assay in stools was also proposed as a noninvasive and economic indicator of food sensitization [5].

Total serum IgE values in healthy children according to their age are shown in Tables 6.12 and 6.13 [76, 82]. A study on 1,160 newborns determined IgE levels at 1, 2, 3, 5, and 6 years of age and the data were compared with the original population-based sample of 4,082 children [82]. Data given in ku/l (1 U/ml=1 ku/l) [82] are more widespread than the GM data [76]. Statistically significant higher total IgE values were found in boys than in girls at each age; this sex difference was not statistically significant within the group of atopic children [82]. IgE levels absent or very low at birth subsequently increase compared with the rise and sum of single sensitizations,

Table 6.12. Normal values of total IgE (UI/ml)

Age levels	GM	Limits	GM ± 2 SD
Birth	0.22	<0.1–1.5	0.04–1.28
6 weeks	0.69	<0.1–2.8	0.08–6.12
3 months	0.82	0.3–3.1	0.18–3.76
6 months	2.68	0.9–28	0.44–16.25
9 months	2.36	0.7–8.1	0.76–7.31
12 months	3.49	1.1–10.2	0.80–15.22
2 years	3.03	1.1–49	0.31–29.48
3 years	1.80	0.6–7.6	0.19–16.86
4 years	8.58	2.4–34.8	1.07–68.86
7 years	12.89	1.6–60	1.03–161.32
10 years	23.66	0.3–215	0.98–570.61
14 years	20.07	1.9–159	2.06–195.18

Data from [76].
GM geometric mean.

Table 6.13. Normal values of total IgE (UI/ml)

Boys					
Age (years)	1	2	3	5	6
Percentiles					
25	2.5	6	9	18	18
50	6	19	25	45	47
75	16	58	72	110	118
90	44	137	197	271	269
95	85	248	297	501	493
Girls					
Age (years)	1	2	3	5	6
Percentiles					
25	2.2	4	7	11	12
50	4	12	16	25	32
75	11	3051	64	80	
90	55	77	116	155	249
95	62	189	206	241	421

Data from [82].

in turn dependent on genetic and acquired factors, but above all correlated with the extent and persistence of contact and the antigenic stimulus (Fig. 6.22).

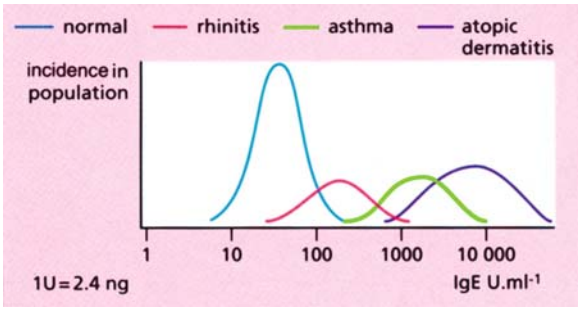


Fig. 6.22. IgE levels in allergic disease. Determination of total IgE serum and cord blood is of some value in allergy diagnosis, even if there is a great overlap between normal and allergic children. In general, however, the more severe the IgE inflammation, the higher the serum IgE

Total IgE Level Determination in Neonates and Infants

Total serum IgE levels in these early ages are nearly always less than 0.02 UI/ml (Fig. 2.10). Since the greater part of assays allows measurements from 0.5–10 UI/ml, it is indispensable to use ultrasensitive assays, that is, RIA (Pharmacia IgE RIA Ultra 50) or ELISA (Pharmacia IgE EIA Ultra 50). Values of >1 UI/ml at birth, >5 UI/ml at 1–3 months and >10 UI/ml at 4–6 months are suggested as probably pathological [127].

Advantages and Disadvantages

As regards predictive values, PRIST has an uncertain clinical significance at birth (Chap. 3), but is a useful tool to identify atopic disease early, before clinical symptoms are evident in children previously manifesting severe reactions, anaphylactic shock or severe AD, and for research purposes in prospective studies aimed at atopy prevention [27]. Total IgE associated with sIgE for Der p and egg white in 6-month-old infants are predictive of a conversion to sIgE values positive for Der p at 5 years, resulting in a reliable screening method [150]. In addition, we have reported valuable results in children with bronchiolitis, CNO, OME and hyper-IgE syndrome [27]. In children, especially preschool children, such an assay could have a diagnostic value significantly higher than in adults [32]. However, the diagnostic value of *PRIST in pediatrics* also has disadvantages, as Table 6.9 summarizes, such as low levels in subjects definitely allergic [120], high IgE levels in nonallergic diseases and in nonatopic children and conversely normal IgE levels in some atopic children [69].

Specific IgE Antibody Determination

sIgE are antibodies specifically directed against specific allergens present in serum of allergic individuals: the allergic interaction of sIgE, linked to mast cell and basophil surface by the Fc fragment, induces mediator release with vasoactive and inflammatory properties. There are discordant opinions on the RAST value in allergy diagnosis: we stress the prerequisite of evaluating case by case, mainly because there is widespread use by nonspecialists, leading to unbalanced interpretations when RAST results are not critically evaluated by experts [4, 37].

Indications

- History of previous episodes of anaphylaxis triggered by a particular food
- Children <2 years because of the discomfort of performing SPTs
- Highly sensitive children
- Disagreement between history and SPT responses
- Severe and/or chronic cases
- Children with dermographism
- Food allergens that are not very well purified and/or standardized
- Allergens not employable for SPTs (toxic, not hydrosoluble or highly sensitizing substances)

Assays for sIgE Determinations

The most commonly employed assays are of radioimmunological or immunoenzymatic types. sIgE are expressed in arbitrary units, according to the commercial kit employed for sIgE determination.

The most common is Pharmacia *Phadebas RAST,* with allergens covalently linked to a solid phase such as a paper disk incubated with a patient's serum specimen. When the serum contains sIgE vs some allergens, these bind to allergens and nonspecific IgE antibodies are washed away. Anti-IgE marked with I^{125} are then added and the amount of measured radioactivity increases in proportion to bound IgE concentrations in the serum sample. We believe an important clarification must be made, also from the point of view of false-positive and false-negative results: RAST is not a quantitative record of sIgE in a rigid sense, but only the binding between IgE antibodies and the allergen bound to the solid phase. In practice, allergen-specific IgE results are reported by different assays in nonequivalent arbitrary units or classes that are not always comparable [183]. Moreover, the results are expressed in RAST units, devoid of any correspondence with PRIST IU/ml. A conventional RAST scoring relates all allergen-specific IgE values to a standard curve divided into classes:

1st class IgE levels <0.35 IU/ml
2nd class IgE levels >0.35 IU/ml and <0.7 IU/ml
3rd class IgE levels between 0.7 IU/ml and 17 IU/ml
4th class IgE levels higher than 17 IU/ml [120]

RAST, long used, has been studied thoroughly in children only by a few authors [141] in addition to our data [97, 136]. Additional sandwich methods exist:

- *Second-generation RAST-type* assays have evolved to provide more quantitative, sensitive, precise IgE antibody results [62]. Modified RAST (mRAST), able to detect lower IgE values, is used in mold allergy [113] and in pollen and mite allergy [184], as are Phadezym RAST (Pharmacia) (PhRAST), Riallergy, RAB and Sferikit.
- Immunoenzymatic assays (EIA) are different regarding the solid phase employed to bind allergens, detector systems, and final scoring methods.
- ELISA, similar to RAST.
- FAST using a fluorogenic substrate for the antiserum conjugated to alkaline phosphatase.
- *MAST-CLA* with a chemiluminescence-based reading allows manifold tests with a single assay, 36 aeroallergens and 36 trophoallergens or 24 aeroallergens and 12 trophoallergens, useful in FA, whereas sensitivity is modest in respiratory allergy [102]. Instead, in asthmatic children, one study has found a 54.5%–93% concordance with SPTs and RAST for Der p, grasses, Fel d, Can d and molds including Alt a [134], and another has found a good agreement with BPT (87%), SPTs (80%) and RAST (74%) [106]. MAST-CLA has 69.2% sensitivity to foods, slightly lower than RAST. However, a greater annoyance, in this and other methods, is the frequent crossed allergy within foods [102]. MAST-CLA sensitivity compared to SPTs may decrease to 54% for CM and egg, with 82% and 93% specificity, respectively, and 77% concordance [177]. Other studies have noted no difference with SPTs and RAST [2]. MAST-CLA is not correlated with total IgE [177] and, considering its high cost, it should be referred to specific cases, for example, when the doctor is faced with a silent history [177] or in doubtful cases [127].
- *Phadiatop* (Pharmacia), qualitative test eliciting results of the positive/negative type. This assay is composed of a mixture of inhalant allergens and is used as a general assessment of allergic responsiveness [81]. It measures the presence of sIgE to the following antigens: Der p, Der f; Phl p, Bet v, Ole e, Art v, Par o; Cla h and cat, dog, and horse dander, so that it is useful as a screening test for atopy. We and others have demonstrated 90%–92% sensitivity, 88%–98% specificity [51, 191] up to 100% [31], 90%–92% effectiveness [31, 90, 191], 100% PPV and 86% NPV [31], with significant differences related to asthma diagnosis [31] (p=0.0001). It is not indicated for children <4 years [156]. The percentage of positivities decreases with age [174]. PP (Pharmacia) now includes food allergens, and is thus positive in 49% of infants aged ≤18 months [155]. In 36 atopic children aged 5–6 years with SPT+, its specificity was 89%, effectiveness 85% and sensitivity 72%, which decreased to 56% to identify clinical atopy [90] and to 22%–40%

Table 6.14. Comparison between CAP and RAST (%)

	CAP	RAST
Sensitivity	94	87
Specificity	96	96
Positive predictive value	97	97
Negative predictive value	92	84
Effectiveness	95	91

Data from [124].

in 6- to 18-month-old infants [91] (Table 6.9). Thus in young babies, it appears less sensitive [45]. The analytical reliability criteria varied in children between 85% and 87% compared to RAST: assuming a 20% prevalence of atopy, the NPV would be 97% and effectiveness 86% [42]. Also because of the absence of false-negative results [31], Phadiatop fared better than PP for the screening of children aged 5–6 years, whereas PP appeared to be more useful in preschool children [90] and for correctly classifying children early with suspected symptoms of allergic disease [31]. Once confirmed by means of PP clinical suspicions, the child should be referred to a specialized division to further refine the diagnosis.

- *CAP System FEIA* (Pharmacia & Upjohn), can be employed for both radioimmunologic and immunoenzymatic assays. The allergen under examination is covalently bound to a hydrophilic carrier polymer, encased in a capsule, which catches all allergen-specific IgE in the sample. Allergen-specific IgE is detected directly with a combination of polyclonal and monoclonal anti-IgE antibodies labeled with β-galactosidase, generating fluorescence: CAP sensitivity is 95% and specificity 98% [22, 123] and is positive in 95.1% of symptomatic, in 88.7% of asymptomatic and in 4.8% of control patients, with sIgE levels significantly higher between groups [122]. Table 6.14 brings together additional CAP results compared to RAST [124]. In patients with pollinosis and OAS, the mean concordance between CAP and RAST was 82%, suggesting a CAP preference [17]. Examining the standard RAST, mRAST and CAP results compared to conventional SPTs, mRAST is the most sensitive, standard RAST the most specific, and mRAST and CAP the most effective [75]. CAP, with the advantage of automation, demonstrates a good correlation with both RAST, history and SPTs [22] and appears useful as a guide for the prescription of environmental controls and SIT [22], and it is not influenced by high levels of total IgE (up to 3,000 kU/l), thereby excluding possible false-positive results, or of sIgE (up to 20,000 kU/l). A cross-reactivity vs other immunoglobulins (Ig) is $<1/20\times10^6$ with no IgGs interference [22]. With the CAP System, levels of sIgE to Der p, Der f, cat and dog danders, *Cryptomeria japonica* and orchard grass were determined. [112]. CAP is more sensitive than RAST in the diagnosis of allergy to Hymenoptera venom [88], and it is also more sensitive to pollens, molds and mites than mRAST [184]. CAP System FEIA results, when compared with DBPCFC outcomes, are generally comparable to those of SPTs in predicting symptomatic FA; by measuring sIgE concentrations with this test, it is possible to identify a subset of children and adolescents who are very likely (>95%) to experience clinical reactions to egg, CM, peanut, or fish with high efficiency and specificity, but not for wheat and soy, which should be improved. However, the values found for SPT were slightly higher [149]. Moreover, there may be an advantage for in vitro test results done with CAP System FEIA, which provides results in terms of IgE for food allergens comparable to DBPCFC studies [149]. Thus the need for OFC could be reduced by about 50% by quantitatively measuring food-specific IgE antibody levels in serum and applying these 95% predictive decision criteria [148], or when the cutoff value of sIgE to CM is of ≥2.5 KUA/l [56]. The performance characteristics of CAP System FEIA at the cutoff level of 0.35 KUA/l were as follows: CM had 0.84 sensitivity and 0.56 specificity, and ALA 0.55 and 0.84, β-LG 0.59 and 0.80, casein 0.71 and 0.75, respectively, PPV was highest for ALA (0.74) and NPV highest for CM (0.81) [56].
- In 98.6% of children, *UniCAP Phadiatop* (Pharmacia), semiautomated, was shown to differentiate positive or negative sIgE antibodies to a variety of inhalants in 2 h and 30 min [186]; in the comparison with ADVIA Centaur, using a panel of food and inhalant allergens, UniCAP had a sensitivity of 81%–100% and a specificity of 62%–70%, ADVIA Centaur of 82%–100% and 52%–79%, respectively. Both register the lowest specificity score with egg and mite allergens and the highest sensitivity scores with cat allergen, the UniCAP also with pollen allergen [137].
- *ADVIA Centaur* is a specific IgE assay, a reverse sandwich immunoassay using direct chemiluminescent technology. The monoclonal mouse antihuman IgE antibody is covalently bound to paramagnetic particles (PMPs) in a solid phase. The anti-IgE coupled with the PMPs capture the sample IgE. Non-IgE is removed by washing. Biotinylated allergen is added in excess and binds to the allergen-sIgE antibody captured on the solid phase [137].
- *Enzallergy,* polyclonal anti-IgE marked with alkaline phosphatase.

There are several automated and semiautomated in vitro methods to identify sIgE, all with varying menus, technologies, and performance. These include *rapid tests,* sandwich or EIA assays utilizing mixtures including the most common allergens, some of which allow sIgE determination in very short times (about 2 h), resulting in useful screening systems:

Last, automated and semiautomated systems have been fixed delivering responses within 3–19 h, namely:

- AlaTOP Allergy Screen EIA and Immulite.

- Arianna, a screening test employing allergenic pollen mixtures selected according to relative local prevalences and controlled with positive sera of local individuals.
- AutoDELFIA (Pharmacia), automatization of DELFIA semiautomated system, the same kits of which are employed because they are also utilizable for IgE antibodies, by performing double-triple marked assays.
- CARLA System with BRIO.
- DALIA is a very sensitive technique selective for specific (s)IgG determination, not influenced by competitive antibodies such as sIgA, sIgM and nonspecific IgG [192], and provides a wide application field for SIT.
- The ENEA II system, a totally automated instrument, permits in vitro assays, even synchronous, of total IgE and sIgE, with great rapidity (a test every 40 s). Evaluating concordance with both history and SPTs as reference values has 83.4% sensitivity, 82.8% specificity and 83.4% effectiveness [132].
- With IgE-Screen, a test on a multiallergen strip for food and inhalant allergen, antiserum is conjugated with alkaline phosphatase and readings are done with a direct optical system.
- Matrix (Abbott) is an automated system measuring sIgE against a pool of allergens, inserting serum in a cartridge. Anti-IgE are conjugated with alkaline phosphatase.
- Profilo provides a simultaneous identification of total IgE and sIgE to groups of various allergen (grasses, molds and mites, trees, animals, herbs).
- Additional automated systems include ACCESS Allergy System, Eurospital Tech, MILENIA AlaStat, Saver Allergy System, Step, etc.

In conclusion, systematic improvements in some assay systems and ongoing monitoring with blind clinical samples are needed to assure that all commercial measurements of sIgE are accurate and reliable [183].

Additional Tests

Allergodiagnostics. For asthmatic anti-inflammatory treatment, a method has been developed to monitor high ECP (eosinophil cationic protein) serum levels in the presence of inflammation and normalized levels following the reduction of the inflammatory process. In children, however, this technique does not appear to correlate with peripheral blood eosinophilia, nor is there as yet a clear relationship between ECP concentrations and variations in lung function (see Chap. 11).

Additional markers of allergic inflammation include [62, 173]:

- Endothelial cells: CD62E
- Eosinophils: EPO, END, MBP
- Mast cells: tryptase
- Monocytes/macrophages: lysozyme, neopterin
- Neutrophils: MPO, elastase, lactoferrin and a not yet identified protein of 40 kD
- Platelets: β_2-thromboglobulin
- T lymphocytes: IL_2-R, IL_5

Eosinophils. Blood eosinophilia *per se* is not diagnostic in an absolute sense, being a characteristic of several conditions, nor does its absence exclude an allergic pathogenesis of the affection under scrutiny (Table 6.15). Nasal eosinophilia (>10% of cells) is instead an indicator of IgE-mediated AR and, especially in children, is associated with SPT positivity (p=0.0159] [19], but it is also found in nonallergic eosinophilic rhinitis

Table 6.15. Causes of eosinophilia

Allergic and pseudoallergic diseases
Allergic contact dermatitis
Allergic rhinitis
Allergic vasculitis
Atopic dermatitis
Black widow spider
Bronchial asthma
Bronchopulmonary aspergillosis
Drug allergy
Antibiotics
ASA
Sulfonamides
Erythema multiforme
Food allergy
Heiner syndrome
Hypersensitivity pneumonitis
Persistent eosinophilia
(+ splenomegaly and lymph node tumefaction)
Serum sickness
Stevens-Johnson syndrome
Tropical eosinophilia
Urticaria-angioedema

Immunodeficiency diseases
Chronic granulomatous disease
Graft-versus-host disease
Hyper-IgE syndrome
Selective IgA deficiency
Wiskott-Aldrich syndrome
Parasitic infestations
Amebiasis
Ankylostomiasis
Ascaridosis
Bilharziosis (+ splenomegaly)
Cysticercosis
Echinococcosis
Fasciola hepatica
Filariasis
Hydatidosis
Löffler's pulmonary infiltrate with eosinophilia (ascaridiasis)
Nematode infestation
Onchocercosis
Oxyuriosis

Table 6.15. (Continued)

Parasitic infestations (Continued)

Paragonimiasis
Scab
Schistosomiasis
Sparganosis
Strongyloidosis
Teniasis
Toxocariasis (Visceral larva migrans)
Toxoplasmosis
Trichinosis
Tunga penetrans

Skin diseases

Angiolymphoid hyperplasia
Bullous pemphigus
Candidiasis, mucocutaneous
Cicatricial pemphigus
Duhring's dermatitis herpetiformis
Erythema exsudativum multiforme
Herpes gestationis
Ichthyosis
Impetigo herpetiformis
Incontinentia pigmenti
Lichen planus
Lymphoid papulosis
Papular urticaria
Pemphigus vulgaris
Pyoderma gangrenous
Pityriasis rubra
Pityriasis versicolor
Prurigo
Psoriasis
Sézary's syndrome
Strophulus

Gastroenteric diseases

Crohn's syndrome
Eosinophil gastroenteritis
Protein-losing enteropathy
Ulcerative colitis
Whipple's syndrome

Infectious diseases

Brucellosis
Cat-scratch disease
Chlamydia infections
(trachomatis: bronchiolitis, conjunctivitis, secretory otitis media; pneumoniae: asthma)
Erythema infectiosum
Lymphocytosis infectiosa acuta
Measles (during the incubation period)
Mononucleosis
Scarlet fever

Autoimmune (connective) diseases

Allergic granulomatosis or Churg-Strauss necrotizing angiitis
Dermatomyositis
Felty's syndrome
Libman-Sacks' endocarditis
Löffler's fibroplastic parietal endocarditis with eosinophilia
Lupus erythematosus
Rheumatoid arthritis
Scleroderma, systemic

Hemopoietic and vascular system diseases

Erythema nodosum
Periarteritis nodosa
Pernicious anemia
Polycythemia
Postsplenectomy syndrome
Schönlein-Henoch syndrome
Waterhouse-Friderichsen's syndrome

Hormone disturbances

Addison's disease
Hypopituitarism
Inanition
Myxedema
Thyrotoxicosis

Neoplastic and myeloproliferative diseases

Acute lymphocytic leukemia
Angioimmunoblastic lymphadenopathy
Carcinomatosis
Cerebral neoplasms
Chronic myelogenic leukemia
Chronic myelosis
Eosinophilic granuloma
Eosinophilic leukemia
Epithelial neoplasms
Grawitz hypernephroma
Heavy chain disease (Franklin's disease)
Histiocytosis
Hodgkin and non-Hodgkin lymphoma
Idiopathic hypereosinophilic syndrome
Kimura's disease
Melanomata
Ovarian neoplasms
Panmyelopathy
Systemic mastocytosis
Wells-Whimster disease

Toxic reaction

l-tryptophan syndrome (myalgia-eosinophilia)
Scleroderma-like syndrome

Miscellaneous

Chronic renal disease
Goodpasture syndrome
Hansen's disease
Hereditary eosinophilia
Hypoadrenocorticism
Hypoxia
Peritoneal dialysis
Pleural effusion
Pneumonitis
Radiotherapy
Sarcoidosis
Thymic disease
Tuberculosis
Wegener granulomatosis

The attributions to different systems is in several cases only probable or tentative.

[14]. This assay may be helpful when in vivo and in vitro tests are negative although history confirms an atopic disease, or if the test cannot be performed for any reason [14]. Concerning the technique, see Appendix 6.5 [14, 59]. Counting the cells is suggested in provoked expectorations; however, it is commonplace that the majority of children swallow the sputum instead of expectorating it [14].

Serum IgGs in FA. As discussed in Chap. 1, serum IgGs in FA are found in pediatrics in nonallergic subjects [37] and therefore are devoid of any diagnostic significance.

IgG Subclasses. Assays are not indicated in general [158], but are useful in children undergoing SIT and in children with recurrent respiratory infections, and in all cases of relapsing broncho-obstructive affections, above all those with high susceptibility to infections. However, we underline that a recent AAAAI Position Statement, as discussed in Chap. 1, excludes any practical advantage from the determination of such antibodies.

Tests of Cell Immunity. Tests of cell immunity deal with the quantitative and functional evaluation of lymphocyte subsets (Tables 1.34–1.41).

Additional Immune Tests. Total IgE levels are not correlated with sCD23 and IL_4 levels [116].

Prerequisites, Advantages and Disadvantages of Diagnostic Tests

All diagnostic tests should possess definite characteristics and correspond to specific prerequisites, which are evaluated by means of *analytical reliability criteria* such as sensitivity, specificity, efficiency and positive and negative predictive values.

The *sensitivity* of a method is defined in terms of concentration (weight/volume), and is represented from a minimal quantity of antigen, distinct from the sample not containing it and is called standard zero, linked chiefly to the method's precision, which is variable from one method to another within the limits of a variability coefficient.

Precision represents the valuation of a method's reproducibility, defined precisely when a series of repeated measures of a single sample yields an identical result within an accepted variability. The comparison between measurements is expressed as a variation coefficient, or standard deviation (SD), an index of the method's precision.

Specificity is the ability of a compound to bind exclusively to a given substance, neglecting possible interferences with other molecules. During total IgE and/or sIgE determination, specificity depends on antigen purity and concentration, but chiefly on the antiserum used. Specificity evaluation is based mainly on cross-reactivity trials.

The *efficiency* of a method is defined from the interval between the value found and the true value stemming from the level of the substance sought.

Following this analysis, an allergy test should have:

- *Sensitivity:* the test should yield a high percentage of positive tests in patients correctly classified as truly having allergen-specific IgE, with the target of eliminating false-negative results (such as a high PPV).
- *Specificity:* the test should supply a high proportion of negative results in patients correctly classified as truly free of allergen-specific IgE; the target is that of eliminating false-positive results (such as a high NPV).

In conclusion, a test should supply a high *efficiency*, providing a high percentage of patients correctly classified among all subjects examined (atopic and nonatopic) based on the above results. Table 6.16 [158] outlines how to calculate these parameters.

Table 6.16. Formulas to calculate sensitivity, specificity, efficiency and positive and negative predictive values

Example

	Effective diagnosis	
Result	Positive	Negative
Positive	a (true +)	b (false –)
Negative	c (false –)	d (true –)

Sensitivity: the percentage of positive test results in all patients having the disease

$$v = \frac{a = \text{true-positives}}{a = \text{true-positives} + c = \text{false-negatives}} \times 100$$

Specificity: the percentage of negative test results in all patients free of the disease

$$v = \frac{d = \text{true-negatives}}{d = \text{true-negatives} + b = \text{false-positives}} \times 100$$

Efficiency: the percentage of patients correctly classified as having or as free of disease

$$v = \frac{a = \text{true-positives} + d = \text{true-negatives}}{\text{No. of cases}} \times 100$$

Positive predictive value: the percentage of positive tests that correctly predict disease

$$v = \frac{a = \text{true-positives}}{a = \text{true-positives} + b = \text{false-positives}} \times 100$$

Negative predictive value: the percentage of negative tests that correctly predict the absence of disease

$$v = \frac{d = \text{true-negatives}}{d = \text{true-negatives} + c = \text{false-negatives}} \times 100$$

Data from [158].

Advantages and Disadvantages of In vivo Tests

Skin Prick Tests

One of the main limits of SPTs is the interpretative problem of false-negative and false-positive results. The *causes of false-negative SPTs* [158] are:

- Potential decreased reactivity in very young infants [99]
- Variations among individuals
- Inadvertent employment of allergenic extracts of poor potency
- Variations from lot to lot of allergenic material
- Improper extract preservation
- Inadvertent employment of inactivated allergenic extracts
- Taking medications modulating the allergic reaction (during or before SPT execution)
- Test negativity in children with a true allergy (17% of children with pollinosis) [41]
- Particular foods: soy in 25% of cases [119]
- Improper test execution

Causes of False-Positive SPT Results

- Use of allergens with preparation defects (altered pH or osmolarity, irritants with low MW)
- Substances causing aspecific histamine release
- Measurable mediators within the extracts [185]
- Excessive proximity of positive tests

– Possible inadvertent contamination by irritants and/or allergens during skin testing

- Skin hyperreactivity (dermographism)
- Particular foods: wheat, fish, soy in 20%–27% of cases (Chap. 9)
- Improper test execution

An apparent false-positive can result from a latent sensitization (not yet clinically expressed) or from persistence of positivity after symptom remission [158].

Besides these specific problems, there are others of a more general type:

- Improper technique: execution done by operators with insufficient experience [163], use of poorly standardized lancets [179], too little distance between positive SPTs possibly resulting in aspecific reaction (axonal reflex) [80]
- Lack of standardization and/or different power of commercial extracts [49]

In summary, *SPTs have advantages and disadvantages* [19, 120]:

Advantages

- Indicate IgE-mediated allergy.
- Their sensitivity is usually high.
- Testing is an easy procedure.
- Results available in 15–20 min.
- Minimal equipment required.
- Inexpensive on a per test basis.
- Children's compliance is good because SPTs are for the most part noninvasive.
- They are safe and reproducible.
- Wide variety of allergens tested in the same sitting [120, 176].

Disadvantages

- Not very useful to detect reactions of the delayed type.
- Painful or uncomfortable, with possible irritations, depending on the child's age and reactivity.
- Time-consuming (15–20 min).
- Dependent on skin conditions (lesions more or less extended).
- Affected by drugs.
- Mild risk of reactions.
- Possible false-negative and false-positive results.
- Subjective evaluation of results.

In FA, SPTs are less reliable. Since they are unreliable with labile allergens, a cross-reactivity between different vegetal species is likely, when foods are cooked and digested, allergen modification ensues, as demonstrated by the classic test of Prausnitz and Küstner (Chap. 9), further resulting in poorly specific results in delayed forms, as in CMA with prevailing gastrointestinal symptoms. While SPT scoring and quantification are not challenging, interpretation related to diagnostic and therapeutic results requires a particular experience in pediatric allergy [120].

SPTs trigger usually local or relevant reactions but with wholly reversible functional inability [7]. Adverse effects of the systemic type are relatively sporadic: only two adults (0.02%) have shown such effects following an intradermal test [92].

Prick + Prick Test

This technique can elicit false-positive results with histamine-rich foods (Chap. 10) and foods with high lectin content; moreover, it is difficult to quantify the allergen to be introduced. A retrospective study has reported six infants <6 months of age subjected to multiple P+Ps, whose generalized reactions responded promptly to epinephrine: the babies were breast-fed and were tested with fresh foods [46].

Patch Test

- Causes of *false-positivity* depend on a high concentration or abundant quantity of test material, hypersensitive skin, use of irritant material, skin lesions in an active phase and/or microbial infection at the site of application, active, intense reactions to straps or patches, etc.
- Causes of *false negativity* include insufficient penetration of the allergen, inadequate concentration, poor

level of patient sensitization, technique defects such as inconvenient site, ineffective occlusion, ineffective tightness, scarce adherence of PT, use of high-dose CSs, etc. [16].

Advantages and Disadvantages of In vitro Tests

Concerning *total IgE* and *sIgE* sensitivity, specificity and precision of radioimmunologic and immunoenzymatic methods closely overlap.

Serum IgE (PRIST)

Advantages

- Useful in symptomatic patients with positive history but with negative tests or inconclusive investigations [179].
- When their values are in the normal range it is a practical, predictive method to establish a nonallergy [9].

Disadvantages

- Serum IgE levels exemplify only about 5% of total IgE, also because their passage into the circulation is transient and they have a short half-life. The remaining 95% of IgE is instead at the tissue level, bound to specific receptors of metachromatic cells.
- IgE concentration is also dependent on allergen stimulation and receptor saturation; therefore if atopic children are only slightly stimulated by allergens, normal PRIST results can be elicited, whereas in wholly healthy children serum IgE may be high, up to 330 UI/ml in nonatopic children [9].
- Likewise, children may deliver a positive PRIST result and respond to FCT negatively [26, 109].
- It is unreliable if employed as the only test for atopy screening [27] and is not correlated with RAST [163].

Radioallergosorbent Test

Like PRIST, RAST does not measure the absolute sIgE quantity, but their binding to allergens attached to a solid phase. sIgE are able to bind to tissue mast cells before being present in serum [45]. This binding is highly influenced by various factors, including type and quantity of allergens, quantity and avidity of sIgE present in serum, total IgE levels, serum autoantibodies belonging to other Ig classes such as IgG antibodies.

Advantages

In addition to the advantages noted for PRIST, the technique has the following:

- No risks for children
- Allergen stability in the solid phase
- Wide range of test allergens [79]

Disadvantages

- RAST measures only circulating IgE whereas SPTs point out allergen-induced histamine release at the skin level.
- It fails to give information on mast cell mediator release.
- It is less sensitive than SPTs.
- Cost-effectiveness is higher than SPTs, especially when several allergens are to be tested.

From the first point arises a lack of correlation that is not dependent on method imprecision and not settled by using purified allergens [190].

RAST also has the disadvantage of possible *cross-reactions,* for example, between several grass allergens (usually testing with a few allergens is sufficient), between Der p and Der f, frequent but inconstant Der p results are more reliable and with greater levels of specific binding), constant instead between *P. judaica* and *P. officinalis* [37]. Consequently, the number of allergens to be tested in current practice should be limited, apart from diverse history indications. In addition, RAST alone may not effectively discriminate between SPT-positive and SPT-negative patients [60]:

Causes of False-Positive Results

- Elevated values of total serum IgE.
- Employment of plant allergens with lectin groups binding IgE antibodies in a nonspecific manner.
- Serum IgG antibodies with the same allergenic specificity of IgE, able to bind to the paper disk and interfere with RAST results because IgE-IgG circulating immune complexes (CIC) are formed, generating a false IgE binding [140, 172], thus a positive RAST for soybean or wheat should be verified with a FCT [26].
- No concordance between in vitro tests and exposure to inhalant allergens [96, 109].

Causes of False-Negative Results

- Anti-IgE and anti-sIgE IgG antibodies that appear as IgGs [69] of sIgE in target organs but not in serum, of anti-food serum CICs, an often physiological occurrence [37]. Such data may explain the negative results of some studies related to SIT (Chap. 13).
- In the IgE early response to Der p, other Der p allergens distinct from both Der p 1 and Der p 2 can be dominant and constitute about 50% of the total [28] (Table 1.74).

• sIgE probably bind to mast cells before they are detectable in serum.

The problem of anti-IgE antibodies is generally by-passed by applying procedures able to restrict IgGs interferences, for instance, because of a preliminary removal of such antibodies.

Rapid Tests

Rapid tests provide a single advantage: their rapidity, but depending on the available models, certain *disadvantages* arise such as the need to check their positive results by means of different tests as well as the same limits other tests present, but at a higher cost [120]. A new test, *ImmuneCap Rapid,* allows the diagnosis of the 20 more diffused allergens, including Can f, Fel d, pollens (grasses), Der p, CM, ovalbumin. The test requires a drop of blood and can be done by a pediatrician (P. Restani, pers. comm., 13. 2. 2006).

The advantages and disadvantages of in vitro tests compared to in vivo tests are listed in Table 6.17 [42, 120, 179], those of SPTs are compared to in vitro tests in Table 6.18 [163, 179], and to history and FCTs in Table 6.19 [179]. Representative data on pathological and not pathological causes of Ig abnormalities are shown in Table 6.20 [179] and a panorama of available diagnostic tests in Fig. 6.23 [179].

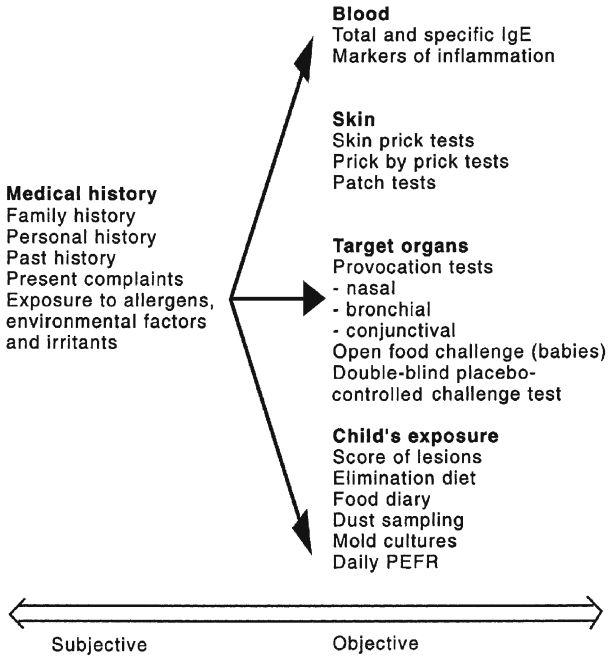


Fig. 6.23. Overview of diagnostic tests. (Modified from [179])

Table 6.17. Advantages and disadvantages of in vitro tests in the pediatric diagnosis of atopic disease

Advantages
Safe
Not affected by skin state or drug assumption
Independent of child cooperation
Less time-consuming
Good reproducibility
Elimination of SPT false positivity and negativity
Single invasiveness
Disadvantages
More expensive
Less sensitive
Done in selected centers
Delayed results
Less sensitive in food allergy
Missed recognition of not proteic antigens
Aspecific antibody entrapment by lectins
Only circulating IgE are measured
Cross-reactivity between inhalant and food allergens

Modified from [42, 120, 179].
SPTs skin prick tests.

Table 6.18. Advantages and disadvantages of SPTs in the pediatric diagnosis of atopic disease

Advantages
Cost–benefit ratio favorable
Excellent for respiratory allergy
Higher reliability
Sensitivity
Long established
Rapid results (<20 min)
Safe and reproducible even in babies <1 year
Technically easy
Disadvantages
Affected by drugs
Dependent on child compliance
Dependent on skin conditions
Difficult quality control
Slight risk of reactions
Time-consuming
Invasive

Modified from [163, 179].

Table 6.19. Comparison between sensitivity and specificity of medical history, in vivo and in vitro tests and provocation tests

Test	Airway	Skin	Anaphylaxis	Gastroenteric	Time necessary for results
History					20–30 min
Sensitivity	++++	++	++++	+	
Specificity	+++	+	++	+++	
Total serum IgE					>24 h
Sensitivity	+	++	+	+	
Specificity	–	–	–	–	
Specific IgE					>24 h
Sensitivity	+++	++	++++	+	
Specificity	+++	++	++++	+	
Skin prick tests					20 min
Sensitivity	++++	++	++++	+	
Specificity	+++	+	++	+++	
Conjunctival provocation test					30 min
Sensitivity	++++				
Specificity	+++				
Nasal provocation test					1 h
Sensitivity	++++				
Specificity	+++				
Bronchial provocation test					1 h
Sensitivity	++++				
Specificity	+++				
Food challenge					2 h
Sensitivity				++++	
Specificity				+++	

Data from [179].

Table 6.20. Allergic and nonallergic diseases associated with immunoglobulin abnormalities in infants and children

High IgA levels
AIDS
Anaphylactoid purpura
Chronic infections
Hepatic cirrhosis
IgA myeloma
Lupus erythematosus systemic
Rheumatoid arthritis
Sarcoidosis
Wiskott-Aldrich syndrome
Low IgA levels
Acute lymphocytic leukemia
Agammaglobulinemia
Ataxia-telangiectasia
Chronic lymphocytic leukemia
Chronic myelogenic leukemia
Dysgammaglobulinemia type III
Heavy chain disease (Franklin's disease)
Hepatic cirrhosis
Hereditary telangiectasia
IgG myeloma
Lupus erythematosus systemic
Lymphoid aplasia
Macroglobulinemia
Malabsorption
Nephrotic syndrome
Otitis media with effusion
Selective IgA deficit
Still disease
High IgD levels
Chronic infections
IgD myeloma
High IgE levels
Addison's disease
Atopic dermatitis

Table 6.20. (Continued)

High IgE levels (Continued)

- Autoimmune diseases
- Bone marrow transplantation
- Bullous pemphigus
- Burns
- Chronic acrodermatitis
- Cigarette smoking
- Cystic fibrosis
- Drug-induced interstitial nephritis
- Erythema nodosum
- Gluten enteropathy
- Guillain-Barré syndrome
- Hepatic cirrhosis
- Infantile polyarteritis nodosa
- Infections
  - AIDS (human HIV infection)
  - Allergic bronchopulmonary aspergillosis
  - Coccidioidomycosis
  - Cytomegalovirus mononucleosis
  - EBV infections
  - Leprosy
  - Pertussis
  - Systemic candidiasis
  - Viral respiratory infections
- Kawasaki's syndrome
- Mononucleosis
- Nephrotic syndrome
- Neoplastic diseases
  - Bronchial carcinoma
  - Hodgkin's disease
  - IgE myeloma
- Parasitosis
  - Ascariasis
  - Capillariasis
  - Echinococcosis
  - Fascioliasis
  - Filariasis
  - Malaria
  - Onchocerciasis
  - Strongyloidiasis
  - Trichinosis
  - Visceral larva migrans
- Primary immunodeficiencies
  - Cellular immunodeficiency with immunoglobulins
  - DiGeorge syndrome
  - Hyper-IgE syndrome
  - Nezelof syndrome
  - Selective IgA deficit
  - Wiskott-Aldrich syndrome
- Pulmonary hemosiderosis
- Rheumatoid arthritis
- Wegener granulomatosis

Low IgE levels

- Familial IgE deficit with recurring sinopulmonary infections
- HIV-1 infections
- Primary biliary cirrhosis

High IgG levels

- Hepatic cirrhosis
- Hepatitis
- IgG myeloma
- Lupus erythematosus systemic
- Rheumatoid arthritis

Low IgG levels

- Agammaglobulinemia
- Chronic lymphocytic leukemia
- Dysgammaglobulinemia type I
- Heavy chain disease
- IgA myeloma
- Macroglobulinemia
- Nephrotic syndrome

High IgM levels

- Congenital infections
- Dysgammaglobulinemia type I
- Hepatic cirrhosis
- Hepatitis
- Hyper-IgM immunodeficiency
- Infectious mononucleosis
- Lupus erythematosus systemic
- Macroglobulinemia
- Rheumatoid arthritis
- Trypanosomiasis

Low IgM levels

- Agammaglobulinemia
- Chronic lymphocytic leukemia
- Dysgammaglobulinemia type II
- Heavy chain disease
- Hepatoma
- IgA myeloma
- IgG myeloma
- Lymphoid aplasia

High levels of all immunoglobulins

- AIDS
- Chronic active hepatitis
- Chronic bacterial infections
- Chronic granulomatous disease
- Cystic fibrosis
- Down syndrome
- Hyperglobulinemic purpura
- Infectious hepatitis
- Intestinal parasitism (mainly IgE)
- Intravenous drug use
- Lupus erythematosus
- Pulmonary hypersensitivity disease
- Regional enteritis
- Rheumatic fever
- Rheumatoid arthritis
- Ulcerative colitis
- Visceral larva migrans

An increase in IgM is found in about 4% of neonates, a third of whom can suffer from an infection, even at a subclinical level. An increase in IgM and IgA during the perinatal period can occur as a result of blood leakage from mother to fetal circulation, with IgM and IgM returning to normal levels within 1 week; if the increase depends on an infection the levels remain elevated in the absence of treatment.
Data from [179].

Table 6.21. Sensitivity, specificity and predictive value of skin prick tests (SPTs) and RAST for cow's milk (CM) and egg in children with atopic dermatitis (immediate and total = immediate + late reactions)

	Sensitivity		Specificity		Predictive value			
					Positive		Negative	
	SPT	RAST	SPT	RAST	SPT	RAST	SPT	RAST
Immediate reactions								
CM	0.88	0.71	0.28	0.56	0.19	0.24	0.92	0.91
Egg	1.00	0.90	0.28	0.59	0.23	0.33	1.00	0.96
Late reactions								
CM	0.83	0.59	0.32	0.60	0.47	0.52	0.72	0.67
Egg	0.91	0.73	0.32	0.65	0.46	0.57	0.85	0.79

Data from [97].

In conclusion, the correspondence between SPTs and in vitro tests is usually 80% [16], with a highly significant correlation ($p<0.0001$) [19, 32]. The analytical reliability criteria show identical values of 0.95–1; however, when excluding false-positive and false-negative results they are equal to 0 and 0.02, respectively, with a 95.6% concordance [159]. Therefore, SPTs can always be done, apart from the above inadequacies, and are preferred in epidemiological studies [59, 163], whereas in vitro tests are recommended when SPTs cannot technically be performed or they elicit undefined results [41]. Employing SPTs with 3,000 BU/ml concentrations, the total predictive value was 91% compared to RAST [108]. However, the Achilles heel of SPT and RAST is in their poor reliability in IgE-mediated reactions: *both fail to account for the late reactions characteristic of FA*. Comparisons between SPTs and in vitro tests are not always concordant: in the young asthmatics reported by Roth et al [144], SPT positivity is 80%, but RAST positivity is 27% and Phadiatop 20%; the latter two show, compared to SPTs, 24% and 31% sensitivity and 60% and 80% specificity, respectively [31]. Table 6.21 applies the analytical reliability criteria to SPTs and RAST for CM and egg in AD children (immediate + late reactions) [97]. However, a direct SPT and RAST comparison shows in general greater SPT sensitivity compared to RAST, independently of the tested allergens [32, 97], including a higher effectiveness for pollens and lower effectiveness for dog epithelia [163]. Therefore, in vivo and in vitro immunological tests make up a combined diagnostic aid [7]. Often we find children, especially infants, with positive clinical history and symptoms, but totally negative to SPTs: we have found a remarkable pair of these patients positive to P + Ps. *SPT safety* has been challenged by a retrospective study showing that the 6 infants out of 1,152 tested over 3 years (0.17% for each year) suffered from generalized allergic reactions after P + Ps with fresh foods [46]. Each infant was tested with several foods (2–4 foods), and the same food was tested in duplicate in the same baby, which doubles the risk of provoking the reaction which can also be generalized. We have done 2,200 SPTs without seeing any generalized allergic reaction [32].

Future Diagnostic Avenues

Recombinant Allergens

Purified RAs are currently available from different foods of plant origin such as apple, celery, cherry, and hazelnut. They might be produced in suitable purity and batch consistency and hence might offer a perfectly standardized diagnostic material. These proteins are much more stable than antigens in food extracts because constituents of the plant matrix responsible for degradation are absent [11]. This study evaluated Swiss patients allergic to cherry and exposed to birch pollen, patients with birch allergy who tolerated cherry, Spanish patients with cherry allergy without birch pollen exposure, and nonatopic controls with SPTs and serum immunoblot analysis. Nonatopic controls had no binding/positive SPT. Patients with cherry allergy from Switzerland and those with birch pollen allergy alone bound primarily to recombinant Pru av 1 and Pru av 4 (related to birch pollen Bet v 1 and Bet v 2), whereas the Spanish patients with cherry allergy bound more extensively to Pru av 3, an LTP (lipid transfer protein). Sensitization to Pru av 3 was positively associated with symptom severity, thus showing that RAs may improve the diagnostic value of allergy tests [11].

CD203c

CD203c (Table 1.2) can be used as a novel specific marker of IgE-dependent activation of basophils. Using RAs and basophils of sensitized individuals, up-regulation of CD203c might provide a reliable basis for a sensitive and specific allergy test. CD203c and a panel of RAs were

applied to establish a novel basophil test that allows for a reliable quantification of IgE-dependent responses at the effector cell level. Incubation of basophils with RAs resulted in up-regulation of CD203c in sensitized individuals (those with a positive RAST result and a positive case history), whereas no effects were seen in healthy control subjects. Therefore, flow cytometric quantitation of CD203c on blood basophils exposed to RAs is a useful approach to determine the allergic state in sensitized individuals and represents a basis for a sensitive innovative allergy test [64].

Provocation Tests

CPT, NPT and BPT are helpful in dubious cases, when there is a clear discrepancy between history and allergometric tests, especially in vivo, for example, in patients with a significant history, when a skin positivity vs a suspected allergen cannot be demonstrated. Obviously, such investigations are not routine, since a specific skill is required, and are feasible only in a specialized university or hospital setting equipped for possible emergencies, thus ensuring the safety of patients subjected to such tests. Both CPT and NPT are relatively easy to achieve, while BPT cannot be carried out when patients do not cooperate; therefore it is necessary that children are 5–7 years old.

Conjunctival Testing

Experimented by Blackley [18], CPT is free of risks, simple to execute and score, reproducible, not influenced by T of test solutions, usually more accurate than SPTs and better standardized than NPTs. There is no absolute correspondence between skin and/or serum and eye reactivity (at the most 70%) because IgE antibodies are chiefly produced in the tears [89]; nonetheless, CPT is utilizable in asthmatic children instead of BPTs [35].

Indications

CPT is employed to meet the following goals: to confirm the sensitization to a given allergen, establish the degree of sensitivity of a shock-organ, evaluate the effects of a drug management, and verify SIT results.

Technical Procedure

Older children are usually seated, younger children commonly lie down. Then they are requested to look upward, not to rub their eyes if there is itching, to avoid the by-product of aspecific irritations. The procedure is as follows: a drop of diluent or normal saline at 0.9% is instilled in the inferior conjunctival sac to exclude an aspecific reactivity. If no reaction ensues within 10 min a drop of a solution of the allergenic extract under study is instilled every 10–15 min. The untreated conjunctiva is used as control. The progressively increasing concentrations start with dilutions in an aqueous solution giving an allergenic extract concentration of 1×10^4/1×10^3 (or from 20 to 2,000 AUR/ml), normally different from that used for SPTs, to a maximum of 1/10, until a positive reaction occurs [3, 35, 139], graduated by an appropriate scoring [139]. Allergic reactions start generally after a few minutes, including itching, tearing, conjunctival injection, and lid-swelling [139], showing the test positivity, confirmed by finding higher levels of mediators in the tears compared to the test start [135]. Tears can be collected and assessed for inflammatory mediator and marker cells [135]. At the CPT end the eye is cleaned with normal saline. The CPT diagnostic efficacy, with the NPT as the reference method, was 89% in adults with SPTs and RAST positive to Der p [138].

Nasal Testing

NPT is more complex, and is usually performed by means of rhinomanometry.

Indications

NPTs are frequently employed when a clear discordance between symptoms, history data and SPT results is noted, or when the doctor and/or the parents have sound motives to mistrust the reliability of potential negative results of previous investigations, and to monitor SPT effectiveness. It is indispensable to previously ascertain that children are in good clinical conditions, free of nasal symptoms, and have stopped all medications, as for SPTs [14].

Technical Procedure

The nasal mucosa is examined by means of an inert substance (for example the diluent) to exclude possible aspecific symptoms. After approximately 10 min, the test is scored: when the resistance measured with a rhinomanometer increases >200% (mean value of at least five measurements), the specific test continues based on SPT positivity evaluated with an end point. Then, 0.2–0.5 ml of allergen extract dilution in a 1:1,000 aqueous solution that is not glycerinated (avoiding Freon propellents) [14] is instilled in the nasal mucosa by means of a pressurized dose inhaler capable of provoking an aspecific stimulation. Alternatively, commercial allergenic extracts can also be prepared in a phosphate buffer. Paying attention that no systemic reactions de-

velop, the allergen concentration is progressively increased at 15-min intervals, until the maximal value (not >1/10) able to provoke a clinical response – itching, rhinorrhea, sneezing, nasal obstruction, serous drainage – or that measurable by rhinomanometry (anterior and posterior), characterized by a 400% resistance increase or a >50% flux reduction. After 6–8 h or more, it is checked a second time to assess whether delayed reactions are present [50].

Occasionally, a metered dose inhaler with 0.4–0.05 ml of a solution containing 10,000 AU of an allergenic extract is insufflated, until a total dose of 800–1,000 AU in both nostrils is reached [10]. The child's responses are measured by a subjective symptom scoring system, referred to the highest or lowest clinical severity, by rhinomanometry, or by dosing selected mediators in subsequent nasal lavages. NPT with a single dose and successive clinical survey have been useful in clinical practice, whereas other techniques can be employed for research purposes [14, 50]. It may be desirable to test both nostrils (concordance = 95%) [159], also because the two may respond diversely [10]. In children with AR, NPT is preferable to CPT, although limited by poor patient acceptance. Moreover, clinical scoring can virtually supplant rhinomanometry [159].

Bronchial Provocation Testing

BPT is based on the assumption that different immunological stimuli (allergens) and nonimmunological stimuli such as acetylcholine, methacholine, histamine, carbachol, cold air, fog, ozone, hyperventilation, and exercise can trigger attacks of wheezing in asthmatic children, but not in healthy children [125]. It consists in PFT performance before and after allergen inhalation (specific provocation) or of substances such as methacholine or histamine (aspecific provocation); BPTs can be completed by an inhalational challenge with bronchodilator drugs.

Indications

Inhalational challenge with aerosolized allergen extracts are a valid instrument in testing for asthma, but for routine diagnosis the indications are limited, since this procedure requires active cooperation. Consequently, it is not normally applicable in children aged <6 years [67]. In addition, it is not always simple to estimate the dose-response relationship. BPTs with chemical mediators could acquire data to monitor BHR (bronchial hyperreactivity). The weight of such appraisal depends on BHR variation ranges: while present in the general population with a Gaussian distribution, asthmatics are at the end of most reactive subjects. Although BHR, as we shall see in Chap. 11, is also seen in other affections, and conversely an asthmatic can transiently be in a state of no reactivity, test interpretation is essentially clinical.

Patient Prerequisites

Patients with basal ventilatory conditions marked by forced expiratory volume in one second (FEV_1) >70% of PV are admitted for BPT. Possible medications are discontinued before the test, for a variable time according to the drug or drugs taken. The factors that can modify BPT results, increasing or reducing individual reactivity, are shown in Table 6.22 [178].

Table 6.22. Factors able to modify the results of bronchial provocation tests, increasing or reducing individual reactivity

Before the test	
Factors increasing the bronchial hyperreactivity	
Exposure to allergens with delayed response	1–3 weeks
Cigarette smoke	2–4 h
Infections of lower and higher airway	6 weeks
Environmental pollutants (O_3, NO_2, SO_2)	days or weeks
Influenza and rubella vaccination	3–6 weeks
Factors reducing bronchial hyperreactivity	
Anticholinergics	18 h
Antihistaminics	48–72 h
β_2-Agonists	4–12 h
Corticosteroids	?
DSCG	8–48 h
Theophylline	12–24 h
Sustained release theophylline	48–72 h
The day of the test	
Airway caliber (reduction increases hyperreactivity)	
Time of day (increased hyperreactivity in the evening and at night)	
The test	
Type of substance with bronchoconstrictor action (allergens, irritants, drugs, etc.)	
Method of administration or nebulization	
Breathing way	
Dosage type	

Modified from [178].

Technical Procedure

As in the case of the aerosol technique, it is difficult to quantify the dose–response relationship, since all subjects above a given age inhale the same quantity of nebulized solution, independently of their complexion, with the exception of young children (1–2 years old). Therefore each clinic or laboratory should fix useful and reproducible parameters to evaluate pediatric results [180]. This explains why specialized techniques for infants and young children are almost beyond reach, reliable for preschool children only in research laboratories [129], whereas textbooks describe PFTs for this age range with minimal variations compared to methods for adults [146]. Thereby, with current techniques, babies aged >1 year always receive the same dose as older children, despite a wide variability in several parameters during their growth, with the net result that 10-year-old children inspire double the adult dose, even if doses are corrected for weight, thoracic gas volume, and the surface area of the air–tissue interface [85]. Consequently, the younger the child is, the higher the level of responsiveness that occurs at a lower allergenic concentration, as with the mediator challenge test [86]. Hence, young children should receive by inhalation a part of the adult dose: what is a right dose? [146]. Further, young babies tend to breathe through their noses, and the resulting lung deposition may be only about 33%–50% of that after oral inhalation, also taking into account their different RRs [85]. The inability to assess PFTs in young children is a serious drawback since many respiratory affections may occur with children of this age group [180]. A recent technique using a computerized lung sound analysis has enabled application of the histamine challenge test to young children unable to perform spirometry [13]. We divide child BPTs according to their age; here we overview children aged at least 6–7 years [43, 86, 101].

Technical Procedure for Allergen Challenge Tests

Substances to be tested are best selected when based on the child's history. After a prior analysis of ventilatory mechanics, inhalations involve either continuous aerosol generation or an intermittent aerosol generation using the dosimeter method, in case of soluble substances or extracts available at a known concentration, starting with very low doses that are progressively increased, following curves of the cumulative type [179]. With insoluble substances or with an irritant activity, exposure in inhalator rooms can be employed. In both cases, a preliminary blank inhalation must be done with buffer or an inert control substance. Only one substance per day can be tested and if the patient has a delayed positive response, a second BPT can be done no sooner than 1 week later, to avoid possible false-positive results due to an aspecific reactivity increase secondary to the first inhalation. Allergen extracts are administered in moderate doses at first, and then increased by twofold increments until the desired resistance increase and compliance decrease is achieved. The usually assessed parameter used to estimate BPTs is the FEV_1, measured twice before and after the blank aerosol, after a series of inhalations every 4 h in the subsequent 8 h [178]. The highest of two reproducible (within 5%) measures of FEV_1 is recorded as the baseline FEV_1: all baseline FEV_1% (FEV_1/FVC) values <80% should be regarded as indicative of baseline airway obstruction [178]. These repeated observations are explained by bronchospastic responses that can be immediate (within 20–30 min), delayed (from 6–8 to 24–96 h and more) or biphasic (immediate, followed by a time interval when PFT values return to normal). BPTs are stopped (and thus positive) after a provocative dose causing a 20% or higher drop in FEV_1 (PD_{20} = provocation dose 20) compared to the blank value. Patients should remain under observation and controlled from the clinical point of view in the interval between the two procedures and over the following 24–48 h, the onset of an acute or late asthmatic attack being foreseeable.

Technical Procedure of Mediator Challenge Tests

BPTs are performed using methacholine or carbachol (aspecific test) with the previously outlined procedure: asthmatic patients react to small doses of methacholine, while allergic nonasthmatic patients react only to high doses. Mediators are delivered in incremental concentrations provoking a 20% FEV_1 reduction. BPT is considered positive when the employed doses are <1,600 µg for methacholine and <400 µg for carbachol [129]. In practice, BPT is positive or negative according to mediator concentration reached; in children positive responses are 94%–95% [43, 129]. Some authors believe that an aspecific test with methacholine is well standardized to elicit BHR even in children aged 7 [43], taking into account both asthma severity and male sex [101]; others have noted differences in responsiveness in asthmatic children, probably related to a larger dose of methacholine for their size, or additional factors, including the airway mucosa permeability and delivery system reliability (dosimeter, nebulizer) [86].

In parallel, histamine is often used; however, with this mediator tussigenic attacks are precipitated even at low doses, while headache and heat flashes gradually become more frequent when the dosage is increased. Cockcroft et al [36] suggested a modification based on a histamine BPT inducing a 20% fall in FEV_1 = PC_{20}. When PC_{20} is >8 (or 16) mg/ml, a diagnosis of asthma is almost always overlooked (92.6% of cases), values <1 mg/ml are instead diagnostic (100% of cases) and those between 1 and 8 mg/ml are intermediate. Data

show 100% sensitivity, 92% specificity, a 34% PPV, and a 100% NPV [36].

BPTs with ultrasonic fog, distilled water, cold air, etc. are rarely employed in pediatrics: a positive response for the first test was 32% [43] and for the second 36% [54].

When BPT is over, children should remain hospitalized until late-phase or biphasic reactions can be ruled out. In case of attacks of violent bronchospasm or spasmodic cough, children are best treated with β_2-agonist aerosols.

The ISAAC has recommended the hypertonic saline challenge test as the method to assess BHR. Two FEV_1 recordings are done as above. Hypertonic saline (4.5%) is nebulized and connected to a 60-cm tube and a two-way valve. The child is encouraged to maintain tidal breathing. The exposure time is progressively increased from 30 s to 1, 2, 4 and 8 min each. After each exposure, two or three reproducible (within 5%) FEV_1 measurements are taken. The exposure time is doubled if the fall in FEV_1 is >10%. The same dose is repeated if the fall is between 10% and 15%. The challenge test should be stopped and considered to be positive when the decline in FEV_1 is >15%. The maximum inhalation period should be 15.5 min and the canister with tubing weighed before and after the challenge test to measure the total dose (g) of inhaled saline. The dose–response ratio represents the degree of BHR, defined as the percentage fall in FEV_1 divided by total dose of inhaled saline (%/ml, 1 g/ml used as density for saline) [94, 115].

Overall Evaluation of Provocation Tests

These in vivo methods are applied as a second diagnostic level, useful for patients when an accurate and detailed history, in vivo and in vitro tests, or other methods have failed to sufficiently clarify or confirm the diagnosis, or there have been multiple positive tests. Such data are relevant when pediatricians register discrepancies between incomplete asthma symptoms and a poorly assessed bronchoreactivity.

In addition, we find several disadvantages to provocation tests such as limited utilization in younger children, latent onset of adverse reactions, the perspective of testing only one allergen at a time, relatively complex and roundabout methods, difficult standardization of the administered allergen amount, quantification and interpretation of allergen response, and the requirement that challenges be undertaken only in specialized centers.

Conjunctival Provocation Test

Positive Aspects

CPT is a fairly easy procedure:

- For diagnostic purposes it is a valuable tool in asthmatic children without eye signs and symptoms and gives clear results, similar to BPT results [35].
- It is useful when both SPTs and RAST are negative despite an intriguing history, or when SPTs cannot be done [89].
- The analytical reliability criteria show values equal to 1, excluding false-positive and false-negative results with 0 values [159].
- Contrary to a demonstrated lack of correlation with systemic allergometric tests [89], there is agreement with SPTs and sIgE: 97.8% [159].

Limitations

- Not always easy to carry out in very young children
- Not appropriate to identify a possible SPT negativity
- Not utilizable for studying the pattern of clinically relevant allergens in multiple sensitizations [89]

Nasal Provocation Test

Positive Aspects

- Free of bronchopulmonary complications [10].
- The analytical reliability criteria show values between 0.94 and 1, excluding false-positive and false-negative results with values of 0.04 and 0, respectively [159].
- Concordance with SPTs and sIgE is 97.8 and 98.8%, respectively, and with CPT is 100% [159].
- Helpful when SPTs are unrealizable or together with RAST are inexplicably negative.
- Adequate control of SIT effectiveness, especially in children.
- Shared use in intranasal SIT [14].

Limitations

- In children, above all in young children, rhinomanometry is difficult to use because of extreme intra- and interindividual variability [14].
- It is time-consuming, since only one allergen can be tested each time.
- Cannot be done when patients suffer from nose signs and symptoms.
- Systemic reactions are not uncommon.
- Can be performed provided that at least 6 weeks have elapsed from the end of pollen season, due to the priming effect (Chap. 12).

- Selected children should be free of other underlying diseases that may confound the interpretation of symptoms.
- It is not yet a sufficiently standardized test [50].

Bronchial Provocation Test

Positive Aspects

- BPT [180] is a tool of great utility for studying the pathophysiology and pathogenesis of asthma and comparing the utility of antiasthmatic drugs.
- It is useful in evaluating children with atypical symptoms such as chronic cough, cough variant asthma, dyspnea, and EIA to confirm or exclude an asthma diagnosis, and all cases in which it is impossible to formulate a reasonable diagnosis, to elucidate BHR impact, either spontaneously or following drug therapy, as well as the effectiveness or ineffectiveness of drug protective effects, as in the case of EIA and SIT results, when for various reasons in vivo and in vitro tests are not available.
- It can be used to investigate selected cases, mainly to persuade patients and their families about the utility of environmental controls, also because *BPT is safe* and no significant morbidity occurs if correctly done in a hospital setting.
- It can be used to correlate pre-BPT FEV_1 with final values, a more precise pattern of risk factors associated with BHR, whether or not symptomatic (the BHR) is [179].

Limitations

- It is not routinely applicable because it is either long and annoying or more valuable information can be obtained from less invasive and less time-consuming tests [154].
- Several studies have assessed that BPT varies greatly in sensitivity (19%–70%) and PPV (11%–76%) in identifying children with wheezing [125].
- BPT is not reliable without an opportune standardization of testing procedures in young children and/or with other airway disorders such as infectious bronchitis, cystic fibrosis, or other affections altering PFTs.
- BPT is best done in specialized clinical settings, with accurate indications and calls for a qualified personnel with experience and well versed in doing BPTs, who are present during the entire duration of testing. Equipment and medications must always be on hand to reverse the possible onset of an asthmatic attack; an emphasis on prompt initiation of emergency measures and with activation of complete first-aid and resuscitation procedure is advisable [93]. A severe reaction was reported in a 22-year-old *asymptomatic patient with a FEV_1/CV of 88%* after 1 min and death after 15 min, despite rapid, aggressive treatment [147].
- A positive test may be helpful to confirm an asthma diagnosis, however, without an absolute value, because different conditions may be associated with BHR such as URTIs [129].
- The degree of reactivity both in normal and asthmatic children forms a *continuum;* therefore, borderline results should be interpreted with a great moderation.
- Considering varying types of challenges, no correlation was found, undoubtedly because of the lack of standardization and the diversity of the acting mechanisms [65], also requiring follow-ups and several visits to the clinic [93].

Additional Aspects to Be Considered

- The dose for a 10-year-old child should be reduced, based on his weight, by 50% compared to an adult dose. As a consequence, the reductions for children aged 0.5 to 1 year should be 75%–80% and 90%, respectively [86].
- Doses that are too high can yield a false-positive result.
- When pollens are to be inhaled, the larger granules (20–30 µm) remain trapped in the nose, while the pollen extract (droplets of 1–2 µm) provokes bronchoconstriction in children with rhinitis [41].
- As much as 42% of AAAAI committee members claim that BPT prescription is unnecessary and several members have suggested simplifying BPT and above all making it less expensive [154].
- The grading of asthma severity is seldom based on PFT values and may be used to fill out the history [93]; others [125] maintain that one BPT is not sufficient to confirm the clinically diagnosed asthma.

Additional Tests

Exercise challenge is suggested for children suffering from EIA and as an asthma screening method [182]: the child is asked to run freely at the speed of 6 km/h for 6–8 min on an equipped track with a maximum slope of 5%, preferably in a dry environment, and a relative humidity <40% and T approximately 22°C. The test is positive when the fall in PEF is >12% and/or in FEV_1 is >15% compared to baseline values. However, if such equipment is not available, the test can be done using a bicycle ergometer or a treadmill test (Fig. 6.24): the child exercises for 5 min, controlling that HR is not >80% for the age; the fall in FEV_1 (≥15%) occurs 5–15 min after the end of treadmill test. Therefore we suggest sequentially measuring RRs 1, 5, 10, 15 and 20 min after the end of the test. When it is necessary to monitor a possible late reaction, RR records are done hourly over the subsequent 12 h. The free running test is objectively the simplest and more asthmogenic [182], while the treadmill test is the most standardized, unlike

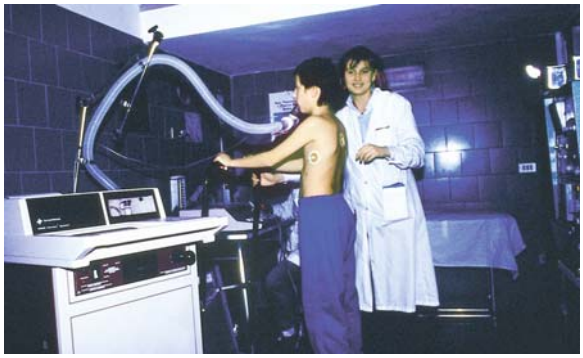


Fig. 6.24. Treadmill test

other exercises, including the bicycle ergometer, and jumps or running up stairs. Environmental and atmosphere trends, as well as dry or cold air, are able to adversely influence the results [129].

Pulmonary Function Testing

PFT is a very valid adjunct to assess the lungs in children with ascertained or suspected pulmonary disorders.

Indications

- To confirm an asthma diagnosis by documenting the reversible airflow limitation
- To quantitate limitation extent by assessing its reversibility after bronchodilator administration
- To monitor responses to therapy with bronchodilators and to follow the course of a child with asthma over time

Strictly speaking, PFTs are not diagnostic tests but they make it possible to define the characteristics of obstructive or restrictive airflow disorders and the type of pulmonary pathophysiological impairment likely present, also ensuring the necessary measurements before BPT is undertaken. The more practically used tests are PEF and spirometry.

Peak Expiratory Flow Rate

Peak expiratory flow rate (PEFR) is the simplest test of lung function, a valuable test to correlate diagnosis with appropriate therapeutic recommendations [68].

Definition

PEFR is the maximum flow rate generated during a forced expiratory maneuver. It is therefore a test measuring the maximum mid-expiratory flow (MMEF), commonly obtained during the first tenths of a second following a forced expiration from total lung capacity (TLC).

Available Devices

PEFR can be assessed by a number of specifically made hand-held devices such as peak flow meters (PFM) (Fig. 5.18), with the following requisites:

- Low cost. Models include the Mini Wright PFM, with two models for normal and low ranges (Airmed), the PFM DHS, the Vitalograph Pulmonary Monitor, the AssessPeak Florometer, and others
- Portable
- Well suited to monitoring children aged 4–5 years and older.

Among these devices, we suggest the mechanical models, which do not require daily calibration, since the first calibration done by the manufacturer is valid, which makes them certainly suited for home PEF monitoring. However, as the accuracy and linearity of different portable PFMs may yield different values when used for the same child [100], for a useful home PEF monitoring, we suggest using the same model in the home and the clinician's office. Alternatively, parents can bring their PFM to the office to compare the results of the two PFMs [168].

Indications

PEFR can be useful as a quantitative and reproducible assessment of lung function in a pediatrician's office; however, its main employment is at home [168]. Given the great interindividual variability of this parameter [57], a single monitoring is scarcely useful to make a diagnosis: it is recommended that home monitoring be done morning and evening (about 7:00 AM and 7:00 PM) to recognize and evaluate each circadian variation in lung function [68], thus allowing the clinician to provide a treatment adequate to the individual child's requirements [168]. When PEFR is measured only daily, it should always be done at the same time of day. If daily measurements cannot be taken twice a day, two or three times a week, then recording readings first thing in the morning and last thing at night of the same day is suggested [68]. In each case, PEFR also evaluates the reversibility of a possible airflow limitation in response to a single dose of β_2-bronchodilator [68]. Obtaining three PEF records is commonly recommended, entering the best one on a diary card, and when children are adequately treated, to establish which are the best personal values with the least circadian variability. The best personal value is the highest PEF value achieved in children under an effective anti-asthma treatment [68]. It must be kept in mind that PEF measures only large airway function, not the small airways, so children with

mild asthma may be underdiagnosed. In addition, 2 weeks may be required to obtain a panel of reliable values. A single daily monitoring does not allow extrapolating data utilizable to detect early stages of airway obstruction [57]. A useful marker of asthma disease is mean diurnal variations (MDV) of PEF, whose values range between 9.6% and 22.6% in children with asthma, with mean/median normal pediatric values ranging from 5.6% to 9.9%. MDV values are normally distributed after logarithm transformation to avoid data dispersion and to obtain a reliable analysis of PEF variability [57]. Pediatricians should therefore be experts in the analysis of both PEF and MDV results before suggesting an instrument to parents. Until this parameter is standardized, a *peak flow diary* can offer the pediatrician a longitudinal record by which disease activity and management efficacy are evaluated [128]; therefore it is advisable to continue to monitor in terms of PEF/PEFR.

Measurement of Flow Rates

All PFMs are portable. Since the maneuver required to perform PEFR is effort-dependent, children, especially younger ones, may need to be coached to correctly perform the test, requiring a notable cooperation not attained in children aged <4–5 years. Time should be allowed for small children to become comfortable with the machinery and to practice blowing with an unattached disposable mouthpiece or other similar tools (Fig. 6.25 a and 6.25 b, c) [180].

Measurement Technique

- Place the indicator at the base of the numbered scale.
- Stand up.
- Take a deep breath.
- Place the meter in the mouth and close lips around the mouthpiece.
- Blow out as hard and fast as possible (a prolonged expiration is desirable when performing spirometry, not in PEFR).
- Write down the value reached.
- Repeat the procedure two or more times as judged by the pediatrician.
- Record the highest value reached (do not calculate the mean of the recorded values).

Normal Values for Male and Female Patients Aged 4–16 Years

Appendix 6.6 [181] shows the equations for the predictive determination of mean PEF values in children aged 4–16 years; Figs. 6.26–6.31 provide the values in males and females related to age (4–16 years) [181], and Table 6.23 [180] the average (50%) related to height (between 1 m and 1.76 m), including forced vital capacity (FVC) and FEF_{25-75} [180].

Interpretation of PEFR Results

A 20% variation from baseline after adequate bronchodilation in subjects in stable asthma indicates an overall worsening asthma, thereby suggesting careful monitoring.

The following is a classification of asthma severity, with the pertinent FEV_1 values in parentheses:

- Mild: PEF >80% predicted at baseline, variability <20% (<100%–≥75%)
- Moderate: PEF 60%–80% predicted at baseline, variability 20%–30% (<75%–≥60%)
- Severe: PEF <60% predicted at baseline, variability >30% (<50%–≥34%)

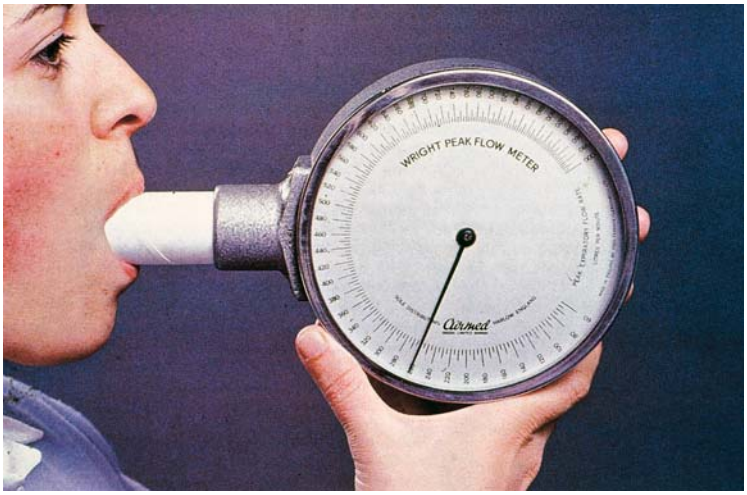
The term "variability" refers to the difference between morning and evening values, or to the different values recorded in the morning within the span of 1 week. It is important, as discussed earlier, to establish the circadian variability that correlates with the degree of disease instability [68].

Management Implications

The zone system has been adapted to a traffic light system based on the percent of the best personal value.

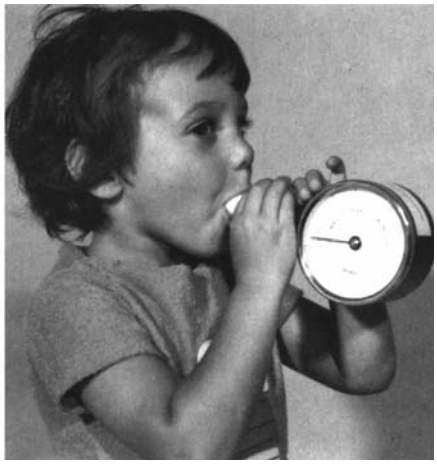
- PEF 80%–100%, *green,* no asthma symptoms, routine treatment
- PEF 50%–80%, *yellow,* possible acute exacerbation and/or inadequate control of overall asthma
- PEF <50%, *red,* medical alert: immediate therapy and/or admission to emergency department

We stress that only children aged ≥5 years can usually undergo a PEF measurement with reliable results [68]. In our experience, organizing the test as a game or a party favor, one can teach PEF maneuvers to children as young as 3 years of age. An example of such coaching might be to ask the child to blow all the candles on a party cake in one breath, or to try a competition on who blows a trumpet louder and longer, etc. (Fig. 6.25 a–c). In case these games do not seem practicable, the doctor could recur to an airway computerized phonopneumography based on the analysis of lung sounds, easily recorded even in young children [166]. As previously mentioned, an early and correct recognition of atopic disease could avoid a series of problems such as those outlined in Table 6.24 [179], thus resulting in a more satisfying approach to asthma management [168].



a

b



c

Fig. 6.25. **a** Normal PEF performed with a PFM to measure PFT. **b**, **c** Use of a birthday party favor to teach PFT maneuvers to young children

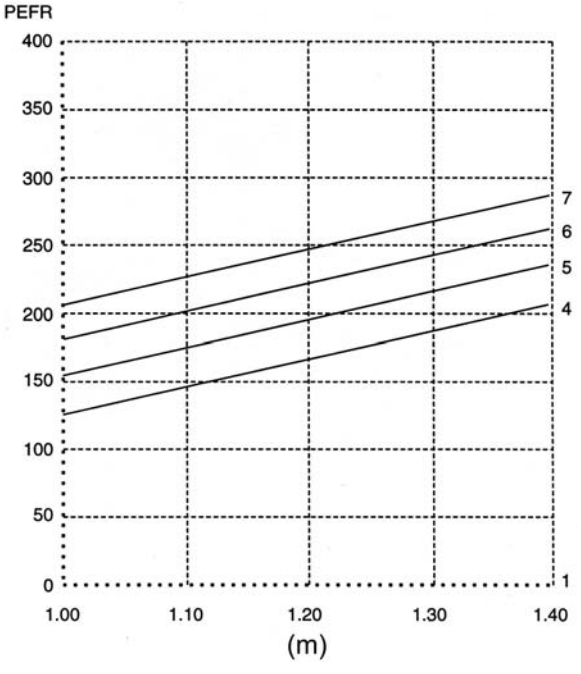


Fig. 6.26. PEFR values (l/min) in boys aged 4–6 years (height in m, age in years). Mini Wright PFM low-range model

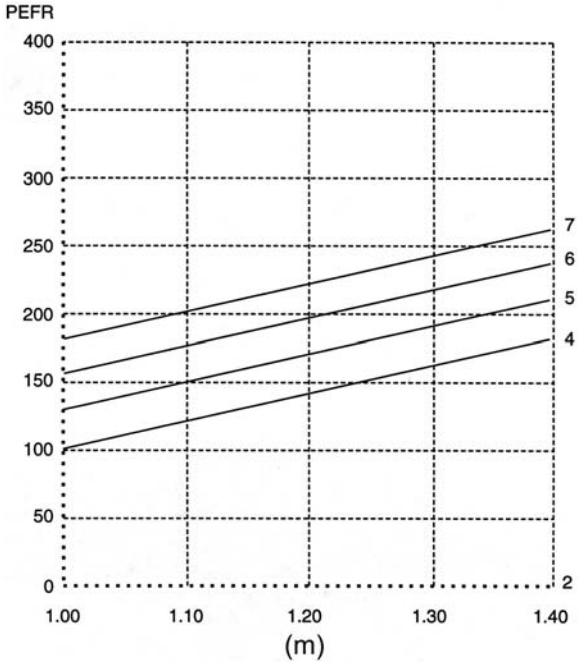


Fig. 6.27. PEFR values (l/min) in girls aged 4–6 years (height in m, age in years). Mini Wright PFM low-range model.

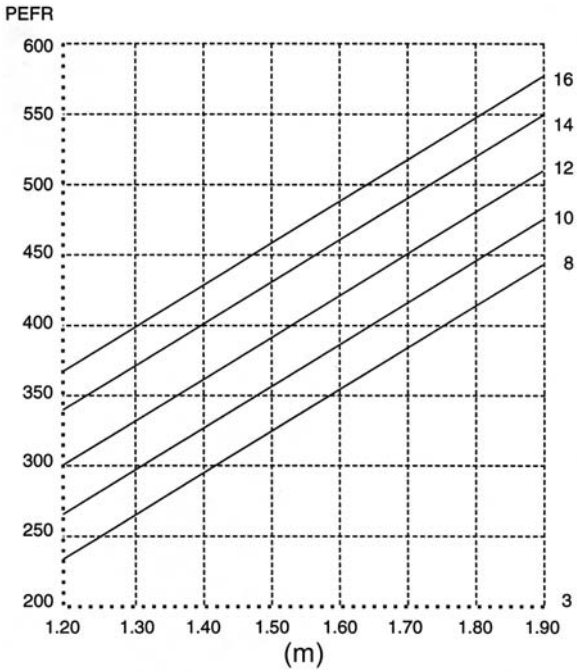


Fig. 6.28. PEFR values (l/min) in boys aged 7–16 years (height in m, age in years). Mini Wright PFM normal-range model

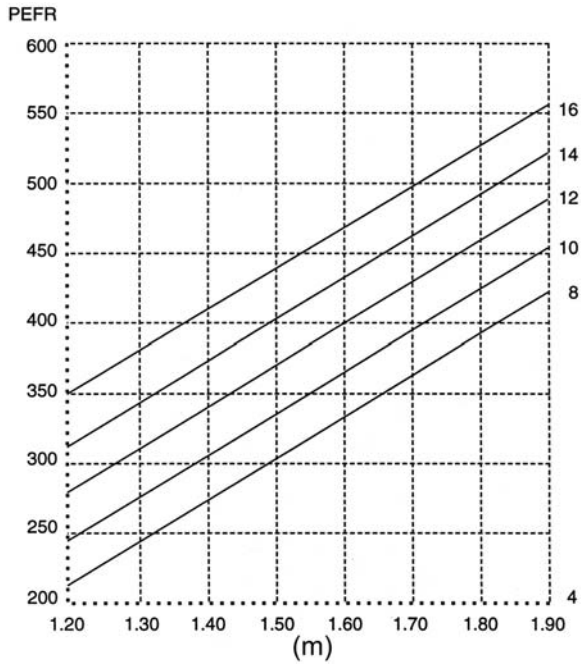


Fig. 6.29. PEFR values (l/min) in girls aged 7–16 years (height in m, age in years). Mini Wright PFM normal-range model

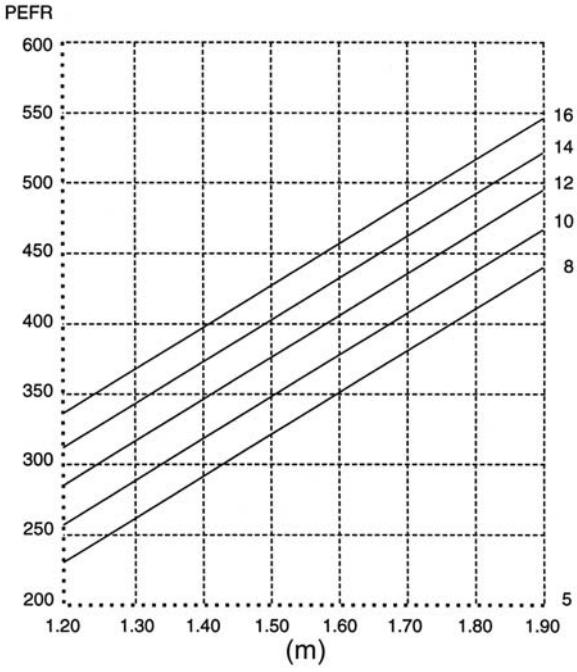


Fig. 6.30. PEFR values (l/min) in boys aged 7–16 years (height in m, age in years). Wright PFM

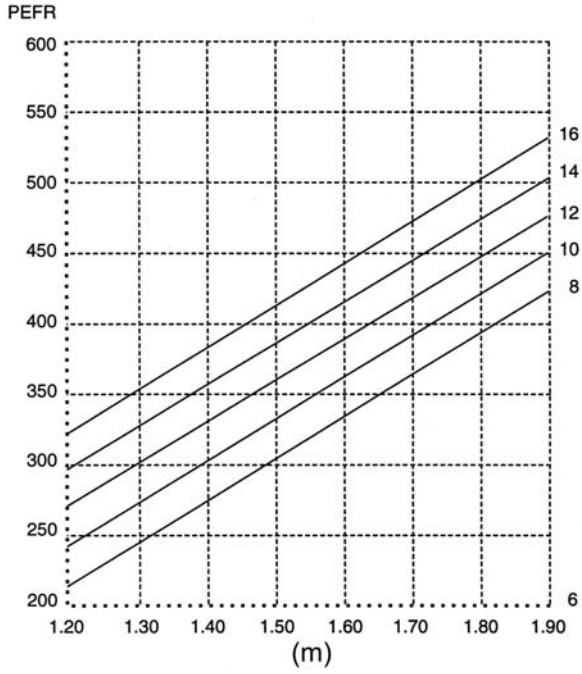


Fig. 6.31. PEFR values (l/min) in girls aged 7–16 years (height in m, age in years). Wright PFM

Table 6.23. Average (50%) values of PEFR, FVC, FEV_1 and $FEF_{25–75}$ in children

Height cm	PEFR		FVC (L)		FEV_1(L)	$FEF_{25–75}$	
	l/min	l/s	Boys	Girls		l/min	l/s
100	100	1.67	1.00	1.00	0.70	55	0.91
102	110	1.83	1.03	1.00	0.75	60	1.00
104	120	2.00	1.08	1.07	0.82	64	1.06
106	130	2.17	1.14	1.10	0.89	70	1.17
108	140	2.33	1.19	1.19	0.97	75	1.25
110	150	2.50	1.27	1.24	1.01	80	1.33
112	160	2.67	1.32	1.30	1.10	86	1.43
114	174	2.90	1.40	1.36	1.17	90	1.50
116	185	3.08	1.47	1.41	1.23	96	1.60
118	195	3.25	1.52	1.49	1.30	100	1.67
120	204	3.40	1.60	1.55	1.39	105	1.75
122	215	3.58	1.69	1.62	1.45	110	1.83
124	226	3.77	1.75	1.70	1.53	118	1.97
126	236	3.93	1.82	1.77	1.59	121	2.01
128	247	4.11	1.90	1.84	1.67	127	2.12
130	256	4.27	1.99	1.90	1.72	132	2.20
132	267	4.45	2.07	2.00	1.80	139	2.32
134	278	4.63	2.15	2.06	1.89	142	2.37
136	289	4.82	2.24	2.15	1.98	149	2.48
138	299	4.98	2.35	2.24	2.06	153	2.55
140	310	5.17	2.40	2.32	2.11	159	2.65
142	320	5.33	2.50	2.40	2:20	163	2.72
144	330	5.50	2.60	2.50	2.30	170	2.83
146	340	5.67	2.70	2.59	2.39	173	2.88
148	351	5.85	2.79	2.68	2.48	180	3.00
150	362	6.03	2.88	2.78	2.57	183	3.05
152	373	6.22	2.97	2.88	2.66	190	3.17
154	384	6.40	3.09	2.98	2.75	195	3.25
156	394	6.57	3.20	3.09	2.88	200	3.33
158	404	6.73	3.30	3.18	2.98	205	3.42
160	415	6.92	3.40	3.27	3.06	210	3.50
162	425	7.08	3.52	3.40	3.18	215	3.58
164	436	7.28	3.64	3.50	3.29	220	3.67
166	446	7.43	3.78	3.60.	3.40	225	3.75
168	457	7.62	3.90	3.72	3.50	230	3.83
170	467	1.78	4.00	3.83	3.65	236	3.93
172	477	7.95	4.20	3.83	3.80	241	4.01
174	488	8.13	4.20	3.83	3.80	246	4.10
176	498	8.30	4.20	3.83	3.80	251	4.18

Data from [180].

Table 6.24. Drawbacks avoidable with a correct and early diagnosis of atopic disease

Inappropriate and/or unnecessary usage of drugs: antibiotics, decongestants, expectorants, antitussives and special foods
Unnecessary and/or protracted dietetic restrictions, potentially resulting in malnutrition
Hematochemical and/or radiological examinations
Adenotonsillectomy
Emotional stress for both the child and family
Absences from school (and from work for the accompanying parent)
Disproportionate cost–benefit ratio
Financial burden

Modified from [179].

Spirometry

Spirometry is the measurement of air movements into and out of the lungs during various breathing maneuvers [39]. In cooperative chest patients, spirometry is a routine clinical examination measuring the volume of air exhaled from the lungs during a maximal respiratory maneuver. The vital capacity (VC) and the functional residual capacity (FRC) are recorded by spirometry measuring the expiratory flow. Even if more testing procedures are combined to assay the potential presence and entity of bronchial obstruction, in monitoring the evolution and therapy effectiveness, spirometry is not inferior to any other test in clinical practice, provided that it is not used as the only test [93].

Definitions

There are five basic subdivisions of lung volumes (Fig. 6.32):

- Tidal volume (TV) is the amount of air inhaled and exhaled during a normal respiratory cycle.
- IRV, inspiratory reserve volume, is the maximal volume of air that can be inhaled over and above the TV: the sum of both volumes is the inspiratory capacity (IC).
- VC is the amount of air that after a maximal inspiration can be expelled with a maximal expiration. It corresponds to the sum of IRV, TV and expiratory reserve volume (ERV).
- RV, the residual volume, is the amount of gas that remains after a maximal exhalation.
- FRC is the air remaining in the lung at the end of a spontaneous expiration, *the only measurement assessed in infants* [6], thus FRC = ERV + RV; while RV = FRC –ERV.

The expiration begins from TLC, the total amount of gas in the lung after a maximal or forced inhalation, that is the sum of TV + IRV + RV + ERV ending with RV [180]. FVC represents the maximal amount of air exhaled irrespective of time (Table 6.23). If FVC is the difference between TLC and RV, the sum of ERV, TV and IRV is the total amount of air that remains after a maximal inhalation at the end of the normal expiration.

The increase in some proportions, RV/TLC = 22% and FRC/TLC = 45%, is an index of respiratory insufficiency of an obstructive type [23].

Figure 6.33 outlines the normal volumes and those bound with moderate airway obstruction.

Additional parameters include:

- FEF, forced expiratory flow
- $FEF_{25–75}$, forced expiratory flow at 25%–75% of VC, more often used than $MMEF_{25–75}$, maximal mid-expiratory flow at 25%–75% of VC (Table 6.23)

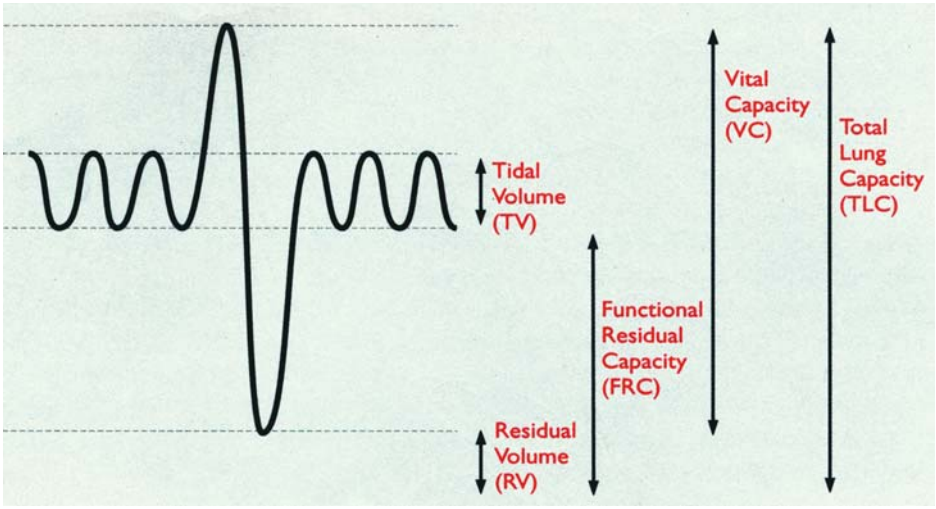


Fig. 6.32. Subdivision of lung volumes

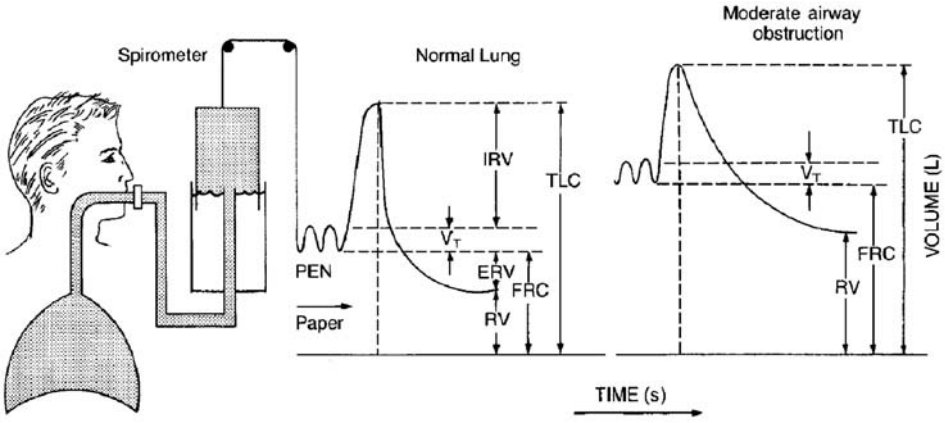


Fig. 6.33. Normal lung volumes and capacities compared with an example of those found with moderate airway obstruction

- FEV_1, forced expiratory volume in one second (in liters)
- Tiffenau index: FEV_1/FVC
- $\dot{V}_{max}$ 75, $\dot{V}_{max}$ 50 and $\dot{V}_{max}$ 25, at 75%, 50% and 25% of VC, respectively

FEV_1, the most frequently employed index, is a maximal forced expiratory maneuver going from full inspiration to full expiration but relies on the child's (aged >6 years) cooperation, since it depends initially on the resistance of large airways and then of the small airways in the second part of a respiratory act. FEV_1 is a sensitive and reproducible expression (with a coefficient of variation between 0% and 8%) of the central and lower airway function, whose reduction is an index of generalized airway obstruction, reversible after bronchodilator administration. FEV_1 expresses the degree of median airway obstruction certainly more accurately than FEF; however, small airway obstruction is scarcely represented.

FEF_{25-75} is defined as the slope of a line connecting the first and last quarter of an FVC, the effort-independent part of FEV_1; it essentially explores the degree of resistance to airflow of lower airways: it is a very sensitive indicator that in case of limited airway obstruction, can be a better measure for following disease in children with a normal FEV_1 [87]; helpful information to quantify variable obstruction can be given in children by $\dot{V}_{max}$ 50 and $\dot{V}_{max}$ 25.

In children, the FEV_1/FVC ratio is a value >85%–90% (percent of PV) and is consistent with normal airflow, since it is the fraction of the VC that is exhaled in the 1st s; the reduction to <80% is one of the best indices of respiratory insufficiency of an obstructive type. However, it is less sensitive to small airway obstruction and can result in an insufficient and/or delayed indication of distal obstruction [23].

In conclusion, FEV_1 compared to previously discussed measures requires a lower compliance, has a greater reproducibility and employs less costly devices [23].

Indications

Spirometry can be performed in children aged ≥6, generally able to cooperate. In selected cases, younger children can undergo the examination [73, 180]. Spirometry should be performed in all true or suspected asthmatics, especially when PEF values do not fit the clinical severity of asthma, to provide adequate information on the child's conditions. It is mandatory that spirometers respond to absolute standards [100].

Interpretation of Spirometry Results

Spirometers offer an immediate digital reading of the main indices, making it possible to visualize airflow obstruction, assess prognosis and quantify the response to therapeutic agents over time. With computerized equipment, the PVs and their relative percentage are automatically measured: the models to be preferred are those displaying flow-volume curves (FVCs), over those showing only readouts of numerical results, by which it is possible to determine whether the child is cooperating. Spirometry does not offer a precise diagnosis, but rather ensures a result setting, based on the belief that the more severe the obstruction is, the more reduced the airflow is during a forced expiratory maneuver, compared to PVs recorded on average in healthy subjects [39].

The lack of FEV_1 responses to a bronchodilator does not preclude a diagnosis of asthma: caveats are a possible persistence of effects related to previous therapy or an inappropriate aerosol administration, in addition to the reflex bronchospasm in response to airway irritation caused by aerosol ingredients and the bronchoconstrictor procedure effect (spirometry-induced bronchospasm) [180].

It is important that results are compared with normal standards for age, sex, race, and height: indeed the normal PVs are different depending on whether sub-

jects are children or adults [85] (Table 6.23). We believe that a comparison should be valid when children are used as their own controls or children of the same or a similar size are compared [85] (longitudinal evaluation).

Pulmonary Function Tests in Children Under 12 Months to 6–7 Years

Approximately one-third of infants, during the first 12 months of life, present a recurrent bronchospasm: independently of the basic disease, PFTs, in particular of flow rates and volumes, are invaluable tools in the management of the basic disease. Flow–volume analysis in severe airway obstruction has led to the understanding that when the curve of forced flows overlaps with FVCs, there is no air reserve left, a problem that during the first months of life can also be observed in asymptomatic infants [83]. As pointed out above, PFTs in children aged <7 years show notable differences: children are thereby divided into three age groups [23, 129], also taking into account the infant's somatic growth and the related functional developmental changes, an issue not facilitating PFT standardization in the pediatric domain [6, 129].

Between 4 and 7 years of life, it is not easy to measure VC, FEV_1 and other related parameters in a reliable and reproducible way, while some cooperation can be attained for the following measures:

- PV (pulmonary volume) at the FRC level.
- BPT of bronchoconstriction or bronchodilation based on Raw (airway resistance), obtainable at this age without excessive discomfort.
- Specific Raw (sRaw) is measured in most specialized centers using whole-body plethysmography in young awake children accompanied by an adult, which yields comparable and equally repeatable estimates of sRaw in young children [77]. Raw measurement can be unreliable since as much as half of the total Raw (expressed in cm H_2O/l/s) in children may be from the upper airways. It can also facilitate the evaluation of the response to bronchodilators causing paradoxical decreases in FEV_1 since it does not require full inflations or sustained expirations, frequent causes of bronchospasm [180]. We note that premature infants have an increased Raw in the 1st year of life (>50 cm H_2O/l/s), predictive of symptoms within the 3rd year of life [58].
- The reciprocal Raw, airway conductance (Gaw).
- RSR (respiratory system resistance), measured by the impulse oscillation technique, facilitates the child's cooperation, since the plethysmographic cabin is absent. However, the entire RSR is not measured, but only the respiratory airway resistance, and this technique is also potentially insensitive to small changes. It is less sensitive than sRaw in detecting Raw changes after methacholine challenge. Thus several methodological issues, including the optimum site for application of the oscillations and the effect on upper airways have yet to be resolved [78].
- The interrupter resistance (Rint) appears more promising for detecting bronchodilator responses than induced bronchoconstriction in wheezy preschool children [130]; however, Rint is not recommended for BHR assessment, since Raw changes may be underestimated during the challenge, because there is no equilibration of pressure changes throughout the respiratory system [55].

Between 2 and 4 years of life, except sporadic exceptions, is the period of non-cooperation to opposition; however, BPTs can be performed, comparing induced bronchoconstriction and dilation to modifications of transcutaneous O_2 tension ($PtcO_2$): several studies have demonstrated the correlation between PFT parameters and $PtcO_2$ in children [170, 187, 188] and infants [164, 167]. These studies are certainly of great relevance, anticipating the diagnosis of asthma to an age level usually escaping such consistent findings, often due to poorly indicative symptoms [23].

Whole-body plethysmography, the helium dilution method (closed circuit), and partial expiratory flow–volume curves (PEFV) have been adapted to *children aged <24 months,* in whom FRC is the only measurable lung volume [6]. The first technique measures thoracic gas volume (TGV), the second one the gas volume accessible in the lungs, and the last technique the maximal flow of FRC [23].

Plethysmography is used to better study lung volumes and Raw, conductance and specific conductance, and to measure gas trapped in the thorax, producing rapid and accurate results. However, the technique could underestimate changes in the small bronchi, unlike compliance assessment. Plethysmography has been appropriately modified, so that the same equipment can also be used for the helium dilution method without modifying the position of the young child [96]. The infant is placed in a supine position inside the body plethysmograph (a closed telephone-booth-like box) and then sedated: only when the infant appears to be in a quiet sleep is a face mask connected to a pneumotachograph and the shutter is sealed around the nose and mouth [96]. Through a mouthpiece the infant performs various respiratory maneuvers. This application takes advantage of Boyle's law, stating that the volume of a gas in a container varies inversely with the pressures to which it is subjected [180]. At a predetermined point in the respiratory cycle, the expiration is blocked for a while, closing the shutter placed between the infant's mouth and the pneumotachometer (Fig. 6.34) [96]. In this way FRC is measured as TGV in synchrony with airway conductance corrected by TGV to calculate the specific conductance [129]. FVC curves can be measured by using plethysmography and a face mask, a method that has gained much interest, since it does not require active cooperation other than a natural TV. Consequently, it can be obtained in awake infants and young children.

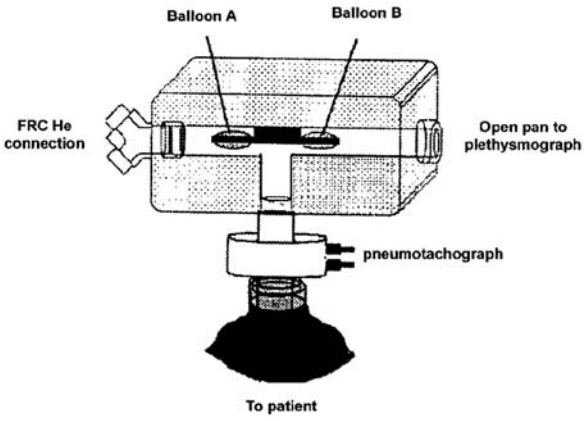


Fig. 6.34. Balloon inflated valve system. Diagram of two-way balloon occluder device permitting redirection of air flow from children to either the dilution or plethysmograph with no movement. The pneumotachograph is positioned distal to the mask as shown in the figure

To obviate the necessity of the face mask, the curves may be measured by respiratory inductive plethysmography [34]. Plethysmography is expensive, and may therefore be restricted to specific centers [96]; the expensive and invasive equipment required can be bypassed with the *pressure sensor method* [12].

The *helium dilution method* requires no infant cooperation; the equipment needed is simple, reliable and relatively economic [129]. The infant is placed in an enclosed system and is attached by a face mask to a spirometer breathing circuit that contains a known volume of air with a pre-set helium concentration (about 10%); the mixture is rebreathed until the helium concentration has remained stable for 30 s. A manometer measures the maximal inspiratory and expiratory flow rates, normally at least 30 cm H_2O, to appraise the neuromuscular component of the ventilation. FRC is calculated considering the initial volume, the mask dead space and the initial and final helium concentration and is proportional to the variations in gas levels [157]. However, in children with gas trapping due to airway obstruction, TGV may be underestimated [129]; similarly, air leaks in the system can induce an inaccurate measurement [157]. To compensate for O_2 consumption during the first minutes of equilibration, O_2 must be added to the circuit during the test. Thereby a third method of measuring FRC can estimate the volume of nitrogen (N_2) washed out of the lungs when the infant rebreathes from a gas-free reservoir, thus estimating the FRC volume (N_2 washout). The amount of N_2 expired during a normal respiration depends on the initial and final concentration in the lungs and on PV [21]. The intensifying use of sophisticated gas analyzers and computers has led to adaptations and refinements of the *N_2 technique,* so that breath-to-breath measurements of expired volume and N_2 concentrations are made; therefore, the procedure may require <1 min in healthy children. This time is significantly shorter than the time required for plethysmographic measurements, which along with the less frightening appearance of the equipment may make it more applicable to infants and young children [21].

However, it is not clear why FRC measurement in infants will differ depending on the technique used, resulting in a greater difference when using plethysmography compared to helium dilution [96]. Even considering that infants are studied in the supine position, asleep and after having received sedation (three notable differences between infants, children and teenagers), and taking into account that FRC values are related to posture, we can speculate that the results may reflect a unique quality of normal infant respiratory physiology, causing a small but significant amount of airway closure [96].

We see that flow can be measured on the forced expiratory curves. In young children, the PEFV curve assessment is done as above with a nose clip and mouthpiece attached to a pneumotachygraph. This technique has been modified to include an inflatable and flexible thoracoabdominal plastic jacket encircling the infant's trunk from the axillae to the anterior superior iliac spines, as originally described for this test referred to as the *squeeze technique*. The bag is connected to a pressure reservoir by a large tubing. The test, called rapid thoracoabdominal compression (RTC), is performed in the supine position 15–20 min after the infant has fallen asleep either spontaneously or sedated [6], applying at the end inspiration in 1–2 s an initial positive pressure of 30–40 cm H_2O to inflate the jacket, causing a partial forced expiration [103]. Using a reservoir that is at least ten times the bag volume ensures that a relatively constant pressure is applied throughout the procedure [129]. Testing is repeated at progressively increasing reservoir pressures (usually from 20 to 80 cm H_2O), until the maximal expiratory flow is reached, finally calculating the mean coefficient of variation (COV) [103]. The amount of intrasubject COV in $\dot{V}_{max}$ FRC has been found to be 37% in the 1st year of life [103]: a value globally similar to that obtained in older children and adults [129]. The technique, helpful to prevent spirometry-induced bronchospasm, measures intrathoracic airway function when a flow limitation is reported, a frequent finding in infants with airway obstruction. PEFV curves have been applied to epidemiological studies of the youngest of infants to determine normal lung growth and development, effects of early lung function, such as the effects of maternal smoke (Chaps. 4 and 24) and BHR. Work done so far has confirmed that males tend to have severe wheezing illness [129].

Another method employs FVC curves compared with the forced expiratory flow–volume loops, roughly ovoid, without or with little peak flow and convex relative to their origin. When an obstruction is present, the loops become triangular with a peak flow, resulting in a straight or concave aspect compared to their origin

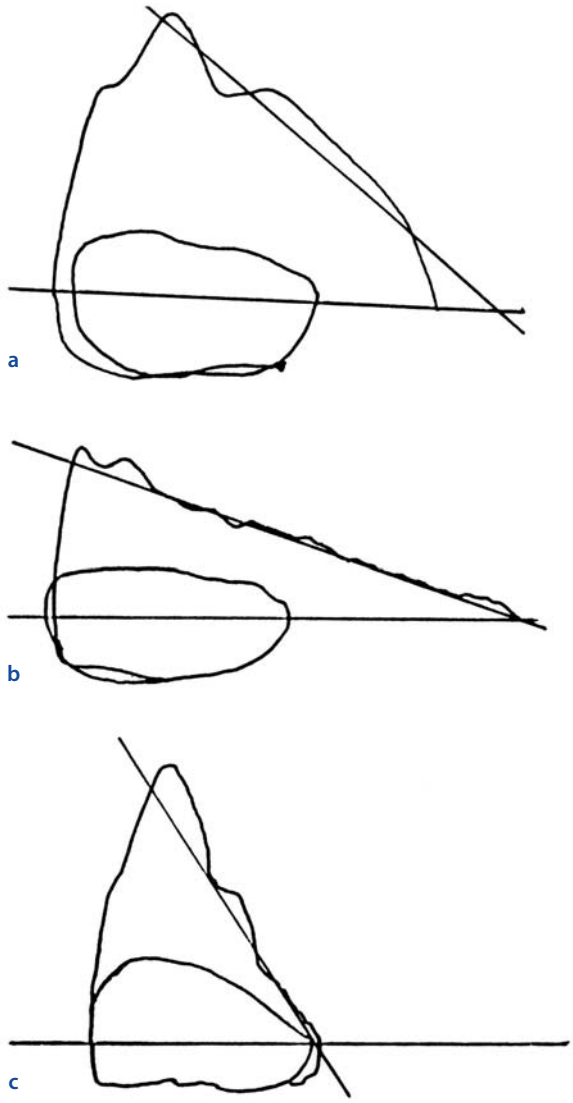


Fig. 6.35 a–c. Flow-volume loops

(Fig. 6.35). The most frequently characterizing indices are the tidal expiratory flow pattern quantified as Tme/Te (percentage of expiratory time to reach peak tidal flow; normal values are between 0.20 and 0.30), and the total duration of an expiration. An early increase to PEF is to be expected in cases of bronchial obstruction, since PEF is also a reflection of muscle strength and effort [129]. Tme/Te may reflect the degree of neuromuscular inspiratory activity at the moment of expiration [6], so the pressure of elastic retraction provokes a greater expiratory flow [23]. Thus the relationship of Tme/Te to total expiratory time may represent a sensitive parameter of great potential, very useful in the setting of severely obstructed children, either in the presence or absence of clinical manifestations, which can be considered predictive of the subsequent development of respiratory dysfunctions [83].

Table 6.25. Mean PaO_2 levels (mmHg) during childhood

Age	Levels
10 days–2 years	72±5
2–8 years	88±4
>8 years	93±6

Data from [23].

The FVC curves show typical patterns in infants with obstructive airway disease (bronchiolitis, asthma), as well as the effect of bronchodilator medications and also a predictive effect of the subsequent development of wheezing [34].

Children <5 years are not capable of exhaling air at a low flow rate in a reproducible way, as they do not understand the instructions and biofeedback signal and cannot exhale over a prolonged time (10–15 s). Short-lasting exhalation at a high flow rate implies that *measurement of eNO at high exhalation flow* does not compromise the power of eNO to detect allergic disease with good reproducibility in children aged ≥37 months [169]. Reproducible measurements of eNO can be obtained without control of expiration flow using a face-mask fitted with a one-way valve on the nasal compartment which reduces the admixture of nasal NO, thus improving the reliability of eNO measurements in young children during tidal breathing [40].

Blood gas analysis measuring the gas dissolved in blood is reliable since a hypoxic state documented by subsequent tests and the delayed appearance of hypercapnia indicate worsening of the prognosis. A useful tool is PaO_2 (partial pressure of O_2) (Table 6.25) [23]. Arterial O_2 saturation (SaO_2, easily measured with pulse oximetry; Chap. 11) as well as transcutaneous arterial O_2 and CO_2 are indirect measures of lung function. SaO_2 is particularly helpful in monitoring acutely ill asthmatic children and acute attacks in young children [34].

In conclusion, we stress that reliable corrections for the size of the child and for air entrainment during inhalation are necessary [85]. In children aged >4.5 and even younger (>37 months) an exhalation flow rate of 350 ml/s is feasible to determine exhaled nitric oxide (NO) *to detect pediatric asthma* [169]. The repetition at regular intervals of PaO_2, SaO_2, and blood gas analysis is a very precise guideline for disease progression and the possible effect of management.

Medicolegal Aspects of Immunoallergic Testing

Several official statements of various societies, including SIAIC [38] and EAACI [49], state that both prescription and/or execution of such tests may induce risks and/or provoke damage to children, concerning the following issues:

- Prescription: absence of specific indications, or presence of specific counterindications
- In vivo execution: superficial, insufficient or absent evaluation of children (by means of history and clinical examinations), including previous allergic dysfunctions and degree of sensitivity to the allergens to be tested, inaccurate identification of children at risk for severe reactions, inadequate equipment in the doctor's office as regards both instrumentation and availability of first-aid drugs
- Risks related to allergenic extracts: defective storage, excessive potency
- Risks related to the child: potential counterindications, even temporary, apart from untoward, unpredictable reactions
- Risks related to incorrect interpretation of results

These precautions are necessary only if the doctor fears that a possible lack of experience, negligence, or imprudence may harm the child.

We emphasize that the examinations should be performed in a facility equipped with the essential supplies and medications available on site and staffed to undertake emergency measures,

Finally, the in vivo tests and SIT can follow a programming schedule, and avoid emergency interventions. In the pediatric domain, we emphasize that FCT should usually be done in a hospital, and that in all cases informed consent should be required from the father, mother, or legal guardian of children to be examined, as shown in the Appendix 6.7 [38]. Since this proposal is adaptable to SIT, in this case doctors shall disregard the section.

Pediatricians and Diagnostic Whereabouts

Atopic diagnosis is usually a challenge to the pediatrician's skill and patience. The diagnostic criteria for allergic disease comprise a wide array of symptoms that make objective appraisal difficult and are often differently assessed depending on the opinion of the examining clinician. Most important, the results of diagnostic tests should not be viewed in isolation. Preferably, the clinical history and physical diagnosis should expedite the diagnosis [62]. The most valuable tools are the pediatrician's eyes, ears and fingertips; however, they are subjective and difficult to standardize. Pediatrics is the only branch of medicine where the history-taking process may depend on secondhand information: infants and little children have either an insufficient language development or the skills to verbalize their disorder, or a poor understanding of their body to help the inquiring pediatrician. To bring about stable compliance, the psychological implications should be taken into account: the unaware child brought to a consulting room will certainly be frightened by an unfamiliar setting, as well as by the presence of other babies moved to tears, while the parents trying to pacify them more or less awkwardly will appear unconvincing, especially during testing, the seemingly unending needles, the coercive measures often called for by hurried doctors, etc. Not only will the pediatrician's experience be disclosed, especially his/her patience and understanding to make the event less startling for the accompanying parents, but also to help and distract, as much possible, the young patient. For example, special software displayed a birthday cake with burning candles on the computer screen: the stronger the expiration, the easier it was for schoolchildren to blow the candles. The cooperation was good, but measurements should nevertheless be performed by trained pediatricians [180].

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Atopic Dermatitis

The First Clinical Manifestation of Atopy

Among the skin allergy diseases, we distinguish atopic dermatitis (AD) and urticaria-angioedema, characterized by IgE-mediated reactions, as well as allergic contact dermatitis (ACD), with prevalent delayed-type hypersensitivity (DTH) cell-mediated reactions, where the allergens reach the skin directly (unlike AD). Urticaria, the most immediate, also occurs in the first phase of AD. Depending on its onset, AD is the first atopic disease in an absolute sense: the dendritic cells (DCs) appear first in the skin and then in the lungs (Fig. 2.24), and CLA (cutaneous lymphocyte-associated antigen) [195] is expressed by 45% of skin T cells [24], but not by lung T cells [196].

The first description of AD could go back two millennia as, according to the historian Svetonius, Emperor Augustus suffered from an extremely itchy skin disease, in addition to chest tightness and seasonal rhinitis. In 1891, Brocq and Jacquet classified AD as neurodermatitis [27], a term that often occurs in the German literature, but this is confusing because one might deduce that AD could be caused by a nervous system instability. The following year, Besnier separated AD from the diseases with itching and described it with the term "prurigo diathèsique," underlining its hereditary nature, the frequent association with asthma and pollinosis in the same patient, as well as its typical skin lesions [16]. In 1933, Wise and Sulzberger coined the term "atopic dermatitis," indicating that it could be considered as the "skin analogous of asthma and allergic rhinitis" (AR) [314]. The term "eczema," although widely employed, is merely descriptive, being a simple transliteration of the Greek word εκζεμα, meaning "boiling out," and is misleading, since it fails to imply both the serum exudation in infants and the dry and scaling lesions in older children. This is why we cannot comply with the term "atopic eczema/dermatitis syndrome" proposed by the European Academy of Allergy and Clinical Immunology (EAACI).

Several factors are implied by AD appearance and subsequent evolution. Recent evidence suggests intriguing aspects of the Th1-Th2 contraposition [269]: aeroallergens such as Der p (also as a contact allergen) and pollens can contribute to AD outbreaks and the study of some staphylococcal enterotoxins (SEs) has attracted growing interest, since they are able to act as microbial superantigens (Table 1.29), in addition to the possible keratinocyte pathogenic role capable of producing a veritable arsenal of inflammatory interleukins (ILs). Since AD is an IgE-mediated disease, blood and tissue eosinophils express CD137 [100]. In high-risk (HR) neonates, it is hypothesized that a drop in IFN-γ secretion is predictive of AD development, and a specific response to bovine proteins predicts the association with food allergy (FA) [302]. Table 7.1 outlines the main characteristics of AD [147].

Definition

AD is a disease with a poorly understood etiology, exceedingly complex and multifactorial, with a chronic recurring course. Due to its complexity, AD is a disease that defies attempts at definition, with no clear-cut marker such as one type of basic lesion, specific histological patterns and typical laboratory data, but with a chronic and inflammatory state accompanied by intensely pruritic lesions, erythematous, exuding or papulovesicular eruption, where phases of upsurge alternate with periods of remissions, and skin hyperreactivity. Particularly in its severe, chronic form, AD is a distressing and even disabling disease, due to the irritation, dryness, sweating, and itching. The itching with nocturnal exacerbations disrupts nocturnal sleep of both infants and parents, while skin lesions can socially marginalize the affected child/adolescent. Persistence into adulthood is possible, even if some features may be absent [36, 96].

Epidemiology

AD is a disease common in atopic infants (Table 5.6), never observed at birth and rarely in the first 6 weeks of life, the mean age of onset generally being around the 3rd month, thus earlier than that of asthma [48]. In genetically at-risk babies, the onset in 48%–65% of cases was in the first 6 months of life, but even before 4 months of life in 66 babies (57%) [48], in 75%–80% of cases within the 1st year, with a male prevalence higher than females: 1.3–1.5:1 (Table 5.5). Table 5.8 stresses the increasing frequency of AD in the last 25 years.

Table 7.1. Immunological and clinical features of AD

Genetic-immunological features
Influence of genetic factors
Increased serum and skin IgE levels
Increased production of Th2 lymphocytes
Decreased CD8 suppressor/cytotoxic number and function
Increased CD23 expression on B cells and PBMCs
Activation of $IL_4/IL_5/IL_{10}$ and other Th2-like ILs
Decreased production of IFN-γ/IL_{12} and other Th1-like ILs
Deficient PBMCs not producing IFN-γ/IL_{12}
Increased basophil releasability
Activated chemokines (TARC, RANTES, eotaxin) by skin keratinocytes and endothelial cells
Persistent activation of macrophages with hypersecretion of GM-CSF, PGE_2 and IL_{10}
FFA altered metabolism
Role of infections
Role of foods and food additives
Role of aeroallergens
Role of CD137 expression on blood and tissue eosinophils
Clinical features
Typical flexural lesions
Irregular and unpredictable course
Severe itching
Skin hyperirritability
Impact of stress and irritant factors

Data from [147].
FFA free fatty acids, *sIgE* specific IgE.

Skin Immunopathophysiology

AD pathogenesis is complex. Therefore it is necessary to focus, beyond the immunological abnormalities, on the particular stigmata characterizing the skin such as xerosis or constitutional dryness associated with alterations of surface lipids and transepidermal water loss and with anomalies of both microvasculature and skin surface barrier function.

The Role of Skin Surface Barrier

The anatomo-functional unit called skin protects the organism from injurious external influences, either passively by means of its basal properties, integrity and impermeability, or actively by developing inflammatory reactions [104]. The main skin constituents are, from the external to the internal layer, epidermis, dermis, and hypodermis. The transitional zone between epidermis and dermis is the dermoepidermal junction. The *epidermis*, a stratified squamous epithelium, as seen in sections perpendicular to the surface, is formed by four main layers, disposed from the skin to the dermoepidermal junction: the corneum, granular, lucidum and basal layers. The last three layers form the *stratum Malpighi*, where 95% of the cells are keratinocytes [10], Langerhans cells (LCs), melanocytes, and less immunocompetent CDs (but IL-producing), disposed in the stratum above the basement membrane, which has no immunological functions being devoid of cells. It is distinctly lamina lucida rich in laminine, lamina densa and sublamina densa, consisting of fibronectin, elastin, collagen and myofibrils anchoring to the underlying dermis. Keratinocytes progress through a program of differentiation as they move up through the skin to become corneocytes, dead bundles of precipitated keratin proteins wrapped in remnants of plasma membrane. These proteins form the cutaneous barrier called the *stratum corneum.* The *dermis*, placed immediately beneath the epidermis, consists of fibrous bundles (collagenous and elastic fibers) and connective cells, in addition to a cementing substance formed by ialuronic acid, fibronectin and proteoglicans. The dermis plays an active role in thermoregulatory phenomena, metabolic and nutritive exchanges and immunopathological events. The *hypodermis* consists of adipose and loose connective tissue and performs functions of mechanical support, thermal protection and energy store [70, 104].

The epithelial integrity is maintained by several mechanisms, including the molecular forces which ensure the cohesion between the cells and their peculiar disposition: the adjacent cells are kept together by cytoplasmic processes, forming intercellular bridges, called desmosomes (*macula adherens*), effectively ensuring the connections between epithelial cells, also through keratin filaments formerly known as tonofilaments, which from one side penetrate inside the cells and from the other converge toward the dense plaque of desmosomes [104]. Epithelial integrity is thus ensured by desmosomes disposed among the keratinocytes; the connection systems between columnar and basal cells are completed and strengthened by hemidesmosomes, which promote the adhesion between cell basal facies and basal membrane by tight junctions (*zonula occludens*), and by *zonula adherens,* with no keratin filaments, located just below the junctions [59]. To understand some typical aspects of AD, especially the significance of dry skin and easy aggressiveness of irritants [70], it is necessary to contend with problems related to defective water retention, increased skin permeability and the resulting transepidermal water loss, a characteristic feature [277]. The *stratum corneum* plays a fundamental role in ensuring the skin barrier function, since

it is composed of epidermal lipids (10%–30%) and has a low permeability, retarding water and electrolyte loss from inner more hydrated layers [104]. Therefore it consists of ≈15 layers of flat anucleate cells tightly linked to one another, thus functioning as a sort of intercellular cement [245] surrounded by complex lipids acylceramides), cholesterol, essential fatty acids (EFA), nonessential fatty acids and gangliosides [159].

Acylceramides, the major components of *stratum corneum* lipids, are disposed within the cells in multiple and parallel layers; they are considered essential to prevent water loss [104]. Any perturbation disrupting the barrier integrity initiates the intervention of protective factors, namely a rapid secretion of lamellar bodies by the *stratum granulosum* cells, an increase in cholesterol and EFA within 1–2 h and in sphingolipid synthesis after 6 h or later [159]. In patients with AD, a reduced total content of stratum corneum ceramides was instead discovered, thereby leading to the hypothesis that such deficiency represents a predisposing factor leading to skin xerosis, a basilar characteristic of AD [116]. The deficiency has been demonstrated in both injured and unaffected skin: with the passing of time it has progressively increased in healthy individuals and more markedly in atopics [57]. Disturbances of epidermal lipid metabolism not only underlie low ceramide content, but are also responsible for the reduced LC-SFA (long-chain saturated fatty acid) and EFA production and for an increased SC-SFA (short-chain saturated fatty acid) production. Given that LC-SFAs are essential components of acylceramides, this reduction is negatively reflected on their synthesis: in particular, an altered LC-SFA/SC-SFA rate is commonly observed in all forms of hyperkeratosis [202]. It is conceivable that another disturbance of acylceramide properties may depend on an EFA dysregulation (EFAD); consequently linoleic acid is replaced by oleic acid in their structure. It has been shown that the barrier existing in the lower part of *stratum corneum,* in the intercellular lipid sheets, is rich in linoleic acid to ensure a proper state of hydration and impermeabilization, thus depending on the incorporation into acylceramides of normal amounts of linoleic acid secreted by keratinosome components of the same layer, which are abnormal in the dry, noneczematous skin of atopics [25]. Linoleic and linolenic acid are EFAs with roles in the normal epidermal barrier structure and function [25]. As a consequence, there is an altered acylceramide biosynthesis and the relative disposition in single intercellular lines, a condition leading to an increased transepidermal water loss [70], as seen in patients with AD compared to healthy controls during the exposure to irritant agents [116], hence leading to dry, scaling skin and to a reduced barrier function [281]. This explains the particular skin susceptibility to irritant agents.

Both in injured skin and in apparently healthy skin there is a high content of *phospholipids* (60%) and EFAs, dependent on the defective acylceramide synthesis, which increases the chance of a shunt toward phospholipid production [245]. Phospholipids predominate in the lower and spinosum epidermal layers and in normal conditions are absent from the *stratum corneum* [163]. Constituting the substrate for phospholipase A_2 (PLA_2), notably increased in AD lesions, phospholipids lead to the production of arachidonic acid (AA) and subsequent pro-inflammatory mediators (Fig. 1.57). It is concluded that allergens including inhalants, microbial agents such as *Staphylococcus aureus* and toxins can easily penetrate through a disrupted skin barrier, provoking an inflammation that results in increased PLA_2 activity and the release of polyunsaturated fatty acids (PUFAs), representing the substrate for both lipoxygenase and cyclooxygenase and inflammatory mediators such as PGF_2, LTB_4. These mediators, in turn PLA_2 activators, are the key players in the aggravation of a vicious circle [163].

Skin Immune System

Recently, the defense mechanisms of skin barrier was credited with a more markedly active role. Interestingly, the skin is able to promote immunological activities autonomously, which can be divided into:

- *Stimulating activities,* induced especially by LCs, which after exposure to different allergens promote allergen-specific T-cell proliferation and their maturation into cytotoxic lymphocytes reactive against allergen-modified syngeneic epidermal cells
- *Modulating activities,* responsible for late and contact reactions and for skin immune tolerance

For these characteristics of the skin, the seat of immuno-specific reactions, the term "skin immune system" (SIS) was proposed [24], to emphasize the integrated system of immunosurveillance formed by lymphocytes, CDs, keratinocytes, etc.

Relationships Between Skin and Immune System

Antibodies and complement diffuse into dermal perivascular layers and thus can reach the epidermis, since the basal membrane does not constitute an obstacle to this diffusion. An immunohistological study has provided evidence that monocytes, macrophages and polynucleated neutrophils (PMNs) are equally able to infiltrate the epidermis under the influx of chemotactic factors, some of which are specifically of epidermal origin [226]. The SIS cell types that are the focus of a great deal of research are mast cells, T lymphocytes, LCs, and keratinocytes [272]. Normal skin contains ≈8,000 mast cells/mm^3; in AD there is a density of ≈20,000–40,000 cells/mm^3, 94% of which are TC (tryptase and chymase-containing) [117]. Mast cells derive from the bone marrow from $CD34^+$ myeloid precursors, distributed along

the dermal vasculature, and differentiated into mature cells migrating toward skin areas, through *HEV* (high endothelial venules) residing in lymph nodes, and toward capillary endothelium and lymphatics of superficial dermis, under the influence of chemoattractants and adhesion molecules, namely CD62L receptor of CD34 [185]. Thus equipped, mast cells participate in IgE-mediated hypersensitivity reactions, so often expressed at the cutaneous level [51, 117], as identified in the epidermis of patients with AD [114], as well as in the epithelium of intestinal mucosa [185]. In the skin, mast cells release histamine in responding either to polymers of L-arginine and L-lysine, or to neuropeptides such as SP (substance P), VIP (vasoactive intestinal peptide), somatostatin and a wide variety of nonimmunological stimuli [51]. Histamine release is extended to eosinophil cationic proteins, two of which, MBP (major basic protein) and EPO (eosinophil peroxidase), are able to inhibit SP-mediated histamine release [186]. Dermal mast cells also dismiss ILs, including tumor necrosis factor (TNF)-α cross-linked to FcεRI [300], in 10 min following IgE stimuli [49]. The direct mast cell activation by IgE molecules and by interactions also by independent ways of CD40-CD154/CD40L, the latter expressed by metachromatic cells and eosinophils, is highlighted by the synthesis of IL_4 (90% by skin mast cells), logically involved in the amplification and propagation of Th2 responses.

In the epidermis in acute and chronic lesions, a low number of infiltrating lymphocytes (CD3, CD4, CD45RO) that are highly positive for HLA class II antigens have been seen, thereby indicating that they are activated [226], because of prior antigen contact, since naive T cells are rarely located in the skin [194]. T cells interact with DCs and allergens that have previously crossed the barrier of the stratum corneum [226]. Even if SIS lymphocytes belong to two phenotypes, the great majority of cells locally are usually T cells, and almost only in AD do T lymphocytes represent an important constituent of the cellular infiltrate [70]. The pattern is completed by *vascular endothelial cells* expressing high CD62E, CD54 = ICAM-1 and CD106 = VCAM titers [297] as well as HEVs, specialized in CD62L expression interacting with CLA [251], likely provided with CD15s (Table 1.50), regulating naive T cell adhesion to HEVs [148]. In the Springer three-step model (Fig. 1.61), the subsequent stage gives rise to MCP-1 (monocyte chemotactic protein-1), another important chemoattractant for lymphocytes, which in this step is a $G\alpha_1$-protein-coupled receptor, while the final step is regulated by CD49d/CD29 (VLA-4) and CD11a/CD18 ($\alpha_L\beta_2$) binding to CD106 and CD54/102, respectively [251] (Tables 1.45, 1.46). Thus, skin-specific adhesion molecules (Tables 1.44) bind preferentially to T CLA^+ (or $MCP\text{-}1^+$) cells, promoting their accumulation in sites of chronic inflammation, while regional draining lymph nodes link the skin with the systemic circulation via afferent and efferent lymphatics [213, 272]. CD62E, most likely

Table 7.2. Cytokines released by epidermal cells in the pathogenesis of AD

Cells	Cytokines
Keratinocytes	IL_1, IL_3, IL_6-IL_8, IL_{10}, IL_{12}, G-CSF, GM-CSF, M-CSF, PDGF, TGF-α and -β, TNF-α
LC	IL_1, IL_6, IL_8, IL_{10}, G-CSF, GM-CSF, M-CSF, TNF-α
Melanocytes	IL_1, IL_6, IL_8, G-CSF, GM-CSF, M-CSF, PDGF, TGF-α and -β, TNF-α

Data from [155, 260].
LC Langherans cells.

driven by IL_1 and TNF-α [213, 251], has the ability to modulate selected subsets of circulating T cells, especially activated $CD45RO^+$ [303] expressing CLA or MCP-1, up-regulating their homing to skin sites and the adhesion to endothelial cells [25]. In detail, CD106 ligand of CD49d/CD29 modulates eosinophil and monocyte migration toward inflammatory sites [20].

Keratinocytes make up about 95% of the cell mass of human epithelium and are responsible for its integrity, protecting the epidermis against external injury [10]. In addition to altering the balance between proliferation and differentiation, keratinocytes participate in immune responses by releasing signaling molecules. They can release complex arrays of proinflammatory factors when provoked by stimuli such as physical trauma, ultraviolet (UV) irradiation, bacterial products, and underlying fibroblasts, triggering the innate immune system. ILs and especially contact allergens allow keratinocytes to recruit inflammatory cells, regulate their behavior, and up-regulate gene transcription of epidermal pro-inflammatory ILs (Table 7.2) [155, 260]. It is not clear whether these are products of a single type of keratinocyte activation or whether they derive from several qualitatively different responses. In either case, these factors convey signals in a paracrine fashion to other cells, including leukocytes, endothelial cells and fibroblasts, as well as in an autocrine fashion to the keratinocytes themselves. Moreover, the hypersensitivity of atopic skin to irritants may result in the increased synthesis of ILs by keratinocytes [253]. Able to induce their own release, regulating the expression of receptors, mediators, integrins and chemotactic factors similarly to LCs, keratinocytes may play a vital role by triggering the start and amplification of lesions [10, 155], facilitating the interaction with adhesion molecules and subsequent influx of immunocompetent cells [253]. Initially, these cells were presumed to be passive targets for toxic substances and for immunological attack from infiltrating T cells [10], but according to recent studies they could play an active role in concert with peripheral blood mononuclear cells (PBMCs) in initiating and maybe directing the mucosal immune response [306]. Keratinocytes interact with PBMCs, probably by an

increased CD54 expression correlated with the lymphoid infiltration [10], and express the CD36 antigen, fibroblast growth factor, Stat3 and PDGF (platelet-derived growth factor) able to repair tissue [4] and release adhesion molecules. Specific *Stat3 gene deletion* in keratinocytes blocks their responses to epidermal growth factor (EGF), hepatocyte growth factor (HGF) and IL_6. Although Stat3-deficient keratinocytes can form approximately normal skin, they are defective in wound healing. While EGF is an important cytokine for keratinocytes, some deficits in Stat3-deficient keratinocytes may also be due to their inability to respond to IL_{20} [19]. Similarly to differentiated cells of diverse tissues, keratinocytes produce several surface antigens, or cytoplasm, such as HLA-A, -B, -DR, β_2-microglobulin, etc., as well as systemic antigens or those with organ specificity (of cellular line), which are found only in the Malpighian epithelium [10]. These antigens can be of three types:

1. Antigens found in all keratinocytes, in whatever differentiation phase, thus elucidating why certain autoimmune diseases are exclusively localized in the skin and Malpighian mucosa.
2. Allergens expressed only by one subset defined as maturational allergens allowing interactions with monoclonal antibodies directed against keratinocyte subsets.
3. Specific antigens characterized by a polymorphism internal to a single species. The repertoire of specific alloantigens, presently studied only in experimental animals, could open new perspectives on the challenge of skin grafts [70].

Keratinocytes lack expression HLA-DR normally, but only in connection with a primary sensitization, when, it is hypothesized, HLA-DR$^+$ concur to enhance lymphocyte activation, driving allergen-specific T cells to develop [306]. However, keratinocytes, despite expressing class II molecules amplified by IFN-γ generated by Th1 T cells, are not able to process peptides [253]. However, no expression of HLA-DR by keratinocytes would pave the way to the Th2 differentiation and explosion of the atopic march [306]. Further studies shall explain whether this is the primary event or the lymphocyte development is the factor upstream of the HLA-DR expression defect. Keratinocytes can be transformed into valid APCs (antigen-presenting cells) via their association with CD80 [179]. IFN-γ expressed by T cells, which up-regulates Fas (CD95) on keratinocytes, thus making them susceptible to apoptosis in the skin. Keratinocyte apoptosis is induced by Fas ligand (CD178) expressed on the surface of activated T cells [3]. Remarkably, the anti-apoptotic genes, *bcl-2* and *bcl-xL*, were expressed at increased levels in AD patients [204]. Recent data show that IL_{30} may induce melanocortins in the skin to protect both keratinocytes and melanocytes from apoptosis [21].

Langerhans cells are CD1a$^+$ (3%–4%), belong to DCs, and since the middle of the 1970s have been identified as

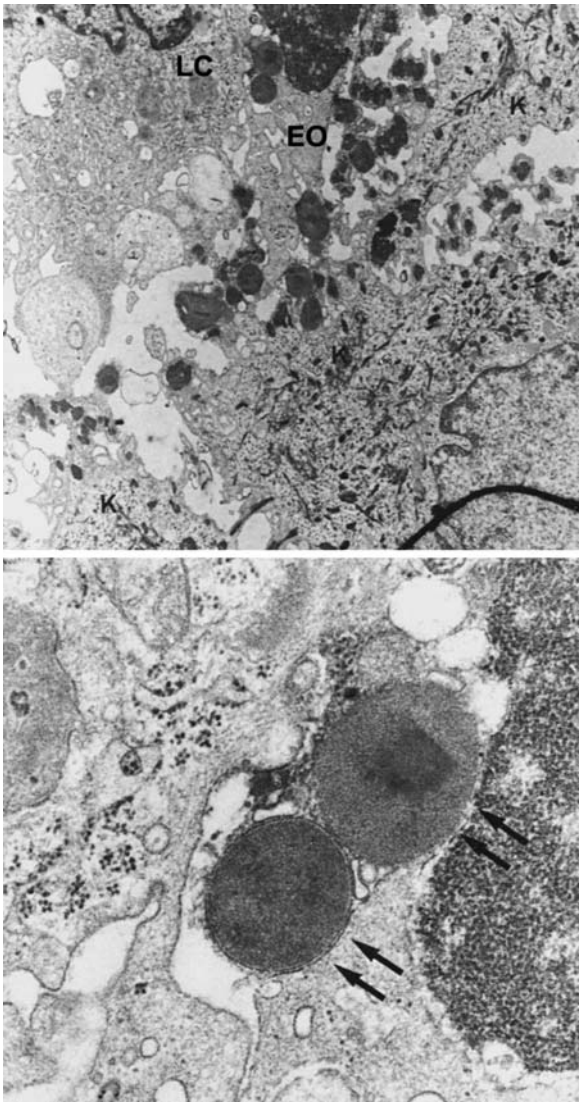


Fig. 7.1 a,b. Electron microscopic section of epidermis in **a** Langerhans cell (*LC*) lying between keratinocytes (*K*) in contact with an eosinophil (*EO*) (magnification 6097×). **b** Magnification showing eosinophil-derived granules (*double arrow*) (magnification ×20,904)

APCs expressing HLA class II antigens, and bear CD1a and CD4 antigens and receptors for IgG and C3b FcR [24, 30]. Viewed as AD-specific, LCs reside in the epidermal suprabasal layer and are in close contact with nearby keratinocytes via an E-cadherin-dependent mechanism [265] (Table 1.51). Ultrastructurally, LCs are characterized by a cytoplasmic organelle with an unusual rod-shaped structure, called the Birbeck granule, originating from the Golgi or from the cell membrane (Figs. 7.1, 7.2) [73]. LC function is still unclear, but they are apparently involved in the intracellular traffic of surface antigens after their endocytosis [30]. Compared to macrophages, LCs have a negligible phagocytic activity; in the skin *the predominant role of LCs is the initiation*

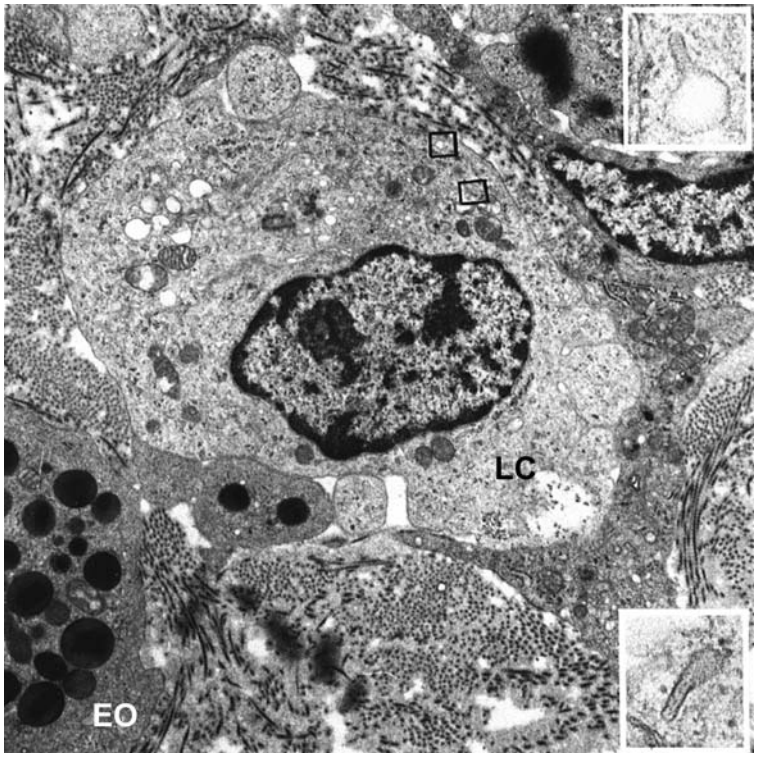


Fig. 7.2. Electron microscopic section showing Langerhans cell (*LC*) in the lamina propria close to the basal membrane containing at least two Birbeck granules (*inserts*), one of them with the typical racket shape; note part of the cytoplasm of an eosinophil (*EO*) (magnification ×13,000, *insets* ×88,000)

of immune responses as APCs for T cells and as stimulant cells in the allogenic reactions between lymphocytes and epidermal cells [90]. LCs also have a surface structured to favor adherence [272], so it is likely that they are the preferential intradermal seat for any substance that has penetrated the epidermis, either exogenous or endogenous; equally important are the IgE antibodies on the LC membrane [28]. Binding of IgE occurs via FcεRII on CD1/IgE$^+$ (studies with CD1$^+$ have clarified that binding occurs via both FcεRI and FcεRII) [18] in different proportions: 6.63±1.92 vs 0.67±1.12 cells, respectively [90]. LCs also express CD11a, CD11b, CD36 and HLA-DR in chronic skin lesions, but not in healthy patients [146, 268], thus increasing the reactivity of autologous T cells in inflammatory lesions [268]. Moreover, CD40 and BcR link to CD80 and CD86 (Table 1.28). Foreign antigens are captured by LCs and other APCs, which migrate and interact with T cells: LC aeroallergen presentation may be very important in sustaining Th2 and IgE responses because LCs overwhelmingly express CD86 rather than CD80 [150]. After capturing allergens, IgE-bearing LCs likely activate memory Th2 cells in atopic skin and also migrate to the lymph nodes to stimulate naive T cells to further expand the pool of systemic Th2 cells [150]. Activated T cells, supported by secondary cytokines from the epidermis, dermis and leukocytes, proliferate and release ILs that promote cellular (Th1, involving IFN-γ) or humoral (Th2, involving IL_4) immune responses. Signals that control IL_{20} expression are unknown; however, IL_{20} receptor ($IL_{20}R$) is upregulated in the epidermis, as well as some leukocytes and endothelial cells [19].

In the epidermis there are *melanocytes* (2%–3%), provided with melanosomes, which are melanin granules subsequently transferred to keratinocytes [59], also expressing ILs (Table 7.2). *Merkel cells* (<1%) function as signal transducers for neurites, and as an unquantified contact site for keratinocytes [105]. LCs and melanocytes are devoid of desmosomes, but have cytoplasmic filaments that protrude into intercellular spaces [328].

The rich vascular set aids the passage of various substances from the circulation: the perivascular area of postcapillary venules in the papillary and deep dermis is the major target for a vascular dilatation that triggers the migration into dermis of intraluminal inflammatory cells, so the dermis contains the highest percentage of cells related to immune responses [328]. In some inflammatory reactions, such venules are similar to HEVs transporting lymphocytes to lymph nodes [328]. Mast cells, PBMCs, T cells and DCs, most of which are LCs, are frequently found next to the endothelial cells and vascular pericytes of these sites. Since they are in close vicinity of epidermis, these structures are literally bombarded by ILs as soon as epithelial damage occurs; as a consequence, the endothelial cells increase their expression of adhesion molecules necessary to T-lymphocyte transmigration [244].

Inflammatory Infiltrates of AD Lesions

Immunohistochemical analysis of the inflammatory infiltrate in AD reveals lesions characterized by epidermal edema, which initially infiltrates the intercellular spaces

leading to spongiosis, and are then grouped in intraepidermal vesicles that dissociate the cells, while the dermis shows a perivenular multicellular infiltrate [321]. In chronic or lichenified lesions, there is more hyperplasia than spongiosis of the epidermis and hyperkeratosis is prominent [226], frequent sequelae of inflammation associated with abnormal hyperproliferation of keratinocytes [10]. Histopathological studies show that in the dermis, in addition to vasodilation, inflammatory edema and demyelination and fibrosis of cutaneous nerves [70], there is a cell infiltrate consisting of PBMCs and lymphocytes bearing a Th2 phenotype, some of which migrate into the epidermis [25]. In addition, most cells in the infiltrate are highly positive for HLA class II antigens, indicating that they are activated [25] in various degrees of degranulation, $CD3^+/CD4^+$ cells, with a few $CD3^+/CD8^+$ cells, usually bearing the αβ TcR [74], and more LCs as detected by the CD1 surface antigen, HLA-DR antigens, and DCs [29]. An influx of elevated basophil and eosinophil numbers suggests, also because of an immunoglobulin absence, the meaningful interference of a cell-mediated immunity (CMI) [226]. During late-phase reactions (LPR) on the coexpression of CD45 (leukocytes) and CD19 (B cells), the B cells in the skin were as high as 50% of circulating B cells after 5–8 h and 25% after 9–12 h, whereas T cells were 16.5% (CD3) [307].

In areas of $CD3^+$ lymphoid infiltrates, as soon as an aspecific stimulus initiates an inflammatory state, physical disruption and infection such as with *S. aureus* can lead to activation of *keratinocytes* [253]. In the first phase of the cutaneous immunoregulatory effects, the permeability increase stimulates both recruitment and transmigration of lymphocytes across the vessel walls and into the tissues. The concurrent secretion of IL_1 and IL_8 from keratinocytes is concretized into a chemotactic gradient, in particular for T cells, inducing their migration into the epidermis, which is CD11a/CD18/CD54-dependent [10]. The subsequent stages of proliferation are characterized by interactions between LCs, keratinocytes and epidermotropic T cells with a resulting dermal infiltrate or granuloma, and recirculation, which autoactivates after both cessation and elimination of the skin insult and weakening of APC activity [272].

To underline the complexity of physiopathological events, several cellular patterns reflecting AD changes have been schematized [226]. In *acute AD* the majority of T cells are $CD4^+$ with a few $CD8^+$, the CD4 in the epidermis and around small blood vessels of deeper dermis, the cytotoxic CD8 T lymphocytes (CTLs) in the superficial dermis, with some cells in the basal epidermal layer [70]. In the dermis, $CD1^+/IgE^+$ LCs are grouped in sparse foci and within the mononuclear cell infiltrate, while in the epidermis they are arranged in a tight network extending from the superficial *stratum corneum* to the basal epidermal layer [147]. Basophils, eosinophils and mast cells that are frequently less degranulated and numerically normal are also found; rare monocytes and macrophages are seen around venous plexus in the dermis [226]. In *subacute AD*, CD4 and CD8 localizations and distributions are similar to those in acute lesions, although in smaller numbers, especially in areas with intercellular edema of the Malpighian layer. B cells [147] are seen only occasionally, and the same is true for NK cells [303] compared to serum levels [307]. The LC number and distribution varies: interdigitating cells are seen in the epidermis and in the dermal superficial infiltrate [70]. In *chronic lesions*, there are more CD8 than CD4 T lymphocytes; the number of CLs is notably increased. They appear as focal aggregates in the epidermis near subepidermal *foci* of inflammatory cells [70], while macrophages dominate the dermal mononuclear cell infiltrate, and the number of mast cells [226] and basic proteins [142] is increased as well.

Pathogenesis

AD pathogenesis remains largely unknown, even if recent progress in asthma pathogenesis offers interesting insights. Indeed AD patients exhibit numerous disturbances of the autonomic system, which are more or less assimilable to abnormalities seen in asthmatics such as an increased α-adrenergic vasoconstriction and piloerection, white or delayed dermographism, a reduction in β-adrenergic inhibition of epidermal mitosis and in lymphocyte and neutrophil responses to β-adrenergic stimulation [192]. Both conditions are characterized by a deficit in G-proteins; however, such diseases are primarily linked by common aberrations of lymphocytes and ILs that may fuel the rise in the IgE level. Further advances in histochemistry and molecular biology have been of invaluable support in unraveling the role of SIS immune abnormalities and shedding light on the study of enigmatic AD [25]. It was not a surprise to find that thymus and activation-regulated chemokine *(TARC) is also active in AD lesional sites:* serum TARC and CTACK (cutaneous T cell-attracting chemokine) serum levels are high in patients with AD and significantly correlated with AD activity [101]. Moreover, in the lesional skin of patients with AD, immunoreactive TARC was positive in keratinocytes in the epidermis, in vascular endothelial cells, T cells, DCs in the dermis, and infiltrating cells. Serum TARC levels significantly correlated with eosinophil number, SCORAD score, serum CD62E levels, but weakly with serum IL_2R receptor (sIL_2R) levels [123]. TARC and CCR4 mRNAs were more positive in acute AD lesional skin [327]; thus *TARC may be an important chemokine in the pathogenesis of AD* [123, 327].

Genetics

The available evidence suggests that AD is inherited with an autosomal dominant or recessive pattern, always associated with abnormal IgE production. How-

ever, the etiology of this disorder remains elusive [321]. The literature on this topic suggests a genetic heterogeneity, since under the label "atopic dermatitis" variously affected individuals coexist, with or without sIgE and with or without an associated respiratory allergy [321]. There are associations with HLA-DQ antigens in children with AD and CMA (cow's milk allergy) [43] and with FcεRI-β on chromosome *11q13*, as in asthma (Chap. 4), or not [54]. Recently, a major susceptibility *locus* for AD was found to map to chromosome *3q21* (by 2 studies), and an association has been found on chromosome *14q11.2* with genetic variants of mast-cell chymase, promoting AD development. Moreover, the role played by IL_4 gene expression may be critical in AD expression. Additional significant AD linkages have been found on chromosomes *1q21*, *13q12-14*, *17q25*, and *20p*, the latter associated with asthma (Table 4.2). The mode of inheritance may express an autosomal dominant trait in association with a maternal pattern of transmission [54, 216]. Genetic findings suggest that the risk of developing AD in infancy may be influenced by *in utero* exposures, including maternal immunologic profiles (IgE) (OR, 2.28), infections (OR, 0.32), and allergic conditions including both AD (OR, 2.46) and active asthma [138]. AD is less likely a monogenic disorder with essentially weak penetrance, but rather the interaction of multiple genes and multiple exogenous or environmental factors that outline a multifactorial inheritance pattern with a polygenic component, thus better encompassing the variety of biochemical and immune abnormalities involved in the phenotypic expression of the disease. Studies have shown an 80% AD prevalence in offspring of two affected parents [25], a 38% and 61% incidence at age 2 years in cases of single or double family history of atopy (FHA), respectively [282]. As far as family risk is concerned, the early onset of skin lesions is significantly increased if both parents are atopic (OR, 2.5–3.4) and the early onset of asthma or rhinitis is increased to a lower degree (OR, 1.4–1.5) (Tables 4.10, 4.11). A demonstration comes from 77% of children with two atopic parents and early AD who were sensitized against aeroallergens at 5 years [14], this could have been predicted in early infancy by Tables 4.10, 4.11. A strong FHA (≥2 atopic family members) was a significant predictor of poor prognosis, (cumulative OR = COR 2.40) [111]. Genetic factors clearly have a decisive role in AD development: extensive studies have noted that in monozygotic twin (MZ) pairs there is a higher risk (86%) of developing AD than dizygotic twins (DZ) pairs (21%), which was no different than non-twin siblings; the certain existence of discordant MZ twins confirms the influence of exogenous factors (Chap. 4). A promising area of new insights could focus the AD association with different genetic conditions, including autosomal dominant ichthyosis vulgaris and recessively inherited anhidrotic congenital ectodermal dysplasia. An alternative to the correlations of the genetic type between these three disorders lies in the suggestion that an epidermal defect predisposes atopic individuals (who would present a different subclinical pattern of disease) to the action of both allergens and irritants that provoke and aggravate AD [321]. Growing experience in bone marrow transplantation has demonstrated clearing of AD and normalization of elevated IgE levels in patients with Wiskott-Aldrich syndrome (WAS), characterized by generally indistinguishable AD, thrombocytopenic purpura, and immune deficit, following successful engraftment. Conversely, atopic donors transfer both atopy and allergen-specific IgE antibodies to nonatopic recipients [235], thus pointing to an alteration of immunocompetent stem cells. Moreover, considering AD and several T-cell PID (primary immunodeficiencies) characterized by high amounts of IgE antibodies leaves open the debate on whether to include AD in a slight pattern of PID [24, 235]. Apart from the indisputable

Table 7.3. Factors suggesting a role for IgE antibodies in AD pathogenesis

1. Personal or family history positive for atopic disease in 70%–90% of children [146]
2. Serum IgE concentrations are elevated in about 80% of children with statistically significant differences [216]
3. Positive immediate SPTs (skin prick tests) and/or RAST to various dietary and inhalant allergens in about 85% of patients
4. Serum IgE levels are highest in children with coexisting respiratory allergy
5. IgE levels are low during remissions and higher during flaring of AD
6. Most children (50%–80%) have coexisting allergic IgE-mediated manifestations such as allergic rhinitis and/or asthma, and food allergy (FA) [192] (Table 7.10)
7. Positive/negative effects of bone marrow transplantation [179]
8. Presence of immediate and late skin reactions after SPTs with allergens, for example after Der p 1 application on the skin of children with SPT+ for Der p 1 [197]
9. Symptoms and food allergen association in 30%–50% of children; 33% based on studies with double-blind, placebo-controlled food challenge (DBPCFC) [33]
10. Improvement of skin lesions during climatic changes (such as a sea resort) [280]
11. Lesion relapse after exposure to environmental allergens and improvement following allergen elimination [197]
12. Decrease in AD prevalence in at-risk babies when food allergens are eliminated during the 1st year of life

Data from [33, 36, 37, 146, 192, 197, 216, 280].

Table 7.4. Evidence against a role of IgE antibodies in AD

A marked elevation in serum IgE is found in several diseases, not necessarily atopic
About 15%–20% of normal children have high concentrations of serum IgE without any clinical manifestation of atopy
About 15%–20% of children have AD without apparent signs of IgE hyperproduction
The synthesis of IgE could be secondary to a functional imbalance of the immune mechanisms controlling it
IgE antibodies are not a prerequisite for the development of skin lesions, since AD can manifest itself even in notatopic children
The typical skin lesion, the wheal, is not a specific marker of IgE-mediated allergic reaction of AD
AD is present in patients with agammaglobulinemia, normal IgE levels, and PTCs negative for allergens, as well as in Wiskott-Aldrich syndrome

Data from [46, 188].

data that, from a clinical point of view, AD patients do not manifest conditions characteristically associated with systemic immunosuppression [25], in AD there are neither severe defects of CMI, nor an increased susceptibility to generalized infections [261]. The prevalence of viral and mycotic infections localized to skin [25] and decreased responsiveness to contact allergens [59] such as dinitrochlorobenzene (DNCB) [147] can result from SIS abnormalities [24]. AD children have shown:

1. *Several immunological abnormalities* (Table 7.1), which can be regarded as the result of decreased activity of cyclic nucleotides such as reduced T-suppressor activity and exaggerated IgE concentrations
2. *Biochemical abnormalities*, including increase in cyclic-AMP (cAMP) PDE (phosphodiesterase) activity, an abnormal cutaneous permeability barrier, where abnormalities of EFA (essential fatty acids) metabolism may explain the dry skin and the increase in transepidermal water loss characteristic of AD
3. *Additional pathogenic factors* such as food, inhalant and microbial allergens; also pollutants are a significant risk factor (Tables 4.16, 4.24)

Several factors suggest a pathogenic role for IgE-mediated sensitization in AD; Table 7.3 [33, 36, 37, 146, 192, 197, 216, 280] summarizes some important features that speak in favor of the *role of IgE antibodies*, which recently has been further strengthened in view of the following points:

- Understanding the function of CD154, which is known to play an important role in *the induction of the synthesis of IgE also found in peripheral tissues such as the skin* (Chap. 1)
- Identification of LCs in vivo [90]
- Presence of IgE anti-*S. aureus* [151] and anti-*Candida* [180] in patients with skin colonized by such agents

The evidence against a pathogenic role of IgE antibodies in AD is summarized in Table 7.4 [46, 188]: the 15%–20% of AD children with no signs of IgE hyperproduction and IgE hypersensitization may form a subgroup of children with nonatopic AD. Furthermore, several investigations confirm that some immune defects contribute to IgE dysregulation: the observation in patients with PID of high serum IgE concentration and clinical patterns indistinguishable from those seen in AD, as well as bone marrow transplantation data [235], suggest that a genetically inherited bone-marrow-derived defect is central to AD immunopathogenesis. These observations lead us to conclude that the cause is outside of the skin [226].

Immune Dysfunctions

Cell-Mediated Immunity

Several lines of evidence suggest that a variety of qualitative and/or quantitative immune abnormalities demonstrated in vitro and in vivo (Table 7.1) are mostly correlated with decreased PBMC proliferative responses to mitogens and antigens [255]. As a consequence, in AD patients T cells express low amounts of ILs [165] and less IL_1 than healthy subjects [20], whereas high concentrations of IL_2R are frequently observed when the dermatitis is most florid and are correlated with the clinical pattern's extension and severity [299]. IL_2R is significantly associated with CD30: the presence of high amounts of sCD30 in atopic children seems to confirm the role of this molecule as an activation marker useful for in vivo evaluation of a Th2 immune response [75].

Perhaps more substantially, the absolute number of T cells appears to be decreased, especially that of T-cell subsets. The analysis of the Th1 subpopulation shows that 52% of T cells are Th2, 44% Th0, and only 4% Th1 [66]. Studies on skin-derived Der p-specific T cells in AD subjects reveal that 42.2% of T clones express the Th2 phenotype and only 11.5% the Th1 phenotype [220], or 70% the Th2 and 15% the Th0 subpopulations [285]. Th1 dysregulation is mainly associated with that of the CD8 subpopulation, since CD8 levels in the cutaneous infiltrate is much lower than CD4 levels [165]. The CD4/CD8 ratio in T-cell clones may be increased up to 200% compared to controls [290]; however, other researchers were unable to reproduce these findings [181, 261]. Studies using AMLR (autologous mixed lymphocyte reaction), which is thought to represent an inducer circuit for the activation of $CD8^+$ effector cells [146], concluded that in T allergen-specific clones of atopic donors 92% were CD4 and only 8% CD8 (11.5:1). Subsequently, employing more precise methods such as flow cytometry (FC) and a particle counter (PC), the ratio was 4:1, with CD4 cell rates significantly higher in chamber fluids than in peripheral blood, while the reverse

Table 7.5. Immune alterations in children with AD (%)

	Active phase Mean±SD	Quiescent phase Mean±SD	Normal Mean±SD
Neutrophil chemotaxis	58.18±18.75	57.13±14.93	89.03±5.19
Cytotoxic activity	20.42±4.41	26.61±4.71	44.74±5.18
	Disease severity (Mean±SD)		
	Mild	Moderate	Severe	Normal
Cytotoxic activity	25.04±1.87	20.39±3.29	16.24±2.77	44.74±5.18

Data from [52].
Affected vs normal, $p<0.0001$.

was true for CD8 lymphocytes [307], even if the CD8 impairment could be attributable to LTB_4 [72].

From a *functional* point of view, the regulation of in vitro IgE synthesis by CD8 cells, and the generation of concanavalin A (Con-A)-activated T lymphocytes in atopic subjects appears to be abnormal [260]. Such a deficit could occur in atopic individuals because of the altered PGE_2 regulation of thymic CD8 subsets, with the consequent reduction of receptors on T cells [48, 165]. FC shows a decrease in CTLs expressing $S6F1^{bright}$ [162], a characteristic that, along with the similar impairment of NK-cell activity and according to the AD active and quiescent clinical stages as seen from Table 7.5 [52], may partially account for the increased susceptibility to viral infections, particularly with *herpes simplex* (HSV) [35]. The selective alterations of NK-cell activity may be the result of the severity of AD more than the dysregulation of IgE antibodies, or of T-cell subsets [255]. In this regard, NK-cell activity can be reduced in vitro by adding PGE_2 and restored by IFN-β and IFN-γ addition [304].

Suggestive findings support the concept that CMI abnormalities are primary, having been observed in neonates with FHA, but without universal results [255]. Recent data suggest that there is no definite data corroborating such a defect: it is true that in healthy infants with AD aged 0–1, the mean level of CD8 cells was 5.5%, but this concentration subsequently increased to 7.5% [303]. In other children aged 0–6, there were no quantitative deficits of CD3, CD4, CD8 and CD19 [160] (Tables 1.34–1.36). On the other hand, it is difficult to demonstrate that AD has an earlier onset when a CD8 T-cell number and function deficiency with a parallel increased ratio of CD4 T cells to CD8 cells is reported at birth or during the first months of life. More crucial are the *qualitative* deficits. Available data suggests an immune imbalance in lymphocyte activation such as the decreased function of CTLs (which again may account for the increased frequency of viral infections), in addition to the increased numbers of non-T non-B cells and the restoration in vitro of both the number and function of lymphocytes [57].

However, AD cannot be simply defined as a clinical manifestation of decreased CMI. More important, immunohistological studies of the cellular infiltrate and studies directed at circulating parameters of CMI indicate a vigorous T-cell activity in AD lesional skin [25]. Increased T-cell reactivity is correlated to the predominant Th2 phenotype, which *down-regulates Th1 cell function and activation.* As Table 1.10 shows, Th2 lymphocytes secrete a wide spectrum of ILs, especially skin-derived IL_4 that affects the switch of immature B lymphocytes to antibody-secreting cells and activates IgE receptors on LCs and monocytes infiltrating lesional skin, automatically antagonizing the expression of Th1 and IL_2R. IgE synthesis is promoted by IL_4 and inhibited by IFN-γ [308]. In addition, there is a *significant relationship between the increased IgE and IL_4 levels.* Consequently several factors are likely to play a role:

- *Increase in Th2 subsets* secreting IL_4 and IL_{10} induced by IL_1, an increase underlying the aberrant IL production profile that promotes the suppression of IFN-γ production [145, 281].
- *Establishment of a Th2-like T-cell IL* production, due to specific clones of allergen-specific $CD4^+$ T lymphocytes with an excess of Th2 [127], which drive an uninterrupted production of both IgE and ILs [155].
- *Skin-infiltrating Th2 cells* after allergen exposure secrete IL_3-IL_6 and GM-CSF, which favor the migration, differentiation, and survival of IgE and eosinophils [25].
- *Increase in PDE activity* by stimulated T cells, which has been suggested to direct differentiation into Th2-type cells, with a high IL_4 production [24].

Interestingly, *the demonstration at the skin level of the concomitant release by PBMCs of atopic donors of reduced quantities of IFN-γ and of substantial amounts of IL_4* [122] was detected only 2 h after the antigenic challenge in house dust mite (HDM) allergen-induced dermatitis in atopic subjects, even before the gene expression [322], and in highly atopic children with a mean age of 5.4 years [267]. Thus in all likelihood, *the relevant expansion of allergen-specific Th2 cells, through the up-regulation of IL_4 levels, has an ongoing impact on the chronic stimulation of IgE synthesis, on the induction of*

Table 7.6. Multiple functions played by IgE in triggering AD lesions

Cells	Mediators/Function	Effects
Mast cell basophils	Histamine, eicosanoids, PAF, etc.	Pruritus, delayed reaction, contact urticaria
Langerhans cells	Antigen presentation, interleukins	Delayed hypersensitivity, regulatory role
Lymphocytes	Interleukins	Mediator release, IgE generation
Eosinophils	MBP, EDN, ECP, etc.	Inflammation

Data from [46].

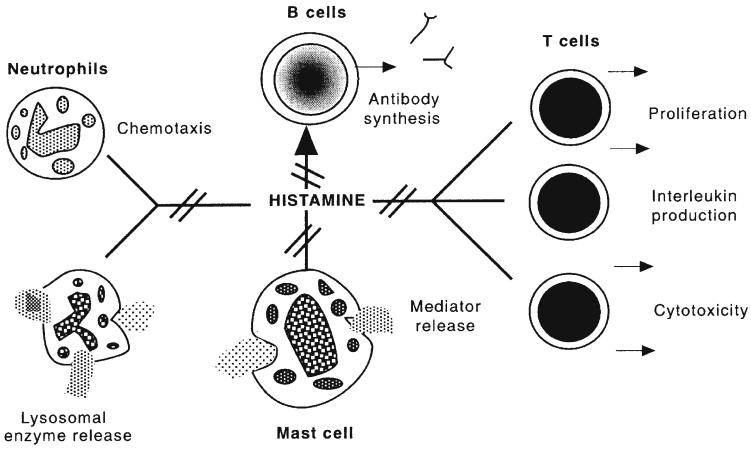


Fig. 7.3. Immunoregulatory effects of histamine. (Modified from [217])

Fcε receptors on B cells and on molecules crucial for antigen presentation, such as monocytes and LCs, as well as on the proliferation of mast cells and perhaps also of eosinophils through IL$_5$. The dominant role of T cells has been confirmed in vivo in children with AD by the spontaneous expression of IL$_4$ mRNA as compared to controls, although in neonates and children <10 years, IL$_4$ secretion was found to be significantly reduced [267]. The proof is that Der p-specific T-cell clones from patients with AD are Th2 cells expressing IL$_4$ and IL$_5$ but not IFN-γ, exactly the opposite of normal individuals [285]. Several studies have found significantly lower IFN-γ concentrations in AD [220, 284] and much higher IL$_4$ levels compared to controls (p<0.0001) [290]. As a consequence, *the Th2 T cells can be viewed as responsible not only for IgE-enhanced synthesis, but also for the high IgE levels in 80% of children*. The significant abnormalities that sanction the exit of Th1 lymphocytes and Th1-like T-cell ILs may be of paramount importance to the pathogenesis either of atopy or AD. A similar immune dysfunction is directly involved in children with WAS or AD [235].

Humoral Immunity: Role of IgE Antibodies

Peripheral B lymphocytes of patients with AD spontaneously produce elevated IgE levels. Since IL$_4$ plays a prominent role in IgE synthesis, its secretion increases in vivo, as shown by anti-IL$_4$ potential inhibition [291]. In addition, B cells of these subjects have an increased expression of the surface antigen CD40 that can be stimulated by anti-CD40 antibodies to generate IgE, also in the absence of IL$_4$, seemingly for a previous exposure in vivo to elevated concentrations of CD40 produced by Th2 T cells along with IL$_{13}$ [155]. Furthermore, IL$_4$ strongly induces the cytoplasmic expression of the ε-chain of FcεRI in DCs and up-regulates the expression of the skin's homing structures E-cadherin and CLA [79]. Thus *the increase in IgE concentrations is a direct result of the manifestations of immediate hypersensitivity and the inflammatory lesions provoked and maintained by mediator release, consequent to mast cell and basophil degranulation and eosinophil action* (Table 7.6) [46]. If IgE levels are so high, clearly the influence of inflammatory mediators released by cells involved in immune reactions will be outstanding. Histamine is elevated in these patients (1.2–5.2 ng/ml, compared to controls (<1 ng/ml) [217] and results (Fig. 1.56) indicate an inhibition of IFN-γ production via IL$_2$ suppression by Th2 T cells. Moreover, monocytes increasing the production of PGE$_2$ reverse the IL$_4$/IFN-γ ratio [48]: consequently, via the almost definitive exclusion of IFN-γ and the deficiency of IL$_{12}$ [145], histamine is able to up-regulate IgE synthesis, thus creating a vicious circle. Histamine has a marked positive effect on NK cells, while it down-regulates the proliferation of B and T lymphocytes and the recruitment of neutrophils and monocytes (Fig. 7.3) [217]. Neutrophil chemotaxis was decreased in 26 out of 34 children (76.5%) and its level was >70% below the control level (Table 7.5).

Several lines of evidence indicate that the intervention of IgE-mediated mechanisms in AD appears to be unpredictable, because AD is not a conventional DTH: the routine histological sections show the characteristics of a type IV reaction, not dominated by IgE antibodies [149]. However, IgE-mediated mechanisms play a role in the pathogenesis of the skin's inflammatory response in AD, as indirectly confirmed by studies which have demonstrated that:

- *An IgE-dependent* LPR can lead to an IgE-mediated cutaneous reaction via a mechanism still triggered by IgE induced by skin allergens that link to mast cell FcεRI.
- *IL_4 release* at the skin level during a DTH could contribute to IgE-dependent cutaneous inflammatory responses being associated with activation of IgE-bearing LCs [20].
- *Children with AD* reacting to DBPCFCs were found to have a rise in plasma histamine compared to children not reacting to DBPCFCs [229] and an increase in skin reactions [230].
- *In areas without lesions,* skin biopsy specimens obtained before DBPCFCs do not reveal eosinophils, which instead infiltrate the erythematous skin several hours later [225].
- *Eosinophils* bind to IgE in AD lesions via both FcεRI and CD23 [263]. The studies so far reported have evidenced that FcεRII is also found on numerous cells ready to respond to IgE, which are very plausibly involved in reactions mediated by cytophilic IgE.

Within minutes of encountering allergens disseminated via the circulation (in ≈50% of cases of alimentary origin), *skin mast cells* found on dermal capillaries become activated by antigen-bridging of IgE molecules, which are bound to the cell membrane by FcεRI and locally release histamine and related mediators [117]. Skin mast cells can also be activated by nonimmunological stimuli such as viral proteins, components of bacterial membrane, anaphylotoxins generated by CICs via complement activation [185] and neuropeptides localized in the dermis, hence confirming more evidently the hypothesis of extended interactions between a peptidergic nervous system and immunocompetent cells: the prospect that mast cells also respond to peptides suggests that they are involved in the neuroimmunological axis [51]. On the one hand, such stimuli further aggravate the inflammation triggered by reagins; on the other hand, they can interfere even in isolation and independently of IgE [51]. Mast cells have an endless activity: it seems that their total number is increased at least in part by an active cell proliferation revealed by mitotic forms at the lesional level [259].

Following the initial, rapid release of plasma histamine and of preformed mediators [225], there is no change in complement activation products, circulating basophil number or total basophil histamine content [229]. Children develop a diffused pruritic, erythematous, macular or morbilliform rash, vasodilation (the initial flare) and increased capillary leakage (formation of an urticarial wheal), characteristic signs of a *type I immune response in the skin* [226]. The reaction peaks within 10–20 min and then the wheal becomes gradually less distinct: by 60–90 min the reaction is diffused, moderately erythematous and edematous, usually no longer pruritic. The initial erythema can occur within 1 h of ingesting an IgE-inducing food [231]. After about 3–4 h, *a mild, diffused pruritus begins and an erythematous or macular eruption heralds the ongoing LPR*. The clinical manifestations are preceded by progressive cellular infiltration, mixed and sequential, in correspondence with the primary lesion. Clinical symptoms are mirrored by the increase in histaminemia: in the first hours it is equal to 13.0±24 [49] to 39±6 [307] ng/ml, coordinately with mediator levels (PGD_2 312 pg/ml in the first 3 h) [49], by 5 h reaches its nadir (<1 ng/ml), by 12 h it rises from 6.72±3.4 [49] to ≈15 ng/ml [20]. LTC_4, LTD_4 and LTE_4 are all very active and potent vasodilators, as are histamine, bradykinin, PGD_2, PGE_2, and PAF (platelet-activating factor), chemotactic for eosinophils [62]. Inflammatory cells are recruited in the first 4 h after initial mast cell degranulation. The expression of adhesion molecules on HEVs is followed by a progressive recruitment first of neutrophils and then of eosinophils in the site of inflammatory reaction [225]. Eosinophils reach the skin 2–6 h after the allergen encounter [29], under the influx of a potent chemoattractant such as MCP-3 [325], as is the case with PAF and IL_8, and reach the greatest concentration with neutrophils within 6–8 h; afterwards activated eosinophils appear to be reduced in number [30] (Fig. 7.4). Eosinophils are thought to contribute to allergic inflammation by the secretion of ILs and mediators that augment allergic inflammation and induce tissue injury in AD through the production of reactive O_2 intermediates and release of their granule proteins [82] (Fig. 7.1b).

Skin biopsy specimens obtained from involved sites after 8 h reveal an infiltration of T lymphocytes (48%), eosinophils (27%), neutrophils (9%) and PBMCs (7%), which increase after 8–12 h essentially around the small vessels; 10%–25% of T cells express CD25 [226]. Numerically, neutrophils are 17×10^5 between 5 and 12 h without hourly differences [307], or up to $11.2\pm6.8\times10^4$ cells at 11–12 h [49]. In patients subjected to challenge, a notable increase in neutrophils is observed in positive compared to negative subjects [227], with ≈74% of the cells at the skin level [307]. Eosinophils and PBMCs total $3–4\times10^5$, significantly increasing from 5–8 h to 9–12 h: they number 9%–10% and 15%–16%, respectively, with significant increases only for PBMCs within the following 24–48 h [307]. At *the LPR peak*, eosinophils constitute >25% of the cells found in the infiltrate (up to $2.5\pm1.7\times10^4$ cells at 11–12 h) [49]. Moreover, $CD4^+$ are found, mostly activated and monocytes able to respond to IL_4 by FcεRII, and, according to some authors, an evident perivascular deposition of fibrin takes place, which persists generally during the following 24–48 h [226].

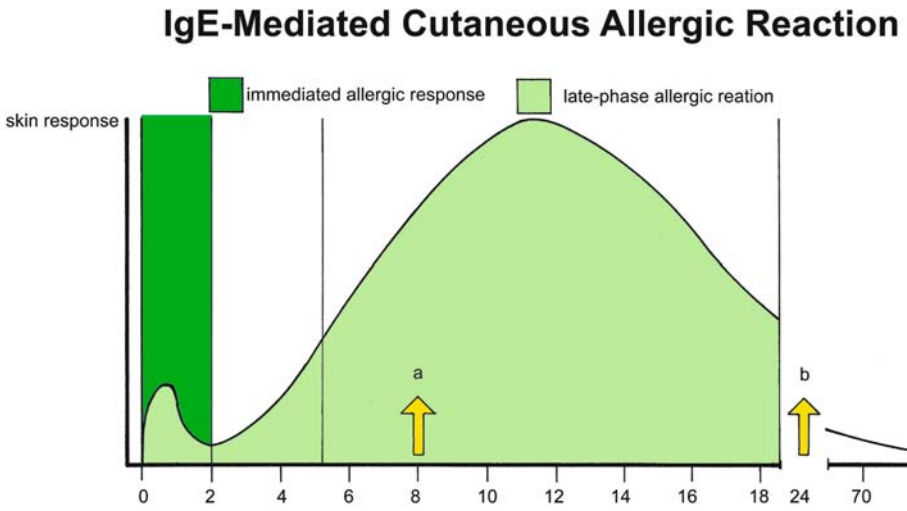
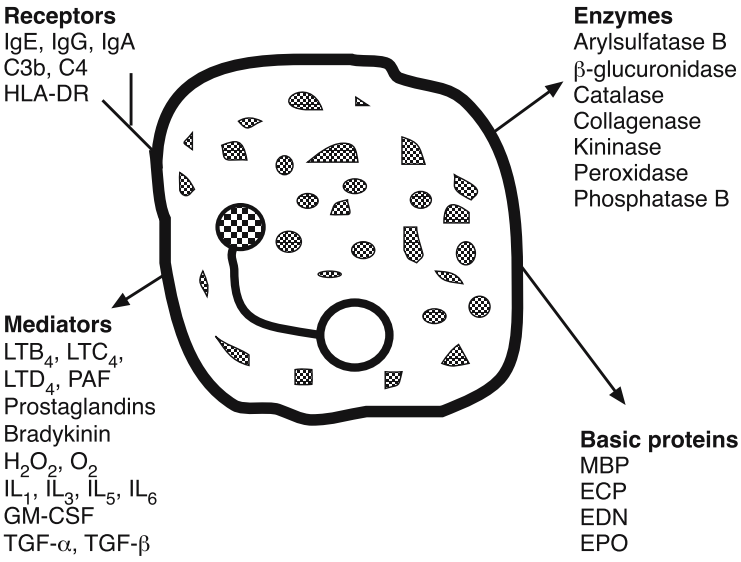


Fig. 7.4. The eosinophils in cutaneous reactions: *a* at 8 h some cells are degranulated without membranes, others have lucent granules together with intact neutrophils; *b* at 24 h the eosinophils appear to be intact, but some nuclei are dissolved

Fig. 7.5. Basic properties of eosinophils. *ECP* eosinophil cationic protein, *EDN* eosinophil-derived neurotoxin, *EPO* eosinophil peroxidase, *GM-CSF* granulocyte macrophage-colony stimulating factor, *MBP* major basic protein, *PAF* platelet-activating factor, *TGF* tumor growth factor. (Modified from [64])



The accumulation of inflammatory cells is promoted by mast cells, endothelial cells and likely by other cell sources that intervene to complete the pathogenic action of mast cells. These cells generate chemotactic factors to attract eosinophils and neutrophils to the serum. *Eosinophils*, sixfold more numerous in skin biopsies obtained from patients with AD vs controls, equal to 709±1,540 vs 115±107/mm^3 [64], present a clear chemotactic response to PAF, LTB_4, C5a, greater if pretractated with GM-CSF, IL_3 and IL_5 [30]. Expression of GM-CSFR β chain and the IL_3R α chain in isolated blood eosinophils was stimulated by IL_4, IL_5, and GM-CSF. Expression of bcl-2 and bcl-xL was also increased after stimulation with IL_4, IL_5, or IFN-γ [204]. In infants and children with AD, *plasma eotaxin levels* are significantly high, probably reflecting the chronic nature of eczematous AD lesions. Eotaxin promotes the selective recruitment of eosinophils [108]. Eosinophils release additional mediators: more PAF, leukotrienes, MBP, EPO, EDN (eosinophil-derived neurotoxin), ECP (eosinophil cationic protein), etc. [142]. MBP and ECP promote mucosal damage; in particular MBP cytotoxic activity is known to damage skin epithelial cells and correlate with disease severity by perpetuating inflammatory reactions [204] (Figs. 7.5 [64], 7.6). The infiltrating PBMCs contribute by producing toxic O_2 radicals [82]. Neither ECP nor MBP serum levels are so abundant in the skin of healthy subjects, contrary to affected patients (MBP 454±90 vs 687±299 ng/ml) [64]. ECP drives eosinophil activation, but not of all cells. Eosinophil analysis in AD has revealed fascinating insights from the recent differentiation of cells with a hypodense phenotype showing signs of activation (Fig. 1.35 c) and correlated with the disease state [157]: in particular eosinophils exhibit a

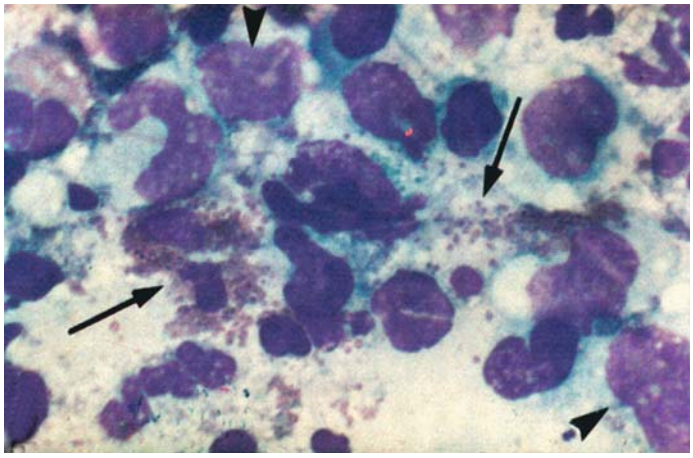


Fig. 7.6. Photomicrograph after a SPT to Der p in an atopic patient 24 h after the intracutaneous injection of PAF. Eight eosinophils are noted (magnification ×400): intensively degranulating (*arrows*), and scattered granules (*arrowhead*)

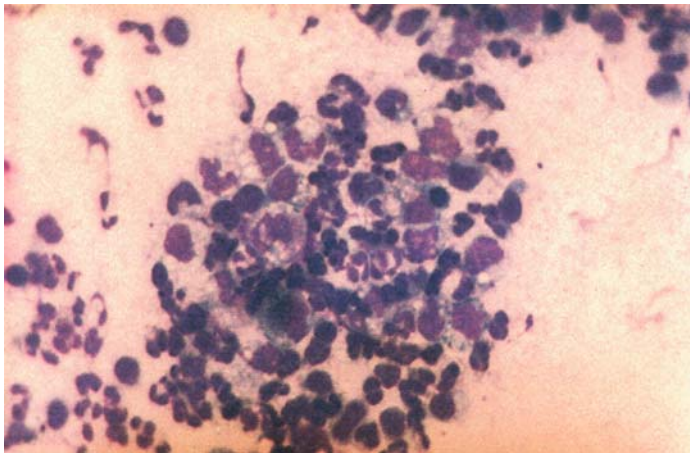


Fig. 7.7. Photomicrograph after the intracutaneous injection of PAF in a control nonatopic patient. The cellular infiltrate is composed of a majority of neutrophils, with a complete absence of eosinophils

normodense profile before challenge and change to hypodense after challenge [225]. *Neutrophils* (Fig. 7.7) are recruited to the site of immune inflammation by NCF (neutrophil chemotactic factor), thus contributing to the maintenance of inflammatory states, developing phagocytic activity and releasing lytic enzymes. No light has been shed on a possible correlation between the entity of their infiltration and DTH extension. However, MBP at this stage can stimulate an increase in expression of CD11b/CD18 and CD11c/CD18 adhesion molecules [172] (Table 1.44); PAF recruits platelets. During the ensuing 24 h, the infiltrating cell population again changes, and later biopsies show a predominance of a parvicellular mononuclear cell infiltrate, namely monocytes and lymphocytes, with *histological appearance virtually indistinguishable from a type IV, cell-mediated hypersensitivity reaction* [73], *type Th2 more than Th1* [148].

An analysis of the AD skin lesions does not allow simple classification into an IgE-mediated LPR vs a T-cell-mediated hypersensitivity reaction. However, in addition to IgE-dependent allergen-induced LPR, there are other mechanisms by which IgE can contribute to cutaneous inflammatory reactions. Previous considerations indicated that allergen-induced mast cell activation could be expressed by a skin LPR, by an extension of the IgE-mediated reaction and that cutaneous LPRs were provoked by mast cells and their chemotactic factors. However, it should be emphasized that there is disagreement as to whether LPR patterns exclusively induced by IgE-mediated mechanisms can be justified, substantiating the controversy on the specificity of the LPR found in involved skin: is this a true LPR? However, there is a clear relationship between the extent of the reaction on the one hand and allergen-specific IgE levels on the other, evidence of the allergen's consistent invasion [64]. Since the levels of PGD_2, the major marker of mast cells, are reduced to basal values, as are those of tryptase [51], it is doubtful that the origin of histamine, following a biphasic course, shows a new peak: the answer is in the further migration of basophils undergoing releasability when stimulated by IL_1. Recent reports have observed mast cell degranulation up to 48 h [185]. Such scientific investigations have re-evaluated the role of LPR, as it seems that it reflects the clinical disease better than the acute phase [142]. However, when LPR reaches its apex several cells are present only to a moderate degree: the neutrophils almost disappear [76]. Neither do they degranulate nor do the amounts of azurophil proteins show differences compared to con-

trols [191]. Eosinophil numbers decrease progressively after 14 h from allergenic stimulation [29]; however, their influx into the epidermis after 24 h is abundant and the infiltration with destructive proteins reaches its apex after 48 h [29, 126]. It was suggested that eosinophils were present but had degranulated during IgE-mediated skin reactions and so were no longer identifiable [125]: routine analysis cannot detect intact cells, although by immunohistochemical analyses their cationic proteins can be identified [329]. It has been hypothesized that eosinophils are associated with a previous mast cell activation [329] or with a preactivation state, making the cells particularly susceptible to the action of IL_5, GM-CSF, IL_3, TNF-α and PAF [125].

By employing immunofluorescent techniques, biopsies of eczematous skin lesions in areas involved in LPR have shown an extensive extracellular MBP deposition in the superficial dermis of atopic skin. MBP was not found in biopsy specimens from uninvolved skin sites in the same patients, a demonstration of local cell degranulation [142]. Similar results were observed testing the skin of pollen-sensitive patients with pollen extract or, alternatively, with buffer [329]. Therefore it is not surprising that in histological sections an elevated MBP content is found, secreted almost exclusively by eosinophils and to a minimal extent by basophils [64]. Cutaneous MBP depositions derive from the activation and degranulation of circulating eosinophils, since MBP is absent in noneczematous skin, indicating that its local accumulation is the result of selective deposition by infiltrating eosinophils and is not caused by nonspecific sequestration in the circulation [225]. Similar findings are not found in healthy patients or those suffering from unrelated skin diseases, such as ACD [127]. MBP releases histamine because of metachromatic cell degranulation, thereby inducing inflammatory reactions, remarkably contributing to the perpetuation of atopic skin inflammation and, in epithelial cell cultures, it provokes *tissue damage similar to that observed in asthma* [64]. ECP titers were significantly elevated in children with AD compared to controls [191, 257], correlated with peripheral eosinophilia, but not with IgE levels [257], nor with granulocyte numbers [126, 191]. In parallel, there is an extended deposition in the dermis of EDN extracellular granules, further stressing the role of EDN in AD [142]. MBP, ECP and EDN levels are closely interrelated, as in tissue deposition, with highly significant differences, above all the correlation between extension of the lesions and MBP levels [190]. Additional severe clinical consequences are elicited by the release of these cytolytic proteins, once deposited locally, since they concur notably to propagate inflammatory responses employing their immunomodulatory ability. ECP, promoting further mast cell histamine release, contributes to destruction of epithelial cells and possibly of keratinocytes [30]. The complete eosinophil degranulation reflects a high degree of activation and conceivably *hyperactivated eosinophils reach the respiratory tree, thus posing the basis of an association between AD and asthma* [238]. Particularly interesting is the hypothesis that eosinophils bind to T lymphocytes via CD54, with consequent, further release of ECP and EPO: their epidermal deposition after 48–72 h may indicate an immunomodulant effect elicited by eosinophils also involving antigen presentation [142] (Fig. 7.4).

Basophils play a key role in both symptoms and DTH pathogenesis: their degranulation is a delayed-type response with the same latency time and clinical evolution as CMI. Recent reports demonstrate that in vitro both IL_3 and GM-CSF, produced by T cells at picomolar doses, favor basophil migration expanding their chemokinetic activity [20]. This stresses that basophil activity should be considered a kind of response consequent to that of T lymphocytes, which form the immunohistological pattern of late reactions [323]. Basophils enrolled by specific ILs [155], chemokines (Tables 1.27, 1.28) and adhesion molecules [20] (Tables 1.20–1.23) accumulate in the seat of cell-mediated reactions, where they influence the inflammatory reactions elicited via chemical mediators with which basophils are equipped, namely histamine and PGD_2, reaching the highest amounts within 11–12 h after a small rise at 8 h, similarly to PBMCs [20]. The findings explain why basophils, histamine and IL_1 are found in the skin windows [20]. In more severe cases, the circulating basophils are primed in vivo, releasing histamine and the potent vasodilator LTC_4, more readily than in children with less severe AD, especially in response to C5a [119].

Here, however, a succinct description of the wide spectrum of eicosanoid contributions to inflammation includes LTD_4 and LTE_4, which cause a skin lesion of the Lewis type: triple response with erythema, vasodilation and wheal persisting for 4 h with a central halo caused by vasoconstriction, accompanied in the case of LTD_4 by dermal edema, evident dilatation of venules and capillaries, and endothelial activation. LTB_4 elicits a transient response followed within 3–4 h by a dermal infiltrate formed in prevalence by neutrophils. PGD_2 produces edema, vasodilation and, like LTD_4, endothelial activation [72].

The *increase in serum IgE antibodies* could elucidate the manifestations of immediate hypersensitivity and the inflammatory lesions provoked and propagated by mediator release; it could be cyclically released by metachromatic cells and also by aspecific mechanisms. However, the elevated serum IgE antibodies cannot account for the eczematous lesions corresponding to a DTH mechanism.

Role of T Lymphocytes

SIS-characteristic T lymphocytes (4×10^{12} in the skin of a normal adult) [25], are the only cells able to directly recognize the presented allergenic peptides of APCs. They have a protracted immune memory, can prolifer-

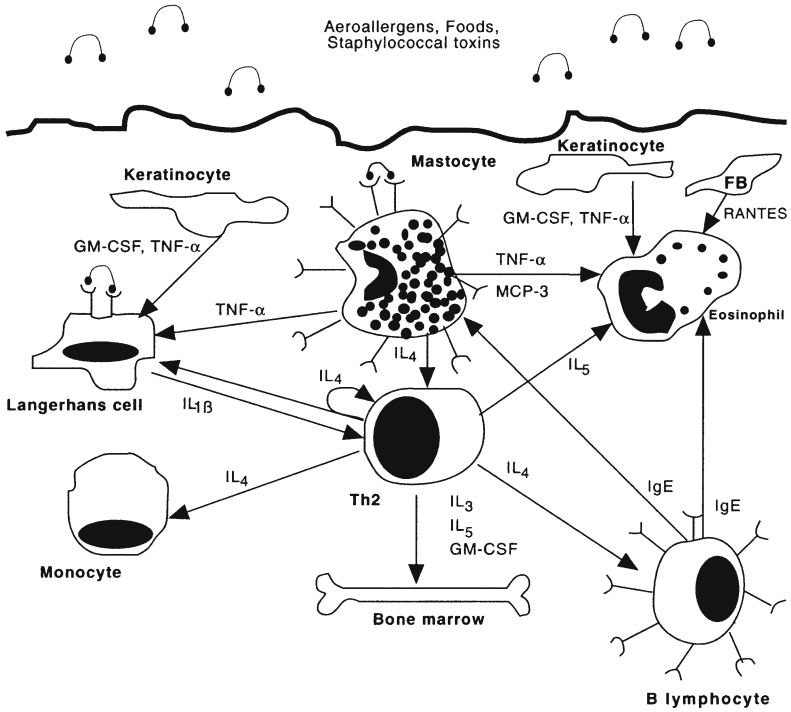


Fig. 7.8. Cell interactions in atopic dermatitis. *FB* fibroblasts. (Modified from [24])

ate very easily, and recirculate to be rapidly recruited to reach target tissues. Their activation is demonstrated by high levels of CD62E and CD49d, expressed by > 90% of skin T cells [307], via the release of different ILs, *relating IgE synthesis, eosinophils, mast cells and basophils.* Th2 cells control IgE synthesis and eosinophil infiltration, exercising a direct effect on immune inflammation, bone marrow production and mast cell maturation and mainly eosinophil maturation, as well as playing a key role in prolonging their local survival by means of IL_{3-5} and GM-CSF [260]. Blood and tissue eosinophils from patients with IgE-mediated allergic responses (thus distinguishing a non-IgE-mediated AD) express CD137, a member of the TNF superfamily. Because CD137 activation blocks GM-CSF–mediated survival in vitro, it can be speculated that the lack of CD137 expression on eosinophils contributes to the excessive tissue eosinophilia [100]. Th2 cells interact with eosinophils and influence their growth, mobility, tissue localization and priming. IL_4 is a growth factor for T lymphocytes in AD; all allergen-specific Th2 clones generate IL_4, IL_5 and GM-CSF, but not IL_2 or IFN-γ. However, IL production becomes significant by adding IL_2 to the culture medium, demonstrating that T cells infiltrate skin lesions after 12 h [30] or after 12–24 h [128]. IL_2 and IFN-γ are primed in normal subjects, but to a trivial extent in patients with AD [292]. A fall in IL_2 levels is also driven by poor PBMC production [126], unlike IL_4 and IL_5 [293]. T-cell infiltrates found in acute and chronic AD skin lesions are in close proximity to the epidermis with subsequent dermal invasion under RANTES (now CCL5) stimulation [325]. T cells consist primarily of $CD3^+/CD4^+CD45RO^+$, which express CD25, CLA and HLA-DR, acquiring specificity in view of the substantial difference in the CD4/CD8 ratio compared to the blood ratio. In children, CD45RO were 87.9%±7.6% of CD4 vs 6%±3.7% of CD45RA [303]; employing CF+CP, the proportion was 9:1 and in blood ≈2:1 [307], a reversed ratio in AD age of onset in infants (Table 2.6). A part of lymphocytes is primed and expresses IL_2R [76], an observation confirmed by an increased expression of $HLA\text{-}DR^+$ from endothelial cells and of CD4 antigen from LCs [126]. Even if there is no clear correlation between extension of clinical lesions and the number of T cells, it is significant that the association between $CD4^+$ and eosinophil number after 24 h is still seen after 48 h [76].

Figure 7.8 summarizes the cell interactions reported in AD, also showing those between keratinocytes and ILs [125]. Although they do not express Fas and Fas ligand, T cells infiltrating the skin of AD patients do not undergo apoptosis, since they are protected from apoptosis by ILs and extracellular matrix (ECM) proteins. Both $CD4^+$ and $CD8^+$ isolated from skin and CLA^+ $CD45RO^+$ T cells isolated from peripheral blood secrete high concentrations of IL_5 and IL_{13}, thus prolonging the lifespan of eosinophil and inducing IgE production [3].

We can speculate that *ILs of cutaneous derivation* may be released during the development of T-cell infiltration in response to allergenic stimuli, *with patterns similar to those of Th2 cells* such as IL_{3-5}, IL_1, and IL_8 endowed with chemotactic activity for lymphocytes. GM-CSF and IL_7 are able to stimulate some T cells as growth factors [155]. Other cutaneous signals to prime T cells are mediated by IL_6, in both the acute and late phase, in close correlation with the eosinophil influx; however, no

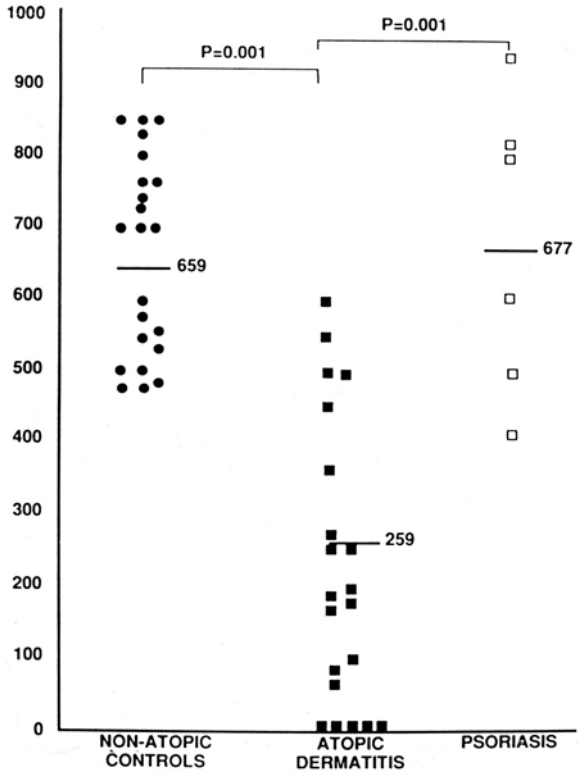


Fig. 7.9. IFN-γ levels in peripheral blood mononuclear cells (PBMCs) of patients with AD. PBMCs from patients with AD produce low IFN-γ levels. PBMCs were stimulated with Con-A (Concanavalin A) for 2 days and then assayed for IFN-γ content; IFN-γ levels of culture supranatants from patients with psoriasis and nonatopic controls are significantly higher than the levels in patients with AD

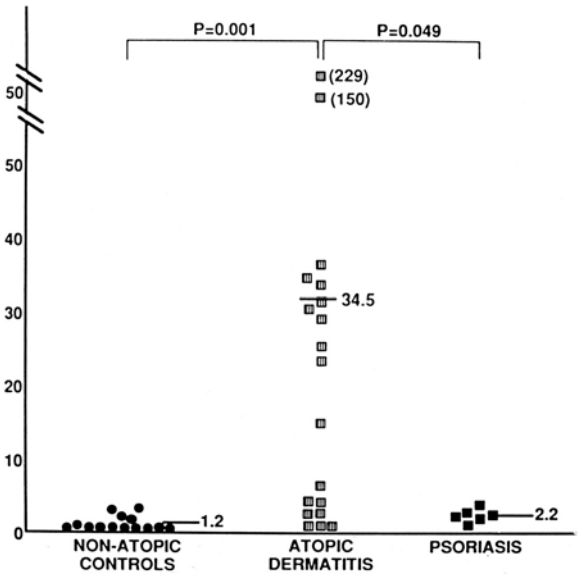


Fig. 7.10. IL_4 levels in PBMCs of patients affected with *AD*. PBMCs of patients affected with AD produce high levels of IL_4. PBMCs were stimulated with Con-A for 2 days and then assayed for IL_4 content; in the supranatants from patients with psoriasis and nonatopic controls, IL_4 levels were significantly lower than the levels in patients with AD

chemotactic activity of IL_6 for these leukocytes has been demonstrated, so we can assume that an association is shared, for example with PAF [155]. Skin biopsies observed after challenge have revealed cells with positive signals only in Th2-like T-cell ILs [128], an unsurprising result since Th2 is the greatest majority of allergen-specific cutaneous CD4 T cells [66, 122, 284, 285]. Indeed PBMCs of patients with AD exhibit negligible IFN-γ levels (Fig. 7.9) and elevated IL_4 levels (Fig. 7.10). Adding an anti-IL_4 to supernatants of patients with AD and controls, IFN-γ concentrations significantly increase only in the controls (Fig. 7.11); instead IgE levels are significantly higher in patients with AD than in controls [122]. Therefore there is a skin LPR mediated by Th2 secreting IL_4 and IL_5, histologically differentiable by the IFN-γ-modulated Th1 LPR [269]. This finding suggests that two histologically indistinguishable cutaneous T-cell-mediated immune reactions exist: the one mediated by IFN-γ-secreting Th1 cells found in conventional DTH reactions, the other mediated by IL_4 and IL_5-secreting Th2 cells and involving allergen-induced CMI [147].

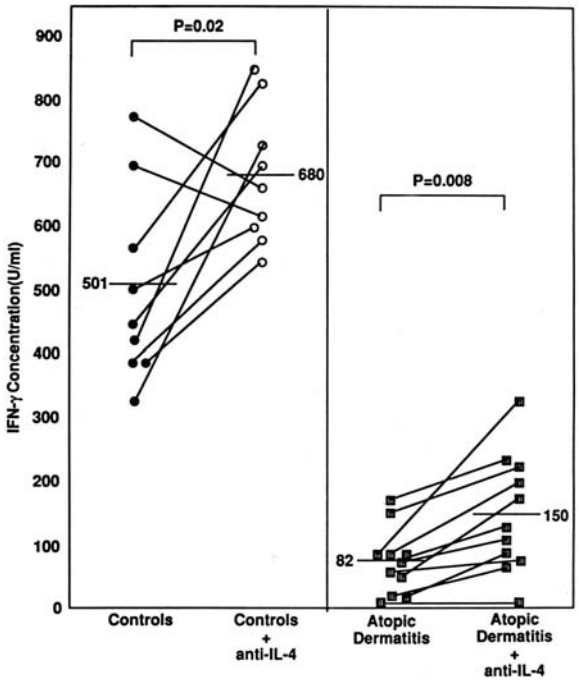


Fig. 7.11. IFN-γ levels in PBMCs of patients with AD. Effect of anti-IL_4 on IFN-γ synthesis PBMCs were stimulated with Con-A for 2 days in the presence and absence of anti-IL_4 and then assayed for IFN-γ content. Anti-IL_4 produced a significant increase in IFN-γ levels in the supranatants from controls and patients with AD. However, in patients with AD, even in the presence of anti-IL_4, Con-A-induced IFN-γ production by PBMSs was significantly lower than the levels in nonatopic controls

How we can distinguish these two phases? Th1 cells found in DTH reactions play the role of effector cells, inducing eczematous lesions [149], and IFN-ɣ is critical for the induction of CD54 by keratinocytes and subsequent accumulation of inflammatory cells in involved skin [86]. The apparent contradiction of LPR with both Th1 and Th2 cells can be unraveled since Th2 cells have been found to be as effective as Th1 cells in inducing skin inflammation [174], recruiting inflammatory cells and inducing IgE via their own ILs [269]. In children with AD and FA, studies on allergen-induced lymphocyte proliferation have demonstrated that in addition to IgE-mediated mechanisms Th1 cells could also be active, producing more IL_2 and IFN-ɣ than in controls [135]. There appears to be a second phase of skin inflammation where Th1 cells predominate in cutaneous lesions [269], and Th1-like ILs such as IL_2 (and sIL_2R) and IFN-ɣ but also IL_4 are found [86, 92, 128]. We can thus elucidate LPR in a biphasic sense, distinguishing a first phase dominated by Th2 followed by a switch to Th1 cells, which worsen the inflammatory state, or show *an effort by the organism to restore homeostasis at the skin level, which fails in subjects with AD* [269]. The existence of both phenotypes can therefore be explained by imagining Th2 cells in an apparent effort to inhibit Th1 cells, to limit tissue damage subsequent to their activation. We have hitherto accentuated the severe IFN-ɣ deficit in at-risk neonates and infants, intensified by PGE_2 and histamine, and record ostensibly contradictory results: on the one hand there is an inhibited IFN-ɣ synthesis parallel to that of IL_{12} by both IL_4 and IL_{10} [145], on the other there is a greater percentage of cells producing IFN-ɣ in children with severe AD compared to normal controls [266]. Data on T cells are still under discussion, active Th2 cells being, in most cases, restricted to allergic reactions, but Th1-like T-cell ILs respond to other allergens, including *Candida* and tetanus toxoid [25], *thus confirming that Th0-cell differentiation into Th1 or Th2 cells is programmed by genetic factors and allergen characteristics* [260], as for Der p 1.

Langerhans Cells

The LC evolutive mechanisms are of indubitable interest. GM-CSF and TNF-α cooperate in LC generation by stimulating undifferentiated hemopoietic precursors to proliferate and differentiate in cells with phenotypic and functional characteristics of human LCs [47]. Evidence has been put forward that, in response to allergens and/or pathogens, the ILs deriving from keratinocytes may prime precursors to transform into effective APCs, establishing an important point of conjunction between immune allergen-specific systems and nonimmune allergen-specific systems [47]. LCs are present in skin reactions 6 h after patch testing (PT) with Der p, exclusively in the epidermis, then in the dermis after 24–48 h [264]. LCs account for some 3 %–8 % of the epidermal cell population; however, based on the increase in number observed using histofluorescence methods, they play a primary role in AD and probably also in the exacerbations, given that they are able to penetrate the skin after epicutaneous contacts, since LCs provoke delayed cutaneous reactions specific to AD patients [272]. In atopic epidermis, $CD1^+$ cells with FcR bind to IgE in patients with elevated serum IgE levels [90]. LCs are HLA class II-positive, bear receptors for the Fc fragment of IgG and C3, and share several properties with lymphocytes localized in lymphoid organs, namely $CD45^+$, $CD25^-$, $CD23^+$, $CD54^+$ [73] (Table 1.28). *IgE of atopic subjects binds to LCs bearing FcεRI on their membrane* [18, 90], this expression is increased by IL_4 and IL_{13}, and *T-cell responses to Der p can only be observed in the case of IgE-positive LCs* [27, 30]. Only FcεRI-bearing LCs [173] in the epidermis carry on the APC function for *T lymphocytes*, as well as the synthesis of various mediators, especially of PGE_2, thus indicating a *correlation between IgE and CMI* [73]. FcεRI on LCs has a higher level in AD than in other skin diseases, since it is proposed along with FcɣRII for the differential diagnosis [315].

LCs are involved in immunological surveillance of the antigens invading the skin, since the $FcεRII^+$ cells, associated with the chemoattracting activity of IL_1, migrate from eczematous epidermal lesions to draining lymph nodes, where antigens undergo processing [253]. However, LCs are equipped with poor phagocytic activity, thus processing may be done by endocytosis, followed by partial degradation and recycling of antigenic peptides [253]. Since the antigen presentation to T cells is the best-known LC function, these peptides are presented to T cells. LCs acquire HLA class II molecules when stimulated by $IL_{1β}$ and sensitize "virgin" T cells when stimulated by GM-CSF [155], presenting antigens directly to Th2 cells [173] and triggering a DTH, as noted above. GM-CSF augments LC activity and functionality and along with IL_1 enhances their differentiation and maturation [272]. Similarly to IgE bound to dermal mast cells, IgE bound to LCs are also specific for aeroallergens, as demonstrated by Der p 1 antigens in close association with LCs after topical antigen application to the skin (see "Role of Aeroallergens"). Evidence has been put forward that LCs play a crucial role in AD pathogenesis. However, an intriguing area is their binding to IgE molecules, since it is unclear whether LCs can produce and activate ILs [272]. It is known that reacting in a specific way with environmental allergens, LCs are involved not only in the induction of skin responses, but also in the regulation of IgE synthesis by B cells, thus delivering *proof of IgE's critical role in the pathogenesis of skin lesions*. It can be hypothesized that via such a mechanism, LCs form a pivotal link between type I and type IV reactions in AD [28]. Furthermore, it is interesting that *$CD4^+$ repeatedly stimulated by LCs may differentiate into Th2 cells* [99]. Interactions of LC IgE^+ (and of macrophage IgE^+) with allergens (and allergen-specific

T cells) may lead to release of IL_1, which stimulates histamine production and promotes proliferation, activation and secretion of other ILs with a pattern similar to that of Th2 cells, as well as of HRF, which primes basophils to release histamine. IL_4 contributes to FcεR increase on macrophages, DCs, LCs and B and T lymphocytes, and is able to induce CD23 on LCs [148]. The activation of IgE-bearing LCs and macrophages by allergens could thereby contribute to the skin inflammation associated with AD. Eosinophils infiltrating cutaneous tissues express CD54 and HLA-DR [142]: the close contact with LCs 24 h after PT [30] thus indicates interactions between these cells. If eosinophils express receptors for both IgE and HLA-DR effectively, they would match APCs as LCs, able to present antigens with a class II-restricted mechanism, and by means of these surface structures, they could be able to drive a lymphoproliferative response.

Interleukins and AD

In summing up the reviewed data on the role of ILs released by epidermal cells in the pathogenesis of AD (Table 7.2), TGF-β and IL_{10} have been identified as inhibitors of several ILs [260], and different patterns have been observed during AD acute and chronic skin lesions. In the first, IL_4/IL_{13} and IL_{16}, and in the second IL_5 and IL_{12} are prevalent compared with uninvolved skin [92, 150], but IL_4 and IL_5 are found after 24 h during the LPR [293]. In neither phase is there IFN-γ expression [148], nor is its production modulated by IL_{18}, unlike that of GM-CSF [26] and even more of IL_{10} [184]. IL_{31} induced by Th2 in monocytes and epithelial cells may be involved in promoting AD development and epithelial responses characteristic of an atopic disease [68]. Furthermore, transgenic mice overexpressing human caspase-1 in keratinocytes – resulting in IL_{18} overrelease – spontaneously develop inflammatory cutaneous changes with high serum levels of IgE and IgG_1 [178], or IL_{18} overexpression may occur independently of IgE or stat6-mediated signaling [136]. IL_{18} causes skin changes in the absence of IgE/STAT6; thus, AD-like inflammation is initiated by overrelease of IL_{18} and accelerated by IL_1 [108]. IL_{15}, a Th1-supportive IL that upregulates IFN-γ, is also down-regulated in individuals with AD and may contribute to elevated IgE [187]. IL_{16}, an IL produced by a variety of immune cells as well as epithelial cells, is a potent chemoattractant for $CD4^+$ T cells in acute skin lesions and may promote a CD4 T-cell infiltration in these lesions, while GM-CSF may enhance eosinophil and macrophage survival in chronic AD lesions [150]. IL_{16} released from LC after allergen-mediated activation through FcεRI may link IgE-driven and cellular inflammatory responses in AD [208]. The significance of ILs in atopic skin is enhanced by the complex network regulating the immune responses in AD: apart from the role, both cardinal and opposed, as discussed earlier, of IL_4 and IFN-γ [308] in the synthesis of IgE and stimulation of effector cells, more ILs are involved in type I IgE-mediated reactions produced by numerous cells including keratinocytes [10]. ILs are important factors that are active in the initiation of immune responses in cutaneous lesions, via modulation of the functions and vitality of LCs as APCs. IL_1 released relatively later in the inflammation sites (10–12 h) may contribute to inviting both adhesion increase and transmigration of neutrophils [20]. IL_3 amplifies mast cell growth and attracts and stimulates basophils; IL_6 enhances the activity of other ILs; IL_8 encourages chemotactic activity on basophils and neutrophils, but inhibits the adhesion to endothelium of both cell types; IL_{10} has a marked effect on the expression of adhesion molecules on endothelium; CD54 and CD62E, and even more TNF-α, prime granulocytes and LCs [155]. TNF-α released by mast cells supports CD62E expression on keratinocytes, facilitating their interactions with $CD11a/CD18^+$ (LFA-1) [300]. IL_4 is an important growth factor for Th2 cells of skin origin: a part of Th2 cells, once activated, leave the atopic skin, reach the bloodstream, produce additional IL_4, and after encountering LCs may stimulate B lymphocytes to generate sIgE in afferent lymph nodes, concurring to elucidate the high IgE levels in children with AD [146]. Moreover, IL_4 regulates IgE synthesis, and is capable of influencing the isotype switching from IgM to IgE/IgG_1 [148]. Table 7.7 outlines the ILs created in AD children by T lymphocytes [308, 309].

Table 7.7. Der p-specific T CD4 lymphocyte clones

IL	Atopic patients	Nonatopic patients
IL_2	+	+++
IL_4	+++	–
IL_5	+++	+
IL_6	+++	++
GM-CSF	+++	+++
IFN-γ	+	+++
TNF-α	+++	+++

Modified from [308, 309].

Interactions Between ILs and Adhesion Molecules

In the first phases of inflammation, keratinocytes releasing IL_1, IL_{10} and TNF-α focus on the regulation of adhesion molecules, chiefly CD54 and CD62E, and are therefore able to influence leukocyte adhesion to epidermal and/or immunocompetent cells and *T-cell localization at the site of lesions*. The prominent role of

CD62E is supported by the evidence that CLA on T-cell membrane is also its ligand, and it is tempting to speculate that CLA with its specific tropism for the skin could materialize the basic clinical expression of AD [149]. As detailed in Chap. 1, CD54 and CD62E mediate the adhesion of all cell types, and CD106 is a source of a selective action for basophils and eosinophils, promoted by IL_4 even in the absence of CD54 and CD62E [20]. Adhesion molecules activate the dermal vascular endothelium, which accentuates the release of CD54, CD62E and CD106, which are found both in the interstitial fluid and in the circulation [24]. Such events allow the adhesion of circulating lymphocytes, $CD4^+CD45RO^+$, and granulocytes to the dermal epithelium; the concomitant secretion by keratinocytes of IL_1 and IL_8/NAP-1 drives a true directional migration of T cells toward dermal sites. CD54 serves as a ligand for CD11a/CD18 and CD11b/CD18, and CD49d/CD29 binds to contrareceptors CD106 and fibronectin [20] (Table 1.44). CD54 binds T cells to epithelial cells, has been shown to serve as a binding site of T-activated lymphocytes to epidermal cells and can promote the transit of inflammatory cells through the endothelium (along with CD62E and CD106) [10]. A similar effect is obtained by stimulating epithelial cells by allergens, demonstrating that the expression of specific adhesion molecules drives the migration and adhesion of various leukocytes and suggesting that endothelial activation in the LPR by ILs plays a cardinal role in the *migration of inflammatory cells at the skin level*. The adhesion molecules allow eosinophils and/or neutrophils to migrate across epithelial layers: only eosinophils express CD49d and are stimulated by RANTES, the most effective chemokine in this site [20], and by MCP-3 [325], released by fibroblasts and basophils (Table 1.55) (Fig. 7.8); thus these mechanisms also drive the eosinophil selective recruitment. Since the treatment of endothelial cells with an anti-CD106 antibody leads to inhibition of eosinophil and basophil adhesion induced by IL_1, but not of neutrophil adhesion [20], it is evident that when CD106 is expressed by epithelium, it represents a further etiopathogenic mechanism, allowing these cells to directly recruit specific cells. Moreover, if the pretreatment with antibodies recognizing CD54, CD62E, CD102 and CD106 inhibit the adhesion of only 60%–70% of basophils, there should be additional ligands on endothelial cell surface with specificity for basophils [20].

IgE and Histamine-Releasing Factors

Different factors are able to drive histamine release: among them is the mediation of increased metachromatic cell releasability secondary to the generation of histamine-releasing factors (HRFs), generated by mononuclear cells or platelets. Some HRFs have been shown to bind to surface-bound IgE molecules and activate and stimulate metachromatic cells to release histamine (releasability). HRFs appear to match CCL chemokines, thus confirming in a hypothetical way that histamine in AD stems from cutaneous mast cells and basophils. It has been demonstrated that a histamine release inhibition factor (HRIF) related to NAP-1/IL_8 at the same time as HRF and HRIF could regulate HRF activity. Peripheral blood basophils of nondieting patients with AD and FA show high spontaneous basophil histamine release (SBHR). In contrast, a SBHR is close to normal in FA patients whose skin lesions have cleared on an appropriate elimination diet for at least 1 year [225]. Subsequently an HRF was reported in children with AD, even those who were FA-free [232]. Consequently, in children with both disorders, circulating food allergens could induce the production of HRF in vivo, which could activate or lower the threshold of metachromatic cell activation. HRF could also account for either the increased basophil releasability seen in some children with AD with a particular severity of clinical manifestations [119] or the increased SBHR production in vitro by basophils in subjects with FA [206]. In these children, HRF could account for the worsening pruritus and cutaneous eruptions [119]. It was postulated that allergic patients have a subset of IgE molecules (IgE^+) [156] that are different from the IgE of normal subjects, to which HRFs could bind in patients with AD associated with FA [225]. The finding that IgE molecules from atopic patients bind HRF but that IgE molecules from nonatopic subjects does not suggests that IgE molecules have a great deal of heterogeneity [156]. These patients could have a hypersensitivity mediated by IgE via HRF, thus presenting sIgE for foods, IgE^+ dependent on the patient's diet, and HRFs produced by PBMCs [225].

Anomalies of IgGs and in particular of IgG subclasses after having found elevated total serum IgG and IgG_4 levels, have been often regarded as a constitutive element of the immune defect of the patients with AD: in about 20% of patients total IgG_4 levels are increased, but the increase is much lower than IgE levels, nor they correspond to 20% of patients with normal IgE levels, therefore the diagnostic value of IgG_4 concentrations in AD patients is very limited. In children the reduction of IgG levels was shown to be related to a higher frequency of relapsing bacterial infections; with children growth serum IgG_4 values normalized, but were not correlated to serum IgE values [1].

The role of *neuropeptides* has received attention that has been recently turned to a feasible neuropeptide involvement on AD pathogenesis, in particular of the ability of SP and CGRP (calcitonin gene-related peptide) ability to up-regulate inflammatory reactions in patients with AD, as well as of PGP9.5 being a marker of noradrenergic innervation, somatostatin, VIP, etc. (Chap. 11), with a significant increase in immunoreactive nerve fibers localized in the epidermis and around dermal vessels. From a pathogenic viewpoint, it is noteworthy that skin mast cells could be activated also by dermal neuropeptides, thus by non-IgE-dependent stimuli [53]. More importantly, a *neuropeptide disequilibrium* enhanced by exposure to road traffic has been suggested that could integrate a neurogenic share of AD pathogenesis [131].

A Concluding Pathogenic Hypothesis

The principal immunological characteristic of AD is the increased production of IgE, a substantial part of which is specific for environmental allergens, including SEs [103]. When IgE synthesis is controlled by CD4-Th2 cells, the preferential expansion of IL_4 and IL_5 producing allergen-specific T cells suggests an abnormal IgE dysregulation in AD. We have stressed that B cells are only rarely seen, if present at all, within the mononuclear cell infiltrates of atopic skin lesions. Therefore, skin-derived IL_4, a potential IgE-inducing function in the dermal lymph nodes, is irrelevant. Subordinately we can postulate that IL_4 induces in B lymphocytes an isotype switching to IgE production, detected in skin-draining lymph nodes and further away as a consequence of spread by the blood or lymphatic circulation, until it reaches AD-involved skin. There is substantial evidence that IL_4 up-regulates CD23 expression on LCs [25] (Fig. 7.8), perhaps an explanation for the presence of IgE on LCs and in infants with AD. Functionally, the expression of FcεRII on skin APCs leads to amplification of the response of Th1 and Th2 cells to minute quantities of allergens. By facilitated antigen processing, only minute quantities of allergens need to be presented to T cells [283]. Thus, serum IgE-complexed allergens in a CD23-dependent system increase processing and subsequent presentation to skin T lymphocytes up to a 1,000-fold, as well as the consequent activation with release of ILs and cytotoxic factors [283]. These results suggest that *cell-bound IgE on LCs* facilitates capture and internalization of allergens into LCs *even before their processing and antigen presentation to T cells.* Moreover, the increased IL_4 production in vivo by T lymphocytes [267, 322] could be responsible for an IgA deficit, coincident with a preferential isotype switching from IgA to IgE.

The activity of Th2 lymphocytes, which infiltrate within the involved skin in response to allergens, is directed to generating a chronic cutaneous inflammation. Of particular relevance is the mechanism based on T response specificity, which in the animal model has been clearly observed in T-cell selective migration from mucous tissues to skin lesions, regulated by interactions between vascular endothelial cells and specific receptors, namely in vivo CLA and CD62L [195, 197] (Table 1.58). CLA^+ T cells are $CD45RO^+$ [194]; in contrast, Der p-sensitive asthmatic patients have CLA^- memory or effector cells [196]. A high number of CD4 cells expressing CLA but not CD62L has been recently demonstrated in children with AD and CMA compared to nonatopic children with different intestinal conditions [2]. That the controls did not express CLA indicates that an elevated concentration of CLA facilitates the leakage of casein-specific T lymphocytes into the skin, thus playing a key role in promoting skin lesions.

Biochemical Dysfunctions

In the last 30 years, consistently sound investigative research that suggests a cAMP dysregulation as a basic characteristic of AD has been put forward. PBMCs stimulated by histamine or isoprenaline produce less cAMP in the skin of patients than in healthy controls. An enhanced intracellular activity of the cAMP-inactivating PDE enzyme activity is mirrored by blunted cAMP levels, which are secondary to histamine release by basophils [60]: thus a correlation between PDE activity, IgE synthesis and histamine release is delineated. Here, however, the conclusions of various researchers are rather conflicting, some stressing that a primary PDE increase is detected in CB (cord blood) PBMCs from offspring of atopic parents, as well as in subjects with remitting AD or AR. Others have put forward the notion that the PDE increase, before and in the absence of obvious allergenic stimulation, is the primary defect, thus the second marker of the atopic constitution, after IFN-γ deficit. However, the enzyme abnormality should be demonstrated to exist *in utero* and in the absence of allergenic stimuli, which in a predisposed fetus could generate IL mediators that in turn would activate PDE [226]. The protein kinase A (PKA) activity was increased and protein kinase C (PKC) activity was decreased in leukocytes of adult patients with AD, an index of increased PDE activity [274]. No significant differences in PDE activity were found between children with AD and normal controls [60], and PDE activity returned to normal in adults with AD following steroid therapy [105]. Another study suggests that an excess of activated Th2 cells promote increased PDE activity via the β receptors, as a consequence of an IgE hyperproduction caused by excessive IL_4 production and histamine release [308]. Additional studies on metabolic anomalies of PDE activity and of cyclic nucleotides may provide further insights on the basic pathogenic mechanism. In Chap. 4, we outlined that the influence of PGE_2 on the immune system can be mediated in part by its capacity to inhibit Th1-like T cell ILs and stimulate cAMP formation, which is reduced in patients with AD.

However, the key role played by *eicosanoids* should not be disregarded: either PGE_2 or LTC_4 directly promote the biochemical abnormalities in addition to cutaneous lesions of AD [72]; the eicosanoid effects on skin are summarized in Fig. 7.12 [217]. A *disturbed EFA metabolism* has been reported in children with AD, putatively secondary to a deficiency in δ-6-desaturase (Fig. 4.30). Such a deficit may depend on exogenous (dietary deficiency, including nucleotides) or endogenous causes (metabolic anomaly or abnormal EFA incorporation into tissues) [236]. EFA deficiency inducing anomalies of epidermal permeability leads to disturbances in some features of AD such as dry and scaling skin, lichenification, and extreme sensitivity to itching to which the skin is predisposed. Since the normal skin

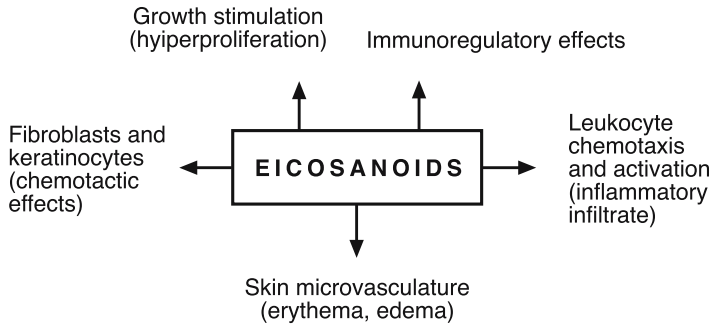


Fig. 7.12. Eicosanoid effects in atopic dermatitis. (Data from [217])

lacks the enzymes necessary to desaturate linoleic acid into AA [236], the epidermal barrier integrity depends entirely on both synthesis and incessant supply by the liver of desaturated metabolites of linoleic acid. This supply is only limited to some extent, the skin is among the tissues that are especially vexed by an EFAD, which may also be considered as a primary defect characteristic of the atopic state. When measuring EFA levels in the cell membranes of red blood cells, neutrophils, monocytes and lymphocytes, it has been demonstrated that AA decrease and linoleic acid increase in monocytes from atopic subjects are at variance with the lymphocytes of the same subjects [211]. These variations in EFA composition in the membranes of immunologically active cells may account for the anomalies found in atopic patients: a reduction in AA levels may be linked to secondary deficiency of PGE_2 reported in AD patients [211].

An increase in linoleic acid has been demonstrated in neonates with high concentrations of CBIgE [256]: such an increase, corresponding to that of IgE antibodies, might indicate a primary, partial inactivation of δ-6-desaturase activity. These findings suggest that EFA composition is genetically determined, therefore representing a basic characteristic and not a consequence of atopy. In our view, measuring EFA serum levels could be a promising approach to a new marker of atopy. In the red-cell membranes of atopic children is found a disequilibrium between high levels of linoleic acid and more reduced levels of polyunsaturated derivatives compared to controls [17], a deficit operative only in erythrocytes, and PBMCs of adults with AD [153]. Elevated concentrations of linoleic acid and of total fatty acids in the atopic skin inflammatory infiltrate can be correlated with an augmented diet supplementation [270]. No differences in γ-linolenic and dihomo-γ-linolenic acid levels be-

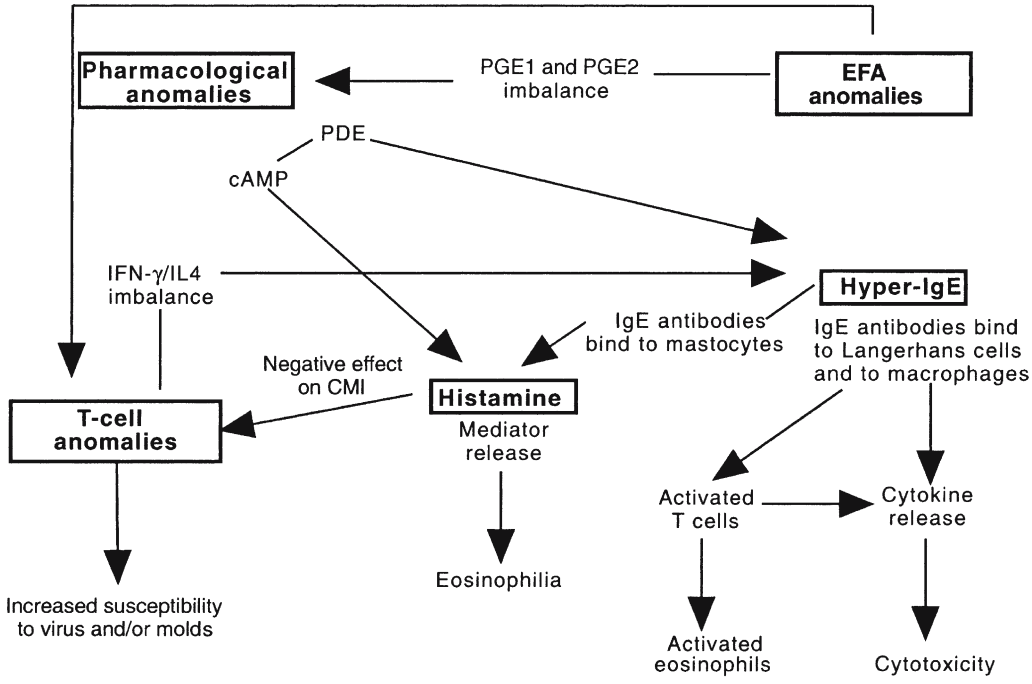


Fig. 7.13. Pathogenetic hypothesis of atopic dermatitis. *CMI* cell-mediated immunity. (Modified from [24])

tween atopic asthmatics and normal controls shows that no inborn deficit of δ-6-desaturase is causative in the atopy pathogenesis [141]. We have suggested (Chap. 4) that these abnormalities may recognize a viral pathogenesis, a further demonstration that this information may have relevance to positive interventions in AD. See Chap. 21 on additional effects of a deficit of EFA and/or nucleotides.

Additional Biochemical or Pharmacophysiological Anomalies

Vasoconstriction. The skin temperature (T) of AD patients is lower than in normal subjects, especially over acral areas: they react rapidly, manifesting a cutaneous vasoconstriction in a cold environment. Such patients show an abnormal response to a cold pressor test (Hines and Brown test), by an excessive elevation of blood pressure when an extremity is immersed in cold water [94].

White dermographism, a nonpathognomonic sign of AD, has been elicited in several dermatoses characterized by erythema; however, it is considered as a useful premonitory sign, since it soon replaces the erythema of triple response and can be elicited on less inflamed skin even in the absence of histamine-induced erythema [94].

Cholinergic responsiveness. A delayed blanch response occurs after intradermal injection of acetylcholine or methacholine, evident only on inflamed skin, similarly to other dermatitis. The mechanism of this paradoxical reaction is controversial: it has been suggested that the subsequent edema develops following a vasoconstriction or a vasodilation. This response can be interpreted as a form of hyperreactivity, assimilable to cholinergic bronchoconstriction recorded in asthmatics [192].

Figure 7.13 [24] summarizes AD pathogenesis.

Additional Pathogenic Factors

Role of Infections

The skin integrity, the most important feature of the cutaneous barrier, means that the skin is resistant to colonization and invasion of microorganisms, unable to pass through juxtaposed epithelial cells, connected by desmosomes. Moreover, pathogen growth is limited by *stratum corneum* relative dryness. However, some bacteria are known to penetrate the skin through the intracellular layers [147]. The reason why atopic patients have an increased proclivity to developing bacterial, viral, and fungal infections, which may run severe courses in AD, is still debated. The potential consequences of defective CMI and the dysfunction of nonspecific immune mechanisms appear to be sufficiently documented by the reductions of chemotactic activity of macrophages and neutrophils, of antibody-dependent cytotoxicity and IL_2 production [35].

There is little doubt that bacteria on the skin surface may also be a key factor and elicit or exacerbate cutaneous lesions, and the pathogenic impact of *S. aureus* is well known, shown at a density of 1×10^6 colony-forming units (CFU)/cm^2 but in greater concentrations when the eczematous lesions are more severe [97]. The unusual tendency of atopic skin to become invaded and colonized by this pathogen has stimulated investigations of the adherence phenomenon. Increased *S. aureus* adherence to corneocytes seems to be both a species-specific and corneocyte-specific phenomenon [55]. Among the receptors responsible for adhesion, fibronectin and laminin are found, whose binding to bacterial wall proteins appears to be irreversible [210]. The reason for *S. aureus* predilection for AD skin lesions seems to depend on the deficiency of defensive mechanisms specific for infection control [210], the dysfunction of skin lipids of endogenous origin [163], and the nutriment offered to germs by both exudate and blood found in inflamed lesions [236]. The specific interactions of *S. aureus* with the immune system is proved by either pro-inflammatory IL production by Th2 lymphocytes or IgA and IgG synthesis reduction in response to *S. aureus* [170]. Reduced sIgA secretion to the skin surface of patients with AD could promote a microorganism colonization [116] and hypogammaglobulinemia G leading to recurring bacterial infections [287].

The following pathogenic observations have been delineated to elucidate the reasons why *S. aureus* is believed to aggravate and/or maintain skin lesions in AD [151, 254]:

- *Induction of an IgE-mediated* response to *S. aureus* or other allergens functioning at the skin surface.
- *Induction of IgE or IgG* directed to the bacterial allergenic material, with a consequent inflammatory skin reaction.
- *Pruritus and scratching* of skin lesions containing *S. aureus* open an epidermal breach, allowing exotoxins and bacterial enzymes to enter the skin of atopic patients.
- *Skin inflammation* caused by SEs A, B, C, D (Table 1.21), which, acting as microbial superantigens, do not require processing for presentation, therefore rapidly stimulating the proliferation of both macrophages and a moderate proportion of T lymphocytes (5%–25%, 1:10,000 for a conventional antigen). This process requires the participation of *HLA class II molecules* restricted only by the specificity of the variable (V) region of the TcR β chain (Vβ) [169] (Fig. 7.14).

Moreover, SE-stimulated basophils of normal subjects do not release histamine. However, basophils of patients with AD react to pertinent SE, but not to other SEs [147]. Recent data show that 80% of children with AD, especially if FA-associated, are sensitized to superantigens colonizing their skin and have sIgE to bacterial toxins, including (Table 1.29): TSST-1 (toxic shock syndrome toxin-1), SEA, SEB, SEC, SED and SEE (staphylococcal enterotoxin A, B, C, D, E, respectively) [169].

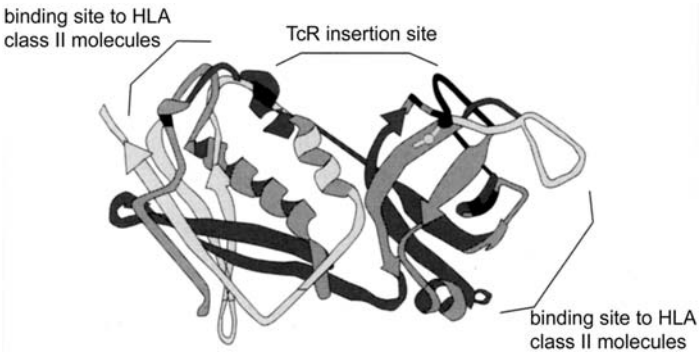


Fig. 7.14. Three-dimensional structure of a staphylococcal enterotoxin B binding site to HLA class II molecules, TcR insertion site and binding site to HLA class II molecules. The figure shows the insertion site for TcR (*top*) and HLA molecules (*side*) allowing the aggregation to related APCs

In particular SEA is capable of activating B cells linked directly to the V_H region Fab, thus acting as a B-cell superantigen [234]. In turn, TSST-1 stimulates IgE synthesis, which is, contrary to SEA, T-dependent, and it is likely that this IgE synthesis develops from a B-lymphocyte polyclonal activation more than from their isotype switching, since even the levels of other isotypes are increased [103]. Characteristically, IgE synthesis is activated by TSST-1 low levels (0.01–1 pg/ml) and inhibited by those elevated (1,000 pg/ml), as well as by IFN-ɣ [103]. In summary, SE ability of binding to HLA class I molecules or stimulating T cells with VβTcR suggests varying mechanisms by which SEs acquire the capacity of exacerbating AD:

- Superficially secreted SEA and SEB penetrate the inflamed skin, where they activate HLA-DR molecules on macrophages or LCs, which produce IL_1 and TNF, both ILs provided with potent pro-inflammatory properties [149].
- SEs putatively prime either a high proportion of Th2 cells (by their VβTcR) to differentiate, thus driving the production of more inflammatory ILs or basophils to release histamine, thus triggering an inflammatory cascade [149] and stimulating IgE production into inflamed skin [151] (Fig. 7.15).
- Roughly 50 % of patients with AD have circulating IgE directed to SEs, namely TSST-1, SEA and SEB, identified at the skin surface, indicating a local immunological sensitization to these staphylococcal SAs [148, 169].
- An analysis of the skin-homing CLA^+ T cells from these patients as well as their skin lesions reveals that they have undergone a TcR V expansion consistent with SA stimulation [32].
- SAs also augment allergen-specific IgE synthesis and induce corticosteroid (CS) resistance, suggesting that several mechanisms exist by which SAs could aggravate the severity of AD [98].
- SEB applied to the skin can induce skin changes of erythema and induration, and the infiltrating T cells are selectively expanded in response to SEB [246].

TSST-1 producers of staphylococci and dermatitis have been found in survivors of toxic shock syndrome, half of whom had IgE levels suggestive of atopy and AD. These findings suggest that exposure to SEs and the consequent immune activation could cause an immunoregulatory imbalance in AD, also because TSST-1 bound to keratinocytes may shift the IL profile, thus emphasizing the role of both IL_4 and IgE [167]. A local inflammation as a result of staphylococcal enzymes and toxic products could activate keratinocytes to express CD54 and secrete a wealth of pro-inflammatory ILs, with further accentuation of the inflammatory state [169]. Recent findings as to the association between staphylococcal adhesion and AD exacerbations point to CD54, leading to increased colonization of epithelial surfaces by *S. aureus*, which propagates to nearby tissues through a variety of enzymes selectively produced. Expressing components capable of modifying the host normal functions, *S. aureus* acts as a highly virulent organism

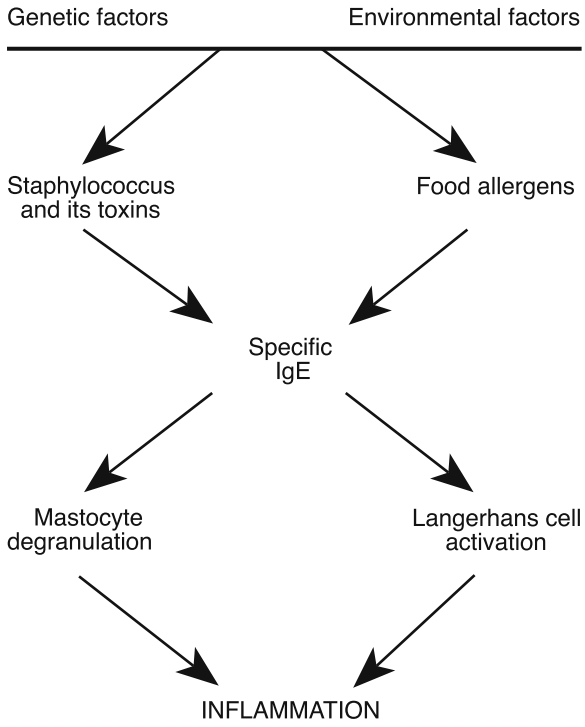


Fig. 7.15. Pathogenic model of atopic dermatitis based on staphylococcal toxins. (Data from [151])

with immunoregulatory properties [169]. Neutrophils, even in a resting state, activate an immune mechanism that produces O_2 metabolites, which are easily stimulated by staphylococcal antigens: reactive metabolites and SEs may cooperate in perpetuating skin lesions [169]. Therefore, as a result of streptococcus-induced sIgE generation, SEs are able to activate either directly by means of sIgE, or indirectly via chemokines, the metachromatic cells and/or other cells equipped with FcεRI, with resulting negative effects on involved skin [144].

Since the original descriptions of a varicelliform cutaneous infection in patients with *eczema vaccinatum* [124], several clinicians have been aware that cutaneous viral infections may run an unusually severe course in subjects with AD, in addition to a raised susceptibility to an atypical incidence of potentially life-threatening disseminated infections [35]. Viral infections may be more frequent in atopic children: EBV (*Epstein-Barr virus*) and *paramyxovirus,* including *varicella-zoster,* CMV (*cytomegalovirus*), RSV (*respiratory syncytial virus*), *mumps virus, measles virus,* etc., may promote a non-specific adjuvant action on IgE synthesis, thus inducing the atopic sensitization, provoking or aggravating skin lesions [254]. In particular, EBV promotes IgE synthesis during the first stages of AD and an increase in antibody titers to EBV has been seen in AD children [254]. EBV activity could be aroused by viral products with IL_{10}-like activity, via IFN-γ inhibition [109]. In adults with present or past AD, Rystedt et al [219] have found greater incidence of multiple recurring respiratory infections (RRI) caused by *Herpes zoster* in subjects suffering from the most severe forms of AD compared to controls. Although antibody titers to EBV are raised in AD [219, 254], there is no statistically significant difference in atopic as compared with nonatopic subjects as regards the outcome of EBV infection; similar results are found relative to HSV, varicella-zoster, CMV, mumps and measles virus antibody titers [254]. During the first 2 years of life, there is a significant association not only between AD and other atopic disease manifestations, but also between AD and RRI manifested in an increased rate of *acute otitis media* (RP 1.13) (ratio of proportion), pneumonia (RP 2.17) and *use of antibiotics* (RP 1.29) [22]. Therefore the infections, in addition to complicating AD, could represent a relevant etiopathogenic factor [35].

Role of Food Factors

Given the importance of the role of food factors, Fig. 7.16 [289] emphasizes that the prevalence of food sensitization is greatest in babies aged 0–2, with SPTs positive for foods in 53% of cases, lower in children aged 3–8 years and 5% in children aged >9 years. DBPCCT studies have found an AD–FA association in 33% of cases [33].

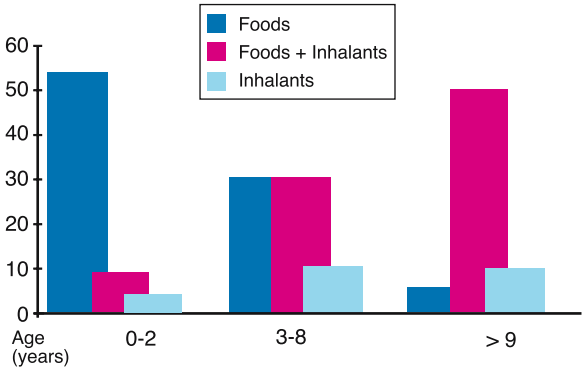


Fig. 7.16. Percentage of positive skin prick tests (SPTs) to food and/or inhalant allergens in children with AD by age group. (Data from [289])

Role of Food Additives

Food additives, mainly HR foods or those containing histamine-like substances, are able to provoke AD exacerbations, even in infants (Chap. 10).

Role of Aeroallergens

Several lines of clinical evidence based on results of different studies have found that the inhalation or contact with aeroallergens can induce pruritus (immediate reaction) and then eczematous lesions (chronic inflammation) [54]. After the first demonstration in 1918 that non-food proteins such as pollen and animal dander were rapidly absorbed through respiratory mucosa and transported to skin mast cells via the circulation [298], other pioneering studies [275, 276] were conducted in the 1950s to ascertain the pathogenic role of inhalants in these patients. Tuft et al demonstrated eczematous lesions in typical sites within 12–24 h of inhaling *Ambrosia* pollens and/or *Alternaria* spores that persisted for several days. An interesting experiment provided conclusive proof that allergens inhaled through the nose may reach the skin by the circulation: a patient who was aware of the rapid onset of pruritus and reddening of the rash on his limbs (within 10 min) after entering a house with a cat was able to remain in the house for more than 1 h without symptoms simply by wearing a mask that filtered the air he was breathing [199]. This data has been confirmed by eczematous lesions that appeared within 1.5–17 h in patients with AD challenged with Der p [279], thus proving that Der p number in mattresses is as high as the rise in relative risk (RR) [7], in close correlation between exposure and subsequent AD onset [11]. Der p finds a rich nurture in skin scales of children with AD, especially in their bedding dust, fallen during sleep [198, 199].

In 1932 Rost reported that AD would improve if patients lived in a climate chamber that was basically dust-free: these children often experience dramatic improvement in their symptoms when living in an allergen-free room such as in hospital wards [214], where inhalant allergens are less common. There the mean Der p 1 level was ≤ 0.2 µg and in the patients' houses > 10 µg in their bedding [233], thus clarifying why pruritus and scratching lesions intensify at night. Figures 7.16 and 7.17 [289] show the uncommon sensitization to inhalants in the first 2 years of life (to Der p a mean of 17%; see Fig. 5.22), although prevailing remarkably from 3–8 years onward and confirming the subsequent high increase for Der p. A significant increase in sensitization and sIgE positive for Der p were found in children born in the autumn, when mites reach their apex [198] (Table 4.12). Later eczematous lesions were demonstrated following aeroallergen application on the skin of patients with AD by an IgE-mediated mechanism. Yet children exhibit PTs positive to aeroallergens in 33%–44% of cases; repeated application of HDM extract on the PT site results in a peak infiltration of skin mast cells, similar to the changes observed in chronic lesions [54]. These observations confirm the action of *Der p as a contact allergen:* thus, a *Der p epicutaneous application elicits a skin reaction,* which more or less resembles a delayed reaction [28]. Der p allergen, once penetrated in the epidermis through skin lesions or epicutaneous application, is capable of binding to IgE bridging mast cells, eliciting an inflammatory reaction strictly distinct from a classic activation. Microscopic investigation of PT lesions revealed an increased proliferation of Der p-specific T cells, able to activate allergen-specific LCs, also without a constant correlation between Der p-specific IgE and test positivity [113]. Delayed reactions to Der p explain both AD onset or worsening in several children: the concordance in these subjects between positivity of immediate and delayed reactions is a further index of the causal link existing between type I and type IV skin reactions [28].

Immunohistopathological investigations have paved the way to new clues, establishing that aeroallergens interfere with AD pathogenesis. Once bound to IgE antibodies, Der p could be presented to activated allergen-specific Th2 cells that generate IL_4, thus stimulating B lymphocytes to produce sIgE in afferent lymph nodes and contributing to the chronic IgE synthesis predominating in atopic children [220]. A parallel, selective accumulation of Der p 1-specific cells (0.4%–2.7% of T cells) is more frequent in involved skin (94% of $CD4^+$ cells) compared to peripheral blood values of the patients [220]; comparable results are obtained by stimulating cutaneous explants with antigen suspensions [181]. These data confirm an inevitable, preferential skin localization of Der p-specific Th2 [284] producers of Th2-specific ILs (Table 7.7). It is not a surprise to find a massive infiltration of eosinophils recruited by IL_5, likely via the T-cell *longa manus* [28]. By topical application of Der p 1, it has been shown that Der p are localized in atopic skin on LC membranes [264] and similarly APCs of peripheral blood LCs present Der p 1 antigens directly to autologous Th0 cells in draining lymph nodes, with resulting Th1 and Th2 clonal expansion [173]. An important and significant pathogenic finding is that mites, via LCs, are believed to select a Th2 response, inducing an inflammatory state able to maintain and exacerbate lesions, also via the demonstrated action promoting cytotoxic effects expressed by IL_4 and IL_5 [284, 285]. T cells of these children respond more intensely to IL_4, on which the proliferation of some T-cell subsets depends [181]. Arguments have been put forward that suggest that repeated exposure to allergens, chronic or seasonal, hasten the development of allergen-specific Th2 cells of skin-derived IL_4 producers, capable of stimulating both growth and local differentiation of additional cloned IL_4 producers [220].

The cardinal role of HDM in worsening AD is emphasized by several reports. Clones of Der p-specific Th2-like T lymphocytes of skin derivation are excellent inducers of IgE synthesis in B cells, which requires the second signal (Chap. 1) provided by several $CD4^+$ of atopic individuals through a T/B, unrestricted HLA interaction [66, 127]. Der p-specific Th2 cells have a greater ability to express CD23 on the APC surface [125]. From a clinical point of view, it is speculated that 40%–50% of patients with high IgE levels specific for aeroallergens have positive PTs to these allergens [198].

The most significant data can be thus summarized:

- Clinical and epidemiological studies demonstrate that *aeroallergen contact may aggravate AD.*
- *Allergen-specific T cells* are isolated in high numbers in SPT sites.
- A part of these T cells are *Th2 cells,* especially if isolated from the bloodstream, with values varying between 11% and 70% and more often *$CD8^+$ cells are very reduced in number* [290].
- These T cells, derived either from the blood or the skin, are able to *trigger IgE switching from B lymphocytes,* probably within regional lymph nodes by virtue of LCs, thus contributing to chronic IgE synthesis.
- Der p-specific cells and Th2-like T cells in skin lesions are not always correlated with serum Der p-specific IgE antibodies or with high total IgE concentrations [66, 290].
- The low correlation is also true for IL levels, for example IL_4 [290].
- However, subjects with Der p-specific IgE antibodies suffer from *more severe lesions* compared to subjects with PT positivity [113].
- It is therefore likely that anti-Der p 1 Th2-like responses are limited to the initial lesions [66]; nonetheless even if further studies are needed to clarify whether such events are specific for AD, it is indisputable that they effectively demonstrate *the role played by mites in AD pathogenesis.*

Aeroallergens other than Der p contribute to skin lesion worsening. In Fig. 7.17, the rate of SPTs to in-

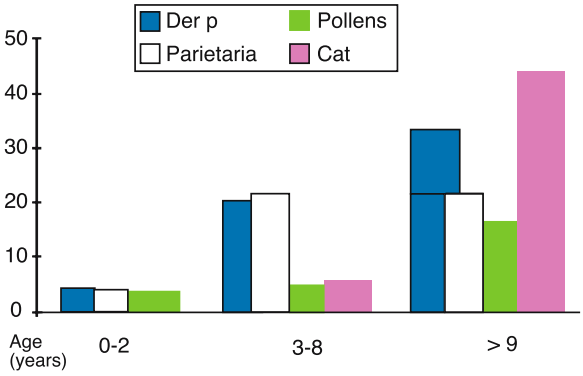


Fig. 7.17. Percentage of positive skin prick tests to inhalant allergens in children with AD by age group. (Data from [289])

halant allergens in children with AD is lowest between the ages of 0 and 2 years, but between 3 and 8 years, pollens increase up to a 20% rate, whereas the low cat sensitization in the first two years (Fig. 5.21) at the age of >9 years acquires an uncommon increase (>40%) [289]. A cat in the home in early childhood increased the risk of persistent AD (COR 2.33) [111]. Aeroallergen sensitization was observed in 66% in moderate AD and 93% in severe AD of children aged 7–15 years [89]. PTs observed in 59 children (mean age, 5.2 years) presenting with AD were, in addition to Der p and Der f (26.8%), positive to garden trees (12.2%), plantain (9.8%), timothy grass, mugwort and damp area trees (4.9% each), and orchard grass (2.44%) [42]. Pollen-sensitized children generally undergo, in spring–summer, a worsening of AD lesions.

Pathophysiology of Itching

Itching is defined as the quintessential feature of AD, and scratching is the major cause of the damaging excoriations, erosions, and lichenification characteristic of AD.

Previously it was thought that itching and pain were transmitted by one type of nonmyelinated fibers [91]. Supporting this view is the observation that itching receptors are correlated with free nerve terminals of nonmyelinated C-fibers, at the dermoepidermal junction. However, studies by intraneuronal microstimulation have shown that two different subsets of receptors and fibers subserve itching and pain. It is feasible that itching mediators are able to act directly on its receptors, indirectly releasing histamine, or directly raising its threshold. As a consequence, the impression of relief, contemporary or subsequent to scratching, would be due to a closure of the so-called gate for pain and itching stimuli arriving in thin C-fibers, via tactile sensations arriving in A-fibers [91, 296].

The mechanisms involved in the pathogenesis of itching remains to be explained, and some observations might help in answering this question [85, 296]:

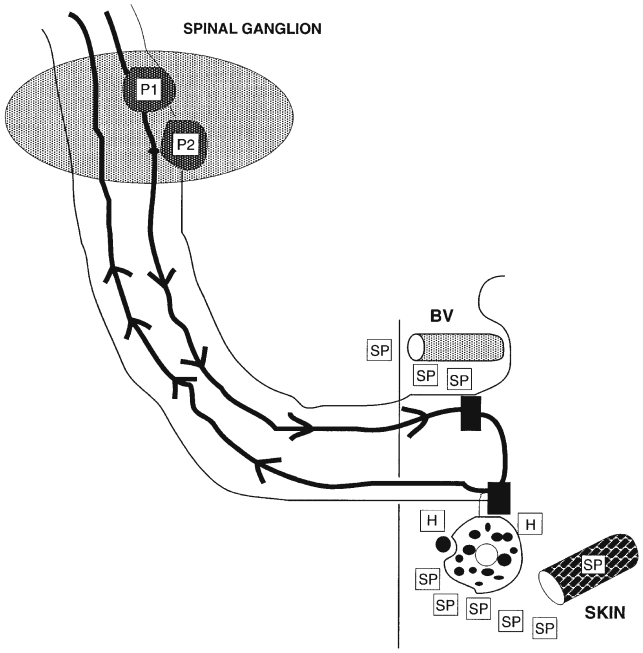


Fig. 7.18. Substance P (*SP*) activates dermal mast cells (*M*), which release histamine (*H*). Histamine stimulates two different populations of nervous cells controlling the pain (*P1*) and itch (*P2*) sensations. By antidromic stimulation of blood vessels (*BV*) vasodilation ensues. (Modified from [91])

- *Histamine* is a classic itching mediator almost exclusively in urticaria, but other pruritogenic factors may be involved as well; likewise serotonin is a weak pruritogenic factor.
- *Neuropeptides* stimulate mast cells to release histamine but little definitive information has arisen about whether neuropeptides are responsible for pruritus.
- *Prostaglandins* stimulate itching by lowering the threshold for histamine in experimental conditions; however, the inhibitors of prostaglandin synthesis such as aspirin have little or no effect in relieving clinical itching.
- In several pruritic skin disorders, T lymphocytes are active. Predictably, *pruritogenic ILs* activated by such activated cells are involved in the AD pathogenesis. This hypothesis is supported by reports showing that cyclosporine A, by inhibiting IL secretion, alleviates itching in some patients with AD.
- *A polymodal nociceptor C* has been shown to play a prominent role in the central itching transmission [296].

However, to identify the central mechanism involved in itching pathogenesis, the more promising advances come from the study of neuropeptides. Several investigators have noted that itching evoked by SP and other neuropeptides is accompanied by wheal and flare reactions, and suggested that itching may be caused by histamine release from dermal mast cells (DM). The pathophysiological mechanism would be based on the following points (Fig. 7.18) [91]: SP activates DMs releasing histamine (H), which stimulates two different populations of nerve cells controlling both pain and itching

sensations (P1 and P2); by antidromic stimulation of blood vessels , the flare response is produced via release of SP and other peptides; SP released from nerve terminals acts directly on vessels and adjacent DMs; the flare reaction reaches its zenith. Thus upstream of neuropeptide-induced pruritus, there is the *SP-mediated mast cell degranulation* which releases H causing vasodilation and activation of sensitive nerves [91]. Therefore, neuropeptides could establish a close point of union between the nervous system and inflamed skin, thus explaining the well-known association between pruritus, skin alterations and emotional stress [85]. Another factor is the frequency of pruritogenic stimuli, accompanied by flare or transpiration, could originate from the finding that C-fibers, because of a threshold lowering, are rapidly activated concomitantly with a skin T increase. However, studies to determine which is the activating mechanism still await basic answers [296].

It seems likely that only the skin is provided with sensory mechanisms that localize the arriving stimuli: this is due to spinal circuits with an inhibiting activity for sensations different from those originating from the central point of the stimulus. Therefore itching is elicited not only by localized stimuli, but especially by stimuli sufficiently expanded and able to provoke a diffused inhibition, different from the moderate response to a punctiform stimulus. Afferent fibers of a small diameter deliver the message to connecting neurones and to synaptic mechanisms, occasioning an hyperexcitability of long duration [85]. As a result of persisting scratching and of consequent mechanical irritation, performed ILs are released by keratinocytes (GM-CSF, TNF-α), which trigger dermal inflammatory responses. Moreover, antigen-specific immune responses in the atopic chronic skin lesions might change into antigen-independent immune responses, which may activate an inflammatory process amplified and automaintained by numerous noxae, not only endogenous but especially exogenous [125]. The fact that itching characteristically worsens at night induces a parallel with nocturnal asthma. As we will see in Chap. 11, the relationship between nocturnal asthma and circadian reductions in plasma adrenaline concentrations can be interpreted as evidence of a permissive mechanism upon release of histamine from mast cells (which could explain the nocturnal fits), and that such a mechanism might help explain the pruritus in AD may be an interesting hypothesis [93].

Irritant Effects

Irritable skin is closely associated with pruritus, even if the pertinent causes are different [277]. The major irritant causes include the following:

- *Defective epidermal permeability* barrier, leading to excessive transepidermal water loss, critical for the penetration of irritants
- *Influence of disease severity*
- *Lowered itching threshold,* unremitting source of irritability, started by minimal environmental changes
- *Excited skin syndrome,* meaning a general increase in atopic skin susceptibility to irritants applied to an unrelated body area [277, 278]

Interestingly, the threshold is not lowered; however, atopic patients perceive as pruritogenic many stimuli that normally are well tolerated by nonsensitive persons: numerous chemical and natural substances may express irritant effects [5]. The chemical products that are the most deleterious are soaps and cleansing agents, which are often very effective in removing lipids and oils from the skin of children with AD, emulsifying them with protective properties, although their moderate use is harmless. Their excessive use makes the skin dry and vulnerable, and the resulting inflammation when improperly used on the skin suggests that they should be employed only by necessity, and thoroughly rinsed [5]. However, lipids are essential in the *brick-and-mortar barrier* of the *stratum corneum;* thus lipid solvents can break down this first line of defense. Natural factors are at first the extreme points and then sudden T changes: undressing of the child, frequent sweating by occlusive clothing, contact with rough fabrics, wool and/or synthetic textiles, inappropriate hygiene and/or bathing with unsuitable detergents, a dry, cold climate or overheated residences, emotional stress, as well as ingestion of sensitizing foods and contact with environmental allergens. Overall, many foods can act as *nonspecific irritants* when coming in direct contact with an impaired skin barrier [83, 247]. UV light abates mast cell responsiveness and eosinophil number during the summer months, but the decreased epidermal water barrier consequent to winter cooling leads to a hyperstimulation of mast cells and nerve terminals [277]. Another cause is humidity: dry skin worsens in a cold environment with reduced humidity compared to a warm environment; therefore dry skin is aggravated in winter [5].

Relapses are inevitable, even if they are attenuated by pertinent treatments; they are generally identified as negative reactions to environmental factors. *Irritant dermatitis* occurs in several atopic children; numerous substances or exogenous factors are implied. Analysis of 300 cases showed that the causes were as follows: winter season 66%, city life 42%, change of residence 29%, foods 19%; 68% improved at seaside and 30% in mountain resorts [236]. There is now extensive evidence that several factors may either precipitate or aggravate AD. These include conditions leading to vasodilation such as hot water, rapid T changes, irritant detergents [36], and preservatives [57].

Clinical Presentation

Clinically AD is a disease characterized by wide individual variations, an erythematous, exuding or papulovesicular eruption, and intensively pruritic lesions; sub-

Table 7.8. Most frequent lesions (%) based on the onset age

Lesions	<1 year	>1 year
Face	58	21
Hands and wrists	47	42
Lower limbs	21	47
Upper limbs	20	27
Buttocks	14	7
Scalp	21	2
Back	11	10
Abdominal skin	10	7
Feet	6	4

Data from [236].

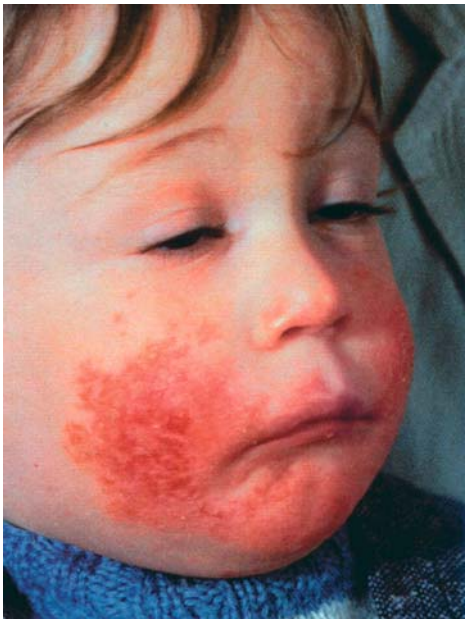


Fig. 7.19. Atopic dermatitis of infants: no clear-cut boundary. When the face is involved, the nasolabial and perioral area are spared (for details see text)

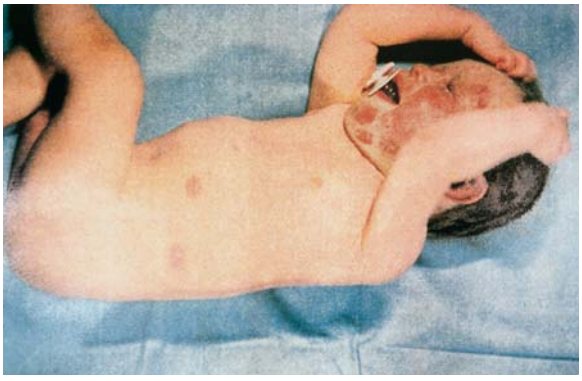


Fig. 7.20. Atopic dermatitis of infants: the marked itching can be seen from the child's posture (for details see text)

sequently lichenification and scaling are more prominent. Scratching often leads to excoriations, blood can leak out of the injured capillaries in the excoriated dermal papilla, which mixes with serum and results in serosanguineous crusts, impetiginization, and secondary infections. The aspect of the lesions during the acute phase is very different from that seen in the chronic phase. For unknown reasons, the distribution of the clinical manifestations differs according to the period of patient's life in which onset occurs; therefore it can be subdivided into three phases [36]:

- Early infantile phase (up to 2 years)
- Late infantile phase (up to 12 years)
- Childhood and adolescent phase (up to 18 years)

Table 7.8 shows the most frequent sites of lesions in children aged <1 or >1 year [236].

In the *early infantile phase* (acute phase), although seborrheic dermatitis may precede and coexist with AD, infants are typically involved at about 3 months of age (47.5%–100% of cases) (Table 5.5), with erythematous-exudative eruption with poorly defined contours and confluent, whose topography is almost pathognomonic (Figures 6.4 to 6.18). The predilection site is especially the face (about 80% of cases), including the cheeks, zygomas, auricles and retroauricular sulcus, with a characteristic sparing of the skin triangle including the nasal, perioral and mental regions (Fig. 7.19). Moreover, lesions may extend progressively to involve the forehead, neck folds, scalp, trunk and extensor surfaces of the limbs, so that at age 18 months the lesions may involve all body areas. The increased drooling occurring with teething can be of further irritation to the inflamed skin [137]. Itching is unremitting and stressful, especially for parents, who undergo long sleepless nights trying to prevent the baby from worsening the disease by scratching the skin furiously. Scratching lesions may also be apparent in young infants who are restless, sleepless, tearful and, to get relief from pruritus, discover how to rub their skin against crib linen or bedclothes by continuous movements, or on their leg surface using the contralateral limb (Fig. 7.20) [320]. The lesions thus provoked expose the skin to bacterial infections, usually by *S. aureus*. The sometime marked oozing causes *hypoalbuminemia* [273].

In the *late infantile phase* (subacute phase) the disease often appears as a continuation and extension of the previous phase and its major feature is lesion chronicity. In 3%–5% of cases, AD arises between the ages of 4 and 6 years. The lesions are more localized, less oozing and more often scaling, and impetiginization occurs on an erythematous basis, but is less exudative than in the previous phase, and eczema of *dorsal folds of the limbs* typically develops (Fig. 7.21), with involve-

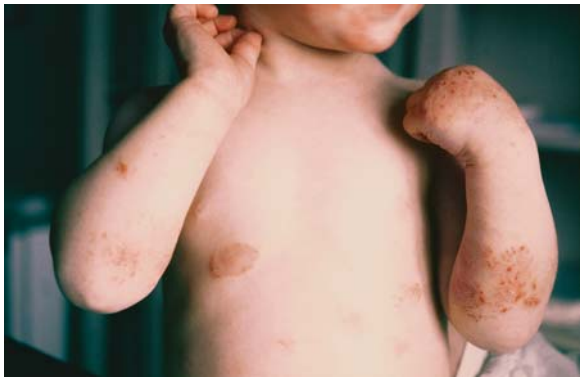


Fig. 7.21. AD of infants: the lesions of the limbs are on the extensor aspects

ment of the antecubital and popliteal spaces as well as of the wrists. Additional predilection sites are the face, neck, legs, ankles, wrists, and hands [94]. When the face is involved, the lesions extend to palpebral and perioral regions, the cheeks (Fig. 7.22a) and the retroauricular areas (Fig. 7.22b), appearing persistently fissured with moderate serous oozing. Pruritus is again unremitting, especially in the acute phases, and children are easily irritable and restless; scratching lesions are frequent, provoked even through clothing. In older children, either the back or the sole of the foot may be involved (assuming the aspect of an ACD), often diagnosed as athlete's foot, while in fact it is a rhagadiform dermatitis [319]. Since the lesions are long-lasting, undergo thickening, dryness and lichenification, skin dryness worsens in cool and dry winter months, and improves in summer. The course is relapsing or chronically persistent and continues into the subsequent phase. A *reversed pattern* primarily affecting the elbows, knees, dorsal hands, and wrists has a less favorable prognosis [288].

In the *childhood and adolescent phase* (chronic phase), AD is the prolongation of infantile phases. The predilection sites are virtually the same: localization to the perioral region, mostly cheilitis with radial fissures, to the periorbital region and neck, and *flexural aspects of the limbs* now being the predominating type of lesion. Lichenification, with skin thickening, greater evidence of fine scaling and xeroderma, is more frequent than the inflammatory component persisting at the periphery of lesions. Dry skin is scarcely elastic. Emotional and environmental stresses (exposure to a cold, desiccating climate) may add a further negative effect. Itching remains unaltered in everyday life, even if scratching lesions are less frequent, but somatic factors are accentuated by significant psychological problems, frequently caused by insomnia or cosmetic disfigurement. On the other hand, psychological factors may also deteriorate the clinical conditions, most likely establishing a vicious circle.

Both the symptoms and the division into phases so far outlined can facilitate the diagnosis, but frequently the typical aspects are not reproduced, especially in subacute and chronic phases, and a persisting progression with wide *interindividual variations* prevails [236], corresponding to the effects of irritant factors. Despite these difficulties and shortcomings, the clinical symptoms will hint at the basic characteristics of AD and have a major impact on therapeutic decisions.

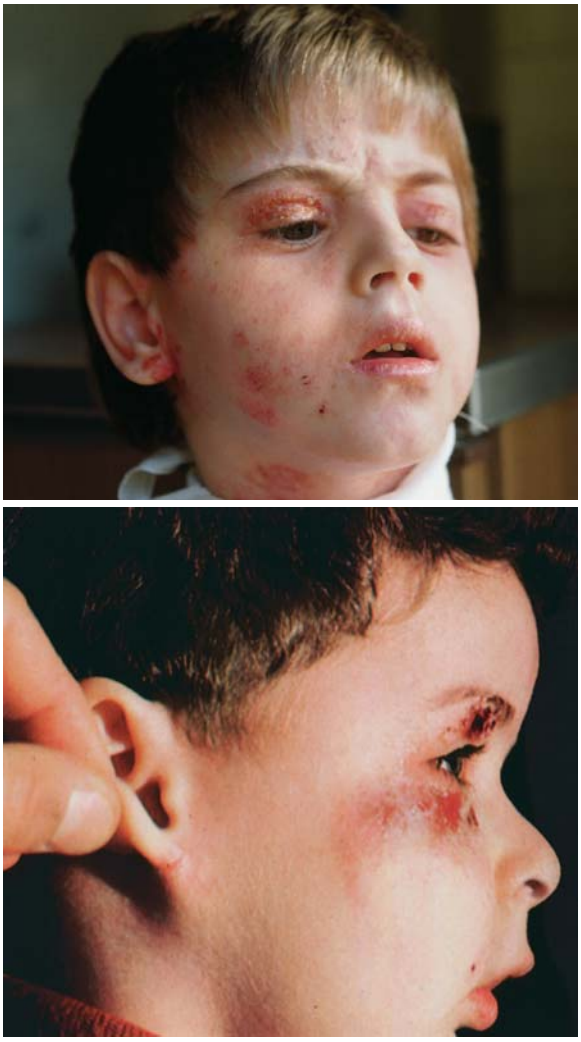


Fig. 7.22. **a** After the first 2 years of life, AD of the face involves the palpebral and perioral regions, the cheeks and **b** the retroauricular sulcus

Additional Clinical Features

Besides full-blown AD, whose features are universally accepted, there are the diagnostic findings shown by Table 7.9 [94, 96, 166, 177, 224, 311]. These findings may help to recognize the constitutional stigmata or minimal forms that should be observable with no technical devices without distinctive symptoms or clues to atopy.

Table 7.9. Diagnosis of atopic dermatitis

Major criteria[a]
Pruritus
Typical morphology and distribution of age-related lesions
Facial and extensor involvement in infants and young children
Flexural lichenification and linearity in older children and adolescent
Chronic or chronically recurrent dermatitis
Personal or family history of atopic disease (asthma, allergic rhinitis, AD)

Minor criteria[a]	
1. Xerosis[b]	15. Anterior subcapsular cataracts
2. Ichthyosis	16. Periorbital darkening[b]
3. Palmar hyperlinearity[b]	17. Facial pallor or erythema[b]
4. Keratosis pilaris[b]	18. Pityriasis alba[c]
5. Immediate, type I skin prick response	19. Worsening of itch when sweating[b]
6. Elevated total serum IgE	20. Intolerance to wool and lipid solvents[b]
7. Early age at onset	21. Perifollicular accentuation
8. Susceptibility to cutaneous infections (*Staphylococcus aureus*, *Herpes simplex*)[b]	22. Food allergy or intolerance
9. Hand and foot dermatitis[b]	23. Course influenced by environmental or emotional factors
10. Nipple eczema[c]	24. Delayed blanch reaction[b]
11. Cheilitis	25. White dermographism[b]
12. Chronic recurrent conjunctivitis[b]	26. Infra-auricular fissuring[b, c]
13. Dennie-Morgan folds	27. Scalp dermatitis
14. Keratoconus	

Three major criteria[d]
1. Family or personal history of atopic disease, asthma, allergic rhinitis, AD
2. Pruritus
3 Typical facial or extensor eczematous or lichenified dermatitis

Three minor criteria[d]
1. Xerosis, ichthyosis, or hyperlinear palms
2. Periauricular fissures
3. Perifollicular accentuation
4. Chronic scalp scaling
History of involvement of the skin creases[e]
Personal history of asthma or allergic rhinitis[e]
History of skin dryness in past 12 months[e]
Flexural dermatitis[e, f]
Onset before age 2[e, g]

[a] Hanifin and Rajka criteria [96] modified by Hanifin [94] (the diagnosis is made according to the presence of *at least 3 major features and 3 minor features*). Hanifin has proposed a list of minor features restricted to numbers 1–6, 8, 9, 11, 27.
[b] Statistical associations suggested in [166].
[c] Statistical associations suggested in [177].
[d] Sampson criteria [224] for infants and young children.
[e] In a refined version [311], child *must* have itchy skin condition for at least 12 months *plus* three or more other features.
[f] In children aged <4 years with history of atopic disease in first-degree relative.
[g] Not influential in children aged <4 years.

Constitutional Stigmata

The following minor criteria are included with the incidence indicated in parentheses [202]:

Dry skin or *xerosis* (48%–98%) may be localized or generalized; the skin is rough to the touch, not inflamed, sometimes slightly scaly, also ichthyosiform; the pathogenesis is uncertain; the association ichthyosis vulgaris is uncommon (2%–6%) [165] (see "Complications").

Hyperlinearity of the palms (and soles) or an increase in palmar creases is frequently referred to (28%–

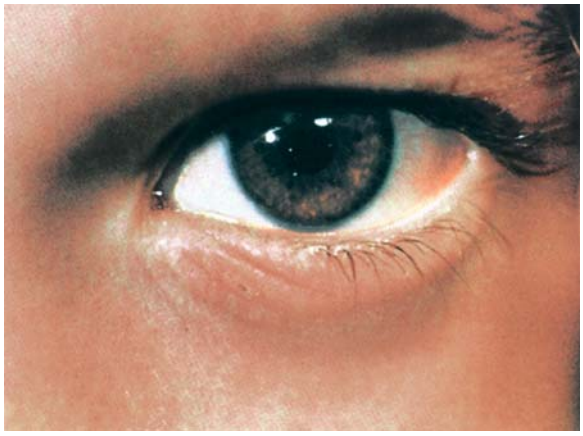


Fig. 7.23. Dennie-Morgan infraorbital folds with AD of the lower lid in a 6-year-old child

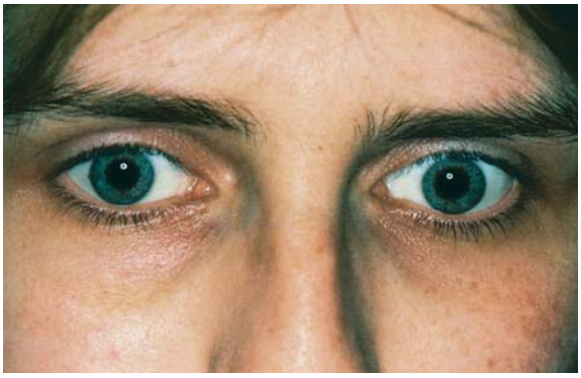


Fig. 7.24. Orbital darkening (for details see text)

Fig. 7.25. Typical Hertoghe's sign (for details see text)

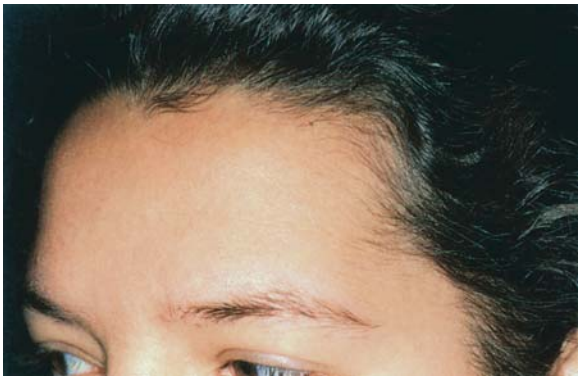


Fig. 7.26. Low hairline (for details see text)

88%). We do not dispose of conclusive data on their interpretation.

Dennie-Morgan infraorbital folds (12%–85%) (Fig. 7.23) [318] are symmetrical creases below the margin of the lower eyelids, usually of both eyes (not to be mistaken for the physiological *sulcus palpebralis inferior*). Considering the folds as closely related to eyelid dermatitis has been proposed; however, they are found more frequently in younger than in older subjects with AD.

White dermographism (38%–100%) is observed more frequently in atopic lesions compared to other types of dermatitis, but also in healthy controls, and in lesions of other chronic inflammatory dermatoses.

Facial pallor (22%–85%) is a diffuse paleness frequent in young patients. Just as white dermatographism is attributable to an abnormal vascular reactivity with a tendency toward vasoconstriction of small blood vessels, facial pallor may occur in patients with white dermatographism following skin rubbing.

Orbital darkening (41%–85%) or allergic shiners: a blue-grey discoloration of the orbital skin with accentuation of the lower orbit (Fig. 7.24) [202]; sometimes there is slight edema. Even if different pathogenic mechanisms have been proposed for AD (a combination of irritation and inflammation) and for perennial rhinitis (edema and/or spasm of the *musculus tarsalis* which impede venous blood flow from the orbital region), orbital darkening is frequently observed in atopic patients with neither of the two diseases.

Hertoghe's sign (Fig. 7.25) [202]: thinning or complete absence of the eyebrows in their lateral aspects.

Low hairline (Fig. 7.26) [202]: distance of ≤3–≤1.5 cm from eyebrows, direct transition from scalp to eyebrows [202].

Minimal Forms

Minimal forms include the following [319, 320]:

Follicular or Patchy Pityriasiform Lichenoid Dermatitis. This is more properly a *keratosis pilaris,* a hyperkeratosis obstructing the follicular orifices appearing in infancy and persisting until it peaks in adolescence. The lesions are common on the extensor surfaces of upper

arms, face, forearms and buttocks with partly confluent areas, where papules that are mildly bran-like, flesh-colored, scaly and nonhyperkeratotic have been noted, with a characteristic aspect of gooseflesh; mild itching is also present. Lesion progression is observed in winter, whereas the summer can lead to improvement or healing of the rash, especially during a seaside holiday. The differential diagnosis involves supracollicular keratosis, follicular hyperkeratosis in the context of an autosomal dominant ichthyosis vulgaris, *lichen nitidus* and *planopilaris* and Gianotti-Crosti syndrome (see "Differential Diagnosis").

Papular Variant. Papules are found on the extensor aspects of the knees, occasionally at the elbows and back of the hands, at variance with the follicular form, more evident in the spring and summer, and are often associated with pollinosis, thus suggestive, from a pathogenic point of view, of an inhaled precipitant such as an AR equivalent or a mechanical component such as friction or rubbing. The differential diagnosis includes the lesions seen in juvenile papular dermatitis, lichenoid eruption by rubbing, and toboggan dermatitis. However, in these conditions both family and/or personal history and sIgE to inhalant or food allergens are positive.

Nummular Variant. The chronically occurring eruption of strictly demarcated, papulovesicular, coin-shaped, excoriated, occasionally exuding lesions covered by scaly crusts leaves the adjacent skin uninvolved; there is itching-scratch succession; it is differentiated from herpetiform and vesicobullous dermatitis.

Localized Minimal Variants. These include:

- *Exfoliative cheilitis* with perlèche: irritant effect provoked by acid foods more than by an allergic reaction [188]; frequent licking to ease the dryness and rubbing of the adherent scales exacerbate the lesion (Fig. 7.27) [318].
- *Retroauricular intertrigo* or earlobe rhagades.
- *Lower lid dermatitis* (Fig. 7.28) [318]: habitually occurring in spring as a pollinosis equivalent, often associated with Dennie-Morgan infraorbital folds and Hertoghe's sign.
- *Nipple dermatitis:* pathognomonic when present (22%–42% or 12%–23% of cases) [215, 217].
- *Tylotic, rhagadiform finger pad* dermatitis (Fig. 7.29) [318]: more frequent in girls and right-handed subjects, characterized by pale erythema, minimal edema, keratosis, scaling, fissuring, accompanying pain may lead to functional impairment in the involved hand.
- *Vulvar dermatitis* is characterized by circumscribed, scaly, erythematous lichenification of the labia, secondary to the severe pruritus of this very sensitive skin, and has a chronically relapsing course [94].

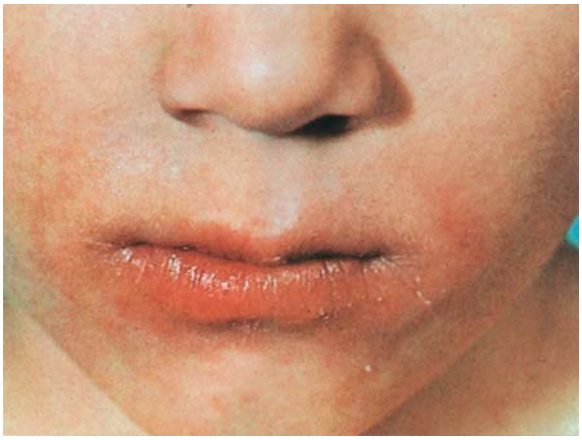


Fig. 7.27. Exfoliative cheilitis with perlèche in a 6-year-old child

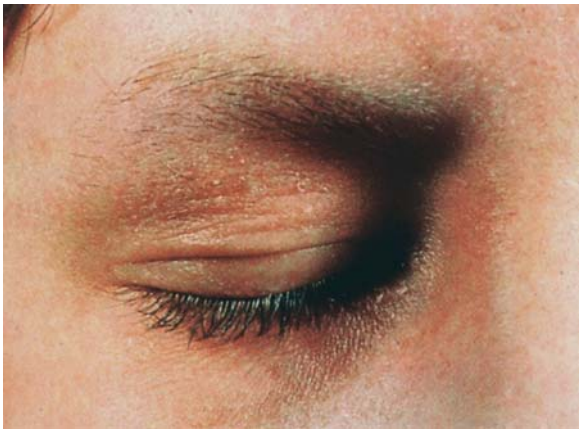


Fig. 7.28. Eczema of the upper lid in a 14-year-old boy (for details see text)

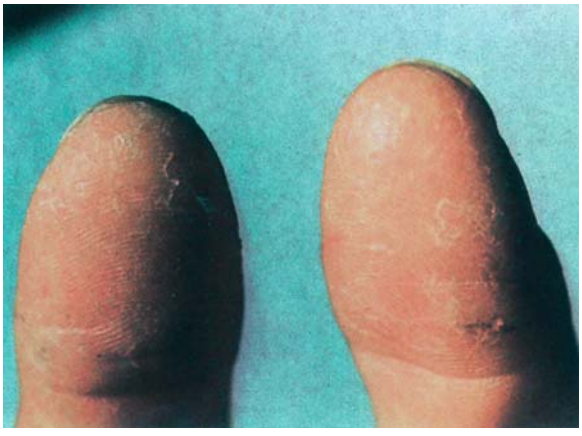


Fig. 7.29. Tylotic, rhagadiform, finger pad eczema

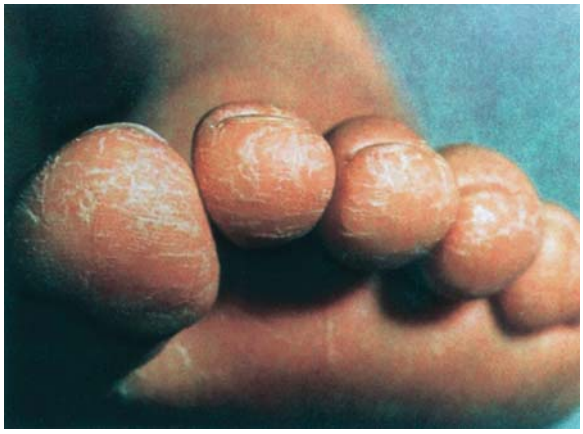


Fig. 7.30. Atopic toes, plantar aspect often misdiagnosed as a foot mycosis

Juvenile Plantar Dermatosis. This is also known as atopic foot dermatitis (Fig. 7.30) [318], more commonly reported in males aged between 3 and 6 years, and more marked in summer and winter months. Characterized by enhanced scaling, and intertriginous and wet areas, it is often misdiagnosed as a foot mycosis and thus treated with antimycotics. Association with AD ranges from 50 % to 74 % of cases. The seat is the great toe, less commonly the forefoot, with erythema, scaling, fissuring, etc., the back of the foot if the shoes are involved. The pathogenesis is also related to repeated microtraumata (frictional ACD), a clammy microclimate due to the wearing of synthetic socks and/or high shoes, and/or contact allergy to parts of shoes. The disease has a mean course of 7–8 years; a complete remission should by expected with adolescence in the majority of cases [319, 320].

As regards the diagnostic importance of both stigmata or variants in AD, in addition to the figures cited so far, only the association of some forms yield highly significant statistics [166, 177] (Table 7.9).

Complications

- *Hypoalbuminemia* in young infants can be the result of extended and markedly exuding lesions [273].
- *Bacterial infections,* whose role has been re-evaluated, especially that of staphylococci and streptococci, which can influence both the severity and tendency for AD relapses. In particular, *S. aureus* colonizes the skin of atopic subjects in 90 %–100 % of cases, and bringing it into close contact with normal skin for 24 h produces eczematous lesions that persist for several days. The lesions are superficial, exuding, with extensive serous weeping, erythematous, with honey-colored crusting, often accompanied by lymphadenopathy and intense itching. Usually AD lesions worsen in the presence of an infection, since *S. aureus* antigens and type I immune reactions are involved in the maintenance of chronic skin inflammations [146] (Fig. 7.31, 7.32). Streptococci also colonize the skin of atopic subjects and provoke infections that often resist therapy. As previously mentioned, significantly higher incidence of *herpes*

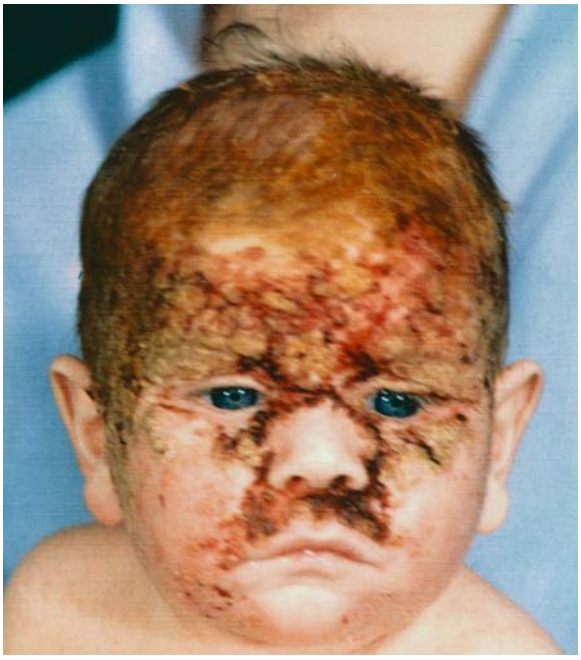


Fig. 7.31. Severe infection due to *Staphylococcus aureus* in a baby with AD as a result of food allergy

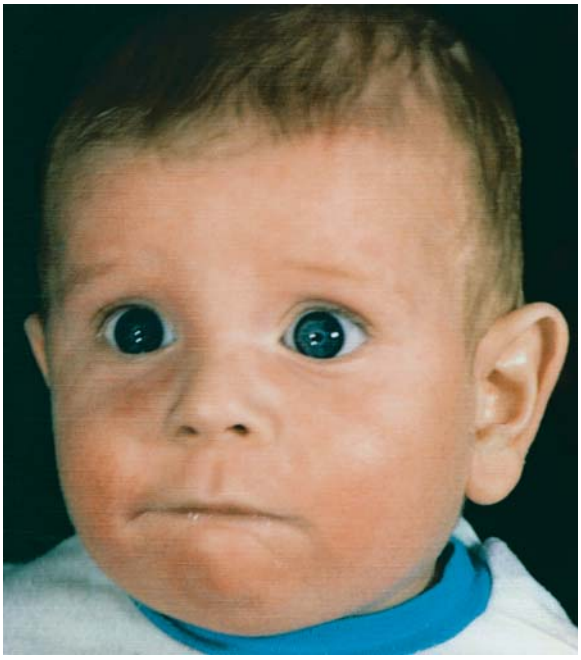


Fig. 7.32. Dramatic improvement in the same baby after 7 days of treatment with erythromycin

zoster and respiratory infections are seen in adults who suffered from a more severe AD during infancy.

- *Mycotic infections* are a more frequent isolation from hands, feet and the nail bed and involve *Trichophyton rubrum* and *Pityrosporum* species in dermatitis of the head (70% of cases in infants), neck, shoulders and mid-thorax, both involved in exacerbations of the lesions [120]. *Pityrosporum* is distinct in the *orbicularis* and *ovalis;* however, the A serotype is from *Malassezia furfur* and B and C are provided with two IgE-binding proteins of 67 and 37 kD [63]. It induces IgE-mediated immune reactions from the age of 4, as confirmed in children with elevated IgE levels by IgE anti-*Pityrosporum*, which correlates well with the course of skin lesions and a poor prognosis [152]. *Candida albicans* is implicated in AD exacerbations, as demonstrated by antibodies anti-*Candida* IgE [237] cross-reacting with anti-*Pityrosporum* IgE via mannans with high PM [69, 180]. *Pityrosporum* infection is diagnosed with RAST, ELISA and monoclonal antibodies [326]. These studies confirm a significant association (p=0.0001) with yeast sensitization [152].
- *Viral infections* [35, 36]:

– *EBV* as discussed in "Role of Infections."

– *HSV* (*Herpes simplex* 1 and 2) usually induces superinfections of eczematous lesions extended to normal skin, sporadically assuming a very severe course. The lesions are characterized by clusters of superficial blisters and intensely itching vesiculopustules probably lasting roughly 2 weeks with subsequent outputs. Ocular complications (herpetic keratitis progressing to blindness) are possible [94].

– *Herpes labialis* is usually rarely considered, but can cause a hemifacial involvement and may be confused with *Herpes zoster.* Characteristic of this infection are its recurrences in different parts of the body, some patients suffer from exacerbations of the lesions concomitant with this virus [94]. Children should not have direct contacts with persons affected with *Herpes labialis* [35].

- *Eczema herpeticum,* whose form of cutaneous type 1 or 2 HSV infection, commonly by heteroinoculation, is not evident <5 years. The localized form is characterized by itching and by papular, vesicular, bullous, erythematous or eczematous lesions. In children, the cluster lesions have a predilection for the face and hypogastric, gluteal and perineal regions; herpetic gingivostomatitis may occur in young male children with active, severe and persistent AD, often after prolonged topical or systemic CS therapy. Disseminated, life-threatening disease may have a poorer prognosis in young children and immunocompromised subjects.

– *Eczema vaccinatum* is a severe eruption by poxvirus, mostly by autoinoculation or heteroinoculation, with vesicles and pustules without seat predilection and high fever. Defervescence occurs within 10 days in uncomplicated cases. Virus dissemination with organ involvement is life-threatening. *Eczema vaccinatum* may by clinically indistinguishable from *eczema herpeticum.*

– *Molluscum contagiosum:* children with AD are susceptible to this infection, which is extensive, recalcitrant, and characterized by roundish, shining, often umbilicated papules, having a diameter of 1–2 mm, with a central body, or *molluscum* body, containing epidermal viral cells with cytoplasmic inclusions. The infection is extenuated by autoinoculation. The risk of developing dissemination of the organism is favored by long-lasting use of CSs; otherwise the infection has a chronic course (1–2 years).

– Virus of human papilloma causes warts, often multiple with a chronic course, which can increase in number by autoinoculation [236].

Additional Complications

- Cold abscesses involving subcutaneous tissue should be differentiated from hyper-IgE syndrome.
- Subcapsular cataract, anterior and/or posterior, for which various types of treatment have been implicated, especially with CSs and PUVA (psoralen ultraviolet A), or herpetic infections [36]. Onset is in early childhood with an incidence of 16% [94].

Association with Other Atopic Diseases

- *Respiratory allergy* (mean, 46.8%), asthma (RP 1.45) [22]. In children with AD, a latent predisposition for asthma has been reported [238] and is significantly higher in children with a smoking mother [175] or in those who have experienced an early age of AD onset [48, 218]. The age of onset is statistically lower in children who are asthmatic compared to nonasthmatic children (p=0.011) [58]. AD can also start within the 1st year and asthma in the following years [192]. Moreover, AD worsens in the spring in pollen-allergic children. As much as 35% of 500 6-year-old children were asthmatics, 50% of those with severe AD and 15% with mild AD [206], showing that asthma begins earlier and more frequently in severe cases of AD [206, 218]. In 14 studies meta-analyzed in Table 7.10 [9, 23, 39, 58, 88, 154, 205, 206, 215, 221, 228, 286, 294], the mean incidence of asthma is 46.8%; in highly selected pediatric populations, it is between 66% [221] and 79% [88]. In up to 95% of these children, bronchial hyperreactivity has been described, PD_{20} (provocation dose 20) is of 0.22 µmol in children with AD and asthma and 2.10 µmol in those with no association [222]. Early AD is associated with asthma at school age, and children with AD and wheeze have a marked loss in lung function. The prevalence of early wheeze was significantly higher in children with early AD compared with children with no early AD (46.3% vs 32.1%, p=0.001) [111].
- *Urticaria* (21%–34.8%) [215] (RP 2.04) [22] is found in up to 43% of children [9]. Urticarial lesions of the immediate type to mite allergens have been reported

Table 7.10. Asthma prevalence in children with atopic dermatitis

Authors (reference)	No. of children	Median age (years)	Asthma (%)	Follow-up (years)
Purdy [205]	93	NS	40	15–20
Vowles [294]	84	NS	50	13–22
Bono [23]	61	10–14	30	9–13
Van Hecke [286]	50	5[a]	28	20
Queille-Roussel [206]	500	5.7[a]	35	5
Businco et al. [39]	56	3	37	5
Corbo et al. [58]	40	12.3	20	NS
Rudzki et al. [215]	365	0.5–16	46[b]	NS
Bardare et al. [9]	92	5[a]	58	5
Guillet et al. [88]	29	3	79	3
Linna et al. [154]	40	11–13	53	11–13
Sampson et al. [228]	205	4.4	59	11
Salob et al. [221]	250	8.4	66	NS
Cantani (in press)	395	4.5	54	3
Total	*2,260*		*Mean 46.8*	

The studies are ordered according to the year of publication of the work.
NS Not specified.
[a] Mean age
[b] 41.8% with one, 50.8% with two atopic parents, and 25.2% with negative family history.

in children; acute and contact reactions are associated with FA or pseudo FA.

- *FA* (30%–50%) has an RP of 3.20 [22]. Children with CMA show cutaneous symptoms in 34%–64% of cases (see "Relationships Between AD and FA" and Chap. 9).
- *Atopic keratoconjunctivitis,* whose incidence is unknown, has an RP of 2.25 [22]. The associated ocular disease involves about 3% of the general population and especially adolescents.
- *ACD* (8.6%–32.2%) (Table 5.9) shows an increased incidence up to 44% of children aged 6 months to 12 years [57], up to 43% of children aged >8 years [168], in atopic children >10 years [61], or a progressive increase: 11% of children <2 years and 58% of those >15 years [108].

Association with Nonatopic Diseases

- *Gastrointestinal disorders:* in children with celiac disease, scaly skin lesions resembling AD have been reported, which may improve on a gluten-free diet. Some patients with eosinophilic gastroenteritis also have AD.
- *Ichthyosis vulgaris:* skin signs are commonly shown in children >2 years of age, rarely involving the extensor limbs and with hyperlinearity of the palms. The disease occurs in 2%–6%. AD changes are seen in >50% of ichthyosic patients.
- *Dermatitis herpetiformis* Duhring: pruritic erythematous or papular lesions are frequently or infrequently associated with AD, but especially with celiac disease.
- *Cystic fibrosis:* an association with AD is observed in 8%–13% of cases.
- *Metabolic abnormalities:* a dermatitis indistinguishable from AD was described in 19%–50% of children with phenylketonuria, principally during the 1st year of life.

Diagnosis

The diagnostic approach to AD includes a medical history, physical examination, and laboratory studies. For the diagnosis of AD associated with FA see below and Chap. 9. The main points are discussed as follows (Table 7.9):

- *FHA* is positive in 60%–70% of cases (Tables 4.9–4.11).
- *Personal history* includes important elements such as the course of AD onset, the foods administered, and environmental and/or emotional changes.
- *Medical examination* is based on the child's symptoms or on the clinical criteria laid down by Hanifin and Rajka [96] (Table 7.9), who recommend taking into account three major criteria and at least three minor criteria. There is a simplified version, with a sensitivity of 80%, specificity of 97% and a positive predictive

value (PPV) of 80% [310]. More adequate for infants and toddlers is the 3+3 criteria [224], because there has been much controversy [166, 177] surrounding the minor criteria [96], which are based on symptoms that are not standardized and do not occur commonly [37]. To assess AD lesions we refer the reader to the diagrams of Figs. 6.1 and 6.2 and, to evaluate the severity of the lesions, to Appendix 7.1 and the SCORAD scoring index shown in Fig. 6.19, which takes into account the morphology as well as the extension (20%), intensity (60%), objective symptoms, pruritus and sleep disturbances (20%) [311]. Figures 6.4–6.18 show a wide photographic documentation useful for the objective diagnosis.

- *Laboratory studies* include several methods, as follows:

– *SPT* or *P+P*, SPTs for food and inhalant allergens, P+P only for fresh foods. When positive (in 75%–85% of cases there is high sensitivity), SPTs may have no absolute correlation with clinical symptoms and the course of AD [147], since a positive SPT to a food is only suggestive of the actual existence of clinical FA. Applying the criteria of analytical reliability (Table 6.16) to SPTs for foods is credited with a high negative predictive value (NPV) with the foods often responsible for immediate symptoms, namely cod, peanuts, etc. As regards SPT use in planning a diagnostic elimination diet, see below.

– In addition to food allergens, it is always appropriate to test, including in infants, *aeroallergens* (major trigger, Der p), adding the PT in children with negative SPTs when an IgE-dependent sensitization is suspected [89].

– *Total serum IgE levels* are elevated in about 80% of children, especially if respiratory allergy coexists, although there is no close correlation between IgE values and AD. In case of need, serum IgE can be determined together with RAST [37], or very elevated values are employed for prognostic screening.

– *RAST* is useful in children with such widespread lesions that it can be impossible to carry out SPTs, and in the case of antihistamine administration. However, a routine use should be avoided [37]. RAST is used to identify food-specific IgE but patients with high total serum IgE levels can have false-positive RAST because of a nonspecific allergen binding [147]. RAST is no more specific than SPTs in detecting clinical sensitivity. We have not always found correlations between sIgE and responses to the diet [37]. A modified RAST has been developed for determination of IgE antibodies to *Candida albicans* [180].

– Clinical–biological studies have investigated the determination of *ECP levels* (normal values, <16 µg/l) as a diagnostic tool [173]. As discussed previously in "Humoral Immunity", there is a correspondence between AD state and ECP activity, but the lack of correlation between ECP levels and those of circulating eosinophils [157], serum IgE [258] and AD severity [157] makes this test unsuitable for everyday diagnostic purposes. ECP may be useful in monitoring the AD course and evaluating the ongoing pathological process [125, 191, 258], as well as for asthmatic children.

– In children with AD and asthma, *LCT_4 is a more sensitive variable* than ECP and/or peripheral eosinophilia to evaluate eosinophil activation state [238].

– Finally, measuring *CD54 levels* has been suggested, as they show a good correspondence with AD severity [123].

Differential Diagnosis

Figure 7.33 [242] shows how to differentiate types of AD according to stage. Moreover, depending on the child's age the following disorders should be taken into account [36, 94]:

Early Infantile Phase

- *Seborrheic dermatitis,* the most frequent, is easily differentiated from AD and often coexists with AD. However, a number of babies with AD develop typical seborrheic dermatitis [94] (Table 7.11) [318, 324].
- *Diaper dermatitis* with secondary eruption, with early age of onset (1–2 months) and localization to the diaper area, with possible extension, even if rare, to the face and/or to deep-seated folds. Itching is absent or mild. The lesions are often superinfected with *Candida albicans*.
- *Acrodermatitis enteropathica,* best-known clinical expression of Zn deficiency and consequent ID, is a disease with an autosomal recessive mode of inheritance. The skin lesions, characteristic and pathognomonic, are initially vesiculopustular and subsequently crusting and psoriasiform, because of insufficient Zn absorption, are preferentially periorificial, and localized to the hands, feet and head. The appearance of lesions when the baby is weaned from breast milk to cow's milk (CM), accompanied by diarrhea and steatorrhea and the regression of both conditions after Zn therapy are differentiating [36] (Chap. 21).
- Rare disorders include

– *Leiner's disease* (scaling erythroderma).

– *Staphylococcal scalded skin syndrome* (SSSS) due to the absence of epidermolytic toxin-producing strains.

– *PID:* hyper-IgE syndrome, ataxia-telangiectasia, WAS, IgA selective deficit, X-linked agammaglobulinemia, severe combined immunodeficiency (SCID), and X-recessive hyper-IgM syndrome. In SCID-Omenn syndrome, infants show a diffuse, exudative erythroderma, often defined as AD, like LC histiocytosis. Nezelof syndrome is characterized by AD-like lesions and very elevated IgE concentrations (Chap. 22). In secondary ID, AIDS and AD may be associated.

– *Metabolic disorders,* including phenylketonuria, Hartnup disease, mucopolysaccharidosis I H, histidine-

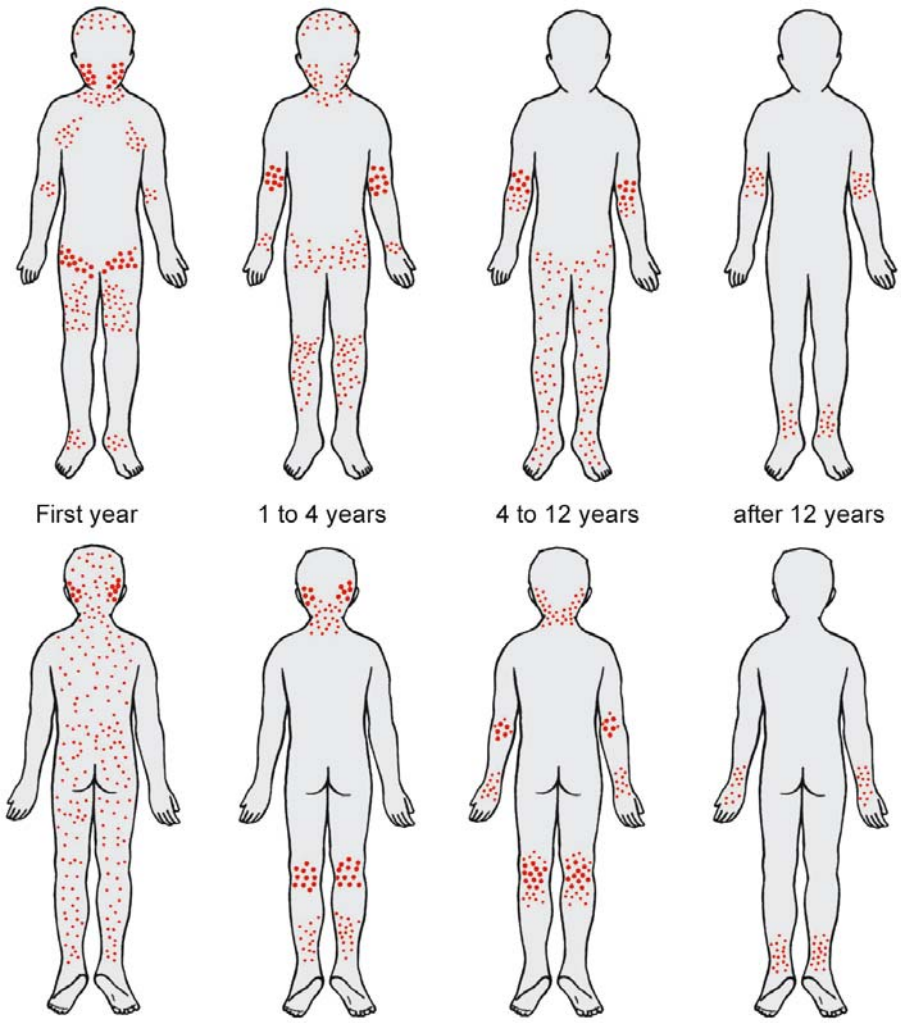


Fig. 7.33. The clinical manifestations are different depending on age. (From [242])

Table 7.11. Differential diagnosis between seborrheic dermatitis and atopic dermatitis

Clinical and laboratory data	Seborrheic dermatitis	Atopic dermatitis
Family history of atopy	Usually lacking	Positive for atopic disorders
Age of onset	Usually <2 months	Usually >2 months
Distribution	Scalp, forehead, nasal folds, neck, axillae, diaper area; in older babies eyelids and eyebrows	Cheeks, forehead, extensor surfaces of limbs; flexural surfaces in older children with limb involvement clearly diagnostic
Lesions	Erythema with yellow-reddish greasy scales	Erythema, papules, vesicles, crusts; no greasy scales
Pruritus	Absent or minimal	Severe, a hallmark of the disease
Skin tests	Negative	Usually positive
Serum IgE	Normal	Usually increased
Specific IgE	Negative	Often positive for foods, later for inhalants
Prognosis	Clears in 3–8 weeks; no recurrences	Chronic, relapsing
Associated allergies	Uncommon	High incidence of associated allergic disease (asthma, rhinitis, food allergy)

Data from [318, 324].

mia, biotin-responsive multiple carboxylase deficiency, and prolidase deficiency occur: some cases have eczematous lesions, mostly insufficiently studied. In trisomy 21, 21 %–56 % of cases may have atopic symptoms, along with those of an ID of the cellular type as well as of a congenital ichthyosis.
– Cutaneous lymphomas (mycosis fungoides, Sézary's syndrome and Hodgkin's disease, T-cell lymphomas) may initially present with eczematous lesions.
– Histiocytosis X.
– Congenital amyloidosis.
– Congenital perceptive hearing loss.
– SIDS (sudden infant death syndrome).
– Netherton's syndrome is an autosomal recessive disorder characterized by hyper-IgE, dermatitis indistinguishable from AD, *ichthyosis linearis circumflexa, trichorrhexis invaginata* and bamboo hairs.
– In cystic fibrosis, there may be an increased prevalence of atopy and of FHA.

Late Infantile Phase

- *ACD,* often confused with back of the foot dermatitis, similarly presenting with erythematous and pruritic lesions. Atopic stigmata are often absent. If a doubt exists, the PT is diagnostic (Chap. 8).
- *Mycosis* of the sole of the foot or *tinea pedis,* where so-called athlete's foot is often diagnosed. A culture of locally collected material may be helpful.
- *Pityriasis rosea* has shown an increased incidence in schoolchildren, of typical oval, pale, erythematous, papular or scaling, isolate lesions, especially of the trunk. The absence of itching and the self-limiting aspect of lesions are diagnostic.
- *Gianotti-Crosti syndrome* in preschool children may manifest with monomorphic, erythematous or papular lesions, nonpruritic, of red to purpuric color and symmetric aspect, affecting the face (especially the cheeks), forearms and hands, occasionally with involvement of the lower limbs, but absent from the trunk, palms of the hands and soles of the feet. The characteristic aspect of the lesions, absence of pruritus and outcome within 2–3 weeks are distinctive.
- *Scabies* can be easily confused with infantile AD because severe pruritus, blisters, and granulomas occur in both conditions, but small papules or pustules are electively localized to the cutaneous axillary and genital folds, extensor surface of the elbows, and interdigital spaces of both hands and feet, and have a distribution that does not predispose to misdiagnosis. The detection of the parasite *Sarcoptes scabiei* or the scabies in other family members is diagnostic.
- *Eczema herpeticum* (see "Complications") culture of isolates allows the differential diagnosis between HSV and varicella zoster.
- *Cutaneous lymphomas* and congenital amyloidosis (extremely uncommon).

Childhood and Adolescent Phase

- *Psoriasis* presents with reddened lesions covered by whitish scales and with very distinct limits, involving extensor surfaces, elbows, knees, etc., and with more or less frequent pruritus. Common linkages have been found with AD and psoriasis in three chromosomes (Table 4.2).
- *Pyoderma* has a characteristic course, with itching less frequent than in AD.
- *Dyshidrotic eczema* manifests with pruritic vesicles of the palm of the hands and soles of the feet, worsened by dyshidrosis and hyperhidrosis.
- *ACD.*
- *Microbial eczema,* characterized by disseminated lesions, nummular, clearly demarcated, measuring a few cm [36, 94].

Other Dysfunctions

- *Mycotic infections* in addition to those by *Candida* and *Pityrosporum* previously described, for example, *tinea corporis* characterized by usually roundish, erythematous or scaling patches, with clear-cut and polycyclic limits and resolution from center to periphery; *tinea capitis,* characterized by roundish patches of atrichia amid normal hairs; and *tinea manuum* occurring with hyperkeratosis on a slightly erythematous basis and also with skin dryness, but the clinical picture is not always diagnostic and should be completed by history and mycological examinations.
- *Molluscum contagiosum* (see "Complications").
- *Impetigo,* a bacterial infection marked by blebs and pustules with erosions and brownish-yellow crusts on exposed skin and on the face around the nose and mouth.
- *Miliary,* a benign and transient skin alteration resulting from immaturity of sudoriparous glands that do not succeed in coping adequately with an external or internal T-cell increase. Reducing the child's excessive clothing eliminates the problem.
- *Insect venoms* produce wheals and/or localized papules.

Table 7.12 summarizes the most common causes of pruritic skin rashes [305].

Table 7.12. Most common causes of pruritic skin rashes

Contact dermatitis
Drug-induced erythema
Exanthematous disorders
Herpetiform dermatitis
Infestations
Lichen simplex chronicus
Mastocytosis
Parasitosis
Psoriasis
Systemic disorders (rare) seen in pediatric age Endocrine disorders Hepatitis Myeloproliferative disorders Obstructive disease of biliary tract
Urticaria

Modified from [305].

Management

The treatment of a multifactorial condition is complex, including dietetic manipulations and environmental measures (Table 7.13). Indications to be followed are different depending on the acute or chronic stage of AD [37]. If FA complicates AD, see Chap. 9.

Avoidance of causal agents and of aspecific irritants is without doubt the first step to be taken. Adopting measures aimed at preventing, reducing, or even minimizing trigger factors is adequate and in certain cases necessary for maintaining control of the disease. To guarantee children a good quality of life and make the condition tolerable, it is vital to control the itch for which children with AD have a lower threshold [36]. In addition, topical treatment should be individualized to control both inflammation and skin infections. The secret of successful treatment in children with AD is to avoid prescribing only symptomatic therapies, but rather to *try to find first the casual factors,* specific to each child. Therefore every form of treatment should be tailored to the individual child [97]. The following points can be used for managing most children:

1. General and local hygienic and preventive measures:
– Itching control
– Child's hygiene
2. Local aspecific cutaneous treatment
3. Medical treatment
4. Specific antiallergic measures
5. Other measures
6. Antiasthmatic measures

Table 7.13. Suggestions for the management of children with AD

Dietary management (Chap. 24)
Favor prolonged breast feeding Favor delayed weaning and solid food introduction Avoid allergenic foods
Hygienic norms
Ensure pruritus control and prevention Ensure skin hydration and protection Minimize too frequent bathing and hot water Avoid detergent or fragrance-filled soaps Reduce skin contact with coarse fabrics in clothing and bedding Minimize excessive sweating Reduce causes of inflammation
Avoidance of irritants
Reduce environmental allergens, including mites and furry pets Eliminate parental cigarette smoke Minimize extreme fluctuations of temperature and humidity
Medical management
Local therapy by topical corticosteroids to combat inflammation and exudation Antihistamine medications to reduce pruritus Secondary infection control by topical or systemic antibiotics
Additional control
Ensure day and night rest Minimize of emotional stress Distract children with games and manual activities

Management should be individualized.

General and Local Hygienic and Preventive Measures: Acute Phase

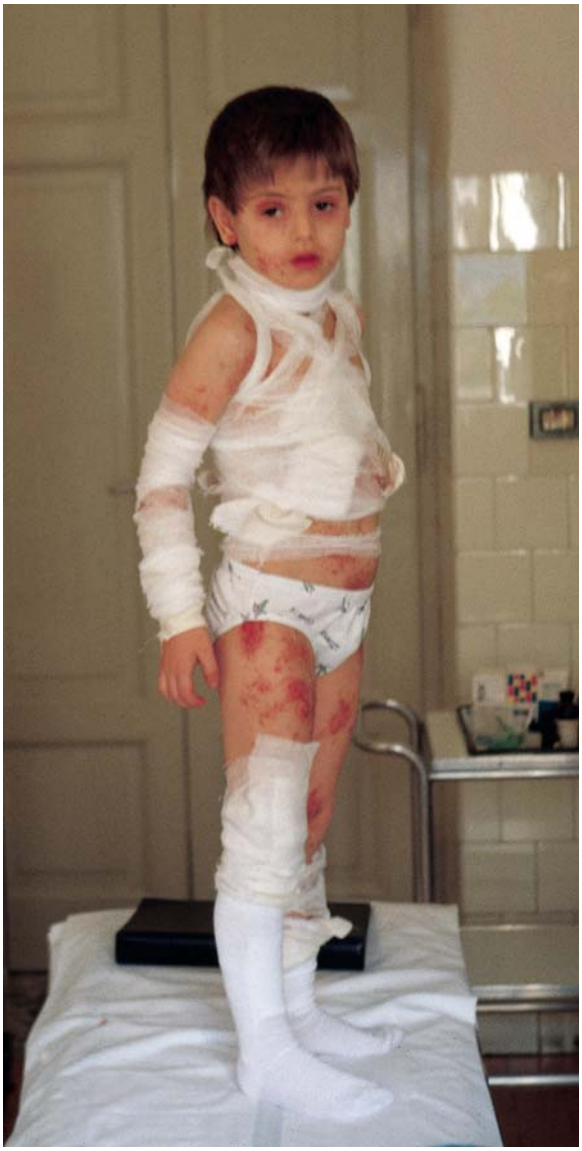
Common sources of skin irritants to be avoided can be summarized into eight main points [94]:

- Woolen or coarse clothing
- Nylon or occlusive clothing
- Plastic mattresses, pillow covers and bedding (Chap. 24)
- Excessive contact with hot water
- Bathing without proper medication
- Exposure to chemicals, above all detergents and solvents
- Vigorous scrubbing in a hot shower
- Contact with dust, dirt, and newsprint

These measures should be individualized, eliminating every factor that aggravates the lesions [5].

Itching Control

- *Reduce the lesions provoked by scratching,* which can be accomplished by trimming and cleansing fingernails, using 100% pure cotton mittens or gloves and stockings to prevent scratching during the night. For this purpose, we also suggest using pure cotton pyjamas [36] and protecting the skin by covering the affected areas with a heavy wrapping or other restraints for infants with AD. Alternatively, the lesions are best bandaged with well-fitting sterile cotton dressings and kept in place with an elastic bandage, after having sparingly applied a low-potency CS ointment and emollients to nonaffected areas [5], also as an effective barrier against the persistent scratching that often undermines therapy. When the lesions are extensive, bandage the child using the above procedure (total-body involvement) (Fig. 7.34 a): for this purpose the common gauze for dressing or tubular bandages may be employed. This wet-wrap dressing can also be achieved by using damp but not soaking wet cotton pyjamas covered by dry cotton pyjamas [5]; severe lesions on hands and feet may be dressed with gauze and bandage wraps. *Occlusive dressings* usually soothe the inflamed skin of children with AD, dry up the weeping exudative lesions, and prevent nocturnal scratching (Fig. 7.34 b).
- *Avoid contact with coarse* bedclothes, wool blankets and pillows, feather and kapok mattresses and pillows, sheets and pillow-cases that are not of pure cotton or linen and unwashable blankets as well as bedding with wool, nylon or synthetic textiles or adherent, heavy, rough clothing. Even in winter, the inherent softness of 100% cotton, free of dye and starch, used next to the skin and for bedding is preferable.
- *Children should only wear* underwear of white cotton (removing every colored label) and pure (not interwoven with wool or with synthetic textiles), or of smooth linen, always with long sleeves. Underwear should not adhere too closely to the skin. Moreover, infants should not be given plastic diapers, instead only those of linen or white cotton, as previously suggested, and these are to be changed regularly. *Avoid sweaters* and pullovers touching both wrists or neck; avoid wool hats, caps, and scarves. Keep children from playing on rugs or on wall-to-wall carpeting.
- *Avoid contact of underwear* and bedding with irritating chemical substances, including fabric softeners and harsh enzymatic disinfectants and bleach during washing. Clothes should be rinsed carefully to eliminate irritating residual laundry detergents, and should be preferably washed with Marseille soap. The irritating and drying effects of many soaps and detergents should be emphasized. Even new clothing and sheets should be washed thoroughly prior to use to remove sizing and other chemicals with which they may have been treated.
- *Avoid physical activities* which require an excessive effort and occlusive clothing (Table 7.13), which with sweating aggravates pruritus. Excessive clothing in AD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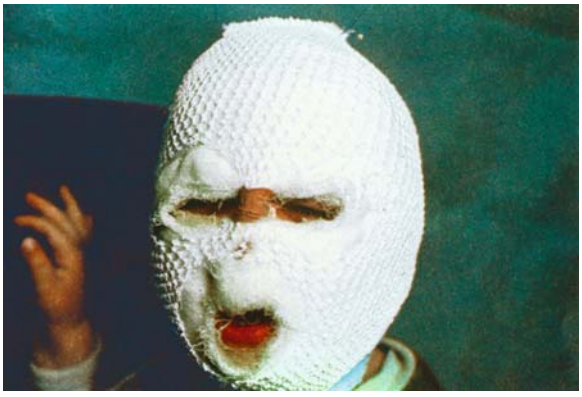
b

Fig. 7.34. **a** The child is bandaged (for details see text) either to avoid uncontrolled scratching or to apply topical medications. **b** The particular bandaging is done to avoid paroxysmal nocturnal scratching: 2 min of uncontrolled scratching can offset 10 days of successful therapy

children may be a hindrance to normal heat dispersion, which automatically is converted into overheating. In healthy children, the cool-warm equilibrium is re-established via both transpiration and evaporation, with a consequent rapid cooling. The alteration of this homeostasis in children with AD results in high amounts of perspiration seeping into inflamed skin, with a notable accentuation of pruritus, via the above-discussed irritating mechanisms [5]. Therefore, we repeat the suggestion of using 100% cotton next to the skin, by far the best choice, because even increased perspiration fails to cool the skin, and evaporates rapidly from the skin surface [44].

- Experience suggests that when the child is undressed, for example during the doctor's visit, both pruritus and scratching increase, thus indicating that the child should remain dressed, as far as this is feasible, also because clothing protects the skin from the excoriations provoked by scratching [5].
- *Avoid indoor overheating* during the winter season, especially in poorly ventilated houses. The indoor T should be kept at 18–20 °C and relative air humidity at 45%–55% to prevent additional skin drying (Chap. 24).

Child's Hygiene

As a cause of persistent skin inflammation, the irritating and drying effects of normal or perfumed soaps, bubble baths and surface-active detergents should not be overlooked (Chap. 8). Although water alone is best [137], mild cleansers (Marseille soaps for washing) may be used, as well as oleate or hypoallergenic soaps, or non-soap cleansers, provided that they are fragrance-free and designed for sensitive skin, and carefully rinsed away. If alternative products are available check their composition carefully, since they may contain CM proteins or other sensitizing proteins [97]. Moreover:

- *Daily bathing* (not showers!) in a soaking lukewarm water (mean T of 36 °C [36], limited to 10–15 min is recommended to optimize hydration of the *stratum corneum;* then rinse the baby carefully with tepid water. Within 3 min of leaving the water, apply hydrating ointments or totally unfragranced creams soon after having dried the baby: waiting longer dehydrates the skin through evaporation of the water absorbed by the *stratum corneum* [94]. The frequency of bathing should be evaluated according to the individual tolerance to water. If the water is rich in limestone, a depurator of reasonable cost can be suggested.
- *The face or neck* can be hydrated by applying a wet cotton towel or facecloth to the involved areas for 10–15 min; hands and feet can be hydrated by soaking the limbs in a basin.

– In AD active phases, it may be useful to have the assistance of another person to occupy the baby's hands.
– We suggest that the skin of the child is dried very delicately: patting not rubbing with a soft absorbent towel of pure cotton, white, smooth and washed as pointed out above.
– If, despite all precautions and efforts, especially during the acute phases of AD, bathing provokes irritations, we suggest reducing bathing to the simplest terms, or employ only tub water, cleansing the body with a dry towel, thus restricting bathing to what maintains the child socially tidy, assuring the mother that frequent washings have more esthetic than sanitary justifications [5]. Other authors suggest using a minimal amount of antiseptic liquid with an insignificant risk of systemic effects, leaving the mother with the impression of washing her child [236].
– Avoid the child soaking in the tub at length and avoid overly frequent bathing since both dehydrate the skin, thus lowering the itching threshold.
– In conclusion, from a practical standpoint the dry skin of children with AD suffers from frequent, prolonged and vigorous washings.

- *Hydrating lotions* or creams should not contain additives, fragrances or other potentially irritating substances. If this is not the case, a commercial preparation that is sure to be tolerated by children can be suggested [236]. Such products are to be employed in particular at the first signs of cold weather to protect the exposed parts of the body.
- *Swimming* is usually well tolerated. However, swimming pools are frequently treated with chlorine or bromine: therefore it is suggested that children shower and use a mild soap immediately after to remove these potentially irritating substances and lubricate their skin [5].
- For *scalp hygiene,* even if the dermatitis is commonly mild, the above measures should be adopted. A mild or baby shampoo in young children and a hypoallergenic shampoo in older children can also be used, paying attention that the shampoo does not come in contact with the skin of the face. For children with an extremely sensitive scalp or more severe or extended lesions, and keeping in mind the scalp's high capacity of absorption, especially in the first 2 years of life because of the scalp's small dimensions, especially formulated shampoos and conditioners are recommended [97].
- Thoughtful attention should guide the selection of reliable *shoes for children* with juvenile plantar dermatosis (atopic feet dermatitis), avoiding excessive heat or humidity to prevent plantar dyshidrosis or other occlusive effects. Therefore, boots, high heeled and rubber shoes should be avoided. The most suitable are those made of natural fibers, replaceable in winter with shoes of pure leather with the following characteristics: the vamp should not treated or finished with chemical substances; the likely glued cover should permit foot transpiration; the internal insole should be of leather or of cork; children should always wear heavy white stockings, avoiding synthetic socks [5].

Local Aspecific Cutaneous Treatment

The principles are different depending on the acute or chronic stages [35, 94, 95, 107, 164]. During the phases of skin dryness and lichenification, an emollient cream or lotion is necessary, taking care that they are free of substances to which the child is sensitive. Their use combined with hydration helps re-establish and preserve the *stratum corneum* barrier. Moisturizers have also been shown to decrease the need for topical CSs [95]. Taking into account an EFA deficit, using creams containing EFA of the ω-6 series is recommended when needed. Next, skin cleansing and hydration is necessary: based on the type of lesions prominent in the three clinical stages, the treatment is adequately adapted to the pertinent phase, with special attention to the itching-scratch cycle. In a chronic condition such as AD, where scratching is the worst evil, skin protection turns into a basic prerequisite [35]. The therapeutic option most favored by dermatologists is topical therapy [5, 107], whose goals we summarize. Their prerequisites are, on the one hand, to lessen the clinical manifestations promptly, and on the other, to painstakingly treat the inflamed skin, thus bringing the skin back to normality again as much as possible. The inherent peculiarities at the *stratum corneum* suggest that there is the inclusion of hydrophilic and/or lipophilic ointments, apt to penetrate more rapidly into this stratum, where hydrophilic and hydrophobic-lipophilic strata alternate. This performs an important reservoir function and allows a sound but constant diffusion of the medications toward the deepest strata of the epidermis. Topical treatment has therefore an elective indication at the level of epidermis, which, being avascularized, is barely reachable by systemic medications [107]. Furthermore, the suggested galenic preparations, or nonsteroid topicals, have the advantage of being practical, free of unwanted side effects, as effective as possible, esthetic and low-cost, and they contain a lesser amount of chemical ingredients added as preservatives, or solubilizers, which can be eliminated by contacting the local dealer. However, it is hoped that we will soon obtain that on the packages of cosmetics and emollient topicals, etc., there will be a clear indication of their composition [107]. As regards preparations in general, since every child has a different tolerance, it is advisable to make a one-sided trial in both cases. The best results are obtained when therapy is instituted promptly, beginning with an accurate cleansing of the inflamed skin: a careful removal of crusts and remnants without rubbing is best applied with the aid of wet compresses, while greasy substances are removed more readily by using suitable solvents. Consequently, different vehicles and excipients are used depending on the AD phase (Table 7.14) and the exposed skin (Table 7.15) [107].

Table 7.14. Stages of AD and rules of topical therapy

Stages of eczema	Management
Acute and exudative	Indifferent bases (oil, water emulsions)
Acute and not exudative	Wet on wet (creams, soft pastes)
Subacute	Mild exsiccating (creams, soft pastes)
Chronic	Hydrophilic to lipophilic (ointments)

Modified from [107].

Table 7.15. Rules of local therapy

Exposed skin	Lotions, ointments and creams during the day; ointments during the night; if necessary (scaling or hyperkeratosic lesions) also ointments during the night
Scalp and hairy skin	Lotions and creams; avoid pastes and ointments, difficult to remove
Creases, folds (intertriginous and diaper areas)	Lotions, powders, creams and soft pastes; avoid fatty excipients which have macerating effect; in the diaper area the use of greasy pastes may be helpful when a highly protective effect is required

Moisturizers are available as lotions, creams, and ointments. Modified from [107].

Acute stage with vesicles, oozing, crusting and erosions: wet on wet hydrophilic packs are suggested, by which an antiseptic and decongestant action is accomplished as follows:

- *Layers of sterile gauze* may be imbued with chlorhexidine hydrochloride 0.01%–0.05% or Burow's solution with Al at 5% (at 1:10–1:40 dilution), squeezing away the excess fluid from the gauze and changing it several times to avoid skin exsiccation. The medications are maintained in place for no more than 20 min and in total should not exceed 2 h/day, but should be used only for a few days because of their drying effect.
- *Oatmeal may be added* to medicated, refreshing baths, it may feel soothing to certain children but increases water absorption. K permanganate ($KMnO_4$) 1/10.000–20.000=0.1%–0.05% is very effective, but $KMnO_4$ can be used only in hospitalized children because it colors the skin brown, especially the nails.
- Packs, creams and soft pastes should be employed in acute but not exudative stages (Table 7.14).

Subacute stage with lesions dominated by erythema, edema, serum papules, oozing scales:

- An exsiccating, lenitive and refreshing action is accomplished using *creams and soft pastes* with a mild exsiccating and anti-inflammatory effect. Mild ingredients should be used. Hydrophilic soft pastes, for example mixtures of water and powders, can be used: water paste with ZnO (zinc oxide) act by virtue of their ointment component in an absorbing, draining and refreshing manner since they favor the transpiration and avoid the local accumulation of warmth, always a negative factor.
- *Moderately emollient action* using oil-in-water or water-in-oil emollients, which when used as vehicles for active ingredients show a high release rate and good penetration into the skin of incorporated ingredients such as basis cream. Moreover, they are useful in removing scales and cleansing the lesions from grease and water remnants. They have a limited occlusive, but not drying effect, thus allowing a sufficient evaporation and transpiration of skin lesions, with an added refreshing effect.

Chronic stage with scaling, lichenification, infiltrated lesions:

- *Vehicling action of active components* by means of *hydrophilic to lipophilic ointments* (water-oil type), for example a simple emollient. Ointments in particular ensure an emollient and deep action, because of an occlusive effect of the lipid phase that creates a state of permanent humidity by preventing the surface evaporation of fluids, thus favoring skin hydration.
- *Refreshing and moderately greasing action* preferably using lipophilic ointments (oil and powders) such as the *Lassar's paste,* based on ZnO, talc, vaseline, lanolin ana. g 25 for lichenified lesions. In lanolin-intolerant children, it can be substituted with an equal quantity of vaseline; Lassar's paste is mild enough to be well tolerated and ZnO helps dry and soothe weeping lesions; both have a greater penetrability and a higher accentuated covering effect than the emulsions. Lotions composed primarily of water and alcohol are not indicated in lichenified lesions [107].
- In both cases, *avoid overly occlusive medications,* which may not be tolerated well by many children: cotton drapes, smooth and less abrasive, are very helpful for bandages (Fig. 7.34), alone for topical medications, or for passive skin protection from contaminants and traumas (scratching, rubbing, etc.) [5].

Pay attention to lipophilic or overly greasy preparations, a possible cause of irritation if applied for several days, not only on the lesions, but also on healthy skin, especially in warm seasons and/or on sensitive skin such as that of the face or intertriginous areas. In treating juvenile plantar dermatosis, avoid antibiotics and CSs; instead keep feet and hands dry and warm, applying vaseline as necessary [164].

Usually, regional differences in the degree of xerosis and grease compatibility are found in atopic skin: the skin on the trunk and limbs tolerates and needs greasier ointments than that on neck and trunk. Moreover, children react to discomforting topicals less by crying than by increased scratching [107].

Medical Treatment

Medical treatment is first aimed at eliminating itching and reducing infections, thus providing symptomatic relief. Pediatricians often prescribe hydroxyzine as the first drug (2 mg/kg/day, divided into two doses), a histamine H1-blocker. Because its antipruritic action with mild side effects of drowsiness persists beyond the normal drug half-life, it is often sufficient and advisable to administer one night dose of 1 mg/kg. Its use is preferentially restricted to limited periods of time, to avoid a possible outbreak of tachyphylaxis (reduced effectiveness of a drug after repeated applications).

Topical CSs are nowadays the mainstay of AD treatment, a very effective pharmacodynamic therapy that also suppresses local inflammation (Table 7.16) [84, 97] and are much more effective when associated with a wetwarp occlusive dressing (Fig. 7.34). Once the remission is obtained, dry skin can be treated with hydrating and emollient creams or lotions as above. Topical CSs are available in several bases, including creams, ointments, lotions such as fluorocortin butylester, which imparts anti-inflammatory and antipruritic effects, similarly to 0.1% methylprednisolone cream [207]. Fluticasone propionate (FP) ointment and mometasone furoate cream (Table 7.16) can be used once as opposed to twice daily [95]. The main points to remember with CS therapy are as follows: use of low-potency products and the minimal quantity necessary to obtain symptom control. Using these drugs without moderation, and/or for continuous long periods may lead to well-known side effects, such as atrophy changes, secondary infections, rebound effects, tachyphylaxis and ACD [170]. Tables 7.17 [107] and 7.18 [84, 97] summarize both advantages and side-effects of topical CSs. *Employing high-potency CSs and on extended cutaneous areas* (Table 7.18) *should be avoided* due to a concrete risk of systemic side effects [84]. CS use may result in a rapid improvement, but they do not solve the problem: following a negligible application of the above-mentioned measures, there is a quick recrudescence of AD as soon as they are withdrawn. Moreover, prolonged or systemic CS treatment may enhance skin susceptibility to specific viral or bacterial infections because of its immunosuppressive effects [35].

Proper use of CSs in AD requires following a number of rules, even before any prescription is given. It is advisable to:

1. Inform the parents and/or the child about the problems related to the disease and those that are foreseeable with the use of CSs.

Table 7.16. Potency of topical corticosteroids employed in children with atopic dermatitis

Indications and preparations	
Group I	
Betamethasone dipropionate 0.05% (cream and ointment)	Diflorasone diacetate 0.05% (ointment)
Clobestasol propionate 0.05% (cream and ointment)	Halobetasol propionate 0.05% (cream and ointment)
Group II	
Amcinonide 0.1% (ointment)	Diflorasone diacetate 0.05% (ointment)
Betamethasone dipropionate 0.05% (cream and ointment)	Fluocinonide 0.05% (cream, gel, ointment, and solution)
Desoximetasone 0.25% (cream)	Halcinonide 0.1% (cream)
Desoximetasone 0.05% (gel)	Mometasone furoate 0.1% (ointment)
Group III	
Amcinonide 0.1% (cream and lotion)	Fluocinonide 0.05% (cream)
Betamethasone dipropionate 0.05% (cream)	Fluticasone propionate 0.005% (ointment)
Betamethasone valerate 0.1% (ointment)	Halcinonide 0.1% (ointment and solution)
Desoximetasone 0.05% (cream)	Triamcinolone acetonide 0.1% (ointment)
Diflorasone diacetate 0.05% (cream)	
Group IV	
Hydrocortisone valerate 0.2% (ointment)	Mometasone furoate 0.1% (cream)
Flurandrenolide 0.05% (ointment)	Triamcinolone acetonide 0.1% (cream)
Fluocinolone acetonide 0.025% (ointment)	
Group V	
Betamethasone dipropionate 0.05% (lotion)	Flurandrenolide 0.05% (cream)
Betamethasone valerate 0.1% (cream)	Hydrocortisone valerate 0.2% (cream)
Fluticasone acetonide 0.025% (cream)	Prednicarbate 0.1 % (cream)
Fluticasone propionate 0.05% (cream)	
Group VI	
Alclometasone dipropionate 0.05% (cream and ointment)	Flucinolone acetonide 0.01% (cream and solution)
Betamethasone valerate 0.05% (lotion)	Triamcinolone acetonide 0.1% (cream)
Desonide 0.05% (cream)	
Group VII	
Hydrocortisone hydrochloride 1% (cream and ointment)	Pramoxine hydrochloride 1.0%
Hydrocortisone hydrochloride 2.5%	(cream, lotion, and ointment)
(cream, lotion, and ointment)	Pramoxine hydrochloride 2.5%
Hydrocortisone acetate 1% (cream and ointment)	(cream, lotion, and ointment)
Hydrocortisone acetate 2.5% (cream, lotion,	
and ointment)	All medications: 1–2 applications/day

Data from the US Pharmacopeia.
The 7 classes are as follows: class 1 is most potent; class 7 least potent; classes 1–3, potent steroids; classes 4, 5, mid-strength steroids; classes 6, 7, mild steroids

2. Avoid overly potent steroids and scheduling long-term local therapies.
3. Never use the agents on thin-skinned areas of the face, neck, diaper and groin area, or axillae, which have a high absorption.
4. Restrict the application to eczematous lesions, using a hydrophil carrier base in the acute phase and an ointment base in the chronic phase.
5. Give parents practical indications on the quantity of ointment to be used, for example applying the agent in strata as thin as possible, to be massaged delicately to favor the penetration into the skin [84, 107].

Additional rules for sparing topical CSs are as follows:
1. Step-down therapy, changing from high- to low-potency CSs.
2. Preference in general for low- or moderate-potency CSs.
3. Daily rotating treatment of different skin regions (no more than 20%/day).
4. Tandem therapy (1–2 applications every morning or night, then alternating with a CS-free basic ointment), or every 1 or 2 days (interval therapy).
5. Limit systemic CSs to short-term treatments, associating topical preparations to prevent a generalized extension of AD (involving >20% of the skin).

When the child improves, use a sandwich therapy, in other words, an antiseptic solution covered by a CS and alternatively by its vehicle, or the vehicle in the morning and the drug in the evening, and then limit the applications to only one in the morning, or an alternate-day regimen [84, 107]. In children with very elevated total

Table 7.17. Therapeutic effects of topical corticosteroids

Anti-inflammatory effect
Via inhibition of:
Eicosanoid synthesis
Cell infiltrate
Via inhibition of receptor expression on:
Lymphocytes
Dendritic cells
Mast cells
Monocytes
Macrophages
Antiproliferative effect
Via inhibition of DNA synthesis on:
Lymphocytes
Epidermis
Fibroblasts
Enhanced accumulation:
Of catecholamine-induced cAMP permissive catecholamine effect
With restored cellular capacity for differentiation
Inhibition of fibroblast functions:
Synthesis of hyaluronic acid ↓
Receptor density ↓
Proline → hydroxyproline

Modified from [107].

Table 7.18. Side effects of topically applied corticosteroids

Epidermal atrophy
Alterations of pigmentation
Atrophy of the epithelium
Increased sunlight sensitivity
Dermal atrophy
Distal phalangeal atrophy
Disturbed wound healing
Ecchymosis
Fatty tissue atrophy
Milia
Purpura
Rubeosis faciei
Striae distensae
Telangiectasia
(Additional complications of crystalline preparations:
Embolia arteriae centralis retinae
Embolia medicamentosa cutis)
Skin and skin appendages
Hair loss
Hypertrichosis lanuginosa
Loss of cutaneous elasticity
Perioral dermatitis
Persistent erythema by reversal effect (tachyphylaxis)
Rosacea
Steroid acne
Vasoconstriction
Cutaneous infections
Acneiform folliculitis
Bacterial, viral, mycotic superinfections
Pyoderma
Tinea (especially *Candida, intertrigo*)
Allergic reactions
Allergic contact dermatitis
Allergic contact photodermatitis
Systemic effects
(see Table 11.3)
Unknown mechanisms
Granuloma gluteale infantum
Stimulation of sebaceous gland secretion
(by enzyme inhibition)

Data from [84, 107].

IgE after an IV methylprednisolone bolus (20 mg/kg/day for 3 days), a prolonged improvement of skin lesions and itching was observed, along with a significant and transient reduction of CD4 compared to CD8 T cells [78]. A *short burst* of a potent topical CS, FP cream, was just as effective as prolonged use of a milder preparation for controlling mild or moderate AD in children. No differences were found between two groups of children for all outcomes. Both groups showed clinically important improvements in disease severity and quality of life compared with baseline [271]. Children aged 3 months to 6 years with moderate to severe AD (mean body surface area treated, 64%) were treated with FP cream, 0.05% bid for 3 to 4 weeks. No significant adverse cutaneous effects were noted. Thus FP appears to be safe and effective for the treatment of severe AD for up to 4 weeks *in children as young as 3 months* [77].

The new nonsedating antihistamines (doses in Table 7.19) [13] may be effective in children, some inhibiting immediate reactions, others not completely reducing IgE-mediated inflammatory lesion size [161]; however, not all interrupt the itching-scratch cycle [296]. In a DBPC study in children aged 6–12 years with positive FHA, cetirizine at a dose of 5 or 10 mg/day for 8 weeks according to their weight has been shown to be effective in controlling symptoms without apparent negative effects [140]. Cetirizine can be employed with success, as in the ETAC study in babies at risk of developing asthma by the age of 5 years as well as levocetirizine aged >6. Loratadine has provided significant daytime relief.

Cromolyn has been shown to be very active, especially in AD associated with FA; however, not all studies share our results [38]. From a scientific point of view, cromolyn has a stabilizing action on mast cell membrane, inhibiting cells in the first line in inflamed AD, namely mast cells, neutrophils, eosinophils and monocytes. In addition to this long-standing base, significant contributions show that cromolyn prevents the in vitro IL_4-induced IgE synthesis and raises the IgG production by B cells [38, 106]. Cromolyn has a neuropeptide antag-

Table 7.19. Pediatric doses of antihistamines and preventive medications

Medication	Dosage
Antihistamines	
Cetirizine	0.2 mg/kg daily[a]
Levocetirizine	1 tablet/day for children >6 years
Loratadine	0.2 mg/kg daily
Preventive medications	
Cromolyn	20–30 mg/kg/3–4 doses 20 min before meals
Ketotifen	Children aged 6 months–3 years 1/2 scoop (2.5 ml = 0.5 mg) × 2 >3 years one scoop × 2[b]

[a] For children 2–5 years old, a cetirizine formulation in drops is available, 5 mg/daily (10 drops) in a single evening administration; if the weight is not much under 20 kg, 2.5 mg/daily (5 drops) may be sufficient.

[b] Reducing after about 10 days to the single evening administration. Children aged over 3 years may also take the tablet formulation: one tablet daily (2 mg), preferably in the evening [13]. Children older than 6 years can take the levocetirizine orally.

Table 7.20. Percent fatty acids in oils from vegetable plants

Fatty acids	EPO	F	B	BC	GS
Oleic 18:1 ω9	0.6	40.4	15.8	10	16
Linoleic 18:2 ω6	72.7	10.4	39.1	48	71
γ-Linolenic 18:3 ω6	8.7	18.9	18.7	17	–
α-Linolenic 18:3 ω3	–	–	4.7	13	<1
Stearidonic 18:4 ω3	–	–	–	3	–

Modified from [121].
EPO evening primrose oil, *F* fungal (Mucor Javanicus), *B* borage, *BC* black currant, *GS* grapeseed.

onist activity, determining a dose-dependent inhibition of SP- and neurokinin B-induced cutaneous edema, as well as the SP in vitro binding to various tissues in concentrations similar to those inhibiting the edema formation [62]. Cromolyn inhibits CD4, CD19 (B cells) and PBMCs expressing soluble CD23 (sCD23) in patients with AD compared to healthy controls [106]. In a cromolyn multicenter study [168], after 8 weeks, positive effects were reported in 59% of cromolyn-treated and in 36% of placebo-treated children. The association of a diet regimen with cromolyn greatly relieved symptoms in children with AD and asthma. Moreover, topical cromolyn (cream or 4% solution) reduces SPT responses and pruritus [193]. Table 7.19 reports cromolyn doses. This is a crucial issue because in a review of 18 pediatric studies that have evaluated cromolyn effectiveness in several atopic diseases, 3 out of 12 AD-related trials, too low doses were used [38]. In a double-blind, randomized, PC trial, children aged 2–12 with AD were treated with a new aqueous skin lotion of cromolyn with a significant decrease in SCORAD scores, improvement of the overall skin condition and reduced use of topical CSs after 12 weeks compared with placebo. Local mild to moderate irritation, redness and burning at the site of application occurred in 11/154 (7.1%) children and were transient. None was reported as severe or very severe. The results of this trial show that this new aqueous-based formulation of cromolyn provides useful clinical benefits in children with AD [252].

Ketotifen has been demonstrated to be active in preventing the clinical manifestations of both AD and respiratory allergy, with the added advantage of simple administration. It shares with cromolyn the mast cell-stabilizing property and has an antihistamine action. In children affected with AD, it reduces the lesion breakthrough and significantly improves the clinical picture after 2–3 months of treatment [13]. In a randomized DBPC study of babies aged 1–36 months, in placebo-treated babies asthma was reported >3-fold as high compared to the active group, thus showing it to be effective in preventing respiratory symptoms [111]. The syrup doses are indicated in Table 7.19.

The evidence that *bacterial infections of the skin* are typically caused by coagulase-positive *Staphylococcus aureus*, which prefers wet and exudative lesions (the serum is a very good culture medium) (Fig. 7.31). Avoiding their extension and worsening requires a vigorous treatment with systemic antibiotics such as both macrolides erythromycin, generally effective for most children (Fig. 7.32) and clarithromycin (15 mg/kg/day in divided doses, from 6 months on), or cephalosporins. The local treatment includes crust removal and application of wet medications on the exudative lesions. However, it is reasonable to bear in mind that broad-spectrum oral antibiotics may affect gut microflora. *Candida* and *Pityrosporum* infection may be treated with antimycotic clotrimazole for topical use and if necessary by systemic route. Severe infections by *Herpesvirus* should be treated with IV acyclovir and less severe cases with topical acyclovir. Topical treatment is with wet compresses and/or lotions with disinfectants.

Based on several *reports on EFA deficit,* the oral administration of evening primrose oil (EPO), containing 72% linoleic acid and 9% γ-linolenic acid (ω6 series), showed conflicting results, especially in children (Table 7.20) [121]. The significant clinical improvement and the concomitant normalization of EFA serum levels observed in adults with AD and serum deficit of both EFA treated with EPO were not associated with both in vitro and in vivo results [316]. A meta-analysis has revisited nine PC studies [171], claiming a particularly consistent response for pruritus, but a paper showing no improvement at all was rejected [8]. However, an essential prerequisite was not established, that is the baseline compa-

rability of treatment groups in terms of disease severity. Some researchers studying breast milk lipid composition in atopic mothers of African [317] and European children [41] with recent onset of AD have shown a reduction of long-chain EFA levels. Other studies revealed that breast milk of atopic mothers provides their infants with normal amounts of ω-6-fatty acids [241]. In Appendix 7.2, the EFA values of 676 European and African mothers are meta-analyzed [41, 134, 317]. Given the normal human milk content of EFAs, valid means and limits have been extrapolated to control the values of affected and healthy subjects. In view of this, on one hand the first could result as normal, and on the other statistical analyses in small samples could reflect physiological variations [134]. No clinical benefit of EPO has been found [15] in children with AD and asthma and in psoriasis [250], because no difference was found in the proportion of γ-linolenic and dihomo-γ-linolenic acids between atopic children and controls [141]. While larger, controlled studies are necessary to determine whether EPO therapy is truly effective, it appears more promising to underline the scientific supremacy of both colostrum and breast milk, which contain elevated levels of EFA (Table 2.14) and nucleotides (Tables 2.16, 2.17). Table 7.20 shows the percent EFA levels also in fungal, borage, black currant and grapeseed oils. The use of EPO has received unanimous opinion [95]. Even if γ-linolenic acid levels were normal in atopic subjects [141], any treatment with this acid should be in prospective plethoric, since no study has provided evidence that AA levels can be restored [141]. Appendix 7.3 shows the recommended dietary allowances of ω6 and ω3 FFA. Concern has been aroused by dietary trans-fatty acids (Chap. 21); however, in no product for children may their level be >6% of total lipid concentration.

Therapeutic advances include two calcineurin inhibitors such as tacrolimus and pimecrolimus [95]. *Tacrolimus,* a macrolide antibiotic, currently marketed as immunosuppressant for transplant rejection, which appears to be safe and effective in children with AD aged >2 years as a topical ointment. It dramatically improved the clinical appearance and degree of itching [137] in more than 10,000 patients worldwide and exhibits potent immunomodulatory properties (Table 7.21) [240]. Tacrolimus 0.03% strength is indicated for children aged >2 years and should be applied twice daily until lesions clear and then twice daily for an additional 7 days [240]. The ointment was more efficacious then 1% hydrocortisone in 624 children aged 2–15 in a randomized DB trial [209]. *Pimecrolimus* cream 1% has similar therapeutic actions. Pimecrolimus benefits were consistently seen at 6 months and sustained for 12 months, providing evidence that long-term treatment leads to better control of AD, reducing or eliminating the need for topical CSs [137].

However, following recommendations made by the FDA's Pediatric Advisory Committee during its Feb. 15, 2005 meeting, the FDA Talk Paper T05-06 March 10, 2005 declared that pimecrolimus and tacrolimus are approved for short-term and intermittent treatment of AD in patients unresponsive to, or intolerant of other treatments and are not approved for use in children <2 years. Although the long-term effect of pimecrolimus and tacrolimus on the developing immune system in infants and children is not known, the Calcineurin Inhibitor Task Force of the ACAAI and the AAAAI concludes that based on current data, the risk-benefit ratio of topical pimecrolimus and tacrolimus are equivalent or superior to most conventional therapies for the treatment of chronic relapsing AD.

Table 7.21. Immune functions elicited by oral or in vitro tacrolimus

Inhibits degranulation
Inhibits synthesis of mast cell mediators and ILs
Decreases the expression of activatory molecules such as CD25, CD40, CD80 and HLA class I and II
Inhibits LC stimulatory function
Decreases FcεRI on LCs
Decreases inflammatory dendritic epidermal cells
Inhibits SEB proliferation on PBMCs

Data from [240].
LCs Langerhans cells, *PBMCs* peripheral blood mononuclear cells, *SEB* staphylococcal enterotoxin B.

"Probiotics" is a general term for nutrition supplements containing one or more cultures of living microorganisms, which upon ingestion in certain numbers (typically lactic acid bacteria and *Bifidobacterium*), have a beneficial effect on the host by improving the endogenous microflora. Prebiotics, undigestible oligosaccharides that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of probiotic-like bacteria, normally present in the gut. Infants who experienced AD while being exclusively breast fed and receiving probiotic supplementation had significant to complete resolution of AD lesions. Intestinal colonization with bacteria is important in promoting Th1 responses and IgA production [137]. Adequate probiotic intervention after antibiotic treatment is likely to improve the intestinal ecosystem, thus preventing the emergence of Th2-shifted immunity after antibiotic treatment. *Lactobacillus* GG is used to manage AD and CMA: in 31 infants aged 2–16 months, it provided a significant improvement in their clinical score and reduced α-1 antitrypsin concentration and TNF fecal excretion [158] and in 35 infants aged a mean of 5.5 months, the SCORAD scores decreased from 19 to 5 units in the study group and from 13 to 8 units in the placebo group [132].

The improvement in AD severity with probiotic treatment in children aged 6–18 months was associated with significant increases in the capacity for Th1 IFN-γ re-

sponses and altered responses to skin and enteric flora and showed a *significant increase in IFN-γ responses* to mitogens and *Staphylococcal* SEB toxin. This effect was still evident 2 months after the supplementation was ceased. Although IL_{13} responses were significantly reduced in children receiving probiotics after 8 weeks, this effect was not sustained after ceasing supplementation [201].

Phototherapy with UVA1 rays has been shown to be very effective if applied in large doses without provoking relevant undesired effects similar to PUVA therapy administered twice weekly [243].

For recalcitrant cases of AD that seem to be unresponsive to any treatment, for desperation therapy of severe forms [65], there are toxic drugs (thymic hormones, IFN-γ, IFN-α and rIL_2), employed in adults, but side effects require their withdrawal. The rationale for using recombinant IFN-γ is the impaired capacity of patients with AD to produce IFN-γ in response to various stimuli. It can correct the immunological disorders of AD, antagonize IL_4 action, and improve NK-cell function, CD8 T cell proliferation, etc. (Chap. 1). In two subjects aged 2–22 months with severe forms, IFN-γ and IFN-α administered in sequence have led to dramatic improvement, without untoward effects other than transient myalgia, headache, or malaise [203]. In other trials with IFN-γ, the clinical benefit was not associated with a fall in serum IgE levels [93, 176, 246], nor with a reduction of IL_4 levels, although in 6 out of 8 patients with elevated IgE there was a significant down-regulation of antibody production [246]. The improvement of symptoms is associated with a reduction in CD25, and it might therefore inhibit an excessive activation of T cells [176]. Five children aged 8–13 during treatment with IFN-α showed an improvement of lesions, and a significant reduction of serum IgE, IgG_4 and eosinophils [130]. Cyclosporine A acts on an early stage of the T-cell activation process, inhibiting NFAT transcriptional activity to block TcR-mediated signal transduction. No therapeutic assay has been done in children to inhibit the isotype switching acting on CD40. Cyclosporine suspension (dose: 5 mg/kg bid) for 8 weeks was given to 3 children with severe AD, refractory to all traditional therapies. The marked clinical improvement reduced their excruciating symptoms without clinical or hematological side effects. The sIL_2R concentration decreased even after 8 weeks of treatment, regardless of IgE levels [143]. Treatment with IVIg (intravenous immunoglobulins) at high doses in children with Kawasaki disease and yearlong severe AD, at a dose of 4–6 g/daily for 5 days, induced the remission of AD lesions within 4–7 days, with a dramatic reduction in serum IgE and in vitro spontaneous IgE production [129].

A DBPC pediatric trial showed that SIT (specific immunotherapy) is effective when protracted [83]; however, the small series studied does not allow the SIT role to be fixed definitively. The potential candidates should have been tested not only in vitro but especially with SPTs positive to Der p. A DBPC trial has experimented SIT with allergen-antibody complexes in adults with AD and Der p hypersensitivity: after 1 year, 82% of patients showed an 83% mean improvement in their symptoms associated with the decrease of Der p-specific IgG antibodies [144]. Chinese herbal therapy [6] is a kind of alternative therapy discussed later (Chap. 16). *Infliximab* monotherapy may be an additional therapeutic option for the management of refractory, severe AD [95].

Specific Antiallergic Measures

Specific antiallergic measures consist in the following actions:

- Avoid food allergens, prescribe a correct diet, and avoid perioral contact with potentially irritant foods.
- Avoid environmental allergens, in particular by admission to allergen-free rooms: Der p, in addition to directly participating in AD etiology, is responsible for the exacerbation and perpetuation of skin lesions induced by different inhalant allergens. The lesional skin is thus devoid of the normal defense barriers easily colonized by Der p, always found in houses and in higher numbers between seasons [199]. From a practical standpoint it is important to see that an inevitable vicious cycle is entered since itching is aggravated by mite-sensitizing substances, with further appearance of scratching lesions and consequent infestation by other mites. Pharmacological treatment usually offers no improvement, whereas an anti-mite strategy pursued aggressively is highly effective in reducing both severity and extension of skin lesions [262]. Infants with very early signs of AD and a positive FHA are candidates for early intervention measures against respiratory allergies [14]. Therefore, if necessary, appropriate removal of mite allergens (as detailed in Chap. 24), centered on thorough cleansing of the potentially Der p-rich bedroom [67, 262] is carried out, also as a preventive measure for the high number of children affected from AD in the first few years of life (44.2%–53.2%) (Table 5.8), who experience thereafter the onset of respiratory allergy caused in 65% of cases by sensitization to Der p [198].

Additional Measures to Be Suggested to Parents

An indirect demonstration is the observation of impressive skin improvements noted when children are admitted to hospital where Der p has an unfavorable habitat and so a lesser survival expectation, reducing its concentration to dust levels lower than 2 μg/g [67].

- In children with widespread cutaneous manifestations and recalcitrant to therapy, the so-called mini-hospitalization [181], or organizing a dust-free climate chamber [233] to calm the skin, should be considered.

Both can be helpful to remove children from an irritant-rich environment and to re-establish control of severe AD [36].

- In summer, let children spend long holidays at the seaside; a cautious exposure to UV rays can be resolvent in children recalcitrant to common treatment [182]. It is known that following exposure to sunlight and to UV rays, flare responses are reduced in July compared with November [278], thus allowing a long period of relief to both children and their parents. In particular, Turner et al [280] have demonstrated that *the more southern the holiday destination the more the child's condition improves. Why then are sunny beaches not actively advertised for this benefit?* In confirmation of this hypothesis, in children who significantly improved on an elimination diet, but still retained skin lesions despite the strict avoidance regimen and the topical treatment, we have evaluated every 3 months in the 1st week of the month the severity score of AD and peripheral blood eosinophils. We have seen that the AD severity score, pruritus and eosinophils per cubic millimeter were significantly decreased in the month of September compared to the start in January (p=0.0001–0.0005). Heliotherapy is known to selectively deplete epidermal LCs in AD lesions [182], an effect attributable to IL_{10} [155], combined with a CD4 T-cell numerical decrease and a CD8 T-cell quantitative and functional increase [278]. Basically, most of the UV radiation is reflected by dry, light drift sand, more than three-fold higher than the reflection from the seawater. Sunscreens should be used to avoid sunburn [252]. However, sunscreens can be irritants, and care should be used to identifiy an nonirritating sunscreen (Chap. 8).
- Avoid excessive sweating, favoring soft, cotton clothing, eliminating coarse, thick fabrics, also taking into account the season, climate and weather. Moreover, avoid sports leading to excessive transpiration.
- Avoid extreme T changes, such as prolonged exposure to the sun's rays, then extreme cold, then heat, etc.; moving rapidly from one environment to another should also be avoided, as large changes in ambient T, above all from cold to hot, and changes in humidity, are associated with deterioration of the skin condition. In addition, skin dryness reduces the barrier function to toxic agents, with a notable risk of lesions after exposure to water and irritant substances.
- According to experts, and taking into account the concurrent role of emotional factors, the causes of stress that create a closed circle should be removed. Owing to their particular and transient psychological structure, children are inclined to be more susceptible to common stress-related problems and their effects. This causes skin exacerbations, often linked to upsets in the mother-child relationship [80]. Parents full of love and understanding should find a routine to be near their child in those frequent critical moments. Parental attempts to stop scratching by physical restraints will surely make scratching longer and more concealed.
- Parents should be advised to distract their children with manual tasks if possible, varying age-related toys such as building blocks, meccano toys, drawing and painting with pencils, pastels (not felt pens), wooden, rubber, and metallic toys, computer games, etc., or with a structured, concrete task. During a structured task, children concerned with task involvement scratch significantly less. During unstructured tasks, children left to their own resources are engaged in significantly more scratching [80]. If the child likes to play with modeling clay and the like and has or had hand lesions, it is imperative that potentially sensitizing substances are not touched; pure cotton gloves can be worn, but only for the shortest time, paying attention to the development of rubber or latex allergy. Reinforcing the child's involvement in appropriate activities means that scratching will be less and of lower duration;
- In children who have severe symptoms and uncontrollable scratching, despite repeated interventions and advice by pediatricians, referral for psychological counseling may be welcomed.
- Growth-related problems are in proportion to asthma development. Height should be more or less normal, depending on the extension of lesion involvement [94]; however, growth retardation is unlikely to be found in allergic children with AD.

Antiasthmatic Measures

Because children with AD often develop asthma, we refer to the measures explored in Chap. 24, stressing the necessity of unquestionably keeping both pets and cigarette smoke outdoors, especially since maternal smoke heralds asthma [175], and reducing HDMs in the environment [11], as suggested in Figs. 4.15, 4.23 and 7.17.

Relationships Between AD and FA

Ninety years ago, it was observed that the elimination of suspect foods from the diet resulted in AD improvement [239]. Subsequently it was demonstrated that various food antigens cross the gastrointestinal barrier and come across skin mast cells within minutes and up to 2 h after ingestion [31]. More recent evidence indicates that IgE-mediated sensitization to food antigens plays a significant role in the pathogenesis of AD and that sIgE to foods are active in 43%–72% of subjects [36, 40, 224, 227]. Data supporting of the relationships between FA and AD are as follows:

- SPT and sIgE positivity to foods.
- Some foods are able to trigger immediate symptoms, including skin reactions, FA expression, generalized urticaria, contact urticaria, gastrointestinal symptoms such as vomiting, diarrhea, colitis, etc.
- Improvement of symptoms after appropriate restriction diets [40].

As regards SPT and sIgE positivity, it is certainly illustrative and relevant that children with AD have a very high prevalence of in vivo and in vitro test positivity compared to children with asthma and/or AR. This clearly favors a specific role of food allergens in AD. However, although food allergens are an expression of FA according to von Pirquet, they cannot yield a definitive proof of FA responsibility in such a disease.

Epidemiology

In children aged <2, the implicated foods are, in order, egg, CM and legumes; in those aged 2–10, they are egg, nuts, CM, legumes, cereals and fruits [200]. In the ETAC study, 32% of children are sensitized to CM (Fig. 5.16) and 45% to egg (Fig. 5.17).
In a group of 89 allergic children aged 7–13, seen by us, 21 (24.7%) had FA.

Pathophysiology

It is widely known that the younger the child, the more enhanced is intestinal permeability in those with AD and FA: as an immediate consequence of food allergen ingestion these proteins are allowed to transfer easily into the blood. The liver is considered a physiological filter and eliminates exogenous allergens; however, it is possible that this role is reduced in infants whose hepatic system, reticuloendothelial system and hepatocytes are immature. Exposure to food allergens, while the immune system and the gastrointestinal tract are still immature, may deleteriously influence the liver. In AD infants aged <6–18 months, when breath tests were used with methacetin – more sensitive than conventional liver function tests – significant hepatic alterations were found. The result was an improvement in both liver function and clinical symptoms, but not in skin lesions [110].

Pathogenesis

Experimental Studies

Several studies have investigated the correlations between AD and FA. As early as 1928, it was demonstrated that ingested antigen, although in small amounts, quickly cross the intestinal mucosa and are disseminated to skin mast cells through blood circulation [31]. Three years later is was shown that crossing the mucosa induced mast cell degranulation at the skin level [301]. Subsequently, convincing evidence for a pathogenic role of FA in AD was shown in infants and children [71], who, 24 h after having been passively sensitized to either fish or egg, ingested both foods and developed a skin reaction at the sensitized site [313]. A child with AD and wheat allergy was hospitalized and subjected to a restriction diet until his skin symptoms cleared and prior to instituting a food challenge test (FCT) with wheat, also to a half-body occlusive dressing: the child showed intense pruritus, but eczematous lesions flared only on the part of his body that was exposed and highly traumatized by scratching [71]. The association between AD and FA was thus proved after having been disputed for nearly a century [239], but the introduction of solid foods anticipates this combined association (Table 4.30), whose predictive value at 12 months is known to foster asthma development [139]. The RR of an infant with AD having IgE-mediated FA is 5.9 for the most severely affected group. As AD severity increased so did the prevalence of IgE-mediated FA, and the frequency of reported adverse FA reactions.

As originally demonstrated by Engman et al [71], the severe pruritus, secondary scratching, and subsequent development of eczematous skin lesions was speculated to be the result of food antigen reacting with IgE-bearing cutaneous mast cell and leading to mediator release, including histamine. This reasoning agrees with results of a classic study on histamine release after FCTs [229]. Only children with AD and hypersensitivity to various foods experiencing positive DBPCCT developed a significant rise in plasma histamine level compared to children having a negative challenge (Fig. 7.35) [229]. Although histamine was the only mediator they tested, it is possible that additional mediators released by mast cells are involved. However, since histamine is rapidly cleared in the portal and renal circulation, only a fraction of histamine released by gut-associated mast-cells will reach peripheral sites. It is therefore likely that the histamine thus released in a sensitized patient may facilitate the entry of larger amounts of antigen by inducing local anaphylaxis. These antigens reach cutaneous mast cells very rapidly, thus inducing a further peripheral mediator release and perpetuating the peripheral cascade of mediators. It has also been postulated that mediators released by intestinal mast cells may induce cutaneous and respiratory symptoms; however, histamine was assayed in plasma draining cutaneous areas [229]. A study on the role played by T lymphocytes, sIgE and the results of elimination diets has elucidated some pathogenic aspects [135].

A 1936 report documented that FA was a contributing factor in AD. It was shown that CM feeding predisposed to the development of AD, but exclusively breast-fed babies had seven-fold more chances of escaping eczema compared to CM-fed babies [87]. In 1953 [81], it was clearly shown that children with FHA had a 75% less likelihood of developing AD and asthma than CM-fed children if in the first 6 months of life they had been fed soy and also excluded beef and egg from their diet. A subsequent DB crossover study demonstrated a marked improvement of AD in 21 2- to 8-year-old children receiving a CM- and egg-free diet. It was noted that 14 of 20 patients responded more favorably to the antigen-

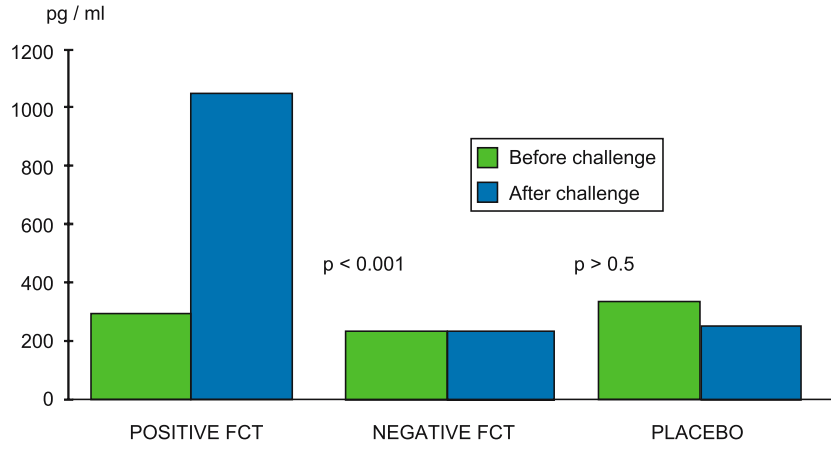

Fig. 7.35. Histamine plasma levels before and after food challenge tests (*FTCs*) in 33 children with atopic dermatitis, with a mean increase from 296±80 to 1,055±356 pg/ml (p<0.001) in children with immediate reaction to FCTs, but not in those with negative FCTs. (Data from [232])

avoidance diet employing a soy formula as a CM substitute, whereas only one had a more favorable response to a non-CM and egg-free diet [7], thus demonstrating with a rigorous method the involvement of CM and egg in the AD pathogenesis.

Clinical Studies

Sampson and his collaborators have dedicated a great deal of scientific commitment to clarifying the role of FA in AD [223–232], and trials by our division have demonstrated a clear correlation between eating certain foods and developing and worsening skin symptoms, thus concluding that the foods more frequently implicated in severe AD are, in order of frequency, CM, egg, wheat and fish [40, 48]. The younger the child, the greater the role played by FA [200]. Characteristically, in two children with eczematous reactions following a positive DBPCCT, the MBP deposition in skin lesions was ascertained [224], thus confirming the role of foods in AD.

Analyzing the data discussed in Chap. 5, we evaluate the most significant results in this area of atopy:

- Sampson reported that over several years 400 children were evaluated by 1,303 DBPCCTs, 447 of which (34%) were positive, 62% for at least one food [223]. Cutaneous reactions developed in 75% of positive challenges, with a clear correlation between food ingestion and development of symptoms in 56% of cases [230]: foods were implicated in 90% of positive reactions in children with AD [227].
- About 40% of 40 children given appropriate elimination diets and re-evaluated after 1–2 years showed food tolerance and improvement of their clinical course [230]; CM was responsible for 81% and egg for 76% of positive DBPCCTs (similar to our values), and fish was included among the 33% of other foods. The 80% of positivity for peanuts and an uncommon 67% for wheat should be noted. The total of DBPCCT negative results was 30.57% [231], in our cases (single blind), the rate was 36.2%;
- Burks et al reported that 75% of 46 children (mean age, 5.7 years) had positive DBPCCT for three foods: CM, egg and peanuts. Moreover, fish and wheat were positive in 8.3% of cases, chicken and soy in 4.2%; the DBPCCT was negative for beef, pork, cereals and chocolate [33]. In conclusion, FA is an important trigger factor in the AD development in one-fourth of children. In a later study in 71 children (mean age, 4.1 years), the incidence related to three foods increased to 81%, with overlapping results as regards wheat (8%), fish (5%) and chicken and soy (3%) [34].
- The role played by foods in the flare-ups of AD associated with FA was studied in 91 children (median age, 4.5 years) in whom after a food elimination diet and a randomized DBPCCT, both AD and associated symptoms became significantly worse on CM and tomato challenge compared to controls, but not on egg challenge. The appropriate diagnosis and exclusion of CM, egg and other foods elicits a positive reaction by DBPCCT, which results in a statistically significant reduction in the symptom score to ≤3 in 74% of children and clinically, a reduction in erythema, lichenification and extension of involved skin [248].
- Only 54 of 96 children (41/54 with FA) completed the diagnostic protocol (elimination diet and FCT): CM and egg were the most offending foods (Fig. 7.36) [12]. The pathogenic significance of these foods has been confirmed by FCT results in 88 children with AD and followed by us for 8 years: only 25 children (28%) tolerated the offending food(s) (p=0.003). Moreover, *24/27 children (89%) showed an association of CMA and AD* (p=0.0000) [47], corresponding to the 93% reported in children aged <2 years with severe AD [88].

Polysensitizations in children with AD associated with FA were found to be SPT- and RAST-positive to different foods that only rarely were clinically prominent [12, 39]. Instead by FCT the rate was of 26.8% [12] or 29% (CM, egg and wheat) [39]; by DBPCCT, 89% of 113 children reacted to one or two foods, 9.5% to three and one child to four [230]. Children given restricted diets often eliminating three foods (CM, egg, wheat) may experience significant improvement in their clinical

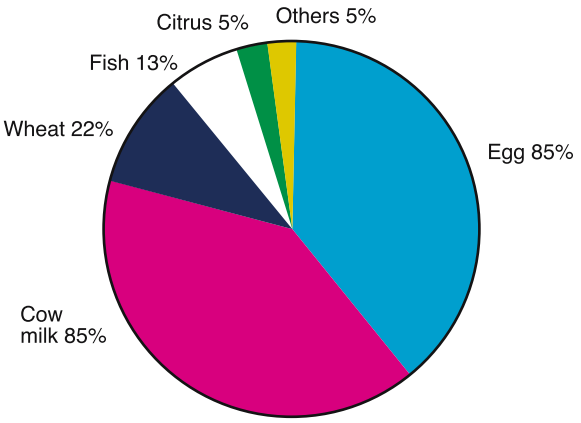

Fig. 7.36. Foods that provoked positivity of FCTs in 41 of 54 children with atopic dermatitis. (Data from [12])

course, and an appropriate environmental control contributes to the avoidance of future flare-ups.

Clinical Presentation

A frequent challenge in these children are the IgE-mediated immediate lesions: symptoms prevalent on the face, such as urticaria-angioedema and above all contact urticaria, characterized by the development of erythema and/or wheals within a few minutes, where the direct contact with food is materialized. The appearance of typically eczematous lesions, usually perioral lesions, of short duration (a few minutes), evident immediately after eating, makes us nearly certain that contact urticaria is the primary diagnosis and CM, egg, fish, fruits, vegetables, and nuts are responsible [88], aggravating AD in 85% of cases [189]. An additional characteristic symptom is acute urticaria originating in seats distant from those where the contact with antigens ensued [230], although the most frequent distant skin reactions occurring within 2 h of ingesting the offending food is an extensive erythematous or morbilliform rash and pruritus [33, 230]. The immune mechanism underlying such reactions, as discussed in "Pathogenesis," is the classic IgE-mediated reaction type I (the type IV triggered by T cells promotes worsening of lesions and/or gastrointestinal symptoms); otherwise the mechanism is pseudoallergic. The probable underestimating of contact urticaria is generally one of the main causes of the poor credit accorded by pediatricians to food-induced reactions in AD [88]. As recently reported, it is significant that some children complain of LPRs (8–12 h) heralded by pruritus and worsening of their lesions; in certain cases there is a continuous progression from one phase to the other, thus allowing a parallel with the biphasic reaction seen in asthma [49]. We propose the data shown in Table 7.22 [45, 263], which demonstrates the triggering of clinical forms in different ways, also by

Table 7.22. Small quantities of offending allergens may be heedlessly or inadvertently ingested by, or may reach food-allergic children by different routes

Negligible quantities of offending food(s) may contaminate other foods:
Because of inadequate cleaning of kitchen utensils, pots, etc.
Because of clumsy attempts to separate or eliminate an offending food from the others
Because of direct or indirect contact between two foods, such as when one uses the same pots or trays to serve different courses that may inadvertently reach the sensitive child:
Touching or licking kitchen utensils, bottles, tableware, glasses, cups, etc. with residues of the offending food
Inhaling the fumes during cooking or baking of offending food
Being unknowingly in the vicinity of or during servings of offending foods in restaurants, at parties, etc.
Being in the vicinity of a person eating the incriminated food
Inhaling food allergens from carpeted and smooth floors
Opening wrappings or cans containing the food or inadvertently touching it
Kissing, or being kissed/caressed by persons who have consumed/touched the food
Failure to eliminate specific components of the offending food:
Lactose can be contaminated with CM proteins
Lecithin can be contaminated with egg or soy proteins
Starch can be contaminated with wheat, corn, tapioca proteins
Gluten-free products may contain other wheat proteins
Unlabeled inclusion of the offending food as ingredient:
Errors in processing and preparations
Unlabeled and/or unspecified restaurant foods
Unknown source of food ingredients: lecithin (egg or soy-bean) starch (wheat, corn, tapioca, potatoes, etc.)
Deliberate/accidental feeding
Hidden bottle at maternity ward
"Grandmother effect" such as egg feeding in dieting babies
Cross-reactivity among closely related foods:
Cow, sheep and goat milk
Chicken, turkey, duck and goose eggs
Protein hydrolysates and CM proteins

Data from [45, 263].

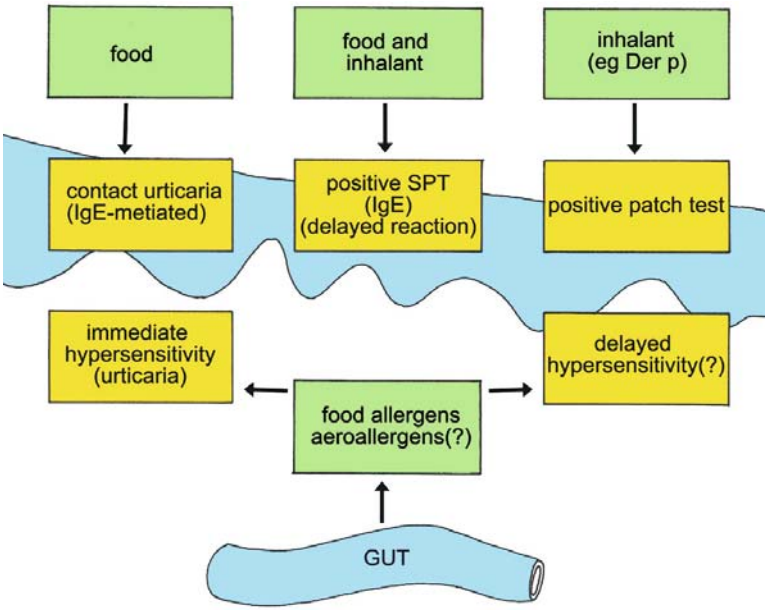

Fig. 7.37. Possible correlation between skin and immediate and delayed hypersensitivity for inhalant and food allergens

skin contact, especially of peanuts [45, 263] and by CM inhalation (Chap. 20).

Possible correlations between immediate and delayed hypersensitivity to inhalants and foods expressed on the skin are outlined in Fig.7.37.

Diagnosis

Diagnosis is based on a careful personal and/or family history. DBPCFCs are the rule, but open FCTs (OFCs) are necessary in small children. As regards SPT and RAST reliability in AD associated with FA, the conclusion is:

- Compared to SPTs, RAST sensitivity is lower and specificity is greater, therefore neither method can be utilized separately at the clinical level. In addition, the RAST NPV lower than that of SPTs is less useful for the diagnostic process [40] (Table 6.21).
- Comparing SPTs and PTs in children aged 2–36 months, 67% positivity was found in immediate reactions to FCTs, 89% was found in late reactions. RAST was positive in 55% of positive FCTs and in 25% of negative FCTs [118]; in children, there may be a dissociation between the SPTs and PTs for inhalant allergens; thus combined SPTs and PTs enhances identification of FA, since the PT is less invasive in infants, even if it contradicts other results [118].
- Because of SPT and/or RAST NPV, they may be used to exclude a priori the onset of immediate reactions to the FCT. Sampson [224] maintained that SPT negativity has a NPV >95; however, his group has acknowledged that in patients with SPT negative to foods it is necessary to reinforce the diagnosis with positive results [212], as we have demonstrated (Chap. 6).
- In 91 children, the clinical outcome of the restricted diet could not be predicted by personal and/or family

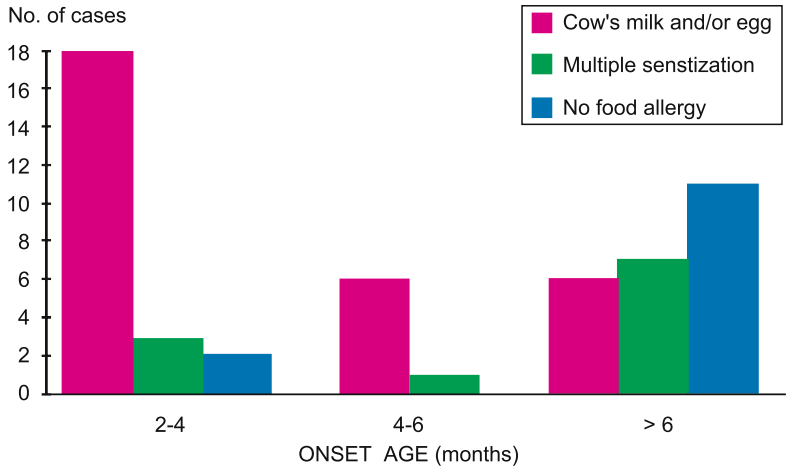

Fig. 7.38. Onset age of atopic dermatitis in 54 children according to the sensitizing food. (Data from [12])

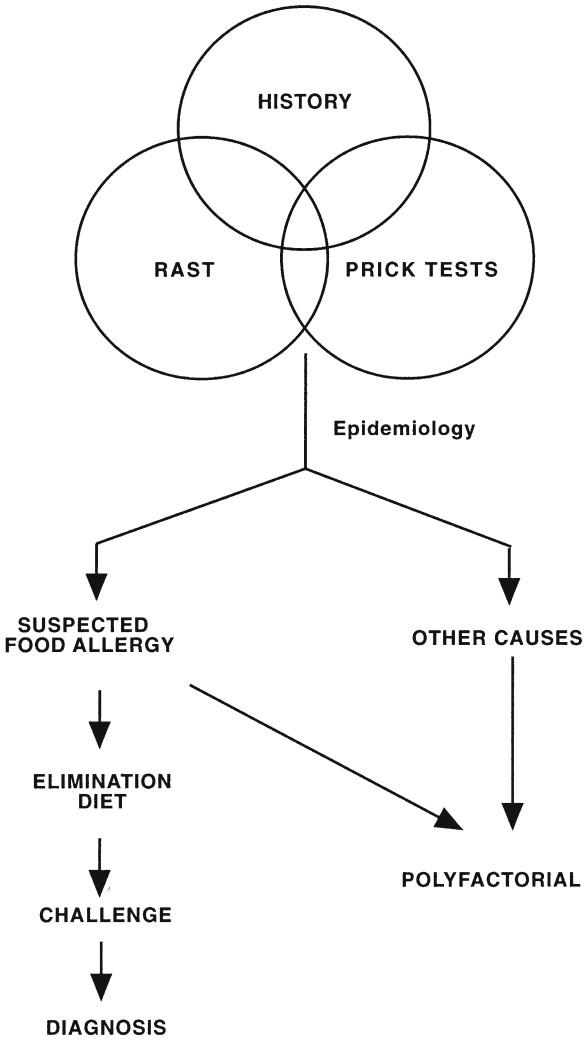

Fig. 7.39. Algorithm for a diagnostic titer to be followed in AD with suspected FA

history, initial SPT results, or total and food-specific IgE. Results of FCTs could not be predicted by initial serum immunoglobulin levels, and no initial level was helpful in planning food diets in those children [249].

To select the food allergens to be tested, it is practical that they be based on history, epidemiological criteria, child age and the diet followed [12, 230], avoiding the foods eliciting positive reactions in 90% of these children [36, 40, 224, 230]. Figure 7.38 [12] shows that if AD onset occurs in the 1st month following birth, CM and egg are usually responsible. If, however, AD onset occurs after this time, it is probable that additional foods were introduced into the infant's diet (wheat, cereals, legumes, fruits, etc.). According to our studies [12, 37], it is easier to identify the offending food when it is consumed sporadically, such as fish, whereas identification proves problematic when the food is a diet cornerstone. Chocolate has been acquitted [33]. Figure 7.39 exemplifies the diagnostic titer to be followed.

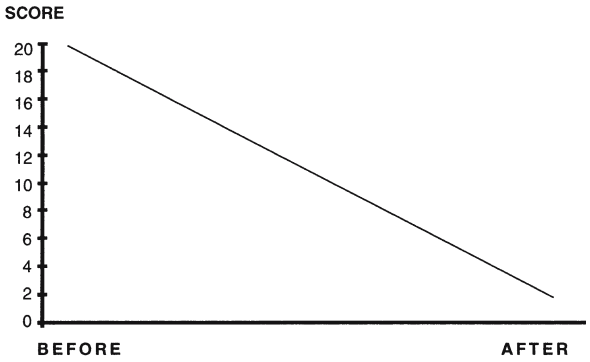

Fig. 7.40. The impact of a 4-week elimination diet on the clinical scores of children with AD. p=0.0001

With older children and adolescents additional foods enter the diet [51]; beyond the infant age, history is often unreliable, especially in predicting immediate reaction, hence the recourse to FCT is frequent. Since the typical reaction of AD is delayed (type IV), the worsening of lesions occurs even after 72 h (often later), thus complicating the individuation of the cause-effect ratio (Chap. 9). A diagnostic elimination diet should be tried if FA is suspected after obtaining a reliable history, medical examination, and SPTs.

Treatment

A standard therapeutic elimination diet eliminating tomato, citrus fruit, and coloring and preserving additives is effective in 80% of children with the assistance of a dietitian [248]. This type of diet should be prescribed to all children with AD to a moderate or severe degree, because of its positive effects [37] (see Chap. 9, including drug therapy). Table 7.22 shows that tiny shares of offending allergens may be negligibly or inadvertently ingested by or may reach FA children by different routes. However, arguments put forward that criticize diet treatment should be reappraised in light of the results of HRF studies [225, 232]. Patients placed on appropriate diets for at least 6–9 months experienced a fall in HRF spontaneous generation, leading to normal SBHR levels and eosinophil density and good clearing of their AD. There is a correlation with the results demonstrated by Fig.7.40 with a very significant statistical reduction in the clinical score in children with AD after 4 weeks of a diet eliminating both CM and egg.

Course and Prognosis

The course of AD is capricious and marked by exacerbations and remissions, which are often unexplained. Figures in the literature for the persistence of AD

Table 7.23. Outcome of atopic dermatitis (AD) associated with food allergy (FA) in 56 children (follow-up 5 years)

Outcome of AD	No. of cases	%	Median age at the last follow-up
Remission	32	57	6 years + 7 months (4–14 years)
Median age 4 years (2–13 years)			
Persistence	24	43	6 years + 9 months (4.7–13 years)
Development of respiratory allergy	27	54	4 years (1–10 years)

Remission vs persistence, p=0.02.

Table 7.24. Outcome of FA associated with AD in 56 children (follow-up 5 years)

Tolerance to the offending foods	No. of cases	%	Median age
To all foods	25	60	
To one or more	6	14	
To none	11	26	
To cow's milk			3.5 years
To egg			4 years
To wheat			4.5 years

Data from [39].
Tolerance vs persistence, p=0.0001.

vary widely. Several babies improve between 18 and 24 months of age, but when AD onset is after 24 months, the overall clearance rate is 50% at ≈10 years; several investigators have shown that children followed up from infancy for 10–22 years have associated respiratory allergy. In the 115 children, 62 (53.9%) developed respiratory allergy [48]. Table 5.8 shows a mean remission rate in one-third of cases, with development of asthma in 48.7% and of AR in 53% of cases: this is the substantial motive by which the ETAC program has been started for the early treatment of atopic children to prevent the onset of respiratory allergy. We complete the data outlined in the Table 5.8:

- Sampson and Scanlon followed up 75 children with AD and FA aged 3–18 years, and 25% of them acquired food tolerance after 1 year of elimination diet and 9% after 2 years. After 3 years of elimination diet, *no other child* was food-tolerant [231].
- In Tables 7.23 and 7.24, we detail the unlucky outcome of 56 children [39]. Subsequently we found that (Table 7.25) [48] 43% of 115 children had not lost their food hypersensitivity at the age of 14, and 37.4% suffered from multiple sensitizations developed in 98% of them within the 4th year; 55% of intolerant children had CMA, 31% egg allergy and 12% wheat allergy [48].
- Guillet et Guillet reported [88] an association persisting in 73% of children aged 2–7 and in 67% of those aged 7–16 also suffering from respiratory allergy in 27% and 33% of cases, respectively.
- Only in 11% of 82 children with early-onset AD was a clearance rate found at 5 years from onset. In the same time span the children developed sensitization to inhalant allergens with a peak increase at age 3 years, and asthma onset in 27% of cases within the 1st year was reported [9].
- In the 88 children, 30 were also tolerant and developed asthma, 10/15 children with CMA and 4/15 children with egg allergy (Fisher = 0.032).
- In the 1,314 children of the MAS study [111] the severity of the clinical manifestations (OR 5.86) and atopic sensitization (OR 2.76) were major determinant of prognosis.

In conclusion, we can affirm that FA could be considered as an unfavorable prognostic factor of AD, being the marker of an atopic predisposition. The data hitherto examined highlight the priority of recommending to the parents of neonates at risk of atopy that breast-feeding be continued up to the 6th month, with a gradual weaning and no early introduction of cereals, to prevent

Table 7.25. Atopic dermatitis and outcome in 115 children (follow-up 14 years)

Outcome	Atopic dermatitis		
	Yes	No	(%)
Cured	38 (68.3%)	52 (96.3%)	78.3
Improved	18 (30%)	2 (3.7%)	17.4
Unchanged	4 (6.7%)	–	3.5

Data from [48].
p=0.0001.

and/or delay the development of atopic disease [40]. In France, the management in schools of children with FA is clearly inadequate and we do not know in how many other nations.

New avenues. Omalizumab (anti-IgE) treatment induces the decrease of FceRI expression in skin mast cells which is slow and associated with decreased actue allergen wheal size.

Pediatricians and AD

Pediatricians should pay special attention to the confidence and cooperation of parents and the information given to them, especially on AD causes and natural history, and the target and limits of treatment. They should explain that since AD is a hereditary condition, no perfect treatment exists, and that the goal is to achieve the best possible control of the clinical manifestations, while awaiting spontaneous remission. Parents concerned with the future of an atopic child are helped to reflect above all on positive events: consequently pediatricians should balance negative statements clearly stating that the prognosis is favorable, stressing the good general health, the psychophysical and intellectual development of their child, analogous to that of friends, the absence of permanent disfigurements, etc. Pediatricians will discern the prerequisites for therapeutic success. Parents need help, understanding, encouragement, and safety, and one should listen carefully to their complaints and enable them to put questions or express concerns without hesitation, especially when the disease appears to be severe: the number and duration of the meetings will depend on the disease condition, response to treatment, degree of anxiety, reliance on planned therapy and serenity stemming from the pediatrician's advice. The establishment of compliance and proper communication between the pediatrician and parents or older children may be the steps that move the field further, the crucial point for achieving lasting success in the treatment of children suffering from AD. Older children and adolescents with AD experience frustration, sadness, and anxiety, which very often exacerbate their disease and increase itching. These children may be ostracized and ridiculed, which can be addressed by a supportive home environment from parents and siblings [137]. Finally, better understanding of the immunological and inflammatory aspects of AD should also provide new targets for therapeutic, dietary and pharmacological treatment.

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Other Allergic Skin Disorders

A Skin Panorama

In this chapter we discuss allergic skin disorders other than atopic dermatitis (AD): the urticaria-angioedema syndrome, allergic contact dermatitis (ACD), protein contact dermatitis (PCD), phytodermatitis, allergic photodermatitis and allergic vasculitis.

Urticaria-Angioedema Syndrome

Urticaria and angioedema comprise a unique syndrome, with the two frequently associated. Angioedema may be isolated: when associated with urticaria it is commonly underdiagnosed. Such disorders are well known to pediatricians and allergists and often are a manifestation of type I IgE-mediated reaction.

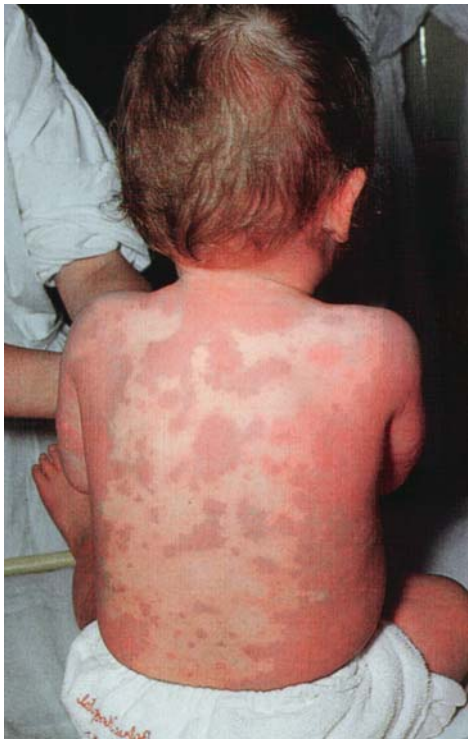


Fig. 8.1. Urticaria (for details see text)

Definitions

Urticaria (Fig. 8.1) is a cutaneous vascular reaction characterized by transient localized areas of edema (lasting from 2–3 min to <24 h) on hairless or hairy skin. Wheals, lesions that affect the superficial dermis, are circumscribed and slightly prominent, surrounded by a variable rings of erythema (flare) with a flat surface or a raised border. Hives have a variable form and size and are accompanied by more or less intense itching. *Angioedema* (Figs. 5.12 and 8.2) is a "urticaria involving the deep dermis and subcutaneous tissues," with deeper and less localized edematous swelling, which affects both skin and mucosal sites, preferring areas of loose connective tissue such as the face, eyelids, tongue, lips, extremities and genitalia, but may occur anywhere. These lesions are typically painful, rather than pruritic. Superficial mast cell degranulation induces wheal manifestations, whereas that of deeper mast cells induces extended vascular edema [117].

Urticaria can be divided into four main types based on a chronological outcome of clinical manifestations:

Acute urticaria (recurring <6 weeks), certainly the more frequent urticaria in pediatric populations

Chronic urticaria (recurring more times for a total of 6 weeks)

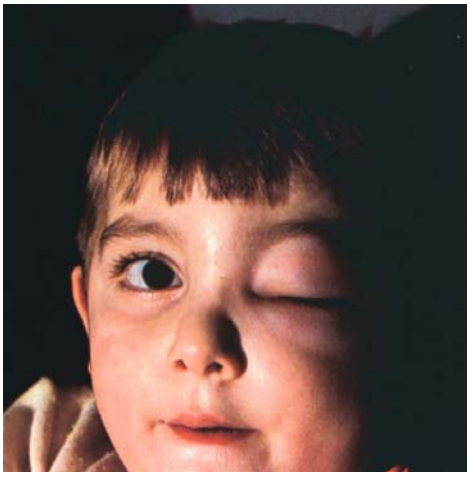


Fig. 8.2. Angioedema in a child sensitive to Fel d 1 after having stroked a cat during a visit to the house of a cat-loving friend

Recurrent chronic urticaria with sporadic manifestations recurring 2–3 times/year

Idiopathic urticaria, usually chronic: most cases are thus considered since no precipitating cause can be identified after having excluded all potential causes of urticaria (70%–80% of cases) [18, 37]

Prevalence

Pediatric urticaria is credited with a 2%–5% prevalence [60], with a total value of 3.9% (Figs. 5.5, 5.9) or at 0.5–1 year (Table 5.5). Isolated angioedema is present in up to 9.6% of allergic children [60]. Higher incidences are found in cohort studies. Urticaria was seen in 28 (49%) and angioedema in 34 children aged 1–3 years (60%) [144]. Urticaria was associated with angioedema in 38.6% of children [29]. Urticaria manifested mostly in male children (59.8%), mainly of preschool and school age rather than the under-1-year age group [13]. Acute urticaria may have an even higher prevalence (91.7%) [13] than that seen in AD: probably its frequency is underestimated since often, above all when caused by foods, a specialist is not consulted, the parents or the patients having recognized the evident cause-effect correlation (Chap. 7).

Classification

Table 8.1 [37, 42, 45, 117] shows the division into hereditary and acquired types, further subdivided into types that are apparently primary and secondary to other disorders.

Etiopathogenesis

Genetic Factors

A genetic influence was demonstrated in 56 children with urticaria-angioedema subjected to challenge with additives: the 25 children with positive challenge showed a positivity, with statistically significant differences, of family (64.2%) and personal history (80%) and elevated IgE levels (40%) [59]. A 67% rate of food urticaria occurred in atopic children [67, 90]. Children are more likely than controls to have a personal or family history (FH) of atopy (FHA) [169]. As a consequence, children affected by IgE-mediated atopic disease are more at risk of urticarial manifestations compared to the general population; likewise dermographism and aquagenic urticaria are significantly more frequent in atopic children than in nonatopic children [60]. Recently, Muckle-Wells syndrome and familial cold urticaria, two rare autosomal dominant disorders, both localized on chromosomal region *1q44* [50] have been found to be associated with mutations, all located in exon 3 of the *CIAS1* gene [62].

Pathogenic Mechanisms

Several pathogenic mechanisms are operative in urticaria, either immunological or nonimmunological (Table 8.1), which may be involved in acquired types of this syndrome.

The urticaria-angioedema caused by an immunological pathogenesis include [44]:

- IgE-mediated urticaria:
  - Foods, inhalants, insect bites, etc.
- Exercise-induced anaphylaxis (EIA)
- Physical urticaria (by external stimuli: cold, sun rays, pressure, vibrations, etc.)
- Non-IgE-mediated urticaria
- Complement and circulating immune complexes (CIC)-mediated urticaria
  - Cutaneous vasculitis
- Serum sickness
- Infections

Urticaria caused by a nonimmunological pathogenesis includes:

- Non-IgE-mediated mast cell activation
- Anaphylactoid reactions
- Chronic/idiopathic urticaria

Immunological Mechanisms

Several pathogenetic mechanisms may involve types I–III immune reactions [45, 175].

Type I mechanism is fulfilled once specific IgE is bound to basophils or skin mast cells. Contact with allergens leads to consequent degranulation, mediator release and development of typical skin and mucosal lesions. This IgE-mediated mechanism is operative on reactions to foods such as cow's milk (CM), egg, fish, wheat, parasites (helminths), some protozoa, insect venom, pollens, pet dander [53, 103], β-lactamine metabolites, insulin, enzymes, sera [227] and latex [112]. T-cell expression of cutaneous lymphocyte antigen (CLA), a unique skin-homing receptor, was selectively up-regulated in patients with CM-induced urticaria and may play an important role in the pathogenesis of this disease [28].

Type II mechanism involves complement-fixing IgG or IgM antibodies, complement activates C3a, C4a, and C5a components capable of directly activating mast cell degranulation. Known examples are the hemolytic reactions after blood transfusions (due to incompatible groups) and immunoglobulins (mainly IgA) and sulfamide administration [227]. This mechanism has been found in physical urticaria such as cold-induced, cholinergic and dermographic types [53].

Table 8.1. Urticaria-angioedema syndrome: pathogenic classification and classification of acquired forms

A. Pathogenic classification

1. Immune-mediated urticaria
   - FcεRI cross-linkage
     - Allergens
     - Autoantibodies
     - Anti-IgE
     - Anti-FcεRI
     - Polyvalent lectins
   - Anaphylotoxins

Most common etiological agents causing IgE-mediated reactions
- Foods
  - Beans
  - Celery
  - Cow's milk and dairy products
  - Fish (cod)
  - Nuts
  - Parsley
  - Seafood
  - Spices
  - Tomato
- Inhalant allergens
  - Animal danders
  - Molds
  - Pollens
- Physical stimuli
  - Cold
  - Exercise
  - Heat
  - Pressure
  - Sunlight, etc.
- Insect venom

More common etiological agents causing immune complex-mediated reactions
- Virus
  - Coxsackie virus
  - Cytomegalovirus
  - Hepatitis virus
  - Infectious mononucleosis
  - Psittacosis virus
- Bacteria
  - *Mycobacterium*
  - *Staphylococcus*
  - *Streptococcus*
- Molds
  - *Candida albicans*
  - *Trichophyton*
- Antigens deriving from neoplastic cells
- Cryoglobulins
- Nuclear antigens
  - LES or other autoimmune diseases

2. Nonimmunological mediated urticaria

Substances with histamine-releasing activity
- Drugs
  - ASA
  - NSAIDs
  - Penicillin
  - Pyrazolone
  - Radiocontrast materials
  - Sulfonamides, etc.
- Proteolytic enzymes
  - Trypsin
  - Papain, etc.
- Substances on the cell surface
  - Biliary salts
  - Dehydrocholic acid
  - Tween 20
- Compounds with high MW
  - Dextran
  - Egg-white
  - Polyvinylpyrrolidone
- Agents causing mast cell histamine release (see Chap. 10)
- Chemically defined substances contained in foods
  - Tyramine, etc.
- Chemically undefined substances contained in foods
  - *Complement activation* (nonimmunological pathway)
  - *Complement activation* (classic pathway)
  - Bacterial endotoxins
  - Immunoglobulin aggregates (myeloma, dermatomyositis)
  - Proteolytic enzymes
  - Staphylococcal A protein
  - Uric acid crystals
  - *Complement activation* (alternative pathway)
  - Lipopolysaccharide complexes (dextran, zymosan, agar)
  - Na dehydrocholate
  - Polysaccharides of cell wall of Gram-positive organisms
  - Polyvinylpyrrolidone
  - Snake venom

B. Classification of acquired forms

1. Apparently primitive acquired forms
   - Drugs
     - ACE inhibitors
     - Amphetamine
     - Antibiotics
     - ASA and NSAIDs
     - Codeine and morphine
     - Curare and derivatives
     - Hemoderivatives, plasma expanders, gammaglobulins
     - Heterogenic proteins (organ extracts, specific antiserum, etc.)
     - High-molecular-weight substances (dextran, polyvinylpyrrolidone, Na dehydrocholate, etc.)
     - Hormones
     - Iodinated radiocontrast materials
     - Local anesthetics
     - Opiates
     - Oral antidiabetics
     - Proteolytic enzymes (trypsin, chymotrypsin, streptokinase, etc.)
     - Radiocontrast dyes
     - Sulfonamides
     - Vitamins (thiamine, polymyxin b)

Table 8.1. (Continued)

- Foods
  - Cocoa and chocolate
  - Cow's milk and dairy products
  - Egg
  - Fermented cheese
  - Fish (cod)
  - Fruits (strawberry, banana, etc.)
  - Peanuts, nuts, hazelnuts, etc.
  - Shellfish, shrimp
  - Tomato
- Food additives
  - Na benzoate
  - Na metabisulfite
  - Na salicylate
  - Tartrazine yellow
- Venoms
  - Insect
  - Snake
- Contactants
  - Cosmetics
  - Topical medications
- Idiopathic urticaria
- Psychogenic urticaria

2. Urticaria associated with other affections
   - Autoimmune disease
   - Cryoglobulinemia
   - Endocrinopathies (diabetes mellitus, hyper- and hypothyroidism, hyperparathyroidism)
   - Infections (bacterial, viral, mycotic, etc.)
   - Neoplastic disease
   - Parasitosis
   - Serum sickness
3. Physical urticaria
   - Aquagenic urticaria
   - Cholinergic cold urticaria
   - Cholinergic urticaria
   - Cold urticaria
   - Contact urticaria
   - Delayed pressure urticaria
   - Dermographism, urticaria factitia
   - Exercise-induced urticaria (anaphylaxis)
   - Localized heat urticaria
   - Pressure urticaria
   - Solar urticaria
   - Vibratory angioedema

Data from [37, 42, 45, 117].
ASA acetylsalicylic acid, *ACE* angiotensin converting enzyme, *LES* lupus erythematosus, systemic, *NSAIDs* nonsteroidal anti-inflammatory drugs.

Type III mechanism is manifest via an interplay between CIC, activated complement and kinins and the anaphylotoxin system, which is seen with serum sickness. This mechanism is observed in childhood infections [7], urticarial vasculitis, Schönlein-Henoch syndrome (SHS), panarteritis nodosa, cryoglobulinemia, hereditary complement deficiencies, autoimmunity etc. [53]. Such a pathogenic mechanism has been considered in food-induced reactions, since elimination of CM from the diet of a patient with angioedema and bronchospasm resulted in CIC disappearance, and reintroduction of CM caused a return to previous CIC levels within 24 h[119]. These II and III types are objectively rare in children.

Several investigators consider that a *type IV mechanism* may be operative via IL_3 and IL_5 generation, which are capable of acting on both mast cell degranulation and eosinophil recruitment, thus explaining some types of chronic urticaria [37], especially eotaxin-driven allergic acute urticaria [95].

The skin is particularly rich in *mast cells*. In Chap. 7 we described their origin in bone marrow and subsequent migration into the skin aided by chemoattractants and adhesion molecules [37]. In immediate-type hypersensitivity, mast cell activation is triggered by allergen cross-linkage of high-affinity IgE receptors (FcεRI) borne on their cytoplasmic membrane and on peripheral blood basophils. Mast cells may be activated by specific allergens or by HRFs (histamine release factors) [46] or chemokines, produced by peripheral blood mononuclear cells (PBMCs) that are part of the cell infiltrate causing skin inflammatory lesions. Cutaneous mast cells are tryptase and chymase-containing mast cells (TC) (Table 1.27) and contain in the granules both types of proteases, tryptase and kinase [194], which are able to induce further degranulation [53]. Tryptase in particular can be viewed as a marker of mast cell activation, since its concentration in lesional skin increases proportionately to histamine concentration [103]. Leukocyte emigration is regulated by vasoactive and chemotactic mediators released from mast cells, inducing a sequential up-regulation of endothelial adhesion molecules (CD62P, CD62E, CD54 = ICAM-1, and CD106 = VCAM), β2-integrins on leukocytes, and ILs on endothelial, epithelial, and infiltrating cells [91]. In chronic/idiopathic forms, T lymphocytes (50%) are characteristically found with perivascular distribution, monocytes (20%), mast cells (10%) and no B lymphocytes [103]. CD4 prevails over CD8 [19]. One hypothesis postulates that there is a predominance of activated CD4, as seen in delayed pressure urticaria (DPU) [103]. However, a more likely hypothesis shows no evidence of CD4 activation, although it confirms the unbalanced CD4/CD8 ratio [19]. CD62E has been found in both groups of patients, but CD54 only in DPU patients [19]. Both CD62E and CD54 act as T-lymphocyte adhesion receptors and their increase found in DPU patients may reflect the inflammatory nature of this disorder [19].

In chronic urticaria, IgG Aabs (autoantibodies) directed against IgE in function of anti-IgE and/or anti-

FcεRI have been detected. These Aabs selectively directed against the receptor α subunit are of the IgG_1 or IgG_3 subclass and effectively fix complement and in the absence of IgE induce basophil and mast cell histamine release in both instances [92]. The number of mast cells is not significantly different compared to controls, so the histamine increase may be due to circulating *basophils* recruited into skin lesions [194]. Moreover, the serum of ≤60% of patients reacting with basophils of healthy donors stimulated in vitro basophil releasability [88, 89, 92]. Consequently, cross-linkage of Aab IgG to FcεRI is a pathogenic mechanism peculiar to chronic urticaria, which operates by stimulating or facilitating metachromatic cell degranulation [88] with consequent histamine release [67]. Such Aabs could correspond to HRFs with molecular weight (MW) >100 kD, equally provided with IgG anti-IgE Aabs able to interact with IgE [252]. These data explain why some patients suffer from severe and ongoing symptoms [89]. Basophil histamine release plays a significantly pathogenic role because basophils, when stimulated with anti-IgE that interact with IgE present on basophils, release less histamine [249], even if the levels are variable between subjects [88, 252]. The easy basophil degranulability acts as an opening key to the gaps between endothelial cells, which allows allergen and CIC to break into the perivascular tissue [225]. The skin histamine content and its releasability are increased in vivo during active disease and in remission. Mast cell release following an allergen challenge is not different between patients with chronic urticaria and normal controls [67]. Therefore, the histamine level increment could depend on a transient functional imbalance more than on an intrinsic mast cell defect [194]. To reconcile apparently conflicting data, we should admit that histamine may derive from mast cell nonimmunological stimulation.

Although *eosinophils* are not the most prominent cell type found in urticarial lesions, several eosinophil-derived mediators, including EDN (eosinophil-derived neurotoxin) and MBP (major basic protein) have been detected in late-phase reactions (LPR) in chronic urticaria between 2 and 5 h after allergen challenge [251]. These cationic proteins have been shown to elicit wheal and flare when directly injected into the skin. Eosinophils may also comprise a part of the inflammatory cell infiltrate, along with neutrophils, in DPU and solar urticaria [121]. Moreover, eosinophils are major LTC_4 producers in allergic inflammation [103].

Histamine plays a central role in urticarial lesions by increasing vascular permeability and intensely itching wheals on clinical grounds [231]. The classic *Lewis triple responses* (Fig. 8.3), produced by subcutaneous histamine injection or by gentle stroking with a smooth object, are paradigmatic of what occurs in urticaria linear erythema; flare and wheal are due, respectively, to vasodilatory (H_1 and H_2 action) and vasopermeability (H_1 action) histamine action, to which is added (for the vasodilatory phase) a local axon reflex with dilation of small perilesional arteries, a neurogenic response of the short-circuit type, sending a stimulus back to the cutaneous nerve endings of the involved area. The increased vascular permeability leads to activation of the plasma kinin-forming system and production of bradykinin, which is especially important in hereditary angioedema. Likely the histamine-mediated stimulation of neural sensorial fibers provokes the release of substance P (SP) and of tachykinins with histamine-release action, with consequent amplification of symptoms [103]. Additional mediators important in the pathogenesis are both PG (prostaglandins) and LT (leukotrienes), deriving from arachidonic acid metabolism, PGD_2 is active in mastocytosis, LTB_4 and LTC_4 in pressure urticaria, LTB_4 and PAF in cold, heat, and cholinergic urticaria [103]. With the advent of LT-receptor antagonists, the LTC_4 contribution to chronic urticaria symptoms is more evident [103].

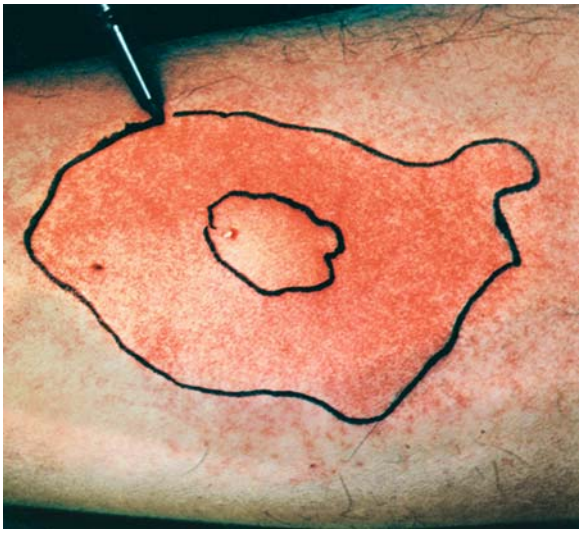


Fig. 8.3. Triple Lewis response: the inner circle is the wheal, the outer one the flare

In other urticarial types, the immunological mechanism is not always identified: allergens may be produced in solar urticaria, both in serum and epidermis, triggered by solar ray exposure, which in aquagenic urticaria in contact with water become soluble in the corneum layer, and diffuse to dermal mast cells. Cryoglobulins, cold hemolysins and cryofibrinogen are observed in some cases of cold urticaria [60].

Serotonin chemotactic factors, kinins, derivatives of tissue and plasma kininogen with an inflammatory action, tenfold more pronounced than that of histamine and MIF (monocyte-macrophage migration inhibiting factor), and 15-HETE (15-hydroxyeicosatetraenoic acid) in LPRs may also participate in the wheal formation process [37].

Immunohistopathology

Urticaria is histologically characterized by dilation of venules and capillaries, tissular edema and a predominant perivascular infiltrate, of variable composition and intensity. The histological peculiarity of urticarial wheals is common to all types, independently of etiology; mast cells and related mediators are the cornerstone of the lesions [166]. The more evident alteration is the dermal edema with vascular turgor, secondary to an increased size of endothelial cells: edema and infiltrate preferentially involve the dermal deeper strata, extending in certain cases to subcutaneous tissues, where flushing and itching stimuli are lacking because of the scarcity of capillary bed and nerve endings. The perivascular cell infiltrate is represented, especially in chronic forms, by T-activated lymphocytes, monocytes, eosinophils and mast cells; the clear-cut prevalence of *neutrophils* is characteristic. Activated T cells are predominant compared to all other cells, followed by mast cells and both B lymphocytes; NK cells are absent [117]. Since this pathology involves the vascular compartment, the etiological agents more frequently responsible are internally transported. In immune processes persistent for >30 min, numerous leukocytes are active, among which neutrophils are disposed between the walls of capillary and post-capillary venules which, if their number increases, may lead to urticarial vasculitis more frequently associated with chronic forms [89]. Eosinophils are seen more rarely, and perivascular infiltrations by lymphocytes expressing the CD4 phenotype are frequently detected [19]. Histopathological evidence in chronic lesions is similar to what is seen in AD and ACD LPRs: vasoactive mediators and chemotactic factors recruit PBMCs, neutrophils, and eosinophils into the cutaneous microenvironment; inflammatory cells in turn stimulate mast cells to dismiss HRFs [46]. It is tempting to speculate whether the initial stimulus comes from activated T cells or from mast cells [19, 103].

Nonimmunological Mechanisms

Several naturally occurring and exogenous nonimmunological compounds have histamine-releasing action via nonspecific mast cell activation. These compounds include (Table 8.1) ASA (acetylsalicylic acid), which, in common with NSAIDs (nonsteroidal anti-inflammatory drugs), generally inhibits the cyclooxygenase pathway, resulting in derailment of the arachidonic acid pathway towards LT production (Fig. 1.57). These drugs have a short half-life; thus possible plasma increments are sporadically detected, unlike in patients with mastocytosis or urticaria pigmentosa, who are primarily susceptible because of an increased skin mast cell population [61]. Nonimmunological activation has been demonstrated in several types of idiopathic, heat- and cold-induced, cholinergic, etc. urticaria [60], which respond to H_1 anti-histamines in the absence of allergens [44]. Urticaria may be caused by a host of agents, including detergents, foods, and endogenous peptides such as endorphins, neuropeptides or tachykinins [131]; but compound 48/80 and codeine cause histamine release only in the skin mast cell [45]. Likewise, physical stimuli may act on peripheral skin nociceptors, thus inducing neuropeptide release. Experimental evidence demonstrates that SP has a vaso-permeabilizing effect on epithelial cells. Tachykinin release into the skin may explain the functional aspects of several types of physical urticaria and the lack of response to anti-histamines and corticosteroids (CSs) [225].

Nonimmunological mechanisms activating mast cells should be re-examined in light of the new acquisitions on neuropeptides, and interactions between the peptidergic nervous system and immunocompetent leukocytes, thus suggesting that mast cells fall in the neuroimmunological axis. SP, NKA (neurokinin A) and CGRP (calcitonin gene-related peptide), via a retrograde axon reflex stimulating mast cells to release histamine (Chap. 10), act moreover on blood vessels. Alternatively the reflex may originate from mast cells, by leading to a tachykinin antidromic stimulation [115]. Abnormalities of skin mast cell responses to neuropeptides may underlie pathological manifestations in several types of cutaneous urticaria, for example in patients affected with cold or heat urticaria, in areas devoid of tachykinins following application of capsaicin (stimulating SP release from sensorial termination and provoking an equal increase in vascular permeability), vascular responses to thermal challenge are reduced. Similarly, patients with chronic urticaria show an increase in cutaneous symptoms in response to codeine. As a consequence, such mechanisms activating mast cells nonimmunologically may have great weight from a physiopathological point of view [166], probably in several cases of chronic urticaria.

Pathogenesis of Hereditary Angioedema

Hereditary angioedema includes [155]:

- Genetic deficiency of C1 inhibitor (C1-INH)
  - Hereditary angioedema type I and type II
  - Five other genetic syndromes
- Acquired forms

The gene for human C1-INH is localized to chromosome *11q11–q13.1* [204].

Hereditary angioedema is inherited as an autosomal dominant trait with incomplete penetrance and is relatively uncommon (0.1%). *Type I* (85% of patients) results from a complement esterase inhibitor deficiency (C1-INH), with MW of 104 kD, produced in liver, monocytes, megakaryoblasts, fibroblasts and placental cells, acting as a regulator of coagulative, fibrinolytic, inflammatory processes, etc. Owing to C1-INH deficiency,

complement is also activated after minimal stimuli, often of a traumatic nature, with formation of C3a and C5a, activation of kinin-like C2 factor, and secretion of more chemical mediators, associated with angioedema onset. In *Type II* (10%–15% of patients), C1-INH levels are normal, since the deficiency is functional. More precisely, in Type I patients, C1-INH-deficient production results from one chromosome 11 gene, which is defined as unproductive; in Type II patients, a gene mutation leads to a functionally inactive C1-INH (MW = 96 kD): therefore the product of a solely normal gene is insufficient to ensure a C1-INH adequate concentration [155]. Consequently, in both cases C2 and C4 are reduced, the C4d/C4 ratio is elevated, and Clq and C3 levels are normal [250]. A *type III* has been recently described in women with normal C1-inhibitor protein, C1-inhibitor function, and C4 levels [35].

Twenty-one children with hereditary angioedema had Type I and five suffered from Type II [70]. In either type, the onset age is between 6 and 20 years in subjects with positive FH [250], or within 2.5–12 years of age [70], but acquired forms develop in adults aged >50 [250]. In 11 members of a family, the mean age at onset of symptoms was 11 years [247]. In children <10, intestinal colic and edema of the extremities are the most frequent manifestations [69]. Edema formation primarily afflicted subcutaneous tissues. Mechanical trauma was identified as a precipitating factor in 80% of children [70]. A 10-year-old child died from laryngeal asphyxia, and an 8-year-old had membrano-proliferative glomerulonephritis [69]. C1-INH deficiency is evident when C1-INH concentrations fall under 15%–20% of normal and occurs frequently in patients with reduced inhibitor activity, in whom the lacking control of complement C1-esterase enzyme activity, and of activated Hageman factor, plasmin and kallikrein, lead to release of vascular permeability factors [60].

The additional five genetic syndromes are as follows [166]:

- *Familial deficiency of C3b inactivator,* autosomal recessive; the alternative pathway is activated with production of anaphylotoxins.
- *Familial deficiency of carboxypeptidase-N,* inactivator enzyme of anaphylotoxins and kinins.
- *Muckle-Wells syndrome,* autosomal dominant, with painful urticarial dermatitis, deafness of the perceptive type and renal amyloidosis.
- *Melkersson-Rosenthal syndrome,* with chronic orofacial noninflammatory tumefaction (tapiroid face), usually limited to lips, characteristic fissured tongue and relapsing facial palsies. Several patients reported with this autosomal dominant syndrome have no FH of the disease [142, 202]. Additives might be implicated in the pathogenesis [202].
- *Episodic angioedema* (from 1 week to 1 month) associated with eosinophilia, periodic attacks of fever, myalgia and oliguria; 8 out of 12 patients were aged between 2.5 and 18 [168]. Eosinophilia is usually associated with an increase in IgE and/or IgM levels; the pathogenesis is based on T-lymphocyte activation with production of interleukins (IL_1 and IL_2R) [168]. An adolescent had similar symptoms and an IL_5 elevation [8]. An increased IL_6 production could be related to blood monocytes and endothelial cells stimulated by an eosinophil mediator [208].

Vibratory angioedema described within the physical urticaria is also hereditary.

Acquired C1-INH Deficiency

Type I acquired C1-INH deficiency (35 cases) has been shown in patients with lymphoproliferative disease, especially B cell-mediated, such as chronic lymphocytic leukemia, macroglobulinemia, essential cryoglobulinemia, or lymphocytic lymphoma. These patients may have a nonfunctioning C1-INH, and extremely low C3 levels, or by C1-INH consumption at a higher level than that of re-synthesis. This is caused by an excessive C1 activation, with consequent C1 reduced serum levels, either via particular CIC (idiotype-anti-idiotope), which appear to fix C1q, or via anti-C1-INH Aabs, which block the normal activity of C1 inhibitor. The clinical manifestations are shared with those of hereditary forms [155].

Type II acquired C1-INH deficiency with C1-INH deficiency and IgA/IgG_1 Aabs directed at C1-INH: the resulting C1-INH functional block could lead to an uncontrolled activation.

Rare cases not belonging to either type may be recorded [155]. Briefly, these deficiencies depend on anti-C1-INH Aabs interfering with normal interactions between C1-INH and proteases, thus increasing C1-INH catabolism. Moreover, there is the risk that some patients may be associated with autoimmune disease, including LES, autoimmune hemolytic anemia, diabetes mellitus, rheumatoid arthritis, etc. [250].

Pathogenesis of Urticaria

Possible types of urticaria [60, 156] include:

1. Prevalently IgE-mediated
2. Complement-mediated
3. Drug- and/or additive-induced
4. Pseudoallergic (by agents directly releasing histamine)
5. Infection-induced

Prevalently IgE- or Non-IgE-Mediated Urticaria

Apparently Primitive Urticaria

Foods are common causes of urticaria in children, the mechanisms are immunological of type I [90], or non-IgE-mediated, or aspecific. There is insufficient proof to confirm a CIC role in urticaria syndrome. Glycoproteins with MW of 10–50 kD are often responsible, since they resist enzyme digestion and heat denaturation. The

foods most frequently implicated are fish (mostly cod), seafood >CM and dairy products >peanuts and nuts >egg >fruits, etc., in some cases with IgE-mediated (55%) or aspecific mechanisms (75%) [90], and acute urticaria provoked by caresses after peanut contact (Table 7.22). In pediatric cohorts, urticaria was secondary to foods from 11% [144] to 57% [90] of cases. In young infants, CM and egg play a role [184], with cases of urticaria and shock by CM, peach and wheat at 4–6 months of life [90]. A wheat allergic boy experienced systemic urticaria and angioedema within 40 min after the ingestion of 9 g of packed rice crackers contaminated by 1.50 µg/g of wheat [137]. Two children seen by us showed severe urticaria while touching or eating a peach. The mother of one boy had urticaria by maize, pollens, and fruit in general. A drop of CM fell on the foot of a 10-month-old child and a generalized urticaria was spread to the whole leg. In two pediatric cohorts, urticaria was caused by CM in 18%–26% [40, 60], peanuts in 63.6%, tomato in 37.3% and egg white in 26.2% of cases [60]. In 67% of 100 children, the foods were implicated in this frequency order: eggs and nuts, fruit, CM, vegetables, fish and shellfish [67]. Fish often induces IgE-mediated urticaria-angioedema in children (60%–92% of cases) [41, 49, 164]; urticaria on the face and arms in one boy following hand immersion in water used to wash codfish and in another after handling a raw peach, complicated the latter by glottis edema [184], have also been noted. As in our cases [41], if a fish-allergic child with AD eats a few milligrams of fish, he does not show worsening AD but persisting angioedema despite an elimination diet (Fig. 8.4). Fish provoked urticaria in 23.8% and urticaria-angioedema in 33.3% of 21 children by *inhalation of airborne particles* [49]. An uncommon case was elicited by a hemostatic sponge of bovine fibrin used in tooth extraction [242]. Immediate reactions have been reported after contact with apple, endive, lettuce, flour, garlic, honey, lamb, pear, potato, turkey, and wheat [184] (Table 8.1).

Inhalant allergens may occasion urticaria since they cross-react with food allergens (Table 1.73), or by direct skin contact: above all, exposure to inhalant allergens in children with AD may cause lesion flare-ups, preceded by urticarial lesions (Chap. 7). There might be a reduction at the threshold by which release of mast cell products can be induced (priming effect) during hay fever season. Grass pollens cross-react with peanut and tomato: in a pediatric study [60]: skin prick tests (SPTs) were positive to grasses more frequently than to Der p. A case similar to peanuts was angioedema, which occurred after stroking a cat (Fig. 8.2).

Urticaria associated with other conditions such as urticaria associated with AD may reach an elevated prevalence in children (Chaps. 5 and 7).

Insect bites and stings cause acute urticaria. Urticarial reactions can be part of a systemic reaction to Hymenoptera stings heralding potentially fatal anaphylactic reactions on subsequent stings.

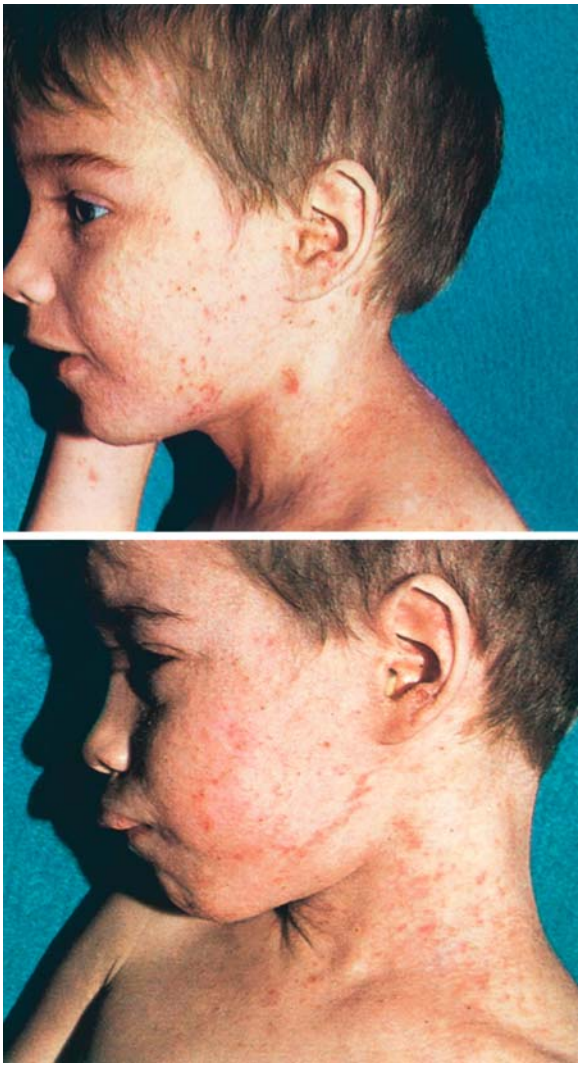


Fig. 8.4. Angioedema provoked by the ingestion of a few milligrams of fish in a fish-allergic child (for details see text)

Complement-Mediated Urticaria

Complement may be activated in different ways in urticaria [45, 157] (Table 8.1).

Classic pathway activation via aggregated immunoglobulins such as IgG and IgM in CICs, and the same mechanism may be active to a varying degree in other forms of urticaria, as well as in cases of myeloma or dermomyositis, etc.

Alternative pathway activation might result from venoms, antigens, radiological contrast media, complex carbohydrates (agar, dextran, polyvinylpyrrolidone, zymosan), polysaccharides and lipopolysaccharides (LPS) of the cell wall of Gram+ and Gram– organisms. Moreover, activated complement C3a, C4a, and C5a anaphylotoxins are capable of triggering mast cell histamine release, thus playing the pivotal role of possible mediators of urticaria [45].

Urticaria Induced by Drugs and/or Presumed Arachidonic Acid Metabolism Abnormalities and/or Additives

The whole spectrum of drugs are the most common causes of urticaria by pseudoallergic mechanisms in 29.8% [13] and in 22% of children [90]. ASA is the most usual and best studied drug that acts directly and even provokes relapses in subjects with chronic urticaria secondary to different causes. ASA was the most frequently used antipyretic [53.8%) [13]. ASA also cross-reacts with NSAIDs in ASA-sensitive patients suffering from clinical manifestations following NSAID treatment, in addition to colorings and preservatives [59]. Other causative drugs were antibiotics (60.9%) [13] or 47.7% [29], antipyretics (35.1%), or a combination of antibiotics and antipyretics (16.2%) [13]. Contaminant penicillin derivatives in CM or poultry meat routinely penicillin-treated may cause immediate allergic reactions or chronic urticaria [156], prevalently in highly sensitized subjects [157]; gastrointestinal symptoms are frequently elicited, but the food challenge test (FCT) is rarely positive [156].

The *additives* (Chap. 10) most often incriminated are as follows [59]: E102 (70%), E110 (64.2%), E127 (35.7%), E160b (60.7%), E211 (57.1%), aspartame (48.2%) and ASA (12.6%). A cause–effect ratio is more evident in chronic urticaria, that is 30%–50% [60]. The pertinent role in children is unknown, since the related FCTs have rarely been done.

Pseudoallergic Urticaria

Several histamine-releaser foods, if ingested in sufficient amount, have the ability to act on mast cells by an aspecific mechanism, thus inducing urticarial reactions also in nonsensitized subjects. In both cases (immunological and nonimmunological mechanisms), the symptoms are practically overlapping, but a nonimmunological response is best defined as a *pseudoallergic* reaction [18, 60]. Urticaria provoked by additives contained in prepared foods is a frequent finding among children consulting in our department.

Infection-Induced Urticaria

Urticaria may occur during parasite and infectious disorders, especially in chronic urticaria [159]. Among infections with helminths, oxyuriasis, and ascariasis are most diffused in infancy [175]. The underlying mechanisms are uncertain, such as urticaria in the prodromes of hepatitis B and infectious mononucleosis (IMN), either spontaneously or following ampicillin treatment [227]. Urticaria has been associated with enterovirus, cytomegalovirus (CMV), and other infections, but clinically these infections are not unlike those of different etiology. Among 44 children aged 1–12 [22 aged 1–2), 90.9% had symptoms of respiratory tract infection, suggestive of viral infection in 79.5% [29]. Infection, either associated or not with drug intake, was the cause in 46 children (81%). The organisms were enterovirus (50%), parvovirus B19 (20%), Epstein-Barr (15%), and mycoplasma (15%) [29].

In conclusion, a cause was suspected in 28 out of 52 children (54%) such as a viral illness (19%), antibiotics (15%), or a combination (35%) [169]. Even if the pathogenic mechanisms appear to be numerous, conflicting with clinical monomorphism, they may actually be related to a few vasoactive factors that play a role of potential mediators [175].

Clinical Presentation

Urticaria (Fig. 8.1) is a skin eruption characterized by raised, erythematous wheals, with defined or serpiginous borders, irregular form (usually oval), color varying from pink or reddish to whitish, surrounded by normal skin or by a bright-red flare, and with variable form, seat extension and duration. These lesions, accompanied by intense itching, are fleeting and resolve completely within 24 h of onset, especially after Hymenoptera stings, without leaving any trace, even if new elements may follow while the old ones clear. Lesions lasting longer should raise suspicion for the diagnosis of vasculitis presenting as urticarial lesions Wheals vary in size (from 1–2 mm to 1–2 cm) and number and in some cases undergo a coalescence involving very large areas (*giant urticaria*) up to 10 cm in diameter and assuming the aspect of a geographical map. Urticaria may be generalized or localized and in the latter case the lesions affect preferentially the trunk and limbs, but also the palms and soles, the face and scalp. An important characteristic is that the color typically blanches with pressure; this simple test is helpful *to differentiate an erythema from a skin hemorrhage* [117].

Acute allergic urticaria is an IgE-mediated allergic reaction associated with systemic anaphylaxis. It is extremely common, possibly affecting about 10%–20% of the general population. Most frequently, this a self-limited disorder [103]. However, excluding drug reactions and insect stings, acute allergic urticaria comprises 2%–6% of the total number of urticaria cases seen in a dermatology clinic [117]. This is an eosinophil-driven disease, as demonstrated by a study on 19 infants and children aged from 9 months to 8 years and recruited from the emergency room while presenting with acute attacks of wheals and itching, accompanied by angioedema in seven subjects. Total serum IgE levels were above the highest normal for their age. The eotaxin values were significantly higher than the controls' corresponding values. As a potent eosinophil selective chemoattractant, eotaxin is a chemokine that promotes the selective recruitment of eosinophils, the major effec-

tor cells in allergic inflammation. Eotaxin is thus implied in the causation of the tissue eosinophilia that characterizes allergic acute urticaria and may also be a biomarker of lesional activity [95].

Angioedema (Figs. 8.2, 8.4) is sometimes referred to as angioneurotic edema, coined by Osler who did not refer to neurosis at this time [158]. Isolated angioedema is rather rare at the pediatric age, is more often a symptom of a generalized anaphylactic manifestation, as when it is caused by an insect sting [175]. In 26 children, pedigree analysis revealed 19 patients with afflicted relatives, and clinical manifestations of the disease first occurred at 2.5–12 years of age [69]. Angioedema was caused by food in 40% (Fig. 5.12), insect bites in 30%, infection in 20%, and an antibiotic in 10% of children [187]. The swelling involves deeper skin layers with fewer mast cells and nerve endings, and is thus painless and nonpruritic. It is characterized by gradual onset of circumscribed bouts of edema, localized in subcutaneous tissue, skin and mucosa, more evident in the face, lids, lips, tongue, upper airways, gastrointestinal mucosa and less in the limbs, involving the mucosa to varying degrees and discomfort, accompanied by extreme weakness. Incidence of angioedema of the head or neck, most often facial was 80%, tenderness or pain 40%, dyspnea 30%, dysphagia (including drooling and spitting) 30%, and hoarseness 10% [187]. The clinical manifestations, self-limited and present in two-thirds of cases in patients up to 13 years of age, are usually preceded by local trauma, also mild, such as local inflammation, minor surgery, dental extraction, fatigue, and emotional stress, sometimes without an apparent cause [60]. It is characterized by recurrent attacks of self-limiting angioedema affecting the face, limbs, gastrointestinal system and upper airways [247]. Additional symptoms are erythematous rashes, pleuritic pains, urinary retention and seizures or hemiparesis, sometimes simulating a cerebral edema. The swelling progresses over hours, commonly increasing over 12–72 h and then subsiding over 1–3 days, leaving a normal-appearing skin [155]. The frequency may vary from a single episode over the entire life to weekly recurrences.

Gastrointestinal exacerbations may cause vomiting, watery diarrhea, colicky abdominal pain and guarding, but in the absence of fever and leukocytosis there is instead enteritis [103]. When the intestinal mucosa is involved, an occlusive syndrome may occur with subintrant colic and other symptoms mimicking an acute surgical emergency, sometimes resulting in unnecessary exploratory laparotomies and even appendicectomy [237], regardless of signs of peritoneal irritation. Extravascular fluid leakage to gut edema can lead to hypotension and hemoconcentration [155]. The most severe complication is laryngeal edema, which, when not treated quickly and aggressively, can be lethal; the laryngeal involvement provokes death by asphyxia [169]. Usually an asphyxial crisis is not suddenly precipitated, because in certain cases the attack, before the real appearance of swelling, is preceded by dysphagia, dysphonia and other prodromic signs such as subjective sensation of pharyngeal prickling, pruritus or burning. Subsequently a sensation of painful tension precedes the edema. These are warning symptoms allowing the timely institution of medical therapy, thus eluding tracheostomy [103]. In these patients, oral surgery represents a particular danger since edema can easily progress to upper airway obstruction. Trauma precipitates facial and airway edema via dental manipulation or adenotonsillectomy. Normal activity such as writing and/or computer typing are causes precipitating hand edema. In rare cases, symptoms can be triggered by infections [237].

Physical Urticaria

Physical urticaria can be subdivided into thermal, mechanical and cholinergic urticaria, with an 8% incidence in children [90]. In some patients more than one type of physical urticaria may be present (Table 8.1, Fig. 8.5) [42].

EIA syndrome with a family tendency, is rather rare and differs from other physical urticaria due to its sporadic occurrence. Four clinical phases have been proposed: (1) the prodromal manifestations are cutaneous (pruritus, warmth, flushing and fatigue), (2) early symptoms include urticaria and angioedema, (3) the full-phase symptoms are respiratory and gastrointestinal and (4) as in phase 1, and less often shock [183]. Occasionally the symptoms occur without a break. Triggering factors are cold and certain foods and drugs [42]. Three clinical forms have been described: one is EIA occurring after jogging started within 2 h of any meal (postprandial EIA), another form occurs only following a specific food ingestion, and the third when no food is identified [190]. High histamine concentrations and mast cell degranulation were detected in skin biopsies [190] (see Chap. 20).

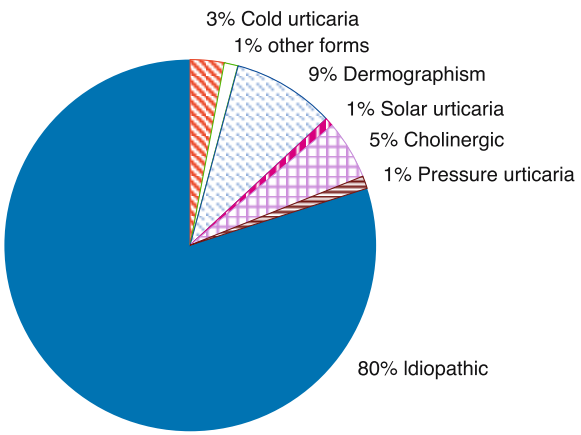


Fig. 8.5. Forms of physical urticaria

Dermographism or dermographic urticaria, meaning literally writing on the skin, is the commonest physical urticaria, which is seen in about 5%–10% of normal subjects without a sex predilection [103]. In 238 children aged 2–14 years, the prevalence was 24%, with a significant predominance of females (33%) over males (16%) [136]. Following the application of a linear pressure or a gentle friction, there is characteristically the production of a wheal (diameter >2 mm) and flare reaction. If the skin is stroked with a wooden tongue-depressor, a fingernail, an instrument with blunted point (a key), or a retracted ball point pen [156], within 2–3 min the usual white line secondary to reflex vasoconstriction is followed by pruritus, flushing and a linear edema, which occur exactly in the distribution of the stimulus [111, 175]. A positive response occurring at a stroke pressure of 3.6 g/mm^2 of skin surface or less confirms the diagnosis. Less obvious triggers are pressure from clothes or leaning against a chair [117]. Three types of dermographism have been reported according to the chronology of the wheal and flare reaction, following a stimulus application:

Type	Onset	Duration
1. Immediate (common)	2–5 min	30 min
2. Intermediate	30 min – 2 h	3–9 h
3. Late onset, delayed (rare)	4–6 h	24–48 h [111]

Symptoms are more common on the trunk and limbs and are differentiated by the classic triple response because minimal stimuli lead to extreme responses. Many subjects are carriers of a mild dermographism, while a wheal response to the mechanical skin stimulus is less frequent. An association between skin and bronchial hyperreactivity (BHR) has been suggested in children with dermographism [136]. In about 70% of patients, a passive antibody-mediated transfer (Prausnitz-Küstner reaction) has been documented, which underlies an IgE-mediated reaction, along with findings of mast cell degranulation histamine release [183] or mechanisms involving IgM antibodies [117].

Variant types of dermographism have been described [67, 183, 233]:

constitutional, its onset is often in infancy and duration may be lifelong.

acquired primitive, with a sudden onset and a duration of 1–2 years.

acquired secondary, associated with cutaneous mastocytosis, upon challenge with penicillin or insect sting, or in patients with hyperthyroidism, scabies, etc.

cold-dependent, the classic wheal is produced only during skin cooling, but not if the skin is stroked when skin temperature (T) is normal.

delayed form, causes burning and pain instead of pruritic symptoms.

Pressure urticaria, initially classified as a rare familial variant of dermographism. DPU appears commonly after 6–24 h or more after pressure has been applied for a period as short as 2–3 min. Pressure areas are diffused and result from prolonged sitting on a nonupholstered seat; wearing tight clothing and straps, watches, belts; wearing tight shoes; or walking, jogging, and climbing ladders. Swelling of the soles of the feet may limit ambulation and become progressively disabling. The typical lesion is painful as it is deep-seated and edematous [183]. The trunk, buttocks, feet and hands are the regions affected in 95%–98% of cases [64]. Reactions persist for 36 h on average and for 86 h at most [64], so that the more delayed types are practically indistinguishable from delayed dermographism [42]. *Immediate forms* are full-blown within 30 s to 4 min. Skin biopsies reveal signs of vasculitis with neutrophils in the lower dermis and subcutaneous tissue 4–5 h after peak swelling, then lymphocyte infiltrate after 24–48 h, thus denoting a cell-mediated immunity (CMI), with tissue eosinophilia but not in the bloodstream [225]. A mechanism elicited by histamine probably coexists, with participation of axonal reflexes and neuropeptide involvement as SP [156]. The increased IL_1 levels may contribute to leukocyte recruitment and systemic symptoms such as fever and malaise [117]. Up-regulation of adhesion molecules CD62E and CD106 has led to the hypothesis that vascular endothelial activation plays an early role in the lesion upsurge [19].

Cold urticaria refers to a group of urticarial manifestations induced by exposure to the cold such as typical (primary, secondary) and atypical, familial immediate or delayed, and acquired systemic or localized:

Primary typical cold urticaria (idiopathic) is characterized by a rapid onset (2–5 min) after a local cooling induced by a blunt decrease, even of 1 °C, of body T, with itching, burning, flushing and edema *limited to cold-exposed skin* (face, limbs) occurring on skin rewarming even after 1 h [42]. An act of short duration is sufficient, such as holding cold objects or drinking cold beverages may produce hand or lip swelling, respectively. Eating cold foods may also cause a glottis edema. Total body exposure to cold such as showering or bathing in cold water, especially in open places with further cooling, may produce such widespread vasodilation that severe hypotension and collapse ensue, and in extreme cases even death by drowning for persons diving into cold waters, often labeled "faintness" [111, 233]. Histological findings often overlap with those of other physical forms. In children aged 12.6 years, atopy was present in 67% of cases, and girls more frequently had cold urticaria, other types of physical urticaria were present in 25% with no familial inheritance; 83% of children had localized and generalized symptoms [181]. Among 30 children <18 years old, the age of onset was at ≈7 years, with a strikingly high rate of asthma (46.7%) and AR (50%), and a FHA of 89.3%; 33% had anaphylactic reactions [4].

Secondary typical cold urticaria is associated with cryoglobulinemia, cryofibrinogenemia, cold hemagglu-

tinins and hemolysins; complement deficiencies; urticarial vasculitis; viral infections (IMN); insect stings or drugs (griseofulvin, penicillin) [175].

Atypical cold urticaria has varying manifestations and syndromes:

1. *Systemic or generalized* cold urticaria has systemic symptoms characterized by giant urticaria and negative ice cube test [110] (ICT).
2. *Two rare familial types* of cold urticaria (20 cases) plus four large North American families [93], both with autosomal dominant trait: in the immediate type mapped to chromosome *1q44*, burning papules or macules appear after 30 min to 4 h associated with chills, fever, arthralgias and neutrophil leukocytosis. In the delayed type, they occur in the skin area of cold exposure after 9–18 h, accompanied by burning with absence of pruritus. In several cases, histological features include mast cell degranulation and an increase in blood histamine levels following cold exposure. In other cases, the IgE-mediated reaction is passively transferred [100, 233]. This syndrome is associated with the *CIAS1* gene [62, 93].

Acquired cold urticaria manifestations and syndromes:

1. *Cold-induced cholinergic urticaria* and cold-dependent dermographism, appearing after cold exposure. These forms are linked to both delayed cold urticaria and systemic types by ICT negativity [233].
2. *Localized cold urticaria* affects certain areas of the body after cold contact under specific predisposing conditions mostly related to cold injuries or insect stings [111].

In most of these syndromes, the most closely studied pathogenic aspect is the participation of histamine; TNF-α-induced mast cell release is relevant, which could be implicated in shock-like clinical manifestations [209].

Solar urticaria (1%–4% of urticaria cases), rare in children [240] and familial cases, affects both sexes, usually when they are <30 [141]. Within 30 s to 3 min after skin exposure to sun or UV (ultraviolet) spectrum light, patients note erythema preceded by, or immediately followed by, pruritus and then wheals (5–10 mm, persisting for 15 min to 3 h, less if patients take shelter in a shaded place [42]. Patients complain of itchy, pricking, tingling, or burning lesions. If a sufficiently large area of skin is exposed, *systemic symptoms up to anaphylaxis* may occur [183]. In about 25% of cases, it is associated with dermographic urticaria or with a history of AD [141]. A rare delayed form is characterized by a 17- to 72-h time-lag for lesion onset [111]. An axon-induced flare encircles the area with changing borders beyond the involved sites [111]. This disorder has been classified into six different types depending on the wavelengths of light to which patients react: only types I and IV can be passively transferred and may be antibody-mediated. In type VI, or erythropoietic protoporphyria, protoporphyrin IX acts as a photosensitizer activated by sun rays with a wavelength of 400 nm [197]. Skin biopsies show neutrophils and eosinophils within a time-lag of 5 min to 2 h, replaced by PBMCs after 24 h [151] and eosinophils degranulate in skin lesions with MBP deposition [121].

Contact urticaria with erythematous and wheal-like eruptions is aroused 30–60 min after normal skin exposure to the offending substance, most often induced by nettles and certain vegetables (see "Phytodermatitis"), some coelenterates such as jellyfish, as well as drugs, foods, cosmetics, fragrances (cinnamic aldehyde), chemical substances (disinfectants and bleaching), cat, dog, horse hair (in order of frequency), etc. [103]. Contact can be achieved via airborne agents, including grass pollen, as in sensitized children walking outdoors during days or in places with notable pollination, or playing in the grass, etc., or indirectly via toy contamination with pollens. Food allergens include egg, CM, tomato, chicken, honey, peanut butter, sunflower seeds, and cooked chick-peas [1, 115], also during their manipulation by children affected with oral allergy syndrome (OAS). Symptoms are usually elicited within 30–60 min; cases with onset delayed up to 6 h have been reported.

There are four clinical forms [117]:

- Contact urticaria, localized, the most frequent, usually caused by foods and pet danders.
- Contact urticaria with angioedema, also with systemic manifestations, often formaldehyde-induced.
- Contact urticaria with asthma, sometimes associated with gastrointestinal and oculorhinitic disorders. the onset is triggered by cephalosporins, various vegetables and processionary caterpillars, whose urticating hairs caused toxic-irritative effects on both skin and mucosa by direct contact as in 60/653 children (9.18%) with 4 IgE-mediated cases (6.7%) [222], and on the airways by aerodispersion (allergen Tha p 1, Table 1.74).
- Contact urticaria with anaphylactic shock, a severe reaction often associated with penicillin, neomycin, bacitracin, etc.

A delayed type of contact urticaria is limited to some families.

Contact urticaria can be categorized into three groups from the pathogenetic point of view [115]:

- *Immunological contact urticaria,* almost always IgE-mediated: Table 8.2 [115].
- *Nonimmunological contact urticaria,* mostly triggered by preservatives or additives employed the world over (Table 8.2).
- *Urticaria characterized by an undefined mechanism* including OAS and protein contact dermatitis; see, respectively, Chap. 9 and the second part of this chapter.
- *Vibratory angioedema,* a hereditary disorder more commonly transmitted as an autosomal dominant condition, arising in children and persisting into adult age, decreasing progressively in severity [42]. Patients complain of pruritus, erythema, edema and wheals of 5–10 mm, within minutes in response to the application to the skin of an even gentle vibratory stimulus and lasting up to 24 h, depending on the stimulus and body sur-

Table 8.2. Substances producing immune contact urticaria and nonimmunological urticaria

A. Substances producing immune contact urticaria

Animals and related products
Amnion fluid, bovine blood, cat and dog dander, cockroaches, pig gut, rat liver, placenta, saliva, serum

Cosmetics
Hair sprays, nail polish, perfumes

Foods
Apple, apricot, banana, beans, cabbage, carrot, celery, cheese, cherry, chicken, chives, cow's milk and dairy products, cucumber, egg, endive, fennel, fish, flour, garlic, kiwi, lamb, lettuce, liver, maize, malt, mango, mustard, onion, orange, parsley, peach, peanut butter, plum, potato, sesame seed, spices, strawberry, sunflower seed, tomato, turkey

Medications
Antibiotics (ampicillin[a], bacitracin[a], cephalosporins, chloramphenicol[a], gentamicin, neomycin[a], penicillin[a], rifamycin, streptomycin[a]), ASA, benzocaine, benzoyl peroxide, chlorpromazine, dinitrochlorobenzene, mechlorethamine[a], promethazine, pyrazolones (aminophenazone[a], methimazole, propylphenazone), tocopherol

Metals
Copper, nickel, platinum, rhodium

Plants and related products
Birch, camomile, castor bean, china, chrysanthemum, corn starch, emetine, hawthorn, latex, lichens, lime, mahogany, marine flora, papain, rose, rouge, seaweed, tobacco, teak, tulip

Preservatives and miscellaneous chemicals
Aliphatic polyamide, aminothiazole, benzoic acid, benzyl alcohol, *p*-hydroxybenzoic acid, chloramine, chlorocresol, diethyltoluamide[a], formaldehyde, gentian violet, lanolin, lindane, nickel salts, parabens, phenylmercuric propionate, polysorbates, sodium hypochlorite, tropicamide

Textiles
Silk, wool

Miscellaneous
Acetone, acrylic monomer, aliphatic polyamide, aminothiazole, ammonia, ammonium persulfate, benzophenone, butylated hydroxytoluene, carbonless copy paper, epoxy polymer resin[a], formaldehyde resin, human sperm, lanolin alcohols, lindane, methylethylketone, monoamylamine, naphtha, naphthylacetic acid, nylon, paraphenylenediamine, Perlon, phosphorus, sesquisulfide, plastic, polyethylenglycol, polypropylene, potassium ferricyanide, sodium silicate, sodium sulfide, sulfur dioxide, terpinyl acetate, vinylpyridine

B. Substances producing nonimmunologic contact urticaria

Animals
Arthropods, caterpillars, coelenterates, corals, jellyfish, moths, sea anemones

Foods
Cayenne pepper, fish, mustard, thyme

Medicaments and chemical substances
Acetic acid, butyric acid, cinnamic acid, cinnamic aldehyde, cinnamon oil, balsam of Peru[a], benzocaine, camphor, cantharides, capsaicin. chloroform, dimethylsulfoxide, iodine, methyl salicylate, methylene blue, myrrh, nicotinic acid esters, nicotinic acid tetrahydrofurfurylesther, resorcinol oil, tar extracts, tincture of benzoin, witch-hazel

Plants
Nettle, seaweed

Preservatives and miscellaneous chemicals
Benzoic acid, formaldehyde, sodium benzoate, sorbic acid

Miscellaneous
Butyric acid, diethylfumarate, histamine, pine oil, turpentine

Modified from [115].
[a] Substances that have caused local reactions and anaphylactic symptoms in skin tests.

face area involved. If the stimulus is appropriately intense a systemic reaction may occur, inciting stimuli are motorcycling, toweling, massaging, and the like. A form with delayed onset (4–8 h) and one acquired idiopathic form have been reported [42, 103, 111].

- *Aquagenic urticaria* is another rare form: 15 pediatric cases have been recorded [129, 146, 163, 236]. Age ranges from 2–15 months [146] to 7 years [163]; eight children had associated symptomatic dermographism [146, 163]. A familial tendency has been observed in 11 families [146, 183], in one family over three generations in association with familial lactose intolerance [213]. Affected individuals develop pinpoint wheals after skin contact with water, snow, or more or less intense perspiration, regardless of the T [183]. The lesions (pruritic wheals of 1–3 mm) predominate on the trunk and, less often on forelimbs, occur from 2–3 min to 30 min after exposure to water [156]. On immersion, seven babies became pale, hypotonic, still and unreactive [146]. The lesions may subside after 30 min [156]; however, recovery took a few seconds after withdrawal from the bath and stimulation [146]. These features may even go unnoticed [156]. Biopsy specimens of the lesions reveal mast cell degranulation and hyperhistaminemia; also a cholinergic mechanism seems to play a role [183]. The sex ratio is unfavorable to females (2:1) at puberty or pre-puberty age [238].
- *Cholinergic or thermolytic urticaria*, also known as generalized heat urticaria, along with dermographism is the most common physical urticaria (5%–7% of all urticaria forms), prevalently affecting adolescents and young adults. Prodromic signs may

Table 8.3. Ascertained causes of chronic urticaria in children (%)

No. of children	Age (years)	Ascertained causes	Physical forms	Infections	Foods	Inhalant allergens	Additives	Drugs	Reference
226	1–14	21.2	6.2	4.4	4.4	2.2	2.6	1.9	[229]
97	4.5	78.5	5.1	2	14.4	8.2	18		[18]

arise within 2–30 min of the triggering event, consisting of pruritus, tingling, warmth, or burning of the skin [183]. The ensuing cutaneous manifestations are erythematous and wheal-like, 1–3 mm in diameter, with surrounding bright red flares. These may become confluent to form intensely itching papular wheals, which appear abruptly after variations in ambient T or changes in body T following fever, intense sweating after vigorous exercise, hot showers or sauna, intense transient emotional stimuli, often accompanied by symptoms of cholinergic stimulation, such as lacrimation, salivation and diarrhea, with the involved area returning to normal after about 1 h [117, 175, 183]. In severely ill patients, systemic symptoms such as angioedema and cardiovascular, respiratory or gastrointestinal signs may be associated with anaphylaxis [156]. A similar picture can be produced in affected individuals by acetylcholine: it is postulated that *pathogenic mechanisms* are based on body T increase and the following skin exposure to a warm stimulus can affect the higher nerve centers [197]. Thus, acetylcholine release may be evoked along peripheral nerve endings, also inducing histamine release, probably by a direct action of cholinergic receptors on mast cells [197].

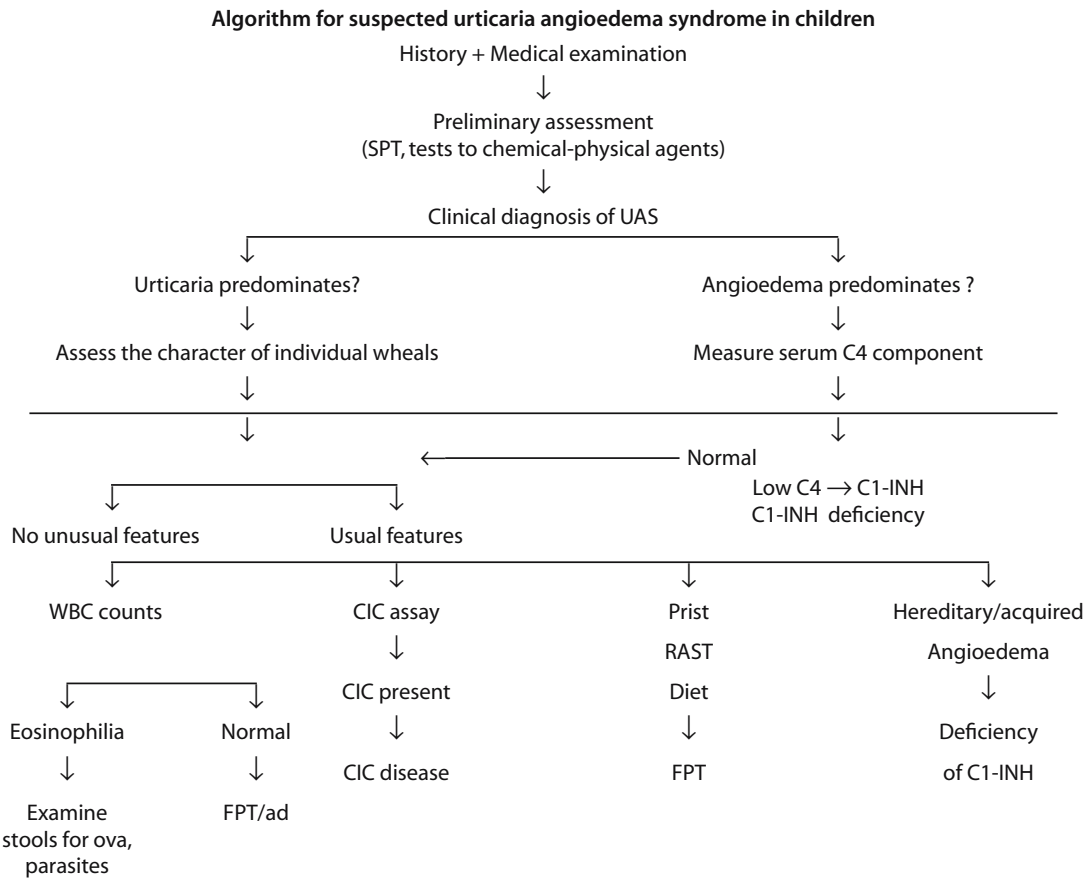
- *Cholinergic cold urticaria* occurs when systemic cold contact produces a form similar to cold urticaria [4, 181].
- *Localized heat urticaria,* also rare (18 cases) is apparent with immediate urticarial reactions within 5 min after heat application, for example warm water [54] at a T of 43 °C and lasting about 1 h, with increased plasma histamine levels [42]. A 5-year-old girl developed sudden-onset episodes of pruritus (after 3 min of heat exposure), redness, and local skin swelling, which resolved within 90 min. Histamine increased at 3 min after heat challenge and then declined [134]. A delayed familial localized heat urticaria is very rare; lesions appear 6–18 h after exposure to localized thermal stimuli and last 12–24 h [111].

The mother of a child seen by us experiences urticaria to both cold and warm objects or even the weather.

Chronic Urticaria

Chronic urticaria is defined as persisting or recurrent urticaria lasting >6 weeks, which may be presently considered the most frequent cause of urticaria [18]. In follow-ups, several cases may progress from acute to chronic urticaria, for example 12 (30%) of 40 children in one study [144]. In three studies on 366 children aged 1–14 [18, 201, 229], several causes were ascertained, summarized in Table 8.3 [18, 229], including physical forms (cholinergic, dermographic, cold and pressure urticaria), infections (parasites and/or streptococci), foods (CM, egg, fish, nuts), food additives (tartrazine, salicylates), aeroallergens (pollens, cat dander) and drugs (penicillin, phenobarbital). In 75% of children, urticaria was attributed to pseudoallergens such as coloring agents, preservatives, saccharin/cyclamate and monosodium glutamate [65]. In 43 children with additive sensitivity (32 also with angioedema), both SPTs and FCTs were positive in 2%–28% of cases [201]. We stress the cases provoked by yeast extract (Chap. 10) and penicillin [157]. In 132 children, in most cases the cause remained unknown (63.6%) [13]. *Chronic idiopathic urticaria syndrome* applies to many patients without an easily identifiable cause; however, frequently these patients suffer from a chronic disease [89]. Recently, two youngsters were reported, one with positive autoimmune markers, the other with juvenile rheumatoid arthritis (JRA), thus opening the door to *autoimmunity in the pathogenesis* of chronic urticaria [54].

Histopathological features show that the preponderant cell types are $CD4^+$ T cells predominant over $CD8^+$ T cells, and that mast cells degranulate [19], thus being considered the primary effector cells in chronic urticaria with a significant increase in number [19]. Significantly increased are intradermal $CD3^+$, $CD4^+$, $CD8^+$, and $CD25^+$ T cells, as well as eosinophils, neutrophils, basophils, and macrophages. T cells from these patients are characterized by a Th0 cytokine profile, with significant increases of IL_4 and IL_5 mRNA and cells positive for IFN-ɣ. A pattern distinct from that seen in the allergen-induced LPR, where IFN-ɣ is absent [248]. An increased histamine release may be induced by nonspecific mast cell activation. It is unlikely that the histamine release is secondary to peripheral blood basophils [45]. Actually, basophils of these patients have a decreased releasability; thus mast cells as the source of spontaneously released histamine in these patients is further substantiated by increased tryptase levels in blister fluid [45]. On skin biopsies, there is evidence of neutrophils within capillary and post-capillary venular walls, but not of structural damage [89]. Spontaneous wheals show expression of CD62E and CD50 on vascular endothelial cells and of CD106 on perivascular cells [89].



Algorithm for diagnostic approach of patients with suspected hereditary angioedema

	C1-INH	C1-INH Funct.	Complement components C1	C4	C3
Hereditary angioedema type I	D	D	N	D	N
Hereditary angioedema type II	N	D	N	D	N
Acquired C1-INH deficiency type I	D	D	D	D	N
Acquired C1-INH deficiency type II	D (60–70 %)	D	D	D	N
Chronic CIC disease	N	N	D	D	D
Idiopathic angioedema	N/I	N/I	N	N	N
ACE inhibitor-induced angioedema	N	N	N	N	N

Fig. 8.6. Algorithm for diagnostic approach of children with suspected urticaria angioedema syndrome (*UAS; top*) and suspected hereditary angioedema (*bottom*). *Top*: *CIC* circulating immune complexes, *FPT* food provocation test, *FPT/ad* with additives, *WBC* white blood cells. (Modified from [89]). *Bottom: 1* inhibitor, *ACE* angiotensin-converting enzyme, *Funct* functional, *D* decreased, *N* normal, *I* increased. (Modified from [155])

Psychogenic Urticaria

Psychogenic factors may exacerbate symptoms of urticaria-angioedema due to diverse causes; however, it is insufficiently understood whether they are primary or associated with other unidentified factors.

Diagnosis

A complete etiological diagnosis (Fig. 8.6) [89, 155], with particular attention paid to the patient's history (Table 8.4) [53] and a thorough physical examination [89, 103, 155, 156, 233] are necessary tools for the evaluation of children with urticaria.

Table 8.4. Flowchart for the diagnosis of urticaria and related forms of angioedema

Features	Manifestations	Diagnosis of urticaria (angioedema)
Genetic factors		Hereditary angioedema, some physical urticaria
Duration of clinical symptoms	<6 weeks	Acute
	>6 weeks	Chronic
Onset related to the time of stimulus application	Rapid	All types
	Delayed	Pressure, cold, cholinergic, contact urticaria
Width of lesions	Few wheals	Cholinergic, aquagenic, solar urticaria
	Arciform	Drug-induced urticaria
	Giant, involving subcutaneous tissue	Angioedema, pressure urticaria
Color	Yellow	Associated with hepatitis B
Localization	Contact and stroking	Dermographic urticaria
	Contact with foreign substances	Contact urticaria
	Photo-exposed sites	Solar, heat urticaria
	Pigmented sites	Urticaria pigmentosa
	Points of pressure	Dermographic, pressure urticaria
Duration of lesions	Short	All types
	Long-term	Vasculitis, familial cold urticaria
Causative factors	Aeroallergens	Acute and chronic urticaria
	Chronic disease	Chronic urticaria
	Cold	Cold contact, familial cold urticaria
	Exercise, foods	Exercise-induced anaphylaxis
	Exercise, stress	Cholinergic urticaria
	Foods, additives	Acute and chronic urticaria
	Heat	Heat contact urticaria
	Insects	Papular urticaria
	Medications	Acute and chronic urticaria
	Natural water	Aquagenic urticaria
	Rx-examinations	Acute and chronic urticaria
	Sunlight	Solar urticaria
	Vibrations	Vibratory angioedema

Modified from [53].

When *hereditary angioedema* is suspected, the history should be complete (FH may be negative in 20% of cases), especially investigating previous recurrent episodes of edema. Diagnosis is made by studying the complement components (C4, C3 and C2) and C1-INH levels (algorithm):

- In asymptomatic periods, the C4 level is decreased and the C2 level is normal; during the attacks the C4 level usually cannot be measured, C2 levels are reduced, C3 turnover is enhanced, and CH50 (50% hemolytic complement) may be reduced.
- C1-INH immunochemical assay: the levels are reduced in 85% of cases; in the remaining 15% the levels are normal or increased, so that functional studies with enzymatic and immunochemical tests should be done [155].
- If C4 and C1-INH are very low, then C1-INH antigenic protein assay should be done to distinguish type I (low levels) from type II (normal) antigen, despite low functional protein [35].

In one family, C1-INH levels were undetectable or low in some patients and CH50 was undetectable in all of the patients. The C4 level was low, and in a 10-year-old boy diagnosis was based on low C1-INH, CH50 and C4, in addition to his FH [247].

Diagnosis has been *successfully made at birth* using cord blood and by examining C1-INH functionality [230].

Diagnosis of *urticaria* (Table 8.4) first requires a distinction between acute and chronic urticaria [44]:

- Genetic factors, atopy
- Aspect of the lesions
- Triggering causes
- Clinical manifestations
- Duration of clinical manifestations
- Associated symptoms

The child's lifestyle should be investigated, particularly food preferences and potential, continuous or occasional drug intake, as well as information on additives, inhalants, recent infections, stress, work, and hobbies [175]. The ability to establish a cause and effect recurrent relationship becomes particularly significant. It should be ascertained whether or not [44]:

- It is a clear-cut type of urticaria
- There is a pathogenic mechanism correlated with what has been ingested, inhaled, or injected

Table 8.5. Foods that contain ASA, benzoates or colorings to be excluded from the diet

Almonds, toasted	Fruit juices
Apple	Grape
Banana	Green peas
Beans	Margarine
Cabbage	Mayonnaise
Carrot	Nuts of all types
Cherry	Onion
Citrus fruit	Parsley
CM, powdered	Peanut
Coca-Cola	Potato
Cranberry	Red, green peppers
Cucumber	Rhubarb
Eggplant	Spinach
Eggs, powdered	Strawberry
Fizzy drinks	Vinegar
In addition	
Processed foods of all kinds, bottled, canned, cased, dry packaged, frozen	
Gelatins, marmalade, soft drinks, ice-creams and sherbets, yogurt	
Caramels of all kinds, chocolate and chocolate puddings (not plain chocolate)	
Bakery foods, candies, cake mixes, chips, puddings and pie fillers, pancakes, wafers	
Cheese, processed cream-cheese, macaroni and cheese of all kinds	
Salad dressings, ketchup, mustard and other prepared sauces (bearnaise, curry, hollandaise, tomato, fish)	
Smoked and frozen fish (anchovy, kipper, sardine)	
Colored toothpaste, chewing-gum	

Diet in use in the Division of Pediatric Allergy and Immunology of Rome, University La Sapienza.

- There is a contact reaction (difficult to discern if AD coexists)
- There is an association with a systemic disease
- There are infectious episodes, past or present

For *food-induced urticaria,* a possible approach involves:

- When the reactions occur <2–3 times/week, it is useful and economic to record the ingested foods in a diary, a practice which focuses the attention of both the family and the child on food factors.
- When the manifestations recur with greater frequency or daily, a diagnostic elimination diet is prescribed, also to detect occult, clinically relevant allergens present in many different foodstuffs. A positive result indicates the opportunity to continue with SPTs or RAST, but FPTs ensure conclusive evidence to be looked for only in children who have a history of previous food-induced anaphylactic shock. A possible therapeutic elimination diet is discussed in Chap. 9.

In *additive-induced urticaria,* countless food dyes and preservatives are suspected [59]. An elimination diet such as the one we prescribe should exclude ASA, benzoic acid and dyes (Table 8.5), frequently prescribed to the children seen in our department: a positive result validates the search for causative additives. (See Chap. 10 for a complete list.) A diet free of ASA and other pertinent additives is similarly helpful in children with drug-induced urticaria [227]. In our department we have noted that almost all children improve by eliminating all prepared foods, with no exception.

In the case of pseudoallergic urticaria, the above-mentioned causes should be chosen among nonimmunological mechanisms. When aeroallergens are suspected, interventions for allergen avoidance should be scheduled. Total serum IgE levels could increase, with a possible peripheral eosinophilia [117].

Physical Urticaria

- *EIA:* it is important to assemble a circumstantial history. Then the patient is invited to run in place or on a treadmill for at least 5–10 min. Positive responses follow the four phases, sometimes a differential diagnosis with cholinergic urticaria may be necessary [190].
- *Dermographism:* by gentle stroking of the skin, in normal responses an immediate blanching followed by a red flare for 10 min is detected, in simple forms a linear wheal and flare appear lasting 10–15 min and in symptomatic forms there are pruritus with linear wheal and flare lasting 30 min to 3 h. In the delayed variant, the wheal decreases within 20–30 min, recurs in the same site by 3–8 h and persists for 24–48 h. The diagnosis is confirmed by using the dermographometer (Fig. 8.7), which is pressed on the skin and a typical response occurs at the pressure of 3.6 g/mm^2 = 3.5×10^5 Pa [117].

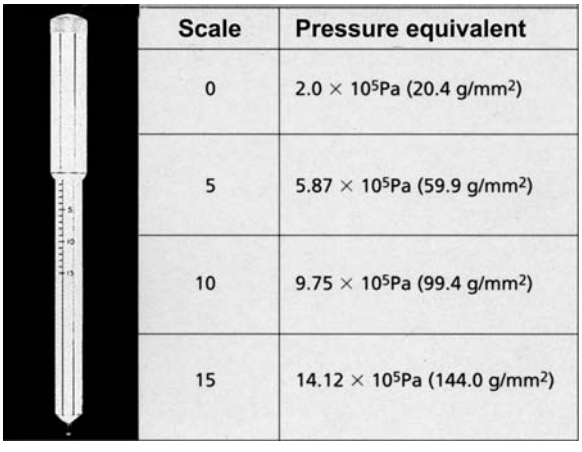


Fig. 8.7. Dermographometer

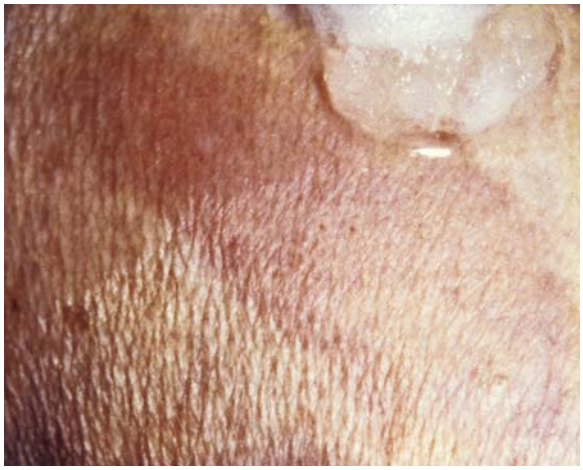


Fig. 8.8. Positivity of ice cube test in cold urticaria

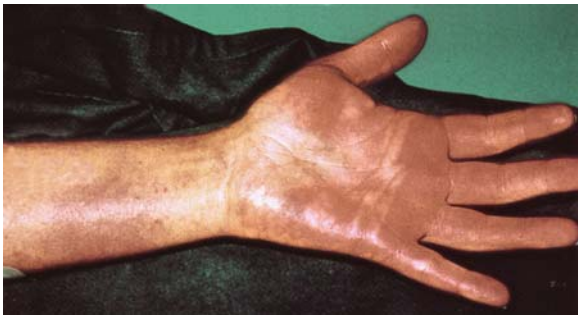


Fig. 8.9. Vibratory angioedema edema and erythema of the palm and forearm after the test

• *Pressure urticaria:* hanging a 6–10 kg weight across the shoulder or thigh for 10–15 min will produce painful swelling at the pressure site after 4–8 h. In younger children the pressure is adjusted according to age. In delayed pressure urticaria, the dermographometer is set at 9.75×10^5 Pa (99.4 g/mm^2) and held firmly against the skin of the back for varying numbers of seconds [117].

• *Cold urticaria:*

• In the localized form, diagnosis is confirmed by placing *an ice cube at 1 °C* on forearm skin for 10–20 min, then rewarming the skin until it returns to a normal T: within 5–20 min an edema appears matching the cube basis (Fig. 8.8). A negative test (and for systemic cold urticaria) is repeated by having the patient stand for 10–20 min in a cooled room at a T of 4 °C [110]. By the end of the test, characteristic wheals and flares develop, especially on the trunk and proximal aspects of the limbs, or giant urticaria [110] in 92% of cases [181]. Children with negative ICT at 10 min had similar symptoms and response to antihistamines as patients with positive ICT. All children with cold urticaria and their parents should be cautioned regarding the risk of anaphylaxis [4]. In selected cases it is necessary to test both cryoglobulins and cryofibrinogen [156].

• In systemic cold urticaria, the ICT is negative and the cooled room test positive. Levels of both histamine and PGD_2 are increased [140, 156]. Measuring blood histamine levels after a negative ICT appears to be a sensitive way to diagnose patients without risk of anaphylaxis during generalized cold exposure [140].

• The prevalence of secondary cold urticaria is 1%; however, we recommend the related screening.

• *Solar urticaria:* a small skin area of 1×1 cm for 1–2 min is exposed to a source of monochromatic light of various wavelengths for 10 min at a distance of 10 cm. If the result is negative the exposure time-lag is increased. Fluorescent tubes, carbon arc, etc., in combination with emission of a broad wavelength spectrum, employing different filters to ascertain which one provokes urticaria [111, 183], are used. A differential diagnosis from erythropoietic protoporphyria requires measuring protoporphyrin and coproporphyrin levels [240].

• *Contact urticaria:* an accurate history and SPTs may disclose some offending agents such as topical drugs, diverse chemical substances, cosmetics, vegetable substances, pet danders, etc. The rub test may confirm the diagnosis, that is the suspected item is rubbed gently on normal skin, and inspected for signs of a reaction at 60 min [117].

• *Vibratory angioedema:* symptoms may be reproduced by gently stimulating the medial aspect of the forearm with a laboratory vortex for 4–5 min or laying a finger-pad on a common tube mixer for 10 min. Positive responders should develop a pruritic circumferential edema, which distinguishes the condition from delayed dermographism and delayed pressure urticaria [42, 111] (Fig. 8.9).

• *Aquagenic urticaria:* challenge tests are performed using tap water compresses at 35 °C or any other T applied to the upper body for 5–30 min. Wheeling is usually evoked at the site of contact with water [129, 238] after 20 min [129]. The condition responded to treatment with UV-B and oral antihistamines [163].

• *Cholinergic urticaria:* to reproduce the eruption, exercise such as jogging on the spot, running on a treadmill in a plastic occlusive suit, running up and down stairs with warm clothes or with an exercise bicycle should be continued until it provokes an increase in perspiration, or by half-body immersion in a hot bath (42 °C) to raise body T. In case of a negative response, an intradermal test is done using 0.05 ml of methacholine chloride at 0.02% or carbachol at 0.002%, which in positive tests induce a localized wheeling surrounded by flushing within 20 min. The test is positive in 33%–50% of patients. However, it is rarely a reproducible test [44, 183].

• *Localized heat urticaria:* a heated cylinder with water heated to 50–55 °C is applied to the skin for 5 min [42].

Chronic Urticaria. Patients are investigated according to the above-mentioned epidemiological data. Following this evaluation, the diagnosis is by exclusion, apart

from cases clearly connected with foods [99]. Neither laboratory data nor SPTs to foods are of value in adults in whom up to 90% of cases remain unassessed [159], whereas the contrary is true in children, both for foods [18, 229] and additives [18, 201, 229]. In a 6-year-old boy with a 3-month history of recurrent, severe angioedema episodes a diagnosis of autoreactive chronic urticaria (ACU) was made after an autologous serum skin test (ASST) [10].

Differential Diagnosis

The differential diagnosis [45] includes the following disorders:

- *Exanthematous infections* in children and EBV, CMV, coxsackie, rotavirus infections, certain infestations by parasites and microbial infections.
- *Papular urticaria,* provoked by insect bites in exposed areas, is characterized by pruritic papules and wheals, with immediate or delayed onset, expressing immunological mechanisms of type I or IV, respectively. The lesions are often located on the lower limbs and tend to persist longer than urticarial lesions, most frequently seen in young children [103].
- *Erythema multiforme,* a self-limited disorder, typically characterized by pruritic urticarial lesions, wholly similar to urticaria in the earlier stages, which last longer and may have a targetoid aspect evolving into bullous lesions. It is more often located on the dorsal surfaces of both hands and feet, frequently misdiagnosed as urticaria.
- *Dermatitis herpetiformis* is seen in children aged 3–7 years. The early lesions have an evident urticarial component. Sites of predilection are the shoulders, elbows, knees and buttocks, often confused with cholinergic urticaria.
- *Urticaria pigmentosa* is a localized mastocytosis occurring in infants and children before the age of 10, with most cases developing in the 1st year of life [12]. Individual macules or papules, and nodular, lichenoid or plaque-like lesions range in color from pink to salmon, and in size from a few mm to several cm, which, when stroked, form a linear pruritic wheal, Darier's sign, the hallmark for clinical diagnosis. The condition usually fades at puberty; a skin biopsy may confirm the diagnosis [45, 61].
- *Juvenile urticaria pigmentosa* occurs at birth or in the 1st months of life and takes the form of a solitary nodule 1–5 cm in diameter (mastocytoma), most often on the back of the hand, wrist, neck, or the lesions may present as disseminated macule, plaques, or bullae, ranging in color from red-brown to yellow-tan. It most commonly fades at puberty.
- *Systemic mastocytosis* is similar to urticaria pigmentosa, but uncommon and more severe. It is characterized by onset in the first few months of life and mast cell accumulation in the dermis, bone marrow and gastrointestinal tract and clinically, by congestion, migraine and hypotension secondary to histamine release [166].

Urticaria as a sign of systemic disease: urticaria may be the only sign of infections caused by bacteria, viruses, yeasts, and molds. Several parasitic infections are causative agents in urticaria and eosinophilia, such as infestations by *Ancylostoma, Ascaris, Echinococcus, Fasciola, Filaria, Schistosoma, Strongyloides, Toxocara, Trichinella,* etc. Urticaria may be associated with malignancy, endocrine disorders (hyperthyroidism, hypothyroidism), and autoimmune disease.

Treatment

Specific management consists in the removal of all offending agents, stimuli, or allergens, and the drug management of possible predisposing or concurrent factors (infections, infestations, etc.), or ongoing problems. A truly specific management is not as yet available, since the etiopathogenetic mechanisms underlying urticaria are largely unknown. A stimulating prospective involves the histamine releasing IgG Aab neutralization by means of soluble FcεRI-α [92].

An *aspecific* or second-line *management* includes the following:

- New-generation nonsedating H_1 *antihistamines,* alone or combined with H_2, are the mainstay of symptomatic management, such as chronic urticaria [192], efficaciously controlled in particular by cetirizine [10], levocetirizine and cimetidine associated with hydroxyzine [192]. Cetirizine and levocetirizine dosage schedules are shown in Table 7.19.
- In a double-blind (DB) study, 31 children aged 2–6 years (mean 3.85) with idiopathic chronic urticaria were treated with cetirizine at a dosage of 5 mg daily, which showed a significantly more rapid resolution of symptoms and itching compared with 31 children treated for the same length of time with oxatomide, at a dosage of 25 mg daily [113].
- Membrane stabilizers such as ketotifen (Chap. 7), which has been found useful in cold-induced and cholinergic urticaria and dermographism after the demonstration that the drug inhibits histamine release at a specific challenge [103]. Similarly, ketotifen was effective in urticaria refractory to antihistamines and in chronic urticaria [101].
- In more severe cases and/or those resistant to other therapies, systemic CSs are required for transient relief [103].
- For pediatric cases of extreme severity, a resort to *Epi-pen* may be suggested [4].

A child aged 10 was treated with ε aminocaproic acid plus montelukast plus ranitidine. This regimen induced a full remission of urticaria in about 48 h. The treatment was gradually tapered in the subsequent months, and after ≈13 months, the boy still remains completely symptom-free [228].

Angioedema. The above-mentioned therapeutic options may be followed. In children with the hereditary form, *danazol* has been demonstrated to be effective at the recommended dose of 20–30 mg/kg/day, with a maximal dose of 600 mg/day [15] for 5 days before and 2 days after the event [35]. Therapy improved serum complement parameters significantly and reduced the frequency and severity of clinical manifestations [70]. Doses of 0.5 g/day of ε aminocaproic acid (EACA) and 1–2 g/day of tranexamic acid are prescribed to prevent episodes in children as needed [35, 69, 70]. Acute, life-threatening edematous attacks are treated by the *administration of C1-INH concentrate,* which achieves the resolution of the edema within several hours [35, 70]. Undesirable adverse effects can be avoided and the *child's quality of life* enhanced dramatically by administering the lowest effective drug dose [70]. In C1-INH deficiency, both primitive and acquired experimental treatment procedures are under study [155]. In cases of laryngeal edema, a tracheotomy will be indispensable. Patients may die as a result of laryngeal edema before a diagnosis is established [247]. Adequate prophylaxis and follow-up care can spare pediatric patients from edematous attacks.

In some patients with Melkersson-Rosenthal syndrome, a remission was obtained with an avoidance diet free of tartrazine and Na benzoate [161], but not in others [142]. Clofazimine, 100 mg, administered orally 4 times weekly for 3–11 months was shown to be effective in several patients [202].

Prevention

A paradigmatic example of *preventive management* is the elimination of stimuli triggering urticaria episodes:

- In *cold urticaria,* cold desensitization by repeated skin exposure to cold until it becomes refractory to challenge has been recommended in motivated patients. A cardinal objective is the prevention of shock reactions during aquatic exposures, including traveling by boat or motor boat, water-skiing and other exposures to cold which lower the body T [233]. In a child with cold urticaria seen by us, protection by a large hat and a scarf was unsuccessful.
- Patients with *cholinergic or generalized heat urticaria* should protect the upper part of their body from excessive heating; also these patients may have recourse to a desensitization with warm baths by gradually increasing water T [44].
- In *solar urticaria* ordinary window glass 3 mm thick will absorb most UV radiation <3,200 Å. Protective garments to cover the skin should be used. Oral β-carotene was useful for type VI of solar urticaria. Types I–V can be prevented, at least in part, by antihistamines or by PUVA therapy [141].
- Subjects with *EIA* should select activities requiring a more modest effort, avoid ingestion of foods and drugs (ASA and NSAID) in the 4–6 h before a planned activity, and carry out physical activity possibly with a partner. Moreover, they should recognize the prodromal signs, to slow down to a minimum or stop exercising [190]. An epinephrine kit (Epi-pen) and medical identification bracelet is mandatory.

In a prospective, DB, parallel-group study of urticaria prevention in 817 children with AD who were 12–24 months of age at study entry, acute urticaria occurred in 5.8% of the children treated with cetirizine, 0.25 mg/kg, and in 16.2% of the placebo-treated children [191].

Allergic Contact Dermatitis

ACD is one of the most frequent skin disorders and the prototype of a delayed type of CMI hypersensitivity reaction: any part of the skin that comes into contact with a relevant sensitizing allergen may be vulnerable. Characteristically, ACD is at first limited to the skin site exposed to the allergen, but the reaction may then spread to other locations. The allergen is brought into skin contact in many ways such as compression, friction, transpiration, humidity and warmth. Lesion severity and persistence are dictated by several factors such as MW, chemical potency, concentration and dose of the allergenic stimuli, site of contact, the frequency, duration and intensity of exposure, and especially the patient's degree of sensitization [82].

Definition

Dermatitis of variable intensity, due to lymphocytes previously sensitized by antigens making contact with exogenous haptens, mostly chemical haptens, at variance with AD, in which allergens arrive by the hematic route. In this reaction, after the first epicutaneous allergen sensitization, Langerhans cells (LCs) take allergens up and migrate from the skin to draining lymph nodes and activate naive T cells in the context of HLA class I or II. Clinical manifestations vary from a transient erythema to edematous papules with vesiculous and occasionally bullous outcome; itching is ACD's distinctive symptom, manifesting with variable intensity [107].

Prevalence

Pediatric ACD is a much more frequent disease than previously reported (Table 5.9). Epidemiological studies supply an ever-growing body of data pushing out the boundaries of the disease, including prematures [76], a *1-week-old neonate* with ACD to the epoxy resin in his vinyl identification band, and a *7-month-old infant* who developed nickel dermatitis to the snaps in his sleepwear [75]. Children as young as 6 months of age may be sensitized to contact allergens with a prevalence of sen-

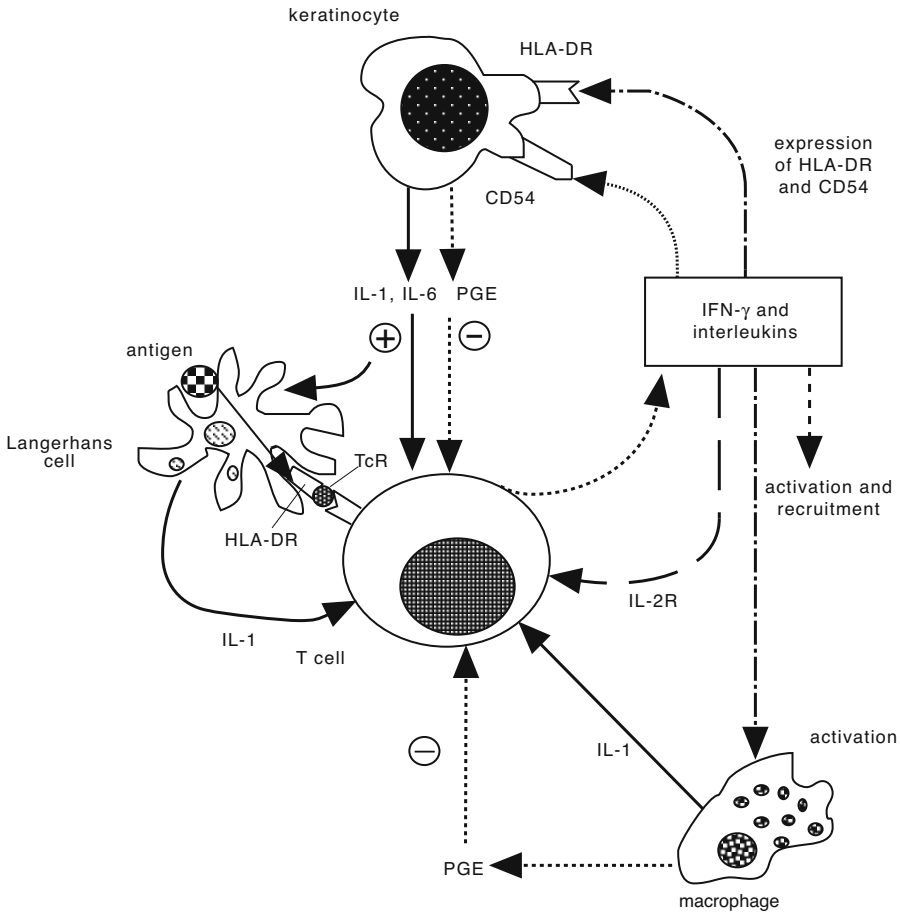


Fig. 8.10.
Cell interactions in ACD.
(Modified from [20])

sitization as high as 24.5% [38]; 43% of children aged 8 (median) suffered from ACD of the delayed type to metals and the like and 6.6% of them to patch-test diagnosed foods, increased up to 20% by an open FCT [154]. The development of contact allergy and ACD increases with age [145, 185] In children aged 3–14, the following positivities were found: 38.8% preservatives, 25% topical drugs and 11%–16.6% fragrances and perfumes [56, 212]. Also, occupational ACD incidence is increased (21.2%) in boys and girls helping their parents in domestic work [7] or work outside the home [85, 199]. These data also complete the ACD natural history in Table 5.9.

Etiopathogenesis

Genetic Factors

Several textbooks assert that ACD is not linked to atopy. On the contrary, atopic sensitization in children and teenagers ranges from 16.7% to 32.6% [7, 56, 85, 199, 212, 214], with peaks of 50% [63], with positive FH between 20.7% [199] and 42.1% [80]. As discussed earlier, a large share of children with AD are also affected with ACD, with a prevalence between 16.7% and 32.4% [7, 56, 63, 85, 212]. Genetics was established by the finding of two monozygous girls with contact allergy, thus suggesting a genetically determined selection process during development of the peripheral T-cell system [206].

Histopathological Aspects

In the acute forms, within 6 h from allergen exposure, perivascular infiltration of lymphocytes and monocytes are seen in the highest dermal layers. Subsequently an intracellular and intercellular edema develops in the epidermis, thus causing spongiosis. Chronic lesions are characterized instead by hyperkeratosis, and in the dermal superficial layers by a dense monocyte and basophil infiltration [107, 138].

Immunological Aspects

Though humoral immunity may participate in ACD pathogenesis, this depends above all on allergen-specific T cell activation stimulated by chemical substances of low MW, which are haptens and therefore need to link with proteins called carriers in the skin before they become antigenic and acquire immunogenicity (Fig. 8.10)

[20]. These circumstances result in a cascade of inflammatory events leading to the development of dermatitis or, after a diagnostic FCT, to a hypersensitivity reaction [20]. ACD can thus be classified as a delayed type of hypersensitivity (DTH) reaction, in other words, a stage of hypersensitivity associated with an increased number of allergen-specific T cells able to invade the peripheral tissues [105]. The series of immune events that induce ACD implies the interaction, at an undetermined site, between T lymphocytes and *epidermal CD1⁺ LCs* (Figs. 7.1, 7.2), functionally immunocompetent ($CD1^+/IgE^+$) cells. Early and important participants in the induction of ADC, LCs act as APCs in the initiation of a type IV immune reaction [207]. Mobilization of dendritic cells (DCs) occurs in response to local generation of proinflammatory ILs such as IL_1 and TNF-α. Activated DCs express high levels of CCR7, HLA antigens, and costimulatory molecules (CD40, CD80, CD86) [33]. Sensitizing allergens contacting the skin activate T lymphocytes. To a subsequent cutaneous contact the duo hapten + carrier is taken up, processed and expressed on the surface of LCs bearing class II HLA-DR molecules [23]. After the allergen-HLA complex presentation, LCs migrate out of the epidermis into the dermis and then into draining lymphatics and on to regional satellite lymph nodes. Within 4–6 h of hapten application, LCs congregate in T-dependent zones; within 18–24 h a great number of LCs are in the draining lymph nodes to present the processed antigen and transfer the sensitization to a large repertoire of specifically sensitized CD4 T lymphocytes bearing CD3 receptors that undergo proliferation and clonal expansion [207]. The antigens interacting with HLA molecules emit the first signal for lymphocyte activation, and the second signal is represented by IL_1 produced by keratinocytes and LCs with the primary function of activating T lymphocytes. This results in the clonal proliferation of antigen-specific memory T cells with the phenotype of $CD4^+$, CD45RO, CD45RA, which yield a number of ILs, including IL_2 and IFN-γ [207]. The coincident presence of both ILs corroborates the conclusion that they are the product of CD4 Th1 specific for contact antigens, in parallel with the delayed reaction of AD [105]. *Keratinocytes* with their HLA-DR antigens and CD54 (Fig. 8.11) act as target cells for activated T cells, and the inflammatory IL they produce attract PBMCs in an allergen-nonspecific way [20]. During the induction phase, the CD4 infiltrated in the dermis proliferate and differentiate into effector cells and release Th1-like ILs (Table 1.10), capable of inducing capillary vasodilation and permeability increase, thus leading to the allergic skin inflammation [207].

Th1 T cells dominate and amplify the generation of cytotoxic allergen-specific CD8 lymphocytes (CTLs), which leads in the final analysis to cutaneous lesions. As seen from studies on the animal model, both mediate type IV immune responses, Th1 with class II HLA, and CTLs with class I HLA. Suppressor CD8 T cells are subsequently generated, although in a lesser number,

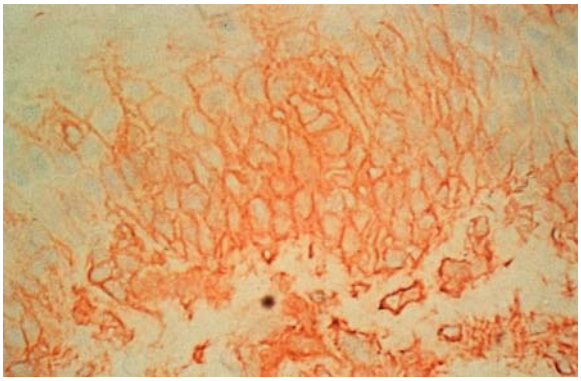


Fig. 8.11. Keratinocytes expressing HLA class II in ACD

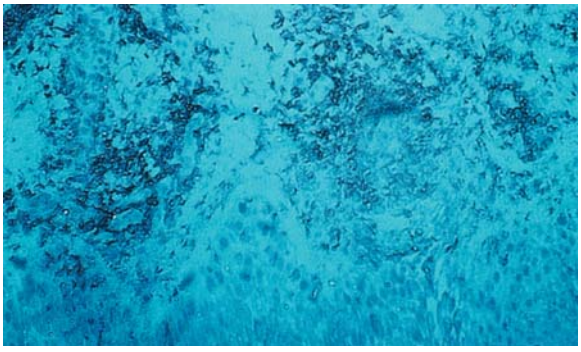


Fig. 8.12. The majority of T cells infiltrating the allergic patch test after 96 h are CD4

thus achieving a balance between sensitization and tolerance, orchestrated by a complex suppressor circuit acting on both afferent and efferent pathways of the DTH reaction [23]. Looked at in more detail, whether sensitization has an effect either on Th1 or Th2 activity depends on the exposure intensity and LC integrity; however, there is no numerical difference between the different T-cell subpopulations in the various phases so far delineated [211]. Intercellular connections are ensured by β1–β3 integrins (Tables 1.45–1.47), which allow cells to migrate into the skin, and IFN-γ-stimulated endothelial cells express CD54, more adhesion molecules and class II molecules [251]. This leads to preferential PBMC migration from the bloodstream to the inflamed skin lesions, and circulating T cells, by expressing their ligands such as CD11a/CD18 and CLA, recognize CD54 on endothelial cell and keratinocyte surface membranes activated by IL_1 and can bind these cells [20, 251]. Moreover, PBMCs of patients with ACD presenting antigens to autologous T cells can stimulate the proliferation of the two different subsets, $CD45RO^+$ and $CD45RA^+$, potentially recognized by two different epitopes. However, $CD3^+$, CD45RA and CD8 T cell responses are absent [23]. $CD45RO^+$ $CD4^+$ T cells stimulated by antigens reach the maximal expression at 96 h (Fig. 8.12), thus suggesting that they are able to induce immediate cutaneous reactions in patch test sites [23].

Table 8.6. Cytokine production by keratinocytes and LC

Cytokines	Keratinocytes	LC
$IL_{1\alpha}$	+	+
$IL_{1\beta}$	+	+
IL_6	+	+
IL_7	?	?
IL_{10}	?	?
IL_{12}	+	?
Colony-stimulating factor		
IL_3	+	+
GM-CSF	+	+
G-CSF	+	+
M-CSF	+	+
IFN-α	+	?
IFN-β	+	?
IFN-γ	?	±
TNF-α	+	+
TNF-β	±	±
TGF-α	±	?
TGF-β	±	?

Data from [58, 128].

Table 8.7. Cytokine production by nickel (Ni)-specific T CD4 lymphocytes in patients with ACD

Cytokines	Allergic	Nonallergic
IL_2	+	+
IL_4	+	+
IL_5	+	+
GM-CSF	++	++
IFN-γ	+	+++
TNF-α	++	++

Modified from [104].
ND not done.

Metachromatic cells are involved in ACD: the cellular infiltrate can contain up to 10% basophils and 40% mast cells, activated by IL released by T lymphocytes, and they amplify the reaction by dilating capillaries [107]. *Basophils* principally are seen transiently: at 48–72 h large numbers of basophils are seen within the skin blood vessels, but their exact role is far from elucidated [107]. *Macrophages* invade both dermis and epidermis also at 48 h, and contribute to calming the reaction by producing either PGE, which inhibit IL_1, IL_2 and NK cells, or LTB_4 inducing T CD8 cells, which further progressively calm the reaction. Therefore both hapten size and its capacity to steadily bind a carrier protein appear to be important factors in immunogenicity [107].

Tables 8.6 [58, 128] and 8.7 [104] show the production of ILs by keratinocytes and LCs [82, 128] and by nickel (Ni)-specific T lymphocyte clones in an ACD response. It is surprising how many ILs are produced by keratinocytes: Ni-sensitive patients produce both IL_4 and IL_5, but the nonallergic patients have elevated levels of IFN-γ. A recent study [82] showed that $IL_{1\alpha}$,and $IL_{1\beta}$ are among the several ILs produced in the skin after contact by an immunizing agent, which potentially primes the synthesis of more chemotactic ILs involved in ACD and IL_{10} [71, 128], which instead inhibits the reaction. In particular the keratinocyte-produced IL_{10} may down-regulate DTH, thus terminating the response, which is instead up-regulated by its inhibition [71]. TNF-α stimulated with UVB propitiates the reaction by its proinflammatory and vasodilatory effects, but may be critical in inhibiting the migration of epidermal LCs [104], which produce few ILs [128]. Chemokines such as RANTES and MIP-1β may recruit a higher level of T lymphocytes, with a further increase in local mediators [203].

A distinctive type of reaction involves *Ni*. Ni-specific T lymphocyte clones recognize the antigen only on the cell surface of LCs in association with HLA class II molecules, whereas they were unable to recognize Ni when presented by monocytes. Therefore, there may be a direct and non-HLA-restricted recognition by T-cell clones; thus Ni might interact directly on the T cell surface structure, with consequent activation and proliferation of these T cells. Since HLA class II molecules are polymorphous and contain mostly three subtypes, all heterozygotic subjects inherit two different alleles for each of these three subtypes, so Ni may be associated with up to six different HLA class II determinants presented by APC, although it is difficult to identify the precise determinant involved [105]. See in the next section that oral Ni administration may have important suppressive effects in the recipients [76].

Etiological Agents

A plethora of compounds hold a top position as ACD causative agents, some, such as dinitrochlorobenzene (DNCB), exercising their action indiscriminately, while others are much more selective. The more frequently induced substances are summarized in Table 8.8 [7, 56, 57, 63, 85, 131, 149, 199, 212], and Table 8.9 [20, 131] shows the sources of sensitization as related to their topography. On the drug panorama, we give concise indications since Chap. 19 discusses the topic at length, and latex dermatitis is analyzed below. We examine the technomarketing particularities related to the most diffused haptens [7, 52, 57, 80, 131, 138, 173, 227].

Table 8.8. Most frequent causes of ACD on a mostly nonimmunological basis and related rates

Substance and references	[57, 131]	[149]	[56, 212]	[7]	[199]	[85]	[63]
Balsam of Peru	3.3–12	3–3.3	3–10	0.9	2.5	0.9	0
Benzocaine	1–4.5	0.6–3.1					0
Chloramphenicol	0.5						
Chrome	5–8.4						
Cinnamic alcohol	2.7–4.8	2.5–4.8					
Cinnamic aldehyde	3.1–5.9	4.1–6.8					
Cobalt chloride	6.3–7.1		3–17	6.6–13.2	10	7.2	5.7
Colophony	3.4	1.7–4				0.9	0.7
Ethylenediamine	2–7.1	3.9–5.6		1.7–5.4	5	0.3	0
Formaldehyde	1–6.8	4.7				0.9	0
Fragrance mix		4.1–7		1.5	3.7	4.2	
Magnesium, inorganic compounds			12				
Mercapto mix			3				
Mercaptobenzothiazole	0.9–4.8	1.5–2.66		1.4–7	3.7		
Mercury				0.7	6.1		
Neomicyn sulfate	2–7.2	4.5–5.9		0.8–1		0.3	1.4
Nickel sulfate	9.7–34	9–12	5.5–37	2.3–35.1	56.2	21.5	14.9
Parabens	1.1–3.5			0.3–0.8		0.6	0
Paraphenylenediamine (PPDA)	3.7–9		3–12	13.6–17		1.8	0.5
Penicillin	0.4						
Potassium dichromate	2.4–11		10	11.3–24.8	10	2.7	1.2
Thimerosal	6.2–8.7	3.4–6.2				11.2	
Topical steroids	1.9–2.9						

Data from [131] on 29,499 patients and [57] on 18,822–20,791 (age unknown) [57, 131]; on 1,370 patients from the US and 780 from Canada [149]; pediatric data on 244 nonatopic and 47 atopic children aged 4 months to 16 years [7]; [85] on 69 reactions in 53/214 atopic children and two studies in children aged 3–16 years [56, 199, 212], in comparison with 34 reactions in 28/143 nonatopic children and 47 reactions in 424 schoolchildren aged 7–12 years [63].

Balsam of Peru: vegetal fragrance containing principally benzylbenzoate, benzoic acid, cinnamyl alcohol, eugenol and vanillin. It is used in:

- Emollients, medical creams and ointments, powders, tinctures, suppositories, healing preparations and for burns (due to antiseptic and/or keratoplasty actions)
- Straps and paints due to its adhesive action
- Perfumes and cosmetics (after-shave lotions, brilliantine, creams, face powders, deodorants, infantile cosmetics, lipsticks, lotions, perfumes, soaps, toothpastes, etc.) as fragrance
- Tobaccos
- Drugs (balsamics, cough syrups, emollient lozenges, topical drugs); liquid cement for dentists
- Foods (as aromatizing in food industry) such as aperitifs, baked goods, bitters, chewing gums, candies, chocolates, cola, cocktails, ice creams, orange squash, pâté de foie gras, sauces, vanilla, products containing

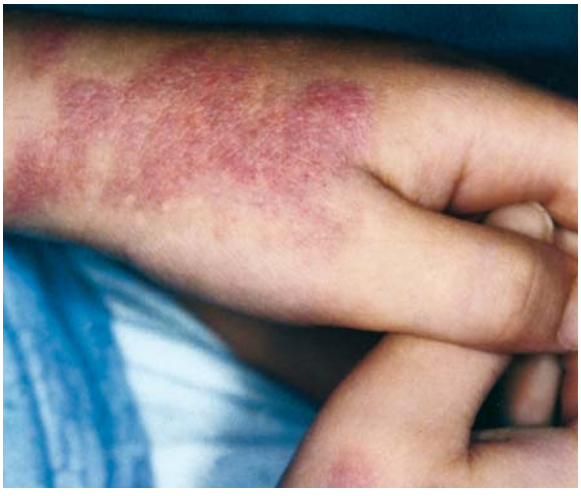


Fig. 8.13. ACD provoked by resin

Table 8.9. Location of common allergenic ACD contactants in relation to body sites

Regions	Allergenic substances
Face	Soaps, face powders, cosmetics in general, perfumes, cleansing milks and creams, shaving creams, razor blades, cosmetic lotions used after and before shaving, crash helmets, frames, nebulizer masks, antiallergic masks (Chap. 11), sun creams (hand dermatitis usually appears first), topical medicaments
Scalp[a]	Hair lotions, lacquers, lotions, brilliantine, hair tonics, anti-dandruff products, hair dyes and curling agents, earrings, bobby pins, hair clasps, wig adhesives, hats, caps
Forehead	Cosmetics, hair lotions and shampoos, hat bands, cap bands, anything applied to hair
Eyelids	Eye and face cosmetics and cleansing milks, facial creams, eye shadows and make-ups, eyelash dyes and curling agents, medicaments: collyrium, eyewashes and ointments, contact with fingertips
Ears	Earrings and ear piercing, pendants, earpieces for portable radio, etc., earplugs, hearing aids, earphones and cellular receivers, ear preparations, topical medicaments, medical eardrops
Lips and perioral areas	Ointments in general, lipsticks, lip protectants (both sexes) and lip pencils, chewing gums, toothpastes, mouthwashes, nail polish (contact with fingertips), foods (see contact cheilitis), toilet paper, tobacco smoke, latex, metals, also related to ear piercing
Neck	Perfumes, necklaces (nickel), garments, sweaters
Armpits	Deodorants, depilatory creams, textile fibers, tinctures, topical medicaments, antiseptics
Arms	Watch cases and related watch bands, textile fibers, metals, plants, tinctures
Hands	Cosmetics, lotions, creams and sun creams, metal jewels, metals, plants, cleansing agents, soaps, leather, rubber and latex gloves, metal, rubber and latex handled objects and materials and irritants encountered at work, topical medicaments
Body	Perfumes, bath salts, cosmetics and detergents in general, metal parts of garments (buttons, zippers, etc.), shoulder straps, brassiere clips, textile fibers, leather, rubber and latex dresses, rubber in elastic of garments, dyes, formalin and resins in garments, douche additives, plants, topical medicaments
Genitalia	Infant diapers, briefs, topical antiseptics, antibacterial and antifungal drugs, disinfectants in general, (women) contraceptive creams or jellies, menstrual pads or tampons, preservatives, deodorants, rubber diaphragms (men), remedies, latex condom, vaginal agents used by partner
Anal area	Textile fibers, topical medicaments, suppositories, remedies and ointments
Legs and thighs	Stockings and drawers, leotards, leather dresses, boots, textile fibers, rubber, clips of garters and other metal parts, depilatory creams, topical ointments and medicaments
Feet	Shoes, rubber shoes, shower sandals and slippers, tinctures, shoe waxes, buckles, textile fibers, deodorants, topical foot remedies, plants

Data from [20, 131].

[a] Since the scalp is relatively resistant to dermatitis, hair preparations generally cause dermatitis mostly on the forehead, scalp edges, neck, and sometimes also shoulders, i.e., on hair-free skin.

citrus peels (orange jam, cakes with candied fruits, etc.), confectionery products with spices, perfumed teas

Colophony (Fig. 8.13): a resin obtained from different conifer species, one of its derivatives is turpentine oil. It is found in:

- Electric insulators, pharmaceuticals, soldering materials, such as patches, plasters, and products of common use, including soaps and cosmetics, in addition to adhesives, depilatories, dressings, mastics, nail polish, shoe wax, stickers, tape, varnishes, watercolors; cleaning compounds (furniture and floors), lacquers, surface coating, paints, paper boxes, resins, and printing inks

Turpentine: oils, resins and varnish solvents, is found in:

- Perfumes, bath salts, soaps, shaving-creams, toothpastes
- Insecticides
- Expectorants by mouth, antirheumatic and rubefacient liniments

Parabens mix: substances with antioxidant, antifermentative and fungicidal power, found in cosmetics, drugs and foods as preservatives; are present in:

- Preservatives found in many dermological creams, pastes, face powders, cosmetics, sun-creams, toothpastes, cleansing milk and creams, hair lotions, lipsticks, soaps

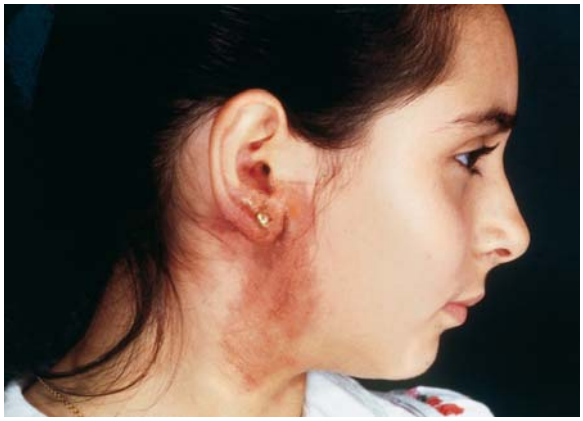


Fig. 8.14. ACD provoked by Ni in earrings

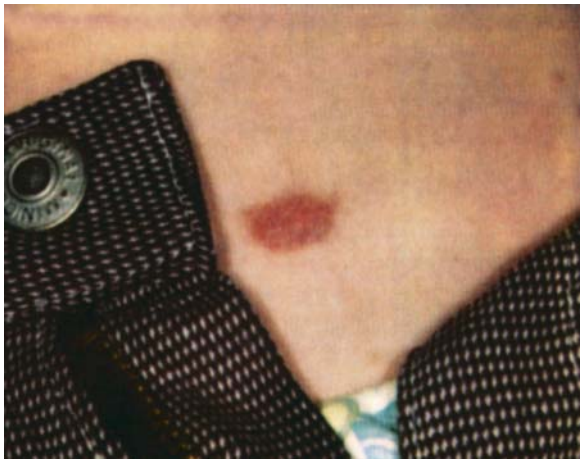


Fig. 8.15. ACD provoked by Ni in fasteners

- Bandages, collyrium, nasal drops, antifungal pomades and for varicose veins, ointments
- Floor wax
- Foods: canned fish, caviar, fruit juices, lemon- and orange-squash, mayonnaise, smoked salmon
- Parabens can also cross-react with compounds of the "para" group (Chap. 19)

Nickel sulfate and chloride (Figs. 8.14, 8.15) is one of the most common sensitizers in our environment contained in nickel-plated manufactured products of common use such as:

- Coins (euro cents), door handles, drawing-pins, earrings, inks, keys, lighters, metallic parts of watch-straps, needles, paper-clips, pencils, pins, safety-pins, scissors, spectacle frames, thimbles, tweezers, utensils, watch-cases, etc.
- Identification bracelets (maternity wards), particular groups of patients, also allergic children.
- Dental prostheses, orthodontic instruments, and low-weight gold alloyed with Ni used in dentistry.
- Clothes accessories such as bobby-pins, buckles, buttons, clips, fasteners, clothing and hair clasps, shoe laces, safety-pins, zippers.
- Costume jewelry since nickel-plating precedes gold-plating (bracelets, earrings, jewelry of white and yellow gold, medals, etc.); some children may be exquisitely sensitive to even Ni traces found in gold jewelry.
- Colorings for cosmetics, for example eye-shadows.
- Curling instruments for eyelashes and hair.
- Door handles, handles of electric appliances, stainless steel pots or enameled pots (especially in blue or green), as well as pottery, insecticides, plastics.
- Dyes, metallic paint, mordents, paints, shoe waxes, wallpapers.
- Young children and girls have a high risk of sensitization at the time of ear, nose, and tongue piercing and subsequently [130] (the Italian press reported on March 12, 2003 the case of a 24-year-old who probably died of a fulminating hepatitis after a tongue-piercing).
- Ni can contaminate foods cooked in chipped pots or pickled in metallic containers or cans.
- Can be found in raw and canned foods, for some of which we give the Ni content in μg/hg [173]: lentils 310, white beans 285, rye 270, peas 225, cocoa and chocolate 220, peanuts 160, raspberry 40, herrings 30, jam 25, potatoes 25, white wine 10, CM and derivatives 5, as well as asparagus, beer, butter, carrots, Brussels sprouts, cabbages, corn flour and whole meal, hazel nuts, margarine, mushrooms, onions, oysters, pears (also cooked), popcorn, raisins, rhubarb, soy, spinach, strawberry, tea, tomatoes, walnuts, yeast (artificial), etc., in addition to traces in cleansing tools.
- It is known that sweating may release Ni ions from metallic objects. The metallic containers for food preservation should no longer affect Ni content, since Ni contact is now avoided by means of an internal coating formed by a layer of acrylic or epoxy polymeric resins, as for enameled pots. Instead, wearing Ni-releasing orthodontic braces induces by immunosuppression tolerance to later Ni contact, provided that fitting of braces is absolutely not preceded by further Ni contacts, for example ear-piercing [130, 221]. We stress that in Denmark *the use of Ni has been banned since June 1989* in belts, buttons, frames for glasses, hair-clips and bobby-pins, costume jewelry in general, watch-cases and related watch-straps, zippers, and any object touching the skin; violation of the ban carries a 1-year sentence of imprisonment.

Potassium (K) *bichromate:* is employed:

- In chromed alloy and manufactures.
- In leather tanning.
- As cloth dye, such as in military green fabric and billiard- and card-tables.
- As anticorrosive in paints, oils and refrigerant fluids.
- As a catalyst in acrylic resin synthesis.

It is contained in:

- Objects of common use such as colored pencils and pens, fragrances and deodorants, magnetic tapes, match

heads, paper money and wall-paper, shaving-creams and razor-blades.
- Bleaches, cleansing creams, detergents in general, floor waxes and other products, porcelain, pots, shoe polish.
- Leather objects, shoes.
- Fixatives, inks, photographic chemicals.
- Rubber objects and artificial flowers.
- Foods in traces (apples, beer, bread, canned cereals, chocolate, eggs, frozen peas, mushrooms, onions, potatoes, plums, raisins, watercress, wholemeal).

Cobalt chloride (CoCl) is found in almost all bichromate materials, moreover:
- In galvanized manufactured products.
- As catalyzing in rubber synthesis, acrylic resins and polyesters.
- As Ni impurities.
- As an alloy in joint prostheses.
- In adhesives, artists' paints, buttons, ceramic and plastic paints, coins, colored pencils, costume jewelry, eye shadows, inorganic pigments in inks, hair dyes, linseed oil-based paints, tattoo pigments, watch straps, wet cement, zippers.
- In Ni-containing accouterments on clothing.
- In anti-perspiration creams, flypaper.
- In vitamin B_{12} preparations and in mineral tablets.
- In foods, namely apricots, beans, beer, beets, bread, cocoa, cabbages, chocolate, cloves, liver, nut, tea, wine [125].

Chrome (Cr) in small amounts is present in cigarettes and in objects of common use:
- Alloys for tooth prostheses, bleaches, cement, ceramic paints, detergents, flypaper, fireworks, floor wax, green felts, matches, military green fabric, pots, razor blades, safety matches, shaving creams, shoe polish, wood ashes and stain, wallpapers.
- As a water and flour pollutant: small amounts are found in mineral waters.
- In foods: apples, beer, cocoa, canned cereals, chocolate, eggs, fish, frozen peas, meat, mushrooms, onions, potatoes, plums, raisins, spices, tea, watercress, wholemeal, wine [125].

In industry:
- As fore-gilding of raw textiles and chrome-tanned leathers (also with Cu and tannins).
- In typo-lithography: auto-implants, fixatives, inks, photographic chemicals, special papers.
- In mechanics: anticorrosive oils and greases, antirust dyes, cooling mixtures for motors, metallic alloys, welding materials.
- In galvanizing processes: aluminium coloring, chromium plating, electroplating, polishing.
- In colors, enamels, dyeing, oil paints and distempers.

Copper (Cu) is a minor sensitizer found in bronze, brass, and other alloy objects, also of common use:
- Pots for polenta, coins, costume jewelry, electric materials, green eye shadow, intrauterine contraceptives.
- Acaricides, antiparasitics, astringents for mouthwash and lotions, fertilizers, fungicides.
- Foods in traces: beer, clams, edible mushrooms, lentils, mussels, oysters, shrimps.

Paraphenylenediamine (PPDA): is employed in:
- Color film developers, inexpensive fabrics and furs, lubricating oils, permanent black or dark hair dyes, dyed textiles, etc.
- As an accelerator and antioxidant: rubber, catalyzers for resin synthesis.
- Antioxidant in gasoline, oils, lubricating, printing inks and cartridges for printers (computer), reagents for photography, lithography and radiography.
- Cross-reactivity with other substances of the para group such as local anesthetics (benzocaine, procaine, etc.), sulfonamide drugs, aniline dyes and PABA (*p*-aminobenzoic acid) used in sun screens (creams, lotions, ointments) and in several cosmetics, including eye shadows, lipsticks, hair balsams, nail varnishes, shampoos. PPDA oxidizes rapidly in contact with atmospheric oxygen, but this reduces its allergenicity only slightly.

Ethylendiamine: stabilizer found in cosmetics and topical drugs (antihistamine ointments, eye drops, nose drops, lotions for itching eczema), in fungicides, insecticides, and preservatives in creams, as a catalyzer for resin synthesis, as well as in anticorrosive and antifreeze agents, solvents, etc. It can cross-react with aminophylline, hydroxyzine, prometazine, etc.

Formaldehyde is a chemical widely used as a disinfectant, while formalin, a 27% aqueous solution, is used to treat allergens to produce allergoids. Formaldehyde antigenicity was recognized as early as 1914 [116]. It is found in:
- Bactericides, denatured alcohol, fabric softeners, fungicides, oral disinfectants, preservatives, and in antitetanic vaccines.
- Cosmetics in general, deodorizers, nail hardeners, permanent fluids, soaps, shampoos, toothpastes, etc.
- Plastic packing (demopac) for foods prepared in advance, from which residual amounts are naturally released.
- Enamels, glues, pastels, tanning agents, tempera painting, in dry-cleaning as a spot remover, in typography, etc.
- Adhesive strips to apply wall-to-wall carpeting, textile finishes.
- Insulating materials, plywood paneling and wrinkle-resistant clothes.

We underline its hidden presence in carpets, cigarettes, insulators, paper tissues and serviettes, towels, and other paper products also for toilet or medical supplies, preservatives employed in the pharmaceutical and other industries, disinfectants also for housework, and as a monomer in butylphenol-formaldehyde resins used in adhesives, corsets, glues, leather goods, products to protect color fading in clothing articles and in underwear elastics or as sizing, crease-resistant and/or waterproof finishes. However, that contained in shampoos

and liquid soaps is a rare cause of ACD since it is rapidly diluted and rinsed.

Mercury (Hg) includes different compounds, all cross-reacting between themselves, employed in medicine and cosmetics:

- Mercurochrome, phenylmercuricborate, etc., are used as disinfectants for small wounds and in lipsticks.
- Thimerosal is a topical antiseptic employed as an ingredient of germicides, cosmetics, eye shadows, preparations for dentists, eyewashes and nasal drops, contact lens cleaning and wetting solutions (Chap. 14), etc., as preservatives in pharmaceutical preparations, including vaccines and sera, and as a rubber accelerator.
- Yellow oxide (HgO) is a topical antimicrobial employed for eye applications and as caustic, cicatrizant, etc.
- Bichloride ($HgCl_2$) is employed in amalgam fillings, contraceptives, electric materials, lubricants, thermometers, etc.
- It is also found in aniline colors, textiles, etc., for leather, felts, furs, wood preservation, in photography and printing.

Topical medicaments

- Benzocaine, a local anesthetic, can be found more or less commonly in medicaments reducing pain, itching, or stinging, cuts, burns, insect bites, rashes, sunburns, toothaches, and in hemorrhoidal preparations, cough syrups, throat lozenges (cross-reacts with PABA).
- Neomycin is present in over-the-counter antibiotic creams, eyewashes, lotions, many skin, ear and nose products, medication powders, ointments and some types of measles vaccines (Chap. 9). It is also a component of deodorants and toilet soaps. Other aminoglycoside antibiotics such as gentamicin, kanamycin and streptomycin may cross-react.

Benzalkonium chloride is a quaternary ammonium cationic detergent with manifold applications as a preoperative skin disinfectant, for disinfection of surgical instruments, and antimicrobial preservative in nasal sprays, ophthalmic medications, solutions for contact lenses (Chaps. 12 and 14), but also in house cleaning products. However, it frequently induces irritant reactions and may cross-react with curariform preparations employed in general anesthesia (Chap. 20).

Disinfectants include (in addition to thimerosal and benzalkonium chloride):

- Betadine, a skin disinfectant used for burn or wound dressings, etc.
- Chlorhexidine hydrochloride, a topical disinfectant found in skin cleaners, gingival washes, cosmetics, deodorants, etc.
- Dequalinium chloride, employed as benzalkonium chloride, is present in throat lozenges.
- Menthol, alcohol extracted from peppermint, is perfuming and revulsive, used also as antipruritic in cosmetics and pharmaceutics or in inhaled medication for respiratory diseases.

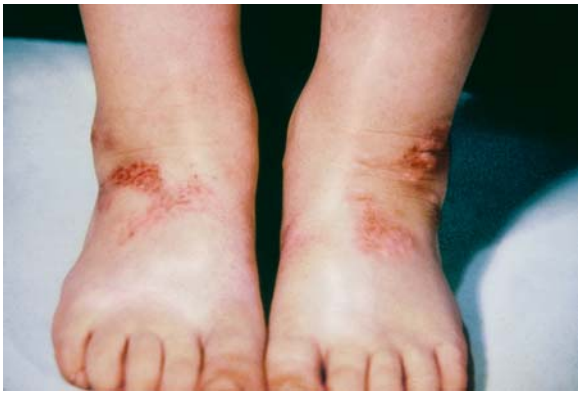


Fig. 8.16. ACD provoked by rubber shoes

- Gentian violet, coloring employed also as an antimycotic mostly in topical applications; however, it is sensitizing, and cross-reacts with bright green.

Fragrance mix is a frequent ingredient of after-shave lotions, beauty creams, cosmetics, soaps, sanitary napkins, shampoos, toiletries, toothpastes, and in household products such as deodorant sprays, detergents, polishes, and solvents. It contains cinnamic alcohol, cinnamic aldehyde, eugenol, geraniol, etc.

- Cinnamic alcohol is utilized as a fixative of mixtures based on jasmine, hyacinth, lilac, narcissus and rose perfumes, as deodorant at 12.5 % solution in glycerin and in the soap industry.
- Cinnamic aldehyde is used to give a cinnamon flavor to foods, drinks, pharmaceutical products (it is a valid anti-mold for syrups) and to formulate synthetic perfumes.
- Geraniol is found in flavors used in foodstuffs and soaps, and is also used in the color composition in rose scale.

Rubber additives (accelerators, antioxidants): essentially thiurams and mercaptobenzothiazole (MBT) and benzothiazole bisulfide (BTS) and diphenylguanidine (DPG). They are found in almost all rubber objects, including boots, galoshes, shoes (Fig. 8.16), adhesives, brushes, condoms, elastic bands of all types, especially for undergarments, electric cords, gaskets, germicides, gloves for housework, physicians, and health care workers, inflatable mattresses, insulating tapes, oil, paints, rubber bands, rubber mattresses, soaps, shampoos, tool handles, etc.

- MBT (mercaptobenzothiazole) is a preservative found in disinfectants, fungicides, bactericides (in foods, creams, soaps, sprays), a vulcanization accelerator, an anticorrosive agent in cutting oils such as antirust and antifreeze fluids, and in tires of black color, such as those of cars.
- Thiurams have fungicide actions, and as bacteriostatics are used in foods, soaps, creams and sprays, and in antiparasitics and pesticides.

• DPG, a rubber vulcanization accelerator, is found in boots, elastic bands for undergarments, gloves, preservatives, and elasticized fabrics (with textile fibers interwoven with elastic), etc.

Epoxypolymeric resins are found in adhesives, coatings, dental cements, glues, and in the inner coating of tins for preserved foods.

Ni sulfate reached the highest figures in two cohorts, from 14.9% [84] to 23.7%, with a peak incidence among children aged 1–3 years of 39% [179]. Two children aged 4–6 elicited positive reactions to Ni sulfate contained in medication [47], a 4-year-old girl to Cu present in toys, and a boy aged 11 to turpentine contained in pastels [80]. Several waxes, rubber, spices and flavoring agents provoke contact urticaria and are summarized in the Appendix 8.1 [184].

Allergic Reactions to Ingested Allergens

Several causative agents of ACD can exacerbate clinical manifestations if ingested with foodstuffs [125]:

Co: Absorption in the gastrointestinal tract appears to be particularly rapid, owing to sudden relapses after FCT.

Cr: Even if we ingest 25–300 µg/day, Cr concentration in the organism is low, therefore the role of Cr contained in foodstuffs is controversial.

Ni: High content in the above-mentioned foods. It is estimated that we ingest 150 µg/day of Ni and the absorption in the gastrointestinal tract is 1%–5%, sufficient to aggravate ACD and provoke urticaria and/or wheezing (Ni asthma) [221]. However, it is unknown how much Ni is released by orthodontic instruments and the like [221]; remarkably, oral Ni administration may induce a high frequency of anergic T cells with persistent suppressor activity [9].

Clinical Presentation

ACD is initially located in the skin sites exposed to allergen contact, often a diagnostic distribution, although it may subsequently spread to other sites. ACD can be divided into acute and chronic forms [138].

Acute Forms

At first the lesions appear on the more exposed skin sites, where the contact is easier. Common sites of ACD are under rings, earrings and sites of ear-piercing, spectacle frames, bracelets, necklaces, coins in pockets, jeans studs, and other sites of metal contact (Figs. 8.13–8.16), and clinically the ears, neck, finger interdigital or dorsal aspects, or forearm back, the hand dorsal region, less the palms, face and less obvious sites are the lower limbs. Within 15–20 min of contact the lesion begins to itch. Dermatitis is characterized by macular erythema and papules due to vasodilation, vesicles or blebs related to intensity of allergic reactions and to the tendency to flood from the contact site. The dermis shows perivascular leukocyte infiltration and edema. In some parts, for example, the eyelids, penis and scrotum, both erythema and edema predominate on the vesicles: above all the edema may be particularly intense. Lesions may remain circumscribed or, due to persisting exposure, spread even to far regions by an involuntary contact or in certain cases by self-contact. By progression of the inflammatory reaction, the skin becomes hyperpigmented, small vesicles appear, which result in exudation, transudation, crusting and subsequent scaling. Itching is a symptom invariably present and mostly intense. The dermatitis may appear later, with different localizations, by hand transfer; palms and soles and scalp are frequently spared, due to either the greater thickness of the corneum layer or an increased barrier function [7].

Chronic Form

If allergen exposure persists, the skin undergoes an epidermal hyperplasia evolving into hyperkeratosis and acanthosis when the leukocyte infiltration spreads via the epidermis and basal or corneum layers, then to the features of long-standing AD, with lichenification, scaling, fissuring and crusts [7].

Systemic forms include pompholyx (vesiculous-bullous relapsing dermatitis, limited to fingers, palms or soles), the baboon syndrome (common involvement of the buttocks, accompanied by an eczema-like and symmetric eruption on the elbows, armpits, eyelids and lateral neck region) [138], following ingestion or absorption of Ni, Cr, Co, due to metal prosthesis [7] or provoked by medications first applied topically and then ingested by mouth [227].

Types of ACD

Studies on the prevalence of localization of ACD lesions in two pediatric case reports have recorded the lesions as follows: generalized in 15.8%–22.5% of cases, if localized: face 8.3%–8.9%, face and neck 42.3%, mouth 35%, limbs 35%, upper limbs 25.2%, hands 13.3%–45.9%, lower limbs 3.6%, feet 5.4%–20%, hands and feet 3.3%–8.9% [170, 185, 199]. Other children had foot eczema in 27.7% of cases [7] more often than on their hands [214] and on the hands in 5%–6% of cases [63]. The 13 main types of ACD are as follows:

Diaper dermatitis peaks at age 9–12 months. The skin may become erythematous and scaly and in more severe cases exhibits a papulovesicular or bullous reaction, which extends to external genitalia and buttocks, usually sparing the genitocrural folds. The lesions may spread

beyond the diaper area to the lower abdomen and to the thighs, often complicated by secondary infection. This is certainly the most common contact dermatitis in infants, the prototype of irritant contact dermatitis (ICD), a reaction to the irritant and protracted action of ammonia deriving from urine left too long in the diaper, overly acidic feces, traces of soaps and detergents from an inappropriate cleansing of the diaper area, deodorants and preservatives in the absorbent diapers, ointments and oils, medicated or not, applied several times a day for emollient and anti-irritant purposes, and frictions, washings and wiping, often favored by the occlusive effect of plastic diapers [7]. This dermatitis is commonly treated, over a long period, with various topical medications, some potentially allergizing, for example, casein in a diaper ointment (Chap. 9).

Contact cheilitis is more frequent than stomatitis because the transition epithelium and lesser moistening by saliva facilitate sensitization. Milder cases are caused by lip dryness, chapping and lip licking, and in most severe cases it is intumescent, with erosions and crusts. Cheilitis stems from irritation provoked by:

- Foods, especially artichoke, carrot, cheese, citrus, fennel, kiwi, mango, peach, tomato, fruit juices, etc.
- Topical medications, toothpaste, lip salve, lipsticks, cosmetics.
- Mouthwash and cough syrups, candied fruits and foods containing menthol.
- Preservatives contained even in cold drinks and ices.
- Nail polish, nail enamel and nail hardening for onychophagists; eyelid involvement is unique for nail-polish sensitivity.
- Saliva may be irritant when children lick or bite their lips too often, or suck candies or chewing-gum [172].
- Lip contact with nickel-plated objects [47].

Toothpastes often contain cinnamon, carnation essence, menthol, eugenol, etc. Lipsticks may contain dyestuffs such as fluorescin and eosin, as well as antioxidants, carbamate cinnamon, lanolin, methylethane, oleic alcohol, and perfumes; lipsticks and lip salve contain carmine and lanolin. From cinnamon is derived an oil cross-reacting with balsam of Peru, benzoin, colophony, vanilla and essential oils of orange peel [138].

Contact stomatitis. The skin, differently from mucosa, has several proteins in the keratin layer, which may act as a carrier for hapten-sensitizing molecules. It is characterized by burning, pain, taste reduction up to ageusia and paresthesia. Unlike the substances previously listed, when the contact involves the mouth cavity, the oral mucosa lesions vary from a barely perceptible enanthem up to a dark red coloration with edema and ulceration, which may limit ingestion of foods and drinks, talking, and occasionally breathing. The direct application of offending allergen may provoke local lesions of the urticarial type, generalized and even anaphylactic. Especially at risk in sensitive subjects are the widely used metals in dentistry [6, 224], such as, Ni, Cr (present in wires and alloy) [224], Hg (capping amalgam) [6], further toothpastes and mouthwashes, rubber, essential oils, preservatives, etc. [6]. Recovery is assured by removing instruments with Ni and/or Cr [224].

Other contact dermatitis of the mucosa [138]:

- Conjunctivitis.
- Contact balanitis is a glans pruritic erythema, often accompanied by preputial edema, derived from soaps, products for personal hygiene, condoms and related detergents and lubricants, too close-fitting underwear.
- Contact vulvitis has an onset with pruritus and edematous, erythematous, oozing lesions, limited to the area of contact with the etiological agent, which may become chronic when it persists for a long time. Common causes are soaps, bubble baths (and related additives), hygiene sprays, products for vaginal irrigation if insufficiently diluted or frequently employed, spermicidals, the partner's condom, perfumed toilet paper, too close-fitting sanitary napkins, underwear, and contact with clasps, hooks, and zippers with Ni.

Shoe dermatitis, caused by rubber and tennis shoes, boots, etc., due to a combined action of Cr and Co (tanned leather), Ni (metal decorations) and Hg, present in the upper and/or lining parts. It affects the sole (59%) and dorsum of the feet (41%) and toes (29%), sparing the interdigital spaces [214]. Most often the lesions are symmetric. Common allergens in rubber shoes and boots are thiomersal [214], MBT, BTS [102], thiurams, carbamix [48], mercaptans and PPDA [56], and a shoe glue with 6.2% of reactions [7]. The condition is worsened by excessive foot sweating.

Clothing dermatitis depends on a wealth of sensitizers, including dyes, fabric finishes, mordents, resins, rubber antioxidants, detergents, especially cleansing solutions, synthetic fibers, wool fabric, linen, garments, socks, shirt collars and cuffs, and elastic in garments. Obese children are more at risk. Nitric substances and poorly fixed dyes may be leached out by sweating. Clothing and accessories are implicated in 17% [7] and 56% [56] of pediatric cases.

Dye dermatitis (known since 1938) [86]. As in the clothing dermatitis, the affected areas are those where contact is prominent: neck, trunk, lateral and thigh posteromedial regions, popliteal space, antecubital space, the upper point of contact of stockings, or where other body parts come into contact with bed linen.

Dermatitis from plastic materials particularly include epoxypolymeric resins, diluent and thickenings used in dentistry (epoxyacrylate, methylacrylate and dimethylacrylate metylenglycol) [6]. Artificial nails based on acrylate (similar to that employed in dentistry) can cause long-lasting finger burning, tingling, and paresthesia related to tactile sensitivity [74].

Dermatitis from emollient and emulsifying agents found in cosmetics and topical medications such as oleic alcohol [127].

Dermatitis from topical CSs may be unsuspected, particularly if the medication is being or has been applied for a pre-existing dermatitis, but it is diagnostic when

the initially improved ACD worsens without any apparent cause [2].

Latex dermatitis, latex hypersensitivity (generalized urticaria by a caoutchouc dental prosthesis), has been recognized since 1927 [198]. Natural latex (a variant of the original term "latice," derived from the Latin root *lact*, due to its milky aspect) is obtained from *Hevea brasiliensis*: 17 allergens are listed in Table 1.74 with a MW between 2 and 100 kD (*Euphorbiaceae* family) and 1% from *Parthenium argentatum* [216]. Hev b 12 may be important as a cross-reactive pan-allergen [25]. The basic product contains natural rubber (33%), resin (2%), proteins (1.8%) and water (65%), to which ammonia is added up to 0.6% to prevent premature coagulation during transportation [216]. During the industrial manufacture, many chemicals, such as vulcanizers, stabilizers, accelerators, antioxidants are added [216]. World production is about 6×10^6 tons/year [193]. The worldwide increase in glove production following the advent of AIDS and now the poultry infection precautions has dramatically increased the prevalence of latex dermatitis [216]. All medical personnel at risk, as well as dentists, laboratory and health care workers must wear rubber (latex) gloves to prevent a potential contact with blood or body fluids throughout working hours, thus causing ACD in their patients [193]. For this reason, the use of condoms and diaphragms has also increased, with a great risk of sensitization [193, 218]. Particularly exposed are operating-room workers because, in addition to the gloves, they have contact with catheters, tubes, cannulas, bags for anesthesia, etc. [114]. Among non-health care workers, there are industrial workers, housewives, customers of restaurants and food sales points, as well as consumers if the shopkeepers and dairy workers use latex gloves during their daily work [97].

Pediatric groups at risk are in particular children exposed to some types of latex orthodontic appliance or undergoing surgical procedures, especially children with spina bifida or other urologic conditions, in whom reactions are common, due either to repeated surgery (contact with surgical gloves), or the frequent use of IV infusion sets, catheters and Rx procedures [193]. In children and adolescents affected with myelomeningocele, which is associated with immature defense mechanisms of the mucous membranes [66], the principal risk factors were the number of surgical procedures, atopy and/or sensitization to latex [167]: 60% of children had reactions outside of the operating room environment [112]. Children become sensitized mainly by direct contact between latex particles and blood vessels and open mucosa, whereas in adults the process takes place transcutaneously or by inhalation of aerosol particles [150]. Tables 8.10–8.12 [97, 109] show how latex allergy also develops to an incredible number of objects in common use [126, 132, 218].

Among the population considered to be at risk are health care professionals, where the incidence is between 2.6% and 16.9%, and in atopic babies (2%) [218]. The increased incidence in children of latex allergy has recently been highlighted [112]. Atopy as a factor that facilitates sensitization [66] is present in a large group of subjects who are not at risk (41%–74%) [97, 112, 196]. Latex-specific IgE have been detected in 0.5%–10.2% of atopic children with IgE antibodies ±1,000 IU/ml; almost nobody was informed of any allergy before the visit [3]. SPTs are positive in 3%–6.8% of atopic children and specific IgE (sIgE) are elevated in positive challenges [152]. The determination of sIgE (CAP) shows that 7.2% of 282 children are allergic. In this sample, the incidence among the atopic population varied between 1.69% and 9.5%, depending on the methodology used [66]. The prevalence of IgE-mediated allergy in children with neurological abnormalities is the highest: sensitization is 18%–41%, with peaks up to 77.1% [112, 193]. Alternatively, atopy is present in 49% of sensitized and

Table 8.10. Common latex sources and number of hypersensitivity reactions provoked in 70 patients: sources of clinical and/or IgE-mediated reactions

Sources	No. of patients	No. of reactions
Surgical and household gloves	69	43
Sticking plaster	11	9
Balloons (any type)	8	6
Elastic bandages	6	5
Rubber contraceptives	5	3
Face masks for anesthesia, diving, underwater fishing	3	2
Stretch textiles	3	2
Shoes	3	2
Insulating materials	2	1
Air mattresses	1	1
Sailing equipment	1	1
Stamps	1	1
Colors	1	1

Table 8.11. Common latex sources and number of hypersensitivity reactions provoked in 70 patients: sources of clinical reactions

Sources	No. of patients
Hot water bottle	1
Baby pacifiers	1
Shower curtains	1

Modified from [97].

Table 8.12. Additional consumer products and hospital latex products provoking clinical and/or IgE reactions

Additional consumer products
- Adhesive tapes
- Baby balls and balloons
- Baby bottle nipples
- Boots
- Carpet backing
- Chewing gums
- Condoms
- Diaphragms
- Diazo-sensitized photocopy paper
- Dress padding
- Dress trimming
- Elastic bands
- Elastic or elasticized parts of clothing
- Elastic stockings and socks
- Foam rubber pillows
- Glue and other adhesive substances
- Gummed paper, envelope
- Handlebars (such as bicycle) and wheels
- Medicine dropper
- Paddles (such as ping-pong)
- Pads
- Panty-hose
- Pencil rubber
- Plasters
- Rain wear
- Racquet
- Rubber bands
- Rubber handles
- Rubber key-case
- Rubber soles and heels
- Rubber tires (such as strollers, roller skates, bicycle, wheelchair, etc.)
- Shoes
- Shoulder pads
- Toy balloon
- Toys
- Tires
- Water toys
- Wooden batons

Additional hospital latex products
- Ambu bag
- Anesthesia/ventilation bags
- Bag straps
- Band-aids
- Bands
- Blood pressure cuff and tubing
- Cannula for IV use
- Catheters (such as balloon, rectal, etc.)
- Dilatators
- Elastic
- Endotracheal, nasogastric tubes, etc
- Enema kits
- Gastrogavage kits
- Heating/cooling blankets, pillows
- Occlusive dressing
- Orthodontic appliance
- Rubber parts of medical equipment: stethoscope, otoscope, rhinoscope, etc.
- Straps for masks
- Tourniquet

Data from [109].

30% of nonsensitized patients, with statistically significant differences related to allergic reactions to latex, in 96% and 30% of cases, respectively [193]. The report of one case of anaphylaxis in 646 surgical procedures over 18 years is reassuring [167], but latex-induced anaphylaxis occurred in 10% of patients >5 [124]. A severe anaphylactic shock occurred in an 8-year-old boy who was undergoing elective surgery for an ileostomy [94]. Type IV hypersensitivity to natural rubber latex may be a problem for a proportion of patients with eczema, particularly on their hands [195]. The incidence of latex allergy in the general population is unknown, but it seems to be about 0.001%, with a frequency of 0.125% in unselected surgical patients [218].

The immunological mechanism is IgE-mediated [3, 43, 112, 196], especially in children: serum sIgE are detected to natural latex [97, 196], SPT, ELISA, and RAST. RAST inhibition is also positive. The allergens are usually peptides found in natural latex [193]. Latex exposure may occur by cutaneous, percutaneous, mucosal, parenteral, and respiratory (from inhaling latex glove powder) routes [97]. By the respiratory route, the reaction is materialized within a few minutes with a progression of symptoms from rhinitis, wheezing, conjunctivitis, facial angioedema, to generalized urticaria and symptoms of anaphylactic reaction up to severe generalized reactions (Table 8.13) [160, 216]. Latex allergens in respirable particulate air pollution from tires rubbing on roads is a cause of significant respiratory reactions [239], as also is cornstarch powder on latex products for glove lubrication [205]. Skin exposure induces prevalently ACD symptoms [97, 152, 193], but the latex-fruit and latex-vegetable syndrome provokes anaphylaxis also in children. Anaphylaxis burst occurred within 5 min in a girl playing ball [126], as well as in a girl and a boy playing with a plastic ball in play areas [73] and in allergic adults [25], and facial edema in a boy blowing up a balloon [196]. Recently, a *profilin* has been identified as a component of natural latex, structurally correlated to profilins of different origin found in foods and pollens [220] (Table 1.72), and associated with cross-reactivity between latex, taxonomically unrelated plants and several fruits and nonfruits (Table 8.14) [22, 36, 43, 126, 160, 176]. A latex-fruit syndrome was reported by 55.9% of latex-allergic patients [36]. In Appendix 8.2, we indicate [36] the employment procedures of some extracts: papain and chymopapain are associated with anaphylaxis, as analyzed in Chap. 20.

Occupational ACD. In children of 13–14 years, the hairdressers among them were allergic to PPD, Ni, thiuram; construction workers to Cr, cement, Co; in food industry workers, some were allergic to garlic and Ni; in the footwear industry the problem came from Cr, Co, and Ni; in the ceramic industry it was Co and Ni [85]. Other working adolescents were allergic to metal accessories (37%), medicaments (8.1%), cosmetics (5.4%) and shoes (4.5%) [199].

Table 8.13. Manifestations and differential diagnosis is immediate, delayed, and irritant latex reactions

Clinical manifestations	Immediate type (type I) hypersensitivity	Delayed type (type IV) hypersensitivity	Irritant type
Causative agent	Latex proteins	Accelerators: thiurams, contact with gloves, powder, surfactants, formaldehyde, etc.	Insufficient hand rinsing, rubber additives, glove powder oxidants, formaldehyde, etc.
Atopy	Yes	Yes	No
Pathophysiology	Skin/membrane contact, invasive procedures, injections, allergen inhalation (powder)	Skin contact	Skin contact
Percent (%) of vulnerable subjects	General 0.0001 %, hospital: non-surgical 3 %–5 %, surgical 7 %–12 %	7 %–18 %	100 %
Onset time	Minutes, rarely >2 h	6–48 h after contact	Gradual, over days
Initial reaction	Itch, tingling, burning	Itch, then pain	Itch or erythema
Dermal reaction, acute	Urticaria	Erythema, vesicles or blisters	Scaling, edema
Dermal reaction, chronic	Urticaria, more extensive	Dryness, thickening, scaling, fissuring, peeling crusts, papules, vesicles	Dryness, thickening, fissuring, scaling, crusts, papules, sometimes vesicles or blisters
Limits of the reaction	Whenever part of the body beyond the contact area	Even beyond the contact area	Limited to the contact area
Facial involvement	Diffused swelling, runny nose	Only if face is touched	Only if face is touched
Respiratory involvement	Rhinoconjunctivitis, wheezing	No	No
Systemic involvement	Nausea, hypotension, anaphylaxis	No	No
Life-threatening	Yes	No	No

Data from [160, 216].

Table 8.14. Cross-reactions between latex, fruits, and non-fruits in patients with related allergies

Fruits	
Apple	Passion fruit
Apricot	Peach
Avocado	Peanut
Banana	Pineapple
Cherry	
Chestnut	Non-fruits
Coconut	Alder
Fig	Buckwheat
Grapes	Celery
Hazelnut	Chocolate
Kiwi	Potato
Mango	Pistachio
Melon	Sesame
Papaya	Tomato

Data from [22, 36, 43, 126, 160, 176].

Skin-diver dermatitis is elicited by the equipment (diving mask, fins, etc.). The effect is caused by constituents such as dithiocarbamates, formaldehyde, isopropyl-phenylparaphenylendiamine, mercaptobenzol, butylphenolformaldehyc resin, thiourams, etc. [7].

Diagnosis

In *establishing an etiological diagnosis,* it is crucial to begin with a careful, exhaustive history, the single most important and cost-effective diagnostic tool, including family and personal history, to be completed at subsequent visits. A clinical history of redness, itching, or swelling, or of unexplained urticaria or anaphylaxis after contact with a specific product suggests that a detailed history can be useful for the identification of allergic children. All children and parents should be questioned.

- Does the child have a history of atopic disease?
- What product was there contact with and how often?
- Is a relation with a particular activity or environment suspected?

- Are diapers, chemical products, detergents, cosmetics, ornaments, etc. used?
- Does the infant use rubber pacifiers or do children play ball?
- What type of clothing, shoes, gloves, etc. are used?
- When a latex allergy is suspected, is there a history of allergy to fruits (Table 8.14)?
- What is the course of the disease?
- Have topical medications been applied for an ongoing episode or an earlier skin disease?
- Was symptom onset after ingestion of foods containing substances that formerly induced a skin disease?
- Is ACD caused by cross-reactivity [56]?

The *medical examination* is essential to exclude other skin conditions with lesion aspect and distribution similar to those of ACD. In younger children the diagnosis, apart from diaper dermatitis, is facilitated by the restricted panel of age-related foreign substances to be uncovered:

- Babies starting to crawl may develop leg, knee and elbow dermatitis, by contact with wax or detergents for floors, components of rugs and wall-to-wall carpeting, etc.
- Babies and young children may touch or caress parents, relatives, or baby-sitters who use cosmetics, fragrances, deodorants, etc.

Points to be considered:

- Little girls have ear-lobes pierced for earrings.
- Children and adolescents of both sexes wear jeans with metal buttons or other trimmings.
- Early use of cosmetics, etc. In Belgian participants, the mean age of cosmetic allergy was 12.4 years; however, earlier cases were detected at age 4 [56].
- Topical medications, also for trivial skin lesions or ear pain, are typically used more frequently compared to adults.
- Similarly, orthodontic treatments are more frequent in children.

ACD diagnosis related to lesion topography (Table 8.9):

- *Face and neck* ACD. This localization may disclose diagnostic difficulties because several agents are potentially acting: contact and photocontact substances, cosmetics and costume jewelry should be taken into account [57]. The application of chemical substances to the scalp can induce manifestations in distant sites such as the face, ears and neck. Shampoos and hair gels may provoke helix reactions. ear piercing facilitates Ni sensitization [47] and is a risk factor for AIDS.
- *Hand* ACD. About 50% of cases involve the hand, generally on the back, whereas the wrist is involved when objects are taken. It is chiefly caused by the use of rubber gloves, cleaning products, cosmetics, but any of these substances can be the cause. Hand dermatitis is frequent in girls and boys helping in housework or in family shops (see protein contact dermatitis, PCD), in girls working as apprentice hairdressers, in boys helping bricklayers, mechanics, barmen, and the like [172, 200], and above all the Europeans using Ni-containing coins: euro cents.
- *Foot* ACD. The typical location on the back and at joints does not offer diagnostic problems. Causative agents are those found in rubber shoes, shoe creams (see description of shoe dermatitis in preceding section) or in the coloring agent both of shoes and stockings.
- *Generalized and/or unusual pictures.* These are attributable to ubiquitous substances such as those in rubber (see the related additives) able to produce lesions at several sites, including the face (sponges for make-up, ear plugs, etc.), periocular region (goggles for motorcyclists, swimmers, skin-divers, etc.), thorax and belly (elasticized underwear), genitals (underwear, condom, diaphragm, pessary, etc.), legs (elasticized stockings), and dyes and other cloth constituents often unsuspected and difficult to diagnose.

Particular contact lesions are caused in children and adolescents of both sexes by piercing, at all ages by substances employed for oral and dental hygiene and in dentistry: soaps, detergents, toothpaste, anesthetics, metallic plates and screws, and ear drops in children [172]. Dentists in turn can show symptoms after use of an anesthetic or by contact with instruments cleaned with formaldehyde [6], a hidden allergen which gathers in ear canals or under rings during washing with formaldehyde-based products. Inhalation of related steams may induce face and periorbital swelling [16].

- *Systemic* ACD. It is objectively rare, often dependent on re-exposure to previously used topical medications, thus sensitizing the subjects up to severe systemic reactions [200] by spread of lesions to sites far from the primary exposed region or by oral, inhalation or parenteral exposure to antigens formerly the cause of cell-mediated contact manifestations [227].

Laboratory Studies

Latex allergy. SPT is the diagnostic procedure of choice not only in young children affected with spina bifida, but also in all subjects with positive history due to frequent exposure to latex, in those allergic to mentioned fruits and in cases of urticaria and/or anaphylaxis by unknown causes [196]. Diagnostic screening is complicated by a number of asymptomatic children with SPT^+ [152]. SPTs are efficient in children [152, 193], in addition to prick + prick testing [126, 152], also with gloves (Chap. 6), RAST inhibition [193], useful for the study of crossed allergenicity [115], and challenge tests, which appear to be correlated to RAST [152]. Latex gloves are the source of great heterogeneity: there are significant (sometimes striking) differences between manufacturers and product lines in the amount of free latex protein that can be released from the glove and the number and types of chemicals used in glove production [196]. Among the stabilizers is also casein [133], so CM allergic children may have false positive reactions to casein [133].

ACD diagnosis is confirmed by *patch test* [14, 77]: Finn chambers [14] or True test [2] (Chap. 6), selecting the haptens that more frequently are causes of ACD (standard series) or those potentially relevant in the surroundings of a single patient (special series) [14]. All children with suspected ACD should undergo patch testing. Dentists should undergo a preventive assessment by this method [6] because of the notable spread of Ni-plated instruments . Patch testing is also helpful to reveal possible cross-reactions or concomitant hypersensitivity to additional haptens [149], and to natural rubber latex [195]. False-positive results may depend on aspecific skin hyperreactivity, following a local increase in LC numbers, or particular conditions of both epidermis and vasculature which contribute to a pro-inflammatory cytokine increase [207].

To verify whether trimmings or worn objects contain Ni, some drops of a diluted ammoniacal solution containing 1% dimethylglycoxin can be applied: the Ni presence is revealed by a bright rose dye [138].

Differential Diagnosis

Differential diagnosis includes the following considerations:

- ICD (Table 8.15) [138] differences with ACD are rare, since both haptens and irritants decrease epidermal LC numbers, HLA-DR, CD54 and CD80 expression is diverse, and RT-PCR (reverse transcriptase-polymerase chain reaction) has shown an impressive overlap between ACD a ICD [82]. The main ICD example in children is diaper dermatitis, but one baby had physical irritation from constant friction due to occlusion by abdominal skin on a metal pin causing physical irritation [143]. Several agents (up to 489 in number) [143] are capable of expressing a direct irritant action on skin cells, affecting the hands in 36.3% and face in 26.4% of cases [143]. Their power depends on the chemical nature, concentration, duration of application and individual predisposition. The principal symptoms are a burning sensation (pruritus in ACD), early onset 1 h after contact,

Table 8.15. Comparison of irritant (*ICD*) and allergic contact dermatitis (*ACD*)

	Irritant	Allergic
Clinical morphology	Acute ICD: erythema, edema bullae, necrosis restricted to the area of necrosis	Dermatitis in acute and chronic ACD can be similar to ICD, but lesions are often spreading, papules and vesicles are seen most often
	Decreasing phenomenon	Increasing phenomenon
	Chronic ICD: lichenification	Kinetics of resolution may be slower than ICD during patch testing
	Erythema, scaling, hyperkeratosis, rhagades with less area restriction than acute ICD	
Histology	In acute ICD, necrosis of epithelial cells may by present	Same as ICD; but no epidermal necrosis, neutrophils usually less prominent
	In delayed and chronic reactions, spongiosis, exocytosis, dermal edema and a mononuclear infiltrate; occasionally, neutrophil-rich infiltrates	
Immunochemistry T cells	Predominantly $CD4^+$ T cells;	Predominantly $CD4^+$ T cells, some
	Some $CD8^+$ T cells, activated by IL_2R expression	$CD8^+$ T cells, activated state indicated by IL_2R expression
Frequency of hapten-specific T cells in infiltrate	Not known	Estimated to be approximately 1%
Number of Langerhans cells	No consistent changes	Decreased then recovery
Morphology	Alterations noted, but are highly dependent on chemical	Alterations noted, particularly with high doses of hapten
Accessory molecules		
HLA-DR	Increased	Increased
CD54	Increased	Increased
CD80	Increased	Increased

Table 8.15. (Continued)

	Irritant	Allergic
Cytokine profiles		
TNF-α	Increased	Increased
IFN-γ	Increased	Increased
GM-CSF	Increased	Increased
$IL_{1\alpha,-\beta}$	Not detected	Increased
IL_4	Not detected	Increased at 24 h, absent by 48 h
Chemokine profile		
IP-10	Not detected	Increased
MIP-2	Not detected	Increased
Transgenic mice		
Overexpression of		
CD80 by keratinocytes	Increased	Increased
CD54 by keratinocytes	Increased	Increased
Knock-out mice that lack		
TNF-α R	Not tested	Increased
CD4	Decreased	Decreased
CD8	Decreased	Decreased
CD28	Decreased	Decreased
Clinical manifestations		
Frequency	Several patients	Few patients
Diseased	All exposed	Only sensitized
Extension of lesions	Only the contact area	Beyond the contact area
Onset of lesions	Within a few hours	24–72 h
Aspect of lesions	Erythema, edema	Erythema, papule, itching
	Scaling, bullae, necrosis	Vesicles
Symptoms	Burning, stinging	Itching

Data from [138].

polymorphous aspect with no tendency to generalization, rapid regression after interruption of contact.

- AD is often associated with ACD: Ni is accused in children with a mean prevalence of 4% [47]; the hand eczema appearing as a dyshidrotic vesicular eruption may be largely associated with an atopic condition [63].
- To differentiate from PCD, we mention that the pathogenic agents are protein fractions of animal and vegetable origin.
- Mycotic infections (see above).
- Several cutaneous eruptions such as those of SLE, erythema multiforme, dermatomyositis, viral exanthems, *pityriasis rosea* have mostly a symmetrical aspect rather than eczematous, while ACD has peculiar locations; for example, in feet ACD involves the back (in psoriasis the sole), with a relative sparing of interdigital spaces (unlike viral infections).
- Dyshidrotic eczema often begins with small congested, pruriginous and relapsing vesicular lesions, located on the internal sides of both hands and toes, but then occurs in the soles of the feet and the palms of the hands.

Treatment

Beyond any rigid schema of the inhibiting effects on LCs, TNF-α [128] and UV rays with an IL_{10} mediation could be utilized for therapeutic purposes [71].

Local or general management aims to reduce the clinical manifestations and avoid possible infectious complications; in both cases a preventive ascertainment of the composition of products for topic or systemic use will eliminate those containing sensitizing substances. Treatment of allergic reactions to latex begins with immediate removal of any identified source of latex in direct patient contact. In Chap. 13 we discuss SLIT desensitization to latex. Treatment is similar to anaphylaxis from other causes and may require the use of epinephrine [30].

In case of acute, congested, edematous, exudative dermatitis, local therapy should be limited to hydrophilic packs with watery solutions at ambient T, repeated from two to four times during the day. A number of solutions with antiseptic and decongestant action, without the danger of unwanted allergization, are detailed in Chap. 7, along with water, oil or glycerin pastes, useful in congested and exudative stages, and inert powders in case of intolerance to wet medications. When the acute and exudative stage subsides, lipophilic ointments such as Lassar paste may be appropriate.

In addition, always for topical use, low or moderate potency CSs can be used (Table 7.16) in different excipients (lotions, creams, gel, ointments), for short-term (7–10 days) exclusive application on the involved cutaneous sites in tandem or alternate therapy, taking into account possible contact reactions [2]. Subsequently, topical nonsteroid medications can be used for the same length of time; this combined treatment should be sufficient to return the skin to a normal state. Oral antihistamines may hold promise in relieving itching (Chap. 7). The newer topical immunosuppressors, tacrolimus and pimecrolimus, may be effective in the treatment [118].

Diaper dermatitis, especially in summer, responds to rigorous hygienic measures, which are useful also for prevention: the use of cloth diapers, washed at home exclusively with Marseille soap.

Prevention

A *unique specific management consists in the prevention* of whatever new contact with responsible or suspected substances, especially if they are capable of cross-reacting with other compounds [7], or when feasible substituting, for example, metal wires in orthodontic prostheses with acrylic ones [224], almost always followed by ACD regression. Unfortunately, labels do not fully report product composition [57]: cosmetics, antigens correlated to rubber, textiles and metals are examples. However, several clinics have a complete display of materials that contain the offending allergens, a great aid for the patient, the doctor and the industry, as well as for an exchange of information between experts in the discipline [57].

People allergic to Ni should exclude from their diet the above-mentioned foods and eat small amounts of carrots, cabbages, cauliflower, cucumber, wholemeal wheat or corn, fresh fruits other than pears, and lettuce. Older children should also exclude beer, coffee and wine. Do not cook foods in stainless steel pots, both new and used, in particular foods containing oxalic acid (spinach), malic (apple), or citric (citrus fruit), because the Ni concentration is increased significantly [125]. For metal-induced dermatitis, it is appropriate to prescribe a diet with a low content of foods that are positive to patch testing, which is needed to be supported in children by open FCT (OFC) [125]. For objects of common use and imitation jewelry containing Ni and/or CoCl, replacements are available in brass or low-carat gold, and drawer and utensil handles of nonmetallic material. Alternatives to perfumed cosmetics and household detergents are the *fragrance-free products.* Those who are allergic to these substances should therefore carry out strict dietetic prevention, together with contact prevention, to ensure a clear-cut clinical improvement of ACD lesions [80]. Women have long been encouraged to prefer Ni-free accessories, and men to give up metal watch bands, etc. But is impossible to give up handling metal coins. *We hope that the countries of the European Union (EU) conform to the Danish legislation.* In our opinion, difficulties will be Ni is practically irreplaceable in the alloys encountered *because* since it combines excellent technical properties and low price. Recently, a group of European companies has obtained a Ni-free international patent and the EU has issued directive 94/27 and thereby defined the Ni limit level at 0.5 g/cm^2 for objects in direct and prolonged contact with the epidermis.

People allergic to Cr should employ leather tanned with vegetable systems, by ascertaining whether tannins are present, and those with ACD to dyes should wear white or plant-fiber clothing.

The data available today show which measures should be suggested to patients with ACD to *latex* [109]. Latex avoidance should be advocated for all individuals with a positive skin or blood test or a positive history [30]. Because latex glove antigen content varies among brands and among lots from the same manufacturers [193], the allergics and surgeons can wear polyvinyl gloves, or gloves subjected during manufacture to washing after pressing and in-process steam sterilized for 1 h at 120 °C [216], or powder-free Biogel Skinsense ™N. *In allergic patients,* premedication with H_1 or H_2 antihistamines or prednisone has been used to prevent untoward side effects, but only as a supplementary measure in addition to discontinuing use of latex gloves, since the results are not always effective [30, 193]. In *atopic children,* surgical procedures should be undertaken with the greatest caution since they tend to be sensitized even by the slightest contact with latex [3, 126]: in children with spina bifida, latex contact should be avoided from birth [193]. In particular, pacifiers should be banished [109], surgeons should manipulate latex gloves outside an allergic patient's room to avoid airborne transmission of latex particles [112], which are also spread by the cornstarch covering gloves and other objects [205], during surgery catheters in silicon (silastic) and for medications tapes and bandages with acrylic adhesives should be preferred [112]. Latex allergy should be investigated not only in multioperated patients but also in children with severe AD, fruit allergy and with urticaria or anaphylaxis by unspecified causes [196].

A new form of treatment consists in asking a latex patient to wear a latex glove for increasingly longer times, or in a type of SLIT desensitization (Chap. 13).

Protein Contact Dermatitis

Definition

PCD is an exquisitely occupational dermatitis seen in individuals exposed to animal and/or vegetable foods: cooks, veterinarians, slaughterhouse workers, housewives, boys and girls helping in family business, etc. [184]. PCD occurs as a chronic eczema accompanied by erythema, swelling and itching on fingers. Immediate recurrences in the same sites are frequent following contact with the causal agent.

Etiopathogenesis

PCD is another area of increasing interest with only a small rate of patients with positive family and/or personal history for atopy [186].

The causative agents are protein fractions of animal and vegetable origin, handled for a more or less protracted period, but the precise pathogenetic mechanism is as yet largely unexplored [1]. It is proposed that apparently unaffected skin facilitates the passage of protein substances via the epidermis, thus producing a spongiosis, an intercellular edema of the skin's spongy layer. Histological studies have revealed the outcome of these lesions, including superficial vesiculation, parakeratosis and lymphomonocyte infiltrate localized in the superficial dermis and in the perivascular seat. The mononuclear infiltrate delineates full-blown T lymphocytes with a clear-cut CD4 prevalence on CD8 T cells. Immunohistochemical studies show an increase in the number of LCs in the vesicles, epidermis and superficial dermis [186].

The list of foods, fruits, and vegetables identified as etiological agents is remarkably long: see Table 8.16 [1, 186].

Clinical Presentation

The vesiculation starts suddenly after contact with the causative agent, also following a long period of manipulations without lesions [186]. After contact, prominent erythematous, pomphoid and vesicular manifestations appear within a few minutes on the back of the hand and/or fingers, with progressive spread of erythema, accentuation of vesiculation and itching [131].

Table 8.16. Protein fractions of animal and vegetable origin more often causative of PCD

Meat and fish
Anchovy
Chicken
Cod
Crawfish
Cuttlefish
Egg
Herring
Lamb
Mackerel
Pork (intestine)
Shrimp
Turkey
Fruits, vegetables, cereals
Apple
Carrot
Celery
Chick-peas
Eggplant
Endive
Fennel
Garlic
Kiwi
Lemon
Lettuce
Oat
Onion
Peanut
Pear
Potato
Red-pepper
Tomato
Watermelon
Wheat
Wheat bran
Others
Baits for hooks (worms)
Pepper
Yeast

Data from [1, 186].

Diagnosis

SPTs are the diagnostic tests of first choice. The reactions show within 10–30 min, small vesicles that are sometimes dyshidrosiform, combined with erythema, and notably itching [131]. Better results are obtained with the prick + prick test, or the similar SAFT (skin applied food test) by employing fresh and raw foods [1]. The RAST may be reliable.

Differential Diagnosis

As discussed earlier, the offending agents are protein fractions of common foods, which distinguish PCD from the haptens responsible for ACD [186].

Treatment

There is no specific management. Affected subjects should protect their hands with polyvinyl gloves that cover the lower forearm or, if possible, should change jobs [1].

Phytodermatitis

Phytodermatitis is a particular type of contact dermatitis caused by plants, especially by chemical substances present in the leaves, stems, flowers, pollens and roots, particularly essential oils, components of the oleoresinous fraction, containing in turn phenols, aldehydes and aromatic alcohol, terpenes, hydrocarbons, aliphatic and aromatic esters, and kinones. The dermatitis is distinguished by the lesions provoked by irritant effects, particular to some plants such as *Urticaceae,* and by injecting histamine, serotonin and acetylcholine at the contact site, often leading to linear or figured aspects and bullous or pomphoid eruptions elicited by toxins of further plants, including *Compositae,* crucifies, lilies and *Ranuculaceae* (directly or following plant cutting) [7].

Allergic Phytocontact Dermatitis

Several agents cause plant contact dermatitis:

- *Catechols,* belonging to the family of phenols, are among the more widespread allergens in the plant world, equipped with an elevated sensitizing power. These include eugenol, used in dentistry and the manufacture of soaps, perfumes and carnation oils; vanillin; pentadecilcatechol, a potent allergen of the *Rhus* genus, including *Rhus toxicodendron,* or *R. radicans* and *R. vernix*, by far the most common cause of plant contact dermatitis in the US, where they are called poison oak, poison ivy and poison sumac, and are a frequent cause of ACD in children via pets, mainly long-haired dogs, which rub against the plants and transmit oleoresin to newborns and children.
- *Terpenes* are contained in citrus, celery, chrysanthemums, and other products such as resins and balsams.
- The plants of the *Compositeae* family (chrysanthemums, pansy, ragweed, sunflower), those of the *Alstroemeria* genus and *Hydrangea* species, and hepatica contain sesquiterpene lactones yielding extended contact phytodermatitis on exposed skin sites, especially in florists [2]. The *pollens* are common causes in the fall season via their allergenic fractions, the oleoresinous and hydrosoluble protein, which by inhalation can cause respiratory allergy. These conditions are differentiated from *photodermatitis* by the season and the lesions with shaded instead of clear-cut limits [7].

Allergic Photodermatitis

Definition

Allergic photodermatitis is kind of CMI to chemical substances that become antigenic when activated by sunlight. When skin is exposed to UV rays, cutaneous symptoms of various types may appear during a time when a patient is exposed to different exogenous or endogenous substances or is taking an offending medication topically, but most often systemically [58, 174].

Prevalence

It is connected principally with newly introduced medications and chemical substances. The manifestations have alternating phases, in epidemic form when a new therapeutic agent is commercialized. Generally as soon as its photosensitizing potential is ascertained, the product is withdrawn and the prevalence abates.

Pathogenesis

UV rays are divided into UVA, with bands comprised between 400 and 315 nm, UVB between 315 and 280 nm, and UVC of <280 nm [58]. UV rays can induce phototraumatic reactions (basically solar erythema) and photodynamic reactions, further classified into phototoxic and allergic variants [58, 174].

Photoallergic Reaction. This immunologically mediated photosensitization is activated by longer wavelengths (UVA 320–450 nm), which alter molecular structure, thus leading to a type IV sensitization. The radiant energy absorbed by a chromophore, for example the photoantigen, produces photochemical changes resulting in a photosensitized molecule, then conjugated with a protein carrier to occasion a complete allergen, against which is directed the immune response mediated by T lymphocytes, as in ACD [58]. In predisposed subjects, symptoms develop after a persistent exposure to sensitizing substances continuing for an adequately long period (up to 1–3 weeks). The wavelength of UV light is elicited by offending chemical substances. The causes are cosmetics based on natural perfumes such as bergamot (Fig. 8.17) and sandal oils and synthetic perfumes (musk and coconut), fragrance ingredients and lotions containing musk ambrette, dyes such as eosin used in lipsticks and the like, known photoallergens in sunscreen products such as oxybenzone, or correlated to PABA and methylcoumarin, disinfectants (halogenate salicylic-aniline: bithionol, fenticlor, etc.) [58, 215], and foods (Table 8.17) [173].

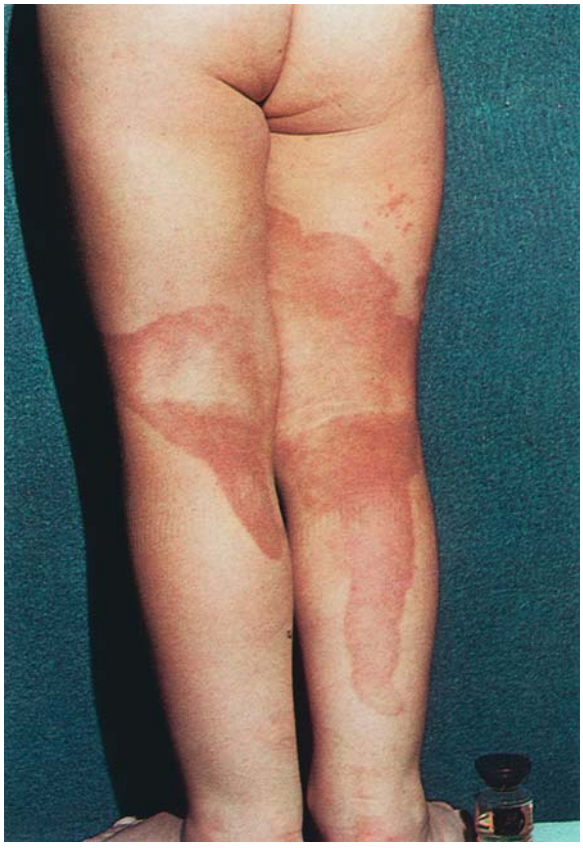


Fig. 8.17. Dermatitis caused by bergamot contained in a perfume

Table 8.17. Foods and food constituents implicated in (phyto)photodermatitis

Foods
Anise
Carrot
Celery
Fig
Lemon
Lime
Parsley
Parsnip
Food constituents
Cyclamate
Dyes

Modified from [173].

Phototoxic Reactions. The cause is a foreign chemical substance reaching the skin either exogenously or endogenously in combination with exposure to a sufficient amount of UV rays, in this case UVB rays, which activate nonimmunological cell damage. The pathophysiological mechanism of phototoxic drugs involves absorption and accumulation of UV energy in the skin. Photo drugs or their metabolites may combine with dermal proteins, acting as haptens solely when a significant dose of UV rays (285–310 nm) is present. When this dose is higher, it is likely that the energy modifies the drug to form reactive metabolites that combine with skin proteins, thus also expressing in this case a complete allergen. The period necessary to sensitization development may range from weeks to months, critical for cross-sensitizations, often triggered by low allergen doses. When it is all put together, the dependable molecule amplifies the skin sensitivity to UV rays. Phototoxic effects occur by damage to the DNA chain such as by psoralens [174]. Systemic medications and chemical substances more frequently incriminated are coal tar and derivatives, cyclamates, demeclocycline, doxycycline, griseofulvin, hexachlorophene, phenothiazine, psoralens, sulfa drugs, tetracycline and derivatives, thiazide, and photosensitizing substances such as furocoumarins (including psoralens), employed for their antimycotic action [138].

By a similar mechanism, *phytophotodermatitis* may develop after contact with plants containing natural furocoumarins, diffused in numerous vegetables: *Umbrelliferae* (celery, parsley, etc.), *Rutaceae* (citrus fruit), legumes, and *Moraceae* [174].

Clinical Presentation

- *Photoallergic reactions* (Fig. 8.17) are elicited after re-exposure to both the photoallergen and radiating energy, taking the aspect of an urticarial eruption appearing after several minutes, or erythematous, edematous, eczematous or exuding, also involving parts of the body not exposed to the sun (≥24 h).
- *Phototoxic reactions* are manifested 5–18 h after the first sun exposure and reach their maximal effect after 36–72 h. Their typical aspect is characterized by hyperpigmentation, erythema, and vesicles. The flare-ups develop in cloth-covered sites not exposed to UV radiation and that are distant from those initially involved.

The clinical picture is therefore various. In certain cases, the reactions to UV rays produced by photoallergens may persist, even after prolonged abstention from sensitizing substances (for example, ambrette) [58], or after eradication of the plant.

Contact photocheilitis is induced after sun exposure by dyes based on fluorescin, eosin, erythrosine, or on above-mentioned natural furocoumarins and psoralens.

Diagnosis

The diagnosis, when not evident from a careful history and clinical examination, requires photopatch testing, which reveals several photoantigens including methylcoumarin, ambrette and oxybenzone [58, 223]. However, the EU has prohibited using ambrette in cosmetics and

Table 8.18. Differential diagnosis between toxic photodermatitis and allergic photodermatitis

	Toxic photodermatitis	Allergic photodermatitis
Prevalence	Common	Uncommon
Mechanism	Nonimmunological cell damage	Immunological sensitization
Immune mechanism	No	T-cell mediated
Clinical picture	Solar erythema	Eczema
Route of exposure		
Topical	++	+++
Systemic	+++	–
Onset	Minute to days after exposure	Days to months, once sensitized 12–24 h after exposure
Occurrence at first exposure	Yes	No, requires a sensitization period
Flare-ups of earlier reactions	No	May occur
Drug-induced chemical modifications	No	Yes
UV band	285–310 nm	320–450 nm
Drug dosage	Dose-related	Dose-independent once sensitized
Morphology		
Erythema	±/+++	±/+++
Edema	+/+++	+++
Papules/papulovesicles	±	++
Blister formation	++/+++	+
Lesion spreading	No	++
Circumscribed lesions	++	±
Histology		
Sunburn cells	+	–
Spongiosis	–	+
Time course	Decreasing	Increasing

Data from [58, 174].

has introduced for furocoumarins a limit of 1 mg/kg in sun-tan lotions and in sun-tanning products in general.

Differential diagnosis takes into account, in addition to phototoxic reactions (Table 8.18) [58, 174], the extended contact phytodermatitis caused by *Compositae* pollens, which differ from photodermatitis since they occur in the fall season and produce the lesions with shaded instead of clear-cut limits.

Treatment

No specific management is available. When it is possible to detect an eliciting substance, allergen prophylaxis is crucial. Patients should avoid sunlight exposure while taking implicated drugs. If necessary they can protect the exposed sites with sunscreens that do not contain PABA esters [215]. Topical treatment is based on packs to alleviate pruritus and burning, and topical CSs if erythema and edema appear not to resolve, rigorously avoiding topical antihistamines, a cause of (photo)allergic reactions when applied on the skin.

Contact Dermatitis by Seawater Organisms

Contact is with toxic proteins of some *Coelenterates,* echinoderms, molluscs, sponges, seaweeds, cercariae, and fish of various types. *Coelenterates* are provided for attack and self-defense with nematocysts containing venomous urticating substances capable of remaining on the skin surface or penetrating it. Symptoms consist, in addition to urticaria and ACD, of linear erythematous or edematous eruptions that are either vesicular or hemorrhagic and toxic reactions. The forms stemming from jellyfish are characterized by erythematous wheals appearing within a few hours, subsiding spontaneously after a few days. Echinoderm prickles elicit pain, burning, erythema and edema; pain is live, pyrotic if caused by venomous substances emitted also by morays, skates,

scorpion fishes, etc. Systemic symptoms induced by *Coelenterates* consist of nausea, asthenia, ataxia, muscle spasms, paresthesia, vertigo, etc. [7]. Lesions by *Coelenterates* should be treated with seawater or salted water, because fresh water is hypotonic and induces nematocysts to burst; it is sufficient to disinfect with alcohol. The more severe fish dermatitis and lesions should be cleaned with seawater then applying a tourniquet; application of hot water (>50 °C, but in practice up to the degree of tolerance) helps to inactivate the poison. Depending on the circumstances, we suggest instituting a local or systemic treatment. Echinoderm dermatitis is best treated by surgically removing the prickles or by dipping in hot water. Prophylactic approaches include the use of suitable shoes in shallow water and avoidance of touching such types of fish.

Allergic Vasculitis

Allergic vasculitis, or hypersensitivity vasculitis, affects small-caliber vessels: this vasculitis is most frequent in children, also considering the objective rarity of this allergy section [34]. The allergic origin with CIC deposits is hypothesized after the report of several immunological dysfunctions and the histological and immunological aspects similar to those seen in experimental models of Arthus reaction and acute serum sickness [34].

Definition

Allergic vasculitides in children is primarily characterized in histopathological terms by inflammation of vascular walls, possibly associated with necrosis and subsequent occlusion of vessels [24]. They are included in a heterogeneous group of vasculopathies involving arteries with different locations and diameters, veins and capillaries, whose vessel walls have in common the typical lesions of necrotizing vasculitis, or are *leukocytoclastic,* always associated with inflammatory cells and perivascular infiltration of polymorphonuclear (PMN) leukocytes.

Classification

Several classifications based on morphology and clinical features have been proposed [11]. However, it has thus far been impossible to formulate a global classification that takes into consideration all anatomical, clinical and etiopathogenetic characteristics. Both the etiology and clinical features are entirely different, due to a spectrum of varying causes: Table 8.19 [11, 17, 34, 122] classifies the principal allergic vasculitides in children. Contrary to adults, children suffer above all from SHS and to a lesser extent from hypersensitivity angiitis [31].

Table 8.19. Main types of allergic vasculitis in children

Primary allergic vasculitis
Acute hemorrhagic edema of infants
Giant cell arteritis
Granulomatous arteritis
Wegener's granulomatosis
Churg-Strauss syndrome
Hypersensitivity angiitis (with no apparent cause)
Younger children
Kawasaki syndrome
Polyarteritis
Schönlein-Henoch syndrome
Older children
Polyarteritis nodosa, classical
Polyarteritis nodosa, cutaneous
Polyarteritis nodosa, infantile
Temporal (giant cell) arteritis
Takayasu's arteritis
Secondary allergic vasculitis
Associated with infections
A. Microbial
Brucella, Yersinia, Rickettsia
Mycobacterium leprae
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Streptococci, staphylococci, etc.
B. Viral
Cytomegalovirus
Epstein-Barr virus
Herpes simplex e zoster
HIV
Hepatitis B virus
Rubella virus
C. Parasites
Plasmodium malariae
Toxoplasma gondii
Disimmune disorders and syndromes
Chronic atrophic polychondritis
Cogan's syndrome
Congenital deficiency of complement components
Cryoglobulinemia
MacDuffie's syndrome
Associated with autoimmune disease
Behçet's syndrome
Dermatomyositis
Lupus erythematous, systemic
Rheumatoid arthritis
Scleroderma
Sjögren's syndrome
Other associations
α-1-Antitripsin deficiency
Contact agents
Cow's milk proteins and other foods
Cystic fibrosis
Drugs
Hemorrhagic rectocolitis
Insect stings
Physical agents (cold, heat, etc.)
Sarcoidosis
Serum sickness

Data from [11, 17, 34, 122].

Prevalence

A study referred to by Athreya [11] points to a 4% incidence, probably an underestimate because only academic centers were involved in this data collection, and pediatricians usually see and treat cases of SHS and Kawasaki syndrome, the most common vasculitides.

Histological Aspects

Dermal inflammatory phenomena are localized prevalently among capillaries; therefore the vasal endothelium plays a crucial role in these cases. The vessel wall becomes a target of immune processes, characteristic of vasculitis [34]. The vasal lesions affect the superficial dermal capillaries; endothelial cells are the seat of edema restricting the vessel lumen. Thus the wall is thickened, infiltrated by fibrin deposits that extend to adjacent connective tissue, leading to fibrinoid necrosis, which derives its name from its tintorial affinity with fibrin, characteristic of necrotizing vasculitis. A perivascular inflammatory infiltrate complicates the wall lesions, which, along with edema, are responsible for the infiltrated character of the purpura, including various amounts of PMNs, whose nuclei undergo pyknosis and disintegrate to so-called nuclear dust. PMN cell decay is known as *leukocytoclasis,* inconstant but with a great diagnostic value [34]. The infiltrate may also include intact eosinophils and mast cells, associated with PMNs; however, PBMCs could be recruited before PMNs. Vasculitis is a cause of dermal hemorrhages, clinically corresponding to purpura, more rarely of local ischemia, responsible for more or less ulcerate bullous lesions. Yet the pattern is not always that of a typical form [24].

Pathophysiological Aspects

CICs have a key role in the pathophysiology of leukocytoclastic vasculitis; additional elements confirm that these vasculitides depend on type III immune reactions, based on serum anticomplement power, hypocomplementemia and above all the observation, by direct immunofluorescence, of complement and/or antibodies in the vessel walls. CICs are not always demonstrable in the vessels; on the other hand, even if CICs are detected in numerous pathological conditions, only occasionally are CICs demonstrated in some forms of vasculites, or there is a parallel between CIC titer and the outcome of vessel lesions. Both antigens and antibodies found in the vessel walls are rarely detected [34].

PBMCs infiltrate in some vasculites, chiefly lymphocytes and partly monocytes, by lysosomal release of digestive enzymes that attack first the adventitia and then the media coat of vessel wall, thus destroying the cellular matrix of these vessels and surrounding tissues. In the absence of PMN and CIC markers, the interference of CMI is also probable: active cells may be NK cells with perforins, T lymphocytes with direct cytotoxicity and/or DTH reactions, leading to increased vascular permeability and the accumulation of more leukocytes and proinflammatory molecules [24]. These pathogenetic models postulate that the endothelium suffers passively from the insult favored by complement activation, resulting in the production of complement breakdown products (C3a, C5a) that are chemoattractants to PMNs and PBMCs. Usually, endothelial cells are protected from potential damage originating from CICs activated by several regulating molecules at various levels of the complement cascade; consequently 90% of CICs are eliminated by the liver (Kupffer and endothelial cells) and to a lesser extent by the alternative pathway. Similarly, if CICs react directly with erythrocytes CIC–erythrocyte complexes are transported to the liver, thus decreasing the risk of epithelial damage [11]. Conversely, the endothelium could also actively contribute to the development of vasculitis in the general context of inflammation [24].

Immunopathogenesis

Although etiopathogenesis remains in great part barely recognized, recent work speculates that CICs, after binding to endothelium, increase synthesis of adhesion molecules on the cell surfaces. Leukocytes carry integrins and selectins, and endothelial cells add CD54 and CD106 to the selectins [78, 148], which are crucial for leukocyte migration between endothelial cells, as analyzed in Chap. 1. A new mechanism mediating the unmatched pathogenetic processes of diverse types of vasculitis is essentially the formation of anti-endothelial cell antibodies (AECAs), CIC deposition and T lymphocyte adhesion to the vascular wall. Several other mechanisms may play a major part, including the release of antibodies against antigens located in the surrounding sites, phagocyte adhesion and complement dysregulation at the tissue level [24]. It is tempting to speculate that CD4 activation by antigens presented by APC in association with HLA class II molecules mediates B lymphocyte activation with subsequent production of ANCA (anti-neutrophil circulating antibodies), formation of CICs and complement activation attracting PMNs also recruited by chemokines (Fig. 8.18) [177]. A set of Aab directed against PMN proteinase 3 (PR3) such as the c-ANCA (cytoplasmic ANCA) have been implicated in the pathogenesis of Wegener granulomatosis with a remarkable predictive significance, thus embodying a key diagnostic role [122]. That these c-ANCA recognize PR3, p-ANCA (perinuclear ANCA), MPO (myeloperoxidase) and the elastase, shown also in patients with polyarteritis nodosa, Kawasaki syndrome and SHS, where they are of IgA class, supports the hypothesis that clinically different disorders might have a

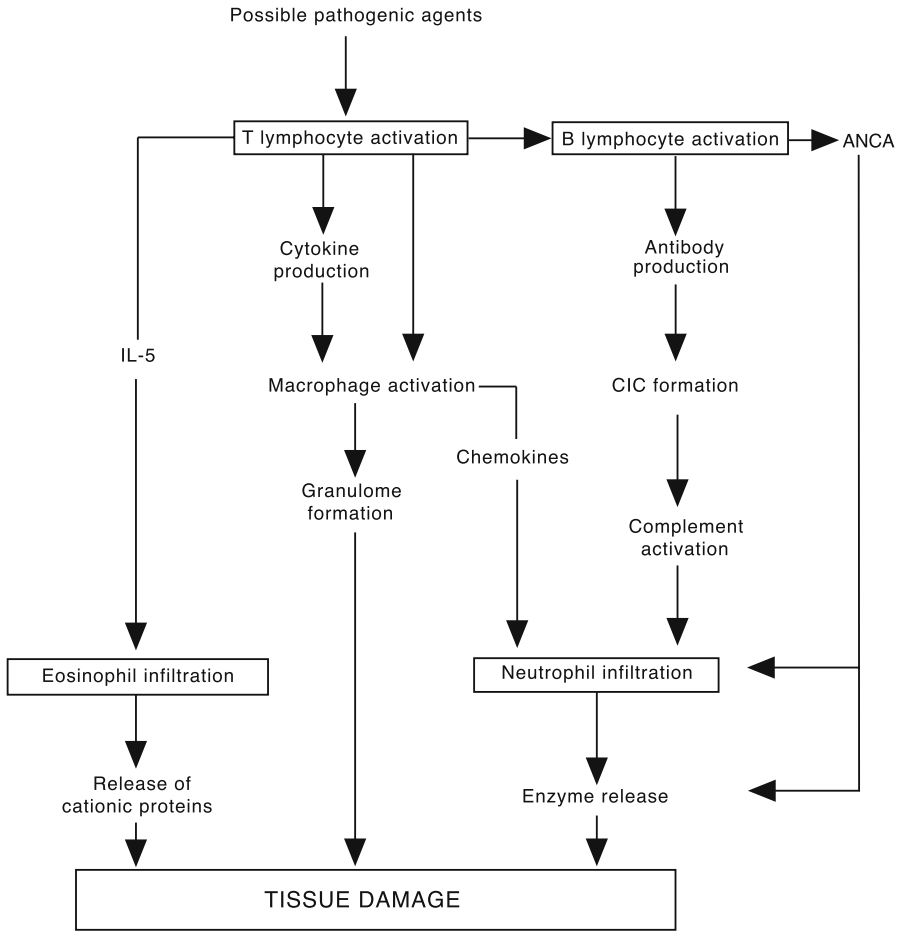


Fig. 8.18. Possible pathogenic events responsible for tissue damage during vasculitis. (Modified from [177])

common pathogenesis caused by antibody-mediated PMN activation [79], and by ANCA involvement in pathogenesis.

Microbial or viral infections may foster TNF-α production and thus the expression of ectoplasmatic antigens such as PR3 and MPO on PMN membrane and epithelial cells [177]. Both proteins and enzyme recognized by ANCA have also been found on granulocyte surface, and are therefore more easily attacked. In addition it is likely that ANCA antibodies bind PMN expressing PR3 on surface, thus causing degranulation with release of free O_2 radicals and lysosomal enzymes on surrounding epithelial tissues, or alternatively bind enzymes released by cells damaged by the inflammatory process, facilitating their transport to the tissues where the enzymes cause further damage [79]. In parallel, the ANCA/PR3 interaction induces, by mechanisms not yet fully understood, an increased CD62E and CD106 expression, which attaching to epithelium modulates the adhesion of PMN [177], CD54 and CD102 [78, 148], which bind their contrareceptors CD11a/CD18, CD11b/CD18 and CD11c/CD18. In this model, the integrins are necessary for leukocyte adhesion to endothelium. Figure 8.18 shows the tissues damaged by a complex interaction of cells, with a prominent role played by eosinophil cationic proteins. Additional etiological factors to be considered are genetic and local factors (lesions differentially affecting one organ rather than another), etc. [122].

Clinical Presentation

Vasculitis disorders share cutaneous and visceral symptoms [34].

Cutaneous Symptoms

Cutaneous vasculitis is a condition characterized by infiltrated palpable purpura (erythema not fading on vitropression), with predilection for the lower limbs, with tendency to symmetry and worsening following orthostatism. Of 130 children, 116 had SHS and14 had hypersensitivity vasculitis [32]. The often associated lesions are mostly polymorphous, erythematous or maculopapulous, well circumscribed, occurring in crops, have dimensions from a pinpoint to several cm, often evolving to form hemorrhagic blisters or necrotic ulceration, lasting from 3–10 days, and affecting in par-

ticular the lower limbs and feet. Certain lesions may complicate the diagnosis such as well circumscribed ecchymotic spots, erythematous papules (palpable purpura), often with an annular arrangement appearing as a polymorphous erythema, which are able to transform into nodules, vesicles, blisters, and/or become necrotic. Preferred sites are gravity–affected areas such as lower half of the legs and buttocks, but also forearms and hands, although in severe cases the face and trunk may be involved. Subcutaneous nodules are small, stiff, often short-lived, most often located on the back of both hands and feet, elbows and knees. Less frequent is *livedo reticularis*. Generally such lesions are asymptomatic, but can be accompanied by pruritus, burning sensation and pain [34].

Additional Symptoms

Articular manifestations are the most frequent and renal lesions condition the prognosis. No relationship is apparent between extension of cutaneous lesions and presence or not of renal manifestations.

- *Musculoskeletal apparatus.* Arthralgias are usually present in >40% of cases.
- *Kidney.* Renal lesions should be investigated, although it has been suggested that the prevalence is not 25%–40% of cases, but <1% of children develop persistent renal disease [11]. Proteinuria is usually associated with microscopic hematuria: symptoms may persist or subside; however, <0.1% develop renal insufficiency. Renal biopsy results are discriminatory as far as the type and severity of injury are concerned.
- *Gastrointestinal tract.* Abdominal pain with nausea and vomiting are frequent (15% of cases); rarely does an occlusive or hemorrhagic syndrome develop.
- *Central nervous system.* Rare associations include hemiparesis, neuropathies and migraine.

Prominent Vasculitis Syndromes in Children

As shown by Table 8.19, the main types of primitive allergic vasculitis in children that most frequently have an immune pathogenesis are the following:

SHS, or anaphylactoid purpura, is the most common among *leukocytoclastic vasculitis* patients, with an estimated annual incidence highest between the ages of 4 and 6 years (99.3×10^5) [81]. SHS peaks preferentially between 4 and 7 years (or between 5 and 6), with a greater prevalence in males and in fall season and in winter [98, 245]. The SHS annual incidence may be 12.9×10^5 children <17 years of age [245]. However infants aged 5–24 months have been reported [5, 188]. It is an immune-mediated disease, paradigmatic of cutaneous necrotizing or leukocytoclastic vasculitis, of small dermal and visceral vessels (<0.1 mm in diameter), which appear to be infiltrated by PMNs with nuclear fragments in various stages of necrosis, releasing proteolytic enzymes resulting in damage to vascular endothelium [11]. The inflammatory and thrombotic process is likely correlated to increased biosynthesis of vasoactive prostanoids [210].

A pediatric study has focussed on the action of these molecules and demonstrated a significant increment of thromboxane A_2 (TXA_2) and PGI_2 in acute stages correlated with the degree of clinical severity [210]. The TXA_2 increase is consistent with platelet activation; a lesser portion derives from activated PMNs and macrophages, while the PGI_2 increase seems to reflect endothelial cell damage by local inflammatory and thrombotic process [210].

The prostanoid, the main metabolite of arachidonic acid, is a product of vascular endothelium and has potent anti-aggregate effects on platelets. It is a vasodilator and inhibits the TXA_2-induced platelet activation on vascular lesions. PGI_2 of endothelial cells influences the interaction between vasal walls and leukocytes, blocking their adhesion after stimulation [210]. Prostanoid synthesis in the vascular cells can be induced by PAF and IL_1, both CIC-stimulated, thus confirming the CIC activity in immune-mediated vasculitis [235]. In the acute stages of SHS, a PGE_2 increase has been shown, probably derived from PMN and macrophage activation. Examined in greater detail, it is unclear whether its action amplifies or suppresses the inflammation characteristic of SHS [210]. On the one hand, it is an active mediator in inflammation, by promoting blood afflux in the inflamed area and thus leukocyte infiltration; on the otherhand, it exploits a local down-regulation by inhibiting lymphocyte activation and antibody and IL production.

In conclusion, both TXA_2 and PGI_2 increase allows a correlation of SHS with other affections and syndromes characterized by an increased interaction between platelets and vascular walls, so that a parallel between a wide spectrum of causes and a virtual uniformity of responses may be advocated [210]. The deposition of IgA and C3 in the small vessels of the skin and renal glomeruli is characteristic. Recently deposition of IgA has been shown in the intestine as well [106]; therefore SHS is considered an IgA-mediated vasculitis of small vessels [11]. The main clinical manifestations outlined in Tables 8.20 and 8.21 [5, 188] show that the skin manifestations, edema and purpura of both scalp and limbs, are characteristically seen in infants, while in older children the frequency of articular (arthritis may be the initial manifestation) and renal complications is increased [5, 188]. The SHS hallmark is the purpuric rash, first macular and then erythematous maculopapules, with hemorrhagic elements (petechiae and ecchymosis), involving symmetrically the lower limbs (Fig. 8.19), associated with edema of the scalp and/or extremities. Depending on the age, abdominal pain and migratory articular manifestations are more or less frequent, the onset of glomerulopathy may even be long after SHS

Table 8.20. Clinical Schönlein-Henoch syndrome manifestations in children aged 0–8 years

Clinical manifestations (%)	Age at onset (years)				
	<2	2–3	4–5	6–7	≥8
Edema	100	62	25	33	25
Facial purpura	83	31	15	0	0
Gastrointestinal	8	54	50	50	67
Renal	17	23	35	25	50
Arthritis	17	69	65	83	100

Data from [188].

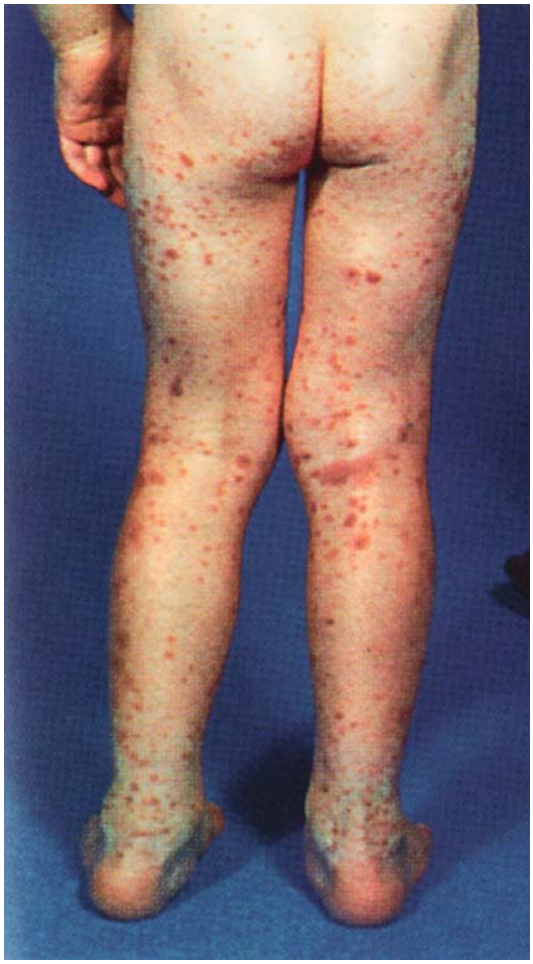


Fig. 8.19. Manifestations of Schönlein-Henoch syndrome in the lower limbs

Table 8.21. Diagnostic algorithm in children aged 0–3 years

Not thrombocytopenic purpura associated with ≥1 of the following symptoms
Abdominal pain
Arthritis
Edema
Gastrointestinal hemorrhage
Glomerulonephritis

Data from [5].

onset. In cases not complicated by nephropathy, the prognosis is good after 24 years of follow-up; however, long-term follow-up of all patients who had severe renal symptoms at onset is needed during adulthood [171].

Hypersensitivity angiitis is another leukocytoclastic vasculitis, clinically similar to SHS, occurring mostly in children, excluding those cases in which the specific allergen (drugs, microbes, virus, insect stings, pesticides, etc.) is easily identifiable. Clinical manifestations include fever, myalgia, and arthralgias without frank arthritis and visceral involvement, and may sometimes resemble the SHS limited to the skin, with which it has in common the character of acute phases *(one-shot disease)*. The small vessels are involved, arterioles or venules, with a variable degree of vessel wall necrosis, localized and rarely occlusive. The cutaneous lesions are typically small hemorrhagic papules, usually in sloping areas and at a similar stages of development. Inflammatory cells are usually PMN [72, 122].

Acute hemorrhagic edema of infancy (Fig. 8.20) is a disease with a benign course characterized by a suddenly occurring cutaneous vasculitis in children aged 4 months to <2 years in good general condition. Large purpuric lesions are distributed on the face, auricles and lower limbs, associated with limb edema, or they are segmental, thick or soft [153]. In some cases, extended trunk lesions may suggest a differential diagnosis with fulminant purpura. Progression is spontaneous and favorable within 2–3 weeks [83, 120, 182], or after a 7-day oral CS course [87]. Vasculitis, essentially cutaneous of the leukocytoclastic type, a (very) young age, the topography of lesions, peripheral edema and common absence of arthralgias and internal organ complications (in any case benign) are diagnostic [83, 98, 120] and should be evaluated as a benign SHS variant [182].

Kawasaki syndrome [108] is the most frequent vasculitis in infancy after SHS. An epidemiological study on

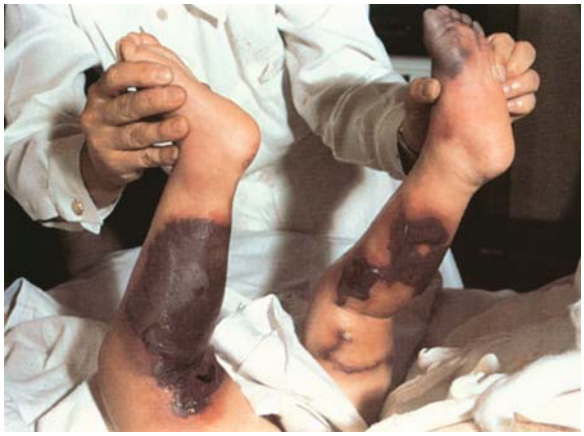


Fig. 8.20. Acute hemorrhagic edema of infancy. The condition subsides spontaneously and is not associated with severe, systemic complications

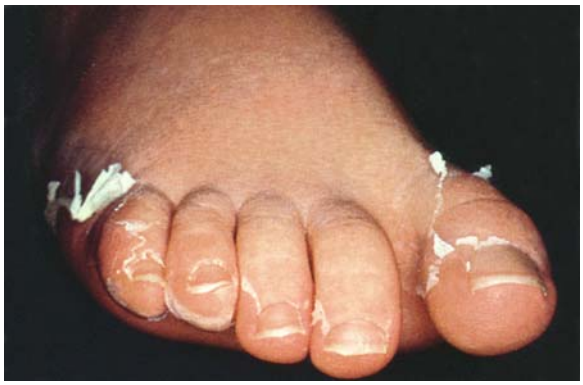


Fig. 8.21. Kawasaki syndrome: scaling of the toes

105,755 cases reported over 35 years ago in Japan [217] showed a prevalence of 4.5–8.5 cases $\times 10^5$ aged <5, with the youngest patient aged 26 days, 0.006% of infants aged 30 days and 1.67% aged 3 months. Among newly treated children aged <5 years, the incidence was 102.6×10^5 in 1995 and 108×10^5 in 1996, with a male-female ratio of 1.37 [244]. The estimated annual incidence was 5.5×10^5 in children <5 years, and was highest in Indian subcontinent Asian children (14.6×10^5) [81]. The incidence rate in Korean children <5 years was ($\times10^5$) 73.7 in 2000, 90.8 in 2001, and 95.5 in 2002. The mean age of onset was 30.5 months [162]. This is a multisystem disorder causing substantial symptoms of vasculitis and mucocutaneous lesions with desquamation, after 2 weeks, beginning at the nail-pulp junction (Fig. 8.21). It is another CIC-induced one-shot disease, characterized by a polyvasculitis of small and medium-sized vessels, especially affecting medium-sized arteries, with a predilection for coronary arteries with thrombosis and formation of aneurysms [72], a particular risk for infants aged <6–12 months [178]. Ongoing antibody synthesis may foster inflammation involving

Table 8.22. Main immunological changes in Kawasaki syndrome

Activated B cells
Activated T $CD4^+$ increase
Ongoing antibody synthesis
Expansion of TcR Vβ2 and Vβ8 regions
Microbial superantigens secreting toxins
Monocyte-macrophage activation inducing:
IL_1, IL_6, IFN-γ, TNF-α raised expression
IL_2R and CD45RO on CD8 raised expression
Endothelial cells expressing HLA class II antigens, CD54, CD62E
Circulating autoantibodies and CIC
Suppressor/cytotoxic T $CD8^+$ decrease

Data from [21, 123, 147, 165, 235].

all three layers of the vascular walls, thus destroying the internal elastic lamina: Table 8.22 [21, 123, 147, 165, 235] reviews the many immunological features consistent with the pathogenesis. Another area of intensifying interest is the demonstration that ILs linked to AECA favor endothelial cell migration, thus modulating the worsening of vascular changes [180]. The changes are multiplied by the high levels of adhesion molecules on endothelium, including CD54, CD102 and von Willebrand factor [78, 148]. Recent data have revealed toxins acting as superantigens (SAs) on Vβ2 and Vβ8 [51, 123, 243], produced by microorganisms of the respiratory or gastrointestinal tracts [51, 147], in particular by *Staphylococcus aureus* and *Streptococcus pyrogenus* secreting TSST-1 (toxic shock syndrome toxin-1) and SPEA/SPEB (staphylococcal pyrogenic exotoxin A and B), respectively [123]. A result still to be weighed is whether CD45RO expression is raised solely on CD8 T cells [165]. There is a tempting suggestion that the intestine encourages causative agent entry such as microorganisms [147, 243] that are producers of toxins with SA properties (Table 8.22). A novel human coronavirus, designated "New Haven coronavirus" (HCoV-NH) was identified in 8/11 infants with Kawasaki disease. Human coronaviruses (HCoVs) have attracted renewed interest because of the emergence of a novel HCoV associated with severe acute respiratory syndrome (SARS). The median time between the onset of fever and the diagnosis of HCoV-NH was 5 days; 7/11 infants and 19/22 control subjects had respiratory symptoms that were consistent with an upper respiratory tract infection (URTI) [68]. Table 8.23 [189] outlines the chief diagnostic and associated symptoms. Useful diagnostic markers could be CD8 T cells [147, 165] and IL_2R for early diagnosis [21].

Polyarteritis nodosa (PAN), divided into classic, infantile and cutaneous PAN [122], is among the least common in childhood. This is another disease that differs in presentation and etiology from that seen in

Table 8.23. Main diagnostic criteria for Kawasaki syndrome and main associated criteria

Main diagnostic criteria
Patients should have at least 5 or 6 main symptoms with exclusion of other diagnosis:
1. Fever with sudden onset, lasting ≥5 days
2. Bilateral nonsuppurative conjunctival hyperemia
3. At least one change of the lips and oral cavity, including hyperemic or dry fissured lips, erythema of the oropharyngeal mucosa, strawberry tongue
4. At least one change in peripheral extremities, including edema of the hands, feet, or both, or erythema of the palms and soles, scaling beginning periungually or generalized
5. Polymorphous rash, especially of the trunk, with varying characteristics, without vesicles or crusts, often itching
6. Cervical lymphadenopathy, not purulent, usually unilateral, ≥1.5 cm
For the diagnosis, in addition to the fever, the child must meet 4/5 criteria
Main associated symptoms (in order of incidence)
1. Urethritis
2. Arthritis or arthralgia
3. Cardiac involvement, especially myocarditis or pericarditis
4. Aseptic meningitis
5. Diarrhea
6. Gallbladder hydrops
7. Obstructive jaundice

Data from [189].

Table 8.24. Hypocomplementemic urticarial vasculitis: clinical aspects

Lesion characteristics
Small wheals
Evident central clearing or dusky coloration
Persistence greater than 24 h
Residual pigmentation following regression
Painful, burning or pruritic lesions
Alternatively, presence of palpable purpura, nodules, ulcers
Clinical aspects
Recurrent, widespread urticarial lesions
Association with arthralgia, arthritis, abdominal pain, fever
potential involvement of synovia, kidneys, gastrointestinal and respiratory tracts, eyes and/or CNS

Data from [135, 226].

adults. More frequent is cutaneous PAN, manifested by purpura, edema, linear erythema, tender palpable nodules, high fever, arthralgias and myalgias, without important organ system involvement. It has a chronic course and a better prognosis than the systemic variants [11, 72, 98]. Biopsy reveals a focal necrosis and inflammation of the walls of small and medium-sized arteries that tend to occur in a segmental way and particularly involves areas where arteries bifurcate [98].

Other types of polyarthritis vasculitis include *Wegener granulomatosis,* featuring the formation of granulomas around blood vessels, with typical lung and kidney involvement, which is currently classified as one of the ANCA-associated small-vessel vasculites distinguished by its predisposition to affect the upper and lower respiratory tracts and kidneys clinically and histologically by the presence of necrosis, granulomatous inflammation, and vasculitis [246]. The presence of PR3, normally restricted to neutrophil α granules, has led to the hypothesis that ANCA-induced neutrophil activation is central to a chain of events leading to T-cell activation and a CMI response involving macrophage activation, and that PR3 expression may be abnormal. The cause may remain unknown, but circumstantial evidence suggests the potential roles of ANCA and infection in the pathogenesis [246]. Recurring infections of upper airways and respiratory infections are prominent clinical manifestations, along with cough, fever, weight loss, arthritis, neuropathy, rash, and splenomegaly. ANCA-associated glomerulonephritis has been diagnosed in 20 children, the youngest aged 11 years, but early recognition and aggressive treatment of these children may prevent end-stage renal disease [219].

Churg-Strauss syndrome, an allergic granulomatous vasculitis also affecting small and medium-sized vessels has a predilection for lungs characterized by AR and/or asthma and presents with fever, headache, myalgia, weight loss, and peripheral blood and tissue eosinophilia exceeding 1,500 cells/m^2. The systemic vasculitis may involve the skin, heart, and peripheral nerves. Kidneys are spared or only mildly affected [122]. Recently three cases have been reported: a 15-year-old girl with catastrophic gastrointestinal vasculitis and multiple colonic ulcers with active bleeding [124], a 10-year-old girl with acute abdominal pain, bloody diarrhea, pulmonary infiltrates, a 6-year history of severe asthma, and marked eosinophilia [26], and a boy with poorly controlled bronchial asthma, AR, recurrent sinusitis and several episodes of hemophthisis since the age of 9, who developed purpuric skin lesions, generalized soreness, and symptoms of mononeuritis multiplex at age 11 [234]. These cases demonstrate that the prognosis is poorer in children than in adults [124].

Table 8.25. Histopathology of hypocomplementemic urticarial vasculitis compared with acute and chronic urticaria

Manifestations	Urticarial vasculitis	Chronic urticaria	Acute urticaria
Inflammatory cells	Predominantly PMN	Predominantly monocytes	Few PMN and monocytes
Location	Perivascular and within vessel wall	Perivascular few within vessel wall	Perivascular
Leukocytoclasis	Yes	No or minimal	No
Endothelial cell swelling	Yes	No or minimal	No
Leakage of erythrocytes	Yes	No or minimal	No

Data from [27, 226].

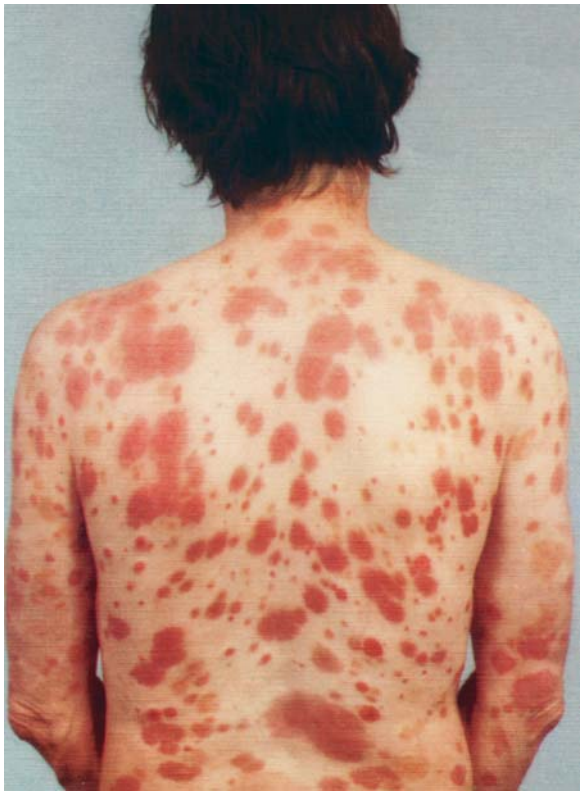


Fig. 8.22. Acute urticarial vasculitis

Wholly particular is *hypocomplementemic urticarial vasculitis,* of which eight pediatric cases are known [39, 55, 135, 232], including 2 identical twins [241] and a girl with SLE [55]. Concordance in identical twins may suggest that the pathogenesis of the disease involves abnormal genetic immunoregulation [241]. CIC-induced pathogenesis has an Arthus-like pattern; there is selective depression of Clq binding IgG and lesser depression of C2, C3, C4, but the levels of Clr and Cls are almost normal [135, 232]. Cl, C3, C4, properdin, IgM, IgG and fibrin are often present in vascular cells and basal membrane. When complement is activated, C3a and C5a are released. These anaphylotoxins with chemotactic action induce *de novo* synthesis of chemokines and ILs and mastocyte degranulation with consequent increase in vascular permeability, followed by LTB_4 release. In both ways, PMNs are recruited, resulting in the tissue damage described above. Clinical features are those of leukocytoclastic vasculitis: clearly delimited erythematous and edematous macules are seen, and are characterized by their relatively fixed aspect, tendency to purpura, are only slightly pruritic, and the possible association with systemic manifestations or an inflammatory syndrome [27, 135, 226]. The lesions resemble urticaria and typically persist for more than 24 h [55]. Tables 8.24 and 8.25 [27, 135, 226] outline the clinical and diagnostic symptoms of urticarial vasculitis, characterized by a wide spectrum of symptoms that may lead to a more severe clinical course that is life-threatening to children, including severe anemia and renal insufficiency [135], and hycomplementemic histopathology, compared with acute and chronic urticaria [27], is useful for the differential diagnosis.

Acute urticarial vasculitis related to postinfectious or drug-induced hypersensitivity starts as transient infiltrated purpura (Fig. 8.22), 2–3 weeks after antigenic aggression; several secondary types of vasculitis are induced by food additives [173, 223], CM proteins [34], other foods and vitamins [173].

Diagnosis

Vasculitis assessment should include history, physical examination, and some of the following items:

- Age and epidemiology, in order of frequency SHS (Tables 8.20, 8.21), Kawasaki syndrome (Table 8.22), leukocytoclastic vasculitis, hemorrhagic edema (Table 8.19) and urticarial vasculitis (Tables 8.24, 8.25), the last being most unusual in this age range
- Recent treatment with potentially implicated drugs
- Palpable purpura
- Maculopapular erythema
- Possible biopsy

Laboratory studies (complete blood count, ESR, CRP, platelet count, kidney and liver function studies, T subpopulations, AECA, ANCA, lymphocyte antibodies, etc.)

Table 8.26. Frequency of c-ANCA, p-ANCA and anti-MPO in several disorders

Disorders	c-ANCA	p-ANCA	Anti-MPO
Polyarteritis			
Classic PAN	Uncommon	2+	2–3+
Microscopic PAN	2–3+	2–3+	2–3+
Churg-Strauss syndrome	Rare	2+	2–3+
Wegener granulomatosis	4+	1+	1+
Generalized			
Active	2+	–	?
Remission	3+	–	?
Limited			
Active	2+	–	?
Remission	2+	–	?
Polyangiitis overlap	1+	1+	?
Idiopathic necrotizing and semilunar-type glomerulonephritis without immune deposits (pauci-immune)	1+	4+	4+
Inflammatory bowel disease			
Ulcerative colitis	Rare	2–4+	Absent
Crohn's disease	Rare	Absent	Absent

Data from [11].

are useful to confirm the diagnosis. Table 8.26 [11] gives the frequency of c-ANCA, p-ANCA and anti-MPO in several vasculitis.

Treatment

The reader is referred to reviews on therapeutic details [11, 226, 235]. Intravenous immunoglobulins (IVIg) were administered to two children aged 2.5–3.5 with Kawasaki syndrome associated with severe AD at the dose of 4–6 g daily for 5 days with remission of hyperthermia (Chap. 7). The IVIg positive effect, to be started promptly and continued for the 5 days of Kawasaki syndrome [244], lies in the inhibition of endothelial cell migration with destructive effects [180], likely favoring the reparative phase.

Pediatricians and Other Cutaneous Allergies

This chapter presents pediatricians with a large number of different diseases and syndromes of childhood, the background to a very extensive and differentiated task. This includes several kinds of foods, additives, drugs, and insects that induce urticaria and the countless and dissimilar physical forms, as well as foods and inhalants inducing angioedema. Since urticaria covers a clinical spectrum, it should not be surprising that several skin disorders have urticarial lesions. ACD involving young children is provoked by buttons, clasps, jewels, cosmetics, topical medications, and the ubiquitous latex with latex-fruit and latex-vegetable syndromes and even in the materials employed by dentists. The most important preventive measure for patients with latex allergy or at risk for it is minimizing direct exposure to latex products, most notably latex gloves. Recent operating room studies indicate that simple preventive measures can dramatically reduce intraoperative reactions [30]. Although rare, the different forms of infantile vasculitis are undoubtedly a challenge for most colleagues. The clinical and laboratory findings are variable, frequently nonspecific or overlapping. Therefore early and correct diagnosis is imperative, but one should never delay initiation of treatment, while trying to finalize the diagnosis. These disorders are therefore found in several chapters of infantile allergy and immunology and will put to the test more than ever not only the diagnostic and therapeutic skill of pediatricians, but also their cooperation in light of understandable parental anxieties. With treatment methods designed both to avoid side effects and limit costs, effective approaches will be centered. However, clinical experience will untie several Gordian knots, thus justifying neither frustration nor polypragmatism, especially when faced by the tempting plethora of current therapeutic options.

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Food Allergy

One Allergy, Several Allergies

Food allergy (FA) was born as cow's milk (CM) allergy (CMA). Only humans began and continue to use milk of other animals to nurse offspring, although Hippocrates recorded gastric upset and hives due to CM and proposed dietetic measures. The use of animal milk to feed children began spreading around the mid-eighteenth century, with a preference for ass or goat's milk rather than for CM [249]. In parallel, the consequent decline in breast feeding became evident from the fifteenth to nineteenth centuries: perhaps "nine months of blood, nine months of milk" [95] were too heavy. Subsequent discoveries in the fields of microbiology and medicine provided a more thorough basis for development of substitutes for breast milk (BM). Comparisons against the advisers of bottle feeding were requested, with unhappy results [95] and also differences in mortality among the BM-fed infants compared to non-BM-fed infants were worthlessly pointed out [249]. Even if at the start of the 20th century the first cases of anaphylaxis [436], one of which was fatal [161], and of CMA [196] were documented in the German literature, artificial feeding increased like an avalanche in the 20th century [249]. This massive progression was also based on data giving the impression that BM did not transfer any immune factor to neonates, although in 1892 Ehrlich had demonstrated the contrary [144]. In the last decades there has been a positive promotion of BM feeding in different countries, and supporting studies since 1985 have again reaffirmed BM supremacy and the several problems connected with CM [538]. Newborn BM feeding is confused with the history of mankind and probably found its first application in the garden of Eden: perhaps for this reason BM feeding has always retained an absolute pre-eminence. For centuries, BM has been the only way of feeding human newborns, and CMA was virtually unknown in infants [64]. It is significant that BM was classically defined as a natural feeding, while CM is mentioned as an unnatural or artificial feeding [95]. Certainly, the occurrence of symptoms stemming from eating foods different from BM, and especially of CM, dating back to ancient times is striking, whereas only in 1918 was it reported that a girl had the so-called BM allergy (BMA) [470]. This is decidedly another demonstration that BM is the ideal food for neonates, and although in the past it was recommended since it provides practical, economical, nutritional, physiological, and psychological benefit, we can say that every day a further protective factor is added to the long list of BM's documented advantages (Table 2.12). Clearly, throughout history not all neonates and infants had BM available, so their mothers were compelled to select a more easily achievable and/or economical substitute: thus the onset of CMA.

Adverse reactions to foods are frequent and common causes of childhood morbidity, and one of the best-known and most frequently encountered problems in current infant feeding practices. However, they are surrounded by considerable controversy to the point that some standard textbooks in pediatrics either avoid mentioning FA altogether or only refer to it in passing in relation to gastrointestinal (GI) or skin symptoms; at times only a few lines are devoted to FA, but no mention is made of CMA, so the frequently divergent attitudes toward FA often result in its underdiagnosis by some allergists or its overdiagnosis by others. In 1980 *Allergies to Milk* was published [23], and in 1982 the specific use of a homemade diet based on lamb meat with CMA infants was first reported, originally devised in 1973 in the Division of Pediatrics of Rome University [403]. What is surprising is that casein may remain active for 2,500 years [110] and egg for 500 years [12]. This chapter also includes oral allergy syndrome (OAS) and bird-egg syndrome.

Definitions

Among the clinical manifestations arising after a food ingestion we include *food anaphylaxis,* a severe classic, hypersensitive reaction, and *FA,* based on a clear cause–effect relationship between food ingestion and onset of allergic manifestations. A reaction to a food allergen by DBPCCT (double-blind, placebo-controlled challenge test) illuminates the pathogenesis [322, 413]. Although the immune reactions can be of varying types, the only immune mechanism to demonstrate FA *is IgE-mediated.* All non-IgE-mediated forms are defined as *pseudoallergic (PA) or food intolerance* [10, 334, 519] (Chap. 10). Actually, FA can be defined as the only allergic disease with a homogeneous etiology, since the causative factors are solely foods or their handling. FA children may be cured by oral desensitization in 84.6 %–87.5 % of cases [380].

Prevalence

Epidemiological estimates are highly variable in children: the values we have ascertained have a mean of 6.6% (Table 5.1) for FA and 2.42% for CMA (Table 5.2). However, in other studies done in selected children to be analyzed below, the positivity rate is much higher, with a prevalence of 23.5%. Usually, elevated values are lowered by DBPCCTs or open food challenge tests (OFCs). Table 5.5 shows that FA's onset is in the 1st year of life in 52%–100% of cases. In a prospective study on children aged 3–6 years, 28% were thought by parents to have had an adverse food reaction, only 8% had their symptoms confirmed with OFCs [257] and in DB trials [41]. In adults, DBPCCTs have corroborated 1.4%–1.8% of the presumed 19.4% [524]. In a similar study on 1,759 infants followed up from birth, the prevalence was 6.7% in 117 children manifesting symptoms indicative of CMA, but only in 39 of them (2.2%) was the diagnosis confirmed by FCTs [224]. The *onset age* is certainly a very significant factor: CMA began on average at 2 months with immediate reactions [140] and 57% of cases occur *in the first 3 months of life*, 75% *in the first 6 months* and 97% in the first 2 years [111] (Table 5.3 and Fig. 7.38). Several investigators rank CMA in first place [47, 140, 224, 397] among allergies, others egg allergy [26, 111, 178, 203, 237, 339, 449]. In 544 children with FA, the highest prevalence of FA was in the range of 1–3 years, for egg, peanut, and CM [386]. A double allergy has been reported, for example, 37% of egg-sensitized children were also CM-sensitized [449].

New sources of increased prevalence have been recently theorized, including characterization of additional allergens (Table 1.74), genetic modifications of natural foods, reports of countless cross-reactions (presence of common epitopes) [81], introduction of exotic fruits, etc., with multiplication of sensitization processes. The potential danger of transgenic foods (Tables 1.78, 1.79) seems to be increased, while genetics has been usefully applied to rice [525]. From this viewpoint, the increasingly more widespread practice in maternity wards of administering unnatural formulas to neonates instead of colostrum (Table 2.19) has facilitated the rise of FA prevalence in geometrical progression.

Pathogenesis

Immune Mechanisms

Foods making up a typical diet consumed by human beings are usually ingested daily, to the point that during a lifetime some 150 tons of food and fluid reach the gut, which has a total surface of about 400 m^2, thus being the largest immunologic organ of the body, and should protect the host against a wealth of foreign substances, while accepting and absorbing various nutrients to feed the host [200]. The gut is an extraordinary organ that has to accept these large amounts of nutrients daily while recognizing and reacting to harmful materials. It is surprising that only few foods are responsible for allergic sensitization. The foods as yet characterized have several allergenic proteins, but very few of them act as major allergens. Moreover, some foods retain their allergenicity irrespective of how they are consumed, fresh or cooked, etc. [1]. If FA first shows up as CMA, the first foreign protein consumed early in life, the following definitions should be retained:

More pathogenetic mechanisms may mediate allergic reactions, either immediate or delayed.

Multisensitizations may lead to different types of immunological activation in the same individual.

A different temporal modulation of external or internal factors may up-regulate the immunogenicity of food proteins.

Our understanding of the basic mechanisms underlying FA is still very limited. There are two important prerequisites: the antigen (a protein with a molecular weight of 10–70 kD) should be capable of crossing the protective mucosal barrier, thus triggering a potentially damaging immune response. In neonates and infants, the pathological transport of antigenic macromolecules through the small intestine may cross the mucosal barrier. The controlled uptake of these macromolecules results from the interaction of several immunological and nonimmunological factors within the lumen and on the mucosal surface [508]. Normally, the specific suppression mediated by T cells shoulders the burden of maintaining the host in a state of non-allergy, and numerous defensive components (Table 2.11) consolidate the epithelial barrier, where IgA antibodies predominate [508]. Several functions of the immune system are virtually immature (Chap. 2), a transient deficit in IgA is present and also the defense by antigen-specific CD8 T cells is deficient [461]. CM as such or contained in adapted formulas is able to induce lesions in a quantified way [24, 477]. These interwoven factors in neonates and infants are found to overlap and vary in genetically established and other occasional and environmental settings, which ultimately act as triggering events: in this way the differences can be explained in both the clinical course and histological patterns, explaining the difficulties met in establishing the diagnosis.

Despite considerable advances in the understanding of allergic responses, the mechanisms regulating GI allergy are not very well understood. The role of specific cells and their mediators in the development of food hypersensitivity reactions continues to be the focus of much debate. Several different immunological mechanisms and various pathogenetic hypotheses have in turn been proposed to explain the intervention of other types of immune reactions [10].

Cross-reactions among food (and inhalant) allergens are a new immunological variant of the pathogenesis. Precise identification of cross-reactive epitopes would

represent a significant advance in the problem of FA, since it would facilitate the choice of hypoallergenic substitutes for the treatment and prevention of FA. Table 1.73 offers a wide selection of cross-reactions among allergens. Table 1.72 details the spectrum of profilins, with an additional mixture of food (and inhalant) allergens, which can also cross-react among themselves. Both occurrences will be highlighted throughout the text, specifying their relative weight.

Type I–IV Pathogenic Mechanisms

As regards the mechanisms involved in the pathogenesis of CMA, varying immunological theories have been postulated, including types I, III, and IV of the well-known Gell and Coombs classification. Therefore, the differences in the clinical course, histological picture, various prognoses, and difficulties in the diagnostic procedure have been explained.

In *type I reactions* there are no doubts: this is the only mechanism that can be demonstrated with substantial evidence. Reactions mediated by mast cells of intestinal mucosa induce mediator release, thus resulting in many clinical manifestations acutely arising after food ingestion, with exclusive localization in the intestine (inducing vomiting and diarrhea), or other sensitized organs including bronchi, skin, etc., with a different expression depending on the amount of antigen that reaches mast cells of other areas [327]. This mechanism is self-maintained, since type I reactions raise mucosal permeability favoring the uptake of ever-higher doses of antigen in both the intestine and the circulation.

Type II reactions occur when specific antibody binds to a surface tissue antigen or hapten associated with a cell and induces complement activation. The products of complement activation promote the generation of several inflammatory mediators, which then leads to subsequent tissue damage. These reactions are explained by the likelihood that a mechanism mediated by antibody-dependent cell-mediated cytotoxicity (ADCC) plays a pathogenetic role in CM-induced enteropathy [410] and a case of antibody-dependent thrombocytopenia after drinking CM [145].

Type III reactions could be responsible for many GI manifestations on antigen exposure within the first 24 h or a few days: in 80%–90% of all children by the age of 3–4 years after CM ingestion, a CIC (circulating immune complex) deposition in tissues has been shown, a sign that CICs are not necessarily the cause of mucosal damage, nor are they correlated with disease [457]. In contrast, CIC levels are elevated in subjects with CMA [372]: this is an Arthus-like reaction with a local effect. CIC microprecipitates passively deposited beneath endothelium of vascular structures of target tissue may provoke acute inflammatory reactions in the gastroenteric mucosa, and reaching the cutaneous superficial microcirculation they cause edema and infiltration of dermal papillae [275]. Macromolecules escaping defense mechanisms face IgA and IgG blocking. Given that IgA antibodies as well as IgE are strategically positioned in the GALT (gut-associated lymphoid tissue), normally in excess of IgA, it is likely that an adequate part of antigen is neutralized *in situ* [508]. Unmodified CICs cross the intestinal epithelium and the IgA barrier, pass through the intercellular junctions, enter the portal circulation and reach the liver, where a part is conjugated with IgG of splenic derivation, but antigen-IgG CICs are cleared by Kupffer cells of the liver. The remainder of CICs bind IgA and form antigen-IgA CICs, and are eliminated by biliary salts; thus symptoms decline and pathological lesions regress [275]. Therefore, antigen-IgA CIC levels in normal subjects appear to be necessary for the induction of specific low-dose tolerance to food allergens [275]. In allergic subjects and with specific IgE (sIgE), eating an offending food may cause a passage of proteins in the circulation greater than that in normal subjects, thus resulting in antigen-IgG or -IgA CICs in a greater amount when compared to controls [96]. We stress that *in infants of a few months the levels of IgA and IgG are low*, hence antigen-Ig CICs are damaged, with the result that macromolecules taken up unmodified are sensitized by allowing the access of minute amounts of allergen at the next contact, with the possible outcome of allergic symptoms [367]. IgE and IgG CICs, in direct correlation with symptom development, are detected in the serum of subjects with FA, however without a support in other subjects with similar manifestations, but negative RAST [96]. Obviously, a straight correlation between food ingestion → CIC increase → appearance of symptoms in the absence of IgE is largely conjectural. Recently the role played by CICs has been re-evaluated after the report of a woman with recurrent urticaria-angioedema, bronchospasm and high levels of CICs in the peripheral blood. These CICs contained antibodies against bovine serum albumin (BSA), and a BSA-free diet induced a rapid CIC clearing with levels returned to normal, but BSA addition to the diet led to reappearance within 24 h [298]. In conclusion, the presence of food antigen-antibody CICs has been demonstrated in FA subjects and in nonsensitive and healthy subjects.

Type IV reactions rely on the more likely hypothesis that postulates that type IV immune reactions do not cover the entire spectrum of CM reactions, since children with CMA may manifest type IV symptoms of delayed hypersensitivity and lack of increased levels of sIgE to CM [275, 508]. On clinical grounds, it is characteristic that late responses to CM occur even after 15 days [82, 328]. Relatively little is known about these reactions: the specialized structures of Peyer's patches (PP) are important in a controlled antigen uptake from the gut lumen, as a cluster of lymphoid cells and in the induction of humoral immune responses [103]. An in vitro lymphocyte proliferation or interleukin (IL) generation by lymphocytes handling food allergens has been demonstrated in children with atopic dermatitis

(AD) and FA [445], especially in younger ones, but cannot be considered as specific, since it is also demonstrable in healthy subjects [245]. In children with CM enteropathy, activated $CD4^+$ are observed in the lamina propria of small-bowel mucosa, much more abundant in comparison to controls, which may play a role in contributing to mucosal damage [349], likely IEL (intraepithelial lymphocytes) and lamina propria cells emigrated from peripheral blood and activated by orally administered antigens [366]. These $CD4^+$ increase in the lamina propria after a FCT [366] or normalize with treatment, but regress to pretreatment levels after challenge [349, 366]. However, even if we can expect that CD4 are active [445], it is still difficult to accept the interference of IL_2 and IFN-γ Th1 producers [280]. The experimental demonstration of the type IV immune mechanism is complex: on histopathological grounds, an increase in cryptic hyperplasia and villous atrophy, epithelial lymphocytes and PBMCs (peripheral blood mononuclear cells) are observed in the lamina propria; along with a notable rise in the number of cells with IgG and IgA antibodies, is seen during relapses, thus leading to the hypothesis of a consistency between those immunohistological findings and the existence of III and IV type reactions [384]. TNF-α induced locally by PBMCs after FCT with CM is co-responsible for epithelial cell damage, especially when these cells function as a barrier: TNF-α acts in synergy with IFN-γ to increase gut permeability [208]. Mucosal damage is severe and extends to the entire GI tract even if the small bowel is involved more. The crucial role played by T lymphocytes has been widely investigated in intestinal explants of fetuses of different ages [310]: epithelial lesions and even explant destruction after mitogen stimulation are observed only in the mucosa of fetuses populated by T cells at age 14–20 weeks. Activation of T cells results in local IFN-γ production and an increase in HLA-DR expression by a tenfold increased rate of crypt cell proliferation, as well as of IEL numbers [311], so that after a 3-day culture the explant mucosa resembles that seen in untreated celiac disease (CD) [310].

The development of a protective or allergic response depends on which T lymphocyte subset has been activated by antigen molecules. However, the delayed maturation of suppressor CD8 T cells plays a role of primary importance in the pathogenesis. A IL_4^{++}, IFN-γ^{--} pattern (Table 1.10) follows Th2 T-cell activation, which stimulates IgE-mediated reactions, but if Th1 T cells are activated a reverse pattern and an isotype switch to IgG and IgA antibodies predominate. Recent reports demonstrating an *IFN-γ total absence in children with CMA at 13–23 months* [466] have confirmed similar cord blood (CB) results (Chap. 3). This explains why Th1 are deficient and express *no suppressor protective function*, and this maturation delay may be another pathogenetic cornerstone since it persists as an IFN-γ deficit in CM-intolerant children [465, 466]. Such deficits are added in a negative sense, because CD8 expresses

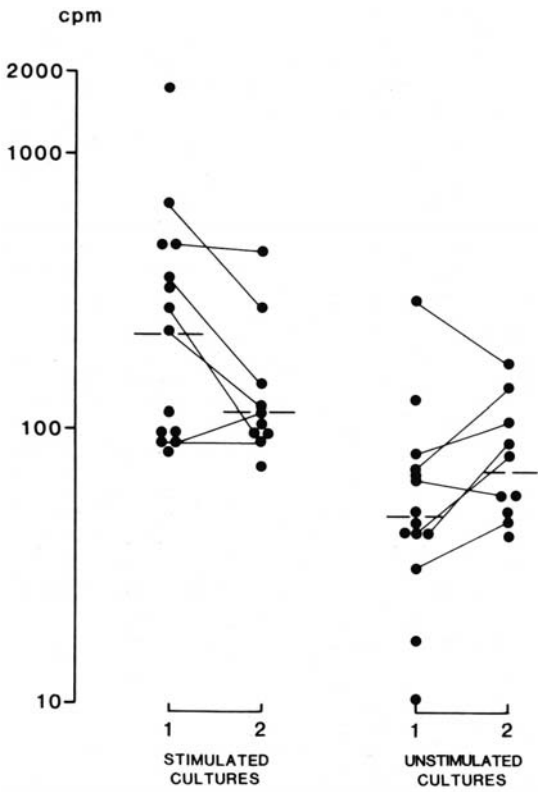


Fig. 9.1. Change in CM-induced lymphocyte proliferation from day 1 (before the food challenge, *1*) to day 8 (after the food challenge, *2*) in children with CMA and gastrointestinal symptoms. Lymphocytes were induced by CM (*stimulated cultures*) or cultured in the absence of CM (*unstimulated cultures*). *Dots* represent individual children, those *interconnected* were evaluated in both cases. *Horizontal lines* indicate geometric mean ($p<0.05$). *cpm* Count per minute

IFN-γ by immune deviation [222]. IL_4 was also wholly absent from the scene [465, 466], recalling previous discussions in Chap. 4. T lymphocytes and ILs generated appear to be dysregulated: at 2–44 months of age both the number and/or function of CM-specific circulating lymphocytes are reduced (Fig. 9.1) [467], and it is therefore likely that these dysregulations also compromise the humoral arm [244]. Although cell-mediated immunity (CMI) mechanisms contribute to several forms of adverse food reactions, there has been no clear-cut and objective demonstration of a food CMI disorder.

Food antigens are the main allergens that cause allergic reactions during infancy and childhood. An important role in the pathogenesis of FA is attributed to food-specific T-cell reactivity. Both in allergen-stimulated PBMCs and at the clonal level, it has been shown that the food-specific T-cell response in patients with FA is Th2-skewed when compared to that of food-tolerant individuals. Because the Th2-like T-cell ILs are important in the pathogenesis of allergic inflammation, these results have suggested a key role for allergen-specific T cells in FA-related symptoms. Recently, CM-specific T cells from

infants with CMA have been shown to express significantly higher levels of CD25 and CD30, both more important T-cell activation markers than CM-specific T cells from infants without CMA. Expression of CD26, a marker of Th1 inflammation, was lower than in normal controls. Following stimulation, antigen-specific T cells from children with FA display an increased expression of cell-surface markers of activation, compared with children without FA [435]. Although it is known that activated T cells of the human intestinal lamina propria are in general high producers of IL_{10}, almost no IL_{10} could be measured in the activated milk-specific T-cell lines. A similar low release was found for TGF-β, both of which contribute to tolerance development in the mucosa.

New insights have been provided into the molecular mechanisms involved in allergic responses of the GI tract. A critical role of *eotaxin* was demonstrated in regulating allergen-induced eosinophil trafficking to the lamina propria of the GI tract, also in the absence of the major eosinophil growth factor IL_5. In the absence of IL_5, allergen challenge promoted partial eosinophil accumulation into the small intestine and a decline in circulating eosinophil levels [542]. Therefore, the accumulation of GI eosinophils is antigen induced, can occur independent of IL_5, and a molecular mechanism explains the dichotomy between peripheral blood and tissue eosinophilia. Furthermore, eotaxin is identified as a critical regulator of antigen-induced eosinophilic inflammation in the GI tract. Eotaxin-1 appears to be more ubiquitously expressed in the GI tract compared with *eotaxin-2,* which is *a potent chemoattractant for eosinophils,* predominantly expressed in the jejunum. By combining the results of comparative experiments between eotaxin-1 and eotaxin-2, both chemokines were demonstrated to have comparable activity in the GI tract [542]. In conclusion, the finding that peripheral blood and tissue eosinophilia can be dissociated in the absence of eotaxin indicates that the relative balance between the expression of eotaxin and eosinophil IL_5 can have profound effects on the relative distribution of eosinophils. As a corollary, underexpression of IL_5 relative to eotaxin can lead to GI tissue eosinophilia in the absence of circulating eosinophilia. Intriguingly, agents that block eotaxin and/or CCR3 may have beneficial effects on modulating eosinophil-associated GI allergies [221].

Humoral Mechanisms

Immune exclusion against CM proteins and tolerance acquisition suffers from significant antibody reduction in infants with CMA (mean, IgA 19%, IgM 15% and IgG 27%) and of IgAs (specific) against β-lactoglobulin (βLG) and casein (11% and 5.5% compared to 60%–50% of healthy controls) [256]. In egg-allergic children, the lymphocyte capacity of producing anti-OVA (ovalbumin) IgAs is deficient up to the 6th year, thus explaining the early onset of CMA [357].

A possible IgG-mediated pathogenetic mechanism has not been confirmed in humans: antigen-specific IgG bound to mast cells are reported in certain patients who release antibodies to pertinent allergens; however, these IgG are characterized by scarce concentrations and short-term effects: thus they fail to play a specific role [429]. Even if IgG_4 show a blocking action to antigens via CIC generation, preventing antigens by competition from reaching target cells or organs, they are present both in healthy and in allergic subjects, so that increased concentrations could exemplify a normal response to antigens, and no pathogenetic role whatsoever has been conclusively ascribed to IgG_1 [59, 212]. The response of involved organisms to a chronic antigenic stimulation is mainly upon IgG_4. This restricted response may be associated with the significant aspect that IgG antibodies may have a protective role for damage due to CIC formation and complement activation when the response is primarily IgG_1 and IgG_3 [96]. Others have not confirmed an IgG protective effect in the first years of life, despite elevated IgG titers in CB and/or in maternal blood [117]. Moreover, anti-CM and anti-egg IgG titers decline in the first 6 months of life, to rise over the subsequent period: an increase in anti-CM IgGs (specific) is a normal event, whereas that of anti-egg IgG is surprising, since eggs are introduced later [117], so we may hypothesize that small doses of egg white presumably found in BM may stimulate an IgG response. However, IgG_4 and IgE immune responses seem to run parallel in some cases, due to a common dependence on Th2 cells.

IgE hyperproduction in atopic children is probably caused by a stimulating effect of IL_4 and IL_{13}, but IgE have been found in CB only in 0.5% of newborns (Table 3.2). However, some infants have elevated titers already in the first months of life, levels that often develop prior to weaning, likely to be in connection with anti-idiotype antibodies from BM.

IgE antibodies to foods are detected soon after birth and develop high titers during the 1st year of life. Based on the data [105, 111], it is not unexpected that high titers of IgE to foods are detected in 14% of infants at 3 months of age and in 31% of infants of 8 months [203]. A transient IgE antibody response to foods is also found in 8% of normal infants, in comparison with 19%–48% of infants at high risk (HR) of atopy [203]. Thus, IgE antibodies to CM and egg white appear frequently in both normal and atopic infants, but elevated titers are found only in atopic infants, thus indicating that predisposed infants are less capable of suppressing IgE antibody responses. However, low levels should not be overlooked, since they can cause severe anaphylactic reactions, whereas moderate titers are often consistent with tolerance acquisition [117]. It is plausible that a massive antigenic load on the GALT favors not only IgE antibody formation, but also formation of IgA, IgG and IgM antibodies with a likely blocking capacity; however, the

titers are very low at the age 7 months [256]. Moreover, the scales may turn to one side or the other depending on the prevalent IL pattern or involved T-cell subpopulation to which sensitized cells are exposed [245].

In conclusion [117]:

The IgE antibodies:

- They are predictive of atopy.
- They appear early and frequently in young infants both in atopics and nonatopics.
- They often appear during breast feeding and before the introduction of causative foods into the diet.
- They are present independently of breast feeding duration and frequently even when breast feeding is exclusive.
- They appear less often in the offspring of nursing mothers on a restricted diet than in the offspring of mothers on an unrestricted diet; however, the effect is limited to the restriction period.
- The IgE response often persists even when all further contact with the allergen is stopped.
- High IgE levels occur in infants with current or future atopy [117].

The IgG antibodies:

- There are mainly maternal antibodies during the first months of life and antibody levels in the infants are not affected by dietetic restrictions during pregnancy and/or lactation.
- Infants with IgE hyperproduction have high IgG concentration, but type I allergy is an IgE prerequisite.
- IgG_4 levels are often increased more than IgG_1 levels: there is a correlation between IgE and IgG antibodies for the same food, contrary to inhalant allergens.
- High IgG concentrations of egg appear to be a good prognostic sign in children with AD [118].
- lgG_4 therefore have no role in pathogenetic mechanisms of FA [117], nor do convincing reports prove an lgG_4 protective function [429], because lgG_4 levels rise during responses to food proteins [212, 429], but no difference in the IgG antibody level was found in patients with positive or negative response to CM challenge [56, 211].

The IgA antibodies:

- Secretory IgA (sIgA) of the intestinal surface and, in lower numbers, IgM antibodies, provide a specific response to the antigens taken up, a response absent in the neonatal intestine where no such antibodies are found.
- The IgA and other BM factors protect newborns from allergic sensitization and thus from FA, especially in infants at risk of atopy, on condition that nursing mothers do not ingest potentially sensitizing foods during breast feeding.
- Antigen-IgA CICs avoid antigens reaching their targets by the same IgG mechanism; however, they have a negligible diagnostic value for an individual patient [275].

Therefore, even if a correlation between IgE and IgG_4 levels has been put forward there is no proof that IgG_4 plays a role in FA pathogenetic mechanisms, nor does a protective function appear to be documented. It can be hypothesized that high IgG_4 levels represent an epiphenomenon of IL_4 that stimulates B lymphocytes to selectively produce IgE and in concomitance IgG_4, likely exemplifying a chronic antigenic stimulation of an immune system not fully controlled and/or subjected to excessive exposures. More important are IgA antibodies that reach breast-fed neonates and infants [275] who are protected from food allergens: only half of infants have measurable levels of IgA anti-CM and/or anti-egg, which are not correlated with IgE and IgG concentrations [203]. In other studies this parallelism is present [117]; anti-food IgM antibodies do not pass the placenta and have a low weight in BM.

Since FA depends on an IgE-mediated mechanism, it is unclear how a sensitization to food proteins is mediated by non-IgE antibodies or how such antibodies interact with the immune system [10].

In Utero Sensitization

The human fetus is able to produce IgE antibodies early (Table 2.3), which cannot act against the fetus. Maternal IgE do not pass the placenta (are absent in CB), which can be traversed by substances with immune properties. Interestingly, the first exposure can be *in utero,* due to intrauterine exposures. The fetus is capable of responding to food antigens ingested by the mother by mounting type IgE antibodies: in the amniotic fluid and CB IgE to CM, egg and wheat have been detected, surely of fetal origin, since the mothers of such children are RAST-negative to the same foods (Chap. 2). Transplacental exchange of immunogenic cells occurs in both directions, an explanation of a possible pathogenetic role of foods ingested by pregnant mothers and transported across the placenta [325]. It has been hypothesized that fetal sensitization is induced by anti-idiotype maternal antibodies, including IgG antibodies directed towards food antigens. The anti-idiotype antibody anti-food antigens, capable of recognizing idiotopes within the paratope, may replace the antigens, mimicking their functional properties, be transmitted to the fetus across the placental filter, and act as antigens in neonates and infants [330] with consequent priming of anaphylactic reactions at the first food administration. Ratner has demonstrated the possible *in utero* sensitization to CM proteins of guinea pigs and postulated that a similar process might even occur in humans [400], a potential explanation for a fetal sensitization to foods previously ingested by the mother, even if these foods are not eaten during pregnancy. No study aimed at verifying the effectiveness of allergy prevention by a CM- and egg-free maternal diet during the last trimester of gestation, not even a randomized study over 5 years, found statistically significant differences on the onset of atopic manifestations in the newborns as related to maternal diet [156]. A fetus of 10–20 weeks already responds to IgE

Table 9.1. Timing of sensitization through breast milk in babies apparently fed only breast milk

Authors/ Reference	No. of babies studied	Age at onset of symptoms	Incriminated foods ingested by the mother (no. of cases)	Tests done and no. of positive tests
Talbot [469]	1	3 weeks	Chocolate	None
O'Keefe [361]	6	>6 weeks	Egg, CM, oat, cod	SPT
Shannon [444]	3	6 weeks–7 months	Egg, oat	None
Ratner [400]	2	2 weeks–7 months	CM	SPT
Lyon [307]	1	3 weeks	Bread, white beans	SPT
Gerrard [176]	81	NS	Wheat (9), orange (8), chocolate (2)	NS
Warner [510]	2	1–4 weeks	CM (1), egg (2)	SPT
Cant [77]	12	3–6 months	CM (7), egg (7), wheat (1)	SPT 12/12
Lifschitz et al [301]	1	31 days	CM	SPT
Cavagni et al [101]	13	1/2–3 months	CM (7), egg (10)	SPT 10/30
Høst et al [225]	9	2–12 weeks	CM	SPT 3/9 RAST 5/9
Cantani et al [92]	21	1–7 months	CM (16), egg (3) Wheat (2)	SPT, RAST OFC 21/21
de Boissieu et al [124]	1	1 month	Egg, pork	Test of intestinal permeability

The studies are ordered according to the year of publication.
CM cow's milk, *SPT+* positivity of skin prick tests, *RAST+* RAST positivity, *NS* not specified, *OFC* open food challenge.

and to a wide spectrum of antigens, inhaled or ingested by mothers during pregnancy, as discussed in Chap. 4. Lastly, CM supplements given in neonatal nurseries are detrimental for *in utero sensitization* in addition to being a risk factor for persistent CMA at age 2.0 years [odds ratio (OR), 3.2%; 95% CI, 1.4–7.8], and early sensitization to egg (OR, 2.8; 95% CI, 1.2–6.6) [408].

Postnatal Sensitization

When human neonates leave the sterile intrauterine habitat, they face a biological status of immunological deficit (Tables 2.1, 2.2); nevertheless a defense barrier should be rapidly organized. In 1918, Talbot [469] reported the development of AD in a 3-week-old exclusively breast-fed infant, obviously a reaction to a food that was never eaten: the atopic manifestations cleared when milk chocolate was completely eliminated from the mother's diet. In the reports *emphasizing BMA,* βLG may be responsible: elevated βLG levels in the milk of several nursing mothers and the serum of their children were found 1 h after BM ingestion [317]. An infant has reacted with vomiting and diarrhea to BM containing βLG levels of 740 pg/ml [17]. More precisely, βLG has been found in BM with frequencies of 18% [462], 44% [314], 47% [238], and 68% [17] of cases; high βLG titers (up to 800 µg/l) have been associated with symptoms of CMA in one child [17]. However, CM hydrolysate formulas (HF) contain even higher βLG rates, contrary to BM [451]. The response depends on the child's immune status: if the child is healthy the allergens will be tolerogen, if atopic, the child will not be tolerant, and the more severe the atopy, the more sensitive the child will be to the allergens. IgE were positive to CM and egg before the official introduction into the diet in 14% of 65 infants aged 3 months [203], and OVA may be detected in the serum of exclusively BM-fed and egg-allergic infants [93]. Actually, BM sensitization is limited to 0.042% of cases [89]. Table 9.1 [77, 92, 101, 124, 176, 225, 301, 307, 361, 400, 444, 469, 510] shows that 153 children have been studied with skin prick tests (SPTs), RAST and OFCs. Minimal doses of heterologous proteins in BM are sufficient to act subsequently as a booster dose, triggering in infants even an anaphylactic shock, frequently labeled as BMA, whereas it is caused by hypersensitivity to CM proteins ingested by their mothers [301, 314] (Table 9.1). Before diagnosing a BMA, one should recall the studies reporting that the exposure to small doses of allergens can sensitize a predisposed individual [247, 248].

The pathogenetic effect of small sensitizing doses disseminated in neonatal nurseries (Chap. 3) agrees with the results of several studies supporting the hypothesis that CMA is significantly more frequent in children who received CM supplements in a period of particular vulnerability, in comparison with infants exclusively fed BM since the first hours of life [78, 86, 225]. Significantly, *only the 39 infants with adverse reactions*

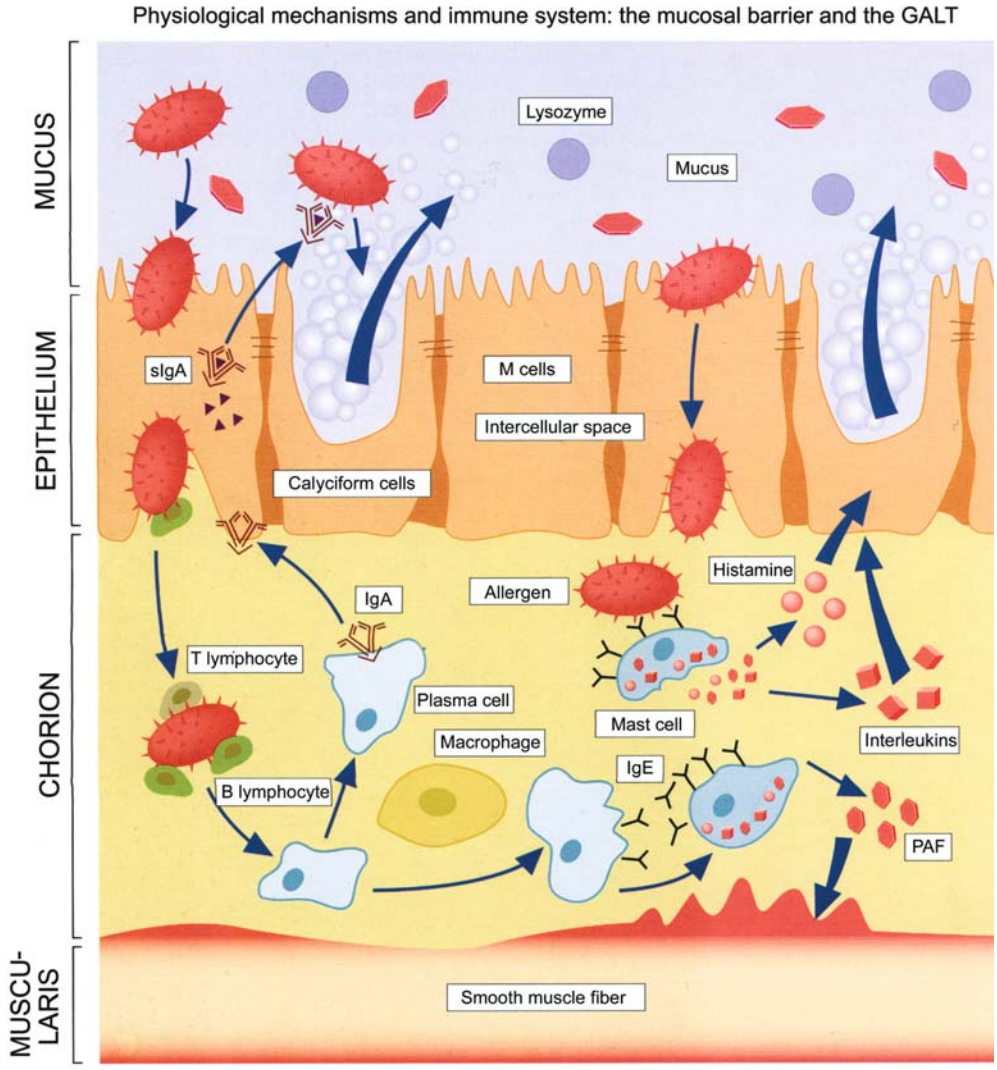


Fig. 9.2. Physiological mechanisms and immune system: the mucosal barrier and the GALT. The intestinal mucosa is not impenetrable: it shows open gates, which allow some molecules to cross its walls, thus penetrating into the blood circulation. *sIgA* Secretory IgA

to CM were fed CM supplements in the first 3 or 4 days of life [224]. Evidently, if neonates with atopic predisposition were not fed potential allergens early in life, the development of an atopic disease would be postponed and the included risk lessened [93] (Chaps. 3 and 24). We are a long way from seeing a complete picture, but the drop in sIgA antibodies in both colostrum and milk in mothers of neonates who will be affected with CMA later on compared to mothers of nonallergic children is significant [431].

Immunology of the Gastrointestinal Tract

To understand how foods, necessary for a child's normal development, provoke lesions in different organs, they should be viewed as a "non-self" that GALT must transform into small molecules to make them immunologically tolerable. When in particular conditions the foods deliver antigenic characteristics, they become capable of stimulating the immune responses, including a high molecular weight (MW) of proteins by which are formed macromolecules; other crucial functions are carried on by microbes, viruses, enterotoxins, etc. [348] (Fig. 9.2) [340].

The GI tract is composed of four concentric layers, which from the lumen inward are the *mucosa* (epithelium, lamina propria and muscularis mucosa), *submucosa, muscularis externa* and *serosa,* specialized tissues containing lymphocytes, macrophages, mast cells [327], and eosinophils [542]. From an immunological point of view, the GI tract can be described as an epithelial tube separating intraluminal immunogenic peptides from immunoreactive cells and antibodies residing in the

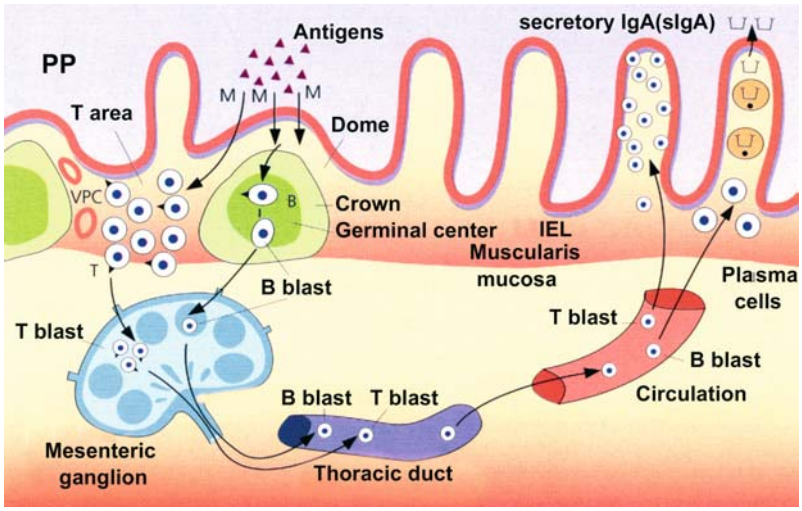


Fig. 9.3. GALT is formed by Peyer's patches (*PP*), intraepithelial lymphocytes (*IEL*) and lamina propria lymphocytes. *M* microfold (cells)

Table 9.2. Lymphocytes and Ig distribution in the intestine and circulation

Lymphocytes (%)	Peyer's plaques	IEL	Lamina propria
B Lymphocytes	60	<2	40
T CD4 lymphocytes	30	15	40
T CD8 lymphocytes	10	80	10
	TcR (% of $CD3^+$) circulation	**IEL**	**Lamina propria**
TcR-γ/δ	0–8	14 (1–63)	4 (0–10)
TcR-α/β	≥100	50–100	60–100

Data from [310, 460].
IEL intraepithelial lymphocytes.

lamina propria. T lymphocytes found in the lamina propria and between epithelial cells form a diffused lymphoid tissue, which has effector functions for the GALT efferent arm [440]. The greatest lymphoid organ of the human body playing a central role in the intestinal immune function includes:

- PP and appendix
- Lymphocytes and plasma cells of the lamina propria
- Solitary lymphoid follicles
- IELs
- Mesenteric lymph nodes, which differ from other lymphoid organs because they do not possess capsule and afferent lymphoid vessels

The sIgA antibodies predominate in the GI tract and are a major characteristic of the human GALT [440] (Fig. 9.3) [343].

Lymphocytes

In the adult intestine (about 9 m long), there are about 10^{10} lymphocytes/m, a striking feature when one considers that all immunoglobulin-producing cells in bone marrow, spleen and lymph nodes combined are as high as 25×10^{10} [310]. The majority of B cells are committed to IgA synthesis and undergo an isotypic switch so that sIgA production in both PP and lamina propria is promoted rather than the IgM isotype [333]. Almost all T cells in the intestinal epithelium and connective matrix of lamina propria use an α/β TcR, and only 10% of epithelial $CD3^+$ are γδ, characterized by a Vγ1/Vδ1 gene rearrangement and in the bloodstream by Vγ9/Vδ2 [310]. About 50% of IEL T cells and 80% of lamina propria T cells are $CD45RO^+$ memory cells; lymphocyte and immunoglobulins (Igs) are summarized in Tables 9.2 and 9.3 [310, 333, 460]. Notably, $CD8^+$ may be positioned nearer epithelium, while $CD4^+$ are found more centrally, in the villi. This location supposedly has the goal of regulating immune response to soluble antigens in the gut [490]. The presence of T lymphocytes is not imperative for the mucosal integrity of GALT: this is supported by the morphologically normal mucosa in infants with SCID (severe combined immunodeficiency) without B and T lymphocytes [457]. As regards lymphocyte circulation (Fig. 1.12), PP B cells, if sensitized, migrate to mesenteric lymph nodes, where they differentiate, returning to colonize even distant mucosal sites including breast [185] (Fig. 2.18).

Table 9.3. Immunoglobulin distribution in the human intestinal tract

Location	IgA	IgA_1	IgA_2	IgM	IgG
Gastric body	74	80	20	12	14
Antrum	80	85	15	8	12
Duodenum/jejunum	70	70	30	17	4
Ileum	80	55	45	11	5
Colon	90	38	62	6	4
Rectum	ND	45	55	ND	ND

IgA, IgM and IgG antibodies are expressed as a percentage of all Ig-containing cells; IgA_1 and IgA_2 antibodies are expressed as a percentage of IgA-containing cells.
Data from [333].
Ig Immunoglobulin, *ND* Not done.

Macrophages

Leaving aside their functions, in the GI tract macrophages are located in subepithelial sites, superficial epithelium, and under the PP dome and reside around efferent lymphatics. Scarce in IELs, these cells are more abundant in the lamina propria, where 80% of cells expressed by HLA-DR antigen have a dendritic cell (DC) appearance, whereas in the colon 60% show phenotypic features of macrophages [311]. Strategically located beneath the surface epithelium, in addition to classic defensive phagocyte functions, they show little evidence of providing costimulatory signals for T lymphocytes [202]. Many of these cells express large amounts of HLA class II antigens and other surface markers associated with phagocyte activity and participate in mechanisms of nonspecific defense, but produce low levels of IL_1 and IL_6, necessary to the local differentiation of B cells. Although macrophages might present antigens to $CD8^+$ IEL in a DQ-specific fashion, they express few activating signals to lamina propria $CD4^+$, since they produce little or no CD54, which is important for T-lymphocyte adhesion via interactions with CD11a, leaving T cells in an antigen-independent state of anergy [442].

Dendritic Cells

Interdigitated in T zones, DCs of oral epithelium, similarly to cutaneous DCs, act as antigen presenting cells (APCs), independently of their origin and antigen administration route [484].

Mast Cells

Distributed throughout the length and breadth of the human GI tract, small in size with a rapid turnover, mast cells increase in number in FA [15]. Mucosal mast cells are T cells, whereas peritoneal mast cells are TCs (Table 1.27) and also may participate in the host defense, because mast cells may recruit inflammatory cells to the site of parasitic infection, etc., but food-sensitive subjects have exhibited food-specific, mast cell-dependent reactions upon ingestion or direct exposure to the appropriate antigen [15]. Mast cell immunological function has been appraised both in children undergoing FCTs, in whom histaminemia underlies degranulation (and that of basophils), and by measuring mast cell protease, a specific marker of cell secretion, or tryptase [365]. T mast cells, localized predominantly in the lamina propria (20,000/mm^3), facilitated by both LPAM-1 (lymphocyte Peyer's patch HEV adhesion molecule 1) and MAdCAM-1 (mucosal addressin cell adhesion molecule-1), are juxtaposed to a true network of neuropeptides, where substance P (SP) operates, and work as sensory receptors of the so-called *enteric mini-brain,* transmitting information on the microenvironment to peripheral nerves and CNS [132]. Data circulate in a bidirectional way, thus also the CNS, through its neurotransmitters, can send impulses to the periphery, where SP is able to evoke mast cell degranulation via subliminal stimuli [132] (Fig. 9.4).

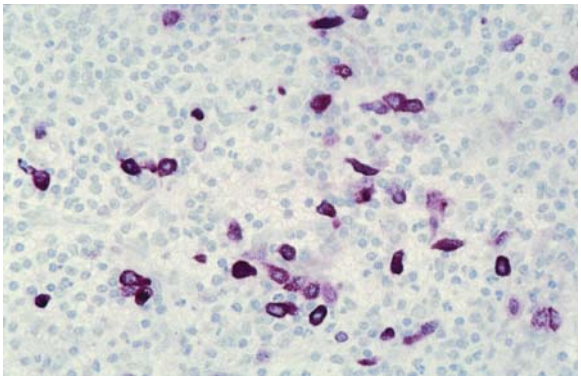


Fig. 9.4. Mast cells in normal intestinal mucosa

Eosinophils

Children with CMA frequently have eosinophilia during CM feeding, with this sign present in 33%–50% of children [385]. Prominent eosinophil infiltration is seen in several diseases to be discussed. New insights have been provided into the molecular mechanisms involved in allergic responses of the GI tract. In the genetic absence of the chemokine eotaxin, constitutively expressed in the GI tract as a key regulator of eosinophil circulation during GI allergic processes, eosinophil recruitment into the small intestine was reduced. A critical role of *eotaxin* was demonstrated in regulating allergen-induced eosinophil circulation to the lamina propria of the GI tract, also in the absence of the major eosinophil growth factor IL_5. Interestingly, in the absence of IL_5, allergen challenge promoted partial eosinophil accumulation in the small intestine and a decline in circulating eosinophil levels. Therefore, the accumulation of GI eosinophils is antigen-induced, can occur independent of IL_5, and a molecular mechanism explains the dichotomy between peripheral blood and tissue eosinophilia. Furthermore, eotaxin is identified as a critical regulator of antigen-induced eosinophilic inflammation in the GI tract. Eotaxin-1 appears to be more ubiquitously expressed in the GI tract compared with eotaxin-2, which is an eosinophil chemokine, induced by IL_4 overexpression. By combining the results of comparative experiments between eotaxin-1 and eotaxin-2, both chemokines have been demonstrated to have comparable activity in the GI tract [542].

Mucosa

The intestinal mucosa quantitatively makes up the largest mediator organ of humoral immunity. GALT discriminates between potentially pathological and harmless stimuli: in one part it elaborates suppressor activity to neutralize ubiquitous antigens, in the other it is capable of responding adequately to stimuli by producing antibodies interacting with potential pathogens, which are eliminated without concurrently eliciting inopportune inflammatory reactions. GALT responses are able first to block the way to and then to eliminate antigenic macromolecules [51].

Epithelium

The intestinal epithelium alone includes 25% cells of lymphatic origin: it is constituted intrinsically by columnar cells capable of absorbing foods, muciparous calyciform cells that produce intestinal mucus and IELs [440]. The effector functions carried out by these cells are exclusion, transport, breakdown and antigen presentation, bidirectional transport, and antibody secretion [311]. The human intestinal epithelium, including the cells overlying PPs, express HLA-DR antigens as PPs, which may therefore *play a role in antigen presentation to the local immune system* by epithelial cells, but rarely in the normal subject [311]. Even if important costimulatory molecules of B cells such as CD80/CD86 and CD54 are absent, it is believed that epithelium is the preferential afferent mode of intestinal immune responses, by taking polypeptides up and activating the CD8: the key ligand is gp180 (CD45RO) of IgSF (immunoglobulin superfamily) [254] (Table 1.4). Instead, in experimental animals the increment of HLA-DR expression, modulated by local IFN-ɣ production by mucosal T cells, up-regulates the enterocyte capacity of to act as APCs [536].

Peyer's Patches

Described in 1667 [386], PPs (Fig. 9.5) are well developed in fetal life and increase in number and size progressively from 50–100 in neonates to 200–300 by late adolescence [311, 460]. The most prominent PP feature is the follicle center, containing centrocytes and centroblasts [185]. The specialized dome epithelial cells derive from Lieberkühn crypts adjacent to the follicles, are part of follicle-associated epithelium (FAE), with a possible function of separating PPs from enteric lumen [311]. PPs are located in the human ileum, but mainly in the mucosa and submucosa, in an immediately subepithelial position, and reach the length of several cm [185]. More precisely, in contrast with various animals, in humans they are of a higher number and size in both the ileum and jejunum, where the follicles number between 10,000 and 20,000. The ileal PPs undergo

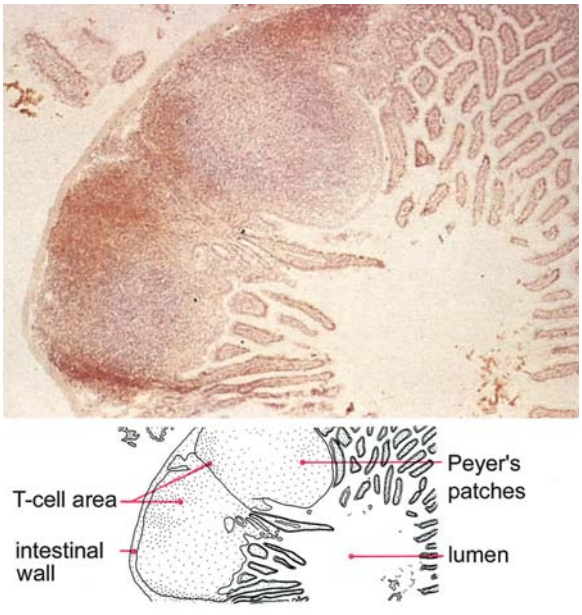


Fig. 9.5. Peyer's patches in mouse ileum, T-cell areas are stained *brown*

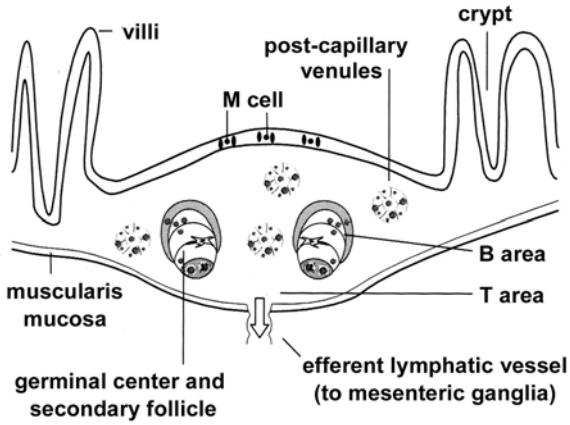


Fig. 9.6. Structure of Peyer's patches

an involution in a way similar to the thymus, whereas jejunal PPs persist in humans throughout their lifetime [185]. Interestingly, *PPs develop during fetal life* when the gut lumen is sterile, thus suggesting that foreign antigens are unessential to start both B cell colonization and expansion within PPs [185]. In humans, PP architecture is comparable to that of lymph nodes, with similar B- and T-dependent areas; the lymphoid follicles with B cells (60%–70%) express sIgA, sIgM and plasma cells, encircled by T lymphocytes (25%–35%) with prevalent CD4 phenotype [461] (Fig. 9.6).

FAE is a one-cell-thick lining layer with fewer villi compared to the epithelium of the surrounding intestine; calyciform cells are few in number and the SC (secretory component) is not seen [327]. On entering the lumen of the small bowel, immunogens present in the gut are sampled by way of specialized M cells (Fig. 9.7), overlying the dome of lymphocytes, which are extremely efficient at sampling antigens infiltrated in the lumen of the small bowel to deliver such antigens to underlying antigen-specific B cells, which are thus transformed into antibody-secreting plasma cells if the appropriate costimulatory molecules are also present [461]. However, B cell activation, as reflected by germinal centers, is not apparent until shortly after birth (Fig. 1.5). M cells are rare in humans, despite countless studies in rodents [185], but are believed to play a key role in the induction of mucosal immune responses, since in the PPs antibody responses against food or microbial antigens are induced [185]. Non-self substances are taken up by M cells, which differ from other mucosal epithelial cells since they have few microvilli, have a poorly developed glycocalyx and no lysosomal organelles [508]. Such data support the view that M cells are especially well adapted for microorganism and endoluminal peptide uptake, which are then transported directly to mucosa-associated lymphoid tissue (MALT), in that since they are probably HLA-DR$^-$, they are unlikely to play a role in antigen presentation [311]. Recent reports stress that M cells are important as a route of antigen entry into GALT. Unlike

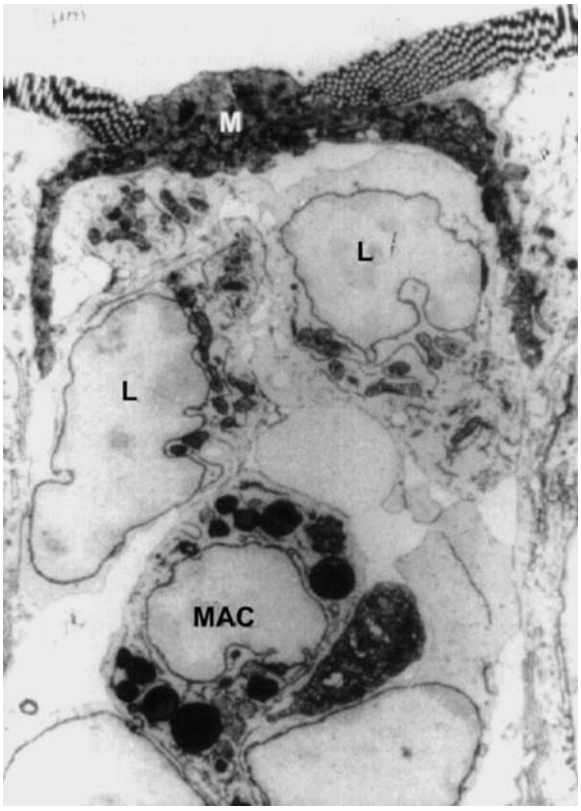


Fig. 9.7. M cell ultrastructure. *M* M cell, *L* leukocytes, *MAC* macrophages

the intestinal epithelial cells that take up soluble antigens, M cells are extremely efficient in sampling and transporting particulate antigens, and their surface appears to be specialized for endocytosis of antigens, which are then processed and presented to immunocompetent cells such as CD8 localized nearby [254]. DCs are present in normal PPs, follicular DCs (FDCs), and macrophages express the HLA class II molecules necessary to present macromolecules to B and T precursors, with consequent activation of these cells. Therefore, the PPs would be for food allergens an inductive site for lymphocyte activation [334].

Antigens of the GI mucosa, via intracellular uptake and ensuing digestion or via intercellular spaces (Fig. 9.8), gain access to PPs, thereby stimulating the local immune system [508]. In turn, B and T lymphocytes reach PP subepithelial and interfollicular zones through small postcapillary vessels of the HEV (high endothelial venule) type [442]. MAdCAM specific of MALT (Table 1.45) is expressed on HEVs of PPs and mesenteric lymph nodes; naive T cells also require CD62L to adhere to HEVs [254]. PPs may have the function of bringing circulating CD4 into contact with intraluminal antigens, and consequently their priming associated with expression of functional CD40 in ileal PP B cells may occur after B cells able to respond are selected, and their expansion is modulated by a combination of

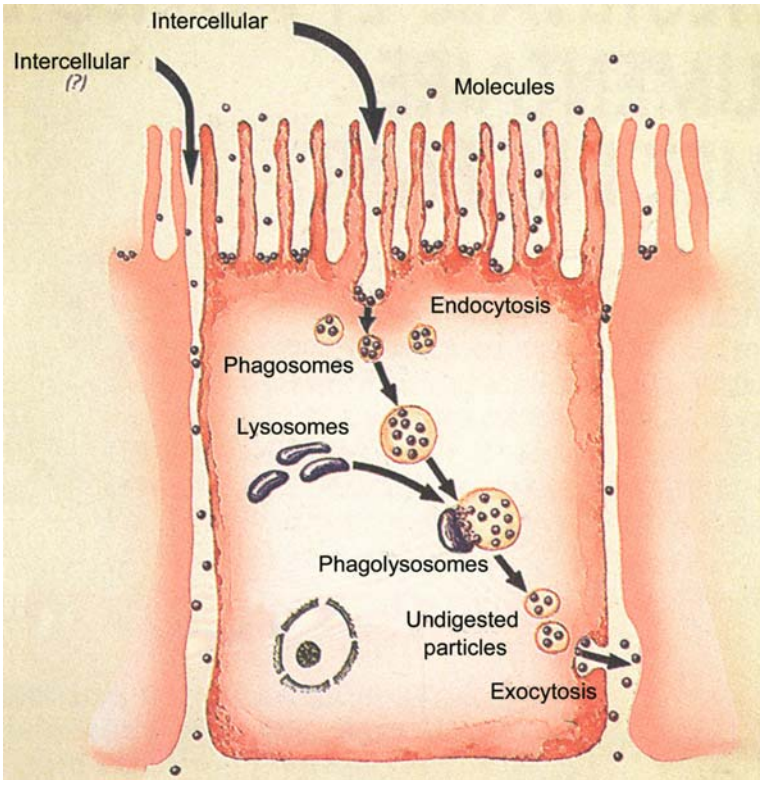


Fig. 9.8. Antigen uptake by enterocytes

ILs in concert with CD154/CD40L [185]. Another prospect is that interactions between B cells and T cells induce the latter to migrate to the periphery via efferent lymphatic vessels, mesenteric lymph nodes and the thoracic duct, consistent with their known migration route. Returned to their final stage, the lamina propria of the intestinal wall, or other secretory areas, CD4 T cells may induce B lymphocyte differentiation in plasma cells-IgA when antigen priming first occurs in the GI tract [275] (Fig. 1.12). B follicles are also found in the colon, but aggregates of follicles akin to those seen in small bowel PPs are not seen in the colon, nor has their function been widely scrutinized [185, 311].

Lamina Propria

Early in life, the lamina propria is infiltrated with B lymphocytes in large numbers committed to IgA synthesis (Tables 9.2, 9.3) and plasma cells (in decreasing order) IgM, IgG, IgE, IgD [457]. The sIgA are the majority, 70%–80%, of all GI Ig-producing cells; the remaining include sIgM and IgE, which amount to 5%, so it can be estimated that ≈80% of all Ig-producing cells of the body are localized in the GI mucosa [51]. In humans, sIgA number some 10^{10}/m of small bowel [51] (Fig. 9.9). Contrary to airways, tonsils and lymph nodes, where IgA predominate, IgA_2 are more numerous (60%) [333]. However, the majority of IgA secreted by plasma cells are dimeric: J-chain expression is more prominent in

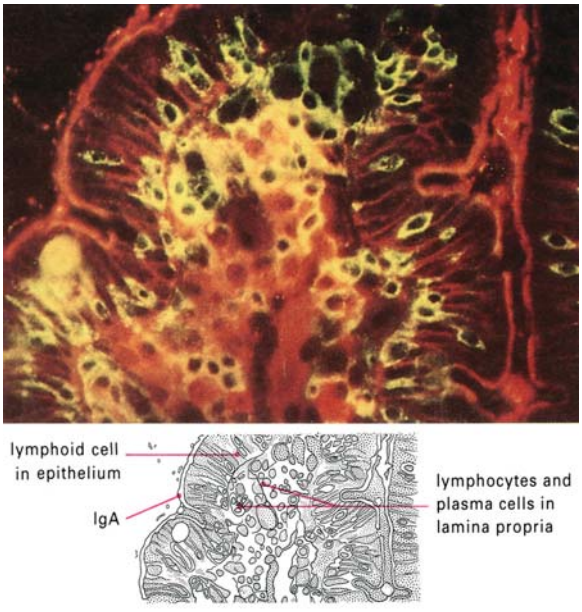


Fig. 9.9. Section of human jejunum showing lymphoid cells stained *green*, in the mucosal epithelium and in the lamina propria. A red fluorescent anti-IgA conjugate stains the plasma cell cytoplasm in the lamina propria and detects IgA in the surface mucus

IgA_2 (100%) than in IgA_1 (88%) [268]. Recently, SC has been found to be a key factor in sIgA and sIgM generation: their expression may be marked by IFN-γ, IL_4 and

TNF-α [442]. IL_4–IL_6 act as B cell terminal differentiation factors [442]. T cells and activated macrophages secrete sIgA and sIgM, which are then actively transported during mucosal reactions. The IgA activity is known to ensure the first line of defense in the context of immune homeostasis [508]: in selective IgA deficiency, IgM dominate in the GI mucosa and IgG are not increased. The sIgA transported in the intestinal environment, once SC binds polymeric IgA, is carried into epithelial cells by pinocytosis. The complex is then transported in vesicular form across the cell to the luminal border. Given its unique structure, sIgA is less susceptible to proteolytic digestion and retains most of its functional capacity while passing along the GI tract [310, 348]. Two different subsets of T lymphocytes are visible, but $CD4^+$ predominate in the lamina propria and in mesenteric lymph nodes [244]. There is lymphocyte differentiation with specific and nonspecific mechanisms either cell-mediated with induction of IgA-specific CD4 and of IgG-specific suppressor CD8, or humoral with B lymphocyte activation and proliferation and IgA raised production [440]. Resident T cells may induce an isotype switch in B cells, so that IgA synthesis is prominent on IgM isotype. Moreover, T cells with Fc receptors for IgA may help commit B_{IgA} development into true plasma cells, a *crucial event in neonates and young infants with no lamina propria IgA* [244]. T and B cell collaboration is underlined by CD2–CD58 interactions, a means of control of IgE synthesis independent of the classic CD40–CD154 interaction (Fig. 1.22a). At this level, the $CD4^+$-to-$CD8^+$ ratio is about 4:1, as in peripheral blood [310]. Roughly one-half of CD8 T cells have cytolytic function and express CD28, 10% are $CD25(IL_2\text{-R})$; very few CD16 are associated with NK cells and CD49a/29 or VLA-4 [244, 310]. In light of these data, lamina propria T lymphocytes possess specific features that distinguish them from subsets localized in other organs and tissues. Homing receptors bind T cells selectively to PP HEVs: this binding is mediated by $\alpha_4\beta_7$ (LPAM-1), with an α chain homologous to α_4 of CD49d/29 (Table 1.45), expressed also by IELs, notably a MAdCAM ligand [202]. The MAdCAM–CD49d/β7 couple is sufficient for this circulation; however, CD62E is absent [254]. The weight of β7 integrin is such that it is expressed in addition to mast cells by human B lymphocytes when they migrate to the lamina propria for terminal differentiation, and the critical role of $\alpha_4\beta_7$ in the existence of GALT should be recognized [504]. In allergic subjects, CD4 is already activated, as demonstrated by T cells expressing IL_2 receptor mRNA (CD25), by HLA class II raised expression and even higher expression of IFN-γ, IL_4 and IL_5 mRNA [245]. CD4 T cells are demonstrated in vitro; there is a reason lamina propria CD4 express more helper activity than suppressor activity as compared to CD4 of other tissues. This activity is exercized upon B lymphocytes, thus modulating in particular local IgA production [202]. Lamina propria contains many mast cells of the mucosal type [15] and can be infiltrated by neutrophils, eosinophils and macrophages.

Solitary lymphoid follicles, presumably functional PP equivalents, are disposed throughout the entire GI tract, especially in colon and rectum; on the whole, they averaged $9/m^3$ of large intestine, usually with germinal centers.

Intraepithelial Lymphocytes

The IEL population (Table 9.2) is not homogeneous: the epithelium lining the enteric mucosal surface contains up to 20% nonepithelial cells, the greater part of which constitute the IEL population, residing in the basolateral surface of epithelial cells that make up intestinal villi, in the interstitial spaces between epithelial cells. IELs can also interact with immunogens that can cross to the epithelial cell surfaces, since they first encounter the myriad of antigens present in the GI tract. Although quantitatively fewer than lamina propria lymphocytes, they are consistent in number, in that in normal humans one of every 5–8 enterocytes and 9–40 out of 100 epithelial cells are IELs, whose numbers increase in inflammatory states [202]. IELs express $\alpha_E\beta_7$ integrin recognized by CD103 (Fig. 9.10), whose expression is amplified by TGF-β1. IELs adhere to epithelium by heterotopic adhesion between CD103 and E cadherin on epithelial cells [254] (Table 1.51). The $\alpha_E\beta_7$ integrin is the only one expressed also by pulmonary IELs and is known to mediate a double T homing. In the IEL subpopulation of the normal human small bowel, 70%–80% are CD8 T cells expressing the CD45RO and CD45RA markers, thus suggesting that they are activated or memory T cells, more than 15%–20% of cells with the CD4 phenotype, half of lamina propria CD4 [51]. $CD2^+$ and $CD3^+$ (Fig. 9.11) are 5%, $CD3^-$, $CD4^-$, $CD8^-$, $CD7^+$ in an unspecified share and CD49a/29 50% [202]. B lymphocytes are virtually absent. CD8 T cells could display a CTL activity, but have phenotype of memory cells, divided between CD45RO and CD45RA [195] (Fig. 9.12). Most human CD8 employ the TcR αβ, but their mediation of oral tolerance is an unproved hypothesis [51]. Animal studies have delineated the IEL effector functions, including NK activity, specific cytotoxicity, IFN-γ secretion, and an intraepithelial increase in HLA class II expression [442].

The heterogeneous IELs are further distinguished by their usage of TcR chains: the thymus-dependent IELs express the TcR αβ chains and thymus-independent IELs express TcR γδ chains (Tables 9.2, 9.3); the latter are few (10%–20%), equally divided between $CD8^-CD4^-$ and $CD8^+$ [202]. Epidemiological evidence suggests that γδ TcR carry on a mucosal defensive function. The γδ IELs also mediate a contrasuppression supporting IgA responses [51], so that IgA antibodies escape suppression as part of a defense system and can confront antigens through interactions between IEL γδ and lamina

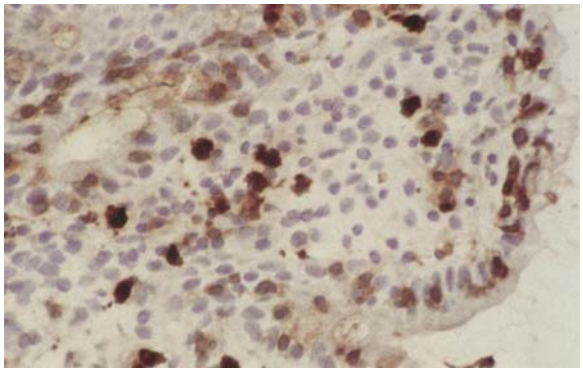


Fig. 9.10. IELs present in the intestinal mucosa demonstrated with CD103 ligand of $\alpha_E\beta_7$

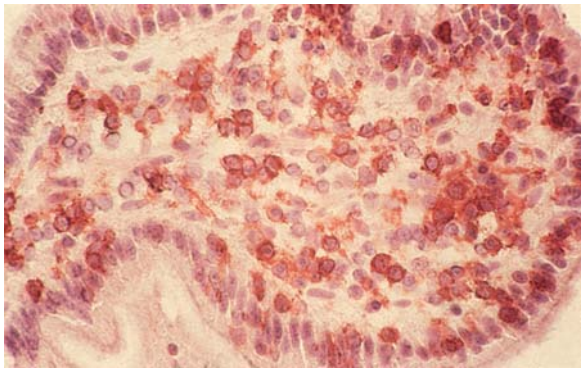


Fig. 9.11. CD3 (*in red*) demonstrating T lymphocytes in both mucosa and submucosa

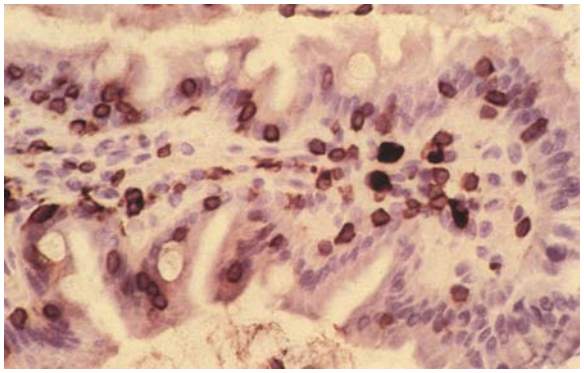


Fig. 9.12. CD45RO demonstrating the antigen-induced phenotype of the majority of T lymphocytes present in the intestinal mucosa

propria $CD4^+$ cells, resulting in an increased production of sIgA [172]. HLA-unrestricted IELs recognize antigens different from αβ TcR, and consequently they could possess an antigen-specific regulatory function [172]. The γδ IELs can regulate the mucosal IgA response, a finding further supported by γδ-knockout mice, which show a pronounced decrease in IgA plasma cells in the GI mucosa and of serum IgA levels [254]. Moreover, γδ may be especially active in the first line of defense against pathogenic microorganisms, function as a protective barrier to antigenic stimuli, or may be scavengers that participate in the elimination of stressed or damaged epithelial cells [442]. In children with CMA, an increment of γδ IEL was shown, characteristic but nonspecific in CD children [278]. Moreover, epithelial expression of IL_7R is up-regulated on γδ IELs stimulated by IL_2 and IL_7 or SCF (stem cell factor) for which IELs express a c-kit receptor [51].

Mesenteric Lymph Nodes

Mesenteric lymph nodes are an integral part of GALT, although actually situated outside of the GI tract. Once activated by immunogens, leukocytes in the gut mucosa can drain to the mesenteric lymph nodes, from which they can gain access to the peripheral circulation. Activated leukocytes also can recirculate back to the mucosa, in that these nodes lie in a vital area for lymphocyte recirculation. Mesenteric lymph nodes also play a cardinal role in the synthesis and maturation of IgA antibodies, also receiving lymph from the whole GI tract via lymphatic vessels running along mesenteric arteries. Germinal centers are more evident in the nodes subjected to antigenic assault. The lymphocytes are mostly naive T cells, a group of immune cells ready to support the weight of immune responses to a new antigenic contact; IL synthesis favors IL_4 with the role of increasing the proliferation of both T and B cells, but inhibiting B-cell differentiation, at the same time encouraged by the low level of IL_2 and IFN-γ activity [245].

GALT Regulation of Effector Functions

GALT is one of the more sophisticated homeostatic mechanisms mainly supporting the immune protection of babies in the first days of life, from systemic and abnormal immunological reactions to a great number of new food antigens, thus promoting a certain degree of oral tolerance. Such specific mechanisms should ensure a strong local defense at the same time, and meet the possible danger represented by the systemic invasion of macromolecules and CICs. Consequently, the GALT is exposed to an unremitting flow of non-self substances with antigenic activity. GALT's crucial function within the MALT is as a central organ with interactions also with other MALT organs such as bronchus associated lymphoid tissue (BALT): for example, oral vaccines are produced to protect children from respiratory infections. Animal studies disclose that oral immunization with *Haemophilus pleuropneumoniae* has been shown to result in an increase in the number of lymphocytes in the bronchoalveolar lavage fluid (BALF), deriving from the migration of gut lymphocytes to the airways [137].

Similarly, the analysis of the distribution of T cells specific to *Pseudomonas aeruginosa*, following intestinal immunization of rats with killed *P. aeruginosa* strains, has shown that T cells appear in PPs, mesenteric lymph nodes and blood and can be found in lung tissues following challenge with live bacteria. Hence, bacteria-specific lymphocytes migrate from the gut to airways, where they reside until re-stimulated by antigens [137]. The same mechanism may be operating in food-induced asthma: food absorption is potentially amplified by the permeability increase common in these cases, which permits allergen passage into the bloodstream and the subsequent action upon the airways. A contrary case may occur, that GI allergic diseases may be related to inhalant hypersensitivity, as in a 5-year-old Der p-sensitized girl who responded with morning vomiting to nocturnal house dust inhalation [434]. Fecal Der p allergens, which to a large extent yield an enzymatic activity, are synthesized and secreted into the alimentary canal, and may prompt an inflammatory response in a nonspecific and nonimmunological way.

The important GALT functions carried out in the host defense or food tolerance are summarized in three closely interrelated mechanisms: immune exclusion, immune elimination and immune regulation.

Immune exclusion is mediated by sIgA, along with aspecific mechanisms that have not yet been elucidated, and has a double objective: sIgA antibodies activate only the alternative complement pathway and are protected from proteolysis by SCs, have the task either of inhibiting the adherence of microorganisms and non-self macromolecules to the mucosal surface or of preventing both contact and ensuing absorption of antigenic molecules through epithelial cells [333], as indirectly confirmed by an increased antigen absorption in individuals with selective IgA deficiency [51]. The process operates in tandem with hepatic clearance cleansing the antigens that have entered [461]. B lymphocytes, after the isotype switch in B_{IgA}, leave the PP and via the lymph and the circulation populate the GALT, where they produce IgA polymers with the J-chain, subsequently handled by SCs at the mucosal level [333]. The predisposition of the immune system to activate chiefly the local IgA response following stimulation by intestinal antigens could prevent severe local changes [440]. Therefore, it is not without significance that IgA has the same antigenic specificity in the whole context of secretory areas: this aspect of the immune response confirms the prevalent concept, which identifies GALT as a sole secretory organ being part of MALT [348].

Immune elimination represents the *second line of defense* to non-self substances that have penetrated the GALT barrier, made up of mucosal IgG and IgM and RES (reticuloendothelial system) cells, including Kupffer cells: humoral responses are mainly based on the action of secretory antibodies, that is, surface IgA and in small part IgM. In adults, IgA are the most numerous and most important of surface Ig classes (Table 1.15), whereas in neonates both sIgA and sIgM are insufficient. No different is the case of 7.6-month-old infants with CMA with antibody levels significantly lower than in healthy infants, thus confirming that IgA serum levels against CM proteins are inversely correlated with the clinical expression of atopy [256]. The elevated IgA_2 resistance to bacterial proteases is a prominent characteristic [333]. Macromolecular ingestion via CD4 T cells stimulates IgA synthesis, thus reducing antigen absorption, and antigen-specific CD8 T cells suppress antibody responses able to activate the complement cascade. Consequently, the antigen cannot link to the specific antibody and since complement fixation is not evoked, no CIC formation and inflammatory processes are triggered [461].

Immune tolerance or *immune regulation* is a specific state of immune hypo- or non-responsiveness of lymphoid tissues against particular antigens determined by prior exposure, and the substance in question is defined as a tolerogen [311]. Experimental evidence on intestinal immunoregulation, because of obvious ethical considerations, comes from laboratory animals; however, there are valid reasons to demonstrate that such mechanisms also operate in human beings [228]. We mention in this context that Dakin was the first to describe the American Indians practice of chewing and ingesting *Rhus* leaves to prevent a severe form of contact dermatitis induced by poison ivy [114], and Besredka demonstrated in a classic experiment that guinea pigs tolerated orally administered CM and were unaffected by sensitization and anaphylaxis when the same food was parenterally injected [33]. Chase established the bases of oral tolerance, which was also named after Sulzberger, who observed that feeding the hapten picryl chloride leads to suppression of responses against a second parenterally administered antigen if the oral antigen was re-fed along with the parenteral antigen [348]. Mice ability to develop tolerance has been shown to occur at around 4 days of life, although a transient defect in the capacity of developing tolerance takes place when mice are weaned [348]. In oral tolerance, studied in man to a limited extent, intact proteins entering the circulation usually cause no clinical response, because T-cell clones responsible for these responses were eliminated or inactivated by a previous contact with the same antigen: this may be the consequence of an encounter in the fetal or early postnatal life, or exposure to very high antigen concentrations *(high-dose tolerance),* or continuous presence of very low antigen concentrations *(low-dose tolerance)* [461]. Tolerance can be typically evoked also by lower doses than the immunogen doses (on the average 100-fold lower [460].

Additional experimental *studies on oral tolerance* have investigated the pronounced antigenic trend to evoke antigen-specific suppressor T cells in PP: it is unclear why suppressor circuits originate in the MALT/GALT system more rapidly than elsewhere; the hyporesponsiveness could be linked to T lymphocytes, in that

B cells remain potentially reactive to T cells transferred from an intolerant animal [460]. It is significant that in normal mucosa, the *antigen-nonspecific* suppressor cells play such a relevant role in the suppression of responses to specific antigens, even if this hypothesis does not receive much support: it has been shown that in animals genetically unresponsive to lipopolysaccharides (LPs), able to elicit in B cells the differentiation into antibody-secreting cells, the mechanism of oral tolerance is inhibited; as a consequence following an OCT (oral challenge test), the responses to selected antigens are increased and those of suppressor T cells wholly absent [461]. The failure of oral tolerance may have an immune background, as demonstrated by a decreased tolerance in autoimmune mice strains: thus we can speculate that an inappropriate reactivity to one or more antigens may condition the development of autoimmunity [461]. The demonstration that PPs contain suppressor cells of IgM and IgG responses, but help IgA function, has shown which mechanism might explain the coexistence of two apparently unrelated responses, a local immune stimulation and a systemic hyporesponsiveness (oral tolerance). Thus, host responses to enteric antigens involve the suppression of IgG systemic responses and concomitant induction of local mucosal IgA responses contributing to tolerance [508]. Low colostral and BM sIgA levels are atopy-associated [431] and in children with an active FA, IgA responses increase only when clinical tolerance to food antigens is acquired [249].

Oral Tolerance: Experimental Data

In normal subjects, the absorption of antigenic molecules via stimulation of inhibiting T cells leads to suppression of IgE synthesis, which is controlled throughout life [222]. In this model, *allergy can be defined* as the consequence of an immunodeficiency that interferes with the mechanisms regulating IgE suppression, which may likely lead to a breakdown of this protective immune response, thus resulting in an immediate hypersensitivity reaction and in clinical injury to the gut [457]. The antigen's foreignness is not exclusively correlated to the chemical structure or molecular conformation, depending in certain cases on the genetic background of both the donor and recipient [460]. Thus, the *antigen immunogen power* also depends on a comparison between the two genomes, and recipients should possess genes capable of recognizing antigens: when a strain is unable to respond to immunogen antigens of another strain, oral tolerance may be under the control of different genes [348]. Acquisition of tolerance and conversely development of food sensitization are therefore a multifactorial process including numerous variables, many of which can be investigated only in experimental conditions.

Minimal proportions (10^{-4}–10^{-7}) of ingested foods reach the circulation of normal infants undegraded after passing across the gut barrier and are eliminated without eventful sequelae [457]. The background of these neutral effects is to be found in states of normality, when a reduced amount of antigen passes across the barrier, but is blocked by IgA and undergoes an intraluminal digestion; as a consequence, only a small portion reaches the portal circulation by perabsorption. However, the low quantity of initially produced IgE antibodies is unable to saturate the metachromatic cells and induce their degranulation, so a repeated oral intake of food proteins commonly induces the development of a *specific immunological tolerance*. It is believed that when no tolerance develops, as caused by lacking or deficient IgA antibodies and/or elevated IgE titers, increased numbers of antigenic macromolecules would cross the barrier, thus making alimentation quite inconceivable [413]. In normal subjects, oral tolerance is ensured by several immune and nonimmune mechanisms, which in the first stages of extrauterine life undergo a progressive physiological maturation [78].

Role of antigen presentation

The epithelium of intestinal villi expresses HLA class II along the brush border and laterobasally, and is therefore in theory capable of taking up and presenting intraluminal antigens to T cells [51]. It is not surprising that APCs play a crucial role in regulating the immunological consequences of antigen uptake. Such a capacity was initially suggested by the fact that agents activating RES appear to enhance APC activity, by preferentially interfering with the generation of CD8 subsets, with a subsequent negative effect on tolerance [348]. Intriguingly, feeding one novel antigen to naive mice will transiently activate RES and inhibit induction of tolerance if mice receive a second antigen [198]: similarly AD may develop after introducing several food solids in the first 4 months of life (Table 4.30). The role of agents capable of activating APCs has been reinforced by IFN-ɣ administration in mice that abrogates induction of oral tolerance [536].

Role of processing and oral tolerance

In mice the processing of food antigens by the gut is necessary for the generation of oral tolerance: if mice are given OVA by other parenteral routes, OVA isso non-tolerogen that it induces DTH (delayed type hypersensitivity) suppression in recipients [53]. Even if studies are mostly done in mice, oral tolerance is induced if foreign antigen is processed by enterocytes associated with HLA class II molecules and presented directly to lamina propria T lymphocytes or IELs via the TcR–CD3 complex, *bypassing APC processing*. In this case, T lymphocytes do not receive a second costimulatory signal via CD28-CD80, but tolerogen (Fig. 9.13) [460]. If there is GALT immaturity of primitive or secondary permeability changes [460], greater in CM-fed compared to breast-fed infants [512], increased amounts of antigen may saturate the scarce M cells and

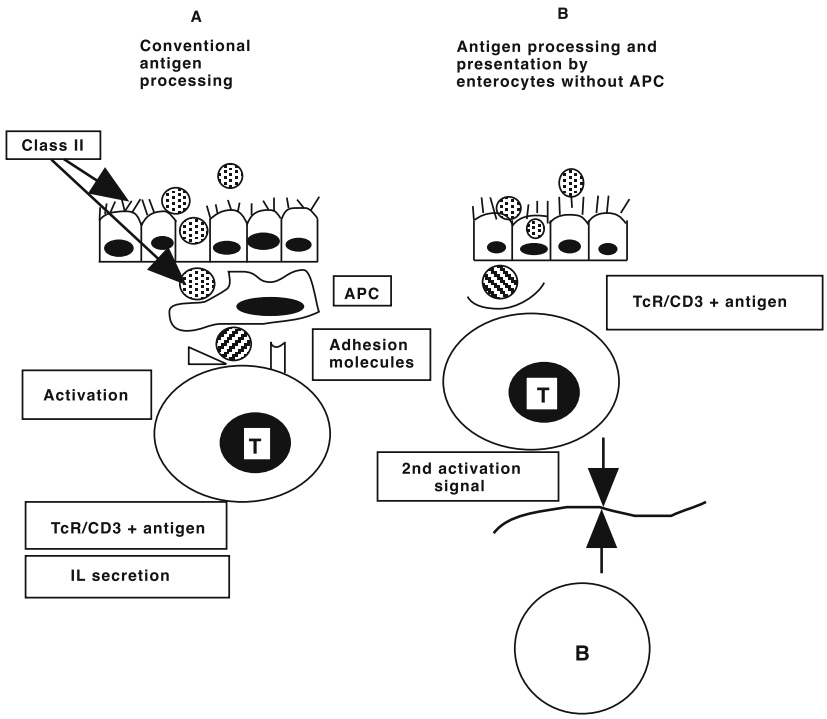


Fig. 9.13 a,b. Tolerance induction. **a** Prevention of oral tolerance: the conventional antigen presentation leads to T-cell activation. **b** Induction of oral tolerance: the antigen is processed by enterocytes and presented directly to T cells. B cells are not provided with a second signal and T cells receive a tolerogenic signal. The role played by Th2 T cells is unknown. (Modified from [460])

by extracellular passage may bypass enterocyte-mediated processing. Unprocessed antigens could be presented to lymphocytes by activated APC in association with HLA class II. In conclusion, instead of tolerance, immune responses are activated that are associated with local anaphylaxis [457].

Suppressor T cells and oral tolerance

Systemic tolerance represents a complex phenomenon involving all types of immune responses, including control of CD4 and CD8 lymphocyte stimulation, antibody regulation and production, and DTH responses; therefore IgE antibody regulation acquires a specific influence [457]. A single feed of antigen proteins induces a systemic hyporesponsiveness of IgM, IgG and IgE responses, as well as of CMI, apparently following suppressor CD8 activation, which may be depleted by cyclophosphamide, with consequent tolerance inhibition [348]. A putative role is assigned to most human CD8 (of IELs) with TcRαβ in the induction of clonal anergy in CD4, thus mediating oral tolerance [51]. However, the full T-cell activation with IL generation and related proliferation requires two signaling events, one via TcR and the other via a receptor for costimulatory molecules. Without the latter signal, T cells mount only a partial response and, above all, are subjected to clonal anergy and do not produce IL_2 on restimulation, leaving them in an antigen-independent state of anergy [442]. Conversely, subepithelial macrophages (APCs), expressing HLA-DQ [51], may activate $CD8^+$ T cells but negate stimulatory signal to lamina propria CD4 T cells [442]. The prominence of CD8 equipped with IFN-ɣ is the cornerstone of immune deviation [222]. However, CD8 T cells are nonexistent or scarce in the neonatal period and later, but IFN-ɣ treatment strongly enhances epithelial HLA expression in mice enterocytes, thus enabling antigen presentation to T cells, and modulating suppression of oral tolerance [536]. Moreover, suppressor CD8 inhibit only systemic T responses and not the humoral responses [348].

Clonal anergy or suppression

Whether at the base of tolerance there is a suppressive [222] or anergizing mechanism [329], the anergy thesis seems to prevail; therefore T suppressors are not necessary to mediate anergy [329], so T lymphocytes that have made tolerogen become capable of suppressing the responses to other antigens that are different from the disease-inducing antigen (bystander suppression) [336]. Small intermittent doses foster active suppression, linked to low antigen dose administration, with high doses causing anergy [329]: thus high doses are required to obtain anergy of B_{IgE} and T cells [200]. As yet, high doses have been fed to infants without appreciable positive effects. The relatively short duration of anergy induced in the neonate by a sudden large intake of antigen could, however, impair the development of necessary suppressor activity or tolerance, a result not without significance in neonatal and infant feeding, since the breakdown of anergy might endanger the MALT homeostasis in response to dietary antigens [200].

In children, tolerance induction is also linked to normal T lymphocyte activity [457]. Instead, a *defect of T-suppressor function* may recur in early infancy, especially when food antigens, always different, come into contact

with the gut mucosa, exhausting the still maturing immune reactive potential. However, oral tolerance is able to reduce the Th2-like profile and enhance the Th1-like profile by inhibiting IL_{10} and TGF-β mediators [38], thus protecting the child from the development of IgE-mediated allergy. Interestingly, after development of CM tolerance, CD25 and CD30 expression was decreased, whereas CD26 expression was increased to normal levels [435]. Although it is known that activated T cells of the human intestinal lamina propria are in general high producers of IL_{10}, almost no IL_{10} could be measured in the activated milk-specific T-cell lines. A similar low release was found for TGF-β, both of which contribute to tolerance development in the mucosa. In this study, CM-specific T cells in children with CMA displayed a clear Th2 profile, and released Th2-like T-cell ILs, especially IL_{13} that promote an allergic response, but failed to release Th3 ILs important for the development of *oral tolerance* [34]. However, Tr1 (T-regulatory 1) and Th3 cells, which are potent sources of TGF, are generated in mucosal lymphoid tissue in response to low-dose antigen and mediate bystander tolerance within the GI tract [450]. The establishment of tolerance during the neonatal period and its relationship with the genetic determination of any individual immune system and the pertinent maturation highlights that *the first years of life may be the most critical in the risk of sensitization.*

Oral Tolerance: Factors that Condition Its Induction and Maintenance

The acquisition of oral tolerance is likely a multifactorial process modulated by many variables [105, 383, 457].

Genetic background

As underlined in Chap. 4, neither in children with CMA, nor in their parents has an association been found with HLA-A, HLA-B, HLA-D and HLA-DR haplotypes, but with HLA-DQ. Evidence for linkage and allelic associations for CMA was not found. We add that a putative immunodeficiency marked by very low levels of T lymphocytes has not been confirmed using more sophisticated methods. The hypothesis of a deficit of IgA evoking a local transient immunodeficiency has been confirmed; however, it should be emphasized by the significant report of reduced concentrations of IgA-secreting cells at diagnosis, which increase during the follow-up, especially in tolerant children [233]. Concerning the low IgA levels, age has a pronounced effect on the response increase, which might be due to a dissonance between essentially low serum levels. Experimental evidence suggests that *infants developing CMA during breast feeding come from a selected population* [225].

Age at first antigen exposure

Children aged 1–3 months (58.4% of cases) had clinical symptoms of CMA [140]. HR neonates or infants are highly vulnerable and susceptible to sensitizing influences. Five out of nine infants developing CMA during breast feeding were from a HR population [225]. With a reduced oral tolerance at the base of infantile CMA, the acquisition of tolerance dependent on the first exposure to food antigens is of paramount importance [199]. It is also believed that the digestive and immunological immaturity of early life induces systemic CD8 T lymphocyte tolerance, but B_{IgE} cells priming at systemic and mucosal sites [228, 416, 508]. Since IgE antibody production depends on the amount of absorbed antigens, in mice as in humans, antibody responses may be suppressed by relatively high doses of food proteins, but primed by the early introduction of low doses [198, 460]. Neonatal rodents were sensitized during the first 1–3 days by a dose of CM or OVA able to induce tolerance in adult animals; however, induction of tolerance could be demonstrated before weaning and before gut closure [413, 509]. Consequently, if the multiplied ingestion of proteins in sensitized animals leads to secondary acquisition of tolerance, repeated doses would be more effective in suppressing hypersensitivity reactions [348, 460]. Giving CM in the first hours or days of life has a sensitizing effect [86, 225, 413, 437]. The selective deficit of oral tolerance is correlated to age in infants, but it is not of a quantitative type, because it can be corrected by transferring mature lymphocytes [383]. This deficit may be correlated *to the inability of an immature immune system to respond appropriately to gut-derived tolerogen stimuli;* and a further deficiency, irrespective of the age, may occur at weaning [413].

Nature of antigens

The larger prevalence of clinical reactions to certain foods is more likely a reflection of infant feeding routine than to be correlated with specific food sensitizing properties [66]. The *allergenicity of food proteins* is wide, as well as their *antigenicity*: evidence suggests that many proteins and antigenic substances are taken up and processed in the GI tube, or modified by industrial manipulations and by such modifications new epitopes may be unmasked, either newly formed or made more accessible [479]. CM antigenic modification (by heat or enzymatic digestion or both) reduces in part, but never totally abolishes the potential allergenicity/antigenicity of the starting proteins; therefore the immunogenicity of modified proteins cannot be excluded completely [63]. Moreover, protein antigenicity may be increased or diminished while being transported via living membranes, and vary from protein to protein, apparently in correlation to specific host conditions and/or to genetic, ambient, adjuvant, and similar effects. The ability to induce an immune reaction is in part *a function of antigen MW:* several antigens with well-defined physicochemical traits are proteins with a high MW; however, small molecules are allergenic, whereas macromolecules such as polyvinylpyrrolidone are not allergenic. Furthermore, an elevated MW is overall a property to promote

allergenicity, which also depends on amino acid (AA) sequence [1]. In several cases, molecules of low MW introduced by the parenteral route may be eliminated so rapidly, especially by urine, that they cannot be an effective allergenic stimulus, whereas other smaller and less immunogen molecules may act as *haptens,* which, by combining with host proteins, confer to these proteins a specific immunogen power [1].

Dose of antigen

The bulk of evidence shows that small doses (ng or µg in rodents, mg in humans) are more sensitizing than larger doses, a µg total dose in rodents primes for ensuing immune responses, whereas mg quantities are able to suppress humoral and cell-mediated responses [348]. In mice the dose required to elicit systemic sensitizations is 1–50 µg protein [348], which affects T-cell responses more than humoral immunity [383]. It is therefore noteworthy that low doses may prime children to develop AD [248]. The dose also depends on the *ideal size* of an allergenic protein, with a MW between 10^4 and 10^6: within these limits is found the MW of main food allergens, including casein, βLG, α-lactalbumin (ALA), Gad c 1, and egg white and tomato glycoproteins (Table 1.74). However, *there is no obligatory minimal limit expressing a protein immunogenicity*, because very small peptides and other molecules, as noted above, may act as haptens. Some CM-protein HFs also have a MW under 1,500 D, compared to allergenic substances with a high MW [297], but small peptides, with a MW of about 3,000 D such as glucagon with only 29 AAs and a MW of 3,000 D, insulin at 3,800 D and in some cases 1,000 D, may retain allergenicity by particular AA sequences, for example, Asp-Glu-Leu-Lys- and Asp-Glu-Asp-Lys- [1]. As a consequence, the allergen physicochemical characteristics are not related to MW. Therefore, immune reaction to a definite food results from IgE antibodies binding to epitopes on allergenic molecules, irrespective of two identical epitopes.

Thermostability

High temperature, in addition to reducing food allergenicity, changes the biological value, in a variable fashion for each component of highly sensitizing protein fractions, without forgetting that in given cases, heat-induced changes may make heated proteins more allergenic than raw proteins, not subjected to industrial treatments. CM proteins are both thermoresistant and thermolabile, for instance casein is stable at 100 °C, and its antigenicity remains unchanged after being heated at 121 °C for 15 min, whereas boiling whey proteins for 30 min reduced immunogenicity [297]. However, it remained dormant for 2,500 years. Infants still react to heat-treated whey proteins. Other allergens are inactivated by digestion, or during cooking. Raw fish, fruits and vegetables may result in sensitizing, but are tolerated when cooked [23]. Each rule has its own exceptions: Küstner, in a classic experiment, reacted to cooked fish, not to raw fish (but did not specify the fish species in question) [395]. Thus cooking formed or made accessible a new antigenic epitope in fish. Patients may have fierce allergic reactions to raw but not to cooked apples, carrots, and potatoes [1].

Concentration of given allergens

Allergen concentration in intestinal lumen is correlated with the degree of macromolecular absorption, which has its weight, as demonstrated by the high incidence of rice allergy (which would normally have an essentially low allergenicity), as in the Chinese and Japanese populations, who eat a high quantity of rice daily [1]. Eighty percent of CM protein is casein and 12% is βLG [297]. Also, CM's intrinsic characteristics, including its widespread use, and early and regular ingestion, are all factors incrementing CM's antigenic power [23], so that infants who are bottle-fed in early life are exposed to a massive and rapid allergen intake at each feeding, which is able to overcome the immune, anatomical and enzymatic barriers, thus representing the first cause of some cases of sensitization [440].

Frequency of antigen administration

Intermittent exposure of breast-fed infants to low doses of a CM formula was correlated with increased IgE production, but continuous and higher-dose exposure to the same formula induced IgG instead of IgE antibody production [163]. Low CM uptake before breast feeding was commenced exposed HR infants to an increased risk of allergic sensitization [225] or to a significant increase in total IgE levels [437].

Antigen transmission

When a sufficient concentration of molecules comes into contact with the cell membrane, invagination occurs at the epithelial membrane and small vesicles are formed. Subsequently, macromolecules migrate within membrane-bound vesicles to the supranuclear region of the epithelial cell where the vesicles coalesce to form large vacuoles. Within these structures, enzymatic degradation occurs; however, allergenic proteins tend to maintain their immunogenicity while submitted to different food transformation stages. Many food allergens, in addition to being thermostable, are acid-resistant (such as tomato), thus undigested macromolecules may escape breakdown and migrate to intestinal mucosa in an immunogen form [275, 440]. The organism is not always capable of eliminating food allergenicity; therefore the *immune barriers* are interposed (Table 2.11), so that peptides are eliminated by binding to BM sIgA [440]. Regardless of such immunological and nonimmunological defenses, there is no complete exclusion of non-self materials, also because normally between the gut lumen and internal ambient of the host there is an exchange of large or small molecules: in this process, both the antigen concentration and exposure timing

concur to establish the antigen load to which the gut is subjected [383].

Antigen absorption
As discussed in the previous section, oral tolerance to antigens taken up reflects the type of processing to which antigens were subjected in the intestine; it is therefore conceivable that any factor that alters the amount or chemical nature of the antigen absorbed from the gut will influence the related immunological consequences. This result can be connected with disorders causing increased permeability to macromolecules in several pediatric conditions such as Crohn's disease and CD, and the resulting increment of antigen-specific antibodies [348].

Antigen persistence
This depends on multiple parameters including antigen nature, structure, presentation and introduction route, host characteristic (metabolism, digestion, immune reactivity, age, etc.). For example, polysaccharide antigens have been found in the tissues 6 months after their introduction, but others, chiefly soluble proteins, are cleared within a few hours [1].

Immunological status of the infant and GALT
During alterations of local or systemic immune system immunocompetence, oral antigen administration increases the rate of secondary sensitization in infants such as viral infections of the respiratory tract. The underlying pathogenetic mechanisms could be represented by:

- Interference with the GALT immunoregulatory functions, such as immunosuppression
- Intestinal mucosa loss of integrity, by physiological or inflammatory causes and/or increased macromolecular absorption into the circulation (Table 2.23)

In the first case, the suggested hypotheses are supported by studies in the animal model [413]; in the second case it is stressed that *in the first weeks of life*, up to gut closure at about 3 months of age, the intestinal and/or immune system immaturity is combined with the deficit involving IgM antibodies from exocrine secretions, salivary sIgA and serum IgA. The mechanisms responsible for antigen macromolecule exclusion and elimination are immature [508]. In newborns, both increased permeability of the intestinal barrier, by altered chemical composition of epithelial cell membrane [413], and *microvillous membrane immaturity,* by tangible lowering of the proteinphospholipid ratio, are all factors resulting in increased antigen attachment and transport [413]. During intestinal absorption, exocyted antigens bind to IELs with the net result of eliciting immune responses [508]. In the vulnerable neonatal period, the development of a protective local immunity is important; however, if sufficient amounts of undigested macromolecules pass into the circulation and if a subject is genetically predisposed, in concert with insufficient mechanisms responsible for oral tolerance, allergic sensitization may develop from the first months of life [440].

Several studies have focused on and demonstrated or not the *increase in intestinal permeability* depending on the method used [138]. The results do not clarify how much such an increase plays an effective role in CMA development in children: the increase in protein endocytosis noted during the CMA initial period seems to be rather a secondary effect of an abnormal immune response leading to mucosal inflammation and impairment of endocytic process [209]. Children with positive FCTs to CM (34%) have serum ALA significantly higher than those with negative FCTs (66%) [253]. The authors hypothesize that in children with CMA, an increased macromolecular absorption depends on small intestinal mucosa damage caused by FCTs, or on incremented passage also during the elimination diet [253]. Another group of infants was evaluated by the lactulose/mannitol test, administered in concomitance with FCTs to CM (before and 3 days after), thus confirming an increased intestinal permeability to antigen macromolecules, evidencing in particular an identity of alterations whatever the manifestations (cutaneous or GI), and suggesting that an increased permeability could underlie a multisensitization [239]. Moreover, agreeing indirectly on the preceding results [209], it was shown that increased permeability is not a primary alteration somehow underlying CMA pathogenesis, but rather an epiphenomenon probably correlated with an altered protein uptake [240] or a local hypersensitivity reaction of intestinal mucosa [460] that amplifies epithelial dysfunction [409]. Intestinal permeability is increased in synergy by PBMC-released TNF-α and IFN-γ in children with CMA following CM challenge [208].

These data show that in CMA there is an abnormal passage of allergens secondary to altered immune response to allergens. Interestingly, experimental animals made hypersensitive to one food and challenged locally in the gut also absorb an unrelated "bystander" antigen: an increased lactulose/rhamnose urine excretion was demonstrated only when both sugars were administered together with the challenge antigen [460]. In infants with active CMA, the jejunal epithelium shows an increased transepithelial βLG [397] and horseradish peroxidase (HRP) transport [208]. However, the increased HRP absorption in an either intact or decayed form [208] might imply an intrinsic defect of metabolic functions of the epithelial cells and their degradation functions. This is a growing field of study and is easily understandable, owing to reflexes that are also preventive.

Finally, there are *numerous pathological causes of increased absorption of food macromolecules* [275] (Table 2.23). An increased permeability may be correlated with alterations of GI mucosa mainly consequent to acute diarrhea, also in children not affected with FA [240].

Cow's Milk Allergy

Allergen Characteristics

Among the foods most frequently responsible for sensitization, some are raw allergenic (carrot), others cooked (fish) etc. Moreover, denaturation processes caused during digestion modify the protein specificity of some products of digestion, which may be different from that of initially ingested proteins [1].

In thoroughly studied foods, numerous substances have been found to be potentially capable of priming for immune reactions, but only one or a few of several proteins are essential causes of sensitization and are called *major allergens*; less important allergens are called *minor allergens*; a high degree of *cross-reactivity* should be kept in mind. The structural and immunogenic characteristics have been identified and exhaustively studied only in a few food allergens, but those so far classified correspond roughly to the principal foods responsible for FA in children (Table 1.74).

CM immunogen characteristics are shown in Table 1.75. CM contains at least 25 separate protein components, equal to 3.3 g/dl (2.8–4.1 g/dl), all virtually unknown to the neonatal immune system, provided with several epitopes and an implicit sensitizing capacity [23]. The serum proteins that can cause hypersensitivity reactions are βLG, casein with 5 genetic variants, ALA with two variants and a polypeptidic sequence in common with egg white lysozyme and BSA (Table 1.75). The treatments to which CM is subjected (pasteurization, boiling, sterilization) modify the thermolabile immunogen components (serum albumin, ALA, Igs), but not the thermoresistant components, provided with a greater sensitizing power (caseins and βLG). The reactivity of circulating IgE against CM proteins detected by immunoblotting has provoked immunological responses in 12 children as follows: α-casein 12, κ-casein 11, β-casein 8, γ-casein 7, ALA 6, βLG 5 [402]. It is likely that techniques of CM isolation and modification elicit the appearance of new epitopes not identifiable with native proteins, since studies on βLG have provided interesting observations on the structural bases of βLG allergenicity and antigenicity (Chap. 1). ALA is absorbed up to serum concentrations of 10–1,000 μg/l of human milk/kg bw. Thus an infant weighing 10 kg may have a serum level from 100 μg to 10 mg (about 0.1–10 μg/ml), both titers being elevated in these infants. Therefore, CM products are potentially capable of provoking sensitizations in children drinking CM and of triggering immune reactions in sensitized children [23]. An unusual type of sensitization may derive from bovine antibodies found in commercial pasteurized CM specific to or cross-reacting with xenogenic products, including rye grass pollen, wheat, Der p, proteins, and *Aspergillus*, likely leading to the production of anti-idiotypic antibodies and anti-food antigens [107].

Clinical Manifestations

Hypersensitivity reactions to foods induce onset of cutaneous, respiratory, and GI symptoms. We divide the clinical manifestations attributed to CM by systems. The symptoms and disorders sometimes believed to be caused by CMA are numerous, but almost none of them are pathognomonic of CMA [23].

- *Systemic:* hypotension, shock
- *GI:* colitis, irritable bowel syndrome (IBS), flatulence, diarrhea, abdominal pain (colic), gingivitis, malabsorption, nausea, constipation, stomatitis, vomiting
- *Respiratory:* extrinsic allergic alveolitis, recurrent respiratory infections, upper airway obstruction, recurrent pneumonia (Heiner syndrome), rhinitis, serous otitis, sinusitis, laryngeal edema, pulmonary hemosiderosis, chronic cough, bronchitis, wheezing, dyspnea, recurrent fever
- *Cutaneous:* alopecia, angioedema, dermatitis herpetiform, eczema, urticaria, purpura, erythematous macular rash
- *Hematological:* iron-deficiency anemia, thrombocytopenia, gross/occult bloody stools
- *Urinary:* albuminuria, cystitis, enuresis, minimal lesion glomerulonephritis, nephrotic syndrome
- *Central nervous:* migraine, epilepsy, attention deficit hyperactivity disorder, restless legs syndrome, chronic fatigue syndrome, tension-fatigue syndrome
- *Ocular:* keratitis, uveitis
- *Various:* rheumatoid arthritis (seronegative), diabetes mellitus, recurrent periostitis, SIDS (sudden infant death syndrome), etc.

In the first part of Table 9.4 are listed the clinical manifestations that are more clearly related to CMA, assessed according to the prevalent immunological mechanisms [413]. The second part is based on nonimmunological criteria, but the literature is not unanimous [23, 430]. Figure 9.14 [29, 66, 86, 182, 200, 225, 237, 293, 339, 349, 439, 456, 500] summarizes the analysis of 13 studies done over about 40 years with the prevalence of more common symptoms and relative ranges. Around 2.5% of neonates experience hypersensitivity reactions to CM proteins during the 1st year of life [439]. In 27 infants with CMA associated with AD [86], at the first CM feeding anaphylactic shock occurred in 4 cases (14%), urticaria-angioedema in 9 (32%), vomiting in 7 (25%), abdominal pain and diarrhea in 6 (21%), respiratory symptoms in 3 (11%), AD worsening in 8 (29%), alone or associated. A 3.5-month-old child seen by us experienced a generalized edema. In 115 infants [92], 41.5% reacted with AD worsening, 16.5% with GI symptoms or with bronchospasm or wheezing, 12.5% with urticaria, 6% with anaphylactic shock, and 6% with angioedema alone or associated with other manifestations. OFCs provoked urticaria in 73% of 467 children aged 3 years (median) [453] and children as young as 8 weeks reacted to CM challenges [237].

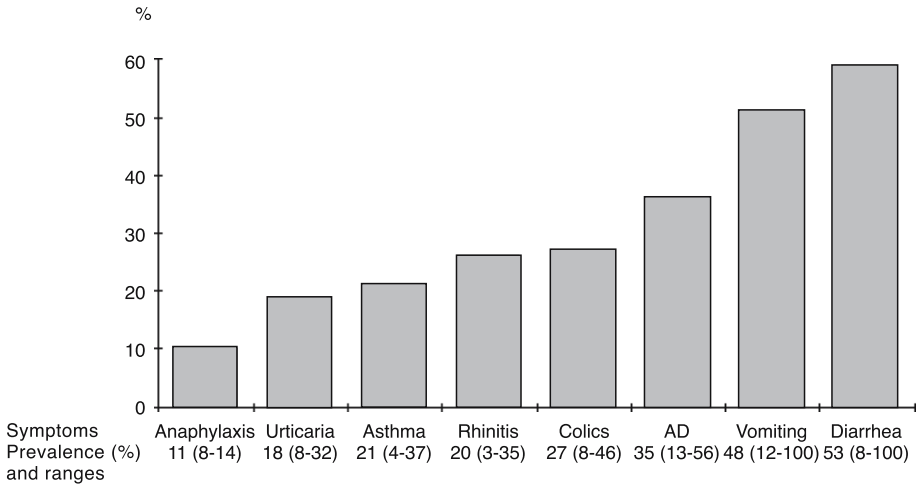


Fig. 9.14. Prevalence of clinical features of CMA. Division between systems: gastroenteric, 45.5%; skin 27.5%; respiratory, 23.5%. AD. (Data from [29, 66, 86, 182, 200, 225, 239, 293, 339, 349, 439, 456, 500])

Table 9.4. Clinical manifestations attributed to CMA

On immunological basis: unanimous
1. Type I: IgE-mediated reactions
a) Systemic
Anaphylactic shock
b) Gastrointestinal
Early-onset enteropathy
Nausea, colic, diarrhea, vomiting, abdominal pain
Allergic eosinophilic gastroenteritis (some forms)
c) Cutaneous
Atopic dermatitis, erythema
Urticaria-angioedema
d) Respiratory
Wheezing, sneezing, cough, rhinorrhea
Rhinoconjunctivitis
2. Type II: Cytotoxic reactions
CM-induced thrombocytopenia
3. Type III: By immune complexes
Celiac disease
Colitis syndrome
Dermatitis herpetiformis
Enterocolitic syndrome
Malabsorption syndrome
Pulmonary hemosiderosis
Sideropenic anemia, sometimes with occult blood loss
4. Type IV: Cell-mediated hypersensitivity
Celiac disease
Dermatitis herpetiformis
Enterocolitic syndrome
Malabsorption syndrome
Pulmonary hemosiderosis
On nonimmunological basis: not unanimous
Attention deficit hyperactivity disorder[a]
Crohn's disease[a]
Eosinophil enteropathy[a]
Gastroesophageal reflux[a]
Infantile colitis[a]
Insomnia[a]

Modified from [371].
[a] Included in the text.

Some authors have reported CMA-related symptoms in children 0–3 years old divided by systems, as shown in Table 9.5 [87, 106, 177, 178, 323, 351, 439, 456, 491, 517]. The mean incidence of GI symptoms is 50%, and 34% when GI symptoms alone are taken into account. Cutaneous symptom incidence is 50% (31%–94%) and the highest rate is encountered in infants 1–6 months of age [275]; in others [224, 456] the incidence is up to 64%–84%, but the groups are less numerous and their age went up to 3 years [224]. The incidence of respiratory symptoms is 19%, but considering only children of 0–1 years, incidence is 13.6%. The incidence shows that *the GI tract is not the only shock organ* [23], but there is always a disagreement due to the known differences among studies [145]. We note that symptoms vary according to age and add that foods follow the same pathway and are often involved in exercise-induced manifestations [270].

The manifestations attributed to CMA [66, 430] and reported in Fig. 9.14 are somewhat typical of FA: they can be local or systemic, the entrance route of allergens is usually oral, but can be inhalatory or by skin contact (Table 7.22). In infants, the pattern of GI involvement is prevalent, sometimes up to atrophy of mucosal villi, but often rhinitis with chronic nasal obstruction can be complicated by otitis, sinusitis, asthma; more rarely recurring respiratory symptoms are recognized, as well as urticaria and AD onset after CM feeding [145]. Risk factors are acute infectious episodes which potentially induce lesions in genetically predisposed children, likely leading to an increased permeability with consequent CMA [240, 509]. Among the patterns reported in Fig. 9.14, GI symptoms are probably the most frequent in absolute terms when we reckon that late onset reactions occurring in 50% of patients involve mostly the GI tract [145].

Table 9.5. Clinical manifestations of CMA related to affected organs in children aged 0–3 years

Authors	Reference	No. of cases (age in years)	Symptoms related to affected organs (%)			
			Skin	Airway	GI	Only GI
Clein	[106]	140 (0–1)	43	10	51	17
Goldman et al	[176]	44 (0–1.2)	20	34	41	
Gerrard	[178]	59 (0–2)	46	44	61	15
Navarro et al	[351]	42 (0–1)	21	0	71	
Stintzing et al	[456]	25 (0–1)	84	40	16	
Ventura	[491]	66 (0–1)	49	8	62	43
Italian group	[517]	148 (0–1)	34	5	61	61
Martin Esteban	[323]	127 (0–0.5)	94	2	35	
Høst-Halken	[224]	39 (0–3)	64	33	59	
Schrander	[439]	26 (0–1)	31	19	50	
Cantani	[87]	28 (0–1)	61	11	46	
Mean			50	19	50	34

Data ordered according to the year of publication.
GI gastrointestinal.

Systemic Manifestations

Anaphylaxis

Anaphylactic shock, the most dramatic symptom of allergy, was the first to be connected with CM in 1905 [161]. The onset of this severe manifestation is usually within a few minutes, but the frequency varies in the literature from one series to another, from zero to 33 % of cases, especially in infants with prevailing GI forms; in Fig. 9.14 the incidence is 11 %. Very typical is the *anaphylactic shock by CM* at the first time CM is met by infants after a more or less long period of exclusive BM feeding, or after low doses: as soon as food touches the lower lip [86, 88], the reaction ensues in infants with pruritus and edema of lip, tongue, palate and pharynx and cutaneous symptoms, including erythema, urticaria, angioedema. and pruritic morbilliform rash [86]. Such reactions may be followed by more severe symptoms such as tachycardia, pallor, cramping, vomiting, diarrhea, shock and respiratory insufficiency due to oropharyngeal angioedema or wheezing and, especially in older children, symptoms of other systems, for example urticaria-angioedema, bronchospasm, nasal obstruction, etc. [145]. The chronology is suggested by Fig. 9.15 [340]. To these cases we add CM-induced anaphylaxis/shock in three infants [41], a child of 4 years [43], a 6-month-old boy [470] and two patients aged 2–17 years [41]. Some children may experience generalized nonfatal anaphylaxis following CM intake [424]. Infants undergoing shock often have elevated IgE levels and should not be subjected to CM challenge [54]; even a SPT should be done in clinical setting and very prudently [92]. A useful model of intestinal anaphylaxis has investigated OVA-sensitized animals to study the effects of repeated daily feeding of OVA antigen. After OVA feeding, serum mast cell protease II (MCP II) levels increased ≈16-fold of normal on the 1st day, and two- to fivefold the normal level by re-feeding OVA in the subsequent 4 days. If OVA feeding was discontinued for 9 days, re-feeding increased serum MCP II levels only fivefold the normal level [55]. This study supports the assumption that a 2-week course of complete food allergen avoidance is necessary to elicit unmatched responses to an OFC.

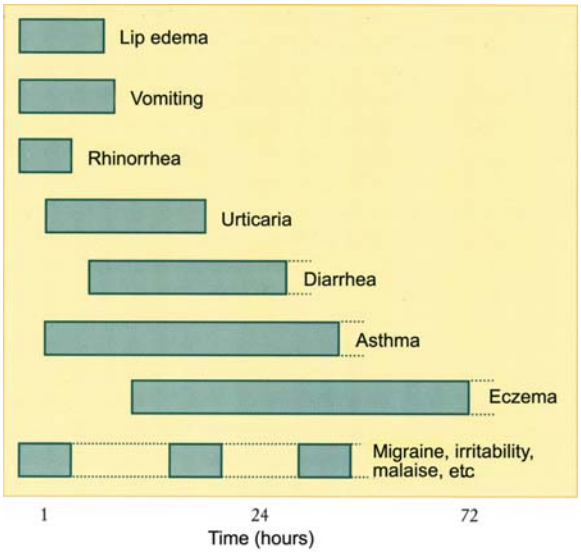


Fig. 9.15. Chronology of clinical manifestations

Gastrointestinal Manifestations

CMA-Induced Enteropathy

CMA is very frequent in babies: two types of clinical manifestations are evident [165].

The acute onset form (80% of infants) [66] is observed in the first 6 weeks of life and is characterized by diarrhea and vomiting in 59% of cases [224]. In 40 infants, the onset was acute in about 75% of babies [131], diarrhea and vomiting were noted in 67% of 54 babies [281], a more dramatic onset was observed in 42 babies, with 93% diarrhea and 71% vomiting [351]. In a series of 100 infants with CMA, 53 had a history of diarrhea and 53 of vomiting, acute diarrhea was more common than chronic [186]. Diarrhea is severe and persistent, the stools being frequent and loose, mucous and watery, with occult or overt blood [186] (a sign of inflammatory colon involvement), often associated with *persistent vomiting* (the second manifestation in order of frequency), appearing within 1 h after the meal. The *diarrhea* may be of short duration (above all when CM is readily eliminated from the diet), or prolonged and resistant to treatment. Severe and/or protracted diarrheal forms may be complicated by dehydration, damaged intestinal mucosa, electrolyte imbalance, weight loss, and malabsorption, accompanied by transient intolerance to several foods before an exact diagnosis is assessed [23, 281, 392]. The diarrhea-vomiting-dehydration association may resemble neonatal necrotizing enteropathy [66, 509]. *Abdominal pain* (gas colic) is present in 26% of infants: the child is awake, or wakes up due to apparent abdominal cramping that does not subside with common medications; crying is unremitting. Often the clinical picture induces us to suspect an intoxication and in extreme cases, even an invagination. Moreover, *systemic symptoms* may be present such as fever and abdominal distension along with projectile vomiting, which may even mimic an acute abdominal obstruction. At variance with other causes of the child's vomiting, *pallor* and shock (and metabolic acidosis) usually accompany vomiting, a cause of massive fluid loss [66]. *Growth arrest* may persist as the only symptom of CMA in 76% [351] to 100% [66] of cases, later complicating the clinical picture, with malabsorption and hypoproteinemia due to allergic gastroenteritis. In more severe cases, protein loss across the intestine may lead to edema, hypoproteinemia or hypogammaglobulinemia, malnutrition, etc. [293], resulting in misinterpreting the diagnosis in some cases.

Table 9.6 outlines the clinical features of 41 infants with chronic diarrhea by CMA, hospitalized in our department between 1973 and 1976 [66], in whom GI symptoms with growth failure had a peak of 100% during the disease. CM elimination and institution of an oligoantigenic diet with lamb meat led to diarrhea vanishing within 1 week and the infants throve again within 2 months. In 21 children with BMA, the symptoms were suggestive of atopy (Table 9.7) [93]. Frequently, following virus-induced mucosal damage after episodes of chronic postinfectious diarrhea, or enteropathies of varying pathogenesis, a *transient lactase deficit* is caused, thus CM may slow down the normalization of intestinal biological functions, with no influence, however, on the child's growth [492]. The differential diagnosis with acute viral enteritis may be demanding in these infants. Likely due to an incorrect treatment of the re-feeding phase more often following viral infectious episodes characterized by profuse and watery diarrhea, especially in younger infants. CM exclusion from the diet does not always induce an immediate resolution of symptoms. The possible elimination of other foods in sequence may imply fallacious diagnostic conclusions as an outcome [54]. Such incentives may be supported by the abatement of symptoms after substitution of CM formula with a soy protein formula (SPF) but the caveat may be that the possible resolution of postinfectious disturbances can erroneously be seen as indirect proof of a possible allergy, not only to CM, but also to other foods previously reintroduced without success [54]. However, lactose intolerance would cause similar symptoms and would improve with dietary changes [439].

Table 9.6. Clinical features of 41 infants with CMA-induced chronic diarrhea

Clinical features	No. of cases	(%)
Onset age 3 months (median)	41	100
Diarrhea	41	100
Growth arrest	41	100
Vomiting	19	46
Bloody stools	8	19
Colic	5	12
Shock	5	12

Data from [66].

Table 9.7. Allergic manifestations in 21 breast-milk-sensitized children

Clinical features	No. of cases
Eczema and vomiting or diarrhea	11
Asthma and vomiting or diarrhea	2
Angioedema and asthma Angioedema and asthma or atopic dermatitis Angioedema and asthma or urticaria Angioedema and asthma or vomiting, diarrhea, asthma	8

Data from [93].

Table 9.8. Time interval between the first known exposure to CM and CMA onset

Authors (reference)	No. of children	Age (weeks)			
		<1–1	1–4	5–12	13–52
Clein [106]	89	39 (39%)	43 (43%)	7 (7%)	
Gerrard et al [178]	59	25 (42%)	16 (27%)	15 (25%)	3 (5%)
Jacobsson and Lindberg [239]	20	10 (50%)	2 (10%)	4 (20%)	4 (20%)
Høst et al [224]	26	19 (73%)	4 (15%)	3 (12%)	
Martin Esteban et al [323]	124	81 (65%)	43 (35%)		
Total	**318**	**135 (42%)**	**104 (33%)**	**65 (20%)**	**14 (4%)**

The studies are ordered according to the year of publication.

Table 9.8 [106, 178, 224, 237, 323] shows the time between the first CM exposure and onset of related allergy: 1 week was sufficient in 42% of cases.

The late onset disease manifests itself several weeks to months later with vague symptoms, generally including failure to thrive, abdominal distention, wasted buttocks, and voluminous, bulky, fatty stools. Even if diarrhea is protracted, steatorrhea is rarely observed, as in the early-onset disease. *Malabsorption* is very usual, likely resulting in growth retardation and malnutrition. In addition to CM, egg, wheat and soy have been implicated with lesser incidence [413]. If gluten already has been introduced into the diet, the picture can sometimes be easily mistaken for gluten-sensitive enteropathy (see "Celiac Disease"), with an *anatomical-pathological pattern* characterized more frequently by subtotal villous atrophy and increased lymphocyte and eosinophil mucosal infiltration; similar histological patterns have also been demonstrated for others forms of FA [89]. *On the scanning electron microscope,* lesions apparently particular to CMA with GI expression are observed, consisting of patchy losses of glycocalix, not covering microvilli homogeneously, leaving them furred and short, a finding missed at standard histological or radiological examination [492].

Colic

Infantile colic, a common problem during the first months of life in otherwise healthy, thriving infants, is of unknown origin. It usually begins in the first 4 months of life and occurs in about 20% of infants. It is a poorly defined syndrome of paroxysmal activity characterized by inconsolable crying, drawing up of the legs, abdominal distention, excessive gas, etc. In children who are exclusively fed BM, several investigators consider colic to be connected with CMA, due to bovine or other foreign proteins present in the maternal diet that escape complete digestion, enter the circulation, and are transferred into BM [17, 272], likely dependent on an IgE-mediated hypersensitivity, which may be a pathogenic factor in some infants [168, 237, 303]. A recent study does not support an association with markers of allergy [100]. CMA has been implicated: colic recedes after dietary CM elimination from the maternal diet, but the effect is only transient [229]; in other cases clinical improvement was obtained with a casein HF [168].

Colitis

The onset of colitis is usually from the first weeks of life to the 6th month, never beyond 10 months: it is frequent in the first 5–6 months of life [217, 367, 492]. Several studies have confirmed that it is caused by CMA [217, 492], or by soy, beef, and other food antigens present in BM, most cases are in BM-fed infants [186, 193, 250, 313], in one study up to 50% of cases [362]. The onset in infants <1 week of age may result from transplacental fetal sensitization by exposure to CM antigens *in utero* [201]. Family history (FH) is positive in 60%–92% of cases [186, 217, 250, 286, 313, 367]. The onset may be acute (<6–12 h after the first CM feeding) [362] as occurred in 36%–92% of cases, with frequent diarrhea containing blood and mucus, vomiting, cramping, weight loss and eosinophilia in 14% of cases [367]. Furthermore, onset may be insidious and protracted, especially if enteropathy and malabsorption are associated, with a clinical picture similar to intractable diarrhea [16]. The symptoms are severe [201]; low doses of CM precipitate bloody diarrhea [217], as well as 5 ml CM or one tin of beef broth [250]. Colon biopsy studies show eosinophils and epithelial cells, as well as alterations of the intestinal mucosa, and although inconstantly, they show IgE plasma cells in the lamina propria, but no lesion characteristic of ulcerative colitis (UC) or CD [250]. In these infants, the age-related titers are low in IgA in 48% and in IgG_2 and IgG_4 in 50%–72% of cases, thus hypothesizing an immunodeficiency [367]. The diagnosis is made when the disease responds dramatically to CM dairy elimination, hematochezia resolves within 72 h and symptoms relapse within hours or days after FCT [186], aided by sigmoidoscopy showing an

erythematous mucosa alternating with patchy zonal lesions of normal aspect, and presence of specific markers of an acute inflammation [217, 250, 313, 492]. A toxic dilatation of the colon in a preterm baby [201] and other macroscopic anomalies in up to 83% of children have been observed [367]. Owing to the marked presence in the lamina propria of more numerous eosinophils compared to controls, prevalent in the colon [313, 362], the syndrome has also been called allergic proctitis [273]. The differential diagnosis includes CM- and/or soy-induced enterocolitis and acute infectious colitis, whereas in the protracted forms the differentiation with chronic inflammatory enteropathy is unreliable [362]. Avoidance of CM and other causative foods leads to a rapid clinical improvement in 77% of cases [367]; however, lesion repair requires months [413].

Eosinophilic Gastroenteritis

Approximately 50% of cases are caused by food allergy and intolerance. Clinically it is characterized by anorexia or early satiety, nausea, vomiting (which can be blood-streaked), postprandial abdominal pain resembling colic, diarrhea, growth retardation or failure to thrive, occult or gross blood in stools, eosinophilic infiltration and peripheral eosinophilia, with histological patterns of colitis, enteritis and gastritis [211, 212, 294, 338, 345, 414, 426]. Some infants and children experience iron-deficiency anemia and generalized edema secondary to hypoalbuminemia dependent on protein-losing enteropathy, but not rarely clinical symptoms, including vomiting, diarrhea, abdominal pain, and growth retardation [345]. Four diverse forms may be related to the predominant layer involvement [338, 340]:

- Mucosal involvement, the most common [345] with steatorrhea, hypochromic anemia, hypoproteinemia and malabsorption
- Muscular involvement, especially of smooth muscles with weight loss, pyloric stenosis, partial obstruction of the large intestine due to gut thickening and rigidity, wholly overlapping with CD
- Subserosal involvement, the least common [345], principally with eosinophilic ascites
- A form combining the features of the above

Another classification, more suited to children, includes only two classes [426]:

- Early onset of symptoms, SPT and RAST negativity, rapid response to CM elimination and outcome toward tolerance
- Late onset, positivity of laboratory testing, ineffectiveness of CM elimination and trend to chronicity

The first class recalls early onset CMA, in which children gradually experience tolerance to sensitizing foods [165, 212, 339, 426].

The IgE-mediated disease (50% of children) is characterized by high IgE levels, sensitization to numerous food and inhalant allergens, peripheral eosinophilia [338, 345, 494] and deficient IFN-γ and IL_2 expression by T cells, which in the intestinal mucosa is 10- to 1,000-fold higher compared to resting blood T cells [245]. In a 6-year-old boy with asthma, but with positive sIgE for CM and egg, a massive extracellular major basic protein (MBP) deposition caused a broad geographic erosion in the gastric body and antrum [277]. In other cases, likely those that are not IgE-mediated, CIC-induced complement activation or IL release by activated lymphocytes denotes a massive eosinophil infiltration in lamina propria of gastric antrum and distal small intestine [54, 345]. The diagnosis is based on the demonstration of eosinophil infiltration in biopsy specimens [294, 345]. The differential diagnosis is with several disorders of peripheral eosinophilia [338] (Table 6.15), considering that eosinophil is a normal constituent of GI tissues [362], and with GER (gastroesophageal reflux), which may be suspected by sharing the unsuccessful specific treatment [263]. Management is by elimination diets, which gives appreciable results [145], especially if based on FCT results [245]. Ten children with an apparently mucosal layer disease failed to thrive despite medical therapy, but symptoms were greatly improved after 5–8 weeks of an AA-based formula with rice [263].

Hemorrhagic Gastritis

The prevalence is unknown and both clinical and histological findings are not well defined. In some cases, characterized by predominance of vomiting, a severe antral form is observed, with an evident eosinophil infiltrate and elevated concentrations of IgE plasma cells [492]. Other cases are associated with the hypertrophic pyloric stenosis, thus raising the suspicion that the disorder is FA-related [186]. Two young babies have experienced hematemesis caused by an eosinophil erosive gastritis or an eosinophil gastroenteritis, which disappeared after CM withdrawal [147, 207].

Vomiting

In the first months of life, persistent vomiting is a typical symptom, never isolated, since it is frequently associated with other GI symptoms, in particular with diarrhea (26% of cases) [281], common in very young infants [392]. Vomiting occurs in enterocolitis, allergic colitis, malabsorption and eosinophil gastroenteritis [23]. It is a very frequent symptom, especially in infants with CMA, likely causing severe metabolic alterations, including dehydration with electrolyte loss; the younger the child the greater the severity. The differential diagnosis includes a wide spectrum of disease: it may lead to a suspicion especially of GER and hypertrophic pyloric stenosis (Table 6.5).

Iron-Deficiency Anemia

Microscopic intestinal blood loss has been recognized for many years and it is considered an expression of a CMA enteropathy. However, iron loss occurs in a substantial group of infants, causing a true deficit in some infants; older children are asymptomatic, although they appear pale and are hypoproteinemic [186, 463]. Certain cases with severe anemia (Hb <7 g) may be associated with generalized edema, abdominal distention, hypoalbuminemia and show an increase as much as 4- to 7-fold of fecal Hb [23]. The relationship with CM has been shown by CM-fed infants of 5–8 months who excreted significantly more Hb than did formula-fed controls [539]. However, it is disputed that the anemia subsides solely with iron therapy, thus excluding immunological mechanisms [23]. Intestinal biopsies of children with severe anemia and hypoproteinemia revealed no histological or immunological abnormalities [186].

Respiratory Manifestations

Heiner Syndrome

This uncommon syndrome, described in CM-fed infants of 13 days to 6 months, consists of chronic symptoms of the lower airways or recurrent disease, characterized by persistent or recurrent pulmonary infiltrates, probably caused by CIC deposits [204]. Additional findings upon several organs may include serous or recurrent purulent otitis media, diarrhea, vomiting, anorexia, poor weight gain, anemia, eosinophilia, and high titers of serum precipitins to CM. Pulmonary hemosiderosis may ensue because of bleeding from the alveolar capillaries and should be suspected when hemophthisis and anemia are present [204]. Most symptoms improved strikingly with a CM-free diet since the disorder may be a result of CMA [480]; in some cases egg and pork have been suspected [296]. Infiltrates apparent on chest X-rays resolved within 4 weeks [495]. IgG, IgA and C3 deposits were demonstrated in immunofluorescence studies on lung biopsies [296]. Antigen–antibody complexes and lymphocyte-mediated immune reactions to CM are postulated in the immune pathogenesis based on the presence of high serum levels of CM-specific IgG antibodies and in vitro proliferative responses of lymphocytes to CM antigens [55].

Asthma

Infantile asthma associated with IgE-mediated CMA is present chiefly in the first 2 years of life, with a 4%–9% prevalence, which increases by taking into account cases of wheezing by mechanisms of the delayed type, and decreases in older children. Figure 9.14 shows a 21% prevalence with a 4%–37% range (see "Association with Asthma"). Severe asthmatic episodes with loss of consciousness and death also depend on *inhalation of CM proteins* by patients with CMA [489].

Rhinitis

Rhinitis can be referred to CMA, especially in infants. The prevalence in children is between 3% and 35% (mean 20%) (Fig. 9.14). Usually it is accompanied by other manifestations and not only respiratory symptoms [23].

Additional Respiratory Symptoms

The following respiratory symptoms have been described [23]:

- Recurrent bouts of cough, dyspnea and fever with a clinical picture similar to that of CF or of foreign body aspiration
- Recurrent pneumonia as such, or associated with chronic diarrhea, in which not only CM but also pork, peanuts and soy have been incriminated as causative allergens
- Extrinsic allergic alveolitis manifests itself with recurrent bouts of difficult breathing, mild chronic respiratory distress, and serum precipitins to CM and egg [495] (Chap. 11)

The prominent characteristic of these syndromes and symptoms appears to be the overlapping clinical manifestations [463]; it seems logical to advance that it consists of one syndrome with a wide spectrum of manifestations, with different degrees of severity and chronicity. Solely from the 2nd year, when the classic acute enteropathy becomes rare and fades away, the diverse syndromes can be independent.

Other Food-Induced Manifestations

Cutaneous Manifestations

CM, egg, peanuts, and nuts are responsible for cases of contact urticaria, less frequently for acute urticaria and/or angioedema: typical is the case of fish [83, 112] (Fig. 8.3). Urticaria and shock due to CM are reported also in infants 4–6 months of age [187]; however, the prevalence is unknown. A young girl seen by us manifested erythema and edema shortly after having been caressed by fingers wetted by CM, or using a CM-containing ointment. Acute urticaria-angioedema may be an early sign of an impending anaphylactic reaction and is often related to food ingestion immediately prior to the symptom onset [145]. A ten-month-old girl seen by us because she was CM-allergic also developed urticaria and edema after her brother kissed her while eating a CM-made ice or drinking CM. It is believed to be the

most common manifestation of IgE-mediated FA and its cause–effect relation with the incriminated food is obvious when symptoms appear within minutes [10].

AD may be present in 50% of children within the first 2 years of life, then the prevalence decreases clearly; the differential diagnosis should take into account all the affections indicated in both Chaps. 7 and 8.

Dermatitis herpetiformis may be mistaken for AD and in 85% of cases is associated with CD, also because of a genetic predisposition denoted by HLA haplotypes in 80%–90% of patients [55]. The diagnosis is based on IgA, C3 and polymorphonuclear (PMN) leukocytes found in the dermoepidermal junction; IgA deposits contain J-chains that indicate its origin in the gut [414].

Colitis Induced by CM and Other Foods

CM- and soy-induced colitis are clinically and histologically similar: endoscopic examinations of colonic mucosa show analogous features in both conditions [55]. Colitis presents in the first months of life in infants, typically secondary to CM and soy hypersensitivity, but these infants usually do not appear ill and are detected because of the presence of gross or occult blood in their stools [186]. Some infants have normally formed stools, lesions are confined to the colon and consist of mucosal edema with evident eosinophil infiltration and IgE antibody increase in the epithelium and lamina propria [55]. In older children, other foods are incriminated such as egg, wheat, fish, molluscs, and nuts [145]. The manifestations are not specific, including fever, vomiting, chronic diarrhea, leukocytosis, carbohydrate intolerance, metabolic acidosis, dehydration and sporadic shock [106, 392]. A subset of children may manifest diarrhea and transient carbohydrate malabsorption, with most of them experiencing alteration of the lactose tolerance test and a rise in fecal carbohydrates as well as PMN elevation in many [392]. In chronic cases, anemia and/or hypoproteinemia may be apparent [55]. DBPC-CT studies are absent; however the disorder generally resolves after 6 months to 2 years of allergen avoidance [55].

Enterocolitis Induced by CM and Other Foods

Food protein-induced enterocolitis syndrome (FPIES) secondary to food ingestion and not specifically IgE-mediated [224], has a probable CD4-mediated pathogenesis [349]. Infants of 1 week to 3 months generally present symptoms in response to food proteins passed in BM or to CM [392, 448] but none of 14 infants developed FPIES during exclusive breastfeeding [358]. Usually the symptoms are isolated to the GI tract, such as protracted vomiting and abundant diarrhea, and can be dramatic enough that some infants may progress to severe illness [358, 392]. The condition can be misdiagnosed as a mucosal enzyme deficiency [178, 392]. Intolerance, in addition to CM, may be to soy [392], which, when employed after severe lesions of the gut mucosa due to CM ingestion, perhaps due to a 30-kD component from soybean, may induce in these infants or young children clinical manifestations similar to CMA [90]. In 14 babies aged <1–7 months at initial diagnosis, 71%–80% with FHA the reactions were to rice (71%), oat (64%), barley/pea/string bean (14% each), squash, sweet, potato, poultry (7% each), >1 grain 50% [358]. In older children, the reactions are more frequently to egg or wheat in 37% of cases [281], but also to peanuts, nuts, chicken, turkey, rice, gluten, sweet potato, squash, string beans, peas, beef, pork, fish, and goat's milk [145, 178, 358, 392, 448, 414, 486, 492]. Among the triggers, oat is a prominent (64%), but hitherto unrecognized cause [358]. The OFC with a SPF was positive in 20.9% of 43 infants with non-IgE-mediated intolerance to CM and/or soy, to both foods in 13.9% of cases [60]; clinical intolerance to soy was noted also in 11.5% of 35 children with malabsorption and CM intolerance [281]. The symptoms can occur within minutes or can sometimes be delayed up to several hours after a FCT [193, 234, 392]. The diagnosis cannot rely on RAST results [84] or SPTs [55] in these children with GI symptoms caused by CM and/or soy intolerance [33]. Thus, the diagnosis is based more usually on symptom resolution within 72 h of elimination of the offending food and on the relapse within 1 h after its reintroduction. FCTs should be done in a medical setting because the symptoms are severe, consisting of protracted vomiting, dehydration, acidemia, hypotension, and anaphylactic shock in 15% [182, 486] to 57% of cases [358]. After the challenge, a biopsy of proximal jejunum reveals hemorrhage, and histology shows flattened villi, edema, increased eosinophil counts (in some cases), and infiltration of PMNs in the lamina propria; the superficial epithelium appears to be abnormal, and in the inflammatory infiltrate both IELs and plasma cells in the lamina propria are increased [384]. Jejunal biopsies disclosing an increased infiltration of mucosal lymphocytes and eosinophils may be wholly similar to biopsy findings in eosinophil gastroenteritis, especially of the first type [294]. Scanning electron microscopy shows poorly visible microvilli because of patchy losses of glycocalix, which leaves villous height abnormally low, with adjacent areas markedly flattened and deeper crypts resembling the ultrastructural pattern of stage II CD, up to total upset of cellular architecture [384, 391]. Villous height is significantly lower than it is in controls, 250 µm vs 380 µm [349]. An increased number of IgA and IgM antibodies in the lamina propria after challenge with SPFs confirms the affinity with CD, and establishes that a similar immune mechanism is potentially involved in soy enteropathy, as confirmed by the local mast cell activation: in biopsy specimens, food antigen-induced mast cell degranulation correlated well with OFC results, but not with SPT and RAST results [186, 430].

Therefore, SPT and RAST negativity in these infants shows that *IgE-mediated hypersensitivity plays no role* in SPF-induced enteropathy, as demonstrated by lack of evidence of IgE pathogenetic involvement [58], and of subjects with IgE-mediated reactions [60]. Breast feeding appears to have an important protective effect against CM and soy-induced enterocolitis [349]. The mainstay of dietary treatment is the oligoantigenic diet with lamb meat [80], whereas casein [16, 264] and whey protein HF are not always reliable, as seen in three infants with chronic diarrhea and dehydration [229, 477], in two of whom notable histological lesions were detected [477].

Celiac Disease

Celiac disease is a common chronic inflammatory disorder of the small intestine with a multifactorial etiology. HLA is a well-known risk factor, but other genetic factors also influence disease susceptibility. Although gluten is a necessary factor for disease development, it is a hereditary condition with a genetic risk of 15% between first-degree relatives, and monozygotic twins showed a concordance of 70%–100% [476]. The analysis of involved genes showed that the strongest linkage with disease was to the HLA *locus 6p*, indicating that *11q*, *5q* and also *2q* are true susceptibility *loci* in this disorder [350]. Chromosomes *15q* and *19q* have also been implicated [537]. The mean incidence in Europe is from 1:4,000 in Greece to 1:300 in Sweden; in northern Europe, 95% of CD patients are positive to *DR17* and *DQ2* alleles; more recently a closer association has been described with *DQA1*0501* and *DQB1*0201* [306]. Roughly 75% of children in central Italy have an association with HLA-DR7 and ≈50% with HLA-DR17 [476]. The *DR7* allele exists in a heterozygous (HET) combination with either *DR3* or with *DR5* [476]. The study of HLA haplotypes reveals that the *DR7* allele is a linkage disequilibrium with *DQA1*0201* and *DQB1*0201* and *DR5* with *DQA1*0501* and *DQB1*0301* alleles; thus individuals with the HET combination to *DR5/7* have the same combination of *DQ* (*DQA1*0501*, *DQB1*0201*) alleles [476]. The enteropathy, which results in malabsorption, is suspected when children present with diarrhea (or steatorrhea) and weight loss 1–6 months after introduction of foods containing *gliadin, the alcohol-soluble portion of gluten* found in wheat, rye, barley and oat [306], with the previously reported histology. Gliadin is responsible for immunopathological alterations, with a shared AA sequence homology with human adenovirus 12. The immunopathogenesis remains unclear; it is likely that in the lamina propria the gliadin antigen is presented to HLA-DQ restricted CD4-gliadin-specific T cells [306], thus leading to the known cascade of events. Also involved is a type IV CMI mechanism. Gluten ingestion induces proliferative activation of TcRαβ CD8 and γδ CD8 IELs [194]; IEL TcRαβ CD8 T cells are markedly increased and express many $CD45RO^+$ memory cells, as well as TcRγδ CD8, thus demonstrating their role in pathogenesis, which is also indicative of the disease [51]. γδ IEL density was significantly greater than in children with CMA [278]. There is hyperactivation of mucosal T cells and release of ILs, which probably contribute either to the increased imbalance of B-cell response or to crypt hyperplasia and enhanced epithelial permeability and HLA class II and SC expression [51].

Urine mannitol and lactulose have been measured as noninvasive means of evaluating the state of the GI mucosa [501]. Subtotal atrophy of the intestinal villi, the classic hallmark of the disease, is not a universal finding, because some patients have subtler lesions that may make diagnosis difficult. Indisputable evidence that the lesion is gluten mediated in theory would require obtaining a biopsy specimen before treatment, another after withdrawal of gluten from the diet, and a third after gluten challenge. In children, obtaining serial biopsies is only reasonable in doubtful cases or in very young children [501]. However, a gluten challenge to establish the diagnosis should not be performed in children, because a relapse may be expected at a much lower gluten load than previously presumed [291]. Gluten challenge significantly increases anti-WGA (wheat germ agglutinin) and anti-gliadin (AGA) antibodies [158], but not anti-βLG and anti-OVA [157], and IgG and IgA levels are not increased, thus showing that the celiac lesion may be a mitogenic response induced by WGA [158]. For a less invasive diagnosis in children who are ≤2 years of age, AGA IgA are measured in the saliva, with a 90% specificity [190], or in the serum (in 98% of patients after the first biopsy and in 81% after reintroduction), or IgG and IgA together (specificity 94% and sensitivity 89%) [157]. In older children, AGA IgA are measured, then anti-endomysial antibodies (EMA) [501] and tissue transglutaminase C (tTG) [288]. tTG has recently emerged as an outstanding tool for diagnosis: in a study in children using human recombinant tTG, specificity was 100%, and sensitivity was 95% [501]. Starting a gluten-free diet (including all foods of the same family of wheat) induces a decrease in antibody levels also to other foods [157]. However, this diet should be continued throughout life [288].

Crohn's Disease

CD is the principal chronic inflammatory condition and affects infants and adolescents, potentially involving every part of the GI tract. The relations to allergy are sporadic, but based on the improvement of symptoms with an elemental diet.

Gastroesophageal Reflux

GER in infants presents with various symptoms, including emesis, anemia, esophagitis, hematemesis, failure to thrive, near miss SIDS and respiratory symptoms, recurrent vomiting, and colic [167]. The possible clinical overlap with CMA symptoms [167] or eosinophilia [263] makes GER an underdiagnosed condition [167, 263].

Insomnia

Approximately 10% of children aged <1 year and 20% of those 2 years of age suffer from insomnia. There is a growing trend to consider insomnia secondary to intolerance (more than to allergy) to CM: however, 42% of young atopics suffer from the same discomfort [258]. Often a 4-week elimination diet under medical supervision, with the assistance of a diet diary, gives positive results. Attention should be paid to the spontaneous resolution related to environmental measures or the sleep routine. Therefore, both diets and FCT should be limited to not otherwise assessable infants.

Recurring Stomatitis

The lesions are seen in up to 20% of children, consisting in superficial ulceration in the oral mucosa, with itching and burning, attributed to local contact with CM, but also apple, citrus fruit, tomato, pineapple, bananas, and walnuts are incriminated [23].

Ulcerative Colitis

UC is limited to the large bowel. CM has been implicated as a triggering factor in several cases, also because CM ingestion causes clinical flare-ups. However, CM elimination is followed only rarely by the resolution in stabilized cases (see Chap. 18 also for CD).

Vasculitis

In infancy Schönlein-Henoch syndrome is better known, but the etiology is scarcely understood: CM is one of the numerous provoking factors (Chaps. 8 and 19).

Allergy to Other Foods

Dannaeus and Inganäs [118] have studied food effects on diverse systems: lemon, tomato and cereals induce only cutaneous symptoms (in 100% of cases), while all remaining foods induce both cutaneous and GI symptoms, but respiratory symptoms are provoked only by CM, egg and fish (8.5%–50% of cases). Furthermore, numerous histamine-rich foods or with histamine-releasing power are responsible for pseudoallergy (PA) (Chap. 10).

Prevalence

The results presented in Table 9.9 are based on 18 studies in 4,657 selected children of 0.1–19 years, with a thorough evaluation and a proper diagnosis of reactions to several foods [28, 29, 46, 48, 61, 111, 177, 178, 203, 225, 237, 257, 270, 323, 397, 416, 520] and are compared to the results of 456 positive FCT in children and adults from three US centers [48] and of 404 European children and adults [520]. The results based only or mostly on parental reports [40, 211] have not been included since they are not comparable with other results. Some foods, also studied in children of a different age, have a wide frequency range, especially egg (7.3%–85%), peanuts (3.9%–31.4%), wheat (1.1%–23%), fish (2.4%–20%), chicken (1.1%–15%), and beef (0.5%–6%). Pork and shrimp are included in five and six studies, respectively, potato in two, and remarkable rates of 3%–30% for orange, 0.6%–9.5% for apple and 0.8%–19% for tomato were found [224]; the records of peach and almond [270] and the logical differences between younger and older children [47, 224, 316] are reported. In babies with an onset age <12 months, the prevalence is highest [322], while at 36 months there are numerous reductions compared to the total [224]. Severe reactions such as anaphylactic reactions are triggered by egg, peanuts, fish, molluscs, nuts, and spices [340]; shock and urticaria are triggered by peach, wheat and egg in young infants [187]. A case of anaphylaxis and a cutaneous reaction to egg [41] and cutaneous, GI, respiratory reactions to peanuts, peas, beans, herbs, vinegar, ketchup, and shellfish [43] have been reported. Numerous cases of food anaphylaxis are discussed in Chap. 20.

Allergy to Single Foods

See Table 9.10 [19, 415] and Table 1.74 for the allergens.

Egg

The prevalence of allergy to egg in selected children is 26.4%, higher than that of CM (23.5%). IgE-mediated allergy was present in 10/191 unselected infants aged 1 year (5%), none of whom had elevated CBIgE levels [267]. The most sensitizing proteins are in egg white, containing 23 glycoproteins, the principal proteins are, in order of frequency [218], OVA (Gal d 2) (54% of total), ovotransferrin (Gal d 3) (12%), ovomucoid (Gal d 1) (11%), ovomucin (1.5%–3.5%), lysozyme (3.4%) and Igs (4%). The egg yolk proteins are lipovitellin, lipovitellinin, livetin (α, β and γ) and phosvitin. The main aller-

Table 9.9. Studies evaluating the incidence/prevalence of cow's milk allergy in selected children aged 0–19 years

Year of publication		1967	1973	1979	1982	1984		1985	1988	1990		1990		1992	1994	1994		1995	1996		1997	1999	1988	1993
Reference		[176]	[178]	[239]	[257]	[203]		[28]	[416]	[225]		[46]		[323]	[270]	[29]		[111]	[323]		[61]	[397]	[48]	[520]
Age (years)		Infants	Infants	NS	0.1–11	0–4		0.3–10.5	5,3 M	1–3	3	<3	3–19	Infants	18 M	1	2	5.4 M	0–6	7–12	4.2 M	0.1	NS	0–51
Children (n)		150	59	20	68	86		54	160	21		74	111	416	112	1.314		355	507	115	165	544	710	404
Diagnosis		H	H	OPC x CM	OPC	OPC		sIgE	OPC	OBC x CM		OPC x CM		OPB	OPC	OPC		H, IgE	sIgE, OPC		DBPCFC	OFC	SPT, sIgE	DBPCFC
				TH x soy	sIgE	8 m	25 m			SPT + sIgE		SPT + sIgE											OPC	
Food	Mean (%)																							
Cow's milk	23.5		7.5	1.9	41	9	0	85	11	100	24	36.3	11.9	19		6.5	9.6	14.3	33.9	14	13.3	8.3	18.9	16.4
Egg	26.4	7.3	12	20	47	22	11	85	38	38.1	28.6	29.8	25	21		8.4	10.5	20.1	25.5	9	31.4	35.7	34.5	11.9
Peanut	14.8			0					24	4.8	4.8	8	31.4		31			3.9	4.4	15.5	25.7	23.6	18.9	1.5
Wheat	7.8	13.3	8.5		23			22	4	9.5	0	4	1.1		3	2.2	3.7		10.6	12.9	4.8	1.5	3.5	2.5
Soy	6.4	36*	10*	35	1	6.6	0	0	5	9.5	0	10.5	2.3		0	1.9	2.9	0	1.6	1.5	2.9	0.8	6	1
Orange	11.5	7	20.3	30	3			5	28.6	9.5								9	0.6	1.5			1.7	1.7
Apple	4.7				7					9.5	4.8				3				2.4	5.7		0.3		1.5
Peach	–														75			5.1	0.9	4.6				1
Banana	1.3									4.8	0	0	1.1						0.1			0.3	0.7	1.2
Almond	9.0														39			2.1	0.8	2.3		0.3		1.7
Nuts	7.3									9.5	9.5	1.6	16.6					7.1	1.2	5.7			4.6	2
Tomato	6.6		6.8		4					19	9.5				3					0.8	0.9			1.5
Potato	–								1														0.7	2.7
Barley	–							2.5																
Rice	5.5	5.3	6.8																3.8	6.1			0.4	0.7
Rye	0.4											1.1	0						0.1				0.9	2.5
Pea	2.5				7			2.5				1.6	1.7					2.3	1	2.3	1.9	0.8	0.9	0.7
Fish (cod)	8.0			15	11			13	7	4.8	4.8	2.4	4		20			17.8	4.4	3.4	2.9	4.3	4.2	7.2
Shrimp	2.8										0	1.7						3.8		9	0.9	1.4	0.9	5.2
Crab	0.8							1	0			1.1										0.3		
Beef	2.8	4.6			6				3	4.8	0								0.5		0.9	0.9	0.9	0.2
Chicken	2.7	2.6	2		15	2				0		1.1							1	1.1	1.9	0.3	1.3	2.5
Pork	2.9		5		10	1													0.3	0.8		0.3	2.2	2.2
Lamb	0.8	0	5						0	0			0						0.5	0			.05	.05

In the study by Høst et al [186], cases of CM intolerance were not taken into account because at 3 years no child was still CM-intolerant. In three studies, the diagnosis of CMA was made with food challenge, but parents reported for other foods [216] or SPT and sIgE were employed [47, 206]. Studies based wholly or mostly on parental reporting were not included. The means were not calculated for foods rarely reported. Cod was almost always reported for fish.

H history, *TH* telephone history, *SPC* skin prick tests, *IgE* total IgE, *sIgE* specific IgE, *OPC* oral provocation challenge, *OPB* idem in double-blind, *M* median, *ND* not done; *NS* not specified, *CM* cow's milk, *CMA* cow's milk allergy, *OFC* open food challenge, *BFC* double-blind food challenge, *m* months specified; * See the text.

Table 9.10. Examples of common food families

Animals
Mammals (meat/milk)
Cow, goat, lamb, pork, ram, rabbit, sheep, squirrel, venison
Birds (meat/egg)
Chicken, duck, goose, grouse, hen, partridge, pheasant, squab, turkey
Fish
Anchovy, barracuda, bass, bluefish, buffalo, bullhead, butterfish, carp, catfish, caviar, chub, cod, croaker, cusk, drum, eel, flounder, hake, haddock, halibut, herring, mackerel, mullet, perch, pike, pickerel, salmon, sardine, shad, snapper, sole, sunfish, trout, tuna, weakfish, whitefish
Shellfish
Arthropoda (crustacea) Crab, crayfish, lobster, prawn, shrimp
Mollusks Gastropoda: abalone, limpet snail Bivalve: clam, mussel, oyster, scallops Cephalopoda: octopus, squid Others: cockle, conchs, polyp
Vegetables
Caper Caper
Compositae Artichoke, camomile, chicory, dandelion, endive, escarole, head lettuce, leaf lettuce, oyster plant
Grass Barley, buckwheat, corn, oats, rice, rye, sorghum, sugar cane, wheat
Ginger Cardamom, ginger, turmeric
Laurel Avocado, bay leaves, cinnamon, camphor
Legumes Acacia, alfalfa, broad beans, black-eyed pea, kidney bean, lentil, licorice, lima bean, mesquite, navy bean, peanut, pea, senna, soybean, string bean, tamarind, tragacanth
Lily Asparagus, chives, garlic, leek, onion
Mint Marjoram, mint, peppermint, sage, savory, spearmint, thyme
Mustard Broccoli, Brussels sprouts, cabbage, cauliflower, celery, cabbage, collard, horseradish, kohlrabi, mustard, radish, rutabaga, turnip, watercress
Parsley Anise, caraway, carrot, celery, coriander, cumin, dill, fennel, parsley, parsnip
Potato Capsicum, chili, eggplant, pepper (green, red), potato, tomato
Sunflower Jerusalem artichoke, sunflower
Fruits
Actidine Kiwi
Apple Apple cider, pear, quince, vinegar
Beech Beechnut, chestnut, chinquapin nut
Birch Filbert, hazelnut
Cashew Cashews, mango, pistachio
Citrus Grapefruit, lemon, lime, mandarin, orange, tangerine
Gourd Cantaloupe, cucumber, honeydew, muskmelon, Persian melon, pumpkin, squash, watermelon, zucchini
Grape Champagne, grape, raisin, vinegar
Mulberry Breadfruit, fig, hop, mulberry
Musa Banana
Myrtle Allspice, cloves, guava, pimento, pomegranate
Nuts Brazil nut, nutmeg, pecan, walnut
Orchid Coconut, date, vanilla
Papaw Papaya
Pineapple Pineapple
Plums Almond, apricot, cherries, nectarine, peaches, plums, persimmon
Rose Bigarade, blackberry, citron, dewberry, loganberry, medlar, raspberry, sorb, sour cherry, strawberry, youngberry

Data from [19, 415].

Table 9.11. Allergy and multiple allergy to cereals

No. of children	Reference	SPT positive to cereals (%)					
		No. of cereals					
		1	2	3	4	5	6
70/225	[488]	40	22.8	17	8.6	8.6	2.9
DBPCFC	[488]	21.4	2.9	2.9			
28/61	[369]	7.1	10.7	10.7	3.6	10.7	57.1
No. of children	**Reference**	**SPT positive to single cereals (%)**					
		Wheat	**Rice**	**Corn**	**Barley**	**Rye**	**Oat**
61	[379]	26.3	23.4	23	9.7		
23[a]	[399]	78.3			4.3	13	4.3

The reduction of incidence by DBPCFC was remarkable [488].
[a] By OFC.

gens, resistant to denaturation and therefore potent allergens, are Gal d 1, recently considered as immunodominant in man [30], Gal d 2 and Gal d 3; Gal d 3 allergenicity is lesser and that of lysozyme is mild. Even if the sensitization is due usually to egg white, small amounts of Gal d 1 and Gal d 2 are also found in egg yolk, and several responses to OVA may be due to contamination of Gal d 1 traces [30]. Egg has been shown to be the most common food to cause allergy, and to be responsible for IgE-mediated symptoms, including urticaria-angioedema, rhinorrhea, wheezing, AD and GI symptoms, as well as severe asthma after remaining dormant for 500 years [12]. Also known are its histamine-releasing properties. No differences are found between cooked and raw egg: the allergens have a stable sensitizing power after heating at 100 °C for 20 min [526]. Egg sensitization may derive from BM, inadvertent inhalation of small amounts of allergen in kitchen fumes (Table 7.22), school floors in up to 41% of cases [139], and from cross-reactivity among eggs of various birds (see below). An unusual case was that of a boy who reacted while in a room next to the kitchen where the mother was cooking with eggs [22].

Egg allergy, often associated with other allergies, can elicit highly variable symptoms. In four pediatric cohorts (160 children), egg provoked cutaneous symptoms in 45%–78%, GI symptoms in 50%–56% and respiratory symptoms in 8%–39% of the children, solely urticaria in 25%, and associated symptoms of the three organs in 41% of cases [112, 166, 328, 481]. In 467 children [453], the rates were 90% cutaneous (84% of urticaria), 20% GI, and 15% respiratory symptoms. Egg allergy was present in 30% of children with AD and FA, FCTs with egg were positive in 45.8% of children with immediate, delayed, or associated reactions [328], or immediate reactions in 72% of cases [453]. In 324 children followed up from birth for 4–15 years and investigated with RAST and Phadiatop Paediatric, 135 (41.6%) at ≤18 months had at least one positive test, 66 to foods (49%), 70% of whom reacted positively to egg and 15% to CM [449].

Cereal Grains

About 40 protein allergens are known, half of which are common to wheat and barley (allergen Hor v 1); others are found in rice. These allergens are found in both the flour and bran. More precisely, the seeds contain four protein fractions: the more antigenic are glutenins, which form 30% of proteins, and globulins [531]. A wide cross-reactivity is seen among cereals, so children sensitive to wheat are also sensitive to other antigens [379], following the taxonomic relations in the following order: wheat – rye – barley – rice – corn – sorghum; but the primary reactivity is more evident between wheat, barley and rye and between rice and millet [531]. Cereal is allergy not always isolated: apart from the related pollens (see below), multisensitization is frequent (Table 9.11) [379, 399, 488]. Children allergic to cereals have SPT+ to rice, corn, millet and buckwheat, thus indicating either a cross-reaction or a true sensitization, since such cereals are suggested as an alternative to children allergic to wheat and the like [488]. The high number of SPT+ in one trial is astonishing, although reduced with DBPCCT [413], and 57.1% of children were positive to six cereals in another trial [379]. Children with positive SPTs to wheat have associated positivities to other cereals in 59%–77% of cases [379]. In younger children, the incidence rises during their growth: among 66 children at about 18 months of age only three (4.5%) were positive to wheat, but after 18 months there were 14 (21.2%) [449]. Symptom location is also diverse, only respiratory in one cohort [481], cutaneous in 86.4% of cases (73.3% AD and 13.1% urticaria) and GI in 13.4% (10.5% diarrhea and 2.6% vomiting) [379]. In an OFC,

there were no respiratory symptoms (Table 9.11). Interestingly the reactions were immediate in 35%, delayed in 61% and associated in 4% of cases [399].

Wheat

The mean prevalence of wheat allergy is 7.8%. Wheat proteins include six main fractions: gluten, albumin, starch, euglobulin, pseudoglobulin and gliadin; α-gliadin is involved in CD. Hydrosoluble fractions (albumin and globulins) are more often responsible for type I reactions, located in the GI tract. Gluten is an elastic protein used as "glue" for baked goods [531]; 11.7% of 102 children who were RAST+ to wheat were also strongly positive to grass pollen [126]. Generally, children allergic to wheat are also allergic to rye and barley, belonging to the same family, *Triticum* [488]. In Europe, wheat allergy is rather frequent, in other countries it is less so, and in statistics wheat comes after fruits and vegetables, or is absent, except in Nordic countries and USA where corn flakes are more often consumed.

Corn

Corn originates in North America: the US produces 50% of the world's corn. It is credited with a relatively low degree of correlation with other cereals, although corn and rice allergens appear to be similar [531]. An insoluble 50-kD protein present in corn flour may act as an allergen in IgE-mediated FA to corn products, because of its stability to cooking and digestion [376].

Rice

The mean prevalence of rice allergy is 5.5%. Rice allergy is more frequent in Chinese and Japanese populations, because rice has been eaten in high daily amounts for centuries, to which the immune tolerance should be established with time. Japanese investigators have identified 16-kD allergenic proteins in both the glutelin and in globulin fractions of rice [511]. Cross-reactions with wheat, rye, corn and rye pollens are known [126, 488]. In Western countries, five anaphylactic reactions (vomiting, diarrhea and shock) in infants 3–10 months old have been reported in the last 20 years [16, 49, 229, 473]: in two cases the FCT was positive [16] and in one lesions similar to enterocolitis were observed [49]. A 4-month-old girl had vomiting, urticaria and shock [230]. Other children had immediate reactions to rice ingestion with shock, but SPTs to rice were negative [102]. In 7% of children, SPTs were positive, whereas DBPCCTs elicited no clinical manifestations [421], corresponding to positive SPTs in 5.3% and 6.8% of two other cohorts [177, 178]. More often, children with enterocolitis show wheat intolerance.

Barley

Barley may induce reactions because its protein antigens are the same as those in wheat, and barley protein fractions show a strong biological cross-reactivity particularly with rye [413].

Oat

Oat-related allergens react slightly with wheat, rice and barley [413].

Fish

The mean prevalence of fish (cod) allergy is 8.0%. The best known and best characterized fish is the cod Gad c 1 (*Gadus callarias*) allergen, which has been purified until the crystalline form was obtained [1]. It is a protein of the parvalbumin group, which controls the flow of Ca^{++} into and out of muscle cells and contains 113 AA residues and one glucose molecule [531]. It is highly resistant to proteolytic digestion and stable when heated to 100 °C for 10 min [1], thus making Gad c 1 a potent food allergen. Parvalbumins are sarcoplasmic proteins from muscle tissue of fish and amphibian; thus Gad c 1 is found in all fresh- and saltwater fish. RAST-inhibition studies have shown allergenic differences of various fish species with Gad c 1 [374], as confirmed by immunoblotting, demonstrating a 13-kD band in several fish species, corresponding to Gad c 1 [32].

Fish allergy seems to be more frequent in European countries with allergy onset in the first 6 months of life in 24% of cases and in the second 6 months in 51% of children [374]. Fish often induces IgE-mediated reactions: urticaria-angioedema (92% of cases), AD (18%), asthma (inhalation during cooking) (14%), vomiting or diarrhea (11%), up to systemic anaphylactic reactions [374]. In 88 children, 60% of those allergic to fish reacted with angioedema [83]. Since the age of 17 months (mean), 21 children aged 2–12 years affected with AD, asthma and asthma and/or rhinitis, when ingesting fish (regardless of which fish was actually eaten) reacted with generalized urticaria and/or angioedema in 85.7% of cases, vomiting in 19% and respiratory symptoms in 4.8%, as well as two cases of OAS (oral allergy syndrome) [112]. All these children had sIgE to 4–7 fish species. Allergic reactions were associated with airborne fish particles: interestingly, when the same children were exposed to *inhalation of fish particles* at a mean age of 7 years, they reacted with cutaneous symptoms (urticaria isolated or associated with angioedema) in 57.1% and respiratory symptoms (wheezing, rhinoconjunctivitis) in 56.6% of cases. Most reactions occurred at home (53.8%), in the kitchen (26.9%), in a fish-shop (15.4%), at the restaurant (3.8%); in 22.2% of cases reactions stemmed from cross-reactions. Methods

of exposure included cooking vapors or fumes from boiling or frying fish and simple exposure to fish [112]. Smelling the fish odor provoked anaphylaxis in two young children seen in our department. Another child tested positive to cod with a wheal = 7 mm diameter, although we prepared a solution 1:10 with saline to test him.

Precisely the fat-poor species, which are commonly prescribed to infants, are the most sensitizing: *Merluccius merluccius* or hake (95%), *Lepidorhombus whiffiagonis* or whiff (92%), cod (91%), *Glyptocephalus cynoglossus* or witch flounder (90%), *Solea solea* or sole (82%). The least sensitizing was *Thunnus alalunga* (albacore) (21.5%) [374]. Children with cod allergy have SPT positive to eel, dentex, sole, perch fish (PF) (85% of cases) and tuna (55%) [127]. Studies with RAST inhibition that have evidenced cross-reactivity of fish-related allergens reported the following data:

- With cod: eel 89%, dentex 75%, PF 72%, sole 66%, tuna 45%
- With dentex: eel 83%, PF 68%, sole 64%
- With eel: PF 79%, sole 75%, tuna 51%
- With tuna: PF 50%, sole 46% [128]
- With salmon: swordfish and sole 50% [374]

Similarly to what was demonstrated for the legume botanical family, fish allergy is often species-specific, and therefore the reactivity to one type of fish does not warrant automatic dietary exclusion of all related fish species [374], as demonstrated by cod-allergic children who tolerated other species [1, 127], despite cod's widespread use and its extended cross-reactions. Canned tuna and salmon, perhaps as a consequence of a prolonged exposure to heat during the production, could be less allergenic than those cooked conventionally [32]. Canned tuna could contain peanut oil and CM [175]. Similarly to egg, RAST-inhibition studies have found Gad c 1 up to 80%–85% in carpeted and noncarpeted schools [139].

Shellfish

Shellfish can be divided into molluscs and crustaceans. These are among the main nonimmunological mast cell activators (Chap. 10); moreover, several highly sensitizing allergens have been identified and characterized by CRIE (crossed radioimmunoelectrophoresis), which very frequently elicit immediate hypersensitivity reactions: 23 to freshwater crawfish and 17 to sea crawfish. Cross-reactions have been found between mollusc-allergic patients and those allergic to crabs, crayfish and shrimp, thus showing common epitopes [526]. Shrimp-allergic patients have sIgE and/or IgG_2 and IgG_4 [346], with elevated levels compared to controls, which tend to persist at length, even if eliminated from the diet [346]. Therefore, elevated IgG_4 levels may reflect a prolonged exposure to mucosal allergens and a normal response to dietary allergens [346]. Interestingly, there is extensive allergen cross-reactivity (Table 1.73), there is a 75% risk in shrimp-allergic patients with crab and lobster [447], as well as with inhalants (Bla g) [113], and recent discovery that Pen a 1 and Met a 1 are tropomyosins involved in actin activation during muscle contraction, shared with Der f 10 (Mag44) and with several food allergens, which may yield severe reactions in those allergic to dust mites, and with a number of other allergens. Finally, a 3-year-old child suffered anaphylaxis after having eaten shellfish [43].

Molluscs

A major cause of adverse food reactions, molluscs include the Gasteropoda (limpets, snails, whelks), Pelecypoda (bivalves) (clams, mussels, oysters), and Cephalopoda (octopus, polyps, squid). IgE-mediated reactions are more frequent to clams, mussels, snails, and oysters. Snail allergy seems to prevail in Der p-sensitized patients and aggravates their asthmatic symptoms [483].

Legumes

Legumes (peanuts, fava beans, lentils, soy, beans, peas, etc.) possess thermoresistant antigenic proteins; cross-reactivity is rather frequent but rare in children. Legumes cause sensitization and allergic symptoms via ingestion (more common), inhalation and cutaneous contact: rhinorrhea and bronchospasm have been reported by inhalation of cooking vapors of chickpeas and lentils [321] and urticaria due to contact with someone eating peanuts (Table 7.22).

Peanuts (*Arachis hypogaea*) are an annual herb of leguminous papilionates, the seeds or fruits of which are one of the most allergenic foods in children. Moreover, they have the property of cross-reacting with chestnut, hazelnuts and other nuts belonging to different botanical families, more rarely with their similar botanical families. Seven peanut allergens (Ara h with a MW between 15 and 63.6 kD) have a protein content varying from 25% to 28%, with three major groups classified as arachin, conarachin (globulins) and albumin.

Peanut reactions (14.8%) are becoming increasingly more frequent, and from the US peanuts have reached a worldwide diffusion with a prevalence that has tripled in the Isle of Wight, up to 3.3% in 1996 compared to 1.1% in 1994 [188]. Peanut allergy has its onset in childhood, in 92% of cases from 1 to 7 years [155]. More precisely, 16.9% at age 1, 27.7% at age 1–3, 39% at age 3–6 and 46.2% at age 6–15, showing a pattern of increase according to the child's age [397], but before 12 months of age it has been reported between 18.3% and 80% (Table 5.5), and up to 100% of cases [155]. Severe reactions have been reported within a few minutes of inges-

tion [155], anaphylaxis, respiratory distress and angioedema *in three children aged 2–3* [43] and acute anaphylaxis, also fatal [424, 528] (see "FA-Caused Death" and Chap. 20). Attention should be paid to numerous *hidden sources* including foods and vitamins for infants and chocolate-containing foods (see Chap. 24). Some authors have found a high incidence of sIgE to peanuts in children without a known exposure [541] and a fact arousing concern, peanut residues in *foods declared as peanut-free,* but contaminated by previously processed peanuts [262]. Fifty-eight percent of children followed up for 5 years experienced adverse reactions from accidental peanut exposure. Regardless of the nature of their initial reaction, the majority with subsequent reactions experienced potentially life-threatening symptoms. The group with isolated skin symptoms (22%) had lower serum peanut-specific IgE levels than the group with respiratory and/or GI symptoms (78%) [485]. Peanuts are often at the heart of a multiple sensitization with varying types of nuts, and many adults have positive SPTs to inhalants [155]; pollen-allergic children also have sIgE to peanuts (23%) [126]. In addition, there is a controversy on the allergenicity of skin preparations containing peanut oil (Chap. 24).

Soy (6.4%) has a protein content of 32%–42% with two protein fractions, globulins and a whey fraction: the main globulins separated by ultracentrifugation are 11S (glycinin), 7S (β-conglycinin), 15S (aggregated glycinin) and 2S (in the whey fraction), the most thermostable and immunogenic [528]. Three glycoprotein allergens, Gly m 1, Gly m 2 and Gly m 3, were identified. Immunological studies show that a birch pollen-related protein from soy, SAM22 (Gly m 4), might cause adverse reactions up to *systemic allergic symptoms* to soy in patients with high IgE titers to Bet v 1 [271]. Examining Table 1.72, we see that both Gly m 3 and Bet v 1 are *profilins,* therefore the two profilins may cross-react and trigger severe clinical reactions. Banana, apple, tomato, fennel, and celery are other food profilins; however soy, either natural or as a GMO (genetically modified organism), is everywhere, and we name only soy lecithin. By the calculus of probability, it is 10- to 100-fold more probable that a Bet v-1-allergic individual eats soy than fennel. In addition, in a study of ten children aged 1–4 with IgE (mean) at 1122.6 UI/ml, the link between CM and soy was found in a 30-kD component from soybean (Table 1.74) cross-reacting with CM caseins. Thus, the molecule could be involved in allergic reactions observed in soy-fed children exposed to CM-containing foods and in CMA children exposed to soy-containing foods [406]. Children without high IgE titers, and no sensitization to birch or to glycinin run no risks. We discuss below several hypersensitivity reactions in infants and young babies [413].

Pea allergens (2.5%) have been studied less extensively, their allergens, PR-1 and PR-2, are found in the hydrosoluble albumin fraction [528]. A cross-reactivity has been observed with the seeds of soy and white beans [532]. This allergy is found in 14% of pollen-allergic children [126] and in a child with CMA [433].

Lentils are allergens that are very common where they are widely eaten: in Spain they are fourth among the causes of hypersensitivity in children; in India they are ranked fifth [532]. Allergic reactions to lentils start usually before 4 years of age; oropharyngeal symptoms and acute urticaria are the most common symptoms through ingestion, and symptomatic reactivity to chick peas is frequently associated [375]. In Chap. 20 we report a case of anaphylaxis to lentils.

It has been demonstrated that legume allergy is not extended to the entire botanical family. In a study on 69 children with positive SPT response to one or more legumes [31], only in two cases did the initial symptoms reappear after DBPCFC with more than one legume; therefore it is unnecessary to eliminate all legumes from the diet. More demonstrative is that, despite the elevated frequency of positive SPT response to peanuts and soy, only 2%–3% of such positive responses have been confirmed by DBPCFCs [46, 421]. Bock and Atkins [46] studied 32 children with peanut allergy confirmed by DBPCFCs and found that ten (31%) had a positive SPT response to soy, but only one (3% of those with peanut allergy) had a clinical reaction to soy. Among 113 children with AD evaluated with DBPCFCs, only one (0.8%) had clinical allergy to both foods, despite 19% reacting to peanut and 5% to soy [421]. In conclusion, clinical hypersensitivity to one legume should not mean that it is sensitizing to the entire family to which the food provoking symptoms belongs; the possible sensitization should be verified by a DBPCCT.

Potato and Related Foods

This family includes the tomato, potato, eggplant, capsicum, etc. Only a glycoproteic fraction of 20 kD of the *tomato* (7.2%) has been isolated with allergenic properties, stable at acid and neutral pH, and thermoresistant. Characteristically, ripe tomatoes are more allergenic than green tomatoes [10]; many 25-mg/kg glycoproteins are found in ripe tomatoes. Isolated allergy is uncommon; 40% of 102 pollen-allergic children were also allergic to tomato [126]. Symptoms linked to its histamine-release power are frequently appreciated.

Potato has seven allergenic fractions at a MW of 16–65 kD, one of which is thermolabile and disappears after cooking at 40 °C, and is usually responsible for symptoms when it is peeled or eaten raw [505]. Nevertheless, a 5-month-old girl experienced anaphylaxis when eating cooked potatoes for the first time [99]. Cross-reactivity is possible with birch pollen [135] and latex (Table 8.14).

Apple and Related Foods

Apple (4.7%) allergens are the pulp proteins, commonly found in fresh fruit juice; they are less resistant and disappear after 48 h if the fruit is stored at ambient T or at + 4 °C. Such allergens are not stable in solution and are easily oxidized, thus explaining why the prick + prick (P+P) method is preferable [135] (Table 6.10). Some varieties express a marked capacity of IgE-mediated binding to an allergen of 18 kD, Mal d 1 [497, 498], more evident in pollen-allergic patients due to cross-reactions with Bet v 1 (birch) [499], Bet v 2 (profilin) [141], Api g 1 (celery) [132], Pru p I (peach) [482], latex (Table 8.14), carrot and potato [135]. SDS-PAGE (sodium dodecylsulfate-polyacrylamide gel electrophoresis) studies have established that 100 g of Delicious apple contain 1–5 mg of Mal d 1, so a mouthful of about 10 g contains 0.1–0.5 mg of Mal d 1, which should be less sensitizing than Bet v 1 [497].

Plums

Peach, apricot, cherries, and plums can induce severe reactions in the pediatric age group, above all when ingested by children sensitized to birch pollen, and can cause cross-reactions with latex (Table 8.14). Pru p I of *peach,* which is mostly in the skin, causes labial and lingual angioedema and/or perioral contact dermatitis and cheilitis, oral and pharyngeal pruritus and rhinoconjunctivitis in many patients [270]. The risk of reactions in peach-allergic children to apple, cherry, pear and plum is 55% [447]. Bet v 1 and Bet v 2 cross-react with Pru av 1 and Pru av 4 (cherry). The study on plum cross-reactions to other fruits (see OAS) has disclosed common epitopes [377], and a case of shock has been reported [413]. Commonly, children may be sensitized to other fruits of this family (Table 9.10) or to strawberries, which in children frequently provoke cutaneous symptoms induced by aromatic substances or dyes [534] and have a direct action on mast cells. sIgE were demonstrated against a 30-kD allergenic epitope present in peach, strawberry, banana, tangerine [503], cherries [377], and kiwi [502] (Act c 1) (Table 1.74). A case of anaphylaxis in a child eating kiwi for the first time was reported to us.

Citrus and Related Fruits

Lemon, lime, orange, tangerine, and grapefruit contain terpene bodies in the skin and pulp (d-limonene, carotene) and can provoke hand dermatitis, *citrus* contact dermatitis and cheilitis and laryngeal edema, possibly related to toxicity of peel terpene bodies or to pesticides (Chap. 10). *Orange* (11.5%) is the cause of anaphylactic reactions, eczema, asthma or urticaria due to seed proteins [534]. In children with FA and inhalant allergy, sIgE to *grapefruit* have been detected [534]. Both citrus fruits and the parsley family contain natural furocoumarins causes of phytophotodermatites (Chap. 8).

Musa and Gourd Family

There is cross-reactivity between banana (1.2%), members of the gourd family (Table 9.10) and profilins (Table 1.72); *banana* induces symptoms in allergic individuals to ragweed pollen because of common epitopes, and latex (Table 8.14).

Cashew

Cashew falls within the hidden allergens: two adolescents died after ingesting foods containing cashew as an undeclared ingredient [424]. *Mango* provokes anaphylaxis and ACD. *Pistachio* antigenic fractions have marked cross-reactivity with mango and cashew [160].

Parsley and *Compositae* Family

Celery, carrot, fennel, coriander, etc. are prominent (Table 9.10). Subjects allergic to *Compositae* have experienced anaphylactic shock by ingesting celery or mustard (Chap. 20). Recently, *celery* allergen Api g 1, with a MW of 17 kD, has been defined [142] by immunoprint inhibition shows shared allergenic 17-kD epitopes with carrot and birch [206]. Analyzing cross-reactivity between celery, carrot, cucumber and watermelon, immunoblotting has demonstrated that all share a 15-kD protein [252]: in both cases the MW recalls that of profilins. Moreover, celery, carrot and anise share allergenic epitopes with fresh vegetables [535]. Api g 1 is part of the profilin family and has antigenic similarity with apple, peach and potato [381]. Celery can elicit cross-reactivity with *parsley,* cabbage, cauliflower and broccoli [535], less often with carrot, cucumber and anise, thus forming the celery-carrot-mugwort-spices syndrome [521].

Nuts, Pine Nuts and Seeds

Walnuts (Juglandaceae) are biologically related to American nuts or pecan (*Carya illinoensis*), and Brazil nuts (*Bertholletia excelsa*) have several 5- to 50-kD antigenic proteins [13]. Patients allergic to birch, alder and hazelnut pollens experience evident reactions after ingesting hazelnuts and occasionally apple or other rose fruits [533]. Generally, pollen-allergic patients report hypersensitivity to walnuts and hazelnuts more frequently than non-pollen-allergic patients [533]. Sensitization to hazelnut was associated with reactivity to birch pollen in 4/11 children [164]. Cases are known of severe reactions to Brazil nuts within 3 min of ingestion,

with cutaneous (angioedema and generalized urticaria), respiratory and systemic symptoms as severe as anaphylaxis [13], caused even by involuntary ingestion [528]. Walnuts provoke fatal anaphylaxis (Chap. 20) and asthmatic manifestations after DBPCCT [359]. Cross-reactions are also frequent: in a group of children, one reacted to DBPCCT to five nuts, one to two nuts and 12 to one nut each; two reactions were positive to pistachio and one to hazelnuts [46]. The risk of reaction in walnut-allergic patients to Brazil nut, cashew and hazelnut is 37% [447]. In the US, cases of systemic reactions and *pine nut anaphylaxis* have been reported in adults (Chap. 20). Two young Spanish girls had two or three repeated episodes of anaphylaxis after eating small amounts of pine nuts, correlated with this nut only after the last episode [231]. Pine nuts are almost unknown in the US, while in Mediterranean countries they are widely consumed as such, or employed in confectionery, sauces and dishes, and are pressed to extract oil.

Cases of anaphylaxis, angioedema and generalized urticaria have been reported to be provoked by *sesame* seeds utilized for vegetarian and health foods, hamburger, salad dressing, as well by bakers [533].

Mustard and Related Foods

Two types of *mustard* allergy, black (*Brassica nigra*) and white mustard (*Sinapis alba*) are implicated. The known allergens are Bra j 1 of *Brassica juncea* and Sin a 1 of *Sinapis alba*, which have elicited type I reactions as well as anaphylactic and type IV (contact dermatitis) reactions: cases of anaphylaxis in children were generated by mustard addition to sausages and sandwiches [344]. *Cucumber* induces sIgE in patients with FA and reactivity to common epitopes of ragweed has been described, as well as to banana, melon and zucchini [150].

Additional Foods

IgE-mediated reactions have been made known to camomile, sunflower honey, muskmelon, pomegranate, kiwi, uncommon fruits (raspberry, cranberry, red and black currant, etc.) and exotic fruits [205, 283, 502, 516, 520], which should be eaten with precaution by children with diagnosed allergy to foods or pollens, because of possible cross-reactions. *Honey* may include pollen allergens: 10 g may contain from 20 to 10,000 pollens [205] and trigger mild [283] to severe symptoms such as generalized pruritus, vomiting and diarrhea [205]. Allergy to other vegetables utilized in bakeries, including sunflower and flaxeeds have been reported [520], and anaphylaxis following ingestion of meat containing papain employed to tenderize meat [534]. Furthermore, the *cross-reactivity* between banana, cucumber, melon and zucchini with ragweed and of almond, apricot, cherry, and peach with birch is widespread [381]. Patients allergic to melon have a 92% risk of reactions to avocado, banana and muskmelon [447]. *Camomile* may cause symptoms in *Parietaria*-allergic subjects and royal jelly in *Compositae*-allergic patients (ragweed and mugwort) [532], the latter up to asthma and anaphylaxis [475]. Cross-reactions are known between *Compositae* and sunflower pollens [159], which have a partial allergenic identity with sunflower oil and mugwort pollens [260], as well as in patients with profilin-modulated allergy to various fruits, between latex and banana, avocado and coconuts (Table 8.14) and other fruits and vegetables [141]. Additional cross-reactions are with poppy seeds and sesame, kiwi, rye and hazelnuts [502] and bird derivatives with chicken egg (see "Bird Egg Syndrome"). We underline that *sunflower* is a cause of cross-reactions [159]. In an infant 6 months old, it caused severe symptoms; the infant ended up in hospital every time he received cereals with sunflower oil [169]. Since the use of this oil continues to propagate, the potential sensitization to mugwort pollens should be taken into account [260].

The medical literature over the years has attributed a varied spectrum of symptoms to *chocolate* consumers. The case of Talbot is well known [469]. Several children had many positive SPTs but DBPCFC to chocolate was negative in all subjects [61, 270]. Since no immunological pathogenesis has been demonstrated, the term "intolerance" is more accurate, and is relatively frequent in children, especially in atopic children, while the hypothesis of cross-reactions between legumes and chocolate belonging to the same botanical family has not been validated [31]. In 4/57 children 1–5 years of age, affected with FA (CM and/or egg) with SPT+ to both chocolate and legumes, and one to legumes, the OFC was negative in all. In the control children, there was one positivity to legumes (p=0.01). Since the 4 children had SPT+ also to legumes, a cross-reaction between chocolate and legumes is very rare, so these data do not warrant dietary elimination of plain chocolate. The cases reported are likely due either to chocolate's pharmacological properties (Chap. 10) or to other constituents, including aromatizing substances (cinnamon, vanilla, nutmeg), soy lecithin (emulsifying and anti-oxidant), cocoa butter and other vegetable fats such as oleic acid. Moreover, nitrogen residues, tyramine, histamine-releasing substances, balsam of Peru, Ni sulfate up to 220 mg/hg, bichromate K, CoCl, Cr (traces) and latex are found (Chap. 8). Hidden CM has been detected in chocolate stemming from contamination by previously processed food items, but CM in plain chocolate is generally not reported on the label and it is unknown whether substituting cocoa butter with different oil types is devoid of risk.

Table 9.12. Prevalence of asthma in children with FA

Authors	Reference	No. of cases	Median age or range (years)	Asthma (%)	Diagnosis
Oehling et al	[363]	284	2–8	8.5	OFC
Onorato et al	[370]	300	6.5*	2	DBFC[a]
Novembre et al	[359]	140	5	5.7	OFC
Meglio et al	[328]	70	2	13.1	OFC
Bock	[43]	279	0.4–19	2	DBFC
Salob	[412]	15	11	6	NS
James et al	[242]	88	7.5	14.8	DBFC
Cantani et al	[86]	213	3	6.6	OFC
Carroccio et al	[98]	91	0.2	8.8	DBFC[b]
Mean				7.3	

NS not specified, *OFC* open challenge, *DBFC* double-blind challenge.
The studies are ordered according to the year of publication.
[a] The six patients with asthmatic reactions were children, whose median age is shown.
[b] Four children reacted to ass milk and four to a casein hydrolysate formula.

Multiple Food Sensitizations

Multiple food sensitizations, contrary to what is commonly believed, are rare [47, 82, 83, 421, 449]: arguments have been put forward that assume that children with several positive SPTs and RASTs should not endure rigid diets that eliminate a wide spectrum of foods. To assess food polysensitization properly, it is necessary to schedule children for FCTs to each suspected food, excluding those with a convincing history of prior life-threatening reactions. These observations underline the difference between humoral (RAST positivity) and clinical sensitizations (FCT positivity). Borderline cases are reported in Chap. 16. The prevalence is highest for 2-food hypersensitivity (24.6%) than for 3-food (7%), 4-food (2.9%) and 5-food (1.8%) hypersensitivity. In 88 children [83], 25 (28%) were sensitized to CM and egg and 4 (4.5%) were also sensitized to wheat. Polysensitivity (especially toward CM, egg and wheat) was recorded in 29% of children [69], and in 43/115 (37.4%) children: to CM, egg and wheat in 27 cases, CM and wheat in 8, CM and egg in 8 [92]. In 128 children affected with AD associated with FA (lasting 1–5 years) [82], we did P+P tests with vegetables and fruits: FCTs were positive to citrus (62%), potato (12%), tomato (24%) and peanuts (11%), whereas no child positive to other foods reacted to a pertinent FCT. Therefore it would be unwise to make a diagnosis of multiple sensitization based only on pseudo-positivity. In 63/113 children with AD associated with FA who underwent elimination diets based on history and positive SPT and/or of sIgE, 20 children (31.7%) reacted to 3 or more foods: 12 of them to 3 (CM, egg, wheat or soy), 5 subjects to 4 foods (the above plus wheat), and 2 children to 5 foods (the above plus fish); in only two children 3 foods (CM, egg, wheat) were eliminated to obtain improvement of cutaneous symptoms [421]. In the study of Bock and Atkins, 52/480 children (11%) reacted to two or more foods, and 10/52 to three or more foods [47], while in 46/66 children with sIgE reacted to egg; 30%–37% also had sIgE to CM and wheat [449]. In 71 subjects with a mean age of 18 years, with FA onset after the age of 10 years, the main SPT results and FCT results were as follows: peanuts, 46 (22%), orange, 21 (6%), carrot, 9 (4%), tomato, 17 (2%), wheat, 10 (2%) and apple, 8 (2%); soy and chocolate yielded negative results [270].

Association with Asthma

Many children react to CM and to FCTs with respiratory symptoms, as results from a meta-analysis on the prevalence of asthma in children with AD and FA (46.8%) have shown (Table 7.10). Respiratory symptoms in Fig. 9.14 were induced only by CM, while, as seen in Table 9.12 [43, 86, 98, 242, 328, 360, 363, 370, 412], asthma has been noted in 7.3% (range, 2%–13.1%) of 1,480 children with FA, but it was also produced by other foods. Our data show that 23/94 children (24.5%) (median age 36 months) with asthma and sensitization to CM demonstrated by positive SPT, RAST and DBPC, reacted with bronchospasm to the administration of CM and five to CM-derived formulas: in five children asthma appeared after administration of very small doses of CM on the lower lip [86]. In a selected group, 5 of 20 children (25%) reacted with asthma to DBFC to CM and HFs [396], and a young woman reacted with asthma to egg proteins that had remained dormant for 500 years [12]. The association of 57%–83% cases of asthma with severity of FA reaction has been demonstrated in 293 children [312]. Many children with

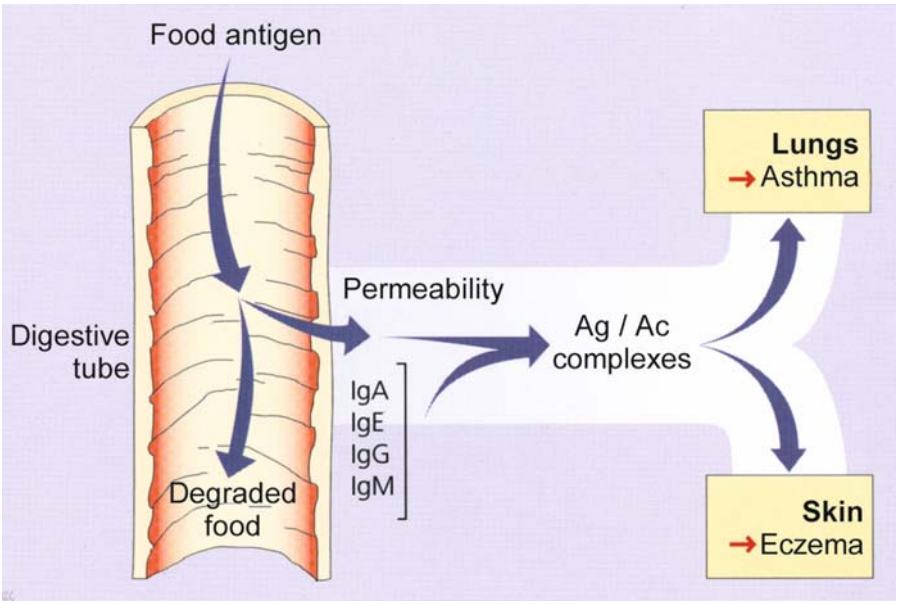


Fig. 9.16. Food allergy and asthma (or atopic dermatitis)

recurrent wheezing that had begun during the first few weeks of life required frequent emergency room visits and even multiple hospitalizations, until elimination of CM from their diet resulted in complete wheezing remission [23].

From the Heiner study it is known that CM [204] or other foods are able to induce respiratory symptoms immediately, due to inhalation, for example of cooking vapors or fumes [112] ingestion during FCTs [88], or exercise, sometimes with fatal effects [424, 528]. A 7-year old boy reacted with wheezing to inhalation of peanut and shrimp [470]. Food and inhalant allergens can share common epitopes, either totally such as garlic powder, crabs and shrimp and fish, or partially such as *Compositae* pollen and related honey, bromelin from pineapple, as well as common epitopes shared by mugwort with celery and tomato with grass pollens [381]. The prevalence of food asthma in children is at the limit of asthma associated with more frequently responsible foods, in order of frequency: peanuts, CM, egg, nuts [44], CM, egg, peanuts [359, 420], CM, peanut, egg [453], egg, shellfish and CM [364], fish [112] and hidden foods [340]. A monosensitization may be present in 77% of cases in a cohort of 163 wheezing children with 56%–83% positive FH of atopy (FHA) and multiple food sensitizations; sIgE were positive to one or more foods, depending on age: 4% <2 years, 24% between 2 and 4 years, and 31% ≥4 years [394]. Cross-reactions are more frequent in older children and adolescents: snail-sensitive individuals experience bronchospasm in 100% of cases and have the highest IgE levels [364] with possible cross-reactions to mites [340] (see OAS). In four OFC/DBPCFC studies in children with FA that we examine below, the rate of respiratory reactions is 28%–59% [47, 55, 242, 416].

To explain the pathogenesis of such episodes, it is hypothesized that the airway uptake of ingested food proteins takes place in two ways [50] (Fig. 9.16) [340]:
1. Across the epithelium, transported by M cells, reaching the respiratory tract systemically (and via the skin)
2. Aspiration and inhalation of food substances, without doubt the less plausible, but it deserves attention

In the first case, food allergens are able to pass across the mucosa at any point of the GI tract, as a result of increased permeability, then into the circulation, thus reaching distant organs, including the skin and airways. These diverse passages could interfere with food immunogenicity and as a result the epitopes can be broken down or hidden epitopes can be uncovered (Fig. 1.18). As a consequence, food immunogenicity is reduced or raised depending on cross-reactions between food and inhalant allergens [381].

Three distinct patterns can be envisaged [50]:
1. Food-induced asthma, caused by adverse reactions to foods, whatever mechanism is involved, immunological or nonimmunological
2. Asthma with FA, in children presenting with both asthma and FA without a confirmed causal relationship between asthma and FA
3. Asthma due to FA when children present asthma and FA causing respiratory symptoms

In conclusion, the respiratory reactions observed during FCTs may be classified as belonging to the second category.

The association is more convincing in the following conditions [540]:
- FH positivity
- Age up to 3 years

- Wheezing present in the first months of life, often in connection with bronchiolitis, especially by RSV (respiratory syncytial virus) or other viruses
- Coexistence of other affections, such as rhinitis, abdominal pains, and above all AD
- Elevated concentrations of total IgE (>1,000 UI/l), especially when concordant with SPT positivity, anaphylactic or urticarious symptoms and/or asthma less controllable by appropriate treatment
- Positive RAST to foods
- Immediate symptoms following FCTs

Asthma may occur in connection with exposure to pollens cross-reacting with foods. The best proof is the remission of respiratory symptoms by an elimination diet and the relapse by DBFCs [50].

Diagnosis

Diagnosis of FA is very challenging and is often not as simple as it might look [19], as we have detailed in Chap. 6, especially the rare compliance (voluntary or involuntary) to elimination diets. A presumptive diagnosis of FA based on medical history, SPT and RAST is acceptable in selected cases of severe anaphylaxis after eating specific foods, when one is in a hurry [82]. The diagnosis is structured on a series of diagnostic procedures outlined in Fig. 9.17.

A detailed evaluation of cutaneous lesions is done before and after the elimination diet, as described in Figs. 6.2, 6.3, 6.19. Additional tests that may be required in selected cases will be elucidated in "Other Diagnostic Tests."

When *history and clinical picture* have yielded valuable clues to a thorough diagnosis, an immediate type is almost always suggested, so that the necessary steps can be initiated. When these first indications are missing (such as in a mute history) or are directed to a food intolerance, the next step consists of seriously considering that children continue the same *diet followed up to the moment of the visit* over 2–4 weeks, with parental help when possible.

A *diet diary* is frequently utilized in adjunct to the medical history to provideg a sequential record of all foods ingested for a specified period and to detect the possible presence of further complaints [23]. Parents are instructed to record serially in a notebook, over a predetermined period and at every meal, the type, time and quantity of suspected foods ingested, specifying the composition of commercial products. They should record the symptoms that occur, even if apparently modest (pruritus, erythema, worsening of lesions, etc.), indicating the day, time and the place if different from the child's home. The symptom occurrence or exacerbation requires returning to the previous diet. The diary is reviewed periodically by the pediatrician or allergist to determine whether there are significant relationships [23, 54]. When unusual and/or apparently severe reactions occur, the parent should inform the doctor quickly: he or she will decide whether or not to change the prescriptions. The partial or complete remission of symptoms shows that these were elicited by one or more eliminated foods. The diet diary is important in that, contrary to history, the symptoms are recorded *on a prospective basis* and therefore independently of poor child or parent memory [59].

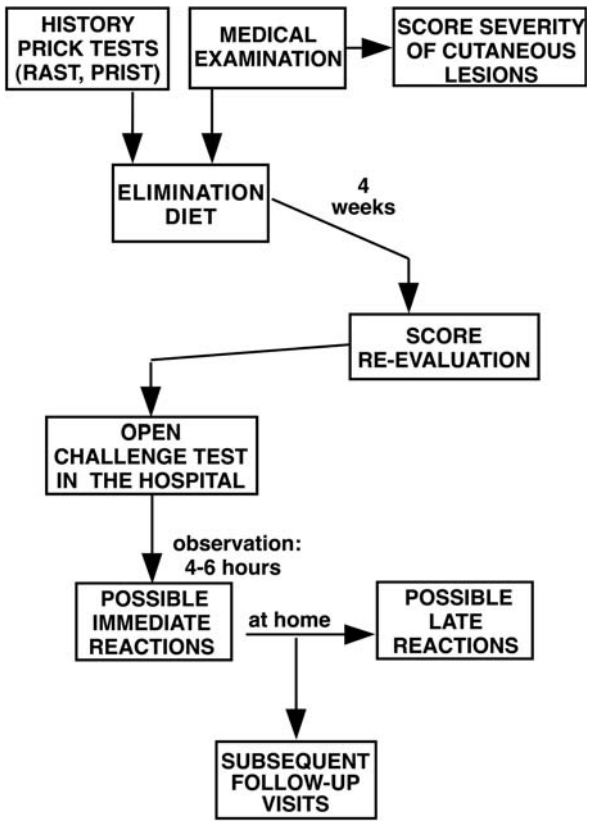


Fig. 9.17. Diagnosis of food allergy

If both pediatrician and allergist agree SPTs, PRIST, and RASTs are done, requiring the number of strictly indispensable allergens. Such tests are a complement also because the results often cause a *diagnostic discordance,* since the auxiliary test negativity and the positivity of clinical data are suggestive of a food intolerance: try in this case the prick by prick test. At the subsequent visit, the results are examined: if the cause–effect relation between administration of one or more foods and recurrence of symptoms is evident, or if these were continuative, or the diary gives eloquent indications, a diagnostic elimination diet is commenced.

The easier cases to resolve are those with mono- or bi-sensitization; provided that the suspected foods are ≤3; their staggered reintroduction should induce the pertinent symptoms, thus confirming the diagnosis.

As mentioned in Chaps. 6 and 7, the *current knowledge limits*, on practical grounds, the *value of SPTs and*

Table 9.13. Sensitivity and specificity of SPT and sIgE in pediatric trials

Allergens	Sensitivity (limits)	Specificity (limits)
Skin prick tests		
CM	0.28	0.80
Egg	1.00	0.25
Peanut	1.00	0.50
Tomato	1.00	0.66
Various	0.00–1.00	0.63–0.90
sIgE		
CM	0.35	0.77
Egg	0.62	0.33
Peanut	0.25	1.00
Tomato	0.14	0.50
Cod	0.91	0.94
Various	0.67–0.77	0.71

Modified from [37].

RAST in the diagnosis of FA, whereas *the necessity of FCT is clear* [179, 270, 328]. An interesting study has meta-analyzed the results of pediatric trials employing DBPCCTs, by examining SPT sensitivity and specificity in five trials with 340 children and of sIgE in four trials with 204 children, as seen in Table 9.13 [37]. The comparison with the data of Table 6.19 shows that SPTs for foods have the greatest specificity, but lack sensitivity, whereas sIgE fare well only with cod [37]. In the "immediate reactor" children, the diagnosis made by a SPT ≥4 to CM was confirmed by a parental challenge [212]. Thus, RAST and SPTs, even if they can yield helpful clues for the diagnosis, should not be the only procedure to exclude or incriminate a food as the cause of symptoms or to select the type of elimination diet, neglecting clinical history, medical examination, food diary, and FCT convincing data.

Diagnostic Elimination Diets

The choice of a diet to start adequately represents a crucial moment for the diagnosis of FA. When data are fulfilled, univocal, etc., or have clear-cut indications, a diagnostic elimination diet is prescribed, which, if followed completely free of any type of the offending allergens, will provide clear improvement of symptoms, and to their relapse after reintroduction of incriminated food. This type of 4-week diet involves omitting the food (or foods) identified with sufficient certainty, inviting the parents to continue to record on the diary the frequency and severity of adverse reactions. The elimination diet is simpler in infants, because only one food is substituted, but is more complex in weaned babies, since the suspected foods to be eliminated are more numerous the food requirements and more differentiated.

Several different elimination diets have been proposed [19]:

Conventional diet. If symptoms occur the foods that more frequently cause symptoms in infants are avoided: CM and dairy products in children eating only CM, also eggs and the foods in which they are incorporated if reactions occurred after their introduction. Otherwise, epidemiological data may be followed based on age: if the child is <6 months CM and egg are avoided, if the age is >6 months often a multiple sensitization exists, hence also wheat, fish, etc. are avoided, always evaluating the specific case and the data on the prevalence.

Advantages: good compliance, minimal financial impact, a good approach when the offending food is not identified but the symptoms are frequent or persistent; thus FA is highly suspected.

Disadvantages: the diet may include sensitizing foods, the effects may be appreciated only after weeks or months, nutritional imbalances may occur, and this diet may be monotonous, thus reducing compliance.

Specific diet. The foods clearly correlated with the symptoms and their onset are eliminated, and by its greater specificity better results are obtained; however, the foods to be avoided are incorporated in common processed foods.

Advantages: most effective, especially in very sensitive children, usually easy to prepare, good compliance, absent or minimal nutritional derangements and financial impact.

Disadvantages: the elimination of highly diffused foods such as CM, egg, wheat, fish, etc., can be problematic, other members of the same food family may need to be avoided, younger babies may be provided with a substitute formula.

Elemental diet. This is suitable for polysensitized children with rather severe symptoms, but for a limited duration, because the formulas without animal or vegetable proteins, replaced by synthetic AA, are thus very hypoallergenic (HA). They often provoke diarrhea secondary to carbohydrate malabsorption due to a 16% carbohydrate content. Because of its look, smell and taste, this diet cannot be proposed to children, especially to infants and older children who prefer fasting to the point that this diet must be administered by *nasogastric tube* [481]. Recent AA products are much better tolerated (see "AA Formulas").

Advantages: easy to prepare, nutritionally adequate, ideal being least allergenic, best for children highly sensitive to several foods, but for a limited duration until the symptoms abate.

Disadvantages: expensive, poor palatability and compliance, monotonous, dietary restrictions at times lead to rebellion, obviously children aim at satisfying their hunger, with understandably irreparable results.

Rotation diet. This might be used in children sensitized to more foods, but to a slight or moderate degree, or to children not able to follow an elimination diet correctly. Members of the same food family are eaten separately: the prerequisite is that, based on food families (Table 9.10), the members of the sensitizing family are eaten at intervals of not less than 1 day; no food may be eaten more often than once in 4 days. This diet is varied and seems to be fairly agreeable. It is based on the principle that allergy to one member of a food family may result in a variable degree of allergy to the other members of the family; however, as regards legumes and fish, this theory finds no confirmation in recent studies [31, 32].

Advantages: satisfactory control of symptoms in children with mild or moderate sensitivity to several foods, reasonable diversification of foods, children may follow tabulated diets.

Disadvantages: necessity of knowing food families and adopting an efficient planning, difficulty of retracing the foods eaten, the sensitizing foods may be masked in processed food products or restaurant servings, etc., unsafe for highly sensitized children, probable failure when the responsible allergen is shared in sufficient quantity in several foods of the same family, and sporadically will trigger subclinical disease by continued intake of small amounts of allergen, adverse reactions to ingestants may require long-term treatment of some children.

Oligoantigenic diet based on foods known to be oligoantigenic: our experience began with the 41 children affected by chronic diarrhea by CMA, hospitalized in our division (Table 9.6), utilizing the effective *homemade meat-based formula* (HMMBF) (Rezza's diet) reported in 1973 specifically for children with CMA [403]. It is prepared with *lamb meat,* fresh or frozen lean meat (free of fat and tendons) that is cut into small pieces, boiled and minced, then mixed with the other diet components (Table 9.14) [80, 403]. We suggest using only one cereal, rice, to avoid a simultaneous exposure to more allergens; Ca (500–1,000 mg/day) must be added to the formula. The HMMBF offers a great advantage since it can be *personalized* by adding other foods gradually (see "Treatment") depending on child age, clinical severity and FCT results [89].

Table 9.14. Composition of home-made lamb-meat diet (Rezza's diet)

Food	Dose	
Lamb (fresh)	g	100
Olive oil	g	40
Rice flour	g	70
Ca	mg	500–1000[a]
Vitamin D	U	400
Water up to	l	1

Cut fresh or frozen lean lamb's meat (free from fat and tendons) into small pieces, boil in a double boiler and mince the lamb meat, then mix it with the other diet components. One liter is for four servings.
Data from [80, 403].
[a] For children aged 0.1–7, see Tables 9.23, 9.24.

The HMMBF (Table 9.15) [80, 403] has an adequate nutritional value, provides 310 kJ/l, (741 calories/l). We have calculated the nutritional value [80], comparing it with ESPGAN guidelines for infant formulas (Table 9.16) [80, 151]. We stress its pleasant taste, the adaptability to individual needs, also in younger infants, and the low cost of its constituents [66]. Lamb meat may be substituted with rabbit, or horse meat, lean pieces of pork, or with chicken [80] (3.1% in Table 9.9) or turkey *only if children are not egg-allergic.*

Rezza's diet is not devoid of a virtual antigenicity (Table 9.17) [80]; however, as far we know no case of lamb allergy has been reported, not even in countries where lamb meat is greatly consumed such as New Zealand, likely because lamb AA sequences are not able to form reactive epitopes. Even if the ovine meat contains serum albumin, this has an elevated thermolability and is denatured by cooking [403], thus explaining its

Table 9.15. Composition of Rezza's diet (per liter)

Foods	g	Calories	Carbohydrates g	Fats g	Proteins g	Ca mg	Fe mg	Na mg
Lamb meat	100	121.7		4.6	20	10	2	100
Olive oil	40	360		40				
Rice flour	70	259	58.8	0.7	4.6			
Calcium	0.5[a]				500			
Total		740.7	58.8	45.3	24.6	510	2	100

If the rice flour is Ca-enriched, calculate the dose for 70 g and subtract it from Ca addition.
Data from [80, 403].
[a] Tables 9.23, 9.24.

Table 9.16. Comparison between Rezza diet and ESPGAN guidelines on infant nutrition

	ESPGAN	Rezza' diet
Energy (kJ)	268–301	310
Proteins	1.2–1.9	2.0
Fat	2.7–4.1	4.5
Carbohydrates	5.4–8.2	5.9
Na (mEq/l)	<12	4.3
Ca (mg/dl)	40	31
Vitamin B_1	0.4	0.12
Vitamin B_2	0.6	0.2
Vitamin PP	3	4.9

Grams per decaliter when not otherwise stated.
Data from [80, 151].

Table 9.17. Advantages of HMMBFs (Rezza diet)

1. Adequate nutritional value
2. No cross-reactivity with CM proteins
3. Lower antigenicity than that of CM proteins
4. Hypoallergenicity
5. Nutritional adequacy
6. Easy availability
7. Pleasant taste
8. Low cost
9. Adaptability to individual needs

Modified from [80].

use in CM-free diets [182]. In one trial, the lamb meat diet was changed in 7.7% of cases [517], but it is unknown whether by a doctor, or the family, or for reasons of poor compliance, or intolerance to ingredients not included in the original diet [403]. Seven reactions to lamb reported in 97 children came from *parental diagnosis* [40], and in most studies reported in Table 9.9, no reactions were found [48]. The total rate of 0.8% is validated by 5% of positive SPTs [178], but when SPT+ to lamb are verified by DBPCCT, they become negative in 100% of cases [416]. For pork (Table 9.9), there is a 2.9% rate in positive SPT, RAST and clinical history [178, 339] but, controlled by FCTs, it is reduced to 0% [416] or to 2% [421]. A reaction to cooked and roasted pork has been documented by OFC (RAST was negative) in a 4-year-old boy with AD and Der p sensitivity [319]. However, some reactions are usually due to the additive penicillin, which is not inactivated by cooking, since it is thermostable [519].

HMMBFs can be used in a number of situations [80].

- *Symptoms of IgE-mediated CMA:*
  - Vomiting
  - Diarrhea
  - Angioedema
  - Urticaria
  - Asthma
- *AD*
- *Intolerance to CM:* used both in diagnosis and treatment of adverse food reactions

Advantages: nutritionally adequate and not allergenic, can be utilized also in children 8–9 years of age, almost always with a good rate of compliance [309], especially since it is personalized and has good palatability, minimal financial impact;
Disadvantages: before personalization it might cause monotony and noncompliance in older children.

In conclusion, depending on the disorder and the age, we suggest the specific diet or the lamb meat diet.

Observations on Diagnostic Elimination Diets

The success of dietary regimens depends on a wide spectrum of factors, including the identification of offending allergens, child compliance, the child's ability to maintain the elimination diet and the assumption that no other factors provoke similar symptoms. For example, if a child reacts to CM the symptoms are resolved after an appropriate diet, he (or she) is diagnosed as having CMA. If instead the child is affected with lactose intolerance she (or he) could suffer from similar symptoms and likewise improve with dietary changes, or could be affected by PA and the symptoms would not change [54].

In the first days of the elimination diet, some concern may arise because of the refusal of new or different foods, even with wholly palatable diets, or a kind of deprivation syndrome or an unexpected worsening of symptoms, which can occur when introducing unrefined sugar, or compensating the possible poor food intake with an increased water supply: this is an example of how important the doctor–family relationship can be. Schoolmates may taunt a child compelled to follow a CM-free diet with their own snacks. Results may be discouraging in cases started after 6 months of age, owing to the frequency of polysensitizations at this age, which a correct and suitable elimination diet is not always able to cope with if specific needs are not taken into account. Especially in weaned children, emotional problems may arise due to negative feelings likely aroused by the diet's impact; thus it is natural that compliance is poor. Mothers are often astonished when such diets are proposed, but they can overcome the problem quickly by discovering that desirable meals can be prepared while following their child's tastes, by varying the procedure of food cooking. Furthermore, families are often confused by opinions that contradict the use of dietary restrictions in growing children, but this misconception can be

averted by either calculating the differentiated calories using Table 9.16 or monitoring the child's weight and height in the Appendices 6: 1–4.

After having correctly followed the diagnostic elimination diet for the prescribed period, the skin lesions are again evaluated:

1. *If the result is positive* (resolution = no relapse), the child is followed up, deciding after 6 months whether SPTs should be repeated.
2. *If the result is negative* and the symptoms persist (are unchanged) or recurrent after an initial resolution, a more restricted diet is resumed for other 3–4 weeks. Then the second diagnostic phase is started, to ascertain that it is not:

– A food intolerance or a PA by additives (Chap. 10)
– Too short a period of elimination
– An elimination diet not maintained exclusively free of all forms of the incriminated allergen.
– A possible persistence of sensitizing allergens
– A food regularly consumed or belonging to the same biological family (Table 9.10)
– Non-food factors

Therefore, in children not improving after a strict diet, the final decision agreed on between both pediatrician and specialist is a FCT in an asymptomatic period, indispensable for diagnostic evidence (Fig. 9.17), also in view of a possible oral desensitization (Chap. 13) in children aged >3 years.

Recent revelations of cattle testing positive for mad cow disease (also known as bovine spongiform encephalopathy, or BSE) in several European countries may continue the practice of recycling nonedible sheep and cattle tissue for animal feed instead of the usual fodder [116]. Moreover bovine meat should not be utilized in allergic children in that it contains cross-reacting antigens with those of CM and BSA found in CM [181]. Bovine meat may induce allergic sensitization [433] as in 11/335 children with CMA: 8 reacted to beef during DBPCFC, and 3 tolerated beef in a DBPCFC and well-cooked beef in an OFC but *reacted to ingestion of less well-cooked beef* [514]. In 12 children with *positive beef-specific IgE* (median 6.23 kU/l, range 0.83–36.6 kU/l, the rate of FH of beef allergy was 67 %. Of the 5 children who underwent OFCs, 3 were positive and 2 tolerated the beef administered [371]. Such results agree with subsequent data that extend beef allergenicity to that of veal when the sensitization involves CM serum albumin and βLG [519], whereas homogenized meat was less sensitizing or thermo-denatured [371] when the meat was well cooked and not rare [514]. The major allergen of bovine meat has been isolated and purified [522], and shows epitopes cross-reacting interspecies with sheep, dog, cat, horse, guinea pig, etc., but *not with lamb* [522]. Children subjected to antibiotic therapy may react by eating *antibiotic-treated meat* and vice-versa.

Food Challenge Test

Pathogenetic Mechanisms

We have mentioned previously a mechanism underlining FCT: intestinal mast cell histamine and tryptase release may be useful in selected cases. A study in 20 children 1–14 years of age [365] has shown significant differences between positive and negative FCTs and immediate and late reactions: histamine levels in positive FCTs were increased about threefold 2 h after FCT and nearly doubled after 4 h. Similarly, raised histaminemia levels matched immediate reactions, raised tryptase levels indicated after 2 or 4 h of IgE-mediated mast cell activation. The interesting reactions are immediate reactions: in children with suspected allergy but not with immediate reactions, only histaminemia was raised, thus suggesting a basophil activation, whereas histamine levels were not significantly increased in children with negative or positive, but not immediate reactions [365]. As a corollary, sIgE antibodies often are not correlated with FCT results [279], and are more often found in children with immediate reactions to CM and/or to egg [103, 284, 398], whereas a lymphocyte proliferation in late-onset reactions was always more evident [398] with suppression of IgE synthesis by IFN-γ [284]. Promising pathogenetic and diagnostic signs were recorded by evaluating circulating eosinophils and ECP levels [354, 464] (see "Other Diagnostic Tests"). Recently, FCT has been shown to trigger systemic release of IL_{10} in atopic children (mean 15 months) with FA, and this IL_{10} up-regulatory effect may reflect a reactive state against the release of inflammatory ILs in children with AD and FA [468].

Challenge Procedure

The provocation test can be done by three modalities:

- *Open food challenge* (OFC) test (both the doctor and the patient know which food shall be tested)
- *Single-blind challenge test* (the doctor knows, but the patient does not)
- *Double-blind challenge test* (neither knows)

FCT is *performed in a fasting state and on an ongoing elimination diet*; if the diet has been stopped, the food to be tested shall be eliminated for 7–14 days prior to challenge [20].

The technique tests foods in the natural state, one at a time, beginning with the most important food. There are no coded rules for the amount of food and the administration rate during the challenge, but it is suggested to start with very small doses to be augmented gradually, also because *after eliminating the offending allergen from the diet, the child may become more reactive* to the point that angioedema and anaphylactic shock occurred after reintroduction of foods previously eliminated [119] in children who had never had such severe reactions [328].

In the majority of cases, *an OFC is done especially in infants and young children* [29, 111, 224, 275], since at this age the symptoms are objective and the effect of suggestion is unknown [21]. Moreover, if FCT is conducted in young children with care and adequate observation, the results of open and double-blind FCTs coincide [255].

A *single-blind FCT* may be employed in infants up to 2 years, but can be utilized in difficult cases such as in weaned babies when diagnostic issues may be essential, or emotional components prevail, or FCT is refused for fear that an agreeable food may be prohibited; but is also useful to escape the parents' emotional influences and their beliefs.

In our opinion, a *double-blind FCT* should be performed in selected cases, chosen by pediatricians among the children with uncertain results, or not diagnostic, even if certain investigators, above all Sampson [415], assert the necessity, for a definitive diagnosis, of performing a DBPCFC, undoubtedly a sophisticated method that often appears to be a necessary prerequisite for publication in international journals [21]. FCTs performed without obstacles certainly contribute to decreasing the number of children labeled as true allergic and, more importantly, the number of parents skeptical of the validity of this diagnostic method [255].

Points to be considered.

- FCTs should always be performed under qualified medical control *in a clinical setting* (university, clinic, hospital, etc.), only if trained medical personnel (preferentially insured) and appropriate equipment for treating systemic anaphylaxis are available, especially if IgE-mediated reactions are suspected, which is entirely unpredictable even in the case of negative history. The practice of instructing parents to perform home challenges should not be undertaken because potentially severe reactions can result [120]. Anaphylactic shock occurred in four children [119] and immediate severe reactions in 18 children [328]: only a drop of CM may trigger such reactions [41, 86, 88].
- A FCT should not be performed in children with prior severe manifestations after eating the food that is to be tested, or with a SPT or RAST highly positive to the food in question. FCT is also not recommended in infants with a severe malabsorption syndrome (unless growth is improved) and in children with lactose intolerance (primary or secondary) or intercurrent infectious illness [21].
- Parents should be informed about possible severe reactions and give their written informed consent (Appendix 6.7). They discontinue antihistamines at least 7 days before the test, minimize corticosteroid use. In case of doubt, FCTs can be repeated in a single- or double-blind format.

Procedure of Open Challenges with CM, Egg, Wheat, and Fish

We follow the following procedure, record-chart in hand (Fig. 9.18) for young children undergoing an OCT by using standardized low doses [472], very low at the start (Fig. 9.18):

1. Check child's personal history and medical examination.
2. A drop of fresh CM, or of an emulsion of raw egg, or a bit of bread or fish (not lyophilized), depending on the food to be tested, are put on the inner border of the lower lip [88]. The drops of CM put on the inner border of the lower lip have been evolved as a labial food challenge (LFC), a neologism indicating an alternative to the FCT [88]. However, LFC is a nonexistient test [397], since we first reported this part of FCT in 1989 [328]. *Using small doses* minimizes the danger of potential immediate severe reactions.
3. After 5 min, 5 ml of fresh CM, or 1 ml of an emulsion of raw egg, or 5 g of bread or fish are administered.
4. After a further 30 min (provided that no immediate reaction arises), the last dose is given, that is 100 ml of CM or half-boiled egg, or 100 g of bread or 80 g of fish.
5. Other foods are similarly tested.
6. After the last administration, the child is observed for 4–6 h (always under strict specialist control in hospital).
7. At the end of observation is again visited.
8. If everything is fine the child is discharged.

If any symptom secondary to FCT is observed, the FCT in hospital is terminated. Others [224] have administered increasing volumes of CM every 2 h, if after the fourth dose (80 ml) no symptoms appeared, the FCT in hospital was terminated. More precise are the doses in milliliters such as successive doses of 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, and 100.0 ml [103, 404]. It is also necessary to evaluate the rate of positive results. In 501 children aged ≈13 months, 992 FCTs were done (OFCs in children aged <1 year and DBPCFCs in children aged >1 year), and only 445 (44.8%) true FCTs but also 10/354 (2.8%) placebo FCTs were assessed as positive [103].

Immediate reactions are defined as those occurring within minutes to 2 h from the first food ingestion, and *late reactions* when the first symptoms occurred after 4 h or more; however, others report that acute reactions develop within 1 [466] or 2 h [56] of commencing the procedure. Goldman et al [182] have suggested doing three subsequent FCTs; however, several authors [20, 21, 224, 353, 439] have not conformed to this proposal, in our opinion also for ethical reasons. In very sensitized children or with severe symptoms, we suggest diluting 1:10 a drop of CM as the first dose, continuing every hour with the following doses: one diluted 1:1, one undiluted drop, ten drops, 10 ml, then 100 ml if no reaction has occurred; the use of reduced doses should be weighed by the doctor caring for these children.

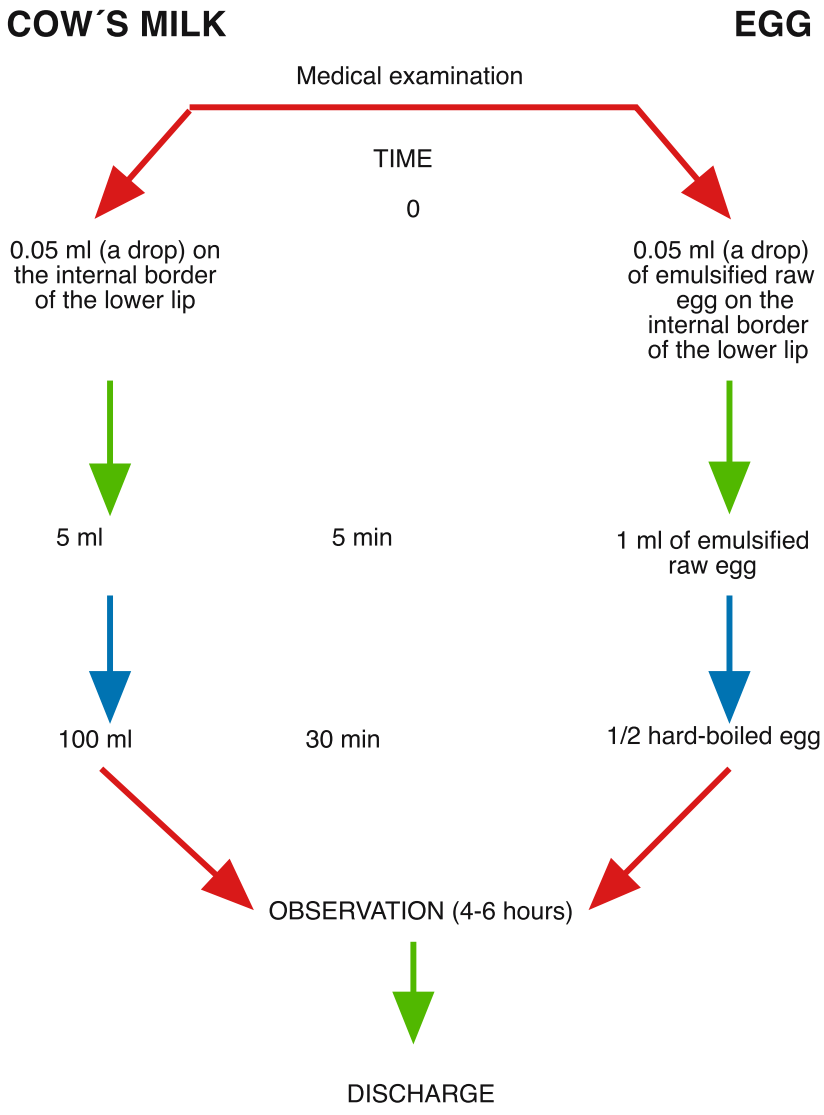


Fig. 9.18. Guidelines from our department for food challenge tests. Note that following the same indications, wheat, fish, peanut, chocolate, etc. can be tested. Appropriate mixtures can be prepared, checking the proportions of food amounts to be tested

Problems may arise in defining the FCT duration and dose chronology since several authors, us included, establish the start of symptoms from the first dose, Bock et al [48] from the last dose, so that different evaluations result, especially if foods are administered on subsequent days, as suggested: day 1, 5 ml, 10 ml, 20 ml, 30 ml, and 60 ml at 30-min intervals; day 2, 120 ml; day 3, 240 ml as a single morning dose up to a normal intake on day 4 [40, 211, 335, 453], so the late reactors [40, 212] react clinically 20 h or more after the normal doses administered on days 3 and 4. In this way, a normal CM intake was only started on days 3 and 4 of the procedure, thus failing to detect immediate responders usually reacting within minutes. Recently, FCTs were done every 48 h, by administering CM, egg, wheat and soy with 20-min intervals in 7 different steps [103]. The duration may also vary from 0.5 to 7 days [248]. Niggermann et al [353] suggest intervals of 15–30 min between doses and agree with us in concluding everything in a single day but extend the observation period to 24 h, and in considering the moment of the greatest dose as the check on late-onset reactions. More similarly, the procedure may last from ≤2 h to ≥24 h [320]. DBPCFCs may be done in two stages: the first day to CM or placebo; if no symptoms ensue, the next day the child begins challenges over a week [18], likely while hospitalized. Several authors have considered as positive the reactions occurring within 24 h [456], or within 3 [21, 517], 7 [40, 212] or 9 days [212]; others judge the FCT as negative only when no reaction occurs within 1–2 weeks [21].

Goldman et al [182] have measured the time elapsed between the start of FCT and the onset of symptoms: 1 h in 39% of cases, 1–5 h in 21%, 6–11 h in 10%, 12–24 h in 17%, 1–3 days in 11%, and 7 days in 1% of cases; so 77% of cases occur within 24 h. However, especially in children with AD, late-onset reactions to egg arise over 3 h to 5 days, reactions to CM over 4 h to 15 days (Fig. 9.19) [328]. There is a difference between immediate and late onset reactions [316]; the first figure refers to immedi-

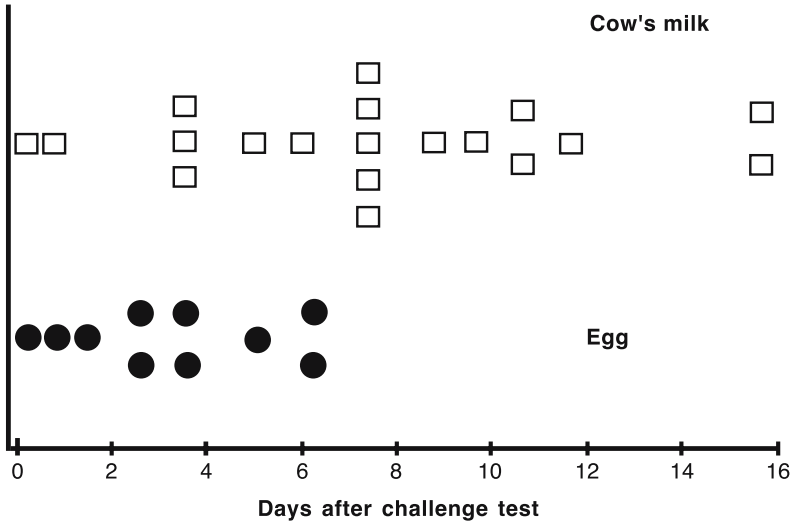


Fig. 9.19. Onset (days) of late reactions after challenge test with cow's milk or egg. (Data from [328])

Table 9.18. Symptoms provoked by OFC/DBPCFC

Authors (reference)	No. of children	Age (years)	Characteristics	Symptoms (%)		
				GI	Cutaneous	Respiratory
Sampson [416]	160	M 5.3	AD + food allergy	43	79	28
Burks et al [57]	46	m 5.7	AD + food allergy	30	96	52
Bock et al [47]	480	3–19	AD + food allergy	56	62	39
James et al [242]	320	M 4.4	AD	41	75	59

M median, *m* mean, results are >100 because patients have manifested more symptoms. The mean shows the following rates: GI 42, cutaneous 78 and respiratory symptoms 44.5.

ate, the second to late-onset reactions, with relative minimal and maximal limits in parentheses:

- Dose-inducing symptoms: 16 (range, 6–27) to 86 ml (range, 70–104)
- Cumulative dose until symptoms: 27 (range, 8–47) to 200 ml (129–270)
- Time from FCT commencement until reaction: 2 (range, 0.1–5) to 31 h (range, 15–46)
- Time from last dose until reaction: 0.3 (range, 0.1–0.4) to 16 h (range, 6–27)

Early reactions were also defined as clinical symptoms occurring within 120 min after administering the highest dose, late reactions as symptoms occurring after 2 h [103]. Late reactions also occurred after ≥5 days [320]. We record many immediate and late onset reactions. The challenge results include the following symptoms (Tables 9.18, 9.19) [44, 47, 57, 175, 242, 416, 420].

GI: nausea, vomiting, abdominal pain, diarrhea (33%); cutaneous: pruriginous, erythematous and morbilliform rashes in the preferred sites, lip edema, rarely urticaria (99%); respiratory: rhinitis, laryngeal edema, wheezing (12%).

Food responsiveness in provoking *respiratory symptoms* has been shown in 121/205 children (59%) with nasal symptoms in 70%, laryngeal symptoms in 48% and bronchial symptoms in 27% of cases [420]. Comparing these data to Bock and Atkins' [47] and Bock's [44] results in asthmatic children, we find differences related to egg and peanuts. In 279 subjects aged 3–19 years [44] with food asthma, 40% had asthma as one of the symptoms noted, 2% asthma as the only symptom. In the first row of Table 9.19, SPT results parallel to Sampson's DBPCCT results [415] are listed: the highly reduced rates are self-evident. In children with AD and FA, immediate or late onset reactions were found in 87% of cases in cutaneous reactions: more precisely, worsening cutaneous lesions, 49%, pruritus, 46%, rash or erythema, 43%, urticaria, 23%, wheezing, 15%, lip edema, 8%, diarrhea, 6% and vomiting, 3% [328]. Relative to DBPCCT, Bock and Atkins [47] and Sampson [415] have recorded, respectively, *4.6% and 1.8% false-negative and 0.9% and 0.5% false-positive results.*

If challenge is positive, on discharge the parents are instructed to continue the administration of foods at home (150 ml of CM, or one-quarter of a hard-boiled egg, or 150 g of bread or pasta or 80 g of fish twice a day) always completing the food diary recording:

- Amount and time of foods ingested
- Occasional breaking (other foods and/or medications)

Table 9.19. Symptoms provoked by OFC/DBPCFC related to the foods tested, divided into overall and respiratory types

Foods (%)	Sampson [416]		Bock et al [47]	James et al [242]	Bock [43]	Sampson et al [420]
Type of symptoms	Overall				Respiratory	
Egg	56	38	25	46	19[a]	51
CM	24	11	23	19	27	28
Peanut	51	24	24	6	28[a]	6
Wheat	15	4	2	5	3[a]	5
Soy	25	5	6	8	3	5
Fish	28	7	3		3	
Nuts			10		15[a]	

In the first row of Sampson's data [416] the SPT results for the same foods are shown.
OFC oral food challenge, *DBPCFC* double-blind, placebo-controlled food challenge.
[a] Shows monosymptomatic patients in Bock's data.

- Ambient and/or emotional variations
- Any late events [328]

Whenever possible, it is useful that the pediatrician observe and diagnose these late-onset reactions. To record the possible onset of reactions, the children return for two visits, after 3 and 7 days or 7 and 15 days. FCT is concluded after 1–2 weeks. The results may be summarized as:

1. Negative → free diet
2. Positive → therapeutic elimination diet
3. Positive, but with other suspected foods → another FCT with an interval of at least 7 days
4. Borderline → repetition of OFC or in single- or double-blind fashion

Procedure of Single- and Double-Blind Challenge

The differences in single- and double-blind challenge tests are:

- Blinding the test to the patient and/or doctor
- The necessity of disguising the food.

The food can be made unrecognizable in various forms, but there is no general agreement on how color, odor, taste and vehicle to be used can be dissimulated in such a case. We disguise CM by mixing it with soy (if children are not sensitized) and other foods diluted in fruit juices prepared at the moment, including nonoffending fruits such as pear, orange, grapefruit, and pineapple; carob powder and syrup are good disguises for cocoa [21]. To disguise peanut and soy the procedure is rather difficult, since different varieties exist of both foods [472]. *Freeze-dried substances* are often utilized. This procedure offers advantages since a whole egg is equivalent to 5 g of freeze-dried food and 30 ml of CM to 3 g [48]. Several foods may be given in a freeze-dried form; however, the presence of additives or preservatives in this test material should be taken into account. The calculation of how much freeze-dried food is equivalent to fresh foods is not always easy. The preparation in opaque capsules with progressively increasing doses of freeze-dried substances certainly ensures blindness, but only older children are able of ingest them. On the one hand, the oropharyngeal mucosa absorbs allergens, as demonstrated by reactions we have observed in the first minutes of an OFC [41] and by test negativity in children with OAS; on the other hand, no explanation can be found for different symptoms recorded after absorption of the capsule in various segments of the GI tract [192]. Therefore, we recommend that blind testing, provided that it is technically feasible, be performed with fresh foods also in older children. However, a 14-year-old boy reacted to DBPCCT with liquid CM and not to that with CM in a capsule [261]. If these manipulations are strictly necessary, it is preferable to apply to specialized structures. In case of negative DBPCCTs, repeating the procedure in the open is suggested to rule out a false-negative reaction [48], which occurs in 3.2% of cases [47, 415]: a 12-year-old boy with CMA and exercise-induced asthma has reacted negatively to two DBPCCTs with CM but with anaphylaxis after eating pancakes made with CM before engaging in exercise [261].

Evaluation of Food Challenge Testing

Our experience shows that there are advantages and disadvantages to carrying out or interpreting the results of challenge testing, which must be examined in relation to SPT and RAST predictive values (PV).

Advantages

The problems related to low sensitivity and specificity of other diagnostic tests are definitively eliminated.

It is the only very helpful test in determining true clinical FA as a food responsibility to a specific pathology (see AD), to get rid of psychological influences and misconceptions on the part of both children and parents.

Positivity rules out clinical food reactivity, and negativity needs to be confirmed by an open test, the cases of false negativity being uncommon and difficult to resolve (see Chap. 6).

It allows a widening of diets that otherwise would be too punitive, leaving children free from unnecessary and harmful food restrictions, thus preventing prolonged use of an inappropriate diet, likely inducing malnutrition [516].

It avoids the assumption that a child is recognized as intolerant only because of persistent SPT positivity.

Disadvantages

It is necessary to perform FCTs in a clinic or hospital setting, with medical personnel and suitable hospital equipment for potential emergencies.

Immediate reactions consequent to FCT are also frequent in children never manifesting such reactions.

Medical history is generally an unreliable indicator of a possible onset of such reactions, notwithstanding a history of negativity.

SPT- and RAST-positive PV (PPV) is rarely helpful to predict these reactions (because of a number of false-positive results that coincide with FCT negativity; however, SPTs and RAST may be a useful means of investigation, especially SPTs, when the onset of severe immediate reactions is suspected by their high *negative predictive value* (NPV).

In our studies related to *immediate and late reactions,* the RAST, PPVs and NPVs are lower than those of SPTs, which similarly were demonstrated to be more specific and less sensitive (Table 6.19).

The absence of immediate reactions following reintroduction of a given food does not exclude the chance of recording late onset reactions (over 4 h, but sometimes over a few days). However, before a definitive diagnosis, each child should be followed with assiduity also in the period subsequent to FCT itself.

A positive FCT merely indicates a cause-and-effect relationship, regardless of the underlying mechanism. A meta-analysis found that DBPCFC results were positive only in 20%–36% of suspected foods in eight pediatric studies [21].

Further Points to Be Evaluated

Hill et al have suggested that a SPT response ≥4+ is equivalent to a positive FCT [212]. More precisely, this equivalence is seen when skin wheal diameter is >8 mm for CM and peanut, >7 mm for egg, smaller in children aged <2 years: 6, 5, and 4 mm, respectively, with a 100% specificity [453]. Among the children seen by us, a 7-year-old male had SPTs positive for ALA (3 cm), β-LG (2.5 cm), casein (12 mm), an 18-month-old child with a SPT = 14 mm to egg-white and a 6-month-old girl with a wheal of 1×1 cm to egg yolk and another with a SPT negative to CM, but with a 2-cm wheal with P+P and CM diluted 1:1 with saline. Another child severely allergic to fish, tested positive to cod with a wheal of 7 mm, *although we diluted the allergen 1:10 with saline*. A group of 11 children had SPTs positive to CM of 8–16 mm [164]. These children have FA and do not need to be subjected to a FCT.

Moreover, for a number of food allergens (CM, egg, peanut, fish), it was possible to define positive DBPCFCs as IgE-mediated, which occur with 95% certainty [419].

Positive results of APT (atopy patch test) to foods in combination with measurement of sIgE serum levels to foods has been suggested to further reduce the need for DBPCFCs in children with FA [404]. Serum food-specific IgE is a useful test for diagnosing symptomatic allergy to common foods such as egg, CM, and peanut since the use of sIgE values could reduce the number of DBPCFCs we need to perform by 40%–50%. If the food-sIgE level is <0.35 kUA/l for egg, CM, and peanut and the SPT response is negative, the FCT can be performed at home unless there is a *compelling history of reactivity.* If the CM-sIgE levels is <15 kUA/l but ≥0.35 kUA/l, we would need to perform some form of CM FCT to determine whether the child is truly reactive to CM [419]. In infants aged ≤1 if sIgE levels for CM are ≥2.5 kUA/l, the FCT *should not be performed* because of sIgE high PPV (positive predictive value) (90%) [174]. Notably, by analyzing the different cutoff points of the sIgE for CM, 2.5 kUA/l had a PPV of 90% and 5 kUA/l had a PPV of 95% [174]. For wheat and soy there is no clear relationship between sIgE levels and the probability of positive FCTs [103].

FTCs, even if they may be inaccurate in FA, are without doubt more reliable than accidental exposures to foods, also hidden, to which children may run into at home or at school, where they are not under their parents' control. The classmates of a severely CM allergic child seen by us waved their snacks in front of him. Therefore, a clinician seeking to avoid nonessential and potentially hazardous FCTs [119] while avoiding unnecessary diet restrictions has a test with a high specificity such as SPTs [453]. Similarly, DBPCFTs may be viewed as unnecessary given an IgE test result above the PV [174].

Surprisingly, children with negative SPTs had positive FCTs [453], and children with positive SPTs had negative FCTs [419].

We continue to observe, for the most part differently from other authors, late reactions not preceded by immediate reactions, with onset even after several days. We deem that only some authors [31, 40, 52] confirm this result. Late onset reactions starting even after 1–2 weeks occasion a controversial issue in the literature since the experiences of each group vary.

Children, once returned home, may find several random and aspecific environmental factors such as changes in emotional or climatic conditions that cannot be verified at a distance which may challenge the diagnosis of delayed reactions. Nor is it always feasible to draw the attention of the family doctor to monitoring and recording such reactions.

In a subset of children with frequent patch test reactivity and total IgE correlated with IL_{10}, a possible explanation of delayed reactions are low IL_{10} concentrations [468].

Physical exercise, including sport, after FCT are firmly discouraged.

Other Diagnostic Tests

Several diagnostic tests are frequently utilized in scientific studies. We do not suggest their use in clinical practicebecause they are poorly standardized, expensive, and more appropriately reserved for the research setting.

- *Intestinal biopsy* may conceptually be an additional diagnostic aid designed to supply helpful indications such as pathognomonic alterations of FA, particularly in cases of CMA with prevalent GI symptoms and for the diagnosis of malabsorption.
- *Peripheral eosinophilia and ECP serum levels.* The count of peripheral eosinophils may be another diagnostic aid, even if aspecific, provided that an elevated number of cells is seen, as well as a significant variation at the start of FCT and soon after, as with ECP at the beginning and 24 h after (Fig. 9.20a) [464], with positivity in children with cutaneous, but not with GI symptoms [354, 464]. However, the ECP increase is transient (Fig. 9.20b) [464].
- *Fecal determination* of ECP, TNF-α and of α-1 antitrypsin widen the diagnostic spectrum being raised before FCT and notably reduced after FCT [316].
- *In total or specific IgG antibodies* and related subclasses, elevated levels are not diagnostic [117].
- *Lymphocyte stimulation test* (LST) and inhibition of leukocyte migration (ILM). Several recent studies have aroused interest for LST [2, 279, 280, 445]: in addition to critical examination of diet results [2, 446], LST is suggested for CMA with prevalent GI symptoms and with late-onset reactions to FCTs (48 h) [279, 280] but not for egg [445] and is not correlated with RAST [279, 280]. It is a time-consuming method and rarely diagnostic, in that doubts have been expressed, either on the technique or the chance of achieving standardization [493]. ILM appears to be equally time-consuming and of scarce practical utility because of excessive variability and rare reproducibility [493].
- For *evaluation of GI mucosa permeability,* the lactulose and mannitol permeability test (LMPT) can be employed as an absorption index of large and small molecules, respectively, and is based on the differentiated assimilation of both sugars. LMPT use can evidence the relative, potential alterations, with consequent digestion of macromolecules (increases after CM load in children with CMA) [239, 341] (Fig. 9.21) [340]. Others have pre-

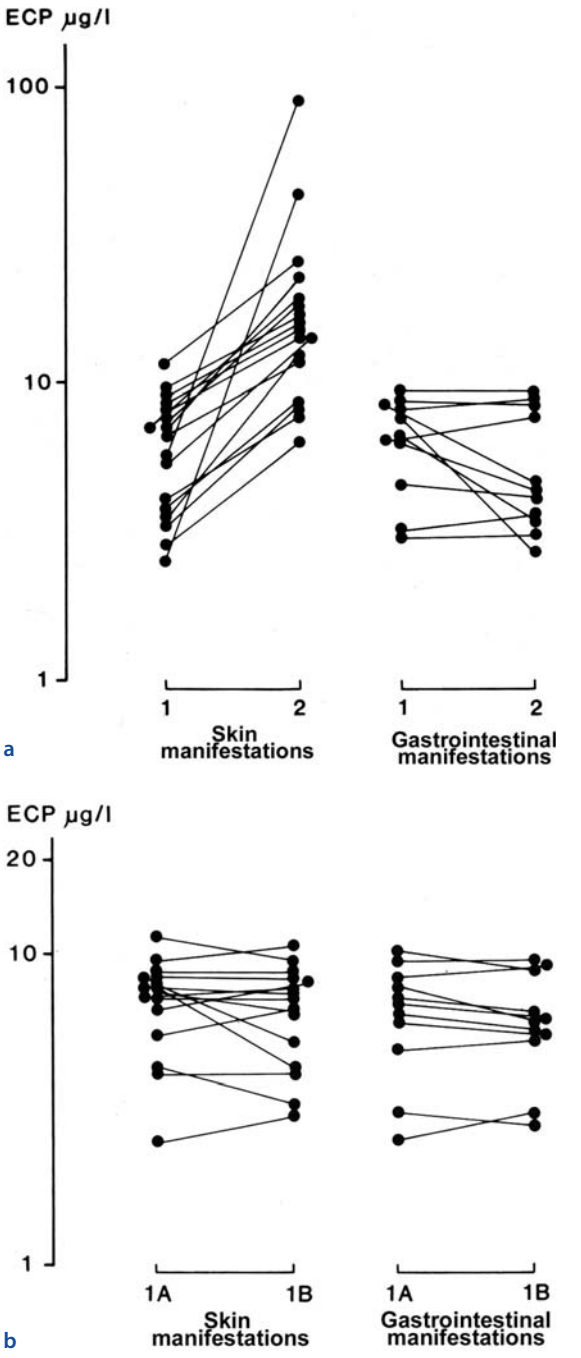


Fig. 9.20. **a** Serum ECP levels (µg/l) before CM challenge (*1*) and 27±6 h later (*2*) in CMA children with skin and gastrointestinal manifestations. During the challenge ECP levels increased from 6.2 µg/ml (mean) to 20 µg/ml (mean), but only in CMA children with skin symptoms (p=0.01). **b** Serum ECP levels (µg/l) taken in two different occasions (*1* and *2*) with an interval of 2–4 months remained unaltered in CMA children with skin and gastrointestinal manifestations

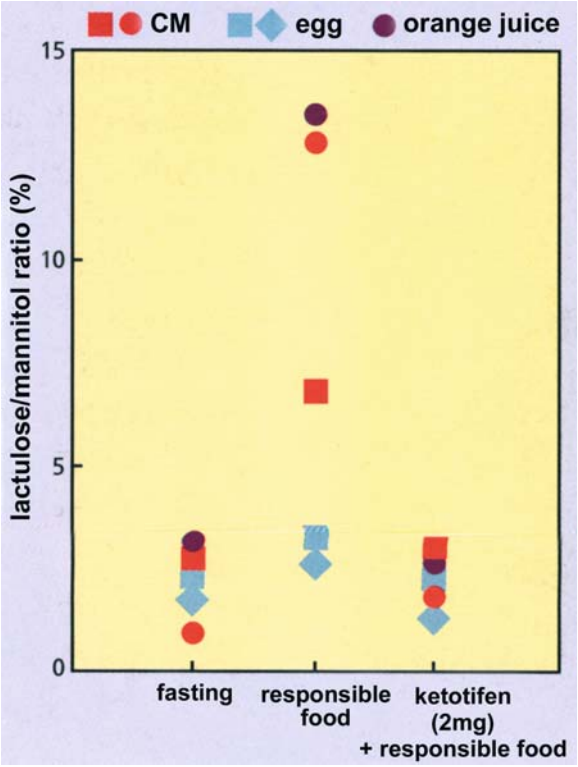


Fig. 9.21. Permeability of the gastrointestinal mucosa as shown by lactulose:mannitol ratio. The patients were tested with CM, egg and orange juice. The ketotifen protective effect is shown

ferred PEG (polyethylene glycol) or OVA [340]. Such methods are subjected to a basic criticism: too different and too small molecules are used in comparison with food macromolecules (PEG, lactulose) or heterologous molecules (OVA), which have immunogen properties and/or may cause CIC formation, and therefore are laborious to determine [340]. The method employing ALA seems to be more useful [253]. The efficacy of this in vivo noninvasive method is demonstrated in the biological diagnosis of BMA [124] and HF allergenicity [24].

Advantages of the Permeability Test

- This is primarily a research procedure more than a clinical tool. A demonstration of its application comes from the above-cited studies, even if further data is needed.

Disadvantages

- More useful in other clinical conditions, such as CD and Crohn's disease
- Necessity of protracted urine collection
- Necessity of employing endoscopic techniques for lactulose and mannitol test, and radioisotopes for EDTA (ethylenediaminetetraacetic acid) and/or PEG

In conclusion, the test is sophisticated, not standardizable, and therefore not of immediate widespread use in clinical practice.

The *differential diagnosis* with other GI disorders is outlined in Table 6.5, structured according to age, as well as CMA diagnosis, also taking into account lactose intolerance that may mimic CMA symptoms and PA [54]. A high incidence of IgE-mediated FA may be found in CD [304]. Enteric infections are capable of disclosing a previous CMA but not evidently clinical, which is revealed by acute gastroenteritis [509]. Münchausen syndrome by proxy should not be neglected (Chap. 16).

Dietary Treatment

It is not known whether diet changes can aid in permanently eradicating FA. Shinoda et al [446] investigated the effect of elimination diets on T cell responses to OVA in hen's egg-sensitive AD patients. The proliferative responses of the patients' PBMCs and $CD4^+$ T cells with monocytes decreased with an egg elimination diet. Similar proliferative responses to a recall antigen and phytohemagglutinin did not decrease after the same elimination diet. The investigators concluded that elimination diets may help reduce the responsiveness of food-sensitive T cells. It has been demonstrated [2, 445, 446] that parallel to good diet results in children with FA (CM and egg), AD and RAST+ [2], both sIgE and proliferative responses of antigen-specific lymphocytes statistically decrease [2, 445], contrary to RAST– children [2], so RAST can be useful to monitor diet effectiveness. Because of diet-positive effects, antigen-stimulated $CD4^+$ express IL_2 and IFN-γ (Th1-like T cells) in the skin and FcεRII on B cells [280], while $CD4^+/CD45RA^+$ increase, which may suppress the ongoing immune responses [446].

Based on FCT results, individualized diets can be prescribed based on the exclusion of sensitizing foods, which, if well conducted, may yield objective results (Fig. 7.40). FA treatment consists in the elimination of these foods: when the causative food is not a source of indispensable nutrients, it can be easily eliminated according to the characteristics reviewed in Tables 9.20 and 9.21 [80]. Success depends on several factors that may be determinant: it is necessary to specify the diet duration and restriction, adopting measures to prevent the detriment caused by inadequate preparation of formulas [516] (Table 9.22) [92, 276], which may also consist in growth retardation and hypothyroidism, reversible after appropriate diets [285]. Compared to controls, children on a diet may show a significantly reduced quantity of Ca and Zn [373], or 25% less Ca compared to the age-related requirements [276], which makes it necessary to oversee the Ca recommended levels (Tables 9.23, 9.24) (Appendix 9.9).

Table 9.20. Prerequisites of an ideal CM substitute

	Soy proteins	Hydrolysates		Elementary diet	Rezza's diet	Goat's milk
		H	P			
Immunogenicity	±	+	+	No	No	+
Allergenicity	±	+	+++	±	±	++[a]
CM proteins (βLG)	No	+	++	No	No	++[a]
Nutritional adequacy	Yes	?	?	?	Yes	?
Pleasant taste	±	No	±	No	Yes	Yes
Low osmolarity	Yes	+	+	+	±	?
Low cost	Yes	No	±	No	Yes	No
Easy availability	Yes	Yes	Yes	?	Yes	±

H highly, *P* partially, *βLG* β-lactoglobulin.
Data from [80].
[a] High cross-reactivity.

Table 9.21. Mean cost of CM substitutes per liter of reconstituted formula

Formula	Content (g)	Price (euros)	Cost (euro)/l
Soy milk			
Isomil	400	16.14	5.44
Nutrilon soy	450	17.9	6.37
Casein hydrolysate formulas			
Nutramigen	460	19.3	6.43
Pregestimil	450	20	6.67
Whey hydrolysate formulas			
Alfarè	400	18.28	6.85
Aptamil 1	900	30.3	5.05
Aptamil HA2	600	18.9	4.72
Nidina HA1	750	24.7	4.94
Nutrilon pepti 1	450	19.5	6.5
Pepti-junior	450	23	7.6
Home-made lamb-based diet[a]			2.76

The collaboration of M. S. Campostrini in compiling this table is acknowledged.
[a] Instead of lamb meat, lean pork or rabbit may be used.

Table 9.22. Possible consequences of inappropriate diets

1. Anaphylaxis
2. Anemia
3. Hypocalcemia
4. Hypoprotidemia
5. Hypothyroidism
6. Hypozinchemia
7. Malnutrition (see Chap. 21)
8. Failure to thrive

Data from [92, 276].

Over the past several years, the number of special formulas has been progressively increasing, but no specialized sources have updated pediatricians and allergists with information on the nutritional adequacy and therapeutic efficacy of such products [276]. Both pediatricians and allergists should therefore develop a critical sense to choose the right formula. The properties of an adequate diet are principally those exposed by Table 9.25 [80], above all the compliance on which depends the child's acceptation.

In Chap. 6, we mentioned nonatopic children who, as a result of erroneous or superficial interpretations, were started on diets by their family, and allergic children put

Table 9.23. Recommended daily Ca allowances

Age (years)	Weight (kg)	mg/day
0.5–1	7–10	500
1–3	9–16	800
4–6	16–22	800
7–10	23–33	1,000
11–14	35–53	1,200
15–17	52–66	1,200
18–29	56	1,000
>30	56	800

Source: LARN 1996.

Table 9.24. Ca allowances that are slightly different from RDA levels

Age	Ca (mg/day)
1–3	500
4–8	800
9–13	1,300

Source: LARN 1996.

Table 9.25. Main properties of an alternative formula for babies with food allergy

No immunogenicity (sensitizing property) between CM proteins and peptides of the alternative formula
No allergenicity (IgE antibodies)
Normal antigenicity (IgG antibodies)
No native proteins from which the formula derives
No cross-reacting with CM proteins and peptides of the alternative formula
Nutritionally adequate according to ESPGAN guidelines
Normal availability
Pleasant taste
Low cost

CM cow's milk.
Modified from [80].

on diets without medical prescriptions. An investigation reports that >50% of 73 children followed diets based on information from the media, books, magazines, radio or TV (51%), friends and neighbors (32%), general practitioners (27%), district nurses or health visitors (20%), personal observations in five cases, and from psychologists, homeopaths, herbalists, or schoolteachers in six cases. Potentially dangerous practices were suggesting goat's milk even to a 4-month-old baby or very restricted diets. Fortunately, no growth retardation, failure to thrive, or nutritional deficiencies were observed [513]. In children with reactions to CM and egg, one-third had strict dietetic limitations on the intake of these foods, which in 20% were unwarranted, and more than one-third of children with confirmed adverse reactions lacked pertinent diet restrictions, in both cases as decided by their parents [143]. A 2.7-year-old child on a highly restrictive diet after a DBPCFC was put on a free diet and 3.5 months later had a fourfold normal weight gain for his age and twofold normal height gain for his age [276].

CM-Free Diets

If CM is the only offending allergen (and the cause-effect ratio is evident), an adequate food substitute is necessary, as indicated by Tables 9.20 and 9.21. The first choice is age-based: an infant is obviously nutritionally vulnerable because CM normally gives almost all necessary nutrients (proteins, carbohydrates, lipids and Ca). In the first weeks of life, CM is a food that represents the main source, when not exclusive, of nutrients of high biological value. From 0.5 l of CM, a two-year-old obtains 100% Ca and riboflavin, 50% protein and 24% energy of the age-related RDA [183]. The high nutritional value is associated with a low cost. CM can be eliminated from the diet of children aged >2 years, without nutritional problems, provided that selected foods, including meat, egg, fish, legumes, etc., supply all necessary nutrients to satisfy daily food requirements [183]. Different measures may be taken, but they should be balanced to be in keeping with the prerequisites established by ESPGAN [151] for correct and adequate infantile nutrition (Table 9.16). Additional parameters to be considered are symptom severity, number of potentially sensitizing foods, alerted by physical examination and previous investigations, family involvement, and availability to observe a diet seriously. Problems may still arise, since an alternative formula represents the only alimentary source and infants should receive all nutrients necessary to a rapidly growing organism, while ensuring good tolerance to ingredients and osmolarity [516]. Consequently, the immunological tolerance is strictly related to the nutritional component and only secondarily different factors acquire relevance, including the infant's and family's compliance [276]. The child's age will determine the diet to prescribe.

Appendices 9.1 and 9.2 summarize the dietary products that must be eliminated in children allergic to CM and/or egg proteins and the dietary products and ingredients for children with potential hidden forms of CM and/or egg proteins [27, 516].

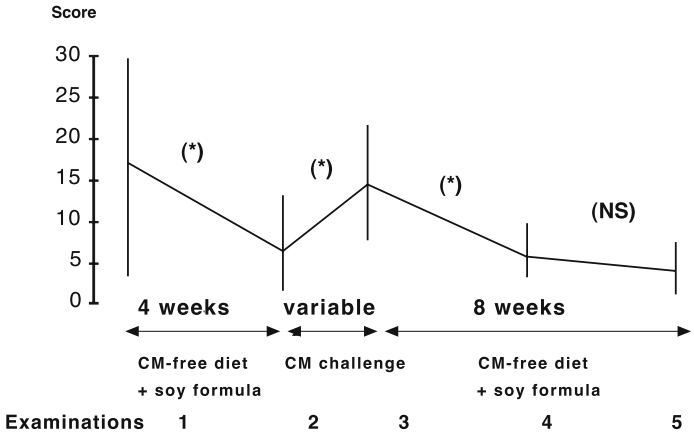


Fig. 9.22. Severity score (mean±SD) of AD in 17 children aged 5–15 months before and after soy-formula feeding and before and after CM feeding. *M* mean, *SD* standard deviation, *AD* atopic dermatitis, *CM* cow's milk, *NS* not significant; * $p<0.001$. (Data from [85])

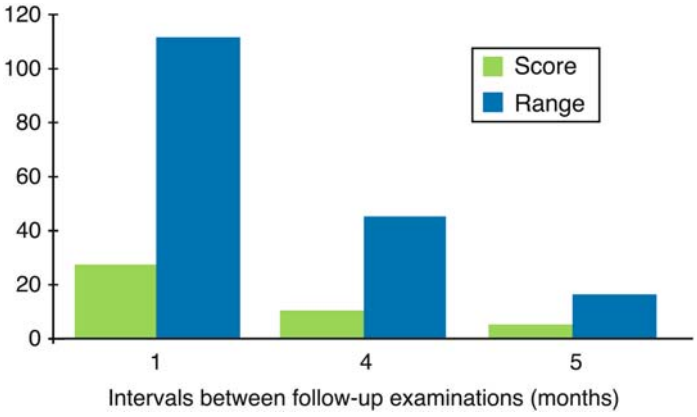


Fig. 9.23. Score severity of skin lesions (geometric mean + ranges) in 17 soy-formula-fed children with atopic dermatitis. $p=0.0003$ between *1* and *4*, 0.0002 between *1* and *5*, 0.000 between *4* and *5*. (Data from [85])

Infant Feeding

The following alternatives to CM can be considered:

1. Breast milk
2. Soy protein formulas
3. Hydrolysate protein
4. HMMBF
5. Amino acid products
6. Goat's milk
7. Mare's milk
8. Ass's milk

Each of these will now be reviewed in more detail.

BM can be highly advantageous when the baby is breast fed and the mother still has BM (homologous proteins) (Tables 2.12, 2.13, 2.20, 2.21). The nursing mother should follow an oligoantigenic diet limiting or excluding ingestion of CM and eggs and completing her diet with Ca (Tables 9.23, 9.24).

Soy protein formulas (SPFs), utilized for many centuries in the alimentation of Chinese and Japanese children [215], were first used to feed US babies with CMA in 1929. Worldwide experience has now accumulated on SPF; we were among the first Italian pediatricians to employ SPFs. Analyzing the paper by Atherton et al [14] studying children with AD on a CM- and egg-free diet, in 14/20 a significant improvement in cutaneous lesions was noted while children were on a SPF diet, which were reduced to 1/20 when they were changed to a common diet. In a study on 17 of 21 SPF-fed children, similar effects were noted on a FCT with CM (Fig. 9.22) [85] and a marked improvement in the cutaneous score when the diet was resumed (Fig. 9.23) [85]. SPFs currently commercialized (Appendix 9.3 shows the composition of the most common SPFs) respond to nutritional criteria of the American Academy of Pediatrics (AAP) [5] and contain purified soy proteins, that is limited to those with the least sensitizing power. The protides are mixtures of vegetable oils (corn, soy, coconut) with unsaturated fatty acids and mean chain triglycerides. The fat is a mixture of vegetable oils, and carbohydrates are represented by maltodextrins, corn, starch, or saccharose, and the RDA of vitamins, minerals and trace elements are usually added, thus forming a sufficiently balanced food [89]. Therefore, the SPFs presently marketable, given the percentage of various nutrients, caloric supply and the described integrations, ensure a valid alternative to CM [64, 73, 85, 89] and, as we were the first to demonstrate, support *adequate normal growth* [85] and a normal bone mineralization [337]. Moreover, SPFs *do not induce immunological deficiencies* even when they are employed since birth and over long periods [71]. Since SPFs are *lactose-*

Table 9.26. Results of studies employing challenge test to soy

Author(s)	Reference	No. of children	Age (years)	Challenge type	Reactions to soy (%)
Sampson et al	[415]	204	5.2 (M)	DBPCFC	5
Bock	[45]	630	<3–19	DBPCFC	2.7
Bock et al	[48]	710	NS	DBPCFC	6
Giampietro et al	[179]	317	0.4 (M)	OFC	3.1
Kivity et al	[270]	52	18 (M)	DBFC	0
Magnolfi	[315]	900	0.1–18	DBPCFC	6.1
Burks et al	[61]	98	0.5–21.9	DBPCFC	2.9
Eigenmann et al	[146]	63	2.3 (M)	DBPCFC,OFC0	0
Bellioni et al	[27]	26	2.8 (M)	DBPCOFC	0
Businco et al	[75]	25	2.9 (M)	DBPCOFC	0
Klemola et al[a]	[274]	80	7.1–2.4 (M)	DBPCOFC	6.25
Rancé et al	[397]	544	1–15	OFC	0.8
Total		3,649			Mean, 2.7

The studies are ordered according to the year of publication.
DBFC Double-blind food challenge, *DBPCFC* Double-blind, placebo-controlled food challenge, *M* median, *NS* Not specified, *OFC* Oral food challenge.
[a] In the study by Klemola et al [274] 5/8 children manifested immediate reactions (urticaria, erythema, and three out of eight delayed reactions; two more children reacted to soy formula as reported by parents who refused DBPCFC studies. The authors considered these as "probable adverse reactions to soy." One child out of eight had positive soy-specific IgE.

free, they are also useful in primary or secondary lactase deficiency and galactosemia [89]. Current formulas are fortified with L-methionine and contain added taurine and iron; and *carnitine,* necessary to mitochondrial oxidation of long-chain fatty acids and essential fatty acids and nucleotides, has been added to some SPFs in the same amount as that found in BM [64].

SPFs have the advantage of offering proteins with which the child has never come into contact [64]. A favorable case may also be that of a sensitization (often of the humoral type) [90], limited to 0%–6% when not evaluated by SPTs and/or sIgE levels, but by DBPCFC or OFC, which was done in 13 trials, in addition to Cant et al, who performed the test in the mother [77]. In Table 9.9, outlining the data of 18 trials, the incidence of soy intolerance varies from 0% to 36%, with a mean of 6.4%, whereas CMA intolerance is 23.5%, as much as 3.7-fold higher [89]. In 12 trials (Table 9.26) [27, 45, 48, 61, 75, 146, 179, 270, 274, 315, 397, 415] *involving 3,649 children,* the mean is 2.7%. Dean et al used a modified RAST [130] and found a 6% prevalence in children with CMA. In 317 children with a median age of 12 months (range 6–120 months), the prevalence of SPF humoral sensitization was 22%, but only ten children (3.1%) responded positively to FCT [179] (p=0.0001) (Table 9.26). RAST has yielded a PPV of 0.06% [179]: as demonstrated in Chap. 6, RAST is also unfit for wheat. In addition, the rate of false-positive SPTs to soy may be as high as 67% [31]. Four cases of anaphylaxis have been reported in children [25, 119, 315, 347], in addition to three poorly documented cases [331, 496, 415]. A child was reported to be fed Sobee [347], which was particularly antigenic [89, 140]. These are a few episodes compared to innumerable children who were and are SPF-fed, a conclusion reinforced by >200 Italian [62] and 1,450 pediatricians throughout the world [251] who have never seen a case of soy-induced anaphylaxis. To our documented invitation to revise their position papers [89], the AAP concluded that the majority of neonates with certain and IgE-mediated CMA can eat SPFs [5], thus in 97.3% of cases. The AAP concludes that most *infants with documented IgE-mediated CMA fare well on isolated SPF* [5] and others add that SPFs are the *first-choice alternative* in such infants [274, 530].

As certain authors stress that "soy protein is as allergenic as CM proteins", it is necessary to state that Eastham et al [140] studied soy *antigenicity,* in that they measured hemagglutinins to soy and to CM, which mainly belong to IgG antibodies [85], as acknowledged by the AAP, affirming that "recognizing that soy protein is antigenic does not mean that soy protein is highly allergenic" [5]. To prove SPF allergenicity, either the capacity of triggering IgE-mediated reactions in sensitized individuals or the existence of peptides with a high MW should be demonstrated [82]. Instead the authors discussing soy allergy [152, 154, 223, 428, 441 and references in 89] quote Eastham et al [140], and stress that soy is as "allergenic" as CM, alluding to studies based exclusively on clinical history and parental reports (never on FCT), which preposterously provoke reactions in

30%–40% of children. Through the meta-analysis of 18 different epidemiological studies (Table 9.9), we conclude that in five studies restricted to history [177, 178] and parental reports [40, 211, 237], the prevalence is 35%, in the remainder <3% [89], thus agreeing with the studies of Table 9.26. The studies with high frequencies [40, 177, 178, 211, 237] do not involve children with colitis and/or enterocolitis, in whom the AAPSNAD (AAP Subcommittee of Nutrition and Allergic Disease) found values of 8%–14% [273]. Therefore, SPFs are indicated at least in these children in the remaining 86%–92% of cases. See "Legumes" for the discussion on soy-CM casein cross-reactions. Some quoted studies failed to employ FCTs also for historical reasons: in the first one [177], there is a retrospective prevalence of 36%, but the intolerance could be due to other substances included in the formula. In the second [178], the authors noted in the five children that when one proprietary preparation was not tolerated, a second one was, suggesting either that the soy protein had been denatured in one and not in the other, or that other ingredients in the formula had caused symptoms. Interestingly, IgE antibodies to a liquid soy formula were significantly higher than IgE to a powdered soy formula: as a consequence, some children may be sensitized, and others receive an epitope-free formula [41]. In another study, 7/20 children were intolerant to CM and soy [237] as stated by parental reports, but 6/7 had multiple sensitizations. Thus the prevalence of monosensitization was *5%*. In studies by Hill et al, the SPFs used up to 1985 contained sucrose, to which many of the infants studied were intolerant [211]. Therefore, Hill et al never stated that 50% of the children with CM intolerance were also reactive to soy. In one review [223], only the studies with a mean incidence of 30% [40, 177, 178, 211, 237] are cited, and the challenge-based trials are neglected (Table 9.9), except one in children on a gluten-free diet [281]. A 30%–32% rate may also depend on a modest [131, 193, 392] or inappropriate sample building, [1] with 20% of OVA-positive "negative" controls [392]. In the studies summarized by Zeiger et al [530], 59% of cases are based on history. In two studies in infants followed up for 2–3 years, 5.1% [224] and 6.6% [203] experienced adverse reactions to soy; however, at 2–3 years of age the prevalence faded, thus demonstrating that the possible allergy is a transient phenomenon. Freier et al [170] utilized SPFs in infants aged >6 months without recording undesired reactions [170].

The presumed soy allergenicity has been widely emphasized by placing it on the same line as goat's milk [4, 136]. Based on our experience [73, 85, 89] and others' [130, 145, 270, 397], there are no scientific data to compare soy allergenicity to that of CM. Surprisingly, either a RAST+ of 22% [179] or SPT+ of 21% [315] and 25% [416] are reduced by FCTs to 2.5%, 6.1% and 5%, respectively, which accounts for the statements highlighting SPT and sIgE [179, 224]. In other words, SPTs suggested an incidence of soy sensitivity as much as 5-fold higher than that shown by DBPCFCs. Even if in children 4 years of age SPT+ prevalence to soy was 0.1% [191], this is not synonymous with soy allergy [216]. The studies restricted to history [177, 178] have a 25% rate of errors and/or forgetfulness (Chap. 5) and the reactions reported by patients [40, 211, 237] are reduced by FCT to 8% [41, 257]. As previously underlined, the prevalence in small cohorts of *infants and children* [407] at a mean of 2 years of age *with intestinal symptoms* is different: 19.3% [61, 131, 193, 281, 299, 351, 358, 385, 392, 396, 425, 530]. Table 9.27 deals with very small selected populations of children, only 7–18 children in 5 studies [131, 193, 299, 358, 392]; the figures vary considerably, from *0% to 57.1%*. In three studies, the results totalled zero, and the diagnosis was made with DBPCFC [299, 396, 425], and in another there was a 14% incidence, but the DBPCFC was done only in 47% of children [530]. Considering the three DBPCFC studies done in these cohorts of children, the incidence of soy-induced reactions falls dramatically to 4.6%, with SPFs defined as a safe alternative for children with CMA [425]. We deem that, to ascertain the true prevalence of soy in CM-induced enterocolitis and colitis, more studies based on DBPCCT and wide pediatric case records are necessary to avoid type II errors. In 392 children aged 0–3 months without CMA soy prevalence (PTC) was 0% and in 191 aged one year it was 1.6%. FHA was positive in 66% of the whole group [269].

In conclusion, the unnecessary restriction of soy may result in a significantly more limited and inconvenient diet, since soy is ubiquitous in prepared foods: soy-based drinks, yoghurts, and ices offer palatable alternatives for CMA children [31].

Experimental data show that no fatal anaphylactic reaction was set off by soy proteins in soy-sensitized guinea pigs (only nonfatal reactions in 30% of cases), contrary to CM in βLG-stimulated guinea pigs (80% fatal and 20% nonfatal reactions [387]), data supported in rabbits by the lesser allergenicity of soy proteins compared to casein [108]. Soy-sensitized guinea pigs undergoing a type IV challenge with soy protein have exhibited no adverse reaction [432] (see the next point below).

Among *hydrolysate formulas* (Tables 9.28 [68], 9.29 [80] and 9.30 [36]) [74, 90] defined as HA, that is less allergenic than CM normal formulas, there are several types deriving from casein or whey proteins, or formed by casein and whey proteins or soy and pork collagen. Table 9.30 specifies the brandnames with which they are available in European and North American markets and Appendices 9.4 and 9.5 specify their components. We were the first to report five exclusively BM-fed infants aged 3–8 months (median, 5 months) with IgE-mediated CMA, who experienced anaphylactic reactions when first fed a small amount of a *whey HF* (WHF), Alfa-Rè (HR). All infants had AD during BM feeding, positive SPTs and RAST for CM proteins and HR; total IgE levels ranged from 45 to 2,990 IU/ml (Table 9.28) [68]. Subsequently, the infants were success-

Table 9.27. Results of studies evaluating the prevalence of soy intolerance in very selected populations of children with gastrointestinal CMA

Authors	Reference	No. of children	Age (years)	Follow-up (years)	Diagnosis	Reactions to soy (%)
Perkkiö et al[a]	[38]	108	0.3	0.1–7.5	FC[b]	4.6
Kuitunen et al	[281]	35	0.7	9	FC[b]	11.4
Powell[c]	[392]	9	5.5	NS	OFC	42.9
Halpin et al	[193]	10	0.2–0.3	3	C.L	40
Délèze et al[a]	[131]	18	5.2 (M)	15	C.L	22.2
Navarro et al	[351]	42	Infants	NS	C	7.1
Burks et al[c]	[61]	43	0.8 (M)	1.5	FC	32.5
Sampson et al	[425]	92	1.5 (M)	1	DBPCFC	0
Ragno et al	[396]	20	2.6	1	DBPCFC	0
Zeiger et al[c]	[530]	93	1.6 (m)	1	DBPCFC	14[d]
Levy et al	[299]	7	1–2	NS	OFC	0
Nowak-Wegrzyn	[358]	14	0.1–7[e]	3	SPT, sIgE	57.1
Total		484				Mean, 19.3

The studies are ordered according to the year of publication.
In the studies by Powell, Burks et al and Zeiger et al, the children were admitted with a suspected diagnosis of soy intolerance. Studies based wholly or mostly on history or parental reporting were excluded.
C clinical, *FC* food challenge, *L* laboratory, *M* median, *m* mean, *NS* not specified, *OFC* open food challenge, *sIgE* specific IgE.
[a] Retrospective study.
[b] Data on food challenges are lacking.
[c] Studies based wholly or mostly on history or parental reporting were excluded.
[d] In 47 % of children.
[e] Months.

Table 9.28. Clinical manifestations of five infants (mean 5.4 months) with CMA experiencing severe anaphylactic reaction following ingestion of Alfa-Rè

Name	D.M.	D.B.	I.D.	M.M.	B.G.
Sex	M	M	M	F	M
Age (months)	6	3	8	6	4
Total IgE U/ml	94	330	2,990	74	45
Prick tests					
BLG	+++	++	+++	+++	+++
Lactalbumin	++	++	++	++	++
Casein	++	+	++	+++	+++
Alfa-Rè	++	++	++	++	++++
IgE to CM					
PRU/ml	12.8	0.46	>17.5	>17.5	>17.5
Alfa-Rè	2.4	>0.35	2.1	0.90	0.78
Challenge test with:					
CM	Asthma	Lip edema	Lip edema	Lip edema	Diarrhea
	Urticaria	Asthma	Asthma	Urticaria	
Alfa-Rè	Asthma	Lip edema	Urticaria	Lip edema	Urticaria
	Urticaria		Lip edema	Asthma	

Modified from [68].
CM Cow's milk, *βLG* β-lactoglobulin, *PRU* Phadebas RAST Unit.

Table 9.29. Immunogenicity and cross-reactivity of HFs, SPFs, HMMBF

	HFs			SPFs	HMMBF
	Whey		Casein eHF		
	eHF	pHF			
Immunogenicity (sensitizing)	+	++	+	±	±
Allergenicity (triggering)	±	+++	±	±	±
Intact CM proteins	+	++	+	–	–
Reactive epitopes	+	+++	+	–	–
Nutritional adequacy	?	?	?	Yes	Yes
Pleasant taste	No	Yes	No	Yes	Yes
Low cost	No	Yes	No	Yes	Yes

Modified from [80].
eHF Extensively hydrolyzed formula, *pHF* partly hydrolyzed formula, *SPF* soy protein formula, *HMMBF* home-made, meat based formula.

Table 9.30. Molecular weight (*MW*) profile and peptide content of hydrolyzed cow's milk formulas

		MW (%)			
Type	**Brand name**	**<1,500**	**1,500–3,000**	**3,500–6,000**	**>6,000**
Extensively hydrolyzed					
Casein	Alimentum (A)	96.5	2.5	0.5	0.5
	Nutramigen (N)	95.5	3.5	0.5	0.5
	Pregestimil (P)	97.0	2.0	0.5	0.5
Whey proteins	AlfaRé (AR)	88.0	8.0	1.5	2.5
	Prophylac/Hypolac[a] (ultrafiltrate) (PH)	83.5	11.5	2.5	2.5
	Nutrilon Pepti (NP)	84.0	12.0	2.0	2.0
	Pepti-Junior (PJ)	85.0	11.5	1.5	2.0
Soy + pork collagen	Pregomin (PR)				
Partly hydrolyzed					
Whey proteins	Beba HA[b]				
	Good Start (GS)[b]				
	Humana HA (H)				
	Nan HA (NHA)[b]	54.0	20.5	7.5	18.0
	Nidina HA[b] (NI)				
	Nutrilon Pepti Plus (NPP)				
	Vivena HA (V)	36	30	29	5.0
Casein and whey proteins	Aptamil HA (APT)	75.0	16.5	5.0	3.5

Additional partial HFs are Enfalac HA (casein and whey) and Milumil HA (whey).
Data from [36].
HA Hypoallergenic.
[a] The same product marketed under different brand names.
[b] Similar products marketed under different brand names in different countries: Good Start in the US, Nidina HA and Beba HA in Europe.

fully fed a SPF [68]. Remarkably, a recent paper demonstrating that even following a blind FCT, the safety of an *extensively casein hydrolyzed formula* (ECHF) (Alimentum, A) in children with CMA [417], corresponding to AAPSNAD prerequisites, provided a safe formula in 90% of babies with CMA (tolerance, 90%; confidence intervals, 95%) for 18 months [4, 273]. This was followed by the documentation of the first case of generalized reactions to the same formula in a 7-year-old girl with IgE-mediated CMA who did not tolerate even another ECHF (Nutramigen, N) and three EWHFs (extensively WHF): HR, Good Start (GS) and ultrafiltrated GS, whereas the girl had no reaction to a SPF [9].

Several papers have proved in vitro and in vivo and with SPT/FCTs the allergenicity of such formulas in children with demonstrated CMA.

In a group of children with immediate-type CMA, Oldæus et al studied the positivity of SPTs to Nutramigen employing casein/whey HFs with an increasing degree of hydrolysis: none of the 15 children (median age, 8 years) had positive SPTs to the regular, high-degree casein HF; however, 5–7 out of 15 (33.3%–46.6%) reacted to the intermediately hydrolyzed preparations [369]. Rugo et al [407] used SPTs, RAST, RAST inhibition and titrated provocation tests in 8 children (aged 5 months to 9.5 years) with known CMA using single-blind challenge tests. Five out of 8 children (62.5%) reacted to a high-degree WHF with symptoms similar to those induced on challenge by whole CM, 4/8 to EWHFs, 4/8 to a whey ultra-filtrated formula that was not marketed; 1/8 manifested only perioral urticaria on challenge with the soy/collagen HF, and none of the children reacted to N and Pregestimil (P).

In 20 children positive to DBPCFC to CM, RAST-inhibition results revealed the following trend: CM > PWHF (partially whey hydrolyzed formula)/EWHF > soy + pork collagen formula and > AA formula [355]: RAST and RAST-inhibition show that 1%–4% of children with CMA have IgE anti-CM against one or more HFs [197]. sIgE directed against P, N and HR were found in 6/13 infants aged 3–32 months with high serum total IgE and CMA as diagnosed by an appropriate challenge [390].

The allergenicity of several infant formulas was investigated using RAST and RAST inhibition on the sera of 15 children (mean age, 7.5 years) with known CMA. RAST was >3rd class to several formulas in 10/16 children (62.5%): seven to the only high-degree WHF (PJ), one to N, two to P and soy formula, and three to Pregomin (PR). The RAST inhibition results were in agreement with those of the RAST [129]. EWHF Nutrilon Pepti (NP) was tested in 32 children with CMA, for comparison with EWHF Prophylac (PH) and PWHF Nan HA (NHA). SPTs were, respectively, positive to the three HFs in 19%, 15%, and 32% of children [180].

A new ultra-filtrated product was investigated in 66 children with CMA (mean age, 1.9 years), who tolerated it on OFC, except four, only one of whom was positive at the first rechallenge and negative at the final one. However, of the 35 subjects with IgE-mediated CMA, 11% (3/28) had positive SPTs and 6% (2/35) sIgE to the formula. The authors suggest performing a rigidly controlled OFC in children with immediate reactions to CM before starting any such formula [192].

Immunoblotting and monoclonal antibodies were used to evaluate the reactivity of circulating IgE of 12 CMA children with residual intact proteins and peptides present in 6 HFs, 8 reactions to HR, 5 to H and PJ, 5 to Nidina HA (NI), three to N and one to PR were recorded [401]. The use of immunoblotting and monoclonal antibodies may evaluate the antigenic activity of CM protein HFs.

Reactions to ECHFs made known since 1944 [181] are summarized in Table 9.31 [7–9, 16, 24, 35, 42, 76, 98, 121, 125, 170, 173, 281, 301, 324, 356, 368, 385, 389, 393, 396, 405, 411, 423, 432, 441, 452, 477, 487, 506]. The reactions observed by Sampson et al on the whole were 28 to N and A; in particular 7 children manifested a classic IgE-mediated reaction to challenge, 9 had what was called a convincing history of immediate reactions because of enterocolitis symptoms, and 10 children with non-IgE-mediated eosinophilic gastroenteritis reacted to casein formulas (Sampson, personal communication).

Additional *reactions to EWHFs and PWHFs* are summarized in Table 9.32 [7–9, 24, 68, 76, 91, 115, 125, 149, 180, 210, 230, 274, 326, 396, 417, 440, 452, 477]. Results of challenge tests in babies tested with CM or NI were similar [91]. The age of the children involved ranged from 15 days [7] to 15 years [26]. As many as 30% of children were intolerant to extensive and 40% to partial HFs [530].

A more severe case is that of an infant with AD fed a soy formula from the 33rd day of life [471]: since no benefit was observed despite normal growth, at the age of 7 weeks, 12 h after the last SPF feeding, GS was introduced, but at the second feeding the baby exhibited dyspnea and diarrhea, and at the third feeding a severe anaphylactic shock with respiratory distress, subsequent cardiac arrest, and death ensued at the 21st h of admission. Postmortem examination showed signs of the shock with GI hemorrhage, necrosis of the Sanarelli-Schwartzman phenomenon type and congestion and edema of pulmonary alveoli. We ask *a posteriori* why a HF was administered whose HA designation had been removed from the label in 1991 [149].

It is also possible that HFs can induce, in addition to immediate reactions, intestinal lesions that both SPTs and DBPCFC fail to detect, while they can easily be documented with light microscopy. In short, two babies aged 15 days and 10 weeks were fed an EWHF and were affected with diarrhea → remission for 10 days → recurrence → resolution when put on a BM diet. The intestinal morphology improved; however, following an OFC with the EWHF showed congestion and inflammatory infiltration of the chorion of a lymphoplasmacytic type, and increased numbers of IELs [477]. Such lesions

Table 9.31. Reactions to highly hydrolyzed casein formulas

Author(s) and reference	No. of cases	Formula	Sex and age (if specified)	Clinical manifestations
Ammar et al [7]	3	N	15 days–13 months	Urticaria, edema, colic, vomiting (OFC)
Ammar et al [7]	9	P	15 days–13 months	Urticaria, edema, vomiting, diarrhea, bronchitis (OFC)
Amonette et al [8]	3	N	10 months–15 years	Stridor, rhinitis, bronchospasm (DBPCFC)
Amonette et al [8]	1	N	F, 7 years	Worsening itch, systemic urticaria
Amonette et al [9]	1	A	F, 7 years	Anaphylaxis
Auricchio et al [16]	7	N	2–3 months	Vomiting and/or diarrhea, hematemesis in 1 case of shock
Barau and Dupont [24]	1	P	F, 8 months	Episodes of diarrhea (history)
Bidat et al [35]	1	A	F, 6 months	Bloating of the face, systemic urticaria
Bock [42]	1	N	M, 9 months	Immediate vomiting, systemic urticaria[a]
Cafferelli et al [76]	1	N	NS	Diarrhea
Carroccio et al [98]	21	N	M (71.4), 10 days to 2 months	Intolerance
D'Netto et al [121]	1	P	NS, 5 months	Vomiting
D'Netto et al [121]	1	A	NS, 9 months	Vomiting, urticaria
D'Netto et al [121]	1	P	NS, 10 months	Vomiting
D'Netto et al [121]	1	P	NS, 9 months	Urticaria
D'Netto et al [121]	1	P	NS, 10 months	Urticaria
de Boissieu et al [125]	6	NS	NS, 4–16 months	Vomiting, diarrhea, one apparent life-threatening event
Freier et al [170]	2	P	NS	Pallor, vomiting repeated for 12 h, somnolence
Galli et al [173]	3	N	NS, 2–48 months	AD worsening, diarrhea
Kelso and Sampson [264]	1	N	M, 8 months	Diarrhea[b]
Kelso and Sampson [264]	1	P	"	Diarrhea (reintroduction)
Kelso and Sampson [264] (reintroduction)	2	A	"	Vomiting within a few minutes
Kuitunen et al [281]	3	N	M, 15 weeks	Clinical intolerance[c]
Lifschitz et al [301]	1	P	M, 31 days	Anaphylaxis
Martin-Esteban et al [324]	2 A	NS	4.6 months (m)	Urticaria, bronchospasm, vomiting within 5 min
Nilsson et al [356]	1 C+W	F	10 months	Obstructive symptoms on three separate occasions
Oldaeus et al [368]	1	M	11.3 years	Severe systemic urticaria
Pettei [385]	1	N	M, 2 months	Repeated hematochezia
Pladys et al [389]	1	P	F, 20 days	Proctorrhagia
Powell [393]	Some cases	P	NS	Diarrhea + eosinophilia and test for hidden blood
Ragno et al [396]	2	A	15–76 months	Rhinitis/wheezing, urticaria (DBPCFC)
Rosenthal et al [405]	3	2 N, 1 P	1 F/2 M, 0.6–1 year	Enterocolitis with hematochezia

Table 9.31. (Continued)

Author(s) and reference	No. of cases	Formula	Sex and age (if specified)	Clinical manifestations
Salmun et al [411]	1	A	4 months	Hives, wheezing, anaphylaxis (epinephrine)
Sampson [423]	28	N	NS	Classic IgE-reactions, CM-enterocolitis[d]
Sampson et al [423]	3	N	NS	2 reactions in 2 children on challenge, 2 after hospital feedings
Saylor and Bahna [421]	1	N	M 3 years	Anaphylaxis + SPT positivity[e]
Schwartz et al [441]	2	N	NS	Labial angioedema (DBPCFC)
Sotto et al [452]	2	NS	1–12 months	Anaphylaxis, generalized urticaria
Sotto et al [452]	4	NS	1–12 months	Vomiting, diarrhea
Tounian et al [477]	1	P	M 3 months	Vomiting, diarrhea
Vanderhoof et al [487]	28	NS	22–173 days	Vomiting, diarrhea, bloody stools, failure to thrive
Vanderhoof et al [487]	17	NS	22–173 days	Abrupt recurrences of symptoms on resumption
Wahn et al [506]	1	NS	NS	Systemic urticaria
Total	172			

M male, *F* female, *m* mean, *A* Alimentum, *C+W* casein + whey (4:6 ratio), *N* Nutramigen, *P* Pregestimil, *NS* not specified.
[a] Probable case, diagnosed with DBFC.
[b] Soy milk resolved diarrhea with permanence of hidden blood in feces.
[c] Three out of four (17.6%) vs 4/35 (11.4%) soy in 54 children with malabsorption owing to cow's milk intolerance.
[d] Personal communication. In total Sampson et al observed 28 reactions to N and A.
[e] For Good Start and Nutramigen (4+) and for Alimentum (3+).

are sometimes discrete, and useful for diagnostic purposes only when they are compared to a previously regular biopsy (which is rarely performed in normal conditions) [477]. Therefore we are confronted with elusive forms, because their diagnosis is difficult with the presently available tests, intestinal biopsy would be necessary, and the differential diagnosis must consider all possible causes of diarrhea in small infants (Tables 6.5).

Reactions that are also severe following SPTs with HFs [115, 421] confirm an event occasioned by cutaneous application of small CM doses [246].

No controlled studies documenting the clinical properties of PR formula are known now, yet the published reactions are few [7, 24, 129, 407].

In conclusion, in the cases reported in the literature, the use of HFs provoked 358 reactions to CHFs, many of which were IgE-mediated, in 172 children aged 15 days to 15 years, (1 case of shock, *5 of anaphylaxis,* 7 of generalized urticaria, 1 apparent life-threatening event, as well as 2 localized reactions), and to WHF in 150 children aged 15 days to 15 years, either extensively or partially (2 cases of shock, *10 of anaphylaxis,* 13 systemic reactions, 3 apparently life-threatening events) [90], and 2 cases of hypotension [76], including 36 children allergic to extensively HF [122, 123, 125]. Moreover, there is the case of *acute anaphylaxis* following the ingestion of casein and whey HFs [35], the unspecified number of significant allergic reactions to GS [149, 441], and those ascribed to a CHF [393] at variance with other studies [74, 153]. Two children after long-term CM elimination with ECHF/EWHF following an accidental CM ingestion experienced loss of consciousness and an other 8 dyspnea, angioedema, urticaria, etc. [154].

We deem it necessary to explain why HFs can maintain a striking allergenicity and immunogenicity, either for the presence of peptides with a high MW or the contamination of CM intact proteins.

The allergen physicochemical characteristics are not related to their MW, in that no defined MW exists below which the peptides are not allergenic [4]. Furthermore, currently available methods do not permit precise calculations of MW distribution in HFs [36]. Thus peptides which should not be allergenic become allergenic by aggregation or cross-binding to other molecules, which may turn out to be antigenic or even allergenic. In any case, the non-antigenicity of HFs can be maintained, above all of finished products, as based on the MW of residual peptides [272]. Certainly the methods designed to estimate the MW profile suffer from inaccuracies, especially as regards the limits of sensitivity, which prevent any exact calculation; consequently the MW distribution declared by a manufacturer is approximate, and

Table 9.32. Reactions to highly and partially hydrolysate whey formulas

Author(s) and reference	No. of cases	Formula	Sex and age (if specified)	Clinical manifestations
Ammar et al [7]	8	AR	15 days to 13 months	Vomiting, diarrhea, edema, colic, agitation (OFC)
Ammar et al [7]	7	PJ	15 days to 13 months	Vomiting, diarrhea, edema (OFC)
Amonette et al [8]	8	GS	10 months to 15 years	3 reactions not well-defined + 5 systemic[a] (DBPCFC)
Amonette et al [8]	8	UGS	10 months to 15 years	8 systemic reactions (DBPCFC)
Amonette et al [9]	1	AR		Profuse rhinorrhea, irritability (DBPCFC)
Amonette et al [9]	1	AR	F 7 years	Rhinorrhea, cough, nausea, agitation (DBPCFC)
Barau and Dupont [24]	1	AR	F 14 months	Challenge >1
Barau and Dupont [24]	1	AR	F 3 months	Challenge >1
Barau and Dupont [24]	1	PJ	F 3 months	Challenge >1
Businco et al [68]	1	AR	M 6 months	Anaphylaxis after receiving one drop of AR
Businco et al [68]	1	AR	M 3 months	Anaphylaxis after receiving 5 ml of AR
Businco et al [68]	1	AR	M 8 months	Anaphylaxis after receiving one drop of AR
Businco et al [68]	1	AR	F 6 months	Anaphylaxis after receiving 5 ml of AR
Businco et al [68]	1	AR	M 4 months	Anaphylaxis after receiving 5 ml of AR
Caffarelli et al [76]	1	PH	NS	Immediate vomiting, delayed diarrhea
Caffarelli et al [76]	1	PH	NS	Immediate urticaria
Caffarelli et al [76]	1	PH	NS	Immediate urticaria
Caffarelli et al [76]	1	H	NS	Immediate urticaria-angioedema, dyspnea, hypotension, delayed diarrhea
Caffarelli et al [76]	1	H	NS	Immediate urticaria-angioedema, dyspnea, hypotension, delayed diarrhea
Caffarelli et al [76]	1	H	NS	Delayed diarrhea
Caffarelli et al [76]	1	H	NS	Delayed diarrhea
Caffarelli et al [76]	1	H	NS	Immediate urticaria
Cantani et al [91]	41	NI	M 27, F 14, 4 months[b]	AD worsening, urticaria, vomiting, wheezing, shock (1 case)
Dalmau and Nieto [115]	1	NS[e]		Bronchospasm requiring adrenalin
Dalmau and Nieto [115]	1	NS[e]		Anaphylaxis, pre-shock state
de Boissieu et al [125]	9	NS	1–16 months	Vomiting, diarrhea, three apparent life-threatening events
Ellis et al [149]	1	GS	9 months	Anaphylaxis
Giampietro et al [180]	1	NP	F 17 months	Urticaria, rhinitis (DBPCFC)
Giampietro et al [180]	2	PH	2 F 17–32 months	Positive DBPCFC
Giampietro et al [180]	9	NI	NS	Positive DBPCFC

Table 9.32. (Continued)

Author(s) and reference	No. of cases	Formula	Sex and age (if specified)	Clinical manifestations
Heyman et al [210]	1	[3]	M 6 months	Profuse diarrhea
Iacono et al [230]	1	AR	F 4 months	Hematochezia, increased circulating eosinophils
Klemola et al [274]	2	NS[c]	F 7 months	Immediate responses, erythema, urticaria
McLeish et al [326]	2	[5]	10 weeks	Hematochezia
Ragno et al [396]	9	AR	15–76 months	Rhinitis/wheezing, urticaria, lip edema (DBPCFC)
Ragno et al [396]	9	PN	15–76 months	Perioral erythema (DBPCFC)
Sampson et al [417]	1	GS		Severe anaphylactic reaction
Sotto et al [440]	2	NS	1–12 months	Anaphylaxis, generalized urticaria
Sotto et al [452]	5	NS	1–12 months	Vomiting, diarrhea
Tounian et al [452]	2	AR	M 2–3 months	Vomiting, diarrhea
Tounian et al [477]	1	AR	M 1 month	Vomiting, diarrhea (reintroduction)
Tounian et al [477]	1	AR	M 1 month	Vomiting, diarrhea (reintroduction)
Total	150			

AR Alfarè, *GS* Good Start, *NI* Nidina HA, *PH* Prophylac Hypolac, *PJ* PeptiJunior, *UGS* ultrafiltrate hydrolysate, *NS* not specified.
[a] Systemic reactions included rhinorrhea, cough, stridor, wheezing, or generalized urticaria.
[b] Hydrolysate whey formula.
[c] Hydrolysate lactalbumin formula.
[d] 4 months (range, 7 days to 15 months).
[e] Nonspecified whey hydrolysate formula.

cannot assess a 100% non-allergenicity of a given HF [318]. Thus the indiscriminate HA labeling, which literally means "less allergenic" than normal CM formulas, and not *non-allergenic*, is unconvincing because it is not quantifiable, hence open to any evaluation [79]. As methods are refined, more data can reveal the greater gap of such HFs, that is the small but detectable amount of residual and potentially allergizing properties can be demonstrated in HFs even with MW >6,000 D [36] (Table 9.30). However, in CHFs there is 15%–20% peptides with a MW >3,850 D and 35%–42% with a MW between 340 and 3,850 D [443]. After protein enrichment by trichloroacetic acid (TCA) precipitation, the presence of high-MW polypeptides was shown in HFs, such as protein bands visible in SDS-PAGE with a characteristic pattern. Partly HFs (H, NI, PR) show the higher amount of polypeptides with a diffused area ranging from 6,500 D and 71 kD, while high-degree HFs have a lower residue (HR, N, PJ) (Fig. 9.24) [401]. A study of 11 WHFs, 7 of which were HA, one based on hydrolyzed casein, and one ultrafiltrated (unspecified) showed a mean 55.5% content of peptides with a MW of 3 kD, 27% 3–5 kD, 13.5% 5–10 kD and 6.9% >10 kD, including two HA HFs *with a 26.3% and 40.6% content* of >10 kD peptides [480], hence all tested products retain some residual antigenicity of one or more of the individual CM proteins [480]. Previously peptides with a MW >5,000 D were not found in A and N, but in GS (Fig. 9.25) [63]. Yet studies done initially in the animal model [273] suggested that HFs even with peptides <3,400 D were not immunogenic and *peptides with a MW between 3,400 and 6,500 D would induce only weak reactions*. The allergenic and immunogenic epitopes that can be detected in CM proteins by humans are not necessarily the same as those seen in a guinea pig, a rabbit, or a lamb.

Taking into account another area of increasing interest are the immunological data on the processing of native protein molecules (Chap. 1), which suggests that an allergen splits into allergenic peptides of low MW, in association with HLA molecules, will be expressed on the cell membrane. The HLA–peptide–APC (antigen presenting cells) complex is recognized by the T-cell receptor (TcR), an event resulting in T and B cell activation; subsequently specific processes lead to IgE synthesis. Children with CMA may react to the partially hydrolyzed whey and casein formulas which contain components able to be bound by IgE, and to the PWHF showing higher protein content and residual allergenicity than any other HFs [345]. *Such processes are operating already in the neonate and small infant* in prospective. The hydrolysis of native antigenic molecules primes the unmasking of epitopes concealed in folded molecular proteins, thus allowing antibody binding and a sIgE antibody response [494]. In normal subjects, the

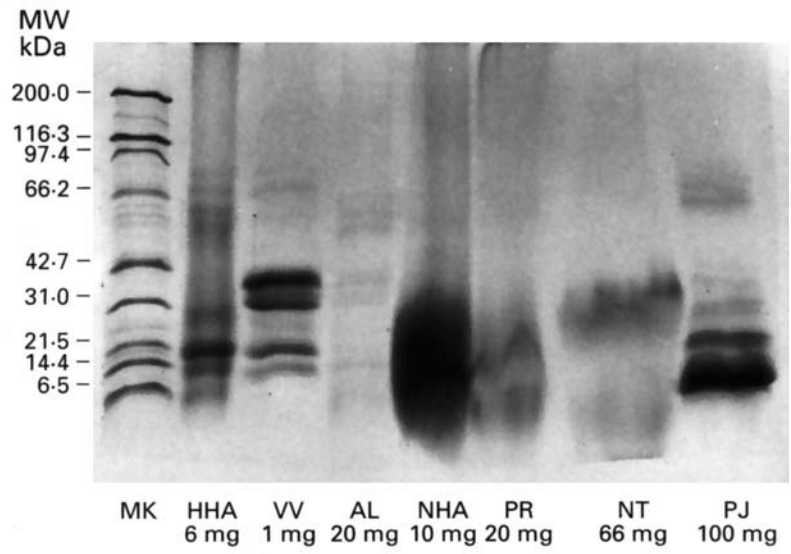


Fig. 9.24. SDS-PAGE of hydrolyzed formulas after enrichment by trichloroacetic acid precipitation (for details see text). *MK* standard solution, *VV* Vivena 2, *AL* AlfaRé, *NHA* Nidina HA, *PR* Pregomin, *NT* Nutramigen, *PJ* Pepti Junior

Fig. 9.25. Molecular weight distribution of three protein hydrolysates (for details see text)

AA sequences provided with structural characteristics essential to forming complexes with HLA molecules, which often are found inside native protein molecules, become available to bind to HLA molecules after the unfolding of protein structures (Fig. 1.18). Although HFs may contain peptides of lower MW than the native protein source, the peptides still have allergenic potency and can be recognized *by the cell-bound IgE of a child with CMA* [290]. But the MW alone does not suffice to guarantee antigenicity for any given polypeptide [416]. Therefore, a "final product" consisting of two or three AA residues (and one epitope) would be more sufficient to trigger allergic reactions in HR children than 7–8 or 12–17 AA sequences with two epitopes clearly recognized by T cells (Chap. 1).

HFs are obtained by heat denaturation and enzyme hydrolysis to reduce MW of whey proteins: one or more enzymes (trypsin, chymotrypsin, pepsin, carboxypeptidase, etc.) are employed for this purpose. Protein *heat denaturation* may cause undesirable reactions such as protein aggregation or precipitation, thus implying lower solubility and reduced digestibility. It is employed in that normally conformational B epitopes are eliminated, thus facilitating hydrolysis, but not of sequential T epitopes [1]. As a consequence, *enzyme hydrolysis* is preferred, more or less extensive in casein or whey proteins, which in specific conditions acts on both types; the subsequent ultrafiltration removes any residuals of elevated MW [407]. Hydrolysis reached a level of 26% in WHFs, 15% in HA formulas (range, 1.3%–32.5%) and 52% in the sole casein formula [480].

It is difficult to understand why there are differences between the hydrolysis employed at the industrial level and natural hydrolysis with which APCs with appropriate HLA markers on their surface activate T cells, thus priming the antibody response [508]. Paradoxically, the result is overlapping, thus the hydrolysis would *activate the IgE response that should instead be repressed* [479]. It is difficult, if not impossible, to affirm that enzyme hydrolysis in vitro abolishes all the epitopes of original

proteins, unless residual peptides are sufficiently denatured by other procedures [297]. As proof, Isolauri et al have found cells secreting IgM against trypsin- and pepsin-digested βLG, suggesting that some antigenic epitopes may resist such treatments [233], and in children with CMA anti-CM IgE antibodies may recognize CM epitopes of one or more HFs [197].

The Küstner case [395] indicates that epitopes not present in the original food protein can be *neoformed* or made accessible by digestion, unless the segments of peptides that resist enzyme hydrolysis are affected and inactivated by adequate denaturation, or some kind of filtration is used to remove the large residual peptides [297]. *Physicochemical manipulations do not allow the abolition of all the epitopes of native protein source* [290], in that the AA sequence characterizing an epitope may resist heat, enzyme hydrolysis, etc.; immunoreactive epitopes concealed inside tertiary structures may be revealed during formula recomposition or after peptide digestion (Fig. 1.18). Lipids and carbohydrates not concerned by the hydrolysis can bind protein structures, building up the substratum to make new epitopes [479]. Obviously, children RAST+ to CM proteins have circulating IgE antibodies, which recognize peptides common to more than one HF [129] and native proteins by cross-reactivity. Haptens can combine with albumin or other protein carriers, so *a peptide not recognized by the immune system will probably never be available* [459].

Consequently, we are skeptical of the idea of eliminating or neutralizing the epitopes of such a peculiar structure: if allergen enzyme hydrolysis is able to reduce its allergenicity, one so selective to destroy the specific epitopes cannot be conceived [290]. Moreover, starting from the assumption that minute amounts of βLG in BM equal to 0.01 mg/l [451] may result in allergenic processes despite the BM IgA, even more the ingestion of HF declared as HA, containing minute amounts of intact protein fragments but in higher concentrations than that found in BM, may induce severe anaphylactic manifestations in children with IgE-mediated CMA, provoked by peptides cross-reacting with IgE to CM proteins, despite enzyme hydrolysis or even amplified by these manipulations. Some HFs are contaminated by undegraded immunogen CM proteins that have escaped enzyme hydrolysis [79]. Residual allergenic components from CM such as casein-unprocessed components and casein fractions could be identified in both the partial and extensive HFs analyzed [133]. There is an unsatisfactory technological separation of caseins from whey proteins [133, 402, 480], and the difficulty in the elimination of immunoreactive epitopes present in casein aggregates after enzymatic treatment [133]. Data shows that *casein formulas contain whey protein residuals* [401, 402] capable of producing precipitant antibodies against at least three whey proteins and casein as well [443], and *whey protein HFs* (HR, Beba HA = GS) and soy + pork collagen *casein residual epitopes* [302, 480], GS up to

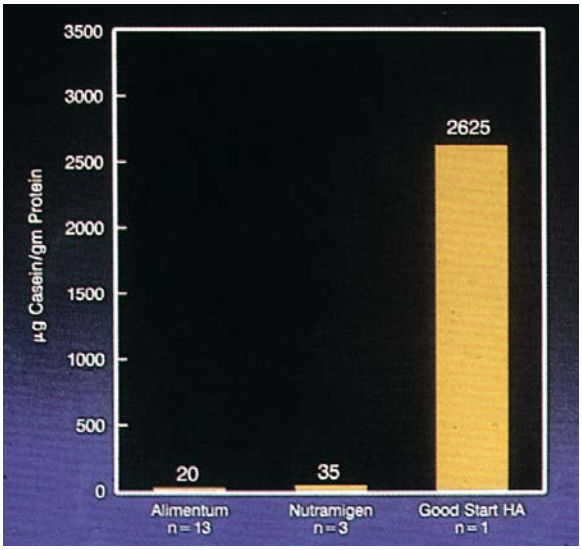


Fig. 9.26. Proportion of immunoreactive casein in a whey partial hydrolysate (Good Start) and in two casein extensive hydrolysates (Alimentum and Nutramigen). (Data from [63])

2,625 µg (Fig. 9.26) of casein/g of protein [63]. An NHA sample has shown bands corresponding to intact whey (ALA and βLG) and casein components (α- and β-casein) [133]. A recent study [220] has shown by inhibition ELISA *that N has a 64.7% whey content and A about 50%, whereas PR has a 36.6% casein content and HR 17.9%*. In a guinea pig model, a PWHF showed a residual sensitizing capacity and a possible casein contamination [388]. An apparent contradiction may be explained because by precipitation methods used to obtain the separation of whey proteins from casein, aliquots of casein may remain in the whey fraction due to casein-derived low-MW peptides, so enzyme hydrolysis is not able to hydrolyze casein completely [297]. Hydrolyzed products may contain 1%–2% or more of nonhydrolyzed molecules; *a single epitope may be sufficient to react with the immune system of highly sensitized children* [455]. Heating casein at a T of 121 °C for 15 min is not enough, and boiling it for 30 min is necessary to reduce its antigenicity and immunogenicity significantly [297]. Casein may resist denaturation, so that fraction α maintains its primary characteristics [23]. Degradation alters in part conformational structures, but IgE binding, almost identical to that of its native form, is preferentially with sequential structures equal to T epitopes, correlated with the elevated allergenicity of fraction κ [402].

Nearly all HFs have significant amounts of βLG detected by ELISA and RAST inhibition (Appendix 9.6) [235, 317, 318, 368], compared to BM [17, 226, 238, 314, 451]. Using ELISA, the immunologically active whey protein levels (IAW) and casein (IAC), either PWHF, or high-degree CHF (in a smaller amount) contain βLG, expressed here in µg/g protein: WHF 1060–1210 IAC

and 140,000–210,000 IAW; CHF, 7.68–12.2 IAC and 19.1–25.6 IAW (compared to intact formulas 400,000–800,000 IAC and 180,000–600,000 IAW) [109]. This study, done using the hyperimmunization method in rabbits [109], demonstrated implicitly the unsoundness of studies done in guinea pigs that previously documented, with no exceptions, only a mild *antigenicity* of whey proteins. Instead, animals sensitized to a WHF after challenge with CM have elicited 8/10 reactions, six of which were fatal [432]. However, if sensitized to an extensively HF, they had no anaphylactic episodes, at variance with 39%–73% of animals sensitized to a partly HF [292].

We have reported that neonates fed HF supplements in the maternity ward in their very first days of life have experienced, at the end of BM feeding, an anaphylactic shock while fed the same formula. Therefore, HFs in that immunogenic category are able to activate sensitization and trigger reactions that are as severe as anaphylactic. So the state of the gut when the allergen challenges the gut and the immune system is probably more important [459].

The bulk of evidence shows essentially that marketed HFs are free of intact proteins, thus guaranteeing their non-immunogenicity (Tables 9.29, 9.30). Great uncertainty on HFs depends on the nebulosity encircling the scientific data related to HFs [129, 136, 292], of no advantage to manufacturers. Not even the documents prepared for physicians contain this information; however, since the technology used differs depending on the manufacturer, at least this information should be delivered to specialists, certainly to the doctor prescribing the formula [427].

In conclusion, HFs *cannot be prescribed for dietetic treatment of children with IgE-mediated CMA* because they are capable of initiating anaphylactic reactions, in that intact or polypeptidic fragments residual from protein hydrolysis are capable of maintaining or precipitating an immunogen activity [402, 480]. Plasma cells-IgE of allergic children may recognize whey proteins in casein [443, 480] and/or casein in whey protein formulas [63, 302, 402], thus explaining the numerical equivalence of reactions provoked by two HFs (Tables 9.31, 9.32). As stated above, the manufacturers of GS had to *remove the HA designation from the label in the US* [149] and based on the data presented here, most manufacturers should submit evidence to document and define their claims of hypoallergenicity. The HA label can also create a false sense of security. Sampson et al [416] recommend that all infant formulas promoted as HA be tested in children with CMA to assess their allergenic potential.

The AAP Committee on Nutrition [4] has stated that *no DBPCCT study has documented the benefits of whey protein hydrolysate, which are not intended to treat children with CM hypersensitivity*. The above-cited AAPSNAD [273] subsequently suggested a definition of the term "hypoallergenic" (accepted by the US FDA), in controlled conditions. A sufficient number of such patients should be enrolled in a prospective study organized in such a way that it can be projected with 95% confidence that 90% of children with CMA will not react to the HF studied [273]. If the 90% level is judged a too low cut-off, using a 95% probability level would require a sample consisting of 120 children with no reaction, but if only one infant reacts to the HF, the group must be enlarged to encompass ≈400 infants (plus 400 controls equally representative), also to avoid the chances of a type II error in the data. However, it seems unethical to increase the number of children only for statistical purposes. In Chap. 24 we will return to such topics and the studies conducted in several countries employing such HFs for atopy prevention in atopic children, also (and above all) from the nutritional point of view.

No HF can offer absolute protection from allergic reactions. HFs have been suggested for children with negative SPTs and RAST, or with GI affections caused by CM intolerance, not IgE-mediated [214, 314], but others disagree since all HFs are not hypoallergenic [419]. We believe that it is more consequential that HFs, if they claim to be effective, be studied in individual children, in vivo in infants with IgE-mediated allergies, and with adequate methods such as RAST inhibition, CRIE, immunoblotting, ELISA, and that studies be organized according to previous procedures [115, 197, 307, 369, 401], or with the patch test that is 3.5- to 7-fold more sensitive than SPTs [235]. Others suggest preferring the lactulose/mannitol test [24], or a FCT with the HF [396], or that the first administrations be under medical control and that during the diet periodic dosages of sIgE anti-CM and anti-hydrolysate are done [322]. The laboratory animal hyperimmunization model proposed to evaluate the immunogenicity of CM protein HFs [109] could reduce costs, risks and the time necessary for DBPCFC studies. Therefore, the task is particularly demanding, because only minimal residual molecular structures are required for antigen recognition by the immune system in previously sensitized children [292]. A recent study stresses that even when infants become sensitized by exclusive breast feeding, there is no justification to change to a HF [352].

A promising base to produce a tolerogenic HF can be found by hydrolyzing a partially whey protein concentrate with both alcalase and papain [518]. A new soybean-whey hydrolysate obtained by the hydrolysis of soybean-whey presented low antigenicity and due to the enzymatic process, an improved digestibility. The combination of high pressure and enzymatic hydrolysis seems to be an efficient tool to reduce or remove the residual antigenicity of soy whey proteins, depending on the protease used. High-pressure technology can supply the ingredients suitable for the HF production [382].

The *meat-based oligoantigenic diets* ensure the reported advantages (Table 9.17) and correspond to established prerequisites for correct and adequate alimen-

tation of children [80] (Table 9.16). The age-related protein/carbohydrate ratio should always be taken into account (Table 9.15). Recently, a rice HF has proven safe when tested by DBPCFC in a study population of 18 children allergic to both CM and soy protein [474]. In 100 children (range 0.18–14.6 years) with a history of immediate reactions to CM and confirmed at DBPCFC, all DBPCFC with a rice-based HF were negative. Therefore a rice-based HF is a possible alternative not only for children with multiple allergies, but also for children with CMA [162], or with clinical reactions to soy and CM HFs. Pediatricians and pediatric allergists are now bombarded with a large variety of information of new special formulas and are confronted with a difficult choice regarding the nutritional adequacy, the immunogenicity and allergenicity of the available CM substitutes. HMMBF may be moderately antigenic, but not immunogenic [80]: therefore homemade formulas may be employed in 99% of cases of CMA, provided that our recommendations are followed [80].

Children with CMA have been reported with good results in the alimentation with *AA formulas* (AAF) [7, 122, 123, 125, 235, 423, 542], with a significant increase in weight and height during follow-up compared to a PWHF [235]. No band corresponding to CM proteins could be detected [133], but in both formulas, unexpected high βLG values were found, in the Nutri-Junior (NJ) formula it was 20-fold that in the Neocate formula [235]. In one batch CM proteins (βLG) were found intact on the same order of magnitude as in the HF used for comparison [235], likely due to residual allergenicity by contamination during the preparation [235]. A 10-month-old girl reacted with urticaria to Neocate [356] and two children with delayed worsening of AD [76]. Recently a new AAF has been marketed, EleCare, that is free of CM and soyprotein.

Although Galen described a case of allergy [305], *goat's milk* has been long employed in infantile alimentation. Even if much more expensive than other formulas [305], recently formulas of goat's milk have been commercialized to feed children with CMA (Table 9.20). Goat's milk, as all animal milks [129], shows a strong cross-reactivity to CM proteins [77, 129, 133, 219] with a risk of 92% in CM-allergic individuals [447]. To RAST inhibition, goat and sheep milk allergenicity is, respectively, 88% and 94% of CM allergenicity [129]. Boiling reduces the allergenicity [23]: this precaution was not recalled by 2/3 parents [513]. Severe reactions to FCTs in 18/20 children aged 3 years (mean) (90%) [305] and in mice with CMA in 100% of cases [219], have confirmed previous data [429]. In 26 children with positive SPTs and CAP results to both CM and goat's milk, DBPCFC was positive to CM, and 24/26 had positive DBPCFCs to goat's milk [27]. In children with CMA or intolerance, 70%–90% showed intolerance to goat's and sheep's milk [97]. Therefore, goat's milk cannot be used to feed genetically predisposed children or those with an IgE-mediated CMA [27, 305]: in a 7-month-old baby in prevention it caused an anaphylactic episode [149] and if children followed by us tolerate goat's milk *this is the proof that they have no CMA*. Therefore, manufacturers should clearly indicate on the label that this is a food not for infants with known or suspected CMA.

Mare's milk can be regarded as a good CM substitute in most children with severe IgE-mediated CMA. It would be prudent, however, to confirm its tolerability by a supervised titrated OFC. Only one child out of 25 had positive DBPCOFC to mare's milk [75].

Ass's milk is intolerant in 10% of children, a 4-month-old girl reacted to this milk with bloody allergy [229].

In conclusion, Tables 9.20, 9.21 and 9.29 summarize the prerequisites of an ideal CM substitute. Apart from the economical factor and the fairly easy availability, its being unpalatable is a cause of frequent refusal in children of >1 year. In two groups of children following a diet supplemented with HFs, the drop-out rates were 38%–56% after 2 weeks and 53%–67% after 6 weeks [308].

Appendix 9.6 shows that a drop of some HFs contains an amount of βLG equal to that found in liters of BM (Chap. 3). As regards maternal levels, is obvious that the studied mothers were not on diets within preventive programs [226, 238, 282, 314, 451]. The more precise data of Sorva et al [451] show that the mean BM level 2 h after a meal of 400 ml of CM is 60- to 420-fold lower than the known levels [226].

Allergy to Single Foods

CM

SPFs are utilized in this age range, also allowing the preparation of CM-free cookies and cakes, thus resolving in older children, when the taste is agreeable, the problem of breakfast and snacks.

The *oligoantigenic HMMBFs* may be utilized with a good index of compliance [309], integrating or varying the diet with an ultimate objective of *personalizing* it as soon as the situation is favorable, amplifying the number of boiled or roasted, or fried foods [80]. Utilizing the food diary and P + P test, the diet can be tailored to the individual child by cautiously adding pleasant foods every 5–7 days such as white potatoes, lettuce, cooked carrots, cabbage, zucchini, cauliflower, Brussels sprouts, rabbit or pork meat, beet, tomato and other greens, legumes, citrus fruit and parsley [80]. While reporting every change on the diary, parents may introduce a new food over a period of 3 days and increase the dose in this time up to amounts normally eaten by their child. Two outcomes are possible: no symptom = food tolerated; the symptoms relapse = return to previous diet up to symptom remission and/or a new directive from the pediatrician. Alternatively a *CM-free diet,* as suggested in Table 9.33, without adding vitamins, whose use is limited in severe cases of AD. For example, the administra-

Table 9.33. Forbidden and allowed foods for weaned children with CMA (see Table 9.25)

Forbidden foods
Bread and pizza made with CM
Candies, cookies, chocolate of all types, ice-creams, whipped cream
CM and dairy products (cheese, butter, yoghurt, etc.)
Cooked ham and sausages in general
Foods containing even minimal amounts of CM (read labels carefully for ingredients)
Lactose-free milk
Margarine (some types)
Meats: beef, calf and young beef
Allowed foods
All raw vegetables, salad, carrots, potato (boiled, baked, fried, etc.)
CM-free cookies
CM-free, home-made soups
Fresh meats: lamb, rabbit, pork (lean parts), horse, ass
Fresh or frozen fish: sole, trout, cod
Fresh or frozen fruit: peeled pears, banana, pineapple; try in different weeks grape, orange, peach, apricots
Pasta of durum wheat (for adults), rice
Raw jam
Rice cream
Soy-milk home-made candies, cakes and cookies (not for soy-intolerant children)
Tea (loose, not in tea bags)
Unrefined sugar
Tap water
Foods may be boiled or baked, or fried
Avoid all foods containing "caseinate"
Ca addition: see Tables 9.23 and 9.24

Diet used in the Division of Pediatric Allergy and Immunology of Rome University La Sapienza.
Chicken and turkey may be added if the child is not allergic to egg.

tion of vitamin E produced proctorrhagia in a girl 37 days old [389]. Appendix 9.8 outlines the nutrient distribution in some special formulas and Appendix 9.9 outlines the Ca content in the main CM substitutes suitable for these children. The *addition of Ca* (Table 9.23) is necessary in all children on a CM-free diet, with the exception of some SPFs.

In all these cases, CM exclusion must follow exclusion of derivatives, extended to all types of cheese, above all goat and sheep cheese [133]. Furthermore, it is necessary to pay attention to *hidden CM sources* and derivatives and/or dairy products also disguised by unintelligible labels: see Table 9.34 [246, 294, 389, 418, 516, 519, 523, 528] *extended to other foods* [516] (Appendix 9.2). Several countries do not require the mention of CM and egg on prepared foods for children which contain either food. CM proteins are always more frequently found in more unexpected cases, such as in old pots [110], gloves (Chap. 8), a diaper rash ointment which has occasioned anaphylaxis a few seconds or minutes after application in two infants with CMA [249, 297], and egg proteins (Appendix 9.1), a substance employed to reduce the lipid content of some foods [418] and therefore able to initiate reactions in allergic children, and in numerous non-food products [389, 516] (Appendix 9.2).

In applying dietetic therapy for allergy to CM and other foods, it is not irrelevant to recall a few important cornerstones, especially the factors capable of affecting the diet's success (Table 9.35) [19], particularly *the compliance of children and their families*: it is often a good idea, also to combat monotony, *to personalize the diet*. Liberalization extensible to allergies to other foods allows restricting the diet only to clearly harmful foods, thus increasing the compliance of young patients and their families [92]. More controls are required by long-term diets.

Egg

To prescribe an egg-free diet, it is indispensable to abolish not only all egg-containing food, but also the meat of animals strictly correlated, and egg shampoo. The albumen, including also OVA and Gal d 1, is the immunodominant and sensitizing allergen, but small amounts of OVA are found in egg-yolk, so both should be eliminated. Tables 9.36 and 9.37 [184, 523] summarize a type of diet for children allergic to egg or egg and CM and suggests how to substitute egg in cooking. Children who are not wheat allergic may eat durum wheat pasta freely (with no CM added) and cookies. See also Appendices 9.1 and 9.2.

The measles vaccination was not recommended to children with IgE-mediated egg allergy based on reports of postvaccination anaphylactoid reactions in two children with egg allergy documented by history. The vaccines employed today are constituted by measles live virus and a cross-reactivity between chick embryo fibroblasts, on which the virus is grown, and egg proteins was suspected, although antigenic diversity was demonstrated [72]. Following studies on surely egg allergic children who have experienced no reactions from the measles vaccine, several authors have vaccinated, without relevant reactions, 1,963 children with manifestations of IgE-mediated egg allergy, while 47 without any contraindication have experienced immediate reactions, virtually independent of egg allergy (Appendix

Table 9.34. Products with potential hidden sources of CM or CM proteins

Bread and breaded foods, including toast bread, bread made with oil, crackers, commercial breads and rolls
Bread, fortified
Breakfast food
Butter substitutes
Candies, especially commercially prepared, also frozen
Candy bars
Canned fish
Canned meat
Canned, dehydrated soups
Caramels
Cereals, fortified
Cheese for vegetarians
Chewing-gums (some types)
Chocolate- or cocoa-containing beverages, also plain chocolate (due to contamination)
Commercially prepared roasted or grilled meat, or *au gratin*
Cottage cheese
Cooked or boiled ham
Dairy foods
Delicatessen meats
Desserts with whipped foods
Doughnuts
Hypoallergenic formulas
Ice creams (also due to contamination)
Instant breakfast, potatoes, etc.
Lactose[a], yoghurt, rennet
Lactulose
Margarine (some types)
Mayonnaise
Meat fillings
Meringue
Muffins
Nougat
Pancakes
Pasta, fortified
Pie crusts
Pizza
Prepared flours
Prepared mixes for cakes
Products for infants and toddlers (soups, pap, biscuits, homogenized foods, etc.)
Puddings
Rarebits
Salad dressings
Sour cream
Sauces and gravies including béchamel, etc.
Sausages (some), Frankfurters (some), and hot dogs
Special nutritive foods: bread, cereals and enriched foods enriched, high-protein beverages, pills or tablets with proteins and/or minerals
Herbal teas (some)
Vegetables, especially creamed and scalloped vegetables
Waffles
Non-foods [389]
Antibiotics
Anti-inflammatory drugs
Drugs containing E and/or P vitamins
Latex gloves (Chap. 8)
Milk-based paints, plasters and wall-paper containing casein (Chap. 24)
Penicillin powder for oral use
Preparations with total pancreas
Products for the hygiene of neonates/infants (ointments, creams, powders, diaper ointments) [246, 294]
Toothpastes for baby teeth

Foods should be home-made if possible, or labeled with all their ingredients, or purchased or eaten only if they are clearly labeled as milk-free. Buy no packaged foods without a complete list of ingredients, and if eating away from home, eat only foods surely free of CM. Furthermore, a D on a product label next to K or circled U indicates CM proteins [516].
Hidden CM may be found even in nondairy creamers, imitation processed cheese, imitation cream cheese, imitation sour cream, and soybean-based ice cream may contain casein [523]. See also Appendix 9.2.
Data from [246, 294, 389, 418, 516, 519, 523, 528].
[a] Lactose-free milk contains CM protein.

9.10 and 9.11) [94, 243, 378]. Sampson and his co-workers asserted that the vaccination is safe, by evaluations based on 95% confidence intervals that 97.5% of vaccinated children are free from adverse reactions [243]. Our calculations on 1,963 children show that systemic reactions occurred only in 0.1% of vaccinations and adverse reactions in 1.7% of cases (p=0.0001) [94]. Recently, the rates were lowered to 0.00035% based on the dos-

Table 9.35. Factors capable of influencing the success of dietary elimination in children with FA

1. Identification of all foods causing FA
2. Achievable elimination of these foods from the diet
3. Elimination of even minimal traces of those allergens in the prepared foods
4. Elimination of possible cross-reactions
5. Exactness and completeness of prepared food labeling
6. Marketing under names generating bewilderment
7. Family factors (compliance, working mother, available funds, etc.)
8. Association with other factors (GI disorders, emotions, exercise, etc.)
9. Concomitant involvement of inhalant allergy
10. Parents' exhaustive information
11. Degree of child's compliance
12. Nature of child's symptoms
13. Variations without physician control
14. Feasible development of malnutrition

Ingestion of fresh food (or frozen at home) is suggested, although industrial deep-freezing is done with more effective methods than household methods.
Modified from [19].

es shipped and 0.00119% on the children studied [189]. No child followed by our division has suffered from immediate reactions to MMR (measles, mumps, and rubella) vaccine and the usual late onset reactions were recorded in sporadic cases [94]. Over 15 years, 18 cases of suspected anaphylaxis were reported in Finland; however, in 10/18 cases the physician treating the vaccinee suspected the reaction to be fainting instead of actual anaphylaxis, so only three of these received epinephrine; therefore the final number of cases was 11, with a 0.0000002% incidence/year [378]. It is therefore likely that the reactions observed by several authors were caused by additional vaccine constituents such as gelatin [189, 266], a component of plasma expanders, which provokes reactions in 0.07%–0.2% of cases and the 0.025 mg of neomycin contained in the MMR vaccine [94]. However, the extremely large amount of gelatin (1.5 to 14.5 mg per shot) [378] in the vaccine is the cause of the reactions, as was definitively proved that 93% of children with anaphylaxis had IgE antibodies against gelatin, and children without adverse reactions all had negative test results for IgE antibodies against gelatin [265]. In 47 children (Appendix 9.11) one case of anaphylactic shock caused by gelatin occurred [265], whereas cutaneous reactions related to neomycin are more frequent, thus explaining the elevated prevalence of urticarious manifestations in vaccinated children: the risk of adverse reactions in children not allergic to egg

Table 9.36. CM-free and egg-free diet

Breakfast
Tea (loose), or SPF, unrefined sugar, CM- and egg-free cookies
Snacks
Banana, bread, raw ham, CM- and egg-free cookies
Dinner and supper
Pasta (normal type)[a], rice dressed with olive oil; try tomato apart
Vegetables[b]: carrot, lettuce, beet, zucchini, green beans, potato in broth or soup (homemade), or dressed with olive oil, or baked, roasted, fried
Meat[b]: rabbit, lamb, horse, pork (lean parts) (boiled, or roasted, or fried)
Fruit[b]: peeled pear, banana, pineapple; try in different weeks orange, peach, apricot
Tap water

Diet in use in the Division of Pediatric Allergy and Immunology of Rome University La Sapienza.
Since egg is necessary as emulsifying in confectionery, an egg substitute may be combined as follows: whole wheat flour 2 tbsp, baking powder 1/2 tsp, oil 1/2 tsp, homemade fruit juice or tap water 2 tbsp.
Data from [184, 523].
[a] Without added powdered CM.
[b] Fresh or frozen only.

Table 9.37. Additional recipes to substitute an egg

Ingredients	Amount
Baking powder	1 tsp
Water	1 tbsp
Vinegar	1 tbsp
Or	
Yeast	1 tsp
to be dissolved in warm water	1/4 cup
Or	
Water	1/2 tbsp
Oil	1 1/2 tbsp
Baking powder	1 tsp
Or	
Gelatin	1 packet
Warm water	2 tbsp

For each recipe, do not mix until ready for use.
A mashed banana may be a substitute for egg in recipes: 2 tbsp for each egg replaced.
Data from [184, 523].

is entirely insignificant: 0.00005% [94]. With modern techniques, it should not be difficult to produce vaccines free of gelatin and neomycin. An egg protein-free vaccine is presently available [378]. Recently two publications [259, 287] have created an *unprecedented confusion and alarmism on MMR vaccination.* Giving MMR vaccinations to all children under controlled conditions in hospital [287] has been suggested, or restricting these precautions to children with cardiorespiratory responses to egg and/or chronic asthma [259]. We stress that cardiorespiratory reactions took place exclusively following SPTs and/or intradermal tests (IDTs) with diluted vaccine [94]. However, extending these recommendations to all children subjected to MMR vaccine appears unmanageable, as it would eliminate all programs in which vaccination is carried out by nurses without doctors in attendance, as occurs in most Third World countries and in almost all public health clinics in industrialized countries. The extreme rarity of severe allergic reactions induced by MMR vaccination supports the view of vaccinating egg-allergic children according to normal vaccination schedules, if necessary with the egg protein-free vaccine [378].

Cereals

A *wheat-free diet* is fraught with difficulties, bread and pasta are widespread foods that children enjoy *throughout the world.* Wheat and cornstarch are found in tisanes, toothpastes, antibiotic products, vitamins, etc. [389]. In specialized shops, biscuits, noodles, bread and other products are found, prepared with rice, soy, and buckwheat flour and starch and leaven for allergic children (all generally reliable) as well as the necessary ingredients for homemade bread and pasta (see Tables 9.38 and 9.39 for suggestions to make bread). As substitutes, tapioca, sweet potatoes, cassava and sago can be employed [531]. Appendix 9.10 lists the hidden wheat sources. *Products for celiac disease* declared as wheat-free may often be contaminated by wheat, even if in very small amounts, as evidenced by immunochemistry and DNA analysis by PCR (polymerase chain reaction) [3], and therefore are not recommended for wheat-allergic children.

Corn allows the preparation of bread and polenta, but is not tolerated by 59% of wheat-allergic children, and thus can be introduced into the diet only after a FCT [379]. Many waxed paper cartons for foods are dusted with cornstarch; therefore all products contained in such cartons, including CM, should be avoided. Corn is used in the preparation of a great deal of processed foods, which may not indicate the term corn (a summary of hidden sources is listed in Appendix 9.10). Instead of cornstarch, tapioca starch and potato starch may be used; check the labels of seed oils to check that no corn is included among the ingredients [379].

Table 9.38. Recipe for bread using wheat-free flour for wheat-allergic children

Ingredients	Amount
Cornflour	1 kg
Olive oil	2 tablespoons
Boiled potatoes (medium size)	3
Lukewarm water	About 200 ml
Salt	1/2 teaspoon
Baking powder	1 tablespoons
Work ingredients together, mixing the baking power with lukewarm water. Let stand for at least 5 h, in a warm place, or all night. Wrap rolls in wax paper and then bake at 220 °C lowering to moderate T after 20 min. Bread will keep for 1 week (or more) in the refrigerator.	

Not indicated for cornallergic individuals.
Diet used in the Division of Pediatric Allergy and Immunology of Rome University La Sapienza.

Table 9.39. Additional recipe with same characteristics

Ingredients	Amount
Rice flour	250 g
Olive oil	2 tablespoons
Sugar	2 tablespoons
Lukewarm water	About 200 ml
Salt	1/2 coffee-spoon
Baking powder	2 teaspoons
Mashed banana or pear	1
Combine all ingredients dry, mixing previously the baking power with lukewarm water. Add oil, banana or pear and lastly water, mixing accurately. Bake the rolls at 220 °C for 15 min, then for additional 45 min at 180 °C. Bread will keep for a week (or more) in the refrigerator	
Wheat substitutions [524]	
Substitutions for 1 cup of wheat flour for wheat-allergic children	
1/2 cup rye flour + 1/2 cup potato flour	
1/2 cup cornstarch + 1/2 cup potato flour	
1/2 cup cornstarch + 1/2 cup rye flour	
1/2 cup cornstarch + 1/2 cup rye flour	
2/3 cup rice + 1/3 cup rye flour	
Substitutions for 1 tablespoon of wheat flour for wheat-allergic children	
1/2 tablespoon cornstarch	
1/2 tablespoon potato starch	
1/2 tablespoon rice flour	
1/2 tablespoon arrowroot	
1/2 tablespoon lima bean flour	

A positive aspect is the reduction in *rice* allergenic activity by genetic engineering that has decreased the allergenic levels of rice grains from 312 to 60 µg/grain, thus creating the basis for hypoallergenic rice. A new variety has also been obtained by hydrolysis of grain globulin fraction, the major allergen; so children who suffer from this allergy may eat rice [511]. The final result was obtained by sequencing the rice genome, thus opening the way to elude rice allergy [525]. We have used rice since 1982 [403] in oligoantigenic diets for allergic children and have never recorded cases of allergy: if the use of rice is widespread as in oriental countries, there should be a high index of sensitization. Similarly to corn, 77% of wheat-allergic children may become allergic to rice [379].

From *barley* is derived malt (often from sorghum), which is employed to flavor some types of bread or breakfast cereals and is also found in powdered drinks to be mixed with CM. Moreover, malt by-products are found in flour for cookies to increase the content of fibers and proteins without altering the taste [531].

Rye is often the first ingredient in commercial cereals; rye flour is frequently utilized in foods for breakfast and if minced, as a component of cosmetics, creams and bath soaps [531].

Several cereals have been transformed by genetic engineering [81] (Table 1.78, 1.79), but we do not know the practical applications.

Fish

Fish often induce IgE-mediated acute manifestations, but also nonimmunological reactions by aspecific mast cell degranulation, not to be confounded with reactions to additives in processed foods. Children with cod allergy (associated with FA in up to 91% of cases) are frequently sIgE positive to several other species, above all caused by notable cross-reactions between Gad c 1 and allergens of these fish [128]. Even if, as previously alluded to, children with cod allergy can eat other types of fish [32], it is a good idea to verify this preventively [289], subjecting children in a specialized center to FCT with the fish to be potentially included in the diet. The fish should be acquired in a well-known shop and never consumed at a restaurant, because of possible contamination with other foods, spices, and additives during the preparation. The consequences of a diet not based on challenge procedures or of involuntary contacts with an often hidden allergen can be potentially harmful, also exposing asymptomatic children to cutaneous, GI, respiratory symptoms and anaphylaxis [289]. If the investigations are not easily feasible, we agree with the colleagues who believe it is prudent to refrain from eating fish, or limiting it as much as possible. Moreover, also for corn-allergic children, oral contact with the glue of some stamps, envelopes, and stickers should be discouraged since the glue may be made up of discards from the preserved fish industry.

Shellfish

A previous anaphylaxis elicited by one species requires an absolute exclusion of every type of shellfish; it is convenient that the block is total when the allergy is IgE-mediated. More common incidents occur with shrimp, *masked allergens* found in cold restaurant, cafeteria, and fast food dishes (Appendix 9.12), often associated with OAS.

Legumes

Allergy to peanuts, soy, and other legumes may be found. Peanuts are sensitizing both fresh and baked, similarly to peanut flour, butter and oil. Numerous are the *hidden sources* of peanuts (Appendix 9.12). Sensitization may be induced by vegetable gums and various flavoring products, but it is unnecessary to exclude several species of legumes from the diet [31]. Peanut allergy may persist over the long term [46]. Since the number of cases is on the rise everywhere, the potential occurrence of incidents from involuntary intake have led us to suggest that these children always be provided with self-injectable epinephrine in case of anaphylactic shock, and if there have been acute reactions in their history, wearing a medical bracelet alert is suggested. The youngest children should be provided with a cellular phone.

In cases of soy allergy, attention should be paid to hidden sources, particularly including prepared foods cooked, baked or fried with soy flour and/or oil, as well as soy lecithin (see the section on chocolate and Appendix 9.12), as with *cocoa* [92]. Lecithin is found in legumes, cereals, nuts and peanuts.

Allergy to Other Foods

In the *potato* family, we found tomatoes rich in histamine positive in 19/80 cases (24%) confirmed by FCT vs only 3/26 of potato (12%), sensitizing only if raw [505]. If cooked potatoes are sometimes not tolerated, this denotes that they were insufficiently cooked, but also cooked potatoes may induce anaphylaxis [99].

The *citrus* family includes oranges (Table 9.12), which usually provokes a PA due to the acids present in these fruits.

Nuts are of numerous types and families (Table 9.12). Cashew was involved in a fatal case, and walnut and Brazil nuts in nonfatal cases [424]. Brazil nuts often provoke reactions by consuming candies, cakes [528] and other foods containing these nuts in a masked form [424] as well as walnuts. These nuts are added to candies and cakes as embellishment, or minced to insert

Table 9.40. CM-, egg- and wheat-free diet (for children with multiple sensitizations not undergoing desensitization)

Breakfast and snacks
Tea (loose) with unrefined sugar – SPF as milk or HMMBF
Rice biscuits or homemade cookies with SPF
Whipped pear/pineapple (homemade) with unrefined sugar
Pear/pineapple jam (homemade) with unrefined sugar
Dinner and supper
Rice, soy, or corn noodles dressed with olive oil
Vegetables (fresh or frozen only): carrot, lettuce, beet, zucchini, green beans dressed with olive oil
Potato in broth or soup (homemade) or dressed with olive oil, or baked, roasted, fried
Meat (fresh or frozen only): rabbit, lamb, horse, pork (lean parts) (boiled, or roasted, or fried)
Fruit (fresh or frozen only): peeled pear, pineapple
Bread made with buckwheat flour, or potato flour or rice flour and cornstarch
Tap water

Add Ca: see Tables 9.23 and 9.24.
Diet used in the Division of Pediatric Allergy and Immunology of Rome University La Sapienza.
Additional data from [184, 523].

Table 9.41. Recommended schedule for monitoring food hypersensitivity in children with FA, not undergoing oral desensitization

Food allergy schedule	Recommended rechallenge
Allergy to CM, egg, wheat	Rechallenge after 12–18 months of food avoidance: if still positive, rechallenge every 2–3 years
Allergy to soy	Rechallenge after 1 year: if still positive, rechallenge every 2 years
Allergy to fish, peanuts, nuts	Rechallenge after 3 years: if still positive, rechallenge every 2–3 years
Other allergies	Rechallenge every 12–18 months

Data from [422].

a tasty ingredient to candies and cakes in general [533]. Hidden sources of nuts and sesame are listed in Appendix 9.12.

Allergics to *hazelnuts,* both raw and toasted (and to rye), are also sensitized to kiwi and poppy and/or sesame seeds [502]. Hazelnuts are widely utilized in nougat dough, an ingredient in candies, ices and pastry [533].

The *mustard* and *parsley* families include celery, anise, cumin and other herbs and spices. Celery cross-reacts with other spices and herbs (see OAS) and cumin is added as a digestive.

Sunflower is found not only in honey, but also in oils, flour (bread, confectionery, etc.), and margarine. As with almonds, the minced seeds are employed as an alternative to peanuts [516].

As discussed earlier, several fruits may induce severe reactions in children. In the case that these fruits are welcome to children, also to provide a satisfactory variation to the diet, the next step is a controlled introduction or personalization.

Appendix 9.12 [25, 27, 207, 276, 301, 390, 478, 516, 523] lists the potential hidden sources of wheat, peanuts, corn, nuts, soy, sesame, coconuts/dates, anise, beef and shellfish.

Multiple Food Sensitizations

Multiple food sensitizations should be suspected when tangible improvements do not follow in adequate time an elimination diet strictly free of CM, egg and wheat. In multiply sensitized children, all the offending foods should be removed from the diet, even if for a limited time. Table 9.40 [184, 523] gives an example of a diet for children intolerant to CM, egg and wheat, which often suffers from poor compliance. In the preparation of meals for sensitized children, use only fresh foods, not processed, or homemade utilizing only unrefined sugar or beet sugar [379]. However, true multiple sensitizations are a rare event, and uselessly subjecting a child to rigid diets eliminating a plethora of foods, often not involved in the pathogenesis, should be avoided [82].

Diet Duration

There are no standardized criteria for the perfect duration of an elimination diet. We have demonstrated that it is unfeasible to pinpoint when the tolerance is acquired. See Table 9.41 [422], for the appropriate moment to do a FCT with the sensitizing food, to verify the potential start of tolerance. CM, egg, fish, shellfish and peanut allergy tend to be the more persistent in FA. To evaluate whether the differentiated reintroduction of the eliminated foods is timely or not, in case of positivity the FCT may be repeated at pre-established terms based on Table 9.41. This scheme is purely indicative, in

that pediatricians caring for these children may decide whether to repeat a FCT after 6 or 12 months, depending on symptom severity. Preliminary data suggest that children with FA symptoms and lower levels of sIgE antibody are more likely to outgrow their reactivity in a few years, so monitoring sIgE levels by CAP System FEIA (Chap. 6) every 1 to 2 years may by helpful in establishing when to rechallenge children [419].

Evaluation of Children on Therapeutic Elimination Diets

Children on prolonged diets should be observed as follows:

- Compliance is obvious to specialists, but the psychological consequences of a restricted diet become more severe the older the children are, while infants up to 1 year of age may accept the monotonous diets without problems. Although initiated despite our suggestions, older children may be deprived of more pleasant foods permitted to their siblings and friends, with the problem becoming aggravated by the unpalatable taste of some popular dietetic alternatives [19].
- Ongoing control of nutritional adequacy of the diet and of the single constituents is important. An inappropriate diet (and even more punitive) may induce diverse disturbances compared with baseline data (Table 9.22). In all cases attention should be paid to the following risks:
  - *Nutritional risks* dependent on a poor caloric supply or of specific nutrients. Based on the data in Table 9.16, pediatricians can check whether the diet prescribed corresponds entirely to ESPGAN prerequisites [151].
  - *Hidden CM sources* and derivatives and/*or of other foods* when country regulations do not require that such foods be mentioned on products for children.
  - *Introduction of sensitizing foods,* both involuntary and voluntary by persons not knowing the effect even of minimal amounts of sensitizing food (see Hamburger et al and Hattevig et al on the problems related to the application of diets, Chap. 24), which may be *cause of anaphylactic shock.*
  - *Small doses* may transform into a cause of immediate hypersensitivity to another allergy that before was asymptomatic [247].
  - Maximal attention should be paid to necessarily integrating Ca and other minerals into exclusion diets.
- Monitor the *hidden sources of penicillin* (Chap. 19);
- Periodically record on growth charts the body weight and height of children based on national standards (calculate the percentiles: Appendices 6.1–6.4), and the parameters of possible signs of malnutrition (Appendix 9.13) [516] (Chap. 21).

Recent data have led us to re-thinking the issue. During an elimination diet without previous problems after CM intake (the last accidental exposure to CM occured *more than a year before the challenge was performed*), 8/11 children developed severe acute allergic reactions to CM after accidental ingestion. During DBPCFCs all 11 children experienced acute allergic reactions to CM. Therefore, there is a considerable chance of developing acute allergic reactions to CM during a CM-free diet in children with AD + FA [164].

Prevention of FA

Even if prevention is discussed in Chap. 24, we comment on issues noted herein and underline the attention required by the hidden sources of CM and dairy products and/or constituents (Table 9.35, Appendix 9.2), and 11 other foods, as shown by Appendix 9.12. In the US, children with CMA had acute and even severe reactions after eating *foods containing unspecified sodium caseinate in products not based on CM*; such traces of CM proteins were not listed on the labels of canned tuna, bologna or hot dog, because according to US regulations sodium caseinate is a natural flavoring [175]. It is therefore necessary to *read product labels carefully* to unmask the hidden CM (Table 9.34). The words on the label depend only on the producers' fairness and not on legal requirements [276]. It is indispensable to remember that:

- Whey proteins or casein (also as caseinate) are important allergenic proteins of CM. Ca caseinate was added to the total parenteral nutrition of the 41 infants with chronic intractable diarrhea [66] (Table 9.6) until they were put on Rezza's diet.
- ALA may be hidden under the words "seasoning" or "flavoring."
- Casein is utilized as an emulsifying agent.
- Whey proteins are added to other proteins as they are considered as fortifying, because these proteins are rich in essential amino acids.

To live with children with FA may therefore be emotionally and materially demanding. The younger ones are not always or almost never able to understand the food restrictions. If they have older siblings, these might be the first to be sensitive to such problems. Practical suggestions include the following:

- Elimination from the home of the incriminated food as such and in all preparations containing it.
- If this elimination is not feasible in practice, warning labels can mark the allergenic foods.
- Pay attention to teasing by older siblings or friends motivated by jealousy.
- Warn all educators in contact with children of this allergy.
- At the restaurant, parents should request precise information on the dish's ingredients,
- Older children should be informed of certain terminology used in food labeling before consuming such foods, but the appropriate sources of this information and the appropriate medical personnel to teach this is currently unknown.

Medical Treatment

In a strict sense, a medical treatment for FA does not exist, also because the dietetic treatment has no substitute. The situation of children with multiple food sensitizations, or in whom compliance with the diet is a major problem, may be ameliorated by disodium chromoglycate (cromolyn), with doses not >40 mg/kg/day, qid 15–20 min before meals (Table 7.19), thus preventing or reducing the symptom flare-ups provoked by sensitizing foods [65]. Cromolyn was administered to 13 children at the dose of 30 mg/kg/day over 7 days: the reactions to foods were efficaciously prevented in six of eight children with CMA and in four of five children allergic to egg [65]. In another double-blind crossover study in 31 children with AD associated with FA, the results were wholly in favor of cromolyn, which was significantly better than placebo in preventing symptom relapses during FCTs [67]. All children had SPT, RAST and elimination-provocation tests positive to sensitizing foods [56, 67, 70]. Otherwise [54] the results were not similar to ours due either to lower doses, or to the short duration of treatment. Among the anti-histamines we use are cetirizine and ketotifen (Table 7.19). The positive effects of *cetirizine* can be of help if the child is invited to a dinner or a party: a preventive prescription will prevent the risk of showing significant allergic reactions in such circumstances. Cetirizine in drops favors the compliance of young children. Allergen avoidance results in some relief; however, we see a dramatic decrease in symptoms after cetirizine is introduced, probably linked to its anti-inflammatory properties [507]. See Table 7.19 also for levotetirizine tablets. *Ketotifen* protects the gastric mucosa after FCT with the responsible allergen [148] and reduces intestinal hyperpermeability and clinical symptoms after FCT with CM and egg (Fig. 9.21) [341]. Administering ketotifen before FCT and measuring the urinary excretion of lactulose/mannitol, the level decreased significantly (Fig. 9.21). *Loratadine* has been demonstrated to reduce significantly the intestinal permeability in children with AD associated with FA [342].

New Therapeutic Perspectives

The most promising aspect is based on oral desensitization in FA, consisting in first diluted, then undiluted foods in progressively increasing doses, which is usually accomplished in 4 months. During treatment, sIgE were significantly decreased, and specific IgA and IgG increased, as well as IL_4 and IFN-γ levels [360]. In a study on 32 children, the success rate was 84.8%: oral desensitization was successful for CM, whole egg, egg-yolk, and fish [375]. See also an oral rush desensitization for CM (Chap. 13). A group of 30 children with recent very severe episodes underwent an original oral desensitization protocol during a 10-day session in the ward. Of these, three children (10%) did not proceed with the protocol because of persistent respiratory symptoms. In a second group of 20 children with less severe allergy, one developed a specific food aversion and two failed to proceed with the protocol (15%). In sum, 87.5% of the children [375] completed this treatment, obviously ensuring a better quality of life for both children and adolescents. An original study on experimental animals, which cannot yet be extrapolated to infantile therapy [171], has demonstrated an effective early hyposensitization to CM by a single intravenous (IV) injection of an IgG-βLG conjugate, abolishing IgE, IgA, IgG immune responses to the protein. The immunosuppression was persistent also after 1 month, and was accompanied by a block of mast cell release. The conjugate thus acted as a tolerogen and the scientific assumption connected with antigen presentation updates the well-known formation of such tolerogen conjugates with IgG, as demonstrated by the hapten mechanism: nucleosides or benzylpenicilloyl bound to IgG prevent in rats IgE formation [171]. We will evaluate the possible practical applications. We recommend tacrolimus and pimecrolimus for children with FA, as reported in Chap. 7.

Acquisition of Oral Tolerance

As pointed out above, standardized and age-related criteria on food tolerance are lacking. It is generally affirmed that tolerance is acquired by 2–3 years of age; however, often IgE-mediated FA tends to persist up to 8–10 years and in some cases up to 14 years. Several data have been reported on the onset age of tolerance to CM or other foods related to 1,286 children (Table 9.42) [26, 40, 66, 83, 92, 93, 98, 106, 122, 212, 213, 224, 227, 230, 234, 241, 281, 439, 454]. Even if 43%–56% of children acquire the FA tolerance within 1–4 years, 53% wait for it up to 5–14 years, and 48.5% and 36.8% develop respiratory allergy and 31% multiple food sensitizations. Although 56% of children acquire tolerance at 3 years of age, many children aged >3 years could benefit from oral desensitization [380]. Even if 16.6% reached tolerance to a limited amount of milk (3.5–45 ml), these children have obtained the primary target: complete freedom from life-threatening reactions caused by the accidental ingestion of offending foods, almost always hidden, or by skin contact [470]. Additional results:

- Tolerance is acquired by children depending on whether they react in an immediate (22%) or delayed manner (59%) [212].
- In 37/41 children with chronic diarrhea with early onset associated with CMA whom we followed up over 4–10 years (Table 9.6), tolerance was achieved in 25/37 cases (67.6%) at a median age of 2 years; 12/37 children (32.4%), at a median age of 6–7 years, were still intolerant to CM; 27/37 children (73%) developed more food sensitizations during the follow-up. Despite a long-last-

Table 9.42. Prospective studies in children on the acquisition of tolerance to cow's milk and later development of inhalant allergy

Age		Tolerance (%)					Allergy (%) to inhalants	Intolerance (%) to other foods
		1 Year	2 Years	3 Years	4 Years	Later		
Authors and reference	**No. of children**							
Unselected studies								
Jakobsson [230]	20	45	60					
Høst et al [224]	39	56	77	87			28[a]	54[a]
Høst et al [227]	39					86 [10 years)	62 A, 52 AR	18[a]
							17 A, 6 AR	0[b]
Selected studies								
Clein [106]	206	80	85	95			80	
Kuitunen [281]	54	100 ([c])						
Businco et al [66]	41		68			57 (>6 years)	73	
Bishop et al [40]	97		28		56	78 (6 years) ([d])	40 A, 43 AR	75
Cantani et al [83]	88					28 (9 years)	39	([e])
Cantani et al [93]	21					23 (14–21 years)	38	
Bardare [26]	92					69 (5 years) [f]	58	25–100
Isolauri et al [234]	37		73					33
James [241]	29			38		45 A, 64 AR [g]		
Schrander et al [438]	88	15	22	51	67[h]			42
Hill et al [213]	42		22 [i]		33			
			59 [i]		31		69	67 (egg) 55 (peanut)
Sprikkelman [454]	93					37 (7 years)	31 A, 19 AR	
Carroccio et al [98][k]	21				52			
	70				78			
De Boissieu [123]	52	15	42	15	27			
Cantani et al [92]	115					57 (14 years)	50	(m)[m]
Saarinen et al [408]	106	61			67 (5 years)		31 A, 66 AR	73 (egg) 26 cereals)
Mean		53%	53.6%	57%	51.4%	53%	47.2% A, 41.6% AR	43%

Table 9.43. Sensitizing foods in 88 children followed up over 8 years

Foods	No. of cases	(%)
Cow's milk	15	17
Cow's milk and egg	25	28
Cow's milk, egg and wheat	4	4.5
Egg	15	17
Fish	5	5.6
Multiple sensitizations (foods and inhalants)	24	27

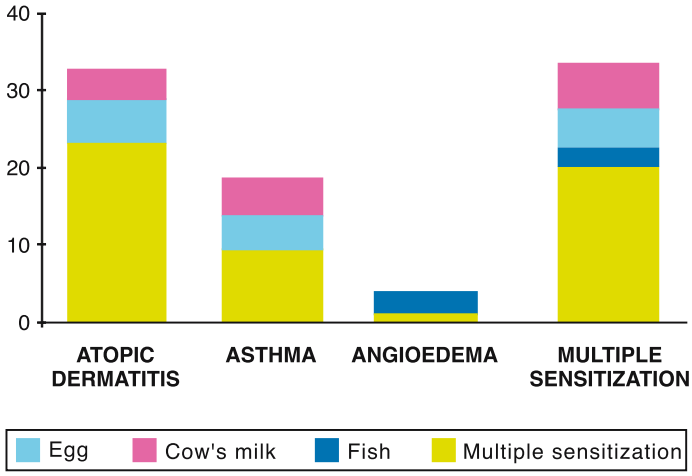
Data from [83].

Table 9.44. Correlation between sensitizing food and development of additional food allergies in 88 children followed up over 8 years

Foods	Development of additional allergies	
	No. of cases	(%)
Cow's milk	10/15	67
Egg	4/15	26
Fish	–	–
Multiple food sensitizations	20/53	37

Fisher = 0.0328.
Data from [83].

Fig. 9.27. Correlations between sensitizing foods and clinical features in 88 children. (Data from [83])



Footnote Table 9.42.

When not specified, the figures refer to asthma. The studies are ordered according to the year of publication.
A asthma, *AD* atopic dermatitis, *AR* allergic rhinitis

[a] In children with IgE-mediated CMA.
[b] In children with food intolerance.
[c] 9% of children recovered from partial/subtotal villous atrophy by 4.5–5 years.
[d] At the final follow-up (after 5 years), 21% of children suffered from AD.
[e] 67% of CM-allergic, 25 of egg-allergic, and 37% of children with multiple sensitizations.
[f] Only 11% of atopic children.
[g] 80% of children were still affected by AD.
[h] At the 4-year follow-up, the remission was based on the initial IgE level (<or >10 kU/l): the first in 90% of cases, the second in 47% of cases
[i] The first group of children had immediate reactions, the second group delayed reactions (l). The second group had delayed reactions at the age of 27.6±7.1 months.
[k] In the study by Carroccio et al, the first line shows the children intolerant to casein HF, and the second line the children tolerant to the same formula.
[m] 55% of CM-allergic, 31% of egg-allergic, 12% of wheat-allergic and 35% of children with multiple sensitizations 90% of cases in the CN-allergic 47% of cases in the egg-allergic.

ing elimination diet, CM intake was followed by immediate symptoms [66].

- In a prospective study on 88 children with FA and 54.5% of them with FHA+, followed up for 8 years (diagnosis based on positive SPTs and/or RAST and OFCs to the offending foods), the results were not very optimistic (Table 9.43) [83]. The more frequently responsible foods besides CM and egg were wheat and fish, some of which seem to induce more persistent and more severe symptoms, such as fish *(angioedema)* and nuts (Fig. 9.27) [83]. At a median age of 9 years, only 25 children (28%) tolerated these foods (p=0.003). As in other studies, 33 tolerant children (38%) developed additional sensitizations (p=0.035): 10/15 children with CMA (67%) and 4/15 of those with egg allergy (27%) developed asthma (Fisher=0.0326). The children with CMA achieved tolerance significantly later than children with other allergies, while remission implied a greater tendency to develop more sensitizations (Fig. 9.28) [83], with a remarkable incidence of multiple sensitizations (Table 9.44) [83]. The persistent intolerance to offending food was significantly manifested with symptoms such as angioedema and AD separately or variously associated (Fig. 9.29) [83].

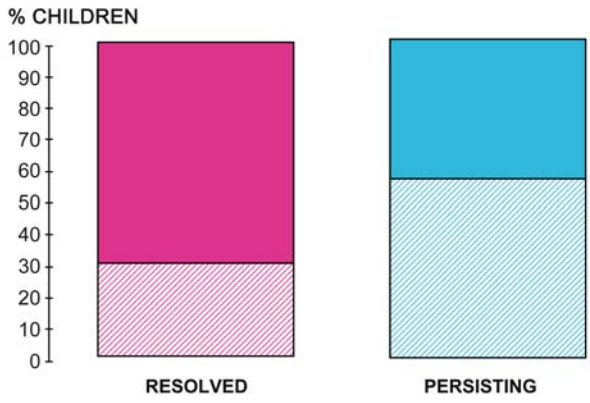


Fig. 9.28. Correlation between tolerance and development of additional allergies in 88 children during follow-up. Fisher, 0.0319. (Data from [83])

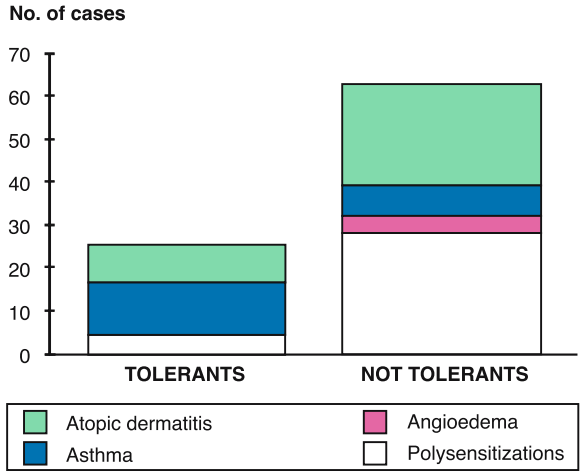


Fig. 9.29. Correlation between clinical features and tolerance in 88 children; p=0.0026. (Data from [83])

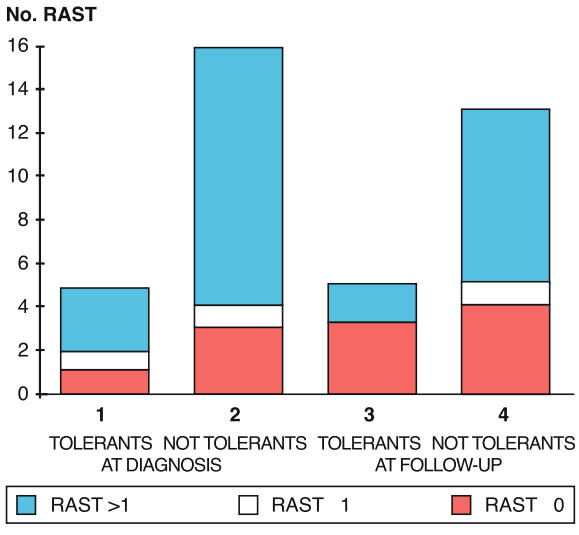


Fig. 9.30. RAST classes to sensitizing foods in 21 children with breast milk allergy at diagnosis (median age, 3 months) and at last follow-up (median age, 13 years), according to tolerance acquisition. *1, 2* NS, *1–3* p<0.0001. (Data from [93])

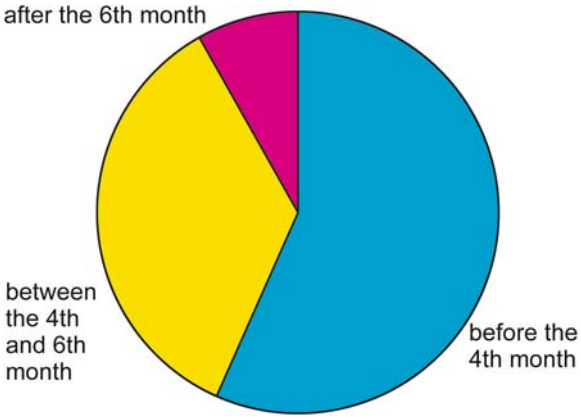


Fig. 9.31. Age at onset of atopic dermatitis in 115 children prospectively followed-up for 14 years; p=0.0001. (Data from [92])

- In 21 exclusively breast-fed children [93] with IgE-mediated FA (follow-up, 10–13 years), 8 of 21 children (38%) developed asthma during the follow-up. Interesting differences between tolerant and intolerant children were shown by RAST classes vs sensitizing foods, particularly indicative for the development of tolerance: at the last check-up, RAST turned negative in 3/5 tolerant children, and in 4/13 intolerant children (p<0.0001) (Fig. 9.30) [93].
- In the 41 children with chronic diarrhea, 2/3 with negative RAST tolerated CM within the 2nd year of life [66], so RAST negativity may be useful for the prognosis of FA;

In 115 children prospectively followed up over 14 years, first examined at a median age of 6 months, the median age for tolerance to CM was 7 years 11 months, to egg 6 years 6 months, and to wheat 7 years 2 months. Only 66 children (57%) acquired food tolerance, but there was the *onset of asthma in 54% of cases*. The median age at *AD onset was very early*, before 4 months of life in 66 babies (57%) (Fig. 9.31), and a large number of both tolerant and intolerant children developed asthma and multiple sensitizations (Fig. 9.32 showing the age-related increase of multiple sensitizations). Table 9.45 [92] shows the correlation between sensitizing food and development of additional food allergies. The study of this correlation (Fig. 9.33) revealed that 58/106 (54.7%) children with CMA developed several sensitizations during the follow-up. Among the improved children, 53 (80%) were <6 months old when AD first appeared, unlike 30 children (61%) with an onset of AD after 6 months (Fig. 9.34) [92]. Table 9.46 [92] shows that a large number of children developed several sensitizations during follow-up. We investigated several factors as predictive of the outcome, including early onset, widespread or untypical (reverse pattern) skin lesions, family history positive for atopy, persistent FA, high levels of total and specific IgE antibodies, association with

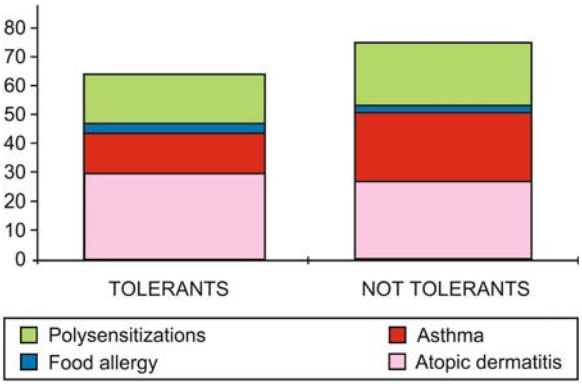


Fig. 9.32. Correlation between clinical features and tolerance achievement in 115 children prospectively followed up for 14 years; p=0.0001. (Data from [92])

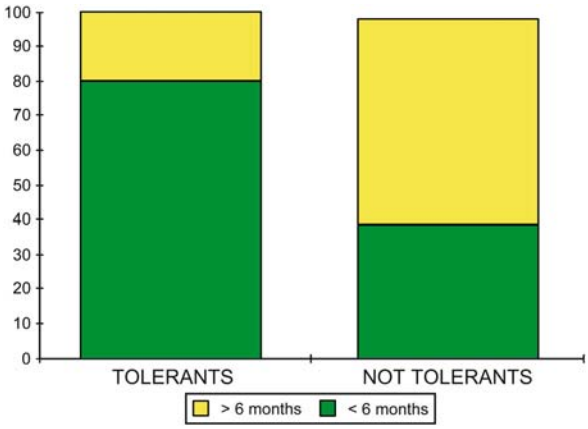


Fig. 9.34. Significant relationship between the age of onset of AD and the outcome of skin lesions in 115 children prospectively followed up for 14 years; (p=0.0001). (Data from [92])

Table 9.45. Correlation between sensitizing food and development of additional food allergies in 115 children prospectively followed over 14 years

Foods	Development of other allergies	
	No. of cases	(%)
CM	58/106	55
Egg	11/35	31
Wheat	1/8	12
Multiple sensitizations	16/43	35

Data from [92].
p = 0.0047.

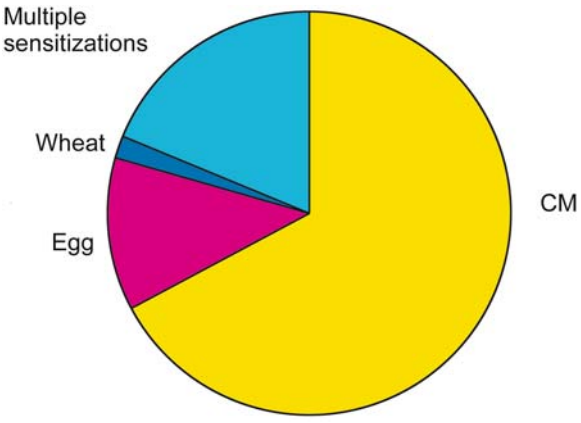


Fig. 9.33. Correlation between sensitizing foods and development of other allergic disease in 115 children prospectively followed-up for 14 years. *CM* cow's milk; p=0.0047. (Data from [92])

Table 9.46. Atopic dermatitis and outcome in 115 children (followed over 14 years)

Outcome	Atopic Dermatitis		
	Yes	No	Total
Cured	38 68.3%)	52 (96.3%)	90
Improved	18 (30 %)	2 (3.7%)	20
Unchanged	4 (6.7%)	–	4

Data from [92].
p=0.0001.

CMA and asthma. All these parameters were significantly predictive of a *long-term morbidity of AD children with CMA* (Table 9.47) [92].

- In 54 children whom we prospectively followed (Fig. 9.35), CM and egg sensitization dominated in the first few months, but there was also an early onset of multisensitization, and after the 6th month onset of AD unrelated to FA. During the follow-up (Fig. 9.36) [69], a significant relationship was found between the outcome of AD and the development of food tolerance: 90% of children with healing achieved a complete or partial tolerance to the offending foods, while 60% with persistent AD did not lose the food hypersensitivity and multiple sensitizations were prevalent.
- In a prospective study on CMA, 5 of 21 children (24%) with IgE-mediated allergy were still intolerant at 3 years (Fig. 9.37) [224] vs none of non-IgE-sensitized children. Adverse reactions to other foods were significantly present in 13 of 21 (62%) allergic children vs 8 of 18 of non-IgE-sensitized children (44.4%), and these were still intolerant at age 3: 8 of 21 (38%) vs 1 of 18 (5.5%), with equal differences in the age-related development of inhalant allergy (Fig. 9.38) [224]. These are concrete differences between IgE-mediated and non-

Table 9.47. Parameters predicting an unfavorable outcome in 115 children prospectively followed up over 14 years

Unfavorable factors	Statistics
Family history of atopy, positive vs negative	$p = 0.0024$
Male sex	$p = 0.015$
SPT results, positive vs negative	$p = 0.0001$
RAST results, positive vs negative	$p = 0.0001$
AD onset before the 4th month of life	$p = 0.0001$
First feeding of a CM formula before the 3rd month	$p = 0.0034$
Correlations	
Between symptoms and development of tolerance	$p = 0.0001$
Between tolerance and development of asthma	$p = 0.022$
Between tolerance and development of additional allergies	$p = 0.0047$
Age of onset of AD and outcome	$p = 0.0001$
RAST class at diagnosis and at last follow-up	
At diagnosis: tolerant subjects vs intolerant ones	$p = 0.0001$
Last follow-up: tolerant subjects vs intolerant ones	$p = 0.0001$

Data from [92].

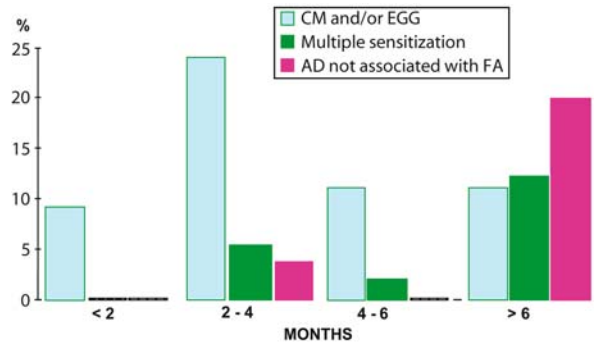
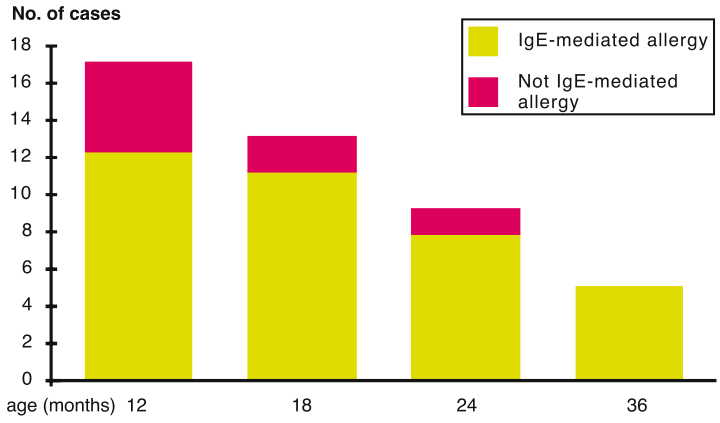


Fig. 9.35. AD onset age correlated to the causative agent in 54 children prospectively followed by us

Fig. 9.36. Outcome of AD and development of food tolerance. (Data from [92])

Fig. 9.37. Prospective study of CMA: age of onset of tolerance. (Data from [224])



IgE-mediated reactions. *In children with early sensitization to CM, the risk of a persistent allergy is high* (62%), as is the risk of developing *adverse reactions, also persistent, to other foods* (54%) such as egg (38%): high sIgE titers to CM and egg could be predictive of such allergies [224].

- Sampson and McCaskill [421] report that 16 of 40 children (40%) given an appropriate elimination diet and re-evaluated after 1 to 2 years showed food tolerance and improvement of their clinical course, but after 3 years no other child was tolerant.
- Sampson and Scanlon [422] extend to 20 years the negative aspects of natural history with 69% of intolerant children: after 3 years of diet *none of the 20 patients* was tolerant.

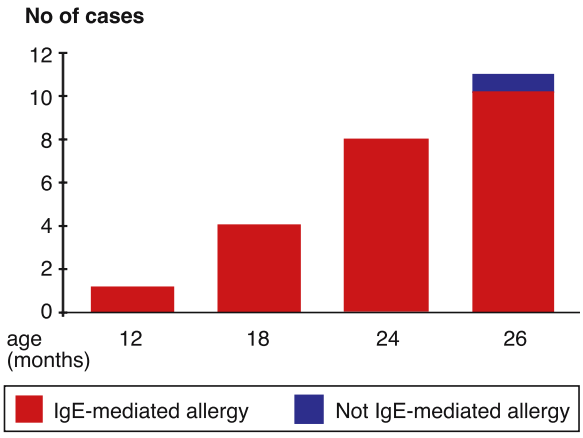


Fig. 9.38. Prospective study of CMA: age of onset of inhalant allergy. (Data from [224])

- James and Sampson [241] confirmed such data, in that only 11/29 children 3–14 years of age (38%) lost their clinical reactivity at the third annual DBPCCT, all affected with AD and FA and/or respiratory allergy; however, they demonstrated a significant decrease in SPT reactivity and had *lower serum IgE antibodies to CM proteins,* both at the initial and final evaluation.
- As seen in Tables 7.23 and 7.24, the tolerance to CM was achieved at the median age of 3 years and 6 months, while the tolerance to egg was achieved at the median age of 4 years and the tolerance to wheat at the median age of 4 years and 6 months. However, 28 children (50%) had respiratory allergy at the last follow-up, 21 children (37%) developed asthma, two (4%) rhinitis, and five children (9%) developed asthma and rhinitis, while 50% with persisting FA did not lose the food hypersensitivity [69].
- A recent study followed 118 children with CMA until recovery and repeatedly measured their sensitization to cow's milk (CM): by age 2.0 years, 60 (51%) children had recovered from CMA. Risk factors for persistent CMA at that age were sensitization to CM at age 1.6 years (OR 6.3), urticaria at diagnostic challenge (OR, 3.3), CM exposure at the maternity hospital (OR, 3.2), and early sensitization to egg (OR, 2.8). By age 5.0 all children with IgE-negative CMA were tolerant compared with 75 (64%) children with IgE-positive CMA. At the final follow-up at a mean age of 8.6 years children in the IgE-poitive group *still had CMA and more frequently had asthma* (31% vs 13%), *rhinoconjunctivitis* (66% vs 21%), *atopic AD* (81% vs 26%), and *sensitization to any allergen* (88% vs 39%) than control subjects ($p \leq 0.001–0.0001$) [408]. In the first 2 years of life 21.5% of 1314 children (MAS study) had early AD, 43.2% of them were in complete remission by age 3 years, 38.3% had an intermittent pattern of disease, and 18.7% had symptoms of AD every year. Severity (OR 5.86) and atopic sensitization (OR 2.76) were major determinants of prognosis. In 33% of these children the onset of wheezing preceded and in 56% followed the AD onset [225].
- Figure 9.39 [26] shows that in 92 children, 62 of whom had FHA+ (67.4%) with AD and allergy to foods and inhalants begun in the first years of life, *54 children (58%) developed asthma, including 25 (27%) with allergy to pollens, 22 (24%) to pets and 39 (43%) with urticaria.* Tolerance was reached to CM in 69% and to egg in 75% of cases vs *0% to fish and dried fruit. At 5 years of age, only 11% of atopic children with AD were tolerant vs 73% of nonatopic children* [26]. The *most dramatic complication is the development of asthma.* A study in 26 children aged 2–9 months at the first visit in our division and at the follow-up at 2–7 years of age showed that ten children tested positive for inhalant allergens (38.5%)

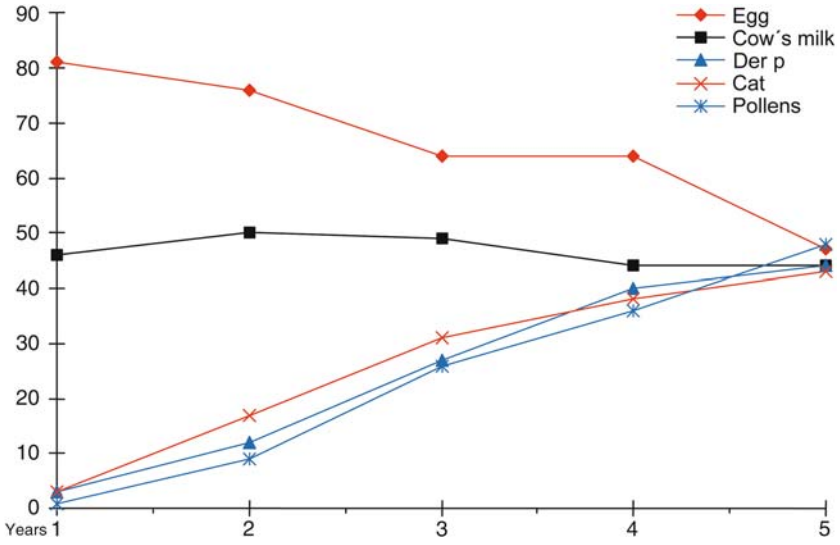


Fig. 9.39. Natural history of food and respiratory allergy in 92 children with atopic dermatitis and allergy to foods and aeroallergens started in the first years of life. (Data from [26])

and had respiratory allergy (asthma and/or perennial rhinitis). All children but two had FHA+ (p=0.0019). Therefore the natural history of IgE-mediated CMA is *less favorable than is usually believed,* especially in atopic children. A 38.5% incidence of asthma at *a median age of 3.1 years* may be explained by the high rate of FHA+ (92.3%).

Egg seems to determine a more persistent allergy:

- In 25 children with allergy verified by DBPCCT, symptoms persisted in 14, in some children from 6–12 years [481].
- Among the children with IgE-mediated CMA, 38% developed IgE-mediated allergy to egg (persistent in 6/8 up to age 3, associated with inhalant allergy), 29% to *citrus* and 19% to *tomato,* with persistent allergy to *citrus* (2/6), *tomato* (2/4) and *nuts* (2/2) [224].
- In 46 children with sIgE to egg within 2 years, 26 (57%) have developed sIgE to inhalants within 4 years, also 19/59 children with allergy to inhalants followed up 12–15 years had sIgE to egg [449].

Persistent sensitization is connected with a significant increase in IgA, IgG, IgM secreting cells after CM challenges [234], or in IgG and IgE antibodies [241], whereas healing is marked by no specific variation, apart from specific IgA antibody-secreting cells [234] and a significant reduction of sIgE to CM [241]. To consider as *favorable markers* the significant reduction of sIgE to CM and the increase in IgA-secreting cells against CM antigens associated with GI mucosal barrier maturation and loss of responsiveness to ingested food allergens, further studies are necessary to confirm such parameters. The maturational process may continue to elicit positive SPTs and sIgE [234, 422], therefore neither can be considered as predictive of tolerance. However, ongoing exposure to allergens may lead to intestinal injury and consequently increased permeability, in turn capable of eliciting more allergic symptoms [234]. Stimulated by CM, PBMCs from infants with CMA released in vitro more TNF-α than those in control infants [208], thus demonstrating that *even during remission CMA may not disappear completely* from the immunological memory of children.

A recent paper provides evidence to contend the prevalent dogma that CMA is a disease of infancy that the infant outgrows after the mucosal immune system matures [278]. Table 9.43 shows that 51.3% of children outgrow CMA between 5 and 8 years of age or that at 8 years have not outgrown CMA [83], yet 48.5% of children have developed asthma and 36.8% AR. In the follow-up of the 39 children [224] at age 10, 21 children with outgrown (86%) OFC-proven CMA developed asthma in 62% of cases and AR in 52% of cases and 18 children with outgrown (100%) CM intolerance developed asthma in 17% of cases and AR in 10% of cases [227]. In 276/1,759 sensitized children (sIgE of ≥ class 2) the rate of current asthma at age 10 was 53% and that of AD 29% [227]. Children with definite CMA had increased densities of IEL CD3, γδ T cells and a higher γδ/CD3 ratio, whereas children with suspected CMA showed a similar trend but without statistically significant differences [278]. However, FA onset has been reported at 10 years of age [270]. Thus, it is confirmed that CMA may exist in school-aged children [270, 278].

Therefore, FA persists for the entire duration of infancy and beyond. The unfavorable markers showing that FA may not be outgrown are as follows (Table 9.47):

- Family history of atopy [26, 83]
- Early onset of sensitization [26, 66, 90, 105, 111, 224]
- Immediate symptoms provoked by food challenge
- Association with persistent allergy to other foods or angioedema or AD, alone or variously associated [83]
- Multiple food sensitization [83]
- High sIgE levels to incriminated foods [64]
- Positive sIgE to egg with high predictive value [203, 449],
- Development of respiratory allergy to inhalants [26, 40, 90, 106, 224, 449]

FA-Caused Death

The cases of fatal anaphylaxis related to CM appeared in the first years of the last century [161, 436] and regained paramount interest after the publication of seven cases of fatal anaphylaxis, four of which occurred *in 11- to 19-year-olds over 16 months* [528], contributing to systematizing this aspect of FA, up to then confined to isolated cases that were difficult to verify. Certainly, the fatal cases by food-caused anaphylaxis are less numerous than those occurring in asthmatics, but are more frequent than those provoked by insect stings [527] and even less foreseeable. Frequently involved are peanuts, a food that is more often sensitizing in Anglo-American children [13, 48, 413], in contrast to what occurs in Europe, where sensitization to peanuts is uncommon: we have elucidated that sensitization takes forms as severe as captious and unexpected [48, 413]. Cod, crab and pecan were also implicated [528] and CM in 50% of cases (Chap. 20). Recently, the medical literature has been enriched by a new chapter: cases of severe anaphylaxis by HFs (Tables 9.31 and 9.32). Sampson et al [424] published six more cases of fatal and seven of nonfatal anaphylaxis in 2- to 17-year-olds, resulting from eating foods prepared with peanuts and Brazil nuts, which are known for their sudden and violent reactions [13, 424]. Neither children nor parents were informed that the allergen triggering anaphylaxis was *hidden in foods and widely diffused candies.* After eating peanuts hidden in a cake, a child was not saved by a quick injection of epinephrine (Chap. 20). Not only children and adolescents and their parents should be informed thoroughly, but also babysitters, day-care and school personnel, as well as restaurant personnel. Allergic children and their families should be warned explicitly but without excessive alarmism about potentially fatal reactions; older children and their parents could venture to the decoding of

symbols, biochemical formulas and labels mostly understandable only to experts. We repeat that children and adolescents at risk should always wear a *medical bracelet* warning of their affection and be provided with a *cellular phone.*

Oral Allergy Syndrome

In 1942 it was observed that pollinotic adults (birch) eating nuts or various types of fresh fruits, especially apples, experienced oral pruritus and other symptoms mostly of the oropharynx [478]. It was subsequently reported that these patients could react to a wide spectrum of vegetables and fruits [135]. This has been named OAS [6]. In this context the first studies on fresh fruits and vegetables with the prick + prick method were done.

The symptoms are disclosed in a polymorphous fashion, mostly within a few minutes of food contact, more frequently with vegetables cross-reacting with pollens [6]. Often the symptoms quickly disappear, but some subjects experience severe manifestations, up to anaphylactic shock. Since these patients have SPTs positive to other foods, OAS is not a syndrome localized to the oropharynx (as stressed by some authors who consider OAS a variety of ACD), but is part of a systemic sensitization.

Epidemiology

Data in children are not available, apart from a substantial trial showing a 1.4% prevalence in children 5–16 years of age (Fig. 5.9) and 3–15 years of age [397] and the *high frequency of SPTs positive to foods as elicited by children with monoallergy to grasses* [126, 343] *and multiple allergy to pollens* [529] compared to adults [6, 39]. Significantly, a girl monosensitized to egg presented OAS symptoms *at age 3 months* [300]; the youngest children had a mean age of 3 [6], 4 [413], or 4.1–4.9 years [126], 11.9±3.8 [529], and 20%–26% of patients were <14 years [6]. In 80 patients, OAS arose in the 1st year of life in 16% of cases, and from 2–12 years in 65% of cases [6].

Pathogenetic Mechanisms

The richness of mast cells and of sensitive fibers of the oropharynx, which has contact with a high concentration of undigested food, could explain the reactivity of this site in allergic subjects: the symptoms occurring within a few minutes indicate that contact with saliva is sufficient to cause food allergen release. An aspecific mechanism has been proposed, based on *lectins* found in raw fruits and vegetables, capable of activating degranulation by an aspecific bridging: by showing a high affinity for carbohydrates the lectins might help food antigens adhere to mucosal cells, in that the consequent increase in permeability would induce a rapid absorption of the antigens released [381]. New knowledge on epitopes common to pollen allergens and vegetables identified in proteins of eucaryote cells shows that the *profilins* [141] induce the presentation of a bivalent epitope to APCs, with ensuing production of IgE antibodies capable of recognizing both pollens and food. Cross-reactive IgE bind to mast cells and a subsequent contact with those particular epitopes surpasses the threshold level and activates mast cells and their degranulation, thus provoking a rapid onset of symptoms [150]. The current mechanism is based on sIgE reacting with profilins, thus explaining the cross-reactivity to common epitopes and between species that are not botanically correlated, including birch, grasses, *Compositae, Corylaceae,* and tree pollens, vegetables (carrot, potato), fruits (apple) analogous to celery and rosacea allergens such as peach [142, 381, 482, 498]. To establish the immunochemical basis of cross-reactions, DNA/RNA technology has recently been used to demonstrate by immunoblotting that patient sera with IgE reacting with Bet v 1 also bind to Mal d 1, Api g 1 (Table 1.74), and proteins of pear and carrot with a MW of 15–18 kD [142]. Using a Bet v 2 DNA clone as a probe, a cross-reaction was shown with the previous allergens, suggesting homology with the pan-allergen profilin, and with cucumber and muskmelon [141]. Therefore, families that are not correlated share common epitopes to which IgE antibodies bind [141, 521]. In certain cases, cross-reactions are caused by IgE contra-glycoprotein epitopes, shared also by pollens and foods, as demonstrated by another major allergen of Lol p IX with glycyl epitopes bound to IgE [482].

Cross-Reactions Between Pollens and Foods and Between Fruits and Vegetables

The pollens of different species within a single family can cross-react with each other and with fruits [482], as frequently occurs between pollens of some families of trees and some vegetables. Several types of cross-reactions are summarized in Table 9.48 [126, 135, 142, 150, 161, 252, 371, 377, 381, 447, 529]. The standardization of fruit and vegetable antigens will facilitate this aspect of OAS.

Clinical Presentation

Onset of symptoms is generally between 2 and 15 min after eating small quantities of the offending food and is characterized by tingling in the lips or mouth, pruritus or lip edema, pharyngeal, palatal and gingival pruritus, facial erythema, a feeling of tightness of the throat, followed after 15–60 min by systemic progression of symp-

Table 9.48. Cross-reactivity between pollens and vegetables

Birch
Apple, apricot, banana, carrot, celery, cherry, fennel, hazelnut, kiwi, medlar, orange, parsley, parsnip, peach, peanut, pear, plum, potato, raspberry, soy, spinach, strawberry, walnut, wheat
Parietaria
Basil cherry, muskmelon
Grasses
Apple, apricot, carrot, celery cherry, corn, kiwi, muskmelon, orange, peach, plum, rye, tomato, watermelon, wheat
Weeds
Camomile, celery, chestnut, chicory, parsley, squash
Ragweed
Banana, camomile, cantaloupe, cucumber, honey, small pumpkin, watermelon, zucchini
Mugwort
Apple, camomile, carrot, celery, fennel, hazelnut, muskmelon, spices, watermelon
Additional possible cross-reactivity between different fruits and between fruits and vegetables
Apple and pear
Apricot, cherry, peach and plum
Celery and fennel
Celery and parsnip and turnip
Cherry and apple
Kiwi and avocado
Muskmelon, avocado, banana, tomato, and watermelon,
Peach and apple, cherry, pear and plum
Potato and carrot

Data from [126, 135, 142, 150, 160, 252, 377, 381, 447, 529].

toms: urticaria, oculorhinitis, palpebral edema, skin itching, asthma, and less often glottis edema [6]. Cases of anaphylactic shock have been reported: 0.3%–3.7% in adults [6, 413]. After 30–60 min, abdominal pain and vomiting may appear accompanied by nausea and vomiting and more rarely by diarrhea and rhinitis, additional symptoms that usually appear after greater ingestion of food [6]. Tables 9.49 [6, 39, 127, 343, 529] and 9.50 [343] outline the individual foods provoking symptoms in children and the rate of various symptoms. In a pediatric cohort, the symptoms were only oral in 50%, only extra-oral in 16.6% and mixed in 34.4% of cases [529]. The severity of respiratory symptoms may vary remarkably from one child to another, depending on the degree of pollen sensitization.

As Table 7.22 suggests, symptoms may be triggered by a simple indirect contact, more often unnoticed [135]. Oculorhinitic symptoms may appear simply by peeling, or scrubbing, or manipulating raw vegetables or fruits, and be aggravated when itching of the eyes and nose cause patients to rub these organs, thereby rubbing high amounts of allergens from the fingers into the nostrils or conjunctiva [134]. We elucidate that OAS is set off only by raw and not by cooked foods, chemically treated, or long refrigerated, with consequent loss or notable reduction of allergen aggression.

Diagnosis

The clinical history is usually suggestive; the patient's history reveals that the typical symptoms appear in the characteristic chronological order of OAS. Generally these symptoms are seen in young pollen-sensitive subjects with allergic oculorhinitis, who suffer from a simple contact with offending foods. The rapid, typical onset of symptoms and frequent exacerbations allow an immediate diagnosis (see Table 9.51) [126, 135, 150, 160, 377, 381, 529].

For SPTs the prick + prick method is preferred [135] to SPTs with commercial food extracts for all raw fruits and vegetables [413], in 60%–80% of cases positive also to crab, cod, CM, peanuts and nuts [6]. CAP System FEIA (Pharmacia) results when compared with DBPCFCs are most often equivalent to those of SPTs in predicting symptomatic FA (Chap. 6).

For good correlation with history, the FCT is done in an open fashion. A DBPCFC in these patients is technically impracticable: oral contact with a great concentration of foods is crucial and these must be absolutely fresh and without additives of any type, but masking aspect, odor and taste would be unfeasible. It is unknown whether food in capsules after bypassing the oropharynx (which nullifies the goal of the test) still causes symptoms [150].

Treatment

A pediatric study has demonstrated that pollen immunotherapy (IT) is unable to influence OAS symptoms caused by fruits and vegetables; however, 46% of children are improved vs 21% of children treated with oral IT and 15% of those who received placebo [340]. Recently, successful pollen IT was shown to lessen oral symptoms in a small group of patients [265].

Prevention

To prevent the onset of manifestations, often severe, it is necessary that children with OAS avoid every type of contact with the foods triggering such reactions. Bet

Table 9.49. Foods provoking OAS in children compared to adults (%)

	Children			Adults	
Food	342	126	528	6	39
Almond	50				
Apple	78				28
Apple, red			17		
Apple, green			26		
Apricot			9.5		
Banana	14		14		
Beans			9.5		
Carrot	43		24		16
Celery			5		24
Cherries	40		12		
Citrus			17		
CM				10	
Cod				18	
Crabs				8	
Dried fruit			21		
Egg				40	
Fennel			9.5		
Fig			7		
Green pea		14	9.5		
Kiwi	19		43		
Melon			14		14
Nuts	79			28	
Parsley	6				
Peach	46		24		23
Peanut		22.5			
Pear	39		5		
Pineapple			5		
Plum	11				
Potato	14		7		8
Radish			5		
Strawberry			26		
Tomato	11	39	21		
Watermelon			14		
Wheat		12			

Table 9.50. OAS symptoms in children

Features	(%)
Palatal itching	81
Conjunctivitis	37
Throat itching/tightness	37
Lip swelling and/or itching	37
Gingivitis	19
Rhinitis	8
Neck itching	6
Abdominal pain	4
Nausea	3
Cough	3

Data from [343].

Table 9.51. Symptoms elicited by OAS in pollinosis patients with FA

Features	Responsible associations
Laryngeal itching	Mugwort, melon, banana
Pharyngeal itching	Birch, apple
	Grasses, tomato, peanut
Lip swelling	Mugwort, melon, banana
	Birch, apple
Perioral dermatitis	Grasses, tomato, peanut
Throat tightness	Mugwort, melon, banana
Urticaria-angioedema	Mugwort, melon, banana
	Mugwort, celery
	Birch, apple
	Grasses, tomato, peanut
Bronchial obstruction	Mugwort, melon, banana
Abdominal pain	Mugwort, melon, banana
	Birch, apple
Anaphylactic shock	Mugwort, celery
	Mugwort, camomile

Data from [126, 135, 150, 160, 336, 377, 381, 529].

v 1- and 2-allergic children may eat apple, pear, carrot and celery cooked >10 min, indicative of their thermolability [141]. Recalling what was discussed in Chap. 6, that pollen-allergic patients may have SPT positive to foods and vegetables and related symptoms by eating such foods during pollination, we stress that this contingency should be taken into account when preparing diets for pollen-allergic individuals.

Bird-Egg Syndrome

A new example of cross-reactions between inhaled and food allergens is based on cases of egg allergy associated with sensitization to avian allergens. Generally these are adults working in the food industries or exposed to household birds, affected with respiratory allergy, with sIgE antibodies against blood serum proteins or antiexcrement of chicken, pigeon and household birds, who acquire a form of FA provoked by the same allergen at the moment of eating egg or chicken meat, even after several years. Six patients with respiratory allergy and sensitization to avian allergens have experienced severe symptoms after eating egg, thus demonstrating cross-reactions between avian proteins and egg, both to egg yolk and egg white [381]. There is therefore a cross-reactivity to egg of birds of diverse species. The case of a feather-egg allergy has also been reported: a child manifested asthma caused by feathers of household birds at the age of 5 and allergy to egg white and chicken 1 year afterwards [11]; the responsible protein of the syndrome seems to have been *α-livetin*, a protein in egg yolk that is the cross-reacting protein responsible for bird-egg syndrome [381].

Pediatricians and FA

Significant progress has been achieved in the understanding of FA. We are a long way from seeing a complete picture and cannot be sure what it will look like when it is all put together. However, the first step is important. The institution of standard definitions for the clinical presentations of FA will render the scientific literature more discernible. Continuing the analysis of doctor–patient relationships, we consider that a large part of the suggestions mentioned at the end of Chap. 7 is pertinent also in this setting, but is extended for a longer time because children with IgE-mediated FA may acquire a food tolerance very late, perhaps beyond childhood, but if tolerance was not reached in adolescence it is very likely that FA will persist even into adulthood. Otherwise pediatricians diagnosing FA in an infant should speculate on the possible onset of future inhalant allergy or multiple sensitizations, with timely initiation of preventative measures, because FA will pave the way to additional and different allergies. We believe that again the doctor plays a prominent part in the early diagnosis of CMA, above all to avoid children being subjected too long to restrictive diets which could negatively influence growth, even psychological. The secret of successful child treatment in FA is: do not only treat the symptoms but try to find the causal factors that are specific to each child! A very promising cure is that of oral desensitization to foods.

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Note added in press:

tTG autoantibody detection in human saliva may be a sensitive method for CD screening (J Pediatr 2004; 114:632–636)

Pseudoallergy and Food Immunotoxicology

Allergy and Pseudoallergy

Several reports have pointed out that there is notable confusion between allergy and pseudoallergy (PA), to the point that epidemiological studies carried out in the United States have shown extremely elevated prevalence values for food allergy (FA), justified solely by a wide confusion with PA. According to the Asthma and Allergy Foundation, 5%–10% of Americans affected by allergy (35–40 million) present symptoms of FA, whereas according to the Federal Register of the Department of Agriculture, it is about 15%, while the American College of Allergists reports rates of up to 25%–50%. According to a group of consultants of the American Food Industry [2], in 14% of 3,700 families there was at least one subject affected by FA or who followed a special diet because they thought they were affected, most often by cow's milk (CM) (30%), fish and crustaceans (16.7%), vegetables (20%), fruit (20%), chocolate (9.7%), egg (7%), meat in general (5.7%), nuts (4%), wheat (3.3%), with an unexpectedly low percentage for peanuts (6.4%) [2]. Out of 18,880 adults interviewed by questionnaire, 1.4% reported reactions to alcohol, 1.3% to caffeine, 6.8% to chocolate, and 0.3% to soy [103]. In a German study, among 2,355 children and adolescents (0–17 years) contacted by mailed questionnaire, 739 (31.4%) responses could be fully evaluated. The result was that 455 (61.5%) reported symptoms related to food ingestion, 284 (38.4%) affirmed reproducible symptoms in the standardized telephone interview. But when 184 (24.8%) participants were fully examined, reproducible symptoms to food were found in 31 (4.2%) entrants and only 26 (3.5%) showed symptoms of FA [70]. Therefore the real incidence of FA has been difficult to ascertain: one major contributing issue is an elevated public perception of FA associating the ingestion of certain foods with adverse clinical symptoms. Well-publicized reports have fueled public debate over these results, the mass media have criticized the food industries economic approach and, as a conclusion of the authors (and by us), the public has become familiar with FA existence, in this case of food PA, while the medical profession has condemned the unmotivated publicity [2]. However, the discussion on the part of doctors is sometimes obscured by ideological argumentation, lack of basic principles on FA and PA, and lack of scientific evidence for several proposed dietary regimens. We believe that the existing debate has always divided the experts, perhaps because of the inherent difficulties in the diagnosis of this condition, that not being allergic escapes the usual classifications. As a result, a number of children are hospitalized suffering from a food intolerance due to indefinite causes.

For decades scientists, doctors in general, dietitians and other experts of nutrition have denounced the presence of allergens in packaged food that are not mentioned on the label [46]. Often, active substances from a pharmacological and toxicological point of view are involved such as histamine, tyramine, serotonin, phenylethylamine. Histamine-producing bacteria (*Proteus*, *Klebsiella*) can also contaminate fish that has not been subjected to rigorous preservation processes: for this reason anaphylactic reactions following the ingestion of fish products have occurred [35]. Furthermore, during the physiological digestive processes the presence of β-casomorphine, a derivative of bovine casein, an opioid peptide similar to codeine phosphate (which directly releases histamine, as well as diamines, polyamines and peptones) [56], has been highlighted in the food canal. Apart from foodstuffs, numerous drugs can set off PA reactions, which are discussed in Chap. 19.

Definitions

The general division into toxic and nontoxic reactions, as proposed by an EAACI subcommittee (Fig. 10.1) [12, 62], includes various non-IgE-mediated forms such as enterocolitic or colitic forms, celiac disease, intestinal inflammatory diseases, etc. [37], which we have clinically defined in Chaps. 9 and 18. Non-IgE-mediated and nontoxic adverse reactions to foodstuffs are defined in various ways [3, 29, 38, 62]:

- *Food intolerance* [12], in which a physiologically abnormal response is identified following the ingestion of a foodstuff or additive [38], for which the ability to set off an IgE-mediated reaction is not demonstrated and includes pseudoallergic, pharmacological and enzymatic reactions (Fig. 10.2) [101]. These are grouped by the general term "adverse reactions to foodstuffs" in the USA, or "food PA" in Europe [101].
- *Food idiosyncrasy*, often called PA to food additives or rather a quantitatively abnormal response to additives

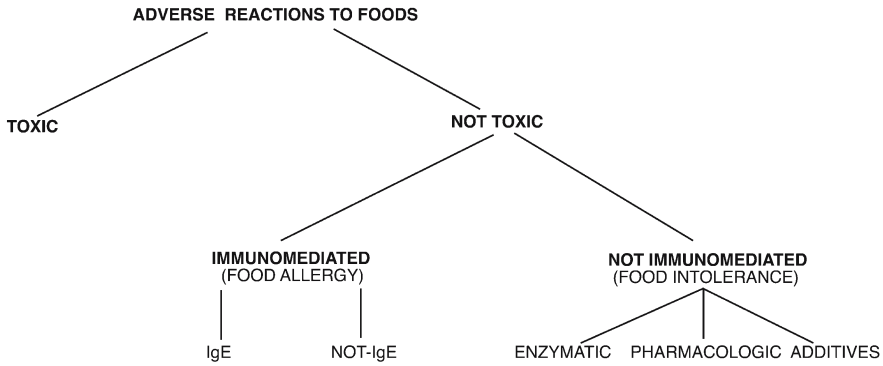


Fig. 10.1. Classification of adverse reactions to foods. (Data from [12, 62])

in particular, which is differentiated depending on its physiological or pharmacological effects: it is similar to hypersensitivity, without, however, involving immunological mechanisms. Notably, the term "PA" is sometimes preferred when referring to reactions to drugs [62]. The EAACI subcommittee includes this PA among the undefined forms in as much as the pathogenic mechanisms are largely unknown [12].

- *Pharmacological reactions,* responsible in the host for a truly pharmacological or similar effect, for example as the result of contact with preformed chemical substances or substances formed spontaneously, which, in subtoxic doses, provoke symptoms in certain intolerant individuals (for example histamine): Moneret-Vautrin proposes the term "false FA" for these reactions [55, 57].
- *Enzymatic reactions* that occur in patients with primitive or secondary enzymatic deficits (for example lactase deficiency) [23].

Separately we discuss:

- *Metabolic reactions* that depend on what effect the substances contained in certain foodstuffs have on the metabolism of certain individuals.
- *Food toxicity* (intoxication), indicating an undesired reaction caused by the direct action of a foodstuff or a food additive without the involvement of immune mechanisms. For this reason, the non-IgE-mediated release of chemical mediators may occur; the toxins may derive from the foodstuff itself or from microorganisms [12, 38].
- *Anaphylactoid reaction,* or rather anaphylactic-like idiosyncratic reactions, with a non-immunomediated release of chemical mediators [3].
- *Immunotoxic reactions* carried out at the immune system's expense by chemical substances foreign to the body, either by accidental exposure as in the case of environmental contaminants, or as a result of their common release into foodstuffs that are then consumed [7]. The role of pesticides (Table 4.21), herbicides and transgenic foods (Table 1.75) has yet to be defined.

For clinical and diagnostic reasons it is useful to consider the non-IgE-mediated, dose-dependent forms in various groups of reactions that often occur in susceptible patients. However, a precise pathogenetic definition is not easy, in as much as various pseudoallergic, enzymatic and pharmacological type mechanisms are often closely intercorrelated [105]. Data available in the literature concern mainly pharmacological reactions and those forms attributable to food additives; enzymatic and toxic reactions are more often considered for a differential diagnosis of IgE-mediated FA. In this environment, the list of substances or harmful foodstuffs is

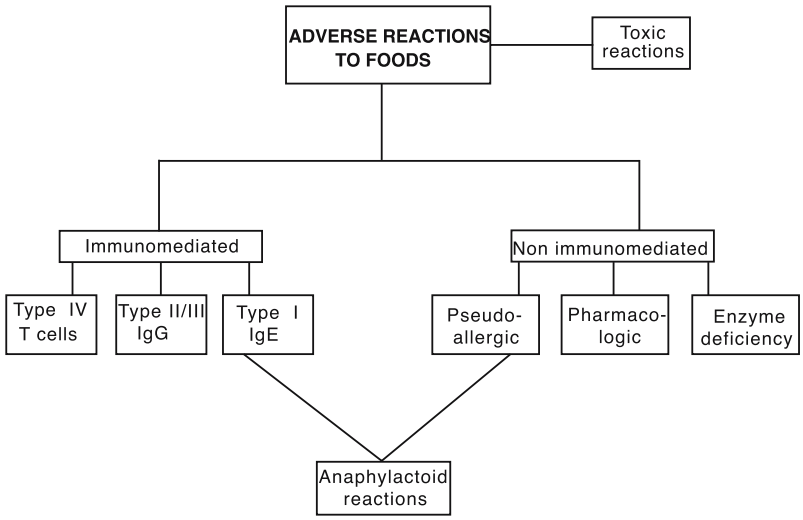


Fig. 10.2. Scheme of adverse reactions to foods. (Modified from [101])

Table 10.1. Prevalence of the reactions to additives. Reported and established prevalence of the reactions to additives (%)

Data and reference	Reported reactions	Established prevalence
18,852 C/A [102]	7.4	0.01–0.02
173 C 5–16 years [33]	18	1.5
333 C 4–15 years [34]	22.7	1.8

A adults, *C* children.
Data from [33, 34, 102].

Table 10.2. Established prevalence of the reactions to additives in two samples of children

Tested additive %	[27]	[80]
Indigo carmine		15.8
Amaranth		10.8
Sunset yellow (E110)	64.2	27.8
Annatt (E160b)	60.7	
Sodium benzoate	57.1	14.8
Sodium metabisulfite		8.3
Monosodium glutamate		8.3
Tartrazine (E102)	50	25.6
Aspartame	48.2	
Erythrosine (E127)	35.7	
ASA	12.5	2.4

Data from [27, 70]

growing daily as new insights are gained, waiting for the authorities to intervene in order to better protect the public, in particular those who are younger and more vulnerable.

Prevalence

The prevalence of positivity for several food additives including ASA is considerable in children [75]. Only for these substances is reliable data available, although studies are characterized by substantial differences between history taking and results, especially when the data are supplied by parents. In Table 10.1 [33, 34, 102], we demonstrate that the prevalence of reactions to preservatives, flavorings, natural and artificial colorings, citric acid, as suggested by parents, was on average 16.3% (up to 22.7%), while with the DBPCFC (double-blind, placebo-controlled food challenge) or the simple blind FPT (food provocation test), it was significantly reduced to 1.1% on average [33]. The values may increase in highly selected pediatric case studies [96]. Botey et al [10] document that 8/82 children (10.9%) had reactions to FPTs as follows: phenylethylamine 4.8%, Na nitrite 3.6%, tyramine 2.4%, while David [25] estimated that 5% of 500 children with severe AD (atopic dermatitis) reacted to the ingestion of tartrazine or benzoic acid. In polysensitive children with severe AD, positive FPT reactions to tartrazine and other food additives have been found [87]. It has already been noted that urticaria-angioedema can be an aggravating factor [80]: 43 children with urticaria, 70% with angioedema, were sensitive to double-blind FPT with various food additives (Table 10.2) [27, 80]; to a lesser extent, out of 120 children tested with seven colorings, 46.4% were positive to DBPCFC [27].

Pseudoallergy to Food Additives

Apicius was famous in ancient Rome for a cook book where he detailed a recipe to preserve the freshness of oysters. It is estimated that 2,000 to 20,000 agents are added to the food (and pharmaceuticals) that we consume. PA to food additives, commercially distinguished into intentional and involuntary, has assumed an ever-growing prevalence in recent years: it is sufficient to consider that they are present in various concentrations in almost all foods and drinks that we typically consume, in order to assure perfect preservation, to inhibit mold growth [94] or to modify the appearance and/or flavor, so as to make the foods more attractive and palatable, especially for children [67]. Others still are used fraudulently: for example, canthaxanthin is added to the diet of farmed salmon to produce a more natural color, or to add up to 400 mg/kg of titanium dioxide (E171) to mozzarella cheese to obtain the same white color as traditional mozzarella made from buffalo milk [26], or to camouflage an incredible number of other foods: even foods for children are not free of such additives [94]. One study found a quantity of aflatoxin, a hepatotoxic furanocumarin derivative produced by *Aspergillus flavus*, to be ten-fold higher than that permitted by current laws (10 µg/kg) in peanut butter sold by 11 of 59 (18.6%) shops specialized in natural foodstuffs vs only 1 of 26 (3.8%) in common shops [26].

Intentionally used food additives are usually permitted by law, which defines a food additive as a substance not normally consumed as itself and not used as a typical ingredient in foods, regardless of whether it has a nutritional value, having been added intentionally to foods for a technological motive, acting as substances which are added at any point during the elaboration to the mass or surface of the foodstuffs in order to preserve the organoleptic characteristics, so as to avoid the spontaneous alteration or to modify smell, taste, appearance, color and consistence, establishing other essential requirements such as:

- Effective necessity
- Short- and long-term absence of toxicity for the laboratory animal
- Absence of suspected carcinogenic phenomena

Table 10.3. Infant foods most frequently containing additives

1. Baby food, powdered or malted milk and other infant food, especially of the ready-to-use type (colorings and preservatives)
2. Bread (whitening additives or to retard oxidation and spoilage: sulfites)
3. Butter and margarine (azo dyes)
4. Potato chips (antioxidants, colorings and preservatives)
5. Fruit juices, beverages, candies (azo dyes, benzoates)
6. Canned, bottled, or frozen foods such as meat, salami, pâté, biscuits, cookies, etc. (preservatives to retard oxidation and spoilage in the prepared foods)

From [67].

- Absence of toxic products deriving from reactions with normal constituents of foodstuffs
- Presence of high purity
- Ease of qualitative and quantitative research in both a purified state and in the foodstuff
- Determination of each recommended daily allowance (RDA) expressed in mg/kg of body weight (b/w)

However, according to EU directives, adopted with the aim of standardizing the international regulations with the adoption of norms valid in all countries, the number of food additives originally allowed has been extended, especially regarding their field of use, in many cases increasing the limit at the maximum allowed dose. Therefore, from a biological point of view the impact of these compounds on the human body, in particular that of a child, cannot be ignored [20], also because the *RDA for babies has not been studied*, especially for neonates, infants and younger children.

The principal uses are aimed at blocking the undesirable activity of microorganisms without affecting the foodstuff's biological value (preservatives or antimicrobials), preventing the spoilage and/or enzymatic browning of some foodstuffs (antioxidants), conferring particular characteristics of appearance and consistency to some foodstuffs (colorings, preservatives, moisturizers, flavoring and emulsifying agents, thickeners), etc. [28]. The foods for children that more frequently contain food additives are summarized in Table 10.3 [67], taking into consideration that the law usually allows the use of a certain number of food additives in infant foods, for example weaning products and in general those for young infants. The foods and drinks commonly containing certain food additives are listed in Table 10.4 [58, 63, 67], and those containing sulfites in Table 10.5 [75, 78, 99]. The hidden sources of sulfites include their use in baked products [63] and in general as antimicrobials and food preservatives, in containers and equipment for fermentation, antioxidants and enzymatic antibrowning [83]; it is hoped that further studies will provide a more complete understanding of this confusion. The hidden sources of thickeners, etc., are shown in Table 10.6 [28] and those of vegetable gums in Appendix 10.1 [91, 106]. The classes of so-called intentional food additives according to the EU are listed in Table 10.7, which includes the division of foodstuffs, specifying in which products they are forbidden. The allowed food additives have been given E numbers, which appear on food labels. Some food additives belong to two categories, for example ascorbic acid, and are divided schematically according to their use into [94]:

- *Acidifiers*

Citric acid (E330), lactic acid (E270), tartaric acid (E334), orthophosphoric acid (E338), to increase acidity by reducing the pH, in order to improve the preservation and the taste, examples: sparkling soft drinks (E338) and nonsparkling soft drinks (E334), sweets, ice creams, fruit juices, cakes (E330), etc.

- *Thickeners*

Carrageenin (E407), cellulose and derivatives (E460–466), Na alginate (E401), alginates (E402–405), Na polyphosphate (E450): in part derivatives of seaweed, they are used to increase viscosity and consequently to prevent separation of food ingredients, examples: cooked ham, meats and tinned meats (E450), puddings, ice creams, mayonnaise, etc. (E401–405).

- *Covering agents*

Bees wax (E901), carnauba wax (E903), to modify the external appearance of the product, for example: citrus fruits, candied fruits, sugared almonds, and dried fruit.

- *Antioxidants*

Disulfites and metadisulfites (E220–224), ascorbic acid and derivatives (E300–304), natural and synthetic tocopherols (E306–309), gallates (E310–312), butylhydroxyanisol (BHA) (E320), butylhydroxytoluene (BHT) (E321), lecithins (E322), to prevent rotting of lipids and the tainting of fruit and vegetables, protecting them from O_2 attack: examples of natural antioxidants are shown in Table 4.22, to which chocolate is added [93].

- *Flavorings*

Amyl acetate, citric acid (E330), benzaldehyde, carvone, citrus fruit essence, menthol, to confer a characteristic aroma to foodstuffs; examples: candied fruit, chocolate, cakes, etc.

- *Colorings*

The principal examples are distinguished here by color:
Yellows: curcumin (E100), lactoflavin (E101), tartrazine (E102), quinolin (E104), sunset yellow (E110).
Reds: carmine (E120), azorubin (E122), lady-bug red (E124), erythrosine (E 127).
Blues: brilliant blue (E131), indigotin or amaranth (E132).
Greens: chlorophyll (E140), cupric complex (E141), brilliant acid green (E142).
Black: brilliant black BB (E151).
Various colors: caramel (E150), carotenoids (E160), annatto (E160b), pigment red (E180), or otherwise distinguished into natural and synthetic, characterized re-

Table 10.4. Foods that may contain the principal additives

Additives	Foods that may contain the additive
Acetylsalicylic acid (preservative)	Alcohols (including beer), aperitifs, cloves, conserve, shellfish, candies, cheese, packaged meat (except seasoned meat), sausages, salami, corn, loaf of bread, potatoes, peaches, tinned or canned foods, grapes; see sodium salicylate
Benzoic acid E210 (preservative)	Soft and fruit drinks, salad dressings, some cheese especially cream cheese, fruit gelatins, low-calorie margarine, industrially produced jams, marmalade and fruit preserves, canned soups, white fish treated with preservatives[a] or refrigerated preserves (anchovies, herrings, sardines, shellfish), drinking chocolate concentrates, ready-made sauces and salads, mustard, ciders
Sodium benzoate E211 (preservative)	Drinks (orange drinks, soda, etc.), caviar and similar products, filled chocolate (not pure chocolate), some cheese especially cream cheese, bananas, fruit gelatins, green peas, licorice, ready-made mayonnaise, gravies and sauces, low-calorie margarine, industrially produced jams and marmalades, canned or bottled fish, fruit juices, common wines
Sodium salicylate (preservative)	Intentional or natural additive present in some foods: apricots, oranges, bananas, blackberry, cabbage, cucumber, cherries, figs, almonds, apples, melons, blueberry, hazelnuts, nuts, green pepper, green peas, tomatoes, plums, raspberry, red currant, strawberry, raisins, zucchini
Methyl para-oxybenzoate E128	Belongs to the same family as benzoate
Erythrosine E127 (coloring)	Red food coloring: candied fruits, canned anchovies, herrings and sardines, ice creams, ice cream bars and popsicles, alcoholic beverages, confectionery cakes, macaroni and vermicelli, mixtures for drinks, medications in lozenges, syrups, tablets
Sunset yellow E110 (coloring)	Yellow food coloring: pickled and salted foods, nonalcoholic beverages, puddings, candy, caviar and surrogates, chewing gum, packed and canned soups, confectionery creams, candied fruit, ice creams, jams and marmalades, salad dressings, ready-made sauces, medications
Tartrazine E102 (coloring)	Foods in oil, soft drinks and sodas, ready-made cakes, industrially made caramel and vanilla custards, fruit candies, sugared almonds, chewing gum, filled chocolate (not pure chocolate), alcoholic beverages, vegetable preserve, cheese-flavored crackers, confectionery creams, potato flakes, cream cheese, ice-creams, ice cream bars and popsicles, fruit gelatins, ketchup, fizzy lemonade (not plain lemonade), industrially made jams and marmalades, pudding and pie fillings, whipped cream, confectionery cakes, orange drinks, concentrated fruit juices, mustard, pickles, nougat, chocolate or vanilla puddings, sandwiches, ready-made hot dogs, filled rolls, sherbets, vanilla, packaged vegetables, fruit yoghurt, meat tenderizers, imitation flavorings and extracts, medications in lozenges, syrups, tablets or inhaled and injected
Sodium glutamate E621 (flavor enhancer)	Processed foods of all kinds (canned, dried, frozen, packaged, etc.), sausages, processed meats of all kinds, seasoned salt, salad dressings, crackers, bouillon cubes, corn pies, dried and toasted fruits, parmesan cheese, meat and fish base, potato chips, canned or frozen fish, dessert salt biscuits, gravies, sauces, toasted seeds, spices, soups: canned and dry mix, tomatoes, tortilla chips
Sulfites (preservatives and contaminants)	Brandy, cornstarch, ready-made or lyophilized crawfish, avocado, other tropical fruits, dried seeds, beer, candied fruits, caramel, sugared almonds, salad dressings, sauerkraut and cauliflower salad, dried cod, fruits and vegetables (frozen, packaged, lyophilized, dried, canned or "dietetic"), seafood, shellfish, shrimps, crabs, etc. (fresh, frozen, packaged, canned, bottled, lyophilized), fresh or dried mushrooms, gelatin, salad including tomatoes especially in restaurants, industrially made marmalades and fruit gelatin, dried cod, cocktail mixture, oysters, spinach pasta, potatoes (raw, peeled whole or cut into slices, frozen, packaged, lyophilized or canned), pectin, fresh fish in general, pie and pizza dough, bakery products, pie fillings (with fruit), common and table salt, gravies, sauces, cider, pickles and relishes based on vegetables, fruit juices, puree and toppings, instant tea, wine, natural and powder sugar, cotton candy, lyophilized and ready-made soups, wine-vinegar; see Table 10.5

Data from [58, 63, 67].

[a] Can be washed off and allowed.

Table 10.5. Common foods that contain or may contain sulfites

Foods	Parts/10^6
Beer	≤10
Canned avocado	
Canned cod	≤10
Canned or dry soups	10
Canned potatoes	≤10
Cider	
Citrus drinks	
Clams, canned and jarred	
Coconut	5
Crackers	≤10
Crawfish	
Dehydrated soup mix	≤10
Dehydrated vegetables	
Domestic jams and jellies	5
Dried fruit (excluding dark raisins, prunes)	1.000–1.200
Dried potatoes	50–99.9
Fresh fruit salad (all types)	≤10
Fresh mushrooms	10–49.9
Frozen potatoes	10–49.9
Fruit juices (not frozen)	≥100
Glucose, syrup and solid	
Grape juice	85
Grapes	1–5
Gravies, sauces	75
Gravies, sauces (canned or dried)	50–99.9
Instant tea	5–10
Lemon juice (nonfrozen)	800
Lime juice (nonfrozen)	100
Molasses	100–150
Malt vinegar	10
Maraschino cherries	50
Mashed potatoes (commercial)	
Pectin	<10–50
Pickled cocktail onions	10–49.9
Pickled peppers	10–49.9
Pickles, relishes	20
Pie dough	7
Pizza dough (frozen)	≤10–20
Salad dressings (restaurants)	
Sauerkraut	10–49.9
Sausage meats	
Shrimp (fresh) and other seafood	10–49.9
Vegetables (fresh, frozen, dried, canned, etc.)	5–30
Wine	100–150
Wine vinegar	50–99.9

Data from [75, 78, 99].

spectively by less or more frequent reactions, and minerals:

Natural: E100, E101, E120, E121, E140, E141, E153, E160–163.

Synthetic: E102–105, E110, E111, E122–127, E130–132, E142, E151, E152, E180, separated into nitrogen-containing (E102, E103, E105, E110, E111, E122–126, E151, E180) and non-nitrogen containing (all others).

- *Minerals*

Ca carbonate (E170), titanium dioxide (E171), Fe oxide and hydroxide (E172), Al (E171), Ag (E174), Au (E175), terra d'ombra (E181) have the unique role of improving the natural color of a foodstuff and of giving it a new color, examples: aperitifs, soft drinks, sweets, etc. Previously only permitted for cakes and ice creams, usually the law allows their use in the majority of products.

- *Preservatives*

Acidic acid (E260) and acetates (E261–263), ASA, ascorbic acid and derivatives (E300–304), benzoic acid (E210) and derivatives, Na benzoate (E211), K benzoate (E212, 213), esters of parahydroxybenzoic acid (parabens) (from E214 to E219), phytic acid, lactic acid (E217), propionic acid (E280) and propionates (E281–282), sorbic acid (E200) and sorbates (E201–205), carbon dioxide (E290), nitrites and nitrates (E249–252), Na nitrite (E250), bromated sesame oil, S dioxide, sulfites and derivatives: sulfurous anhydride (E220), Na sulfite (E221), Na hydrogensulfite (E222), Na disulfite (E223), K disulfite (E224), Ca sulfite (E226), K hydrogensulfite (E227), diphenyl (E230), orthophenol (E231) are used to retard oxidation and spoilage, that is to prevent the oxidative decoloration of fresh and preserved foodstuffs, preventing their deterioration and prolonging the period of preservation. Furthermore, they are used as antimicrobials to disinfect food containers, in as much as they impede or delay the growth of microorganisms. Examples are shown in Table 10.4. Recently the use of E220 and of sulfate in general was extended to foodstuffs in which it was previously forbidden, for example in sausages and salami, and the doses of E249–252 in prepared meats and salami was increased.

Table 10.6. Main hidden forms of thickening, gelling, stabilizing, emulsifying additives

Thickening, gelling, stabilizing additives
Adragant and arabic gums
Agar
Carob seed and guar flour
Carrageenans
Fruit pectins (also modified)
Natural and modified starch
Orthophosphates
Phosphates and polyphosphates
Propylenglycol, alginate
Emulsifying additives
Lactilates
Lecithin
Mono/diglyceride esters of fatty acids
Salts of food fatty acids
Stearoyl
Tartrate

Modified from [28].

- *Sweeteners*

Aspartates, dextrose, fructose, glucose, saccharin, saccharose, sorbitol, and inverted sugars are frequently used substances, some of which are free of nutritional qualities as substitutes for common sugar (saccharose) in various cakes.

- *Emulsifying agents*

Glycerin (E422), lecithins (E322), salts of food fatty acids (E470) make it possible to obtain or maintain for a period of time the uniform dispersion of two phases which cannot normally be mixed such as a fatty substance with an aqueous solution (typical characteristic of emulsifiers). Examples include ice creams, margarines, cooked ham, etc.

- *Flavor enhancers*

Citric acid (E330) is used more as an acidifier, monosodium glutamate (E621) to enhance or improve the flavor and fragrance of foodstuffs, although free of aroma. Examples are given in Table 10.4.

- *Gelling agents*

Agar-agar (E406), carob seeds, locust gum (E410), guar gum (E412), adragant gum (E413), arabic gum, acacia gum (E414), vegetable gums, pectins (E440) are used to confer the consistency of a gel, for example in puddings, canned meats, deserts, jellies, brawn, chewing gum, above all sweet products in which they can constitute 40%–50% of the product, for example sweets or jams, where gelatins and pectins allow a reduction in the quantity of sugars.

Table 10.7. Classification of different additives

Acidifiers
Acidity correctors
Antiagglomerants
Antifoaming agents
Antioxidants
Colorings
Covering agents
Emulsifiers
Flavor enhancers
Flour additives
Fusion salts
Gelling agents
Leavening powders
Modified starch
Preservatives
Stabilizers
Texturing agents
Thickeners
Classification of food products based on additive content
Foods that may contain all additives permitted by law
Foods that may contain only some additives
Foods that should contain no additive: 1. Butter, CM, cream, daily products obtained by living ferments, such as yogurt 2. Honey, nonemulsifying oils and fats of animal or vegetable origin 3. Raw, nonprocessed foods, with exceptions including fresh fish, fruits, vegetables, fresh and chopped meat 4. Dried pasta, sugar 5. Tea and coffee (not instant and aromatized) 6. Mineral water

Source: European Community.

- *Nutrients*

Vitamins A and D, vitamins of the B group (thiamine, niacin, riboflavin), ascorbic acid, Fe, and K iodide are generally added to balance a lack induced by medical treatments.

- *Melting salts*

Na citrate (E331) is used in foodstuff preparation, for examples in cream cheese.

- *Flour food additives*

They are maturing and ripening agents, to whiten flour for bread and complete its maturing process.

- *Rising agents*

Tartaric acid (E334) and $NaHCO_3$ determine the rising of baked goods (except bread).

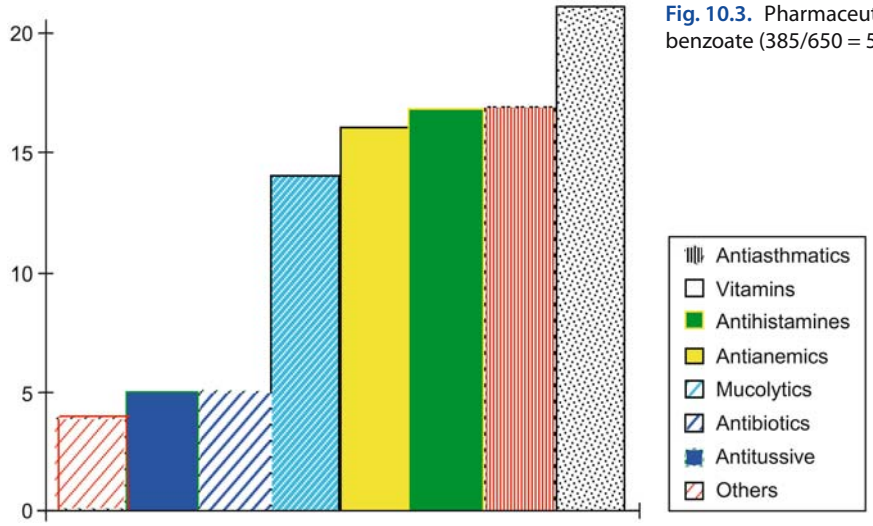


Fig. 10.3. Pharmaceutical preparations containing Na benzoate (385/650 = 59.2%). (Modified from [61])

- *Surfactants*

Methylcellulose, mono- and di-glycerides, and Na phosphate are added to fluids in small quantities to reduce the surface tension, modifying the foaming or wetting qualities.

Regarding colorings, the more restrictive countries seem to be Bulgaria, Russia, Taiwan and Hungary. However, food additives are often used by industries without the necessary guarantees. In one study conducted in Great Britain it was found that 19% of drugs analyzed contain different food additives, 12% of which were colorings and preservatives [68]. In the United States, a wide-spectrum study was conducted on 102 different medicines, sold with or without prescription, including analgesics-antipyretics, antihistamines-decongestants, cough and cold medications, antidiarrheal and theophylline syrups. Although 90% of drug instruction leaflets indicated the presence of food additives, the type was not specified in 35.3% of aroma enhancers, in 24.5% of preservatives, in 10.8% of colorings and in 2% of sweeteners [46]. Such additives, although declared, can be involved in many cases with adverse, pseudoallergic and anaphylactic reactions [61, 68] to fluorescin, sunset yellow, and tartrazine [46], which are also widely used in pharmaceutical products for oral use (Fig. 10.3) [61] (see Chap. 19). Since the triggering dose of Na benzoate is 100–200 mg and the average content of oral drugs examined is 1–2 mg/ml, one can deduce that the use of these drugs should be avoided, at least in asthmatics [61].

Pathogenesis

The mechanism of action of food additives seems to be pharmacological in nature; however, the frequent involvement of histamine would lead to believe that non-IgE-mediated immunoallergic mechanisms are also involved. In patients with symptoms of positive challenge to E102, the levels of histaminemia, methylhistaminuria and urine PGE2 and TXB2 were elevated [60]. Therefore, it cannot be excluded that, like haptens, food additives bind to biological proteins to become allergens and provoke type I, III and IV reactions [100, 101] (Fig. 10.2), as recently observed in an IgE-mediated allergy case [42]. It is, however, very likely that the mechanisms responsible are multiple, including the following which derive from various studies [27, 65, 74]:

- Inhibition of cyclooxygenase, with consequent inhibition of prostaglandin synthesis and unbalancing of the arachidonic acid metabolism toward the production of leukotrienes
- Activation of mast cells and basophils with release of histamine and other mediators, demonstrated in vivo at the gastric level following oral challenge with Na benzoate [74]
- Cellular anoxia determining the inhibition of protective enzymatic activity with a consequent increase in mucosal permeability, which in turn facilitates the passage of allergens
- Alteration of neuronal membrane permeability with interference in their conduction
- Variation in neuropeptide levels [65]

The pathogenic role carried out by ASA [68] is paradigmatic, to which the following actions are related:

- *Virtual inhibition* of cyclooxygenase pathway with a consequent block in the synthesis of prostaglandins (especially PGE), prostacyclins and thromboxanes. This results in the arachidonic acid pathway derailment toward the production of leukotrienes.
- *Direct histamine release* activating mast cells and basophils.
- *Release of substance P* (SP), which orchestrates the vicious circle involving histamine with consequent
- *Release of neuropeptides* by histamine with an increase in serum levels, from which is derived a new impulse to maintain and perpetuate the reaction [7].

Another mechanism involves sulfites, which when orally ingested can determine asthmatic attacks: in the stomach they are transformed into disulfites and sulfurous acids H_2SO_3, which, when they are dehydrated to SO_2, pass from the circulation to the airways [36, 64]. Other pathways include inhalatory, solicited by opening packets of dried fruit or with bronchodilatory aerosols, subcutaneous (adrenaline), and intravenous (corticosteroids) [15]. The pathogenesis is thought to originate from the action of SO_2 on tracheobronchial receptors, which induces a cholinergic reflex, or from the partial deficit of the sulfite-oxidase enzyme, which converts SO_3 into SO_4 [95]. As far as E621 is concerned, glutamic acid is the precursor for the synthesis of acetylcholine, whose level abruptly rises following ingestion of foodstuffs that contain high concentrations, therefore a reduced metabolism of E621 is hypothesized, probably due to a lack of vitamin B_{12} [66]. If this deficit is confirmed, it would be necessary to consider the possible oxidation action of the vitamin, capable of blocking the sulfite-induced bronchospasm in four out of five asthmatic children [4]. Many points relative to PA to food additives require further clarification: for example tartrazine sets off asthmatic crises in 10%–40% of patients with ASA-induced asthma, not inhibiting, as far as can be seen, the cyclooxygenase [58] for which the cross-reactivity with ASA remains difficult to evaluate. Various mechanisms have been highlighted that define a possible role of this food additive as a hapten, which is antigenically efficient following binding to a suitable vector such as a host protein in the presence of a catalyst, whose absence would result in an unstable bond [90].

Clinical Features

Food additives can provoke adverse reactions, or aggravate preexisting illness [64, 65, 67, 89]: anaphylaxis, asthma, chronic or recurrent urticaria-angioedema, perennial rhinitis, behavior disorders such as childhood hyperactivity, Chinese restaurant syndrome and various others highlighted in Tables 10.8 and 10.9 [46]. We stress that 80% of parents blamed food additives and preservatives for the behavior disorders of their children [1].

Sulfites, in particular Na metadisulfite (Table 10.5), used for fresh and preserved foodstuffs, apart from asthma can cause facial erythema, cyanosis, urticaria and/or angioedema, laryngeal edema, lipothymia and potentially fatal anaphylactic shock, especially in asthmatics [66, 99].

Monosodium glutamate, widely used to enhance Chinese and other foodstuffs, at a dose of 3–6 g provokes the Chinese restaurant syndrome, with the features of acute acetylcholine intoxication [66]: severe pulsating migraine, heat flushes, sweating, hypotension, or asthma with alterations in pulmonary functions [94] (≤3 g are sufficient to produce symptoms in sensitive individuals [31].

Table 10.8. Adverse reactions to colorings

Colorings	Adverse reactions
Brilliant blue (E131)	Weak bronchoconstriction
Carmine E120	Allergic cheilitis, asthma
Eosin	Potent photosensitizer
Erythrosine (E127)	Potent photosensitizer
Fluorescin	Urticaria-angioedema, syncope, anaphylactic shock
Ponceau red (E124)	Bronchoconstriction
Quinoline yellow (E104)	Contact dermatitis
Sunset yellow (E110)	Anaphylactoid reactions, anaphylactic shock, retching and vomiting, belching, abdominal pain, angioedema, vasculitis, purpura, cross-reactivity with ASA, sodium benzoate, acetaminophen, and other azo dyes
Tartrazine (E102)	Anaphylactoid reactions, urticaria-angioedema, asthma, contact dermatitis, rhinitis, hyperkinesia in hyperactive patients, eosinophilia, cross-reactivity with ASA, sodium benzoate and indomethacin

Modified from [46].

Table 10.9. Adverse reactions to sweeteners

Sweetener	Adverse reactions
Aspartame	Cross-reactivity with sulfonamides, granulomatous panniculitis, renal tubular acidosis (with large amounts), urticaria-angioedema
Lactose	Diarrhea and malabsorption in lactose-intolerant children, flatulence, vomiting (see also the text)
Saccharin	Cross-reactivity with sulfonamides, diarrhea, gait disturbances, nausea, papular skin eruptions, pruritus, urticaria, wheezing
Sorbitol	Abdominal pain, flatulence, osmotic diarrhea, poor absorption of active drugs
Sucrose	Cariogenicity, increased degradation of active drugs

Modified from [46].

Aspartame (composed of L-aspartic acid and L9-phenylalanine), 180 times sweeter than sugar, used in multivitamin tablets, fruit syrups, fizzy drinks and iced tea, can induce urticaria, gastrointestinal symptoms and

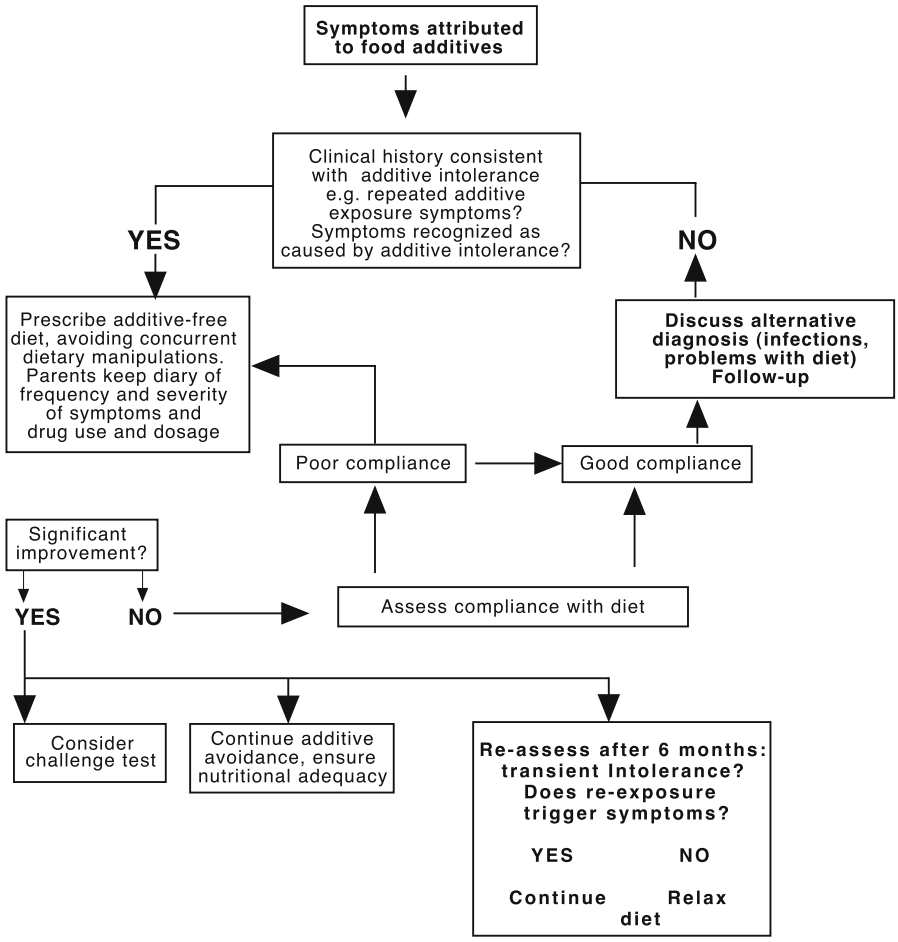


Fig. 10.4. Suggested algorithm of diagnosis and management of suspected food additive intolerance. (Modified from [68])

headaches. However, it does not have side effects as mentioned, neither regarding behavior nor intellectual activity [98] (Chap. 16).

Benzoates (Table 10.4), present as preservatives in commonly used foodstuffs as well as in natural foodstuffs such as many fruits, are responsible for 11%–15% of recurrent chronic urticaria cases [94]. Following challenge, increases in histamine levels in the gastric mucosa have been found [50].

The symptoms induced by *ASA* include shock, asthma-rhinitis and urticaria-angioedema syndrome. Based on pertinent reports, ASA can rightly be considered an important marker for food additive PA.

Gelatins and so-called fruit gums that contain gelatins (Appendix 10.1) [91, 106] also give cross-reactions with IgE-mediated mechanisms, causing symptoms that range from urticaria [91] to anaphylactic shock (Appendix 9.10).

Diagnosis

The diagnosis of PA to foodstuffs and additives is complicated by the great number of incriminated substances and their pathogenic mechanisms; varying the latter from one compound to the next does not favor the availability of a diagnostic test, rendering the certain identification of responsible agents somewhat laborious. Our advice is to act first by exclusion for a possible FA diagnosis.

The first step is composed as always of establishing an accurate personal and family history of atopy (FHA) (Fig. 10.4) [68] based initially on epidemiological data in ≈3- to 8-year-old children who are involved with AD, including symptoms induced by histamine such as urticaria-angioedema, AD exacerbations or sporadic cases of asthma or rhinitis [54]. The history taking will then be focused on highlighting factors that favor digestive alterations such as the use of drugs, dietetic products, laxatives, digestives, irritants, yeasts or a possible infection or infestation.

The compilation of a detailed qualitative and quantitative food diary will enable orientation regarding food habits and preferences for determinate foodstuffs or drinks. It is good practice to advise parents to record the quantity consumed also and above all *when not at home,* noting in detail all foodstuffs eaten with relative amount, the composition, the date of preparation and/or expiry of all prepackaged products, specifying whether boxed, frozen, etc. The responsibility of the

physician is ascertaining whether there has been a high ingestion of foodstuff rich in histamine or agents which release it [10]: if a group of foodstuffs or a single food is suspected, it will be a useful strategy to keep the packaging labels [90].

Specific in vivo or in vitro tests do not exist for PA to foodstuffs or additives; if skin prick tests (SPTs) are positive [52], the diagnosis of a possible FA will follow, if negative, or if clear results for the diagnosis are not obtained (as often happens), an elimination diet rigorously free of food additives and drugs (see "Treatment") will be carried out for 2–4 weeks, or an oligoantigenic diet (Table 9.14], or one followed by a FPT (if necessary double-blinded), also using the food additives presently available, carefully taking into consideration any potential masking [96]. It is good practice to eliminate all food additives and other drugs as indicated [54] (Chap. 6). However, the possible improvement obtained with a restricted diet remains limited, on the clinical side, since it is impossible to establish which eliminated substance causes the hypersensitivity; therefore in children showing improvement, it is necessary to proceed with a follow-up FPT [90]. Since it is difficult to obtain information regarding the upstream pathogenic mechanism, even when these tests allow a cause–effect relationship to be established, the pediatrician must evaluate the opportunity of advising such a test [14].

In particular, in patients aged 3–18 years, free of symptoms, the FPT is performed using a single substance at a time based on the weight of the patient [67]. Some authors administer nonincreasing [41] single doses [27, 80], except for E102 [80]. The doses used in children of 3.3–14 years [27, 80] are 0.1 mg for E110, E122, E123, E132 and 0.1, 0.5, 1 mg for E102, 100 mg for E211, E621 and ASA and 10 mg for E223 [80], or 5–10 mg for E102, E110, E127, E160b, 50–100 mg for E211 and ASA and 20–40 mg for aspartame, with a larger dose used in children weighing ≥20 kg [27], without exceeding 50 mg for E102, 200 mg for sulfites and 5 g for E621 [78]; others use different dosages [21, 41, 78, 94, 96]. In children aged 1.4–14.4 the initial dose was 50 mg of orally administered ASA and subsequent doses were administered at hourly intervals. The maximal cumulative dose was 10 mg/kg b/w [44]. Each substance is given as a gelatin capsule (provided the child is not sensitive), or dissolved in 20 ml of water for the younger children who are unable to swallow the capsule [27]. In asthmatic patients it is necessary to begin with reduced (1/10) doses and spirometric tests are performed at the beginning and then every 30 min until the end. At the first manifestation of reaction, the test is terminated and considered positive. It is necessary to monitor vital signs and check if no clinical signs of a reaction appear [44]. The patient remains under control up to 6 h from the last administered dose to record any possible late reaction [67]. Commercial preparations are available with doses of 5 mg for E102, E127, E160b and E223, 50 mg for E211 and E218 and 100 mg for E621 [40, 101]. Attention must be paid however to the behavior of the parents, some of whom refuse to accept the FPT negative response and continue an elimination diet for their children [25, 68]. A further point to consider is the frequent FPT negativity following a positive result [68]: the possible conclusion is that some transitory reactions are not evidenced, following a delayed FPT response, and can remain underdiagnosed [49]. It has already been reported that late reactions may present only after 24 h, 48–72 h or more.

The most frequent differential diagnosis is with IgE-mediated allergy; PA characteristics are:

- Dose dependency
- SPT negativity
- In vitro test negativity
- Prausnitz-Küstner test negativity (lack of transferability)
- Possible onset of symptoms at first contact with the causal agent [12]: in this case the common allergy tests are sufficiently reliable only when the symptoms are manifested shortly following the ingestion even of small quantities of food additives or of the foodstuff containing them.

Taking into consideration Tables 10.3 to 10.6 it is necessary to differentiate both other food additives as well as numerous gastrointestinal diseases mimicking food intolerance [48].

Treatment

First, the responsible foodstuff or additive and the drugs containing the incriminated additives must be eliminated from the diet (Fig. 10.4). The lack of rigorous diagnosis often renders the diets in these children difficult to follow, above all when the foodstuffs to be excluded are numerous and/or normal constituents of the childs diet. In the younger child, the Achilles' tendon of elimination diets are day-care centers, which cannot always ensure attentive cooperation. In PA to food additives (Table 10.10) [68], we prescribe the diet listed in Table 10.11 [41, 58, 67, 75], specifically free of food additives, to be rigorously followed for a minimum period of 6 months, also excluding drugs containing food additives. Since families have often pointed out the practical impossibility of excluding so many foodstuffs, we add the alternative advice of only using fresh foodstuffs and meals prepared at home and not industrially. Depending the case, elimination diets will be established based on the data in Tables 10.3, 10.4 and 10.10 (food additives), 10.4 and 10.5 (sulfites), 10.6 (thickeners, etc.) and 10.11 (tartrazine, azo dyes, and monosodium gluconate). With correctly predisposed diets that favor the collaboration of the young patient, matchless results are obtained for urticaria, so that after some months the child's diet can be liberalized without inducing acute reactions [43], with positive results varying from 53 % to 89 % of cases [43, 52], even in hyperactivi-

Table 10.10. Suggestions for an additive-free diet (see Table 10.4)

Prefer fresh foods. Check thoroughly the label on the packet of frozen fruits and vegetables. In case of doubt, it is best to avoid these foods.	
Always check the additives listed on the food label.	
Avoid brightly colored soft drinks, sweets, cakes and instant convenience foods.	
Avoid all foods with a label stating "permitted coloring and preservatives" without any specification.	
Avoid non-food products with coloring and preservatives, such as colored toothpaste, throat pastilles and colored lozenges and medicines.	
Whenever possible, avoid the following additives or groups of additives:	
E100–119	Colors
E200–299	Preservatives
E300–321	Antioxidants
E322–494	Emulsifiers and stabilizers
E621	Flavor enhancers
E905–907	Mineral hydrocarbons
Check the diet's nutritional adequacy.	

Modified from [68].

Table 10.11. Foods to be eliminated for a diet without tartrazine, azo dyes, and monosodium glutamate (unless homemade)

Foods that may contain tartrazine
Alcoholic beverages
Cake mixes and icings
Candies, especially orange, butterscotch, lemon, or lime flavored
Canned vegetables
Caramel and vanilla custard
Catsup
Cheese
Cheese-flavored crackers and snack foods
Chewing gum
Concentrated fruit juices
Filled chocolate unless pure chocolate
Fizzy lemonade (except plain lemonade)
Frankfurters
Fruit drinks
Fruit yoghurt
Gelatins
Hot dogs
Ice creams and sherbets
Imitation flavorings and extracts
Jams, marmalades
Jellies
Lemonade
Macaroni and cheese: canned, dry packaged, or frozen
Meat tenderizer
Medications
Orange drinks
Penny candies, caramels and chews, fruit drops
Pickles and relishes
Pie fillings
Puddings and pie fillings
Salad dressings
Seasoning salt
Soft drinks
Stewed fruit sauces, fruit gelatins
Vanilla, butterscotch, or chocolate puddings
Whips and dessert sauces, such as vanilla custard
Foods that may contain azo dyes (unless homemade)
Bakery foods except plain rolls, crackers, cheese puffs, potato chips
Cake and cookie/biscuit mixtures
Candied fruit
Canned anchovies, herrings, sardines, caviar, cleaned shellfish
Caviar and surrogates

Table 10.11. (Continued)

Foods that may contain azo dyes (unless homemade)	Foods that may contain monosodium glutamate
Chewing gum	Accent
Colored toothpaste	Ajinomoto powder
Confectionery cakes and creams	Bouillon cubes
Cream in powder form	Camembert and parmesan cheese
Herrings and sardines	Canned or frozen fish
Ice creams	Chinese restaurant food in general
Jams and marmalades	Fried chicken
Macaroni and vermicelli (certain brands)	Gravy
Mayonnaise, salad dressing/cream; sauces, including ketchup, mustard, ready-made sauces, including béarnaise, hollandaise, curry, fish, tomato, white onion, and parsley sauces	Herbs and spices
Mixtures for drinks	Low-calorie, low-fat diets
Nonalcoholic beverages	Meat and fish base
Packet and canned soups	Potato chips
Pickled and salted foods	Processed foods of all kinds: canned, dry packaged, and frozen
Puddings	Salad dressings
Purees except pure tomato puree	Sauces
Ready-made sauces	Seasoned salts
Red food coloring	Soups: canned and dry mix
Salad dressings	Soy sauce
Waffle and pancake mixes	Spices
Yellow food coloring	Tomatoes
	Tortilla chips
	Wonton soup

In addition, colored toothpaste, medications in lozenges, syrups, tablets.
Data from [41, 58, 67, 75].

ty syndrome (Chap. 16). However, the diet often is in vain because the food additives turn out to be present in many foodstuffs regularly consumed by children, while the label declares simply the presence of *"legally permitted colorings and preservatives,"* followed by a series of names and numbers that give no information to the common consumer, although colorings have been responsible for illness reported for some time [94], or the word "flavorings" is found without specifying *whether they are natural or artificial* [46]. Another imprecision has been introduced: "as necessary" refers to the ascorbic acid added to fresh and minced meat, but the use of ASA has no regulation. An even more serious point is the indication of the expiry date applied to easily decaying foodstuffs such as packaged milk-cheese products, which are therefore not naturally fresh: this simply means that the product is *enriched with food additives to ensure its use without immediate danger* (only for nonallergic individuals) by a given date, but with possible worsening of symptoms, as can be seen sometimes with CM products. We note that it is *not obligatory to mention the packaging date,* which should be necessary. Market research shows that many food-processing industries have complied with the obligation imposed by law [32] and therefore presently produce foodstuffs free of food additives, but in the pharmaceutical preparations a similar compliance is not always found [46, 68]. It is important to answer the fears of parents in a comprehensive and exhaustive way, who are often alarmed by nonscientific publications on the dangers of food additives, also to avoid that the child be unnecessarily subjected to a long series of laboratory examinations or even punitive diets that usually result in malnutrition [49]. We trust that the pertinent Ministry will intervene urgently, also highlighting the specific problem of nonfresh products, in order to protect the at-risk section of the pediatric population, following the example of the Swedish government that has prohibited the use of tartrazine [49].

As such data accumulate, it is likely that adverse reactions to food additives will rival or surpass the importance of adverse reactions to inhalants in clinical immunology and allergy [46].

Pharmacological Reactions

Pharmacological reactions are caused by chemical substances present in foodstuffs, naturally or as food additives, or locally synthesized in the intestine by microorganisms [56].

Pathogenetic Mechanisms

Histamine, one of the best-known and most active mediators of allergic reactions, and other vasoactive amines such as tyramine, dopamine, phenylethylamine, norepinephrine, and serotonin, present in high concentrations in certain commonly used foodstuffs, constitute the predominant substrate of food PA [100]. The amines, produced by bacterial decarboxylation, act directly or indirectly on the intestinal wall by releasing catecholamines; furthermore, they are synthesized by intestinal bacteria [66]. Mast cells in turn can release an abnormal quantity of chemical mediators by aspecific degranulation (non-IgE-mediated), in part due to the effect of substances called histamine releasers. Similarly, interactions between foodstuffs and the autonomous nervous system can take place [56]. Table 10.12 [20, 55, 56] summarizes the foodstuffs rich in histamine (and tyramine) and Table 10.13 some of the more common nonimmunological mast cell activators [21, 65]. Tyramine, a releaser of histamine from mast cells and basophils, has an exogenous origin (namely, foodstuffs) or is endogenously produced by the conversion of casein, serine, globulin into tyrosine, by means of pepsin and proteolytic intestinal enzymes, or it is produced by tyrosine-decarboxylase from intestinal bacteria. The basic oligopeptides and proteolytic enzymes act directly on mast cell membrane, whereas lectins cause degranulation by binding to the carbohydrates present on the Fc and activating specific IgE [66]. The type of diet can influence, either directly or indirectly, the intestinal content of histamine or of other biological amines. However, the fundamental pathogenic role is not only related to eating certain foodstuffs and therefore to the increase in intestinal levels (for example in fermented cheese, tinned fish, tuna, sausages, etc.), but also to the synthesis of excess histamine by the normal flora (for example of the colon) due to the abundant and repeated ingestion of foodstuffs rich in starch or cellulose [66]. A diet rich in carbohydrates indirectly increases intestinal levels, since the endoenteric bacterial degranulation, by eliciting irritating organic acids and gas, causes a disequilibrium in the intestinal flora. Foodstuffs rich in potato flour (potatoes, cakes, biscuits) favor histamine synthesis by fermentation [56, 90]. Some bacteria can trigger reactions similar to those that are IgE-mediated: for example *Proteus morgani* or *Klebsiella pneumoniae* possessing the enzyme histidine-decarboxylase of food or bacterial origin are able to produce histamine by decarboxylating histidine [82]. Normal levels are usually low; however, if these bacteria grow on foodstuffs rich in histidine, the production of histamine may be multiplied, for example when fish is contaminated with histamine-producing bacteria, especially when stored in the refrigerator [82]. With cheese, histamine is formed more slowly, an important requirement is the presence of histamine-producing bacteria [79].

Table 10.12. Main foods rich in histamine and/or tyramine of common use in children

Histamine-rich foods	Content in µg/g when known
1. Bouillon cubes	
2. Fermented cheese[a] (emmenthal, gorgonzola, Roquefort, parmesan, camembert, cheddar, gouda, brie, edam, etc.)	Up to 1330
3. Fermented drinks	20
4. Tinned foods	10–350
5. Vegetables	
Avocado	23
Eggplant	26
Pickled vegetables[a]	
Sauerkraut	160 mg/kg
Spinach[a]	30–60
Tomato (catsup)[a]	22
6. Meat	
Cured pork sausage	225
Meats	10
Pork liver	25
Salami, in general[a]	160–280
7. Fish especially when tinned or smoked	
Anchovy fillets[a]	44
Anchovy, fresh	
Herrings[a]	60
Mackerel[a]	
Salmon	7
Sardine[a]	16
Shellfish, fresh	0.2
Shrimps	
Tinned anchovy fillets[a]	33
Tinned smoke herring's eggs	350
Tinned tuna[a]	20
Tuna, fresh[a]	6

[a] Foods to be eliminated for a histamine-free diet [92].

Table 10.12. (Continued)

Tyramine-rich foods	Content in µg/g when known
1. Chocolate[b]	
2. Fermented cheese	
Boursalt	1116
Brie	0–200
Brick, natural	524
Camembert	20–2000
Cheddar	120–1500
Emmenthal	225–1000
Gouda	20
Gruyère	516
Parmesan	4–290
Provolone	38
Roquefort	27–520
Stilton blue	466–2170
3. Fresh cheese	
Cheese, white	Traces/none
Mozzarella	420
4. Vegetables	
Cabbage	
Cauliflower	
Eggplant	3
Fava bean	
Potato	1
Spinach	1
Tomato	4
5. Meat	
Beef liver (stored)	274
Chicken liver (stored)	100
Meat extracts	95–304
Ripened game	
Salami	
6. Fish	
Salted dried fish	0–470
Pickled herrings	3030
Caviar	
Tuna	
7. Fruit	
Avocado	23
Banana	7
Figs	
Grapes	
Orange	10
8. Miscellaneous	
Brewer's yeast	1500
Soy	1.75
Wine (red Chianti)	25

Data from [20, 55, 56].
[b] Chocolate contains methyltyramine.

Experimental studies in vivo, in healthy volunteers, either by instilling histamine directly into the intestinal lumen by the duodenojejunal tube, or before undergoing cholecystectomy, after instilling 1.75 mg/kg of histamine into the duodenum, resulted only in mild tachycardia and facial flush lasting <5 min. Repeating the test in patients with food PA, 60% noted pruritus, tachycardia, vasomotor migraine, etc., lasting for 10–30 min [57]. On the contrary, in healthy subjects, the ingestion of a large quantity of foodstuffs rich in histamine is not sufficient to evoke ill effects, given that histamine levels >50 g/100 g of food are necessary to elicit adverse reactions [85]. The susceptibility threshold can be reduced in certain patients, who thereby react to small amounts of mediator [105].

Under physiological conditions, several *protective mechanisms* exist in the liver and intestine, which restrain the passage of high concentrations of histamine into the circulation, thus explaining the low levels found in normal individuals (1 mg/ml) [55]. The first protective mechanisms are represented, in the intestine, by the mucins of the gastric juice with an elevated histamine-binding activity, by the glycoproteins of the glycocalyx and by bacteria capable of acting on the imidazole nucleus of histamine to split it; a second line of defense is due to the monoaminoxidase, diaminoxidase and methyltransferase enzymes, distributed throughout the gastrointestinal tract, which inactivate the histamine escaped from the first filter, and to histaminase present in lamina propria eosinophils; when necessary the hepatic filter comes into play whereby hepatic methyltransferase is activated; finally, the barrier formed by serum proteins with histamine-binding activity intervenes [55].

A second pathogenic mechanism could be represented by the local degranulation of intestinal mast cells, which are abundant in the lamina propria, due to the action of nonimmunological activators (Table 10.13) or to autochthonous instability, or finally to an increased threshold for the excitability of the histamine receptors.

Table 10.13. Main nonimmune activators of mast cells

Additives

Foods

1. High content of tyramine (see Table 10.12)
2. High content of histamine-releaser substances
   - Cabbage
   - Egg white
   - Fermented cheese
     - (gruyère, parmesan, camembert, etc.)
   - Fish (especially if canned or smoked)
   - Papaya and other exotic fruits
   - Peaches
   - Pineapple
   - Pork meat
   - Potato starch
   - Seafood in general
   - Shellfish
   - Strawberry
   - Tomato
   - Vegetables with high content of lecithin
     - Legumes (fava beans, lentils, peanuts, peas, etc.)
     - Nuts

Medications

1. Anesthetics, IV

   Antibiotics and chemotherapeutics
   - Chlortetracycline
   - Kinin
   - Neoarsphenamine
   - Neomycin
   - Polymyxin
   - Propamidine
   - Stilbamidine
2. Anti-inflammatory/antirheumatic
   - ASA?
   - NSAIDs?
3. Antihistamines
   - Chlorpheniramine
   - Cimetidine
   - Ranitidine
4. Substances acting on the circulatory system
   - Amphetamine
   - Antazoline
   - Apresoline
   - Atropine
   - Hydralazine
5. Blood derivatives and plasma equivalents
   - Dextran
   - Plasma expanders
6. Enzymes
   - Chymotrypsin
   - Phospholipase A and C
   - Trypsin
7. Iodinized contrast media
8. Muscle relaxants (tubocurarine and derivatives)
   Substances acting on the CNS
   - Apomorphine
   - Codeine[a]
   - Heroin
   - Morphine, etc.
9. Psychopharmaceuticals
10. Vitamins (thiamine)

Activators of the complement systems

Myelomatous proteins
Snake venom

Mucopolysaccharides

Neuropeptides

CGRP, NKA, SP

Animal and insect secretions

Chemical substances

A23187
Basic oligopeptides
Ca-ionophore
Compound 48/80[a]
Detergents
Disinfectants
Peptones

Physical stimuli

Physical exertion
Hypo- and hyperosmolarity
Sunlight
Pressure variations
Optic radiations
Temperature changes (warm, cold)

Venoms

Melittin

Additional substances

Organ extracts
Gammaglobulins
Vaccines

Data from [21, 65].
CGRP calcitonin gene-related peptide, *NKA* neurokinin A, *SP* substance P.
[a] Only at the skin level.

All these phenomena cannot be appreciated in the healthy individual, also because of the a lack of specific studies, and the notion that mast cells are particularly rich in histamine and ready for release brings us back to the previous mechanism. A possible interaction of intracytoplasmatic Mg has come under scrutiny, since a cellular Mg deficiency is observed in 45% of patients. In animals, the defect increased skin mast cell releasability [57]. However, it has been postulated that patients intolerant to histamine containing foodstuffs may have a diamino-oxidase deficit [92].

It is noted that in mast cells various substances can cause axonic reflexes through amyelinic fibers, thus releasing neuropeptides, including SP, capable of inducing histamine release from mast cells. This leads to an increase in epithelial permeability, in particular of the small subepithelial vessels, with increased passage of antigenic macromolecules. Histamine provokes a further SP release, closing the vicious circle orchestrated by neuropeptides and mediators: this mechanism, being also a characteristic of FA, is not specific to PA [55, 56].

Finally, food PA could be provoked by alterations in the regulation of histamine homeostasis dependent upon a deficit of defensive mechanisms; the possible causes can thus be derived from one or more of the following:

- Alterations in the intestinal mucosa, both histological and functional, that would allow an increase and anomalous passage of food macromolecules.
- Functional alterations of one or more of these mechanisms, in which a dysfunction of the monoaminoxidase or methyltransferase enzymes could be involved; a genetic-based anomaly of a mechanism underlying the predisposition to an increased and abnormal release of histamine, or to a hyperactivity to it [56].

As a consequence, each one of these dysfunctions creates the conditions by which, following the excessive introduction of specific categories of foodstuffs, a fertile ground for food PA is determined, with mechanisms which are at times direct, at times indirect. The intraluminal effects of histamine cause an increase in the absorption of histamine-releasing foodstuffs, with a consequent persistence of symptoms.

Clinical Presentation

Methylxanthines, including caffeine, and the vasoactive amines contained in more or less widespread foodstuffs (Table 10.12), above all histamine, which, being thermoresistant, can pass unharmed through the cooking process of the foodstuffs containing it [56, 90], can provoke migraine, palpitations, flush, urticaria, abdominal pains, etc. The methylxanthines, although possessing modest neurobehavioral effects, can stimulate the central nervous system and, by their effects, cause abdominal pains and temporary and/or continuous headaches [88]. Chocolate, which affects even breast-fed infants [81], in addition to tyramine, also contains phenylethylamine, which causes headache and theobromine with its theophylline-like effects. Therefore it must be immediately eliminated from the diet of migraine sufferers [21]. Triggering quantities of methylxanthine contained in common drinks and foodstuffs are summarized in Table 10.14 [35] and the effects of caffeine in Table 10.15 [24]: drinking too many cups of coffee can cause irritability, labeled as behavior disturbances [24]. Significant symptoms are also induced by tyramine: by an overload or an individual susceptibility, the pharmacodynamic properties are manifested in the form of vessel and smooth muscle fiber constriction, as well as release of histamine, provoking urticaria, abdominal cramps and vasomotor headaches [3]. Apart form the foodstuffs shown in Table 10.12, substances naturally contained therein, or derived from cooking or frying, have histamine-releasing activity: certain aliphatic aldehydes such as acrolein (produced from the decomposition of glycerin), formaldehyde, proteases such as bromelin and papain present in pineapple and papaya (cross-reactions), lecithins in legumes, cereals, nuts and peanuts [58].

Table 10.14. Significant amounts of methylxanthines contained in common foods/drinks

Food/drink	Amount	Equivalents (mg)	
		Of caffeine	Of theobromine
Cola can	350 ml	55	
Espresso coffee	75 ml	70[a]	
Cup of tea	200 ml	40[b]	
Chocolate bar	100 g		200

Modified from [35].

[a] Depends on whether it is a weak or black coffee.

[b] Depends on the time of infusion, after 20 min equals the caffeine content of a cup of coffee.

Table 10.15. Main effects of both coffee and caffeine

Increased vigilance to both simple visual and auditory stimuli
Decreased motor reaction time to both simple visual and auditory stimuli
Caffeine may slightly affect more complex tasks and fine motor coordination
Significant effects on the EEG spectrum and on visual and auditory evoked potentials
Coffee ingestion 30–60 min before going to bed often increases sleep latency, reduces total sleep time and significantly worsens subjective evaluations of sleep quality
Increase of stage 2 sleep and decrease of stages 3 and 4 sleep
The number of involuntary awakenings by a loud tone at stage 2 sleep is frequently increased after caffeine consumption
Pronounced effect on mood exercised by caffeine
Caffeine may lead to effects possibly misdiagnosed as an anxiety disorder

Data from [24].

A wide variety of symptoms can ensue systemic: shock, quite rare (>2% of cases); skin and mucosal: urticaria-angioedema syndrome (50% of cases), skin rashes; respiratory: asthma, rhinitis; gastrointestinal: dyspepsia, nausea, vomiting, diarrhea, abdominal pain; hyperactivity.

These disorders are usually observed in 3- to 8-year-old children, in particular when there is FHA+. Apart from typical histamine-dependent symptoms (urticaria-angioedema), there may also be exacerbations of an aphthous stomatitis or AD, in this case not due to FA. Since the eczematous skin is rich in mast cells (Chap. 7), histamine is released from this site in greater amounts than elsewhere and consequently itching will induce the child to scratch, thus aggravating the lesions. If histamine effects are not too pronounced, the child, once healed of the AD, will be able to eat the pertinent foods without provoking any cutaneous reactions. Instead the older child (>8 years) becomes progressively less sensitive to histamine, therefore incriminating foods can be ingested in normal amounts without causing adverse reactions. The patient will, however, maintain the (false) notion of being allergic to some foods that, if tested appropriately, will turn out to be negative [55]. Although adolescents and young adults who are monosensitized to pollens complained of urticaria, abdominal pains and itching in 20%–30% of cases, the symptoms were confirmed upon challenge in 5%, 2.5% and 0% of cases, respectively [9].

Diagnosis

Diagnosis is difficult because of the several foodstuffs rich in histamine, tyrosine, histamine releasers, frequently contained in snacks, orange drinks, ice creams, fish sticks, salamis, tinned foods, etc.

Treatment

Diets free of histamine and/or tyramine can be prepared according to Table 10.12; if the diet is followed regularly, the positive results are more frequent in children than in adults [68].

Enzymatic Reactions

Enzymatic reactions can also reproduce FA after ingesting particular foodstuffs [38]. They are typically seen in individuals with congenital or secondary enzyme deficits [3, 35, 53]. The most common is primary lactase deficiency, frequently seen in Afro-Asian children, while European children can develop a secondary deficit [40]. Apart from this deficit, all other congenital errors of metabolism are rare in this field [23]:

Table 10.16. Types of lactase deficiency

Types	Characteristics
Congenital	Present from birth, probably autosomal recessive, very rare
Acquired, late onset	Hypolactasia of various degrees, autosomal recessive, manifested in adolescents or young adults, marked racial predilection
Secondary	Transient hypolactasia secondary to small intestinal disease: celiac disease, malnutrition, immune defects, inflammatory disease, enteritis, etc.

Modified from [6].

- *Lactase,* an enzyme necessary for the metabolism of lactose found in CM, the only carbohydrate present in mammals' milk and absent in all other foodstuffs, *lactase deficiency* is rare in its congenital form, transient in premature neonates and more frequent as an acquired form in adulthood [13, 72] (Table 10.16) [6]. In infants it can result in gastrointestinal symptoms such as meteorism, flatulence, cramps and diarrhea, mistakenly attributed to FA in carriers of a *transient deficit* of the enzyme caused by an acute episode of infectious diarrhea [14], or in individuals with low levels of lactase (from the 3rd year of life to puberty), who present overlapping symptoms following the ingestion of modest quantities of lactose [3]. Diarrhea can occur in individuals lacking lactase or saccharase who abuse so-called dietetic foods [50]. By anticipating breast feeding of the premature child to the 4th day of life increases lactase [76]. The lactose breath H test may help to differentiate between symptoms caused by CMA and a lactase deficiency [35].
- The neonate affected by *galactosemia* may present vomiting and diarrhea from CM: early diagnosis is imperative [31].
- In breast-fed babies with *phenylketonuria,* occasional vomiting and irritability may be observed following meals [31].
- Malabsorption of carbohydrates can be identified by clinical symptoms such as watery diarrhea, abdominal distension, cramping pains and excessive flatulence because of the increased osmotic charge of the unabsorbed carbohydrates and gas production from colonic bacteria. Distension of the colon therefore ensues, with a consequent increase in motility and reduction of the transit time, a pattern often observed in *irritable bowel syndrome* [23]. Similar symptoms can be provoked by formulas enriched with modified starch [47].
- *Malabsorption of saccharose-isomaltose* and of glucose-galactose, both objectively rare, can also result in the outline of symptoms characteristic of disaccharidase deficiency [23, 48].

Table 10.17. Malabsorption presenting as food intolerance

Malabsorbed carbohydrate	Foods containing the malabsorbed carbohydrate	Clinical manifestations
Lactose	CM and dairy products	Lactase deficiency
Fructose	Fruits, soft drinks, berries	Malabsorption
Sorbitol	Peach, apple, pear, plum, diet foods, sugarless gum	Malabsorption

Modified from [48].
CM cow's milk.

- Comparable symptoms are also provoked by deficits in the metabolism of *fructose* and *sorbitol* (Table 10.17) [48].
- The increased use of both sugars as artificial sweeteners in infant formulas [47], packaged foodstuffs, especially low-calorie foods [22], and drinks and fruit juices [39] has multiplied the cases of intolerance and malabsorption.

Metabolic Reactions

Metabolic reactions are prevalently reactions in hypersensitive individuals; however, normal individuals who consume or chew excessive quantities of a given food or vegetable are also at risk [53]. On the other hand, food metabolic reactions are also observed after the ingestion of completely normal quantities of habitual, harmless foodstuffs in individuals who become sensitive following drug intake (monoamino-oxidase inhibitors, isoniazid) [31], to malnutrition, aspecific intolerance, or congenital enzyme deficits, as mentioned in the previous section [35]. In contrast, symptoms can manifest in well-circumscribed territories and in certain cases also in the general population [35]:

- *Avidin,* a vitamin agonist contained in raw egg white, can cause a dermatitis in individuals lacking biotin, but also a normal and balanced diet can sensitize these individuals [35] and the reactions can be mistakenly diagnosed as allergic.
- The presence of *amines* following an excessive ingestion of bananas, green tomatoes, avocados, or pineapple can cause sporadic headache and hypertension, while cheeses and chocolate can have a more serious effect [54].
- In areas with an endemic *lack of iodine* (I) the excessive ingestion of cabbage, turnips, radishes and mustard, containing goitre-producing substances (thiocyanates, goitrin, β-5vinylthiooxazolidone) can cause thyroid hypertrophy [37].
- *Elementary diets,* especially if rich in methionine, isoleucine or threonine, can cause nausea and/or headache [31].
- *Tryptophan,* an essential amino acid naturally present in many foodstuffs and a vasoactive mediator of serotonin, if taken in large doses as a therapy for insomnia, premenstrual pain, depression or weight loss, causes a syndrome which in an early phase presents myalgia, arthralgia, edema and eosinophilia of more than 1000 cells/ml and in the chronic phase scleroderma-like cutaneous involvement, peripheral neuropathy and diffused paresthesia [8, 77].
- Eating *spinach and carrots* and other vegetables, or drinking water and cured meats, in which nitrites are formed by the action of nitrate-reductase caused by high-nitrate-content fertilizers, long life at room temperature, especially if cut, may cause methemoglobinemia, as well as cyanosis. For higher levels anemic anoxia is seen [86].

Among the more metabolic aspects, serious effects on behavior (aggression, hyperkinesia, also severely antisocial behavior) and on the intelligence of children have been attributed to an excessive intake of carbohydrates [51], suggesting the existence of a possible allergy to sugar [51]. On one hand, studies conducted according to the DBPC methodology have cast doubts on the existence of such disorders [98] (Chap. 16); on the other hand, it has been demonstrated that these postulated effects of carbohydrates can be balanced by a normal diet of proteins [58]. Similarly, it has not been confirmed that hypoglycemia may cause states of delinquency [5].

Toxic Reactions

Toxic reactions (see "Immunotoxicology") are defined as such if recognized as harmful universally or limited to certain genetically predisposed individuals, but also healthy individuals, *especially children* [38], who present a particular intolerance. These reactions recognize an extraimmunological pathogenesis since the release of mediators is caused by a direct action on mast cells. They depend on natural toxic substances or contaminants (bacterial and chemical) present in foodstuffs and are classified into [53]:

- Natural substances distinguished into naturally produced endogenous substances and exogenous substances deriving from the earth, water, microorganisms, etc.
- Contaminants of foodstuffs, or otherwise toxic or infectious agents, or industrial agents (see "Immunotoxicology").

Toxic Endogenous Substances [35, 37]

- *Amanita phalloides*, whose ingestion, even in tiny quantities, causes potentially lethal effects.
- Digitalis (*Digitalis purpurea*), a plant with leaves and seeds with well-known effects.
- The ingestion of calderine grass (*Senecia vulgaris*) and of the heliotrope (*Heliotropium*) in fields and uncultivated earth, rich in pyrrolidine alkaloids (dihydropyrrols): indirect exposure derives from honey when bees collect pollen from such plants or the milk of cows that graze on toxic weeds, or for an involuntary substitution of herbs for infusions with *Senecio longilobus*; the target is the respiratory apparatus [86].
- Fruits and leaves of the *wild apple tree* (*Datura stramonium*) causing hallucinations [53].
- The ingestion of *rhubarb leaves* (rather than the stems) causes a serious corrosive gastritis due to high oxalates and glycoside content [26].
- *Peas and parsley* contain photosensitizing psoralens and nutmeg and carrots contain myristicin, which are perhaps hallucinogenic [21].
- We point out for tourists under 15 years of age that there is a possibility of an intoxication (toxic hypoglycemic syndrome) caused by the ingestion of unripe fruits of the widespread *akee tree, Blighia sapida*, in Jamaica and western Africa. The fruits contain hypoglycin A, a hepatic toxin with hypoglycemic activity and an inhibitor of hepatic gluconeogenesis, and with an effect that limits cofactors for the oxidation of long-chain fatty acids. Symptoms consist of hypoglycemia, profuse vomiting, convulsions and coma with an occasionally fatal outcome.
- Children's hand-to-mouth behavior can turn out to be negative only if they suck or chew even tiny amounts of *leaves, fruits or berries* present at home, in the garden or in the countryside, such as holly, azaleas, hawthorn, eucalyptus, ficus, lily of the valley, oleander, hydrangea, poppy, potus, and rhododendron, with clinical characteristics (diarrhea, vomiting, edema of the glottis, hypotension, arrhythmias, apnea, convulsions, coma, etc.) that require *clinical observation.* Other rarely toxic plants include cyclamen, ivy, philodendron, geranium, mimosa, Christmas star, tulip, mistletoe (but with analogous clinical manifestations and need for clinical observation) [11, 30]. Just touching several plants can cause itching, intense pain, localized or diffused dermatitis, while the ingestion of a yew berry can cause the child to die [11].

Furthermore, the general public ignores the duplicity of the foodstuff, harmless for some, poisonous for others [16]: that the very same natural foodstuffs contain toxins [26] or, if eaten daily, can become toxic if cooked in a different way, or also if abused [35, 37].

- Following ingestion of large quantities of cabbage, turnip and other vegetables of the same family, even when not living in regions lacking I, goitrogenic compounds are ingested, such as thioglycosides [37].
- Foodstuffs containing *cyanogenic glycosides* (lima beans, cassava roots, millet sprouts, the pits found

Table 10.18. Toxic substances present in vegetable foodstuffs

Substances	Chemical origin	Food sources	Main effects and symptoms
Agaritrin	4-hypoximethylphenylhydrazine der	*Agaricus bisporus*	Mutagenic effects
Alkaloids	N,N,-dimethyltryptamine	*Mucana pruriens*	Hallucinations
Cycasin	Methylazoxymethanol β-glycoside	*Cycas revoluta*	Carcinogenic
Favism factors	Pyrimidine-β-glycoside	*Vicia faba*	Hemolytic anemia in G6PD deficients
Furocoumarins	Benzofurans (psoralens)	Celery, citrus fruits, figs, parsley	Photosensitization
Gossypol pigments	Gossypol	Cotton seeds	Hepatic damage, hemorrhages
Hemoagglutinins	Proteins (MW 10,000–124,000 D)	Legumes	In vitro erythrocyte agglutination, reduced nutrient absorption
Protease inhibitors	Proteins (MW 4,000–24,000 D)	Cereals, legumes, potatoes	Inhibit protein utilization, pancreas hypertrophy, growth retardation
Lathyrogens	β-Aminopropionitrile, diamino-propionic acid derivative	Chickling (*Latyrus sativus*) (legumes)	Skeletal deformities, myalgias, paralysis of the lower limbs
Saponin	Glycosides	Asparagus, beans, peanuts, soy, spinach	Hemolysis

Data from [37].
der Derivative, *G6PD* glucose-6-phosphate dehydrogenase, *MW* molecular weight.

in some edible fruits such as apricot, cherry, bitter almond, apple, pear, peach, plum, perhaps also chick peas, maize, sweet potato, etc.), nontoxic *per se* but when, on contact with digestive enzymes and the β-glycosidase of the bacterial flora, they are metabolized and release cyanuric acid in the stomach in variable amounts, from 0.003% to 3.6% depending on origin. With *chronic ingestion,* cytochrome oxidation is inhibited with suppression of cell respiration, resulting in ataxic neuropathy, blindness and death. As far as fruit seeds are concerned, it is sufficient for a child to ingest two to five seeds to put his life at risk and ten bitter almonds to cause death [11, 30]. The toxic effects can be eliminated by thorough cooking, avoidance of eating large quantities, or cultivating varieties with lower content of these toxins [50].

Commonly eaten foods may be toxic if excessive quantities are consumed [35, 37]:

- *Green potatoes* have high concentrations of glycoalcaloids, including solanine, with an algicide part, considered very toxic (symptoms: headache, incoherence, hallucinations, vertigo) [95].
- Substances that are considered harmless such as *licorice* can cause hypertension, water and Na retention if abused.
- Foodstuffs consumed in uncommon ways: insufficiently cooked *raw red beans* can result in gastroenteritis [35].

In Table 10.18 [37] other naturally toxic substances present in foods are listed.

Exogenous Toxic Substances

Exogenous toxic substances (see the immunologic effects in Table 4.17) are produced during the phases of storage and preservation of foodstuffs, during which mycotic contamination is almost unavoidable. They include the mycotoxins [37]:

- The *aflatoxins,* toxic products of foodstuffs with an elevated sugar and grease content, contaminated with mycetes of the *Aspergillus* species, even for fraudulent reasons [26] such as badly preserved dried fruit and cereals, as well as peanuts, figs, wheat, corn, almonds, millet, hazel nuts, walnuts, Brazil nuts, pecans, filberts, coconut and pistachio nuts [35]. Symptoms of intoxication are anorexia, weight loss, jaundice, encephalopathy (Reye's syndrome), hallucinations, etc. [53].
- *Patulin,* produced in particular by *Aspergillus* and *Penicillium*, is found in fruit and fruit juices. It produces acute and chronic toxic reactions.
- *Metabolic products* of the mycetes *Fusarium*, *Trichoderma*, *Cephalosporium*, etc., contaminate millet, barley and wheat, the pathological effects are headache, vertigo, shivers, nausea, vomiting, diarrhea and visual disturbances.
- If *mycotoxins* contaminate cereals, residues can pass into meats and the milk of the animals that consume them [37].

Toxic-Infectious Contaminants

The following are considered as pathogenic agents: microbial toxins, or organisms that, contaminating foodstuffs, cause symptoms of poisoning, often confused with those of FA:

- *Bacteria and their toxins: Clostridium botulinum, Staphylococcus aureus, Vibrio cholerae, Campylobacter jejuni* [35];
- *Scombroid fish poisoning* caused by the ingestion of tuna, mackerel and similar fish that is not fresh or is poorly conserved, or even tinned, with rapid symptoms (15–60 min) of giant angioedema urticaria, tachycardia and anaphylactic shock, absolutely indistinguishable from IgE-mediated reactions but with rapid improvement after therapy, in which high concentrations of histamine are seen from histidine decarboxylation, caused by aerobic and anaerobic microorganisms [59]. It has been challenged that histamine is not involved with the active scombroid fish toxin [19]; however, it is clear that the toxin is involved in increasing the toxic power of histamine [85]. Other symptoms involve the gastrointestinal apparatus: nausea, vomiting, and diarrhea. Sometimes disturbances of the cardiovascular system are present, hepatic toxicity, neuropsychic disturbances, etc. [59].
- Eating other fish products can also turn out to be toxic [37, 86]:
- *Lamellibranchs* such as oysters, mussels, clams and similar molluscs acquire toxicity from marine algae [31].
- *Tetrodotoxin,* contained in the liver and ovaries of certain species (among which the puffer fish, *Arothron hispidum*), causes oral, lingual and pharyngeal paresthesia, nausea, vomiting and diarrhea, in certain cases complicated by ataxia, and respiratory paralysis, which results in death in approximately 60% of cases.
- Poisoning by eating *crustaceans contaminated with dinoflagellates,* producers of a powerful neurotoxin, saxitoxin, produce muscular paralysis (the toxin binds to Na channels in nerve cell membranes and blocks neurotransmission).
- *Red seaweeds* contain carrageenan, which may be used in low-fat hamburgers: it is toxic in experimental animals, and especially immuno-suppressive, accumulating in macrophages [86].

It should be pointed out that only 1% of sea creatures have been studied for their chemical properties and potential harmfulness.

Finally, vitamin excesses can cause the following [35]:

- *Vitamin A:* irritability, vomiting, endocranial hypertension and death.

- *Vitamin D:* anorexia, nausea, vomiting, diarrhea, migraine, polyuria and polydipsia in acute form; edema, pallor, weight loss, fever and constipation in chronic form.
- *Vitamin K:* in pregnancy, hyperbilirubinemia in premature neonates. Other food sources of this vitamin are represented by turnip, lettuce, cabbage and spinach.

Diagnosis

It is necessary to stress the past history (if the child is old enough) as well as the present history, in as much as the harmful action of toxins, even in minimal quantities, often lasts a long time. It is necessary to examine the possible correlation between food habits (with preference to choice as much as cooking methods), the purchase from usual or occasional suppliers, whether there has been refrigerated storage, possible exposure in restaurants or school meals, the exposure to environmental chemical substances and the development of symptoms. Certain foodstuffs such as plums and other fruit, onions, cucumbers, beans, etc., can induce gastroenteric symptoms, often diarrhea, by irritation rather than pharmacological action and they are to be considered in a differential diagnosis with FA [54], in the same way as scombroid fish poisoning [59]. Lactose intolerance, apart from occasionally being a confusing factor in the diagnosis of FA, can mimic irritable bowel syndrome; even when the two disorders coexist in the same patient, it is appropriate to treat the primitive disorder of motility [14]. Laboratory examinations are chosen based on symptoms, if necessary using the FPT and investigating organs and function also from an immunological point of view [53]. Such investigations also become significant for the *differential diagnosis,* which should aim to identify the mechanism in action and the exact background of cutaneous and gastrointestinal symptoms, so as to make the evaluation of the only possible cause possible [29]. The distinction is also based on time courses: while anaphylactic reactions explode only a few minutes after ingesting the foodstuff, the urticarial reactions manifest within a few minutes to a few hours and the toxic reactions within minutes to several hours. As a comparison, gastrointestinal disorders require several hours to manifest and an infection 8 h to several days [57].

Anaphylactoid Reactions

Anaphylactoid reactions are reactions clinically similar to anaphylaxis [104], caused by the excessive ingestion of foods and/or food additives. Basically they are non-IgE-mediated mechanisms: they can also be caused by pharmacological, metabolic and toxic reactions to foods [101]. They are dealt with in Chap. 20. For the differential diagnosis, see Table 10.19 [35].

Table 10.19. Diagnostic clues for food-related anaphylactoid reactions

IgE-mediated food allergy
IgE-mediated allergy to a given food
IgE-mediated allergy to a food contaminant (such as, penicillin)
Food pseudoallergy
High histamine levels in the food
Scombroid fish (tuna, mackerel)
Raw or canned fish contaminated by *Proteus* or *Klebsiella*
Cheese contaminated by *Proteus* or *Klebsiella*
Sulfites
Monosodium-L-glutamate
Idiopathic anaphylaxis

Modified from [35].

Immunotoxicology

By the term "immunotoxicology" we mean the study of the immunotoxic potential of chemical substances that are foreign to the body (xenobiotics) on the immune system (Chap. 4): this is the discipline that organizes the toxic effects of environmental agents to different components of the immune system [7]. Such agents include all components of the environment external to the human body, from the most simple chemical compound to the most sophisticated microbial agent. Immunotoxins are included among substances consumed, *even in low doses,* for long periods of time, such as those considered as foodstuffs, either intentionally (food additives) or involuntarily (contaminants), with potential risks to the immune system [18]. In fact, the most important characteristics of xenobiotics cannot be imagined: immunotoxic substances exercise their effects at doses notably lower than those normally expected [7].

Various actions and other mechanisms are present, often with toxic consequences: accidental contaminants of foodstuff may also include heavy metals (Hg, Cu, etc.) or others such as Al, As, Cd, Co, Mn, Mo, Pb, Se, etc.; symptoms of poisoning are caused when foodstuffs or acid drinks are prepared in containers made of Cu, Sn or galvanized [7]. Refer to Table 4.12 for the probable effects of xenobiotic immunotoxins on the immune system and Table 4.18 for the environmental agents that compromise the immune system of experimental animals [73].

We briefly review the effects of *industrial contaminants* or rather toxins produced during production processes [53]:

- *Heat:* carcinogenic substances
- *Decolorants:* methionine sulfoxime (produced by proteins containing methionine treated with NCl)

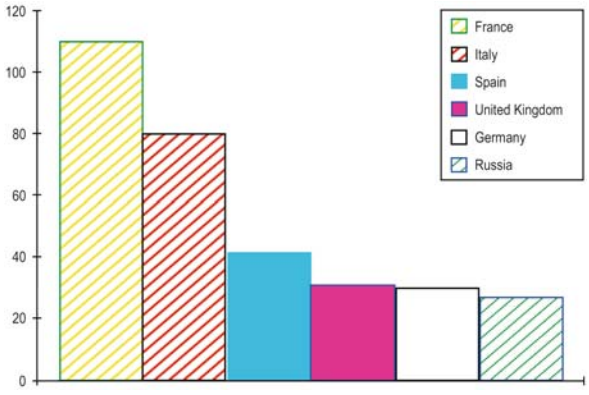


Fig. 10.5. Pesticide use in some European countries ($t \times 10^3$). (Data from FAO)

- *Industrial solvents:* trichloroethylene
- *Sterilizers:* ethylene oxide

A particular case concerns polychlorinated dibenzofurans and diphenyls [86], once used widely as electric insulators and for resistance to heat: only several years later was the toxic potential of *dioxin* to man and the environment realized, also for the prolonged existence *in loco* and the incorrect incineration of home and industrial products; less known is that *smoking is a third source,* for the whitened paper of the cigarette, or for the pesticide used to treat the tobacco. The accidental contamination of rice oil with a commercial diphenyl has caused an epidemic in Japan know as *"oil disease"*, with skin alterations, blepharo-conjunctivitis and, in the lungs, asthma-like symptoms [86].

- Toxic contaminants [53, 73] (Tables 4.7, 4.8, 4.17)

Pesticides (organic phosphates), especially persistent organic pollutants (POPs) (Table 4.21), also used as herbicides and insecticides, may be responsible for liver and central nervous system symptoms, as well as alterations in immunoglobulin levels. Pesticides, with Italy the second largest user in Europe (Fig. 10.5), are found on fruits and vegetables, especially on those without a smooth surface, such as strawberries, or with thick skin such as citrus fruit, *Cucurbitaceae,* etc. We point out the notable toxicity of diphenyl and orthophenol, used as preservatives on the external surface of citrus fruits, bananas and cheeses, even though they should not penetrate into the pulp of the fruit. However, the peel of citrus fruits cannot be used without checking prior to the production of cakes, jams, ice creams and various other foods.

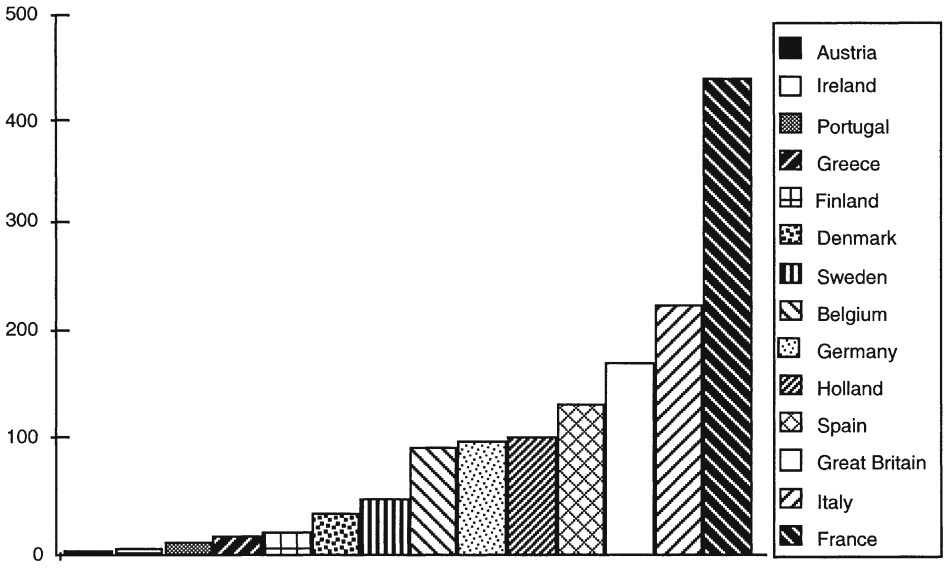
Herbicides (compounds with a chlorophenothane base) can induce neuropathy, and fungicides (pentachlorophenol) can induce hepatopathy. As far as paraquat is concerned, the risk of ingesting contaminated foodstuffs is remote as long as the edible plants are disinfested with truly nontoxic products [86]. A further molecule, lindane, caused an alarm when foodstuffs were found that contained quadruple levels of it: this is to be ascribed to the uncommon persistence of the polluting agent and it is strange that its use has not already been banned by the authorities.

Animal-based drugs (penicillin) are present in traces in meats, CM and cheese products.

Minerals such as Ni, frequently found in foodstuffs (Chap. 8), yeasts and molds that contaminate fruit, vegetables and cereals [28].

In Tables 1.78 and 1.79 it is noted that *genetically modified foods* (GMFs) are obtained from plants modified for protection from insects and tolerance to herbicides, as well as from plants and substances defined as nontoxic to man that deactivate the herbicides. Long-term studies are necessary on humans to evaluate the safety of such foods. Figure 10.6 illustrates how widespread GMFs are.

Fig. 10.6. Number of fields where transgenic cultivations are experimented. To be noted the second place of Italian fields. (From Legambiente, personal communication)



Food Immunotoxicology

In the intestine, the ingested immunotoxins theoretically have the capacity of harming the GALT, in particular in the mucosa, which, for more or less prolonged chronological periods, can come into contact with potentially high levels of xenobiotics during the processes of absorption, altering the local immune response and thereby favoring the setting off of allergic reactions, as well as increasing susceptibility to infection [18]. It should be noted that these products are capable of polluting and, in the case of environmental accidents, affecting the mother's milk, with understandably serious consequences [70]. *Four out of 14 neonates* have presented hemorrhagic forms in the first 4 weeks of life: the *breast milk had levels of dioxin* (TCDD) that were significantly higher than those in the other ten children [45]. In addition to the TCDD, there were also several halogenated pesticides and industrial chemical products [71]. Food safety is threatened by TCDD derived from animal feeds, formed from fats of dubious origin (perhaps even *residues of car oil*) and from ground *animal carcasses;* the permitted levels are unknown. Table 10.20 [70] shows the relationship between concentrations of certain xenobiotics in maternal milk and blood measured in certain Italian areas (see also Chap. 21). In general, the levels that pass into breast milk are reassuringly lower than the concentrations present in the maternal plasma; however, atopic breast-fed children can have immunotoxin levels that are four times higher than those of nonatopic subjects [97]. The levels present are usually very low and somewhat far from the doses considered dangerous, and depend on various factors, including the dose of xenobiotics received by the mother, the absorption, the distribution, the metabolism and the elimination of the single compounds, both regarding the mother and the breast-fed neonate [69]. Therefore, although sometimes there have also been *prenatal exposures,* there is little evidence to suggest that toxic effects are seen in infants [71]. The negative effects of the POPs are represented by their wide distribution, for which they can be found in areas distant from the place of release (Chap. 4). Let us re-examine, under an immunotoxicological profile, a few xenobiotics for which we have listed the clinical effects in Table 4.3, while in Tables 10.21 and 10.22 the immunotoxic effects of some food components are listed [18]. In this context, even more harmful effects are experienced by those individuals weakened by malnutrition (Chap. 21).

Table 10.20. Xenobiotics in breast milk above detection limits

Caffeine	0.8
Chloramphenicol	0.55
Ethanol	0.9–0.95
Iodum^{131}	65
Nicotine	0.17
Streptomycin	0.5–1.0
Theobromine	0.7
Theophylline	0.7

The high iodum^{131} value in milk/plasma rate means that iodum, due to its specific properties, tends to accumulate in breast milk.
Data from [70].

Diagnosis

From the patient's history, particular activities can be revealed, or places frequented may be discovered, or particular experiences, restaurant meals, etc. This may be the beginning of a careful investigation.

The immunological investigations to be carried out include in general:

- SPTs

Table 10.21. Immunotoxic effects of some food components

Substance	Examples[a]	General effects
Additives	BHA, BMT	Reduced T-dependent humoral response
	Carrageenins	Increased IgE response
	Na benzoate	Reduced aspecific defense (phagocytosis, intracellular killing)
Contaminants	PBB	Numeric reduction of B and T lymphocytes
	DES	Reduced macrophage function
	Dioxin	CMI reduction (neonatal exposure)
	Malathion	Immune response suppression
Artifacts	Benzopyrene	Altered immune response
	DMB	Reduced T-dependent humoral response

[a] *BHA* Butylated hydroxyanisole, *BMT* Butylated hydroxytoluene, *DES* Diethylstilbestrol, *DMN* Dimethylnitrosamine, *PBB* Polychlorinated biphenyl compounds
Modified from [18]

- Lymphocyte counts and their subpopulation (Tables 1.33–1.34)
- Lymphocyte stimulation
- Immunoglobulins (Table 1.15)
- Specific antibodies (tetanus, pneumococcus, etc.)
- Bactericidal activity
- T lymphocyte-mediated cytotoxicity
- Macrophage functionality
- Complement

Treatment

Apart from the interventions for pathological forms, which are dealt with according to the organ or apparatus involved, the best kind of therapy is *prevention,* based on one hand on the strict control by the governing organisms involved [32] and on the other on the renunciation of "foodstuffs that man invented departing evermore from the natural state simplicity" [17], prophetic words even more significant today.

Pediatricians, Pseudoallergy, and Immunotoxicology

Nonallergic adverse reactions to foods may account for the majority of food-related reactions, and children may be more vulnerable than adults. Children face risks that were neither known nor imagined decades ago. Although recognition of PA dates back to several years ago, a wealth of clinical data suggests that the constellation of food additive intolerance represents an emergent PA manifestation, and a relevant pathogenetic factor, commonly affecting children and adolescents. Considering the harmful effects of food additives on health benefits, one intriguing challenge should be to interpret the medical consequences of a directive of the European Union that has liberalized the use of additives even in foods for neonates, infants and toddlers. However, governments should critically and regularly update food labeling legislation, inducing producers to specify the food additives employed, abolishing the general wording "permitted by law." Pediatricians and primary-care physicians are a front-line source of information and guidance for families, and are thus in a unique position to suggest that the parents of children affected with atopic disease exercise meticulous control on foods and beverages for their children, referring children and adolescents in whom PA is suspected to specialized hospital departments, to ensure an exact diagnosis and appropriate treatment. Several pharmacological, enzymatic, metabolic and toxic reactions may be misleadingly labeled and therefore they need an accurate setting. Moreover, immunotoxicology is concerned about an emerging issue, the potential hazards of transgenic foods while it cares for breast milk, the most natural of all foods.

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Asthma

Pediatric Asthma

Asthma has been recognized as a disease since the earliest times. In the *Corpus Hippocraticum,* Hippocrates used the term "ασθμα" to indicate any form of breathing difficulty manifesting itself by panting. Aretaeus of Cappadocia, a well-known Greek physician (second century A.D.), is credited with providing the first detailed description of an asthma attack [13], and to Celsus it was a disease with wheezing and noisy, violent breathing. In the history of Rome, we find many members of the Julio-Claudian family affected with probable atopic respiratory disorders: Caesar Augustus suffered from bronchoconstriction, seasonal rhinitis as well as a highly pruritic skin disease. Claudius suffered from rhinoconjunctivitis and Britannicus was allergic to horse dander [529]. Maimonides (1136–1204) warned that to neglect treatment of asthma could prove fatal, whereas until the 19th century, European scholars defined it as "nervous asthma," a term that was given to mean a defect of conductivity of the ninth pair of cranial nerves.

If, from a clinical point of view, asthma can be defined with a fair degree of precision, much doubt still surrounds its etiopathogenesis, although the important role played by inflammation has been largely clarified. To this day, asthma still retains the characteristics of frequency and unexpected severity that can negatively affect a child, leading to considerable concern in the family. Asthma begins early in life [305] within the 1st year of life (Table 5.5) with the developing immune system interacting with environmental influence [305]. Moreover, cases of pediatric asthma are increasing, as can be seen from epidemiological figures (Tables 5.10, 5.12), even though cases may be differently labeled, especially when symptoms are mild or moderate, and subsequently often *underdiagnosed* [446]. A large body of epidemiological evidence shows that allergic disorders in the pediatric population represent a current major therapeutic and preventive challenge for pediatricians. The surprising upsurge of severe asthma is occurring mainly in infants and children (Figs. 5.1, 5.3, 5.8). Until recently, asthma was defined as a disease fundamentally characterized by a state of bronchial hyperreactivity (BHR). In the last few years, it has been concluded that BHR and bronchoconstriction could be the result of the inflammation primed by Th2 T cells [340]. This change in our understanding has stimulated new lines of research and led to a new therapeutic approach [340]. Progress has derived from information gained by new investigative methods such as the study of bronchoalveolar lavage fluid (BALF) cells, using immunohistochemical and molecular biology techniques, and widespread use of more precise fiberoptic bronchoscopy, especially in children [501]. Asthma is a disorder *involving all bronchial structures* and depends on a complex interaction between the respiratory tract and inflammatory cells, mediators and adhesion molecules. Release of mediators by metachromatic cells primes both activation and migration of inflammatory cells that cause various degrees of airway obstruction over relatively short periods of time, alterations in the mucociliary system and hyperreactivity of the bronchial smooth muscles. It follows that both inflammatory cells and their products play a key role in provoking an airway inflammation, capable of triggering severe symptoms in predisposed children when specific and aspecific stimuli are present. *Pediatric asthma differs from adult asthma by its etiopathogenesis, treatment and prognosis.* Studies reporting that the future prospects for treating asthmatic babies and children are optimistic [604] have not taken into account the severity of childhood symptoms [264]. The understanding that events during both fetal and postnatal life may be the major determining factors of chronic asthma is also making progress [589].

The classic concept of asthma is that of a chronic inflammatory disease with a multifactorial pathogenesis, characterized by a state of hyperresponsiveness to various stimuli and a level and intensity that for the most part do not cause disturbances in healthy subjects, which consequently leads to a diffuse airway obstruction, either partially or fully reversible, either spontaneously or following treatment. The confusion still existing in terminology (Chap. 5), the reluctance of doctors to use the term "asthma" and of parents to accept it, *serve only to delay the diagnosis and treatment*. It has long been debated whether two forms of asthma exist: allergic, or extrinsic, and infection-induced, or intrinsic. Some [61] have not confirmed the existence of the latter and others [552] have not found any difference between the IgE values in normal subjects and in subjects with so-called intrinsic asthma. Probably many patients thus classified may be sensitized to unknown

allergens and/or are not included in the usual allergen battery of tests. For example, patients from Catalonia showed 8.5% of sensitization to an unusual pollen of the profilin family, perhaps an emerging pollen (Chap. 6). This chapter also includes bronchiolitis, extrinsic allergic alveolitis (EAA), and allergic bronchopulmonary aspergillosis (ABA).

Definitions

Asthma. To date asthma is a *severe pediatric disease,* affecting a great number of infants during their very first years of life (Table 5.5), which requires early and specific cures. Pediatric asthma should be viewed as a syndrome of lung dysfunction with an imbalance between the forces that maintain airway patency and those forces that operate to narrow or close the pediatric airway [274]. Apart from symptomatic management, pediatric asthma can be cured by anti-IgE, antileukotriene drugs (anti-LT) (see "Leukotriene Modifiers") and SIT (specific immunotherapy) or respiratory desensitization.

Canny and Levison have suggested that any child, in any age group, who has ≥3 episodes of afebrile bronchospasm should be considered as suffering from asthma until the contrary can be proven [79]. This is the definition we and others also prefer [574]. However, the number of asthmatic episodes is not always a helpful guide: 50% of children suffering ≥4 attacks at age 4–5 years had no symptoms by the time they reached the age of 10 [471]. Asthma is a chronic lung inflammation, whose characteristics may be summarized as an acute onset of symptoms with bronchoconstriction (clinical data), reversible either spontaneously or with appropriate treatment (pharmacological data) [465, 594], accompanied by BHR to diverse stimuli (functional data) and by an inflammation of varying degree, which conditions its persistence, duration and severity (biological data) [465, 739]. Asthma can be clinically defined as an *airway response to different stimuli, with paroxysmal dyspnea, wheezing and coughing, variable in form from mild to severe,* or ultimately status asthmaticus [594]. Asthma is characterized by lymphocytes and eosinophils infiltrating in the submucosa. chronic airway inflammation leading to BHR, mucous gland hyperplasia, microvascular leakage, mucus hypersecretion, thickening of the subepithelial collagen layer, epithelial desquamation, mast cell degranulation, airway tissue hyperplasia and hypertrophy, causing variable airflow obstruction [31, 739] that requires long-term recovery.

Wheezing. Wheezing is an onomatopoeic word reproducing the sound made by air rushing through narrowed airways in a flow that is no longer laminar but turbulent, which may also be defined as wheezing and/or dyspnea.

BHR. BHR is the particular ease with which the bronchi constrict in response to stimuli [154]: specific stimuli, the allergens, which provoke bronchospasm in a restricted number of subjects defined, therefore, as allergic; aspecific stimuli cause wheezing even in nonallergic children [603] suffering from bronchial disorders of various types [722] and even in healthy children, if the intensity is at least fivefold stronger than in asthmatic children [335]. BHR is therefore dependent on a markedly lowered threshold of the bronchial response to causative factors, which results in exaggerated bronchoconstriction, following which the bronchi constrict too readily and excessively [154]. Bronchoconstriction depends partly on inflammation, partly on smooth muscle spasm, on the mucosal edema and on the modification of the secretions, with formation of viscous plugs that often fill the bronchioles [255]. Some authors, in consideration of the fact that BHR occurs in the presence of nonallergic stimuli [636], propose the synonym "aspecific bronchoreactivity," which, however, ignores the specific forms [179, 481]. In fact, nonallergic stimuli work through specific, though different, mechanisms, so it seems imprecise to apply such terminology. We believe that it seems clearer to define BHR by the agent that provokes it [238].

Prevalence

Asthma is the most prevalent chronic breathing disease that affects subjects of all pediatric ages, but begins in the first few years. In 85% of cases, it begins in children by the age of 1 year, 80%–85% by 5 years of age (when statistics begin) and 94%–97% within 10 years. This is confirmed by 10.8% of new cases in the 2nd decade of life (Table 5.5); therefore, 90% of cases occur in infancy, considering that sensitization to inhalants is established very early [98] and that cases of sensitization from the 1st year of life onward occur very frequently. During 1991, there were approximately 1.6 million visits for pediatric asthma care. Asthma accounted for 16.9%±9.0% of all pediatric emergency department (ED) visits [121]. During 2000, a nationwide survey among 437,873 Chinese children aged 0–14 found a 1.69% prevalence in children aged ≥3 years and of 0.23% in those aged ≤3 years [100]. Among these children are those with AD (atopic dermatitis) who in 21%–79% of cases, subsequently develop asthma (Table 5.8). The prevalence is also increasing as in our study in 592 children compared to controls (Chap. 5), documenting an 88.8% increase in severe pediatric asthma. In 411 children (220 males and 191 females) ranging in age from 7 to 13 years, we have found asthma in 31.5% and AR in 25.8% of them. Respiratory allergy affects children even more than food allergy (21.7%). Moreover, pollutants represent an adjuvant factor in the onset of respiratory allergy [521] (Figs. 4.26, 4.27) and we wonder whether similar interactions increase its prevalence.

Defense Mechanisms in the Airways

BALT (*Bronchus-associated lymphoid issue*) and Specific Defenses

The respiratory apparatus's first and qualitatively most notable line of defense against excessive local immune responses is the defensive barriers, both physical and immunological, that range from safeguarding the tight junctions among epithelial cells to IgA secretion in the bronchial lumen. The respiratory epithelium is subjected to a virtually uninterrupted exposure to environmental antigens present in the 8,000–12,000 l of air inhaled daily by an average person [520]. The key factor in the maintenance of airway immunological homeostasis is the immune system's ability to discriminate between intrinsically inoffensive antigens and/or those associated with pathogenic microorganisms, and the exclusion of the latter from the respiratory apparatus by the mucociliary system censoring mechanism, before they reach the underlying immune system. The defenses are not always well equipped and penetration of small quantities of allergens can be viewed as a normal occurrence [533].

Parallel to that which has been described in Chap. 9, a *common mucosal immune system* (MALT) exists, to which BALT is connected, located in the respiratory mucosa [40]. It has been hypothesized that in newborn babies and healthy children BALT is absent, or at least reduced [54] and that it could be an inducible system that develops in the airways of infants and children, especially in concomitance with exposure to antigens and pathogenic agents [193]. Several studies on humans [40] have, however, confirmed the existence of BALT. Anatomically, it consists of a well-developed lymphatic system originating from the NALT (*nasal-associated lymphoid tissue*) at the airway entrance, and continues with follicles and submucosal lymphoid aggregations distributed along the whole respiratory tract up to the bronchoalveolar junction [40] (Fig. 11.1). The tissue is covered by a layer of flat epithelial cells, with few B lymphocytes, but essentially with T lymphocytes ($CD3^+$, $CD4^+$ or $CD8^+$), grouped in clumps of lymphoid and follicles distributed in a seemingly homogeneous manner over the entire epithelial layer in the main bronchi of the pulmonary lobes, principally at the bifurcation with the bronchioles [193]. The most common lymphocytes are *intraepithelial* (IEL), situated close to the basement membrane (BM), generally not affected by pathological changes typical of epithelial structures and, as with GALT, CD4 T cells dominate over CD8 T cells [193]. Even in the lymphocytes distributed under the BM of the epithelial layer, T subsets are more numerous than B cells [193]. Specific to IEL is CD103, widely expressed on T cells, of which up to 80% are CD45RO. As in the intestine CD7 expression is frequent; expression of CD25 is lower [520]. The TcR with γ/δ chains plays a prominent

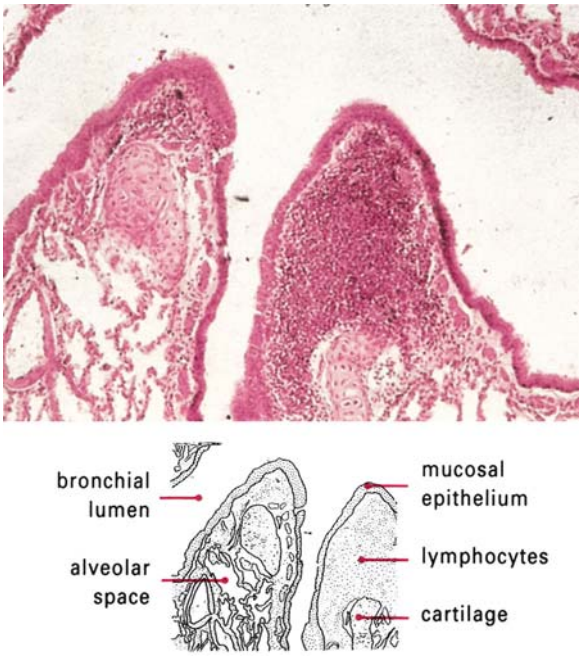


Fig. 11.1. BALT. Section of lung showing a diffuse accumulation of lymphocytes in the bronchial wall

role in the pulmonary *first line of defense* [250], and there is greater evidence, on the other hand, of TcR αβ, which display a large number of Vβ genes [520]. Seen in this light, it is important that in asthmatics the γδ are reduced in the circulation parallel to eosinophil increase, even if the two phenomena seem to be unrelated: nevertheless, low γδ levels in the airways could be detrimental (see Chap. 1 for further details).

The lymphocytes passing from blood flow to the airways reach BALT, devoid of afferent lymphatics adhering to the HEV (high endothelial postcapillary venules), which in BALT function similarly to the mesenteric lymph nodes where B and T cell numbers are similar [40]. BALT follicles contain small and medium lymphocytes but lack capsules and germinal centers characteristic of true lymph nodes [40]; T and B cell rates are similar to those of GALT: 20% of T and 40%–80% of B cells. This rate is reversed in both lymph nodes and circulation: 70% of T and 15%–20% of B cells [533]. Mast cells and macrophages with APC (antigen-presenting cells) activity are present in BALT; therefore antigens can be directly presented to lymphocytes in this location [520]. Additionally BALT might function as a repository for immunoglobulin (Ig)-bearing cells, but because it is poorly provided with plasma cells, further B-cell differentiation should occur outside the BALT; therefore, B_{IgA} migrate directly to the lamina propria along the respiratory tract to produce antibodies for the mucosal surface [533], that is in the NALT, which appears to be the principal supplier of B precursors in the upper airways [54]. All Igs are diffused along the airways: plasma

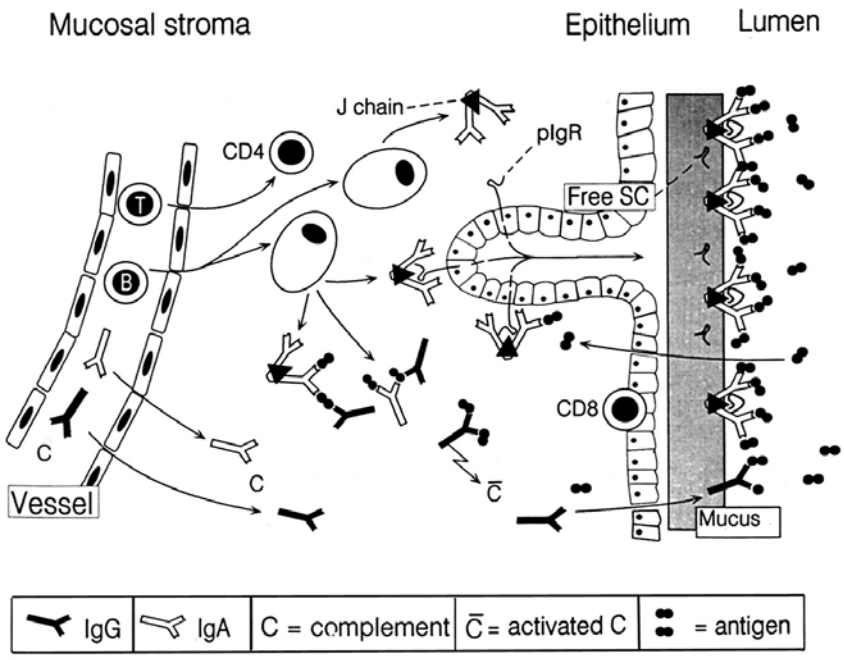


Fig. 11.2. Schematic representation of immune homeostasis in the airway mucosa. Mucosal sIgA antibodies act as a first-line defense by performing antigen immune exclusion. Antigens circumventing this barrier meet mucosal IgG antibodies. The resulting immune complexes activate complement, and inflammatory mediators most likely formed locally. However, the adverse inflammatory development is usually inhibited by serum IgA and by locally produced polymeric and monomeric IgA. Antigens may be returned in a noninflammatory way to the vasal lumen by pIgR-mediated transport mechanisms. The final homeostasis depends on the profile of adhesion molecules expressed by vascular endothelium, normally facilitating preferential extravasation of B and T cells belonging to the MALT (for details see text). *pIgR* Polymeric Ig receptor, *sIgA* secretory IgA

cells of the lamina propria pass into the lumen across the BM and epithelium. In the upper airways, IgA antibodies predominate, with the capacity of neutralizing the RSV (respiratory syncytial virus) *Rhinovirus* and influenza virus and of agglutinating microorganisms, increasing the mucociliary clearance. In the trachea and bronchial tree secretory IgA (sIgA) are 10% of the total proteins; progressing to the alveoli, IgA decrease as low as 5%, while IgG increase up to 10%–15% [533]. Under normal conditions, the respiratory immune system is able to suppress immune responses triggered by allergens circumventing the barrier, in that a successful sIgA defensive system is ready [54]. As seen in Fig. 11.2 [54], sIgA are generated from locally produced polymeric IgA (pIgA), armed with J chain, which is transported by the pIgR (polymeric Ig receptor) to the lumen along with the SC (secretory component) [54]. Consequently, immune tolerance is established after the first contact with non-self agents with a two-phase mechanism related to genetic factors, but, mainly because of environmental factors capable of negatively affecting the immune system, a sensitization with the same mechanisms that we have seen play a part in food allergy (FA) can occur [54].

Nonspecific Defenses

Nonspecific defenses are essentially represented by *bronchial secretions* that block and neutralize soluble toxic substances and by the activity of the mucociliary system that propels them upward. Bronchial secretion emitted by the submucosal glands and/or by the goblet cells, and enriched by transudation fluids or exudation of blood origin, is made up of glycoproteins originating in the mucosa, arranged in a type of fibrillar network and of a component, more precisely a fluid, containing anti-infective substances (Ig, lysozyme, lactoferrin) and C3 [648]. Additionally, the bronchial secretion performs a major role by hydrating the inhaled air and maintaining the ionic balance. The *mucociliary system,* already in place in the first days of life, is made up of ciliated cells, each possessing, on average, 200 cilia, 5–7 µm long and 0.25 µm wide. Each *cilium* is formed structurally of nine pairs of contractile longitudinal microtubules [648], connected two by two by means of nexin links, and arranged in circles around a central pair. All the tubules converge toward the tip of the *cilium,* forming a hood connected to the ciliary membrane. Two dynein bridges catalyze hydrolysis of ATPase and transduce the energy into a mechanical force in the form of ciliary bending. The cilia act on the mucus blanket shed from the mucosal glands and especially that from goblet cells. At the moment of the active phase of the beat (15/s), the mucus is driven by the ciliary tip toward the oropharynx at increasing speed from the periphery toward the trachea, accelerating from 2–4 µm/min. At the alveolar level, the alveolocapillary membrane is covered with surfactant, produced by type II epithelial cells and nonciliated Clara cells, situated on the branching border between bronchial ducts and bronchioles, which synthesize the surfactant A-C proteins. This substance, having surface-active properties, changes the surface-charge properties, making foreign particles less viscid and therefore more easily cleared [648]; in addition, it promotes the intervention of the phagocytes. Ciliary clear-

ance is substituted in the distal airways by that of macrophages and by cough reflexes [648].

The *alveolar macrophages,* in the presence of antigens, pollutants, or chronic irritants, pass from a state of quiescence to one of activation and, if the stimulus persists, to one of intense phagocytic activity, with IL (interleukins) production. In particular, inorganic substances are taken up and transported mechanically to the mucociliary system, while organic substances are decayed by cytoplasmic and lysosomal enzymes, and microorganisms are phagocytized and subjected within the phagosomes to the action of free radicals and cationic proteins [330]. Macrophages can also have a deleterious effect on the airways, by releasing enzymes with lytic activity, oxidants and proteases normally neutralized by anti-proteases and by ILs produced by macrophages. Free radicals, ozone (O_3) and nitrogen dioxide (NO_2) can, in turn, alter the local protease/anti-protease equilibrium. Lastly, the macrophages have the role of controlling T-mediated activation of the immune system: in subjects with BHR, but without symptoms of inflammation and asthma, macrophage hyperactivity may result in the suppression of local T cells, while a reduced activity could be fundamental in triggering T lymphocyte hyperactivation and up-regulating chronic inflammation, which could further exacerbate BHR up to a pattern of full-blown disease [503].

Alterations of the Defense Mechanisms

- *Immaturity.* At birth not all the mechanisms contributing to the protection of the airways have reached their full functional potential. The mucociliary system develops very early during intrauterine life and proves to be fully suited to the mechanical purification of the particles *inhaled from the very first hours of life.* The alveolar macrophages, on the other hand, are late in reaching the airways. This is because they are immature cells endowed with phagocytic activity, but without an oxidizing metabolism or cationic proteins able to ensure efficient bactericidal action. In addition, the factors of acquired immunity have not yet been stimulated and therefore, the appropriate protection is temporarily taken care of by the maternal antibodies.
- *Constitutional disease.* Diverse genetically transmitted diseases share the same characteristic of appearing in the first years of life with recurrent respiratory infections (RRI), for example, cystic fibrosis (CF), primary ciliary dyskinesia, etc.
- *Immunodeficiencies (IDs),* which reduce the airway resistance (Raw) to infections, including primary IgA deficiency, chronic granulomatosis, etc. (Chap. 22).
- *Deficiency of α1-antitrypsin.* The primary deficiency lies in an insufficiency of the anti-protease defense, corresponding to phenotype ZZ, which favors the development of emphysematous lesions.

Genetic Factors

Asthma can be considered a multifactorial disease in that different genetic and environmental factors contribute to influencing its phenotypic expression [340]. The pathogenesis is consistently related to genetic factors, family history (FH), and the particular role of atopy, intimately interrelated.

Four sets of data have emerged recently: a clear-cut genetic component *of 125 genes related* to the causation and progression of asthma symptoms (Table 4.2), *IL_{13}* overexpression [321], *IL_{12}* deficient expression [415], *and Th1 phenotype* downregulation due to reduced expression of T-bet (Th1 transcription factor) [188] while GATA-3 is overexpressed [328], which is implicated in the Th2 development [430].

Several studies on the genetics of asthma confirmed the linkage between asthma and genetic markers on 13 chromosome regions including *5q* and *11q*. Recently, the *ADAM33* gene encoded on chromosome *20p13* (Table 4.2) was found to be associated with small-airway remodeling in patients with asthma. *ADAM-33* polymorphisms may accelerate the proliferation of smooth-muscle cells and fibroblasts, leading to BHR and subepithelial fibrosis [582]. IL_{13} is thought to be especially critical in asthma (Table 1.5): normal signal transducer and activator of transcription 6 (STAT6) expression in epithelial cells is both necessary and sufficient to IL_{13} to induce BHR, eosinophil inflammation, mucus production, lung emphysema, and other central features of asthma in the absence of inflammation. However, mice lacking STAT6 were protected from all pulmonary effects of IL_{13} [321]. Two new ILs are more destructive. The IL_{17} family resulted in bronchoalveolar lavage neutrophilia and inflammatory gene expression in the lung. In addition, intranasal administration of IL_{25} protein resulted in the production of IL_4, IL_5, IL_{13}, and eotaxin mRNA in the lung, marked eosinophilia in the BALF and lung tissue, eosinophil chemotaxis and activation mast cell stimulation, epithelial cell hyperplasia, increased mucus secretion, and BHR [269]. In CRH (corticotropin-releasing hormone) deficiency, such as in CRH knockout mice, increased levels of IL_4, IL_5, IL_{13}, IFN-γ, RANTES and eotaxin in BALF were observed, thus increasing asthma severity [588]. FP in vitro impairs IL_{13} production by PHA (phytohemagglutinin)-stimulated PBMCs (peripheral blood mononuclear cells) from asthmatic and control subjects [150].

Thus the pathogenesis is associated with ILs, namely IL_{12} (down) and IL_{13} (up). Children heterozygous (HET) for IL_{12}B promoter polymorphism (associated with reduced IL_{12} gene transcription) have a greater risk for progression to severe asthma, irrespective of disease cause, but with no difference between nonatopic and atopic children with asthma [415]. As a consequence,

everything is ready to up-regulate aberrant Th2 responses in atopic children, probably leading to a class switch to IgE antibody formation.

Outcomes in adult asthma may be determined primarily in early childhood. In an unselected birth cohor, >25% children had wheezing that persisted from childhood to adulthood or that relapsed after remission. At age 21, 26.9% had continuing symptoms of asthma; 14.5% had persistent wheezing from onset with no remission, and 12.4% had relapsed after remission. The factors predicting persistence or relapse were sensitization to Der p, BHR, female sex, smoking, and early age at onset [554]. It was previously found that at 25 years 88% of symptomatic subjects had BHR, a proportion statistically higher in the asthmatics than in the group of controls (12.8%) [218]. The reason is that a very early presence of eosinophilic inflammation and even remodeling of airway wall occur early in the natural history of pediatric asthma and are present well before asthma would be diagnosed based on clinical symptoms [501]. That the pattern of asthma during childhood predicts outcome is confirmed by the Melbourne study in the original children followed-up at age 42. Most children with persistent asthma (70%), frequent asthma (69%), and infrequent asthma (69%) had severe asthma into adult life and reduced PFTs [264].

Stimulating findings have opened up an unexpected facet of asthma pathogenesis. Studies suggest that TNF-α, a proinflammatory IL that participates in the inflammatory reaction in asthmatic patients, or nearby genes, including those in the HLA region, may contribute to the development of asthma in the Japanese population [449]. Platelet-activating factor (PAF), also implicated in the pathophysiology of inflammation in asthma, is degraded and inactivated by PAF acetylhydrolase: its deficiency is found more frequently in children with atopic asthma [275]. Polymorphism in the activation-induced cytidine deaminase gene might be associated with the pathogenesis of atopic asthma and the regulation of *total serum IgE levels in children aged <3.* The related deficiency leads to a complete defect in class-switching, resulting in a hyper-IgM phenotype and lack of IgG, IgA, and IgE. An area requiring further study is on the role of CC16 (Clara cells), which is secreted in the airway epithelium and plays a key role in inhibiting airway inflammation, but CC16 levels are reduced in patients with current asthma due to increased levels of its *A38G* allele [325]. This allele was associated with increased BHR *at age 1 month* and increased risk of asthma at age 6 [324]. It might be an intriguing candidate gene determining asthma severity in children with the CC16*38A phenotype by increasing Th2 IL production in their airways [242].

Even if the existence of a *genetic predisposition* to asthma has been proved (Table 4.2), the precise mode of hereditary transmission is not yet clear; according to several investigators it could appear to be of the autosomal dominant type. The same model has been attributed to BHR, because of the significant difference between parents of asthmatic children (50%) and those of healthy children (10%) and, based on hyperreactivity to histamine, between nonasthmatic atopics, nonasthmatics (33%), and the general population (6%) [179]. In twins, the concordance rate of asthma (about 50%) is 14.7%–19% in monozygotes (MZ) and 4.8%–8.7% in dizygotic (DZ) twins [174]; 44.5% of asthmatic children have positive skin prick tests (SPT^+) vs 20.7% of healthy children [209]; also, total IgE and sIgE (specific) to inhalants are significantly high in asthmatic children, but normal in controls [433], indicating that genetic predisposition is an essential prerequisite, that increased BHR risk is associated with atopy and that severity is correlated to that of atopic manifestations. Although total serum IgE tracks with age, children who are predisposed to persistent wheezing and early sensitization to local aeroallergens already have high levels of IgE at age 6 [305] and 9 months [572].

Martinez et al [383] clearly indicate that a FH of asthma imposes an increased risk for childhood asthma and that elevated serum IgE levels measured during the first year of life are associated with subsequent asthma [383]. Total serum IgE levels were high at age 6 months in a cohort of 150 children at risk for developing asthma as offspring of mothers with asthma; IgE levels were still significantly higher for the asthmatic children at age 6–8 years with a GM (geometric mean) = 38.32 as compared with nonasthmatic ones (GM = 12.28). IgE levels were higher when the infants were 6 months of age [305]. Within the same family group, some members can have BHR and elevated IgE, others only high levels of IgE [229]. As mentioned in Chap. 5, the risk factor is significant in relation to positive FH (FH+): the prevalence of asthma has increased from four- to tenfold compared to subjects with negative FH [174]; if it is specifically related to asthma, children have an increased risk of becoming asthmatic within the 2nd year of life [746], with a BHR-atopy association correlated to age and to FH [122]. In our everyday work, we see that almost all asthmatic children have a positive FH, and in those with severe asthma, such as the above-cited 592 children, the rate surpasses 95%. A prospective cohort study has shown that even in the absence of respiratory symptoms FH+ children and those with personal atopy could have impaired PFT (pulmonary function tests) early in life [360]. Asthma in adults is usually associated with an elevated PRIST (paper radio immunosorbent test) and with SPT+ to allergens, and therefore *with an IgE-mediated mechanism.* The concept that IgE was central in allergic asthma [305] was stressed by data from a population-based study, which showed a close and highly significant relationship between asthma and serum IgE levels [63]. Conversely, the existence of the two forms of asthma has not been confirmed because, once IgE levels have been standardized according to sex and age, the intrinsic forms were brought back to an IgE-mediated mechanism [63]. Even in children [65, 572] *asthma and*

BHR are associated with elevated concentrations of IgE and atopic sensitization. Additionally, *BHR is already present in newborns and in 2- to 10-week-old infants*, before acquired factors can come into play [745], *in 6-month-old at-risk babies* [553], but also in 8% of apparently normal babies [746]. In early life, children of atopic parents and those with personal atopy have impaired PFTs even in the absence of respiratory symptoms, but with a significant interaction between history of maternal asthma and the child's atopic status [360]. These findings have up to now been observed only in adults and children with significant respiratory disease. The importance of serum IgE in determining BHR is decisive in the pediatric field [64]. If the association of BHR with the FEV_1/VC (vital capacity) rate is operative only in asthmatic young people with at least moderate levels of IgE, then IgE influences the given relationship by interacting with BHR. Asthma associated with BHR increase is independent of all other factors considered. Introducing IgE levels and PFT results into this calculation, all other diagnostic and/or clinical parameters play a substantially secondary role [64]. Therefore, *in infancy, BHR appears to be associated with atopy,* with FH+ of atopy (FHA) and asthma (indicating that it is hereditary) and *with wheezing beginning very early* or after 2 years of age.

In additional studies on pediatric cohorts, a significant correlation between BRH, asthma and atopy was found in 90%–100% of cases [574] and the degree of atopic sensitization was assessed by SPT+ to several allergens and BHR in asthmatic children [65]. Agreement on a positive correspondence between BHR and atopy is not unanimous [574]: this was confirmed in two longitudinal studies [4, 107]. Moreover, BHR is significantly more often found in atopic children with wheezing than in nonatopic children [108]. The correlation is absent in other children [108] and in adults followed up from age 7 [289]; infants at risk aged 6.5 months have reacted to the histamine test with BHR independently of wheezing [106]. Atopy can nonetheless be considered the main independent determining factor that contributes to BHR and even acquires a predictive character when accompanied by altered PFTs [574], in that subjects atopic from birth are those affected with more severe BHR in childhood [666]. Certainly, in childhood an airway inflammatory reaction manifests itself early, strictly related to atopy, which underlies on the one hand BHR and on the other – whether directly or not – PFT reduction [574]. The even greater relationship between atopy, elevated IgE levels, infant asthma and BHR seems to us especially significant [16]. Another possible pathogenic factor is the finding of IgG subclass deficiency, with or without associated IgA deficiency, in children with chronic and/or severe asthma, especially if accompanied by RRI. Table 11.1 summarizes the intimate links between infant asthma and atopy.

Table 11.1. Pediatric asthma and atopy

Asthma is present in a high rate of parents of asthmatic children
In the parents and relatives, atopic disease and SPT positivity to allergens are increased
Up to 90% of asthmatic children have one or two atopic parents
If children suffer from atopic asthma, asthma is even more frequent in their family
The risk of asthma is related to the atopy degree
Asthma severity is correlated to severity of atopy
Early onset of atopic asthma is predictive of subsequent BHR severity
Allergic rhinitis is present in 28%–61% and atopic dermatitis in 50% of asthmatic children
More than 80% of asthmatic children have IgE specific to one or more allergens
Even in the absence of specific IgE, the challenge test with allergens may be positive in some children
Asthma frequency was the exception in developing countries before the arrival of an industrialized society

BHR bronchial hyperreactivity, *SPT* skin prick test.

The pathogenic role of allergens as propitiatory of asthmatic attacks is expressed not so much in immediate reactions as in being the cause of long-term inflammation. Instead, symptom worsening over a short period is more easily released by irritants such as O_3, physical exertion, variations in T (temperature), etc. [333]. However, none of these factors has been formally identified as the cause of airway inflammation [499]. Often, intercurrent respiratory infections are able to amplify not only the clinical features, but also the chronic inflammation, because of the increase in the airways of eosinophils and cationic proteins, including MBP (major basic protein) [158, 341, 477]. This is significant, given the positive interaction between Rhinovirus infection and childs response to allergen exposure [341].

Other details regarding the child:

1. BHR can be present in children with AD, with greater probability in those displaying an early onset of AD.
2. BHR associated with an increase of total IgE levels can be present in children who have never suffered from asthma.
3. Altered PFT can constitute a factor of asthma risk in the first 3 years of life.
4. The onset of atopic asthma in infancy is liable to continue into adulthood if not diagnosed in time and treated appropriately.

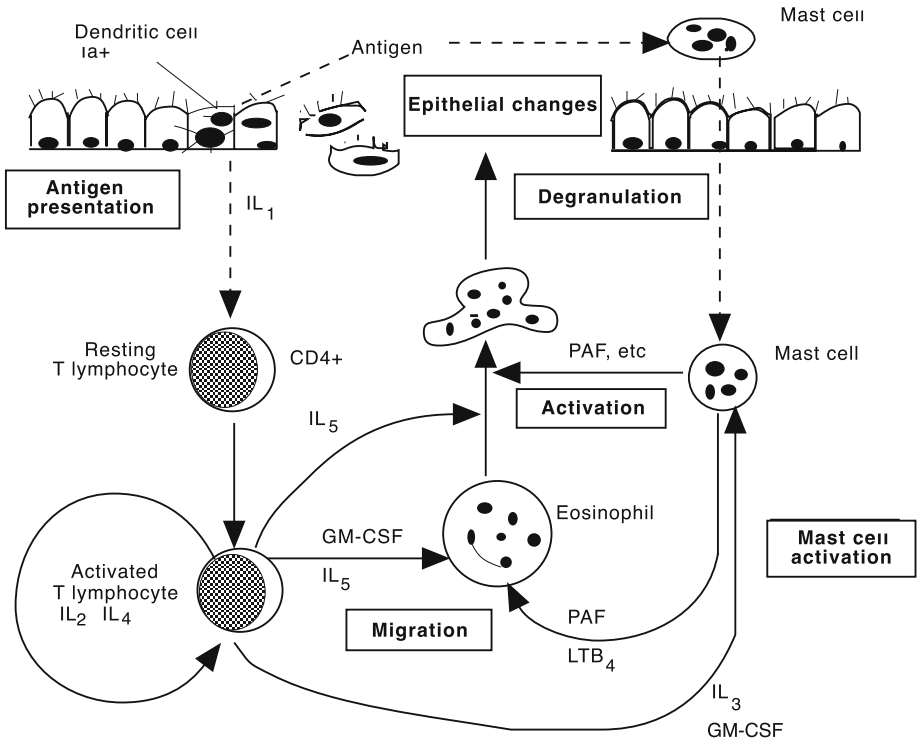


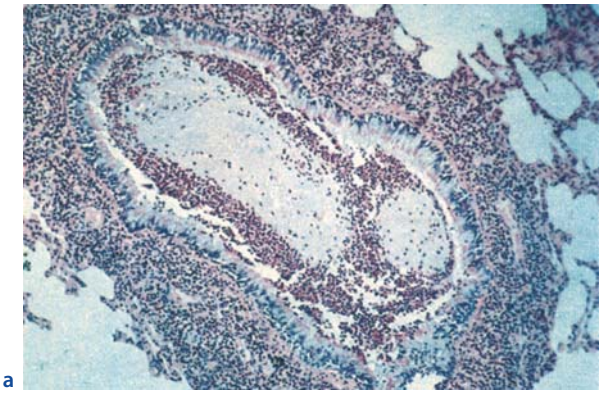
Fig. 11.3. Subsequent phases of the asthmatic immune inflammation, showing the interactions between T lymphocytes, mast cells and eosinophils, and the consequent changes involving the epithelial cells. (Modified from [194])

Pathogenesis

Role of Immune Inflammation

Airway inflammation is a characteristic feature of asthma and contributes significantly to many features of this disease. No doubts remain regarding the pathogenic role of airway inflammation (Fig. 11.3) [194], either in severe, prolonged attacks of asthma or in children with chronic disease. Since 1906 [173], *anatomopathological studies* of patients who died after an asthmatic attack have highlighted the presence of numerous changes in the bronchial mucosa [255, 516, 600] (Figs. 11.4, 11.5):

- *Clear inflammatory response* with subepithelial infiltration of inflammatory cells, eosinophils and activated lymphocytes, with class II antigens, mast cells and macrophages more numerous than in controls.
- *Desquamation* of the bronchial epithelium with shedding of epithelial cells from basal cells and swollen ciliated cells.
- *Hypertrophy and hyperplasia* of smooth muscle.
- *Edema* of the mucosa and the submucosa as a consequence of increased permeability of the microvasculature and plasma leakage.



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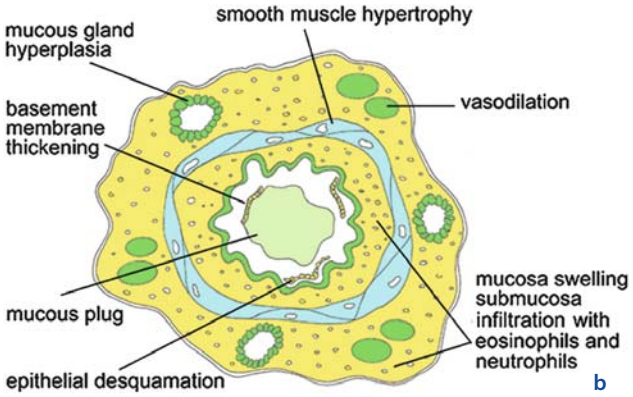


b

Fig. 11.4. **a** Histopathology of bronchial mucosa from a subject who died from asthma, showing intense infiltration with eosinophils and mononuclear cells, inflammation and thickening of basement membrane. The lumen of the bronchiole is completely occluded with mucus, eosinophils and cellular debris. **b** Pathological changes in asthma: diagram of cross-section on an airway in severe asthma showing the characteristic hypertrophy of smooth muscles

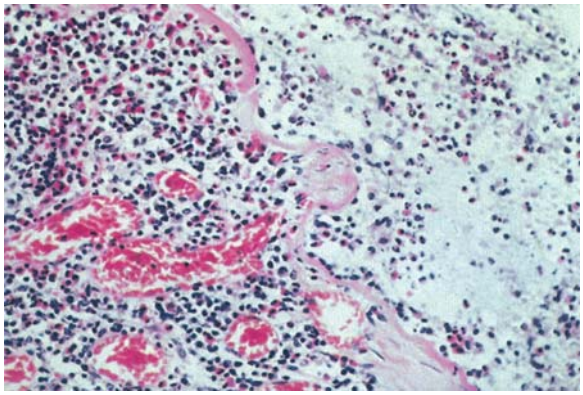


Fig. 11.5. Denudation of the epithelium in an asthma death. Epithelial cells are absent; only the thickened basement membrane remains, with hyperemia and eosinophil infiltration

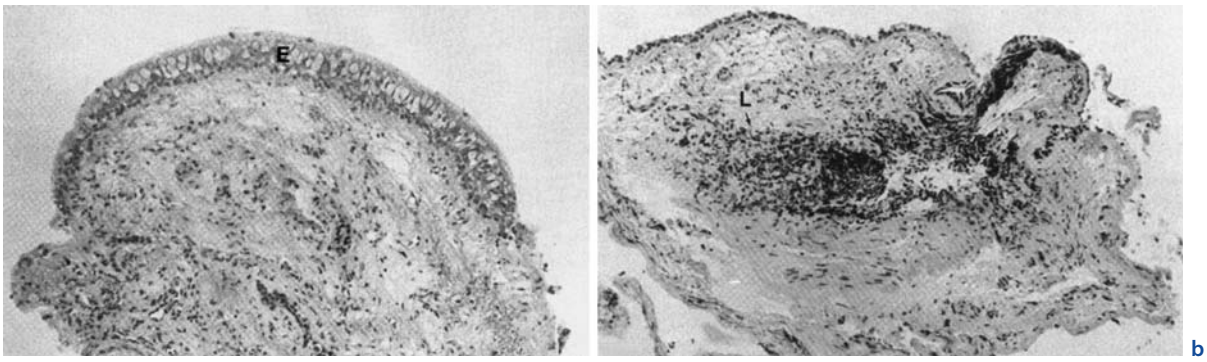
- *Mucus hypersecretion* with mucus plugging in the airways (also containing desquamated epithelia, lymphocytes, Charcot-Leyden crystals (CLC) and Curschmann's spirals) up to the segmental bronchi and bronchioles.
- Numerical increase of *epithelial goblet cells.*
- *Mucosal gland hyperplasia.*
- *Fibrotic pseudo-thickening* of the BM by deposits of interstitial collagen under the epithelium.

The end result is a further compromise of the airway lumen [340].

In these studies, however, a greater number of neutrophils and a lesser number of eosinophils may be present, while fibroscopy has made it possible to demonstrate that asthma is prevalently a chronic airway inflammation in which eosinophils and metachromatic cells play a primary role in provoking tissue damage: the bronchial surface *is left naked by epithelial desquamation,* from which the remodeling processes originate, regenerative of the epithelium with squamous and calyciform metaplasia [53]. If adults with mild forms of asthma show a severe pathological picture, and deceased subjects suffering from asthma during life are found to have relatively normal bronchi [287], the damage found in specimens from lung biopsies in children with asthma in remission were similar to pathological specimens of others of the same age who died from status asthmaticus and even of patients who died of causes unrelated to asthma [255]. Figures 11.6 and 11.7 [50] show the differences between the bronchial state of a healthy subject and that of an asthmatic one during the pollen season. It should be emphasized that consistent damage of a chronic airway inflammatory process caused by more than one cell type is observed in subjects with newly diagnosed asthma, having asthma for <1 year (Fig. 11.8) [327], even after 2–3 months of asthma duration, showing a greatly decreased number of ciliated cells in the airway epithelium as compared with those in the control subjects [328].

If anatomopathologists underline the inflammatory aspects, clinicians favor that of spontaneously reversible bronchoconstriction, or reversible following specific treatment, whereas physiologists consider the increased BHR as the dominant factor. Even if the concepts of reversibility and of hyperactivity have long attracted a great deal of attention, the idea that asthma should be regarded as a pulmonary inflammation has only recently been given its due attention. Based on recent research, BHR is related to the degree of airway inflammation: as such, both tissue damage and BHR can find a unifying pathogenetic mechanism in the interaction of the different mediators discharged by different cells [255]. It does not, however, seem to be caused by the basic inflammation, since it does not regress with corticosteroid (CS) therapy [94].

Figure 11.9 [499] illustrates the etiology of asthma, BHR and bronchoconstriction. Figure 11.10 [287] shows the relationships between T and B lymphocytes and eosinophils in acute and chronic asthma [274]. Figure 11.11 [513] illustrates the physiopathological correlation and Table 11.2 [162, 538] the main differences between immediate asthmatic reactions (IAR) and



a b

Fig. 11.6. **a** Bronchial biopsy specimen of a control subject. The well-preserved mucosa is made of normal epithelium (*E*). Occasional inflammatory cells are observed in connective tissue. **b** Bronchial biopsy specimen of an asthmatic subject during seasonal pollen exposure, showing a partial lessening of epithelial cells and inflammatory cells in connective tissue are abundant, mainly lymphocytes (*L*). ×250

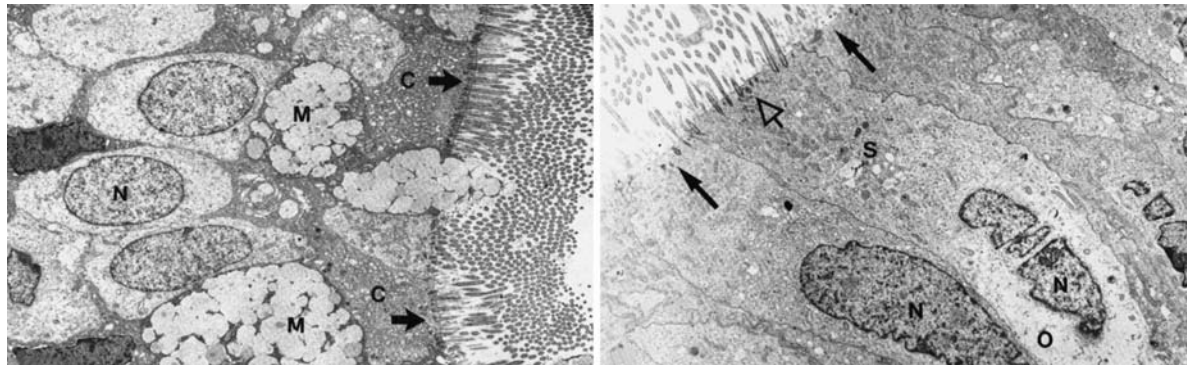


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Fig. 11.7. **a** Electron micrograph of a bronchial biopsy specimen of a control subject, showing ciliated (*C*) and mucous cells (*M*), with several cilia and basal corpuscles (*arrows*). Nuclei (*N*) were ovoid in shape with vesicular chromatin. ×3,400. **b** Electron micrograph of an asthmatic subject out of pollen season with an area of ciliated cells. The chromatin is dense with irregularly shaped nuclear membranes. One cell (*open arrows*) shows a modest number of cilia, but two other cells (*black arrows*) show almost no cilia. The smooth endoplasmic reticulum (*S*) is dilated with associated cellular edema. ×4,600

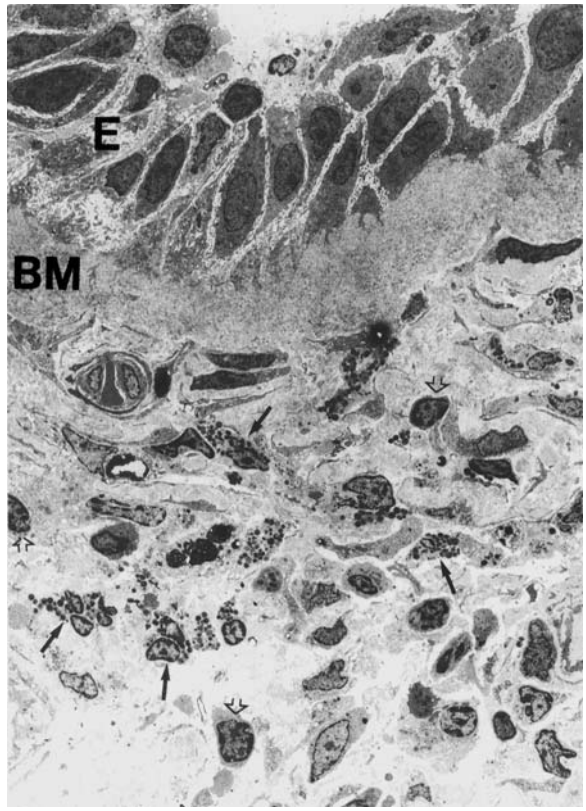


Fig. 11.8. Electron micrograph of a bronchial biopsy specimen of an asthmatic subject with asthma for less than 1 year. The airway epithelium (*E*) is damaged, and the subepithelial basement membrane is thickened (*BM*). A strong inflammatory reaction is seen in lamina propria with eosinophils (*black arrows*) and lymphocytes (*open arrows*). ×2,000

Table 11.2. Differences between immediate and late asthmatic reactions (IAR and LAR)

	IAR	LAR
Time	Minutes	4–12 h
Duration	1–2 h	1–3 days
BHR increase	Present	Absent
Mechanism	Bronchoconstriction	Inflammation
Rate	35	25
	(associated 40)	

Modified from [162, 538].
BHR bronchial hyperreactivity.

late asthmatic reactions (LAR), whose characteristics are summarized in Fig. 1.9. The immediate phase is summed up in IgE production in response to allergenic stimulus (Fig. 1.40). On the next encounter, in the atopic child, the interaction between allergens and sIgE linked to receptors on effector cell surface initiates a complex chain of biochemical events leading to cellular activation, culminating in mediator release within a few minutes of the allergenic stimulation, and, at the same time, to the appearance of clinical manifestations [207] (Fig. 11.11). Parallel with this, in the airways, 3–12 h after allergenic exposure, recruitment of eosinophils, neutrophils, basophils and PBMCs, which constitute the primary histopathological infiltrate in status asthmaticus, starts the delayed reaction ultimately resolving in chronicization. A close relationship between basophil number and histamine serum level, and between eosinophil number and MBP concentration [283] is evident. Eosinophil number, 30 min at most after antigen challenge, reaches its peak after 4–6 h and, in individuals without LAR, after 24 h [538], so it is assumed that their transition occurs from the mucosa to the secretions. The pathogenesis of this second phase no longer focuses on mast cells, which, nevertheless, participate in releasing ILs and chemotactic factors, but rather on ef-

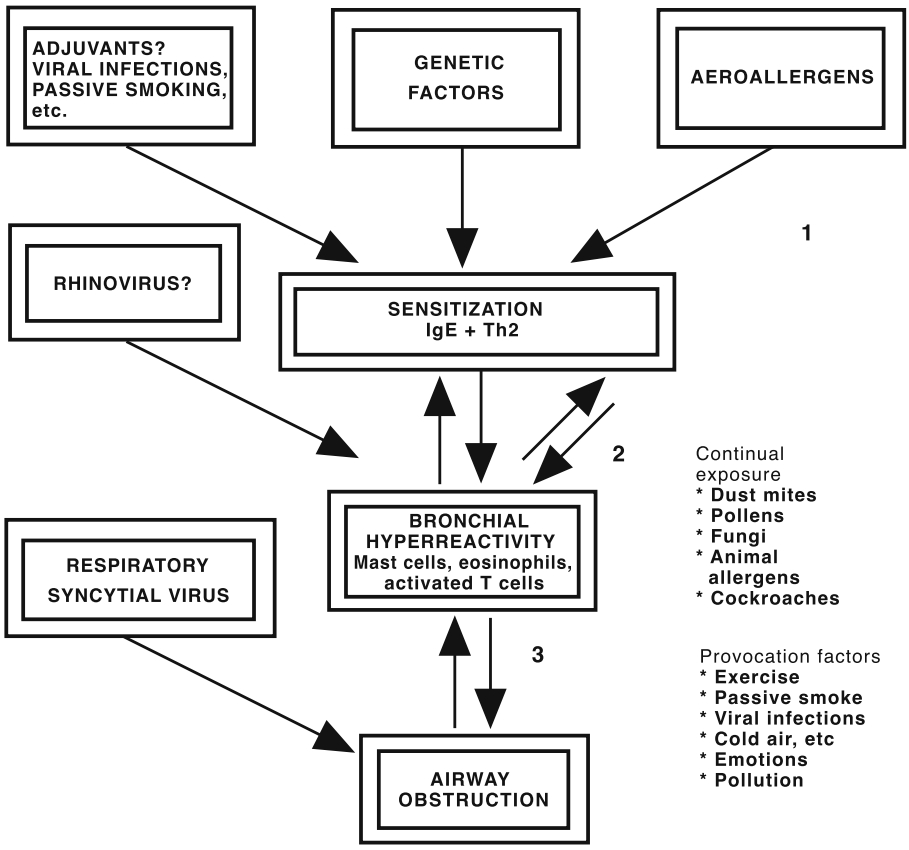


Fig. 11.9. Pathogenesis of asthma and bronchial hyperreactivity (BHR) and interactions with bronchial obstruction. *1* The first phase is asymptomatic and several children with serum IgE levels do not experience disturbances, but persistent exposure may cause bronchial inflammation with BHR. *2* The second phase is symptomatic; however, children may have normal lung function. *3* The precipitant factors are the allergens and aspecific stimuli, including passive smoke, exercise, etc. The susceptibility to viral infections is a challenge for pediatricians. (Modified from [499])

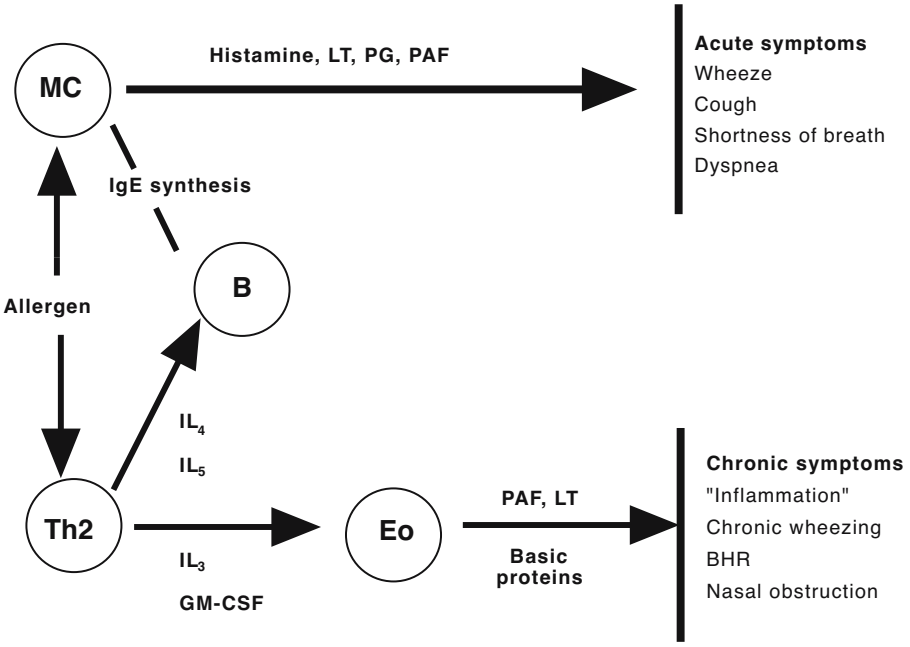


Fig. 11.10. The role of mast cells (*MC*), T lymphocytes, B lymphocytes (*B*) and eosinophils (*Eo*) in acute and chronic inflammation in asthma. *LT* leukotrienes, *PG* prostaglandins, *PAF* platelet-activating factor. (Modified from [287])

fector cells such as basophils, neutrophils, and, above all, eosinophils, as is demonstrated by cationic protein concentrations [442], ECA (eosinophil chemotactic activity) and NCA (neutrophil chemotactic activity) [240] during a BPT (bronchial provocation test) with allergens [240] up to 24 h later [442]. This role is a prerogative of specific Th2 lymphocytes, ILs, and factors they secrete, that is LTB_4, PAF, IL_3–IL_6 and the chemotactic factor of CD8 T cells [258]. On the other hand, a relative absence of IL_{10} characterizes the airways of asthmatic

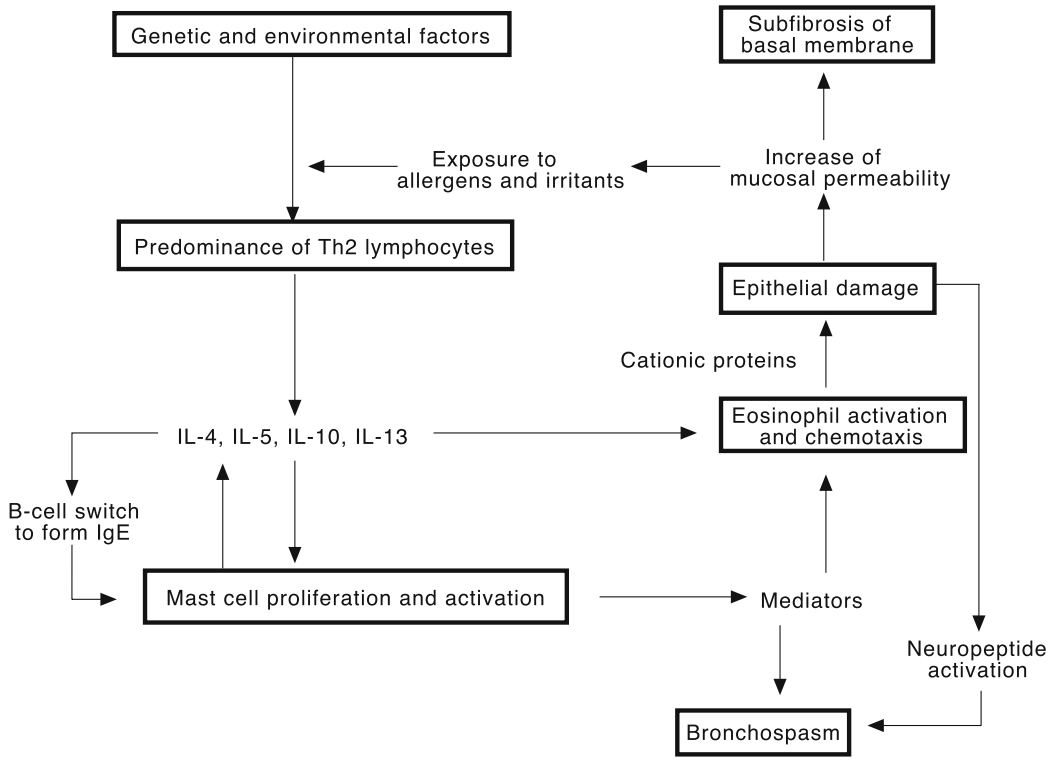


Fig. 11.11. Asthma physiopathology and pathogenesis. (Modified from [513])

sufferers, potentially increasing the severity of allergic inflammation [384]. The role of platelets still remains to be elucidated. On the basis of the latest information available, this reaction appears to be suited for further studies of the physiopathology of asthma and to assess the therapeutic potential of new drugs [162]. Both in asthmatic adults [435] and in children [255], inflammation is usually present. Moreover, a pediatric study has revealed an intimate correlation between activation of both eosinophils and mast cells and BHR [187].

A single pathogenic mechanism does not exist at the basis of the inflammation and consequential BHR; rather, it is likely that the orchestrated action of mediators, their products and cells attracted in this process can play a salient role in provoking BHR [340]. From a histopathological point of view, interesting progress has been made possible by the study of BALF cells via fiberoptic bronchoscopy, which can be performed even in nurslings, using models as small as 1.8–2.3 mm in diameter [737], and which can also be achieved, in severe cases, using a noninvasive method such as a neonatal catheter [182]. Using these techniques, immune cytochemical research has demonstrated that, in asthmatic children, neither the discriminatory presence of cell types nor their quantitative increase as compared to nonasthmatic atopic controls (Tables 1.40, 1.41) have been found. Studies of BALF cells and lung biopsies have revealed that a great number of $CD4^+$ [15, 236] are activated by the expression of CD25 and of class II HLA molecules [691]. In atopic asthma sufferers, these T cells have a Th2 phenotype, which is notably relevant in the pathogenesis of allergic disease, especially of the airways, so a great deal of asthma is associated with the activation in the bronchi of IL_3-IL_6 and GM-CSF (granulocyte-macrophage colony-stimulating factor), a pattern compatible with Th2 activation [524–526]. IL_{13} hyperproduction [321] could also bias the differentiation of T cells toward a Th2 phenotype through its ability to modulate IL_{12} production from APCs. Whether as a result of exaggerated Th2 cytokine production or as a primary defect, lack of IL_{12} can clearly influence immune responses toward a skewed Th2 response [676]. Furthermore, alveolar macrophages, bronchial epithelium, eosinophils and mast cells express these and other ILs, whose secretions lead to the pathological findings observed in asthma [15, 236, 691]. Some CXCL and CCL chemokines (Tables 1.54–1.57) act on both basophils and eosinophils; the former could represent the key mediators of immune inflammation. In immune inflammation, it is not easy to draw a balance of the importance of the different cell types or of the mediators involved. It seems more evident that they act together, each armed either with its own weapons or those shared with others, releasing a complex cascade of harmful events [73]. Of no minor importance are the notable alterations experienced by the *postcapillary microvasculature:* it is known that, in addition to the explicit damaging action carried out by powerful mediators, even vagal nerve stimulation, through neuropeptide release, secreted by neuroendocrine tissues of the airways, including substance

P (SP), can contribute to the vasodilation in the microvasculature. In turn, edema contributes to different worsening changes, including the inhibition of mucociliary clearance. In addition, proteins become available that form the substrate for anaphylotoxins of complement derivation and kinins [287].

Role of the Inflammatory Cells

Different cells are involved in immune inflammation.

T Lymphocytes

Characteristics [537]:

- *The tissues of atopic sufferers* show an accumulation of Th2 capable of orchestrating immune inflammation; consequently, Th2 predominate in the BALF of atopic asthmatics.
- *Activated Th2 cells* are found in blood and airways of asthmatics and their expansion is preferentially allergen-induced.
- *Th2 produce proinflammatory ILs* and, in particular, IL_3 and IL_4, with a major role in the synthesis of IgE, IL_5 and GM-CSF, whereas IL_2 and IFN-γ are absent.
- *Th2 are involved* in the enrollment of eosinophils, the primary effector cells of inflammation [537].
- *During asthmatic attacks,* there occurs in children an expansion of allergen-specific Th2 clones, accompanied by very high production of IL_4, as demonstrated by an increase in CD23 on B cells and on $CD4^+HLA\text{-}DR^+$ [200] (Fig. 11.12) [513].
- *In BALF of nonasthmatic children,* CD8 T cells are more numerous than CD4 T cells (Tables 1.40, 1.41), while in atopic pollen-allergic subjects the proportions are more than reversed: CD4 85% (Th2 48%, Th0 39.5% and Th1 12.5%), CD8 15%, allergen-specific T clones 27% [141].

Thus an imbalance between Th1 and Th2 lymphocytes primes the Th2 or allergic phenotype, whereas the Th1 phenotype may be underexpressed. A reduced expression of T-bet, in T cells from airways of asthmatic patients compared with that in T cells from airways of nonasthmatic patients, suggests that loss of T-bet might be associated with asthma pathogenesis [188].

In a normal lung, T cells, on the order of 1.5×10^8, are in direct contact with aeroallergens [263]. However, in moderate asthma, their number may not be increased (Fig. 11.13). In the mucosa of lower and upper airways, *dendritic cells* (DCs) have been found, whose percentage in BALF cells is 1% [693] and whose number is greatly increased in atopic sufferers [412] and in asthmatic smokers [533]. DCs form a continuous intraepithelial reticulum interdigiting with epithelial cells, similar to the skin process [619]. They do not protrude through the epithelium, being confined by the tight junctions; consequently the allergens that are not removed from the mucociliary clearance must cross the mucosal layer and penetrate via the junctions to encounter immunocompetent cells [533]. DCs send the start signal to T cells during the sensitization phase and can also act as synergistic agents of their activation process in the airway walls during IAR. They are equipped with FcεRI-α that can facilitate allergen uptake and internalization, enhancing their potential role in the induction and amplification of chronic airway inflammation [660]. Having elevated levels of class II HLA, in vivo they efficiently trap aeroallergens and in vitro are skillful APC for

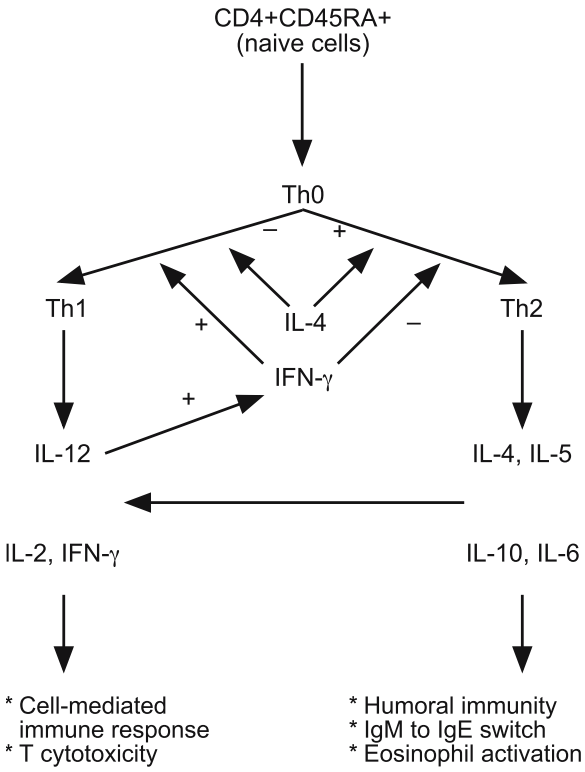


Fig. 11.12. Activation and differentiation of T lymphocytes in asthma pathogenesis. (Modified from [513])

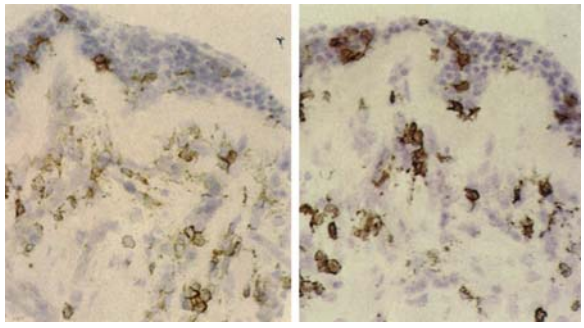


Fig. 11.13. Epithelial and subepithelial T-cell subpopulations (**left**) and CD4 and CD8 (**right**) in a bronchial biopsy specimen of an mild asthmatic subject: in mild to moderate asthma T cells are activated, but their total number is unchanged

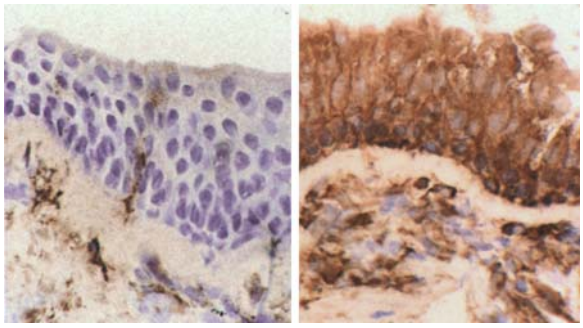


Fig. 11.14. HLA-DR (immunoperoxidase demonstration) in the bronchial epithelium of normal (**left**) and asthmatic subjects (**right**)

IELs [263], where they are CD1α-positive only for 30% [693]. They also are able to transport antigens, by migrating from the respiratory epithelium to the regional lymph nodes, where they undergo a maturing process, acquiring a greater immunostimulating activity [263]. In this way, they provide for antigen processing and presenting to T lymphocytes, thereby involving HLA-DR molecules, up-regulated in asthmatic subjects (Fig. 11.14), which, by linking to allergenic peptides, form bimolecular complexes easily recognized by TcR-CD3 [258]. Even in the bronchial mucosa in correspondence with lymphatic structures, *M cells* similar to those found in the intestinal epithelium situated over Peyer's patches have been described. In the airways, they could perform a similar function, transferring antigens from the bronchial lumen to lymphatic structures, even if they do not play a primary role in mucosal immunoregulation [537]. CD8 T cells induced by antigen encounter are in turn activated in the regional lymph nodes that drain the upper respiratory tract [262].

Lymphocytes accumulate in the respiratory tissues *during the 1st hour* of allergic reaction: in LAR they are attracted by specific receptors located on T cells, as well as on mucosal capillaries and epithelial venules, and activated in sites of immune inflammation, they induce phenotypic alterations in endothelial cells [524]. The predominant clones of allergen-specific T lymphocytes of atopic asthmatics, which are Th2-like, are able to produce elevated levels of IL_4 [535]. Recently, GATA-3 overexpression was found to be implicated in Th2 development in human T cells and IL_5 promoter activity in $CD4^+$ T cells of asthmatic patients was enhanced by GATA-3 [286]. In asthmatic patients, more cells from BALF contain mRNA for IL_3–IL_5 and GM-CSF (whose genes are associated in a gene cluster of chromosome 5), and reduced IFN-γ levels compared to controls [530]. More precisely, using *in situ* hybridization, and the immunofluorescence technique on BALF cells from asthmatic subjects, there is an evident imbalance between the reciprocal proportions of IL_4 and IFN-γ in tissue infiltrates of allergic inflammation [530], with a significantly larger number ($p<0.01$–$p<0.001$), in comparison with controls, of cells showing elevated mRNA concentrations for IL_4 and IL_3, CD3-positive (that is T lymphocytes, predominantly CD45RO), and no difference for IFN-γ [530]. IL_3–IL_5 and GM-CSF are associated with Th2, eosinophil accumulation [531], bronchoconstriction and Aas score [532]. This picture is compatible with the predominant activation of the Th2-like subset, the promotion of an IgE response and generation of pro-inflammatory mediators [524].

In asthmatics, a predominance of Th2 and Th2-like T-cell ILs are reported. T lymphocytes are therefore capable, even supported by the four classes of chemokines, of directly coordinating the accumulation and activation of specific effector cells at the mucosal surface. In particular IL_3, GM-CSF and IL_5 (with the aid of CD54, = ICAM-1) appear to be very active in attracting and activating eosinophils; GM-CSF and other T-like ILs are active in the differentiation of monocyte-macrophages, IL_3 and GM-CSF and metachromatic cells [524]. In this context, the presence of mast cells and basophils assumes great relevance, given that their precursors are able to produce IL_4 for Fcε receptor cross-linking. IL_4 synthesis modulates that of IgE antibodies, by acting directly on B lymphocytes, or by activating Th2-like $CD4^+$ cells. The latter produce on the one hand IL_4 (and therefore IgE) and on the other IL_5, finally closing the inflammatory cycle [535]. Since 24 h after BPT with allergens the majority of ILs originate from T cells [531], we may conclude that expanding allergen-specific Th2 lymphocytes directly orchestrates both the development of bronchial inflammation by producing specific IL and the *continuous synthesis of IgE*, able to elicit decisive influxes on inflammatory cells [525]. Titers of B cells, CD19 and CD23 markers are found to be increased compared to controls [552].

Examination of lymphocyte subsets in asthmatic individuals shows that $CD3^+$, $CD4^+$, $CD8^+$ and $CD45^+$ are present in notably greater numbers than in nonasthmatic atopic subjects and in nonatopic controls [15]. The marker CD25 (receptor of IL_2, IL_2R) [15, 119] and of HLA-DR and CD49a/CD29 (VLA-1) in greater numbers than in normal controls denotes that such activated cells are able to contribute to the pathogenesis [119]. Soluble IL_2R (sIL_2R) is a marker of their activation, with particularly elevated levels in asthmatic children compared to healthy controls and adults [408]. Even in the pediatric age group, high percentages of both CD4 and CD8 peripheral blood T cells express activation markers: thus T-cell activation as compared to controls is found [153, 207, 252, 590], with significantly higher differences regarding the increase in absolute numbers of CD4/$CD25^+$, CD4/$HLA\text{-}DR^+$ and CD8/$CD25^+$ [207], of T-cell specific markers CD23 and CD25 [252], HLA-DR antigen [590] and PEF-correlated CD25 [153]. Associations between eosinophil activation, $CD4^+$ cell numbers, and a high correlation between CD4-Th2 and eosinophils and severity of asthma [691], are totally *confirmed by the evidence in children of CD4-Th2 and CD8 displaying*

CD25 and HLA-DR positively correlated with disease severity (p=0.03) and with a significant increase in total eosinophilia (p=0.01) as compared with the controls [207]. Remarkably, the proportion of CD4 T cells expressing the *memory cell marker CD45RO* was significantly elevated in both atopic and nonatopic asthmatics compared with controls, whereas the naive marker CD45RA was expressed by a high proportion of CD8 T cells only in the nonatopic asthmatics compared with controls. Moreover, high rates of these CD4 and CD8 T cells express mRNA encoding IL_5, but only CD4 expressed mRNA encoding IL_4 in asthmatic children compared with the controls [208]. The relationship between cells displaying IL_5 and activated eosinophils in bronchial biopsies in asthmatics [236, 340] (Fig. 11.10) indicates that high levels of IL_5 distinguish between severe asthma [119] and moderate asthma, where they cannot be measured [153], considering that CSs reduce their transcription, together with several others, being capable of inhibiting in atopic asthmatics T-cell activation markers in BALF and in peripheral sites [723] and DC as well [412]. For these reasons, *T lymphocytes,* with all the cells and factors they stimulate, above all eosinophils, can be considered as the key cells that *bring into effect, sustain and perpetuate the immune inflammation,* the catalyst element that induces BHR in relation with endogenous and exogenous factors, and triggers the series of events that lead almost inexorably to asthma [287]. This is especially evident when the impossibility of establishing a state of tolerance through Th1 T cells results in the sensitization to environmental allergens [262], guided by the Th2 in the critical phase of developing the immune system in infants. Tables 1.40 and 1.41 summarize normal data on lymphocytes and other cells in BALF in nonasthmatic children.

Mast Cells

Characteristics [537]:

- Are present in airways of asthmatics in increased numbers and in various stages of degranulation, but are absent in normal subjects
- Respond to allergens in an IgE-dependent manner with high-affinity IgE receptors (FcεRI)
- Have a key role in driving the IgE-mediated reaction
- Directly express CD154 as basophils do, with the immediate implication that *synthesis of IgE occurs even in peripheral tissues, other than in germinal centers of lymph nodes*
- Produce short-lived mediators and inflammatory ILs

Mast cells derive from bone marrow precursors as regulated by the same ILs that are important contributors to the allergen-specific airway inflammation [537]. Their greater concentration is in more peripheral airways, where they localize prevalently in the sub- and intraepithelial layers, and in the bronchial lumen [258]. A striking increase in the number of mast cells was in the bundles of smooth muscle, and in patients with asthma this number was correlated with the hallmark of asthma, BHR [55]. In asthmatics there is no *instability of mast cell membrane,* which could facilitate degranulation, an event that can be mediated not only by encountering sIgE, but also by sIgG (specific) and anaphylotoxins. Similarly, there is no *functional heterogeneity,* since mast cells of the human airways belong mainly to the T type, even if both phenotypes can coexist in the same site with different levels (Table 1.27). Mast cells participate in the *immediate phase of allergic reactions:* they are found in the BALF of atopic asthmatics taken 15 min after allergen challenge in the *degranulation stage* [258], often in proximity to the small vessels and nerve endings and intimately correlated with smooth muscles, with spontaneous *release of histamine* and eicosanoids [73]. Once the degranulation has started, it is possible that allergen encounter with superficial mast cells provokes an initial mediator release that increases tissue permeability, with consequent enlargement of the intraepithelial tight junction network, thus allowing a greater number of allergens to reach the layers below, where a strong group of mast cells are to be found [283]. The array of primary and secondary mediators further increase the permeability of the epithelial barrier, permitting the entrance of plasma proteins and blood platelets [283]. This is how the true asthma allergy explosion is set off. Consequently histamine, PG, thromboxanes (type A_2), LTs (leukotrienes), PAF, bradykinins (BKs), granule derivatives and chemotactic factors are crucial for developing an inflammatory stage of immediate reaction [287]. As well as inducing adhesion, diapedesis, directional migration and activation of eosinophils and neutrophils, they also recruit other cells that are fundamental for the establishment and self-maintenance of inflammation, such as monocyte-macrophages, blood platelets, more eosinophils, and neutrophils to the injured site [283]. Mediators participating in the triggering of asthma affect the infiltration of leukocytes, probably aided by afferent vagal stimulations, affecting the bronchospasm as well as mucosal gland secretions, which are unrelated to symptom severity [283]. ILs, whose secretion is associated with histamine release and mast cell proliferation (probably associated with IL_3 and IL_4 generation), are in turn able to amplify the vasoactive phase of immune inflammation [73]. Mast cells, as well as sharing with basophils the CD154 production with a direct effect on IgE synthesis, express mRNA for IL_4 and for IL_5, whose release, together with IL_3 and IL_6, is triggered by an IgE-mediated mechanism [287]. Mast cells, however, appear to be *the only cells producing IL_4 in the airways of asthmatics:* this could indicate their possible intervention in LARs, which, however, has not been fully confirmed. On the other hand, the mediators seem to be correlated to IARs, as demonstrated by the increase in histamine levels in circulation and the increase in histamine and tryptase in BALF of atopic subjects a few minutes after allergen

stimulation, and by increased urinary methylhistamine in the ensuing hour [283]. Tryptase in BALF is correlated to PC_{20} (concentration of methacholine inducing a 20% fall in FEV_1), but not in serum after TPB with allergens [240], which could suggest that mast cells, along with their mediators, represent the key cells in a child's BHR, given the possibility of a tryptase-induced up-regulation of bronchial smooth muscle tone [187]. Mast cells could also have the function of controlling the immune inflammation by releasing repair mediators such as heparin [467] and LTB_4, which is highly chemotactic for both eosinophils and neutrophils [54].

Basophils

The characteristics of basophils are as follows [376]:
- They produce MBP.
- They express CD154 as do mast cells.
- Anti-IgE modulate their allergen-induced activation [581].
- PAF selectively induces them to release histamine.
- They have an important relationship with the CCL, lesser with CXCL chemokines.
- IL_3 induction produces IL_4 and activates MCP-1 (monocyte chemotactic protein-1), stimulating their degranulation.

Basophils share with mast cells the IgE-mediated degranulation process, with histamine and LTC_4 release that makes them powerful inflammatory cells; however, they do not release PGD_2 and are unique since they contain MBP along with eosinophils (Table 1.25). Together with mast cells they are high histamine producers, which release after stimulation by PAF, C5a and anti-IgE IgG [581]. Basophils release a greater quantity of histamine in asthmatics than in allergic rhinitis (AR) sufferers [376], even if anti-IgE IgG could down-regulate histamine release [581]. IgE-mediated *basophil releasability* has a clinical relevance in the pathogenesis of airway reactivity, since they are increased in atopic asthmatics: symptom severity is correlated with basophil responsiveness in vitro [376]. Lymphocytes and other cells promote their maturation and differentiation as well as mediator secretion, favored by various ILs such as IL_1–IL_3 and GM-CSF. In particular, IL_3 and GM-CSF induce their migration in vitro in picomolar doses and increase their chemokinetic activity and adhesion [742]. Basophils express, together with eosinophils but not with neutrophils, the integrin CD49d/CD29, equal to VLA-4, counter-receptor of VCAM-1, equal to CD106, which allows adhesion to the endothelia [561]. The parallel with eosinophils is strengthened by the community of receptors for IL_5 that become active in picomolar doses also on basophils [249]. Recently, it has been established that the *basophil role seems to be decisive in LAR,* coinciding with that carried out in the pathogenesis of AD: 19 h after the challenge, an increase in mediators released by these cells has been noted [73]. Of equal importance is the link with CCL chemokines and with MCP-1, MCP-2 and RANTES (now CCL2, CCL8, and CCL5, respectively), which stimulate basophil migration (Tables 1.54–1.57), while the CXCL with a function identical to IL_8 and CCL5, promote their adhesion, to which several integrins contribute (Tables 1.45–1.49).

Eosinophils (Figs. 11.3, 11.4, 11.8, 11.10, 11.11)

The characteristics of eosinophils are as follows (see Table 1.24):
- They are *markedly increased in number* in blood and airways, with statistically significant differences in asthmatic children compared to healthy controls [254, 639].
- They are *equipped with numerous integrins,* CD45, CD59 and HLA-DR among the inducible molecules.
- Dependent on IL_3, IL_5 and GM-CSF, as well as on TNF, PAF and IL_1, for development, activation and survival [460] (Table 11.3) [22, 284, 466].
- An *increase in the bronchial mucosa* of mRNA for IL_5 (eosinophilopoietic) [236] associated with GM-CSF (IL para-eosinophilia) [59] indicates its presence and activity [59, 236], and also the significant connection with a number of activated T cells [236].
- They are *laden with cationic proteins,* which once released may be highly toxic to airway tissues.
- Better than any other cell, they represent the common factor *able to correlate IgE hyperproduction* and involvement of metachromatic cells, macrophages and of themselves in the immune inflammation [526].
- Some studies, but not all, show the *hypodense phenotype* and the correlation of ECP (eosinophil cationic protein) levels with clinical manifestations.

Eosinophils also are *heterogeneous,* as far as density and activity are concerned. Their growth from eosinophil precursors is highly dependent on a set of ILs. IL_5 and PAF not only activate eosinophils but transform the normally dense cells into a hypodense phenotype (Fig. 1.35 c) that responds equally to chemotactic signals [354, 460]. Numerous studies have evaluated the possible role played by such cells, more cytotoxic [73], with more receptors for IgE and IgG, that produce more LTC_4 and more active, from a metabolic point of view, in patients with delayed responses to inhalants [538]. Such data is suggestive, but it is not yet possible to transfer them automatically to the pediatric field since it could more simply be related to immature forms [408], scarcely increased in number [254, 408], an effect inhibited by nedocromil sodium [578]. Moreover, the increase in hypodense cells can depend on artifacts resulting from techniques that use dextran or gelatin, or on the significant increase in eosinophilia in asthmatic children, therefore an epiphenomenon [254]. Thus, notwithstanding recurring indications, density appears to be an overly imprecise sign to be useful to recognize a pathogenic role in the pediatric field as an index of cellular activation.

Table 11.3. Functional correlations between eosinophils and interleukins (*IL*)

Function	ILs	Effects
Leukotriene production	GM-CSF, IL_3-IL_5	Increase
Phagocytosis	GM-CSF	Increase
Proteoglycan synthesis	GM-CSF, IL_3-IL_5	Increase
Superoxide production	GM-CSF, TNF	Increase
ADCC	GM-CSF, IL_3-IL_5	Optimal increase
	TNF	Modest increase
	IFN	Delayed increase
Adhesion	GM-CSF	CD54 increase
	TNF, IL_1	EAEC increase
Degranulation	GM-CSF, $IL_1\beta$	Increase
Density	GM-CSF, IL_3-IL_5	Reduction
Survival	IL_5	Optimal increase
	IL_3	Modest increase
	GM-CSF, TNF, IFN	Low increase

Data from [22, 284, 466].
ADCC antibody-dependent cell-mediated cytotoxicity, *EAEC* eosinophil adhesion to endothelial cells.

Eosinophils have, equal to mast cells, a great number of FcεRI and FcεRIIb (CD23), which demonstrates their *crucial role in atopic IgE-mediated disease*. In fact atopy contributes to infantile asthma by the mobilization and activation of such cells [408]. As can be seen from the data in Table 1.24, eosinophils can be primed to express HLA-DR in vivo, implying the presence of HLA class II molecules: therefore they are APCs able to present antigens to CD4 T cells. The modulating and regulating role carried out at the level of the immediate hypersensitivity reactions is also demonstrated by the increased presence in the IAR sites and by the ability of some of their cellular proteins to inhibit or degrade the mediators of the immune reaction [679]. Especially EPO (eosinophil peroxidase) can degrade LT, and PGE_1 combined with PGE_2 have been credited with being able to inhibit histamine release [679]. Additionally, eosinophils contain great quantities of VIP (vasoactive intestinal peptide) and produce SP. The mature cells synthesize PAF, which causes an increase in cytotoxicity and superoxide generation [758].

Immunohistochemical studies on BALF cells have shown that *eosinophils are in motion 30 min after the antigen encounter* [15, 217] and that their relative percentage, along with that of ECP in BALF, in the majority of patients, is correlated with the severity of asthma (Fig. 11.15) [51]. In fact they predominate over other cells in BALF, together with MBP, ECP and neurotoxins produced by them [531], data significantly documented in histological specimens of patients who died from asthma [255]. Other studies done to evaluate the differences between asthmatics and healthy controls have made it possible to ascertain that in asthmatic subjects, eosinophils appeared in the degranulation stage under the BM and among the basal cells, with a *significant parallel between the presence of eosinophils in a degranulation phase and epithelial damage*, whereas they were not activated in controls [51]. The asserted verifications of such alterations even in normal subjects can be explained by the potential damage provoked by the biopsy per se and in the preparation of the microscopic specimens [15]. As at the peak of cutaneous reaction, eosinophils are markedly reduced during exacerbations in asthmatic children [605].

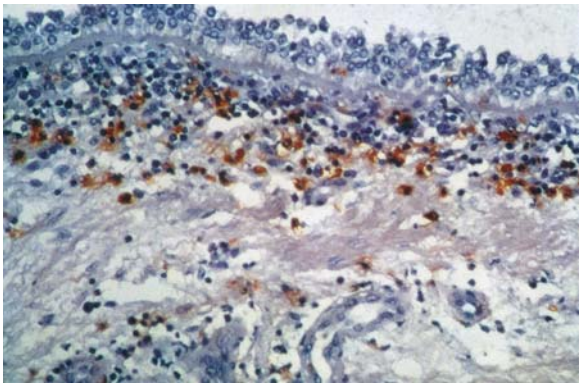


Fig. 11.15. Eosinophilic inflammation in asthma

Numerous lines of research have given further evidence that activated cells and granule products are *implicated in the tissue destruction* that accompanies and

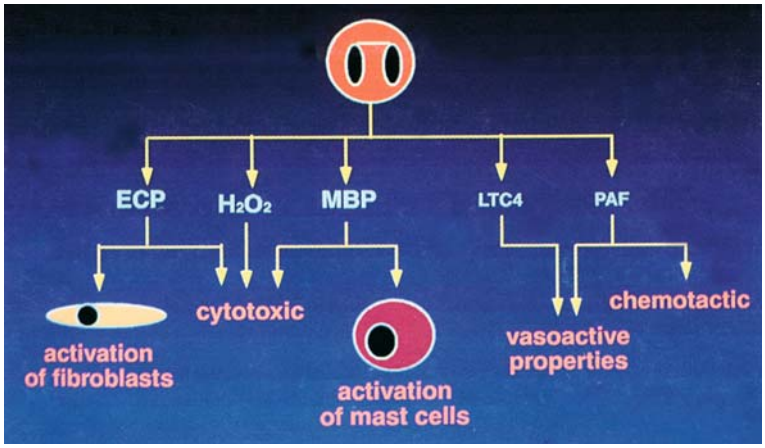


Fig. 11.16. Deleterious role of eosinophils in asthma. *ECP* eosinophil cationic protein, *LTC* leukotriene, *MBP* major basic protein, *PAF* platelet-activating factor

amplifies immune inflammatory changes (Fig. 11.16) [52]. Moreover, as a result of the change in volume and composition of the secretions of the airways that follows, *the mucociliary clearance is compromised* (by the parallel effects of LTC_4). In particular, MBP has a cytotoxic effect on airway epithelium, up to the point of dys-epithelialization [217]. The protein in concentrations of 100 µg/ml provokes in vitro ciliostasis and cell exfoliation within 12–48 h, and, if the dose is increased fivefold, the *denudation of the superficial epithelium* and of the nerve endings appears, whereas with a level of 700 µg/ml, within 30 min, the ciliary beat frequency is inhibited by 20% and the zone of activity reduced by 40% (Fig. 11.17) [217]. Experimental research has proven that EPO, in the presence of H_2O_2 and halide, tangibly damages the tracheal epithelium [51], while MBP stimulates the tracheal smooth muscles into producing PGE_2 and in secreting chlorides [217]; additionally it increases its reactivity to acetylcholine and to histamine, if the epithelium above is normal. If this is not the case, its action is negated [57]. MBP can neutralize the heparin anticoagulant activity [217]. Other highly toxic products derived from the granules play a significant role in determining the epithelial lesions during inflammation, such as ECP, ten times more cytotoxic than MBP, EDN (eosinophil-derived neurotoxin), free O_2 radicals, eosinophil-activating factor (EAF) and eosinophil cytotoxicity enhancing factor (ECEF), two factors empowering the eosinophils and their cytotoxicity [679]. An aggravating role is played by complement components, first C5a, which, like PAF, stimulates ECP and EPO release and the production *de novo* of toxic O_2 radicals. Given that components and deposits of the complement components are present in the bronchial secretions and in airway mucosa, it is likely that C5a not only stimulates eosinophils, but also contributes to their activation in tissues close to the inflammatory foci [749].

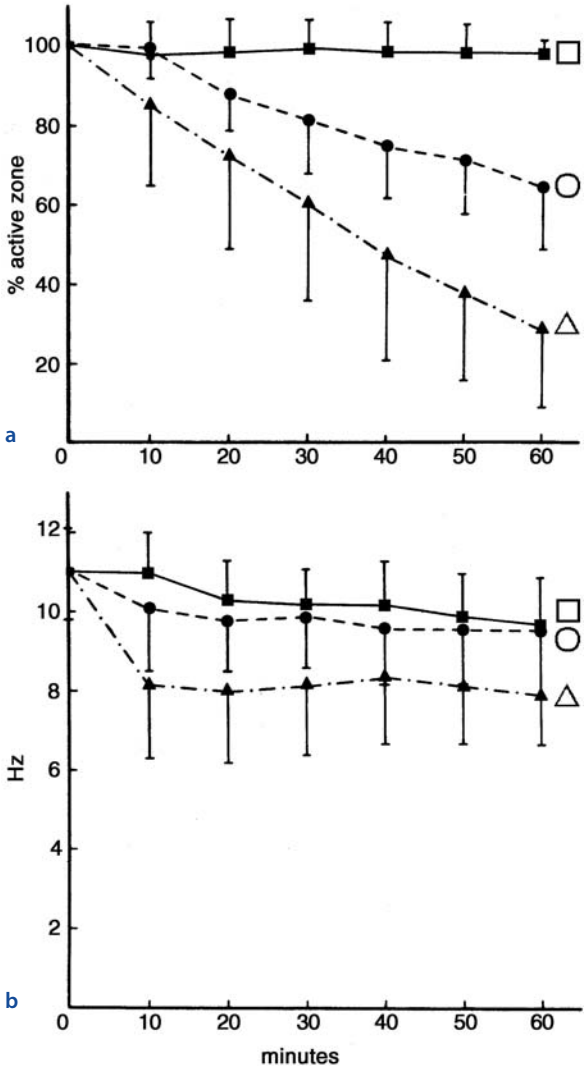


Fig. 11.17 a,b. MBP effect on ciliary activity (**a**) and on ciliary-beat frequency (**b**) in rabbit tracheal explants treated with 1.0 mg/ml of lysozyme control solution (*squares*), MBP 100 µg/ml (*circles*) and 700 µg/ml (*triangles*). The decline in percent active zone in **a**, after addition of MBP 0.1 mg/ml and 0.7 mg/ml became significantly different from control at 20 and 10 min; in **b** the decrease in ciliary-beat frequency is significantly different from the control at 10, 20, 40 min for MBP 0.1 mg/ml and from 10 min onward for MBP 0.7 mg/ml

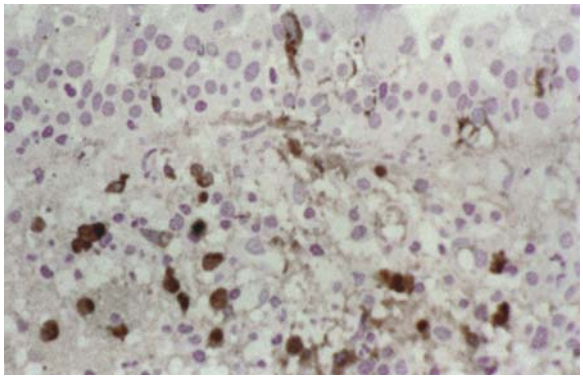


Fig. 11.18. Immunohistochemical demonstration in an asthmatic patient of migrating eosinophils stained for ECP (*in brown*)

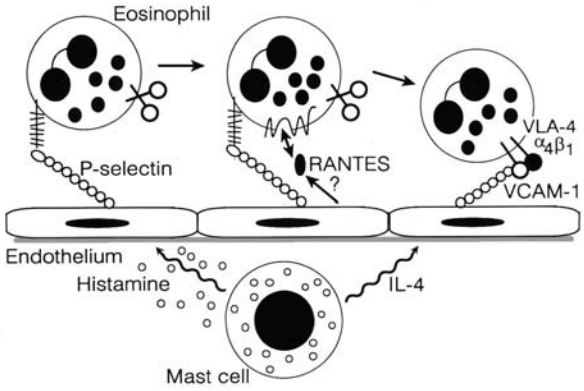


Fig. 11.19. Mast cell putative role on eosinophil transendothelial extravasation. Histamine released by degranulation mediates translocation of preformed CD62P to the endothelial surface and CD62P is involved in eosinophil adhesion. IL_4, another mast cell product, induces CD106 expression that binds to $\alpha_4\beta_1$ on eosinophils

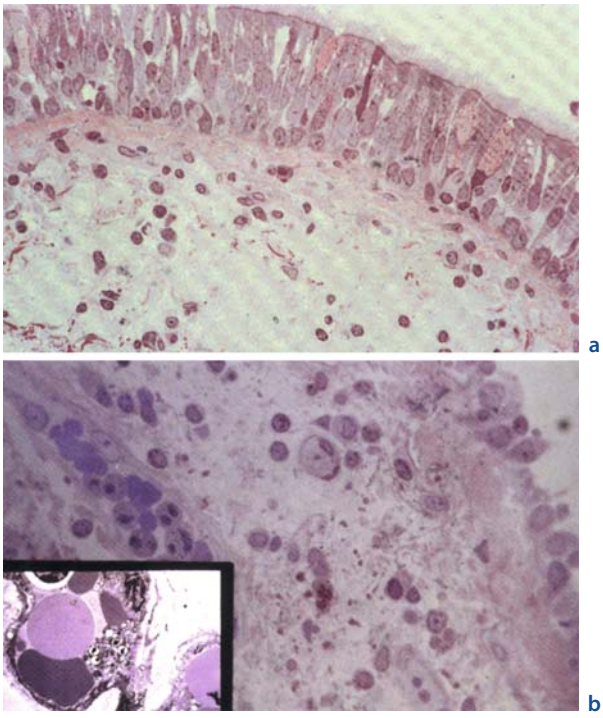


Fig. 11.20. Characteristic aspect of the bronchial mucosa achieved by fiberoptic bronchoscopy from normal (**a**) and asthmatic individuals (**b**): the comparison amplifies the extensive inflammatory infiltrate of the asthmatic biopsy and highlights the eosinophil-endothelial interactions (*inset*)

The role exercised by eosinophils in causing inflammation therefore appears dominant, as demonstrated by a significant increase in asthmatic adults in both number and ECP levels (Fig. 11.18) and in the correlation between the two concentrations, with statistically significant differences compared to controls [442, 678]. In this regard, data is controversial in atopic children [240, 442, 627, 639, 756], making asthma monitoring problematic given that *the rates decrease with treatment* [756], but not when asthma is stable [756], or in asymptomatic children [240]. More convincing seems to be the significant increase in FEV_1 after treatment, correlated with an ECP decrease, whose levels could be predictive of the results of treatment and of eventual relapses [755]. Such discrepancies are only apparent when one recalls that ECP levels depend on those of active eosinophils when inflammation is present and not on total eosinophilia. *BHR is therefore secondary to the epithelial damage caused by eosinophils and their cationic proteins*. In fact, at BHR resolution, eosinophils remain unvaried numerically, but with a reduced activation, as proved by the normalization of EPO levels, which is accompanied by the transitory increase in neutrophils and in the levels of MPO (myeloperoxidase) [229].

There exist *ample interactions with ILs* (Table 11.3) *and adhesion molecules* (Tables 1.44–1.49 and Fig. 1.59). There are three highly selective phases: expression of CD62E, CD62P and CD62L ligands (Table 1.50); activation of CD11a/CD18, CD11b/CD18, and CD49d/CD29 (VLA-4=$\alpha_4\beta_1$); and transendothelial transmigration through adhesion molecules of the Ig superfamily (CD106=VCAM-1, CD54 and CD102= ICAM-1 and -2) (Table 1.4), with a putative mast cell role in binding them to eosinophils through CD62 P and IL_4 (Fig. 11.19) [54]. Like eotaxin-1 and -2, RANTES and MIP-1α induce eosinophil chemotaxis during transendothelial transmigration [563]. The adhesion of only eosinophils and basophils to small-vessel endothelium is mediated by the interaction of CD106/CD49d/29, which therefore plays a primary role by recruiting cells to be sent toward inflammatory sites in vivo [561]. Let us remember that on a cutaneous level the CD62E selectin facilitates eosinophil and/or neutrophil adhesion, allowing them to migrate through epithelial layers, suggesting that endothelial activation, in the late stage, and due to ILs, covers a primary role in the directional migration of inflammatory cells [258] (Fig. 11.20). The glycoprotein

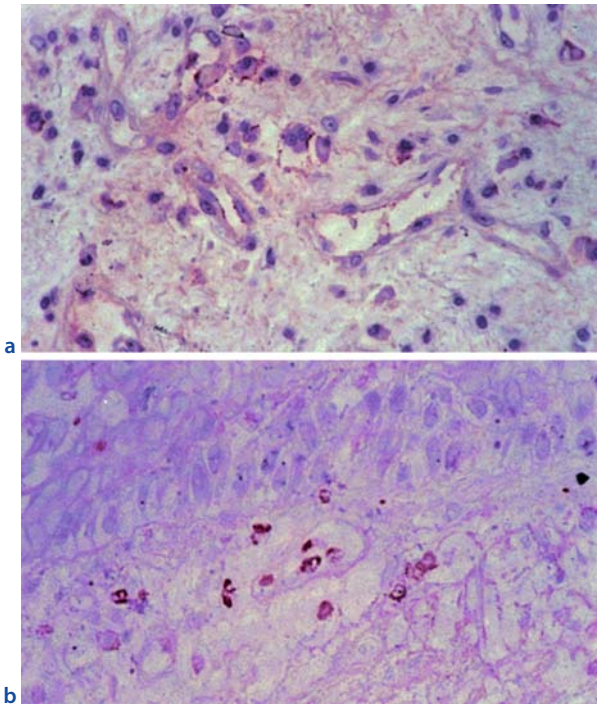


Fig. 11.21. Basal CD54 expression on endothelium of subepithelial blood vessels in a patient with symptomatic asthma (**a**) and of CD11a/CD18 receptor on T lymphocytes and eosinophils in an asthmatic bronchial biopsy specimen (**b**)

membrane of the eosinophils interacts with CD62E; thereafter eosinophils are linked via β1 and β2 integrins to endothelial cell membrane and activated under the influence of IL_3, IL_5 and GM-CSF produced by lymphocytes and mast cells [561]. Eosinophils adhere to endothelium in vitro, IL_5 induces them to express on membrane CD11a/CD18 CD54, they express their ligand on HEVs, and eosinophils are also bound to CD102, CD62E and CD106, which can contribute to their migration to the inflamed tissue in vivo [233]. They adhere to epithelium activated by IL_1 or IL_4; IL_4 can synergize with IL_1 or TNF in promoting the eosinophil link and successive transmigration [563]. Linking to CD102, CD62E and CD106 is crucial in encouraging their accumulation in the airways of asthmatics, and is so characteristic as to be able to consider the levels of CD54 and CD106 as activation indexes that are more sensitive than those based on ECP [233].

Activated eosinophils *migrate to mucosal sites* and through the action of IL_5 and PAF progress to the bronchial epithelium [460]. Elegant data indicate that only IL_5 is correlated with eosinophil enrollment and degranulation. IL_5 promotes the differentiation of mature eosinophils from precursor cells and a current hypothesis suggests that along with GM-CSF and IL_1, it can stimulate their degranulation, even in pollen-allergic subjects during the pollen season [87]. IL_5 is also able to prolong their survival for 24–48 h, with a lesser role compared to other ILs, and to activate them in successive phases, along with elaboration of lipid mediators and PAF activation [460]. In particular [563], it carries out a chemotactic action on eosinophils. Moreover, since it is a growth factor inducing pro-inflammatory modifications in these cells and acting on B lymphocytes, it increases IL_4-induced IgE synthesis, making it possible to conclude that IL_4, after its release from IgE-activated mast cells, can foster eosinophil recruitment and differentiation, both in blood and in the airways. As pointed out previously, the comment that IL_5 regulates eosinophil adhesion, an event underlying a fundamental role in their persistence in airway tissue, is relevant [460].

Studies on animals have widened the field of experimental knowledge, indicating that CD54 expression is induced on *endothelial and epithelial cells of the airways* in the hours following an inflammatory stimulus [81] (Fig. 11.21), that repeated antigen inhalations concretely increase CD54 manifestation on tracheal vascular epithelium and endothelium with a consequent rise in BHR and eosinophils in the airways, and also that anti-CD54 monoclonal antibodies reduce neutrophil adhesion and migration and inhibits (partial) in vitro eosinophil adhesion to endothelial cells [230]. Anti-CD54 delivered in vivo lessens or eliminates eosinophil infiltration and aspecific BHR induced by methacholine [230]. These experimental data clearly indicate that CD54 can play a substantial role in BHR pathogenesis [705], while blocking it by a monoclonal antibody can provide a therapeutic approach for the treatment.

Monocyte-Macrophages

Pluripotent monocyte-macrophage cells are implicated in the pathogenesis of asthma, performing important functions in chronic inflammation, due also to their long life-span (about 180 days). Similarly to mast cells, they are divided into two subpopulations: low-density cells that appear in a state of activation, and high-density cells that are in a state of quiescence.

Immunohistochemical studies on cells taken from BALF have shown evidence that:

- Their numbers are higher in atopic asthmatics than in healthy controls.
- Many cells carry monocyte markers, suggesting that they are of recent derivation from blood monocytes.
- They are provided with receptors for IgE (FcεRIIb) and therefore are activated [330, 693].

Monocytes

The characteristics of monocytes are the following [330]:

- They produce ILs stimulated by endothelin-1 (ET-1) and endothelin-4 (ET-4) [129].
- They liberate thermostable factors.

• They express HLA class II antigens and activation molecules CD11a and CD11b.

Such expression is suppressed by hydrocortisone in sensitive subjects, but not in those who are resistant [330]. They migrate in the alveolar cavities as a consequence of a series of factors related to endothelial microvasculature and chemotaxis phenomena [537]. In the airways, they actively participate in inflammation, secreting numerous ILs with pro-inflammatory activity such as IL_1, TNF, M-CSF, chemokines as well as thermostable factors that stimulate eosinophils and neutrophils to release LT and ECEF. Monocytes in asthmatics are activated, as demonstrated by IL_5 production in atopics with peripheral eosinophilia; they produce GM-CSF in vivo that, on its own or in concert with other ILs, can induce bone marrow progenitors to generate new colonies [330]. Monocytes show a striking ability to produce $O_2^{\cdot -}$ and, when they are linked by FcεRIIb to the receptors, can activate NADPH oxidase, thereby playing a significant role in chronic inflammation by their ability to release toxic products, in part through their linking to CD23-IgE [144]. Co-expression of $CD14^+/CD23^+$ is significantly increased, while the specific CD14 marker shows no change [552]. ET-1 and ET-4 are potent stimuli for monocyte production of TNF-α, $IL_{1\beta}$, IL_6, IL_8 and CM-CSF [129].

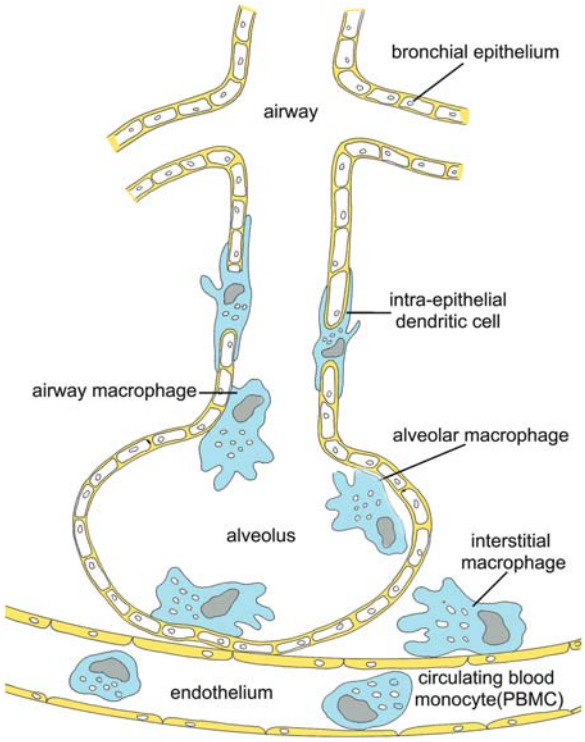


Fig. 11.22. Airway macrophages are significantly increased in asthmatic subjects

Macrophages

The characteristics of macrophages are [537]:

• They are increased in number in asthmatic airways and BALF.

• They can produce proinflammatory ILs such as IL_1, IL_6, IL_8, TNF, and also PGs, LTs, PAF, NO (N oxide) and O_2 free radicals.

• They express FcεRIIb.

It is hypothesized that the majority of active macrophages derive from circulating monocytes [97] (Fig. 11.22). The lung alveolar macrophage is the primary phagocytic cell in the alveolar space [533]. Although their numbers are significantly increased in asthmatics [503], their proliferation in the airways is generally negligible, leading to underestimating their contribution to asthmatic inflammation [330]. On the contrary, they are equipped with HLA class II molecules, becoming, in this association, the predominant cell type in the airways [537], and they are able to process and present antigens to histocompatible CD4 T cells, even though alveolar macrophages are APCs of little effect [287]. After interacting with CD4 T cells, active macrophages pass to a quiescent stage, which precludes the perpetuation of the inflammation [97]. They synthesize a *wide range of mediators*: eicosanoids of both metabolic cycles, including LTC_4, LTD_4 and LTE_4, PAF, and β-glucuronidase. Moreover, they produce O_2 free radicals, pro-inflammatory ILs of toxic effect on the bronchi, various proteins and peptides, C5a, PDGF (platelet-derived growth factor) with consequent release of other mediators (Fig. 11.22) and, if Ca is present, HRF, and they also participate in LARs [564]. In contrast to their ability to release factors impairing ciliary function, macrophages also express a potent capacity to synthesize ILs that may up-regulate ciliary beats [648]. In 75% of cases, macrophages react with GM-CSF [330], which activates them, stimulating their response to IgE via CD23, so IgE occupying the CD23 molecule on macrophages leads to a potential loop for allergens to activate macrophages [537].

As effector cells they are charged with clearance of cellular debris ingestion and killing of microorganisms. They have the ability to migrate to sites of inflammatory reactions where they produce growth factors, bioactive lipids, free O_2 radicals, NO and nitrites [537]. In particular, the marked production of NO [443] may have important effects on vascular and bronchial smooth muscle tone and on bronchial epithelial cells: therefore, the role played by macrophages in airway inflammation [212] is significant. Alveolar cells synthesize elastase and metalloproteins, capable of breaking down macromolecules of the extracellular matrix (ECM), among which elastin is an element in connection with the elastolysis found in asthmatics [53]. As regards the relationship with other cellular types, they contribute to the recruitment and immunological activation of granulocytes, attracted by LTB_4 and IL_8/NAP-1 (neutrophil activating peptide-1), of eosinophils by PAF, GM-CSF and IL_5, of neutrophils by IL_8/NAP-1 and of mast cells [287].

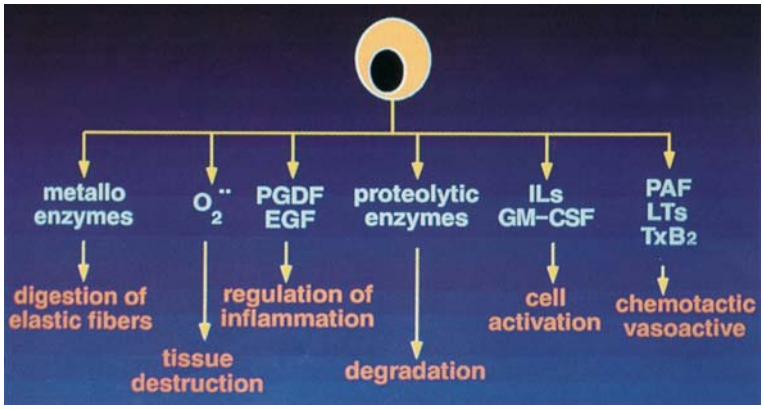


Fig. 11.23. Macrophage role in asthma. *EGF* epidermal growth factor, *GM-CSF* granulocyte macrophage-colony stimulating factor, *IL* interleukins, *LTC* leukotriene, *PAF* platelet-activating factor, *PDGF* platelet-derived growth factor, *TxB* thromboxane B

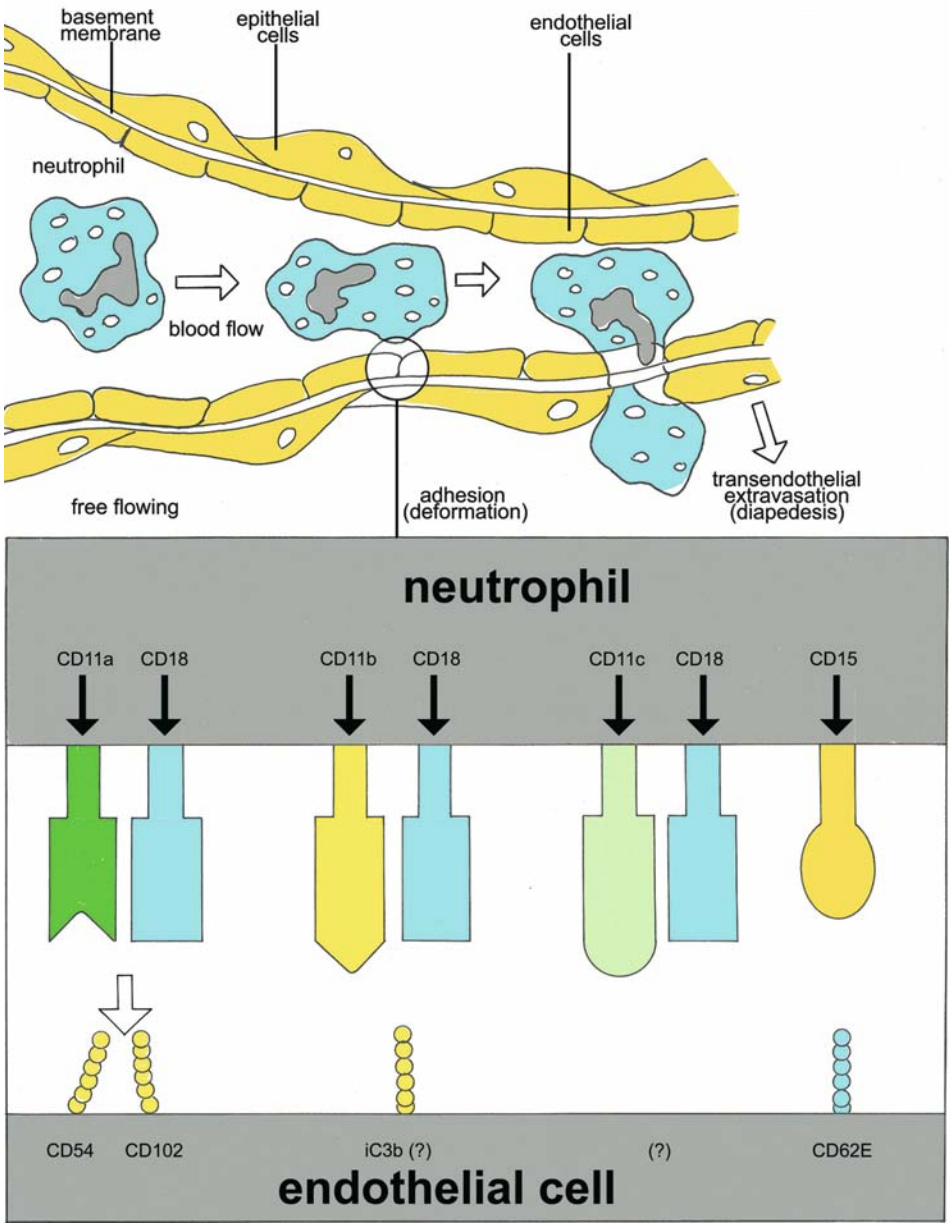


Fig. 11.24. Neutrophil extravasation

To summarize, macrophages participate in regulating the inflammation by releasing ILs and growth factors, and in the growth and activation of mast cells and eosinophils. All these effects stimulate the release of vasoactive mediators capable of provoking bronchoconstriction and mucus secretion. Recently, it has been reported that macrophages produce CCL chemokines such as MIP-1 and -2, MCP-1–3, and that alveolar cells are activated by MCP-1, with increased levels in asthmatics [330]. It follows that even *macrophages could play the role of key cells in immune inflammation* [52], as depicted in Fig. 11.23. Moreover, the virtual involvement in the fibrosis process in the reparative stage [53] demonstrates their wide powers. Finally, they constitute the only cell line that does not undergo the positive effects of therapy [212].

Neutrophils

The characteristics of neutrophils are:

- They can have *cytotoxic enzymes* and toxic radicals of O_2.
- They appear to be *more active in LARs.*
- They enter into close contact with *selectins and integrins.*
- They secrete numerous *ciliostatic substances.*

Neutrophils are active in acute asthma in both IARs and LARs, as shown by the presence in both phases of *neutrophil chemotactic factor* (NCF). Neutrophils accumulate in the sites of IgE-mediated acute allergic reactions within a time that varies from 30 min to a few hours. Therefore, these effects are carried out after the initial action, when NAP-1 and NCF come into play, promoting an accumulation of inflammatory cells that prolongs the inflammatory effects of allergen challenge. Their primary role seems to be that undertaken in LAR, depending on the time needed to produce NCF. Neutrophils are found at the center of inflammatory lesions because of two specific cell sites: the cytoplasmic membrane and the intracellular granules, which contain a vast array of digestive enzymes (Table 1.23). Elastase, collagenase and gelatinase, which attack the vasal endothelium of the respiratory apparatus, are particularly cytotoxic. Not all authors are in agreement regarding the attribution of a specific role to neutrophils [53, 600], even if IL_{17} secreted by T cells could involve them in the airways (Table 1.5).

Recently, however, a better understanding has been gained of the role they play in immune inflammation, which is summed up in the stages described above and which require the recruitment of CD11a/CD18, CD11b/CD18, β2 integrins of neutrophils, which link to their counterparts on endothelial cells, CD54 and CD102 [420] (Fig. 11.24). Furthermore, in vitro studies have shown that the duo NAP-1/IL_8 [266], stimulated by CD62E, directs neutrophils to transmigrate through the endothelium [420], and that SP directs them toward inflammatory sites, joining with VIP and somatostatin in modulating their functions, in concert with various ILs [88]. As underlined in Chap. 1, PAF and SP favor neutrophil adhesion to the walls of vascular endothelium and their successive migration in the interstice of respiratory airways of asthmatics, where they can produce several negative effects on both structure and function [88]. In a mouse model, the link between allergen-induced T cell activation and neutrophil influx induced early IL_{17} mRNA expression in inflamed lung tissue, concomitant with a prominent bronchial neutrophil influx [243]. In asthmatic children, the manifestation of CD11b, localized in specific granules, is also increased in the neutrophils, thus carrying out an important role in their migration together with eosinophils: the induction of complement receptors, in particular of CD35, could constitute the first step in the inflammation [37]. The *negative effect of neutrophils on ciliated cells* caused by proteases such as elastase, by oxidants that impair their motility, as well as by bioactive lipids such as PAF that up-regulate mucociliary clearance [413, 648], has now been confirmed.

Epithelial Cells

The role of epithelial cells is to provide passive support to sensorial receptors, which are opportunely protected by tight junctions and regulate the hydroelectrolytic perireceptor environment, also ensuring, by means of mucus and *cilia,* the clearance of abnormal substances and performing antimicrobial, antioxidant and antiprotease activities [648]. According to recent information, they also play an immune role as APCs, as they are equipped with FcεRII and class II antigens that they can present to CD4 T cells (Fig. 11.13), and express enzyme pathways for cyclooxygenase and lipoxygenase. Oxygenated metabolites have robust effects on smooth muscles, nerves and glands, as well as on inflammatory cells and epithelial cells [52] (Fig. 11.25). Due to specific phospholipases, these cells release eicosanoids, NCF and endopeptidase, able to degrade neuropeptides and fibronectin that mediate cell adhesion together with integrins, and *participate in epithelium regeneration* [525]. Epithelial cells contribute, therefore, by means of these mechanisms, to the pathogenesis of asthma, promoting both infiltration and local activation of granulocytes and T lymphocytes [374].

The epithelial cells also produce:

- *IL_1,* an IL_6 synergistic component of T lymphocyte, IL_8 growth and activation [374], IL_{10}, IL_{11}, and IL_{16} [189]
- *NAP-1/IL_8,* chemotactic for T cells, neutrophils and above all eosinophils, in contrast with normal subjects [33]
- *Growth factors:* TNFR-1 (CD120a), endothelins, lipids, NO, PDGF [189]
- *Adhesion molecules* such as CD54, CD102, CD62E and CD62P, ligands of the counterparts on T lymphocytes (CD11a, b/CD18, CD49d/CD29) and CD62L

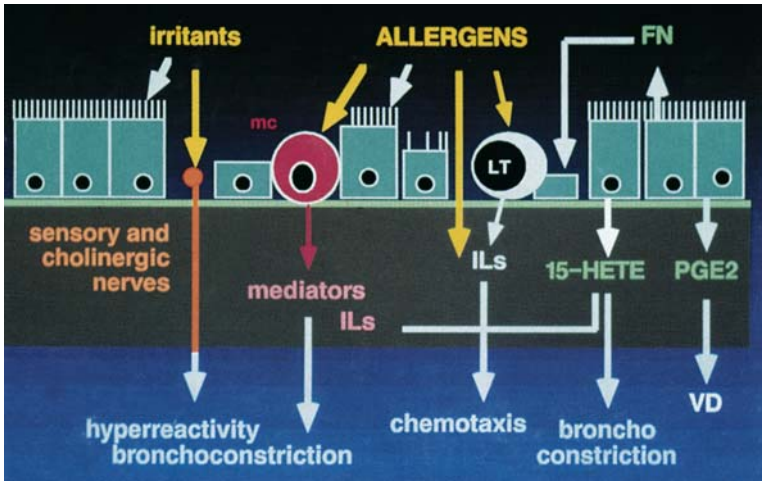


Fig. 11.25. Putative role of epithelium in asthma. *FN* fibronectin, *IL* interleukin, *LT* T-cell, *MC* mast cell, *VD* peripheral vasodilation

- *Chemokines:* MCP-1 and GM-CSF specific for monocytes, MCP-4, GRO-α and GRO-ɣ [189]

The report that $IL_{1\beta}$ produced by monocytes induces an increased synthesis of IL brought about by epithelial cells denotes the possible existence of an amplifying network among epithelial cells and monocytes/macrophages.

Recently the effects in the airways of ET-1, provided with a powerful bronchoconstriction action – less so in endothelin-2 and -3, whose synthesis is codified by an equivalent number of genes identified in the human genome – have been defined [431]. Bronchial epithelium of symptomatic patients produces larger quantities of it compared to asymptomatic patients. ET-1 binding sites are present in bronchial and bronchiolar muscles [4]. Additionally, such peptide stimulates the lipoxygenase activity with an increase of LTs, with both chemotactic and constriction effect on smooth muscle [4]: in a pediatric cohort it reflected the degree of effort required by asthma [12]. In confirmation of its autochthonous production, ET-1 levels in BALF are high; the output is regulated by TNF-α, IL_1, lipopolysaccharides (LPs) (endotoxin) [431] and histamine [4]. Consequently, the airway epithelium, like vascular endothelium, plays an important, double role in regulating the muscular tone of the area of interest, releasing both constriction factors, such as ET-1, as well as the epithelium-derived relaxing factor (EDRF) [431], identified with NO [10].

The potentially harmful activity of epithelial cells also comes about through 15-HETE, a biological mediator that stimulates the infiltration of inflammatory cells by inducing the release of mucosal glycoproteins, influencing in leukocytes 5-lipoxygenase activity and stimulating in asthmatics a premature bronchoconstriction in response to inhaled allergens. They are therefore activated cells, which, with their products, can play a deleterious role in the genesis and persistence of bronchial inflammation and BHR (Fig. 11.25), while their changes are the heart of remodeling [491].

Endothelial Cells

The vascular endothelium, in addition to contributing to the passive barrier and also *taking part in airway remodeling* [490], seems to be involved in asthma pathogenesis and in inflammatory processes associated with its severe forms. It is considered a true endocrine gland, with a surface made up of about 10^3 cells corresponding, in an adult, to about 1.5 kg of tissue [112]. Its cells locally regulate the inflammatory cells that, to reach inflammation sites, must cross endothelial walls. This process is articulated in two phases: first adherence and then passage through endothelial cells. The regulatory activity consists of impeding the adhesion and aggregation of blood platelets and other blood cells, maintaining bloodstream regularity by endothelium-released NO, with an action that is both vasodilatory, especially at the arteriolar level, and antiaggregating along with PGI_2 [10]. Once activated, endothelial cells induce:

- An increased expression of *adhesion molecules*
- Production of *GM-CSF, IL_8 and RANTES,* potentially reduced by inhalation of beclomethasone dipropionate (BDP)
- Production of *chemotactic factors* for neutrophils and T lymphocytes, and of PAF and IL_5 [654]

Basophils adhere to endothelia by means of CD49d/CD29 [561], and TNF-α and IL_1 induce CD54 on endothelium (a prerogative also shared by macrophages) and CD62E for leukocyte adhesion. Activated endothelium could be capable of self-protection against TNF-α negative effects, which exposes the barrier to O_2 toxicity, thus increasing its permeability [654]. Nor should one forget the complex interactions between subsets of lymphocytes and endothelial tissues in the control of lymphocyte recirculation (Fig. 1.5). Hence, it is not out of the question to also attribute to these cells a participation in the inflammatory process.

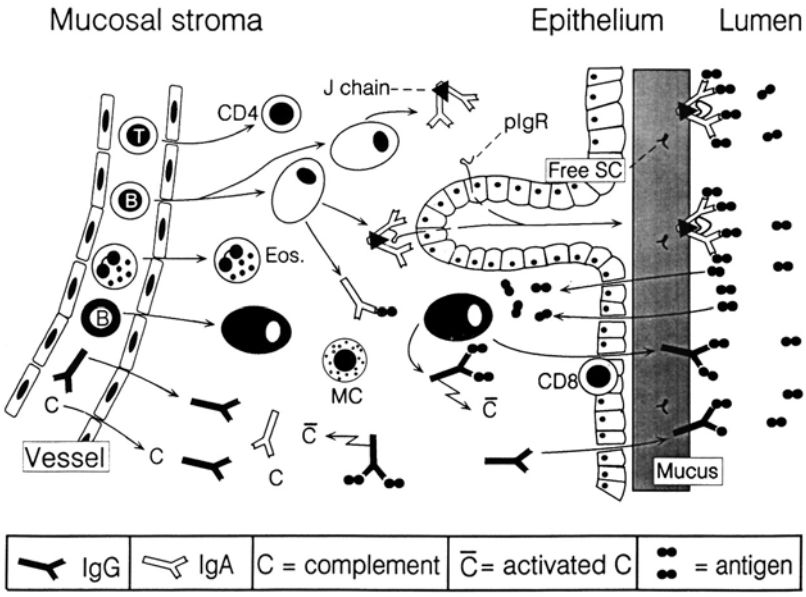


Fig. 11.26. Altered immunological homeostasis in respiratory mucosa. The altered mucosal homeostasis is reflected by distorted B lymphocyte accumulation because of aberrant lymphocyte extravasation, and emigration of mast cells (*MC*) armed with IgE and partly primed eosinophils (*Eos*) is facilitated by cytokines modulating the profile of endothelial adhesion molecules. A second line of defense is set up in the mucosa to perform immune elimination, including local IgG production to limit dissemination of foreign antigens. Due to the proinflammatory IgG properties, a vicious circle may develop with further increase in epithelial antigen penetration, complement hyperactivation, massive phagocyte recruitment and inflammatory mediator release (for details see text)

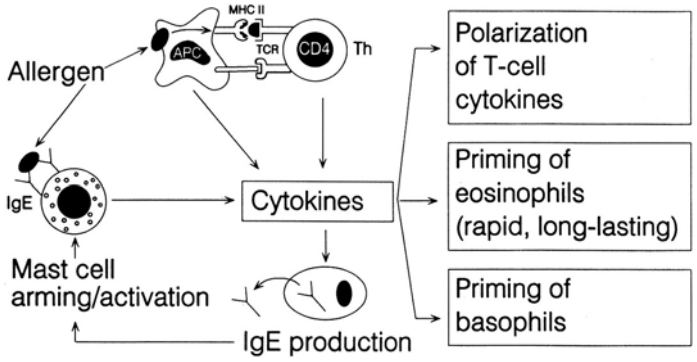


Fig. 11.27. Central role played by IgE and mast cells in development of late-phase allergic reaction. Processed peptide is exposed by APC to T lymphocytes in the context of HLA class II molecules, while intact allergen cross-bridges IgE antibodies on the surface of mast cells. Cytokines generated by CD4 and mast cells enhance preferential IgE production triggering mast cells, thus polarizing T-cell cytokines to a Th2 pattern (for details see text)

Blood Platelets

Blood platelets provided with FcεRII migrate by diapedesis to inflamed tissues, acting as effector cells in the immune inflammation. An inappropriate activation leads to eosinophil recruitment by the expression, either singularly or in combination, of PF4, PAF, PGE_2, and thereby to BHR and asthma. In asthmatics, thrombocytopenia, an increase in associated circulating blood platelets in the microvasculature, of blood platelets in BALF, of PF4 levels and thromboxane B_2 (TXB_2), as well as the prolonged bleeding time and a reduction in the time needed for blood platelet regeneration and survival have been identified. These cells liberate TGF-β and PDGF, the former being able to stimulate fibroblast proliferation, the latter smooth muscle hypertrophy. βTG (β-thromboglobulin) generates NAP-2 and PF4, chemotactic for neutrophils and monocytes/macrophages, respectively (Table 1.57), suggesting platelet involvement in infiltrating and perpetuating structural alterations which, in the long run, lead to subepithelial fibrosis and smooth muscle proliferation at the basis of BHR [743]. The hypothesis that βTG and PF4 perform the role of platelet-activation markers has not been confirmed [743].

The Role of IgE

As already noted, the production of IgE is slight in the respiratory mucosa which, instead, is populated by mast cells armed with IgE in the epithelium and connective tissue, which, along with basophils, produce CD154, whereas eosinophils have the CD40–CD154 duo (Chap. 1). In making a comparison with Fig. 11.2, in the mucosa a contrast with the alteration of immune homeostasis can be noted (Fig. 11.26) [54], provoked by an excessive local accumulation of B lymphocytes following a combination of an aberrant extravasation of lymphocytes, an increase in vascular permeability and an excessive exposure to antigens by the local immune system. All this is transformed into a worsening and perpetuation of the inflammation, laying the foundations for a chronic disease of the mucosa [54]. The increased production of IgE and mast cell positive regulation play a role in the outcome of the late reaction which cannot take place without T cell cooperation (see "Role of the Inflammatory Cells") (Fig. 11.27) [54].

Parallel to the suggestion of interpreting to interpret AD as a failed attempt by Th2 T cells to inhibit Th1 T cells to restore local homeostasis, asthma could be the

result of *a failure of the normal defense of the organism to control IgE-mediated immune responses* [263].

Role of the Mediators

A host of mediators, some preformed such as *histamine* (Fig. 1.56) and others generated *ex novo,* participate, directly and indirectly, in inflammation and bronchoconstriction [162, 538]. Many of those initially released are widespread, far from the discharge site to go toward metabolization or inactivation. Histamine, BK and PAF are at the basis of IAR (Table 11.2); others such as PGs, thromboxanes (TXs) and LTs provoke both IAR and LAR. Other *inflammatory factors* are PGD_2 and TXA_2, which produce vasodilation, LTs, which induce mucus secretion and increase vascular permeability, and factors released by neutrophils, basophils and eosinophils, discarded by mast cells after allergen/IgE-FcεRI interaction and drawn back to the site of immune inflammation by chemoattractant factors (BCF, NCF, ECF, LTB_4). The peptide LTs such as LTB_4, discarded by stimulated neutrophils, monocytes and macrophages, LTC_4 by mast cells and eosinophils, are *powerful mediators of asthma attacks.*

Lipid Mediators

PAF is a vasoactive mediator released by a range of cells that carries out a bronchoconstriction activity after exposure to a specific allergen. It has chemotactic and activating action on neutrophils and, above all, on eosinophils. Additionally, it increases eosinophil cytotoxicity, provoking the release of granule content; it stimulates $O_2^{\cdot-}$ generation (less in neutrophils), promoting the release of MBP and ECP cationic proteins and, in blood platelets, the flow of Ca ions and aggregation [758]. PAF linked to specific receptors can stimulate complex mechanisms of intercellular transduction, by which it induces G proteins and C protein kinase activation, and inositol phosphate and intracellular Ca turnover increase, the result being the secondary activation of phospholipase A_2 with subsequent discharge of arachidonate by membrane phospholipids, with subsequent production of a mediator cascade, including TXA [607]. For eosinophils, it is the most potent chemotactic factor which causes, in vivo, their accumulation in the airways, amplifying their activity both in IAR and LAR. Additionally, TXA promotes their adhesion to human vascular endothelium, stimulating eosinophils to synthesize LTC_4, an effect amplified by the fact that, when eosinophils come into contact with PAF, they release further quantities [607]. Eosinophils can attract other cells through PAF, with an increase in vascular permeability and bronchoconstriction, to the point *of destroying the bronchial epithelium of asthmatic children* [558]. Exudation of serum proteins in tissues – also due to a combined action with LTC_4 – is at the forefront in contributing to the amplification and maintenance of long-term immune inflammation [607]. The selective attraction-activation eosinophil ratio as also documented by the orchestration of cationic proteins, and powerful mediators, makes PAF the *leading player in eosinophil inflammation,* able to reproduce the entire sequence of typical asthma events. PAF plays a notable regulatory role in pediatric asthma re-exacerbations, as demonstrated by its marked increase during acute asthmatic attacks and by the parallel decrease after SIT [265], and by higher levels in asthmatic children, as compared to asymptomatic children and controls [558].

Eicosanoids

Eicosanoids are secreted in the airways by mast cells, alveolar macrophages, neutrophils, eosinophils and epithelial cells [362]. Among those derived from mast cell membrane, via the arachidonic acid metabolism, the second class of cysteine derivatives in particular possess a spasmogenic action, while LTB_4 carries out a chemotactic action. Lipoxygenase leads to the formation of various PGs, which, together with TXA_2, cause bronchoconstriction, milder if induced by PGE_2.

LTs are assuming an ever more important role in the pathogenesis of asthma as based on new experimental data (Fig. 1.57). Inhalation of cysteine derivatives provokes an acute bronchoconstriction that is prolonged and stronger than that caused by histamine in asthmatic sufferers, whose upper air tract is particularly sensitive to LT bronchospastic actions [387]. Higher levels in BALF and urine of asthmatics during a severe, acute crisis can always be noted. LTs also exert a sensitizing action on the airways, making them vulnerable and susceptible to BHR to triggering agents such as allergens, intense physical activity or cold air [387]. Additionally, LTs increase vascular permeability, mucus hypersecretion and the production of IL_1 by monocytes [362]. LTE_4, in particular, recruits eosinophils and neutrophils in the ratio of 10:1, able to increase their numbers in only 4 h [329], while LTC_4 reduces the activity of respiratory cilia in vitro [709], a negative effect confirmed by the report that pretreatment with cromones inhibits LTs [578]. LTB_4, on the other hand, possesses striking chemotactic activities for neutrophils, eosinophils and monocytes, also regulating the expression of membrane receptors and of IgE-FcεRII. Recent data show that concentrations of exhaled LT and 8-isoprostane (a marker of oxidative stress) are increased in atopic asthmatic but not in atopic nonasthmatic children. Exhaled LTE_4 concentrations are reduced by 18% by inhaled CS treatment whereas LTB_4 and 8-isoprostane are not reduced [588].

Prostaglandins PGF_{2a} and PGD_2 are powerful bronchoconstrictors. PGE produces bronchodilation in healthy subjects and bronchoconstriction in asthmatics. PGI_2 has little effect on smooth muscle but, since it

Table 11.4. Airway effects of the mediators implicated in asthma

Mediators	Bronchocon-striction	Airway hypo-secretion	Permeability increase	Chemotaxis	Bronchial hyper-reactivity
Acetylcholine	+	+	–	–	–
Adenosine	+	?	?	?	–
Bradykinin	+	+	++	–	–
Complement fragments	+	+	+	++	–
Histamine	+	+	+	+	–
LTB_4	–	–	±	++	±
LTC_4, D_4, E_4	++	++	++	?	±
NKA	++	+	+	–	–
PAF	++	+	++	++	++
PGD_2, $PGF_{2\alpha}$	++	+	?	?	+
PGE_2	-	–	–	+	–
O_2 radicals	+	?	+	?	–
Serotonin	±	?	+	–	–
Substance P	+++	++	±	–	
TXA	++	?	–	±	?

Modified from from [22].
TXA thromboxane A, *PAF* platelet activating factor, *NKA* neurokinin A.

Table 11.5. Effects of mediators in the airways

Effects	Mediators
BHR	ECFs, NCF, HETEs, LTB_4
Bronchospasm	Histamine (H_1 receptor), LTC_4, LTD_4, LTE_4, PGD_2, PGF_2, TXA_2, BK, PAF
Epithelial desquamation	H_2O_2, OH^-, $O_2^{\bullet-}$, proteolytic enzymes
Mucosal edema	Histamine (H_1 receptor), LTC_4, LTD_4, LTE_4, PGE, BK, PAF
Mucus secretion	Histamine (H_2 receptor), LTC_4, LTD_4, LTE_4, PGE, acetylcholine, C3a, C5a

Modified from [283].

has a striking vasodilatory activity, can cause, in concert with other mediators, edema and therefore BHR [362].

Tables 11.4 and 11.5 [22, 283] summarize the mediator activities and the pathological events for which they are responsible.

Role of Cytokines

These immunotransmitters, able to induce their own release besides that of other mediators and of regulating the expression of the receptors, have specific effects on immune inflammation acting on both growth and differentiation of the progenitors of hematopoietic cells, which are released at the sites of immune inflammation. ILs (Table 1.5), many of which can be found in BALF, are released [58, 334]:

- many that can be found in BALF are released by [58, 334]: IL_3–IL_5, IL_9, IL_{11}, IL_{13}, IL_{17}, IL_{25}, and GM-CSF by Th2 lymphocytes and mast cells
- IL_2, IL_9 and IFN-γ by Th1 lymphocytes
- IL_1, IL_6 and TNF-α by macrophages
- GM-CSF, IL_3, BaDF, NGF by epithelial cells
- GM-CSF, IL_6 by fibroblasts and endothelial cells (see the complete list in Table 1.5, also including IL_{16} and IL_{17})

Allergic airway disease is associated with skewed Th2 cytokine production, although the underlying cause of this aberrant immune response is not well understood. GM-CSF, IL_3 and IL_5 prolong eosinophil survival and stimulate their degranulation. GM-CSF stimulates granulocytes to generate LT and PAF, basophils to release histamine and neutrophils and macrophages to secrete more cytokines, among which are found IL_1, TNF, G-CSF and M-CSF [58]. All ILs act according to what is summarized in Table 1.5, in concert with the newly released and activated factors (Fig. 1.56), perpetuating the allergic inflammation and forming the molecular basis of its becoming chronic. IL_4 plays a highly important role in promoting the isotypic switching of B_{IgG} cells into B_{IgE} cells and, as a consequence, increases the concentrations of total IgE and sIgE recorded in allergic asthma. IL_6 en-

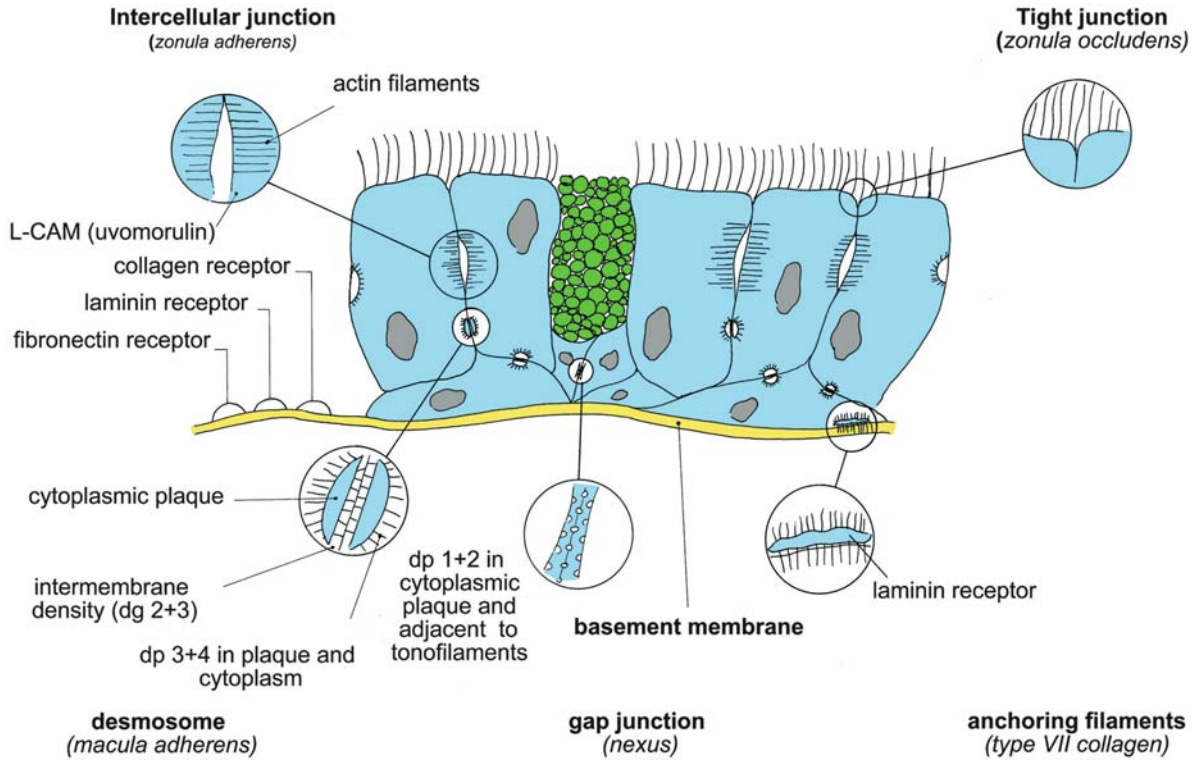


Fig. 11.28. Intercellular adhesion molecules responsible for maintaining bronchial epithelium integrity

hances in B lymphocytes IL_4-induced production of IgE antibodies. All these data reinforce the opinion that ILs perform a leading role in propitiating in the airways the conditions that trigger asthmatic manifestations. Wild et al [721] found that the administration of IL_{18} increased the production of ragweed-specific IgE and IgG_1 in serum in a mouse model of allergic asthma, effects consistent with the support of a Th2 phenotype. Furthermore, intranasal application of IL_{18} together with ragweed increased the production of BALF eosinophilia, suggesting the stimulation of an allergic sensitization when coadministered with an allergen [721]. On the contrary, IL_{11} has a profound inhibitory effect on antigen-induced inflammatory responses in the lung. This inhibitory response is associated with marked diminution in eosinophil recruitment, Th2 cell accumulation, Th2-like T cell IL production, and antigen-induced endothelial cell CD106 expression.[692]. Thus, IL_{11} is an important mediator of the remodeling response in the asthmatic airway and its elaboration reflects, at least in part, an attempt at healing and repair in this setting [752].

Airway Remodeling

In all inflammatory diseases the processes of recovery begin early in the course of asthma (premodeling). They start in the first stages of cell denudation and lead either to restitution to a former state (*restitutio ad integrum*), which does not leave a residue, or leaves changes in connective tissue deposition and to permanently altered airway structure which, in its permanent state, constitutes cicatrization [681]. Epithelial BM collagen deposition and thickening is already apparent in asthmatic children before the age of 3 years, compared to symptom-free children [501]. Normally, healthy epithelium is in contact with cylindrical cells, contact which is characterized by tight junctions in such a way that the adjacent cells oppose an impermeable barrier to the intercellular passage of macromolecules, inhaled pollutants, infectious agents, and other particulate matter. The mucosa generally absorbs small molecules as well as proteins by paracellular means, but when exposed to foreign agents it responds with plasma protein leakage. However, persistent allergic airway inflammation in asthma is accompanied by airway remodeling changes, including hyperplasia of airway mucus glands, myofibroblasts, smooth muscle and vasculature, and the thickening of the airway wall with subepithelial fibrosis [261]. The prospect of developing irreversible airway obstruction should prompt early treatment decisions [189]. IL_{11} induces an airway remodeling response in the asthmatic airways characterized by tissue fibrosis, deposition of types I and III collagen, and myocyte and myofibroblast hyperplasia [752].

It follows therefore that the remodeling process can also involve other cells such as leukocytes and ECM [491].

Fibroblasts

Their biological activity is regulated by a range of cytokines and growth factors:

- IL_1, PDGF and TGF-β stimulate their proliferation and collagen synthesis. Furthermore, IL_1 can activate T lymphocytes that respond with TNF.
- GM-CSF induces the formation of CD106, CD54 or CD62E and is able to stimulate the eosinophils either directly or through the induction of IL_5R (CD125].
- IL_1, TNF, TGF-α, histamine and heparin regulate mast cell number and function, modulating their content of proteoglycans and enhancing the filling of mast cell granules in the bronchial mucosa.
- IL_8 has an important role in directing inflammatory cells in the bronchial mucosa [534].

The fibroblastic activation may be responsible for the deposit of interstitial connective tissue under the epithelial BM [534]. During the initial processes of recovery, fibroblasts proliferate actively, secreting proteoglycans and collagen. These cells, which perform a vital role in the secretion of ECM components, can change phenotype in response to environmental signals and differentiate in myofibroblasts [534]. Macrophages, lymphocytes, mast cells and eosinophils, cells which probably participate in various ways in ECM fibrosis and/or remodeling [53], are always almost present in the granulation tissue. As the recovery process progresses, tenascin, a glycoprotein, qualitatively increases, while fibroblasts and the newly formed vessels diminish [189]. Myofibroblasts, deposits of fibronectin and laminin, and collagen hyperplasia are also found. Even in case of restitution to a former state, the regeneration of the submucosa is always, in part, abnormal [53].

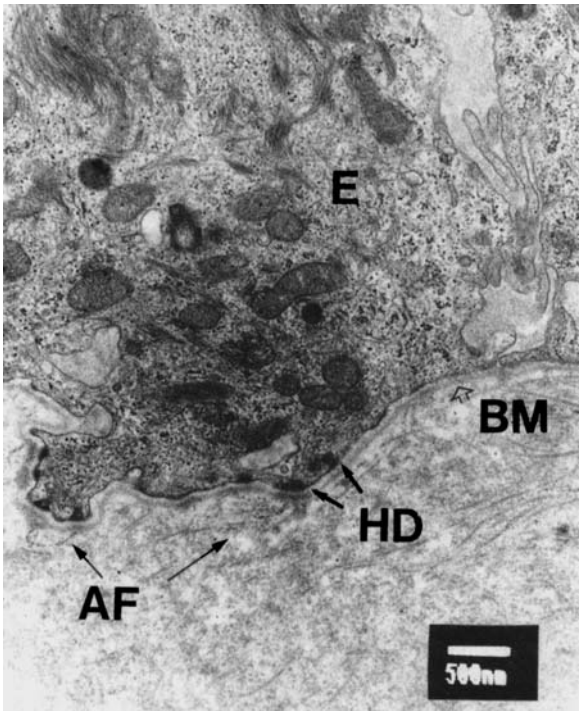


Fig. 11.29. Electron micrograph of thickened subepithelial basement membrane (*BM*). Basal cells possess hemidesmosomes (*HD*), by which they adhere to the superficial part of BM. Anchoring filaments (*AF*) are part of adhesion complex. (*E*) Airway epithelium

Epithelial Cells

Epithelial cells release a wide spectrum of molecules participating in airway repair including:

- Fibronectin, growth factors
- Cytokines including IL_9, IL_{11}, IL_{16} and IL_{18}
- Chemokines such as GM-CSF and eotaxin
- Adhesion molecules such as CD40 and CD54 [681]

The bronchial epithelium is actively engaged in defense of the airways by secreting mucus and many specific and nonspecific cytoprotective molecules that trap and inactivate inhaled components. If the asthmatic immune inflammation is protracted over time, the adhesion mechanisms are compromised, which should ensure the maintenance of epithelial integrity, represented by desmosomes, hemidesmosomes and tight junctions (*zonula occludens*) and *zonula adherens,* which is located immediately below the intercellular joints (Fig. 11.28) [327], as well as adhesion molecules such as β_1-integrins and CD62E [413]. In some cases, a pseudostratified epithelium can be observed with increased goblet cells and vacuolized ciliated cells, often devoid of *cilia* [51]. This tissue often exfoliates, with separation of mucosal cells, leaving the basal layer exposed but intact, though weakening the connection system between columnar and basal cells. It is clear, therefore, that to induce the effects described, destructive activities must take place, concentrated in both space and time (Figs. 11.30, 11.31) [52, 53]. Epithelial damage could depend not only on the cytotoxic effects of eosinophil-derived basic proteins and oxidants, but also on neutrophil intervention which, in vitro [413] and in vivo [677], have the ability of producing similar effects, as well as the exfoliation and detachment of the epithelium from the BM [413, 677]. In addition, epithelial injury is mediated by exogenous factors such as air pollutants, viruses and allergens as well as by endogenous factors including the release of proteolytic enzymes from mast cells [260]. It has been suggested that such changes could also originate from an abnormal response of epithelial cells, stimulated by leukocytes [413]. Moreover, IL_{13} hyperproduction promotes subepithelial fibrosis and thickening of smooth muscle layer [321]. The epithelial response to these stimuli in asthma may be impaired despite up-regulation of CD44 capable of enhancing presentation of EGF (epidermal growth factor) ligands to EGF receptors (EGFR) [260].

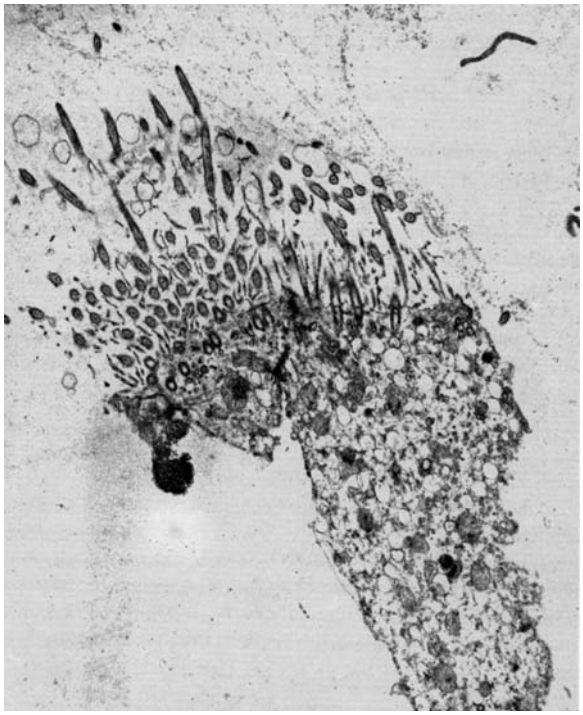


Fig. 11.30. Desquamated epithelial cells undergoing necrosis recovered by BALF from the airways of an asthmatic patient after an exacerbation

However, *regenerative activities* begin with the *re-epithelialization of the denuded surface,* as demonstrated by various stages of ciliogenesis related to nonciliated superficial epithelium, and by a quantitative increase in goblet cells in the ciliated epithelium. The speed and rapidity with which restitution to a former state begins and continues is surprising. Vascular endothelial cells proliferate, forming granulation tissue, the newly formed vessels have open junctions that permit the release of proteins and erythrocytes, the microvasculature also responds by recruiting neutrophils [490]. It is likely that bronchial epithelial cells originally involved with the lesion are able to initiate these reparatory activities, producing chemotactic factors for intact epithelial cells and for PMNs [600]. Facilitated by an adequate hydraulic pressure, the plasma proteins enter the lumen of the tight junctions of the intact cylindrical cells that circumscribe the denuded area [491]. These cells respond immediately, leading to a supposed intervention of specific factors in vivo: substitute secretory cells, both ciliated and basal cells, undifferentiate, become flattened and migrate beyond the membrane – *a process which takes place rapidly in the 1st min following denudation (about 3 µm/min)* [490]. A fibrin and fibronectin gel, also rich in neutrophils, contributes in covering up the stripped areas where it is continually enriched with plasma until the epithelium has been regenerated with the tight junctions [491] (Fig. 11.32). The *fibronectin,* present in the ECM with a great number of binding sites for the cells and other molecules, appears to participate in epithelium regeneration since it is responsible for cell adhesion, a mediator role that is shared, via different receptors, with collagen and laminin [600]. However, the simple restitution of epithelial cells has the potential of being involved in many aspects of structural alterations, present in human airways, that are at the base of symptoms similar to asthma (Table 11.6) [491].

Extracellular Matrix

ECM is a complex and dynamic meshwork influencing many cell biological functions such as development, migration, and proliferation. ECM plays an essential supporting structural role, which differs somewhat in the three physiological zones of the lung: the proximal conducting airways and vasculature, the distal gas-exchanging respiratory zone (alveoli), and the intervening transitional zone (respiratory bronchioles). In the conducting airways, the ECM of the rigid cartilage (composed largely of proteoglycans and collagen) and the more flexible interstitial tissue support the adjacent epithelial and smooth muscle cells. ECM allows some mobility to

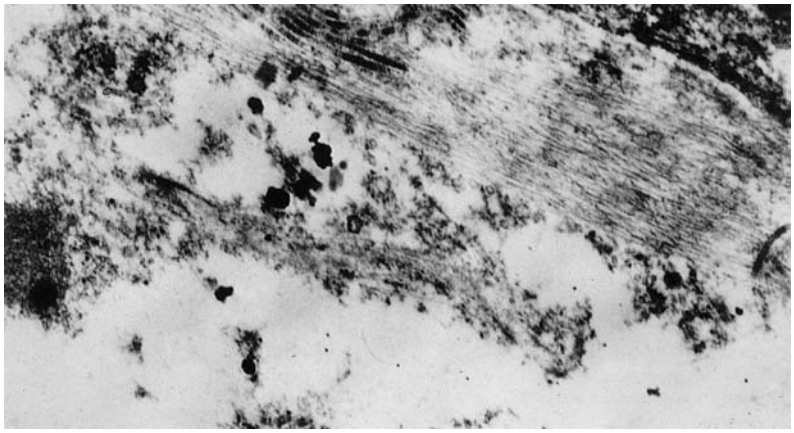


Fig. 11.31. Elastolysis in asthmatic airways: electron microscopic study

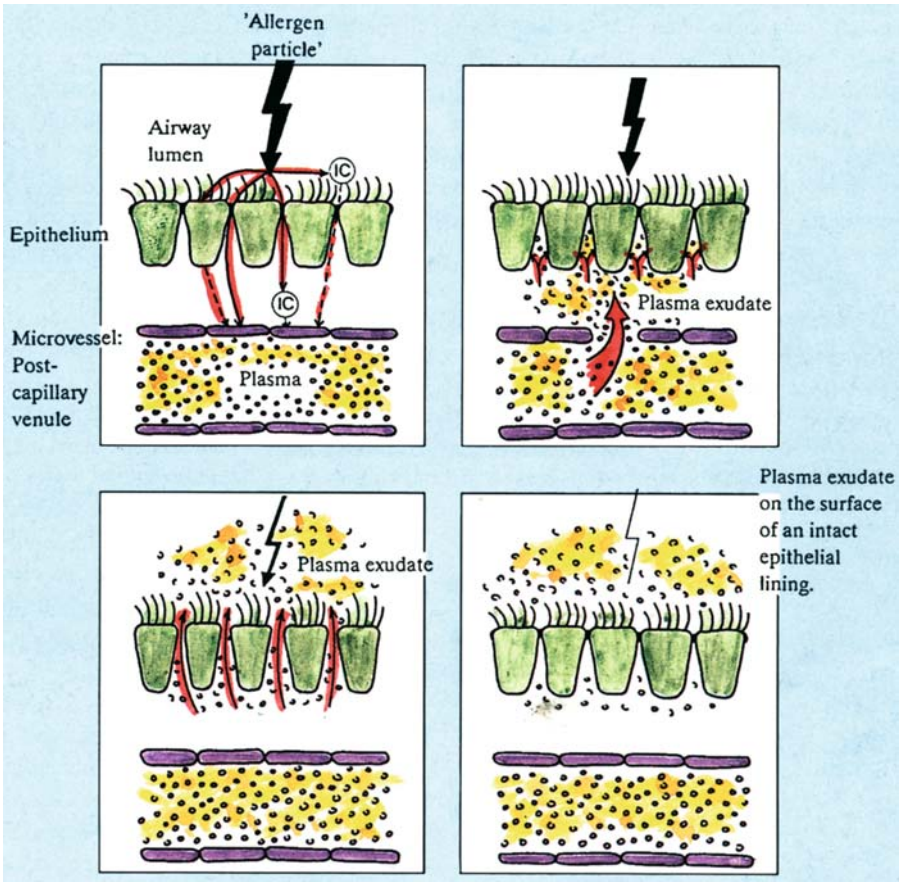


Fig. 11.32. Plasma exuded through barriers in the airway microvasculature and mucosa. Plasma leakage from subepithelial microcirculation multiplies its solutes and expands in volume. It surrounds the basolateral aspects of the epithelial cells; by increasing the hydrostatic pressure the exudate may compress the sides of these cells. At a certain pressure, the tight junctions at the apical pole of epithelial cells would separate due to the increased pressure load. Plasma leakage through the ensuing holes in the venular wall. Tight junctions are reestablished as soon as the interstitial pressure returns to normal levels

Table 11.6. Asthma-like effects aroused by reparation processes

Pathophysiology	Leukocyte activity	Structural alterations
Plasma leakage	Eosinophil traffic and activation	Epithelial metaplasia
Secretion	Neutrophil accumulation and activation	Adhesion molecules of plasma origin
		(Pseudo) thickening of basal membrane
		Proliferation of fibroblasts and/or smooth muscle cells

Modified from [491].

regulate airway and vascular diameter and acts as an essential stabilizer for preventing airway collapse during expiration. In the respiratory and transitional zones, the ECM is more dynamic to accommodate the constant fluctuations in alveolar volume that accompany inspiration and expiration. Macromolecules constituting ECM are secreted locally and consist of fibrous proteins (fibronectin and laminin) embedded in a hydrated polysaccharide gel containing several glycosaminoglycans including hyaluronic acid. *ECM is a dynamic structure,* and an equilibrium between ECM synthesis and degradation components is required to maintain its homeostasis [681].

Basement Membrane

The BM of surface epithelium is composed of two layers: the basal lamina (referred to as the true BM) and the *lamina reticularis.* The basal lamina is of normal thickness in asthma. However, thickening of the lamina reticularis is a characteristic feature of the asthmatic bronchus, occurring early in the disease process [681]. Studies of postmortem reports initially led to the conclusion that in asthmatics the BM becomes thicker as a result of edematous collagen deposits, with fibrils of a plexiform appearance. Electron microscopy (EM) and immunohistochemical investigations subsequently made it clear that BM is normal. Airway thickening be-

neath the BM occurs with collagen deposition and other ECM proteins, including fibronectin and tenascin in the connective tissue layer surrounding the blood vessels, and alveolar interstitium [261]. The degree of collagen deposition into the BM lamina reticularis in children who underwent fiberoptic bronchoscopy is such that airway fibrosis evolves in parallel with eosinophilic inflammation before a clear clinical diagnosis of bronchial asthma could be made [501]. Instead, reticular BM thickness or inflammatory cell number determined in ultrathin sections of endobronchial biopsies were not present in 53 infants aged 3.4–26 months with severe wheeze and/or cough, with reversible airflow obstruction [548]. In healthy subjects, BM, independent of asthma etiology, gravity and duration, shows a *pseudothickening* [51] caused by a deposit of collagen III, V and I (to a lesser extent) and of fibronectin in the *lamina reticularis* situated below the BM [534], confirming that at the basis of this process, is the *activation of contractile myofibroblasts* in the subepithelial site, rather than an epithelial dysfunction [53]. Since BM appears to be the main structure appointed to the regeneration organized by connective tissue, forming a sort of framework for parenchymal cell replication, its structural alterations, in the airways of asthmatics, can result in an ECM deficiency of the submucosa that could be at the basis of airway remodeling.

Elastic Fibers

Most patients with asthma have an abnormal superficial elastic fiber network with fibers appearing fragmented. The deeper layer of elastic fibers is also abnormal in most patients with asthma: fibers are often patchy, tangled, and thickened. EM studies show that a severe elastolytic process occurs in patients with asthma, and in some patients, disruption of fibers has been observed.

Role of Bronchial Hyperreactivity

The pathogenesis of asthma (Fig. 11.11) is in large part identified with BHR which, together with epithelial alterations, secondary to immune inflammation, constitutes the causative element in triggering the chain of events that result in an asthmatic attack. On the whole, BHR measurement could represent a useful means of identifying in advance subjects at risk of PFT worsening, well correlated with severe manifestations and treatment implications. Nevertheless, in children a noticeable variation in clinical symptoms is found, which is why, in practice, the usefulness of measuring BHR is somewhat reduced. Furthermore, the uncertainty of a certain correlation of this parameter with the clinical state and its diversity over time renders BHR not very reliable as a diagnostic test for selecting an adequate treatment and for determining a prognosis [108]. Other authors have demonstrated that reduction of activated T cells and CD25 is correlated with that of BHR [119] but with an improvement of FEV_1, the number of eosinophils and probably of IL_5 [665] decreases.

With the aim of prospecting the nosological picture, it should be clear that [110, 603, 722]:

- BHR is not synonymous with asthma, neither is it systematically predictive of an asthmatic state. Rather it is one of the factors that suggest asthma presence, without possessing the characteristics of a diagnostic symptom *in se* and *per se*.
- BHR is not, therefore, the only component of the physiopathological mosaic of asthma. The etiopathogenic mechanisms of mucus hypersecretion and of bronchial edema are equally noteworthy factors not to be ignored in the overall picture [238].
- BHR does not lessen with age [344] as previously suggested [414] because a small child is more susceptible to BHR also because of airway caliber reduction [584]. BHR is present even in chronic airway disease such as CF [186].

Virtually all asthmatic children have BHR, but not all children with BHR suffer from asthma [108, 476]: more precisely it can be present in apparently healthy children and absent in others who are asthmatic, while nonasthmatic subjects can manifest signs of BHR – for example if faced with a cold air challenge [238]. It is also possible that BHR is present without any ongoing symptoms, which can be ascribed to patients unable to describe correctly the symptoms during initial history [496]. On the other hand, transitory BHR can be present in children with viral infections; therefore exogenous events increase BHR in both healthy and asthmatic subjects [753]. Even exposure to small allergen doses can trigger BHR, without, however, altering airway caliber [722], whereas in asthmatic children the opposite occurs: *continuous or repeated exposure to aeroallergens induces a BHR increase,* causing an almost unnoticeable progressive increase, persisting for weeks or even months. This gives rise to the premises of a vicious cycle, consequently subsequent exposures, even to nonallergic or banal stimuli easily provoke BHR. Even if inflammatory injuries caused *in loco* are numerous and severe, when administering histamine parenterally bronchoconstriction is likely to occur [167]; therefore what results is the product of a series of joint causes.

At present, the contribution of environmental factors is considered important in eliciting BHR, as demonstrated by a greater asthma prevalence in southern countries, where SPT+ to aeroallergens is less [287]. The pathogenic links between damage provoked by pollutants, mediators, inflammation and BHR are harder to characterize [238]. Strongly suggestive in this regard is that BHR may be possibly generated by O_3 with a potent inflammatory action on airways. Injected in test animals, it induces NCA and of LTB_4 formation in epithelial tissues, provoking in the respiratory mucosa a PMN in-

filtration from the microvasculature which, once activated, release TXA_2 able to sensitize the smooth muscles or the nerve terminals, thereby causing BHR [287, 317] (Fig. 4.22). O_3 induces BHR, be it through oxidizing damage [317], in concentrations of 0.12–0.15 ppm [606], or through indirectly stimulating the tachykinins to induce BHR [315]. As a result of BHR, the threshold of bronchial response to various stimuli that unleash factors with bronchoconstriction effects, is noticeably lowered. Among these, the role of mediators should be considered. As seen in Tables 11.4 and 11.5, those capable of inducing BHR are many: PAF and PG, and, to a lesser extent, TXA and LT, even if it is clear that there is not only one but a *combined interaction at work* [283]. These factors are therefore capable of causing a clinical response, increasing and/or maintaining BHR, as a result of which vicious cycles are born between clinical response, immune inflammation, triggering factors and symptoms. BHR degree is often correlated with disease severity with important clinical implications, when these are clearly the important correlation in children between BHR to histamine and the increase in mast cell tryptase and of the number of eosinophils/mm of BALF [187]. As opposed to other children, serum tryptase levels did not increase after BPT with allergens [240]. Recent studies suggest that depletion of IL_{12} [415] and overexpression of IL_{13} [321] increase susceptibility to development of BHR, even in the absence of inflammation [308].

To better explain the pathogenesis, various theories have been put forward based on the intervention of endogenous and exogenous factors.

Endogenous Factors

Among the endogenous factors, the following may be enumerated [154]:

1. Autonomic nervous system (ANS) alterations
- Deficit of adrenergic receptors
- Vagal hyperactivity
- Altered neuropeptide release at the sensory terminals
2. Anomalies of smooth bronchial muscles
3. Anomalies in airway epithelium
- Desquamation of the epithelium
- Loss of EDRF
4. Changes in the biochemical homeostasis
5. Effects of NO

ANS Alterations

Several inflammatory mediators effecting neurotransmitter release by airway nerve endings, or able to act upon ANS receptors, have been identified. Conversely, neuromechanisms could contribute to the inflammatory reaction at the bronchial level. It is therefore possible that some BHR mechanisms are linked to an imbalance of the lower airway nervous control mechanism, entrusted either to the sympathetic (adrenergic system) or parasympathetic system (cholinergic system). These systems are usually in balance: the cholinergic system promotes bronchoconstriction; the adrenergic system, in contrast, modulates bronchial smooth muscle relaxation. At the present time, the ANS airway innervation is considered to be far more complex. Given that sympathetic fibers do not directly innervate smooth muscles, in addition to the usual adrenergic and cholinergic fibers, fibers belonging to a third system called NANC (nonadrenergic and noncholinergic) have been reported, which include both e-NANC (excitatory noncholinergic/nonparasympathomimetic structures) and i-NANC (nonadrenergic/nonsympathomimetic inhibitory structures) [21].

Cholinergic Systems

The fibers of cholinergic nerves travel along the tenth pair with synapse in the airway parasympathetic ganglia, spreading to smooth muscles and submucosal glands. Stimulation of vagal receptors present in the airways provokes one of the most powerful bronchoconstriction reflexes, besides mucus secretion. In particular, mechanical or pharmacological stimulation of vagal terminals occasion, by reflex action, characteristic asthma symptoms and signs such as coughing and rapid, shallow breathing. Cholinergic innervation of upper airways is most dense, thinning out in the periphery. Animal studies have shown that stimulation produces little effect in the lower airways [21]. Given that in humans the muscarinic (M) receptors are equal to parasympathomimetic, which are diffused even in the lower airways, investigations to ascertain whether in asthma there was an increase in their activity [719] were made. This view point is supported by a study showing that many stimuli having a bronchospastic action, such as S dioxide (SO_2), histamine, PG and BK, also affect afferent receptors, therefore inducing reflex bronchoconstriction inhibited by anticholinergics. There could also be an increase in neurotransmitter activity in cholinergic ganglia, either as a result of other neurotransmitter or mediator release, or because postganglion nerve terminals carry out an action favoring acetylcholine release [18]. Given that adrenergic nerves are able to inhibit this production via receptors β or α_2, it is likely that a deficiency in adrenergic responses is reflected in cholinergic tone increase by an increase in muscarinic receptors or others related to them. Asthmatics have an exaggerated bronchospastic response to cholinergic action, but an analogous effect can also be brought about by other spasmogens; therefore an isolated deficit of muscarinic receptors with inhibitor action is unlikely.[21].

The presence in vivo in humans of five muscarinic receptors (only the first three are found in the human lung), each encoded by different genes, has been

reported. Stimulation of such receptors results in bronchial secretions and in smooth muscle contractions, and correlated G proteins have also been identified and cloned [318]. Specific studies have clarified the functionally active types [77, 719]:

- M_1, located in the parasympathetic ganglia, submucosal glands and in alveolar walls, regulating both vagal tone and mucus secretion. They are inhibited by pirenzepine.
- M_2, located on the postganglion nerves in the presynaptic position, with autoreceptor functions, inhibit acetylcholine release and, consequently, the reflex vagal bronchoconstriction actions. They are blocked by gallamine.
- M_3, located in the bronchial and bronchiolar smooth muscles in the postsynaptic position and in submucosal glands promote muscular contraction and mucus secretion. They are inhibited by hexahydroxyl-diphenidol.
- M_4, located in the postganglionic cholinergic nerves, airway smooth muscle and alveolar walls, inhibits acetylcholine release.
- Human skin fibroblasts also express M_2, M_4, and M_5.

In particular, M_1, like α_1 adrenoreceptors, are linked to Gq, and activate phospholipase C (PLC), from which results the turnover of phosphatidylinositol-bisphosphate (PIP2) and Ca^{++} release, whereas M_2 are connected to Gi containing Gi_2 to bind to GTP; the stimulation of the receptor inhibits adenylcyclase and modulates the ionic channels for K^+ and Ca^{++} [21].

The inhibiting activity is not operative in asthmatics, probably due to a *functional deficiency of the M_2 receptors*, which could be expressed by an exaggerated cholinergic activity because of the loss of a normal acetylcholine release retroinhibition, thereby explaining the sometimes dramatic bronchoconstriction action of β-blockers in asthmatics. In fact, a block of β-receptors could abrogate the antagonistic actions of cholinergic nerve activation, thus achieving an excessive acetylcholine release, which is not autoregulatory in asthmatics [21]. Therefore, nonselective antagonists of muscarinic receptors, for example, atropine or ipratropium bromide (IB), are able to mediate both bronchodilation (M_1 and M_3 receptors) and bronchoconstriction (M_2 receptor) [525].

The potential deficits of the parasympathetic cholinergic system affecting BHR are the following:

- Increased cholinergic reflex activity
- Stimulation of the efferent nerve terminals, exposed as a result of inflammation
- Vagal hyperreactivity
- Increased acetylcholine release via ganglionic or postganglionic effects
- Smooth muscle hyperresponsiveness or hypersensitivity to acetylcholine-mediated cholinergic activity
- Possible decrease or dysfunction of protective M_2, which turns into exaggerated cholinergic activity

The M_2 deficit is confirmed by the simple observation that pilocarpine, their antagonist, blocks SO_2-induced bronchoconstriction by limiting the acetylcholine, whereas in asthmatics such a block does not occur. It is significant that influenza virus selectively damages M_2 receptors rather than M_1, probably by an effect of neuraminidase action on M_2 sialic acid residues, providing a valid mechanism to explain increased BHR in subjects after viral infection [77].

Adrenergic Systems

The bronchodilator effect of adrenergic stimulation seems to depend on the basal vagal tone. The adrenergic receptors are of the α type, divided into three α1, subdivided in A, B and C, four α2 further divided into A, B, C and D, and type β, with β1, β2 (present in the lungs with a relationship of about 1:3) and β3 with a more limited diffusion. Also G proteins have been cloned for these receptors, α_1 to Gαq, α_2 to Gαi and β to Gαs (which, together with G_{i2} form the four subfamilies of the α subunit) [318]. Under normal conditions, the sympathetic tone (specifically the β receptors), represents the main balance unit by antagonizing the vagal tone and diminishing vagus-induced bronchoconstriction. The β-receptor antagonists have no effect on the cholinergic tone of a healthy subject; on the contrary, they cause bronchospasm in asthmatics with a greater efficacy the more the vagal tone is raised, α stimulation produces mucus secretion and, above all, mast cell mediators are discharged. β2 receptors are widely distributed over the bronchial area: they can be found on smooth muscle fibers and in epithelial and glandular cells. In asthmatics, due to a poor reactivity of the adrenergic system, it is likely that the main role in regulating the bronchomotor tone is played by circulating catecholamines. Epinephrine inhibits the bronchoconstriction action of histamine, acting as a true circulating hormone, and could play a protective role with regard to bronchoconstriction agents. In asthmatics and other atopic subjects, a *deficit of β-adrenergic receptors* and a parallel *α-receptor hyperresponsiveness,* which could be expressed into blocking the balance of vagal action, has been demonstrated. A supposed *genetic deficit of β-receptors in asthma* has remained an enigma since the beginning [636], even if this anomaly can be hypothesized in a few patients [502]. The postulated interconversion between β and α receptors has been negated by studies with monoclonal antibodies on receptor structures [90]. Generally, potential deficits of the adrenergic system can be summarized as follows:

- An increase in α receptors in the airways
- A decrease in α-receptor antagonists
- A quantitative and/or qualitative decrease in β receptors
- Anomalies in receptor splitting, with α, M_1 and M_3 increase causing a reduction in the number of β and M_2 receptors

Table 11.7. NANC system and potential functions

Action	Innervation	Site of action	Neuropeptides
Bronchoconstriction	Sensory C-fiber axon	Smooth muscle	NKA/SP
	Microganglia	Smooth muscle	SP
Bronchodilation	Parasympathetic	Smooth muscle	VIP/PHM
	Microganglia	Smooth muscle	VIP
Gland secretion	Parasympathetic	Glands	VIP/PHM
	Sensory C-fiber axon	Glands, microganglia	SP
	Microganglia	Glands	SP+VIP, GRP
Vasoconstriction	Sympathetic	Arterioles	NPY
		AV anastomoses	NPY
Vasodilation	Parasympathetic	Arterioles	VIP/PHM
		AV anastomoses	VIP/PHM
	Sensory C-fiber axon	Arterioles	NKA/SP
		AV anastomoses	CGRP

Modified from [18].
AV Arteriovenous; see Table 11.8 for other abbreviations.

Thus it appears that the postulated β-receptor deficiency is secondary to asthma, probably as a result of the inflammation. Additionally, if the increased α receptors were of any significance, the α blockers could have a therapeutic effect, which, however, does not happen; consequently these possible anomalies have few clinical implications. Finally, given that treatment with β-blocking drugs can yield bronchoconstriction in asthmatics (but not in normal subjects), a deficit of this system is excluded [77].

Given the existence of a certain amount of polymorphism related to some β_1 loci, for example at codon 16 (Gly 16), which is more evident in subjects with nocturnal asthma [661], it has been hypothesized that this phenotype predisposes to the nocturnal reduction of the β_2-adrenergic function and therefore to the scant effect of β2-adrenergics on the clinical symptoms. It has also been suggested that substituting glutamic acid with Glu 27 (glutamine at codon 27), which has a receptor with reduced suppression, is associated with a bronchoconstriction reduction [234]. In conclusion, G protein studies are justified by a possibly better understanding of the action mechanism of related drugs.

NANC and Neuropeptides

The functions of NANC, composed of naked nerve fibers that are found free in the submucosa, are summed up in Table 11.7 [18]. NANC was originally described in the gastrointestinal tract. Given that the respiratory system also derives from the cephalic portion of the archenteron, it is logical that NANC and related peptides can be found in both systems; they are, however, present in all organs and can be produced by cells other than CNS cells [21]. It has been suggested that anomalies of the NANC system, in its two components (e-NANC and i-NANC), *represent another important component in asthma pathogenesis.* Numerous stimuli release neuropeptides [18, 90] or tachykinins in the respiratory tract, neurotransmitters that send signals not only to nerve cells, but also to other cells or systems. So-called *neurogenic inflammation* is based on the contribution of numerous neuropeptides to the pathogenesis of a major part of the anatomopathological lesions thus far reported, thereby determining a series of biological responses during the recurrences of acute asthmatic attacks such as vasodilation, plasma extravasation into postcapillary venules, an increase in vascular permeability, exudation of capillaries in the bronchial lumen and edema development, contraction of bronchial smooth muscle cells, mucus hypersecretion, coughing, activation of inflammatory cells and their adhesion to endothelial cells [426] (Fig. 11.33).

Bronchoconstrictors (e-NANC System)

As seen in Table 11.8 [18], several tachykinins released by sensitive nerve endings belong to e-NANC: SP, NKA, NKB (neurokinin A and B), CPS (capsaicin), NPY (neuropeptide Y) and CGRP (calcitonin gene-related peptide) [21].

SP is released by afferent nerves to sensitive amyelinated terminations known as C-fibers. SP binding sites are diffused throughout the smooth muscles of the whole tracheobronchial tree. Injected intravenously, in humans SP provokes an evident vasodilation, probably

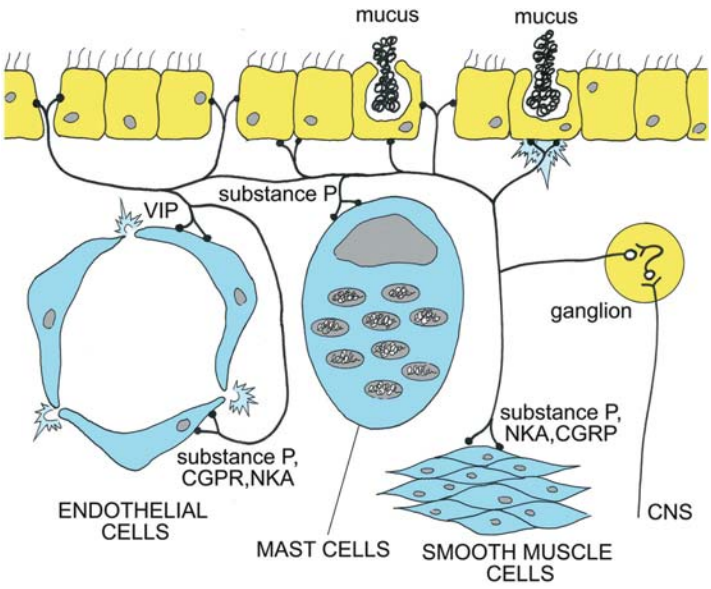


Fig. 11.33. Neurogenic inflammation (*left*) and VIP localization to the airway nerves and adjacent blood vessel. *CGRP* calcitonin gene-related peptide, *NKA* neurokinin A, *CNS* central nervous system, *VIP* vasoactive intestinal peptide

Table 11.8. Neuropeptides in human airway mucosa

Trigeminal and dorsal root ganglion cell sensory-motor neurons	
CGRP:	calcitonin gene-related peptide
GRP:	gastrin-releasing peptide
Tachykinins	
NKA:	neurokinin A
NKB:	neurokinin B
SP:	substance P
Postganglionic parasympathetic neurons	
ACh:	acetylcholine
PHI:	peptide histidine-isoleucine
PHM:	peptide histidine-methionine
PHV:	peptide histidine-valine
VIP:	vasoactive intestinal peptide
Postganglionic sympathetic neurons	
NE:	norepinephrine
NPY:	neuropeptide Y (tyrosine)
Neurons of undetermined origin	
ANP:	atrial natriuretic peptide
CALC:	calcitonin
DYN:	dynorphin
ET-1:	endothelin-1
ET-2:	endothelin-2
ET-3:	endothelin-3
ET-4:	endothelin-4
ENK:	enkephalin
GAL:	galanin
NT:	neurotensin
SOM:	somatostatin

Data from [18, 76].

because of the antidromic activation of local axon reflexes that affect the sensitive C-fibers and also other tachykinins, while PFT alterations result from smooth muscle constriction. SP could, therefore, be the e-NANC neurotransmitter [18]. The presumed mechanism of axon reflexes is that, in response to harmful stimuli in the airway lumen, nerve fibers in the epithelium layer release SP, while other fibers of the same axon, by supplying different targets, release SP by antidromic activation. In this manner, stimulation of epithelial nerve fibers can provoke bronchoconstriction and extravasation of plasma proteins with an axon reflex by SP release near airway smooth muscles and postcapillary bronchial veins [333]. In particular, SP bronchoconstriction activity, at least in vitro, is fulfilled antidromically when reflex cholinergic bronchoconstriction is present, as a result of exposed afferent nerve endings situated under desquamated epithelium, especially those of C-fibers [426]. As well as affecting the muscle, SP also affects bronchial mucus production, stimulating both the submucosal glands to an increased secretion, as well as the myofibrils surrounding glandular ducts to increase their secretion. Furthermore, SP increases mucociliary clearance in the airways [362]. Other evidence suggests that SP induces aspecific degranulation of cutaneous and peritoneal mast cells, but not those of the airways, and causes T-lymphocyte proliferation, neutrophil and alveolar macrophage phagocytosis [90], and enhances neutrophil [88] and eosinophil chemotaxis [90]. Thus, we understand how *BHR to SP in asthmatic children is correlated with asthma severity* [429].

NKA-induced bronchoconstriction, which in the airways travels in the same fibers containing SP, is dose-dependent [126] and stronger than that provoked by SP, which, in turn, is more powerful as a vasodilator and vasopermeabilizer. Therefore, in the present state of our knowledge, NKA and SP exercise a bronchoconstriction

action (e-NANC) that can clash with i-NANC, which has a bronchodilator effect, as well as with EDRF, with an effect on bronchial smooth muscle equal to NO [10]. Epithelial denudation can involve the loss of these factors, thereby increasing reflex bronchoconstriction – an effect also mediated by tachykinins contained in microganglia [90].

CPS stimulates SP release from sensory terminals and, to the same degree as SP, provokes bronchoconstriction and an increase in vascular permeability. Furthermore, it suppresses not only the inflammatory effects of airway vagal stimulation but also the effects provoked by tobacco smoke and chemical irritants [90].

CGRP is a peptide codified by the same gene as the thyroid C-cells which, in turn, codifies calcitonin. It is located in the sensory nerves where SP is also present. It performs a notable vasodilator action on smooth muscle, strong and persistent, associated with eosinophil infiltration, potently contracting in vitro human airway smooth muscle [96], acting in synergy with immune inflammation mediators, including PAF, BKs and LTB_4. It has been credited with a role in blood flow regulation in tracheobronchial small vessels [18] and in APC inhibition [525].

NPY has no direct effect on tracheobronchial smooth muscle contraction, while it stimulates bronchial gland secretion and is a long-acting constrictor of vascular smooth muscles.

The effects of tachykinins are mediated by specific receptors in such a way that each of them activates, by preference, a distinctly separate receptor: NK-1 are activated by SP, and NK-2 by NKA [90].

Possible deficits of the e-NANC system comprise the following mechanisms:

- Enhanced noncholinergic excitatory activity
- Diminished degradation of tachykinins
- Stimulation of the afferent nerve endings in which travel neuropeptides, exposed as a result of inflammation
- Smooth muscle hyperresponsiveness or hypersensitivity to neuropeptide bronchoconstriction activity [96]

Bronchodilators (i-NANC System)

Human PHM (peptide histidine-methionine), its homologous PHI (peptide histidine-isoleucine), the equivalent in many mammals and VIP, have the same localizations and analogous functions (they are codified by the VIP gene in the same pro-hormone and they have in common >50% amino acid structure) [90]. They have a bronchodilator activity, more pronounced with VIP, with an effect 50 times greater than isoproterenol [21]. i-NANC works via parasympathetic nerves containing VIP and/or PHM, these being the only neuropeptides with a bronchodilator action.

VIP, most probably an i-NANC neurotransmitter, is found in parasympathetic ganglia (efferent ways), localized in the cholinergic motor nerve endings in smooth muscle bundles, submucosal glands and bronchial blood vessels. In test animals, VIP has been shown to modulate histamine-induced tracheal smooth muscle contractions, kallikrein, PGF_{2a}, LTB_4 and NKA, an effect not inhibited by adrenergic or cholinergic receptor activation nor by cyclooxygenase blocking activity [90]. Also characteristic of VIP is an abundant distribution in both upper airways and nasal mucosa, but not in bronchioles, which is why its bronchodilator action is greater in regulating the caliber of large airways, and is practically wholly ineffective in small airways. In consequence, the therapeutic effect in the asthmatic patient is deceptive, either because specific VIP receptors are scarce in this location, as has been said, or because of the effect of peptidases (for example tryptase) capable of degrading VIP which are released by inflammatory cells present in asthmatic airways [333]. It is characteristic that tryptase degrades VIP with bronchodilator effect, but not so SP, thus promoting bronchial reactivity, causing in prospective BHR and bronchospasm [96]. The prevalent conclusion is therefore that i-NANC exercises a modulator activity of cholinergic effect rather than a direct bronchodilator action [96].

Potential alterations of the i-NANC system foresee the following mechanisms:

- Shortage of nonadrenergic neurons with inhibitor activity
- Increased degradation of VIP/PHM
- Reversible blockage of nerve ganglia or nerve endings with nonadrenergic inhibitor activity
- Numerical reduction of VIP/PHM receptors [96]

What, then, is the role of neuropeptides? In a healthy individual at rest, the adrenergic, cholinergic and NANC effects are balanced for a perfect bronchopulmonary homeostasis and there is a balance between the bronchodilator mechanisms (β-adrenergic system + VIP/PHM) and bronchoconstrictors (α-adrenergic/cholinergic + SP/NK/CGRP) [90]. As shown in Table 11.7, NANC displays opposing functions: broncho- and vasoconstriction, and broncho- and vasodilation. Confronting the neuropeptide multiform actions, the host generates numerous defense mechanisms. If, on the one hand, VIP reduction/inactivation could contribute to asthma pathogenesis, on the other, the neutral endopeptidase 24.11 (NEP, CD10) is capable of inactivating neuropeptides in bronchial epithelial cells. The contrasting effects of NKA and SP on the one hand and PHM and VIP on the other have already been mentioned. There could be present anomalies of the intrinsic microganglia, the efferent parasympathetic fibers containing VIP, and the VIP receptor system [18]. On the other hand, the effects of bronchoconstriction NANC hyperactivity could, in some patients, play a negative role, such as a hypersensitivity of sensitive nerves and of axon reflexes, amplifying inflammatory responses by releasing factors stimulating such sensitive endings, or an increased smooth muscle sensitivity to bronchoconstriction tachykinins [426]. Its sensitivity to O_3 or to

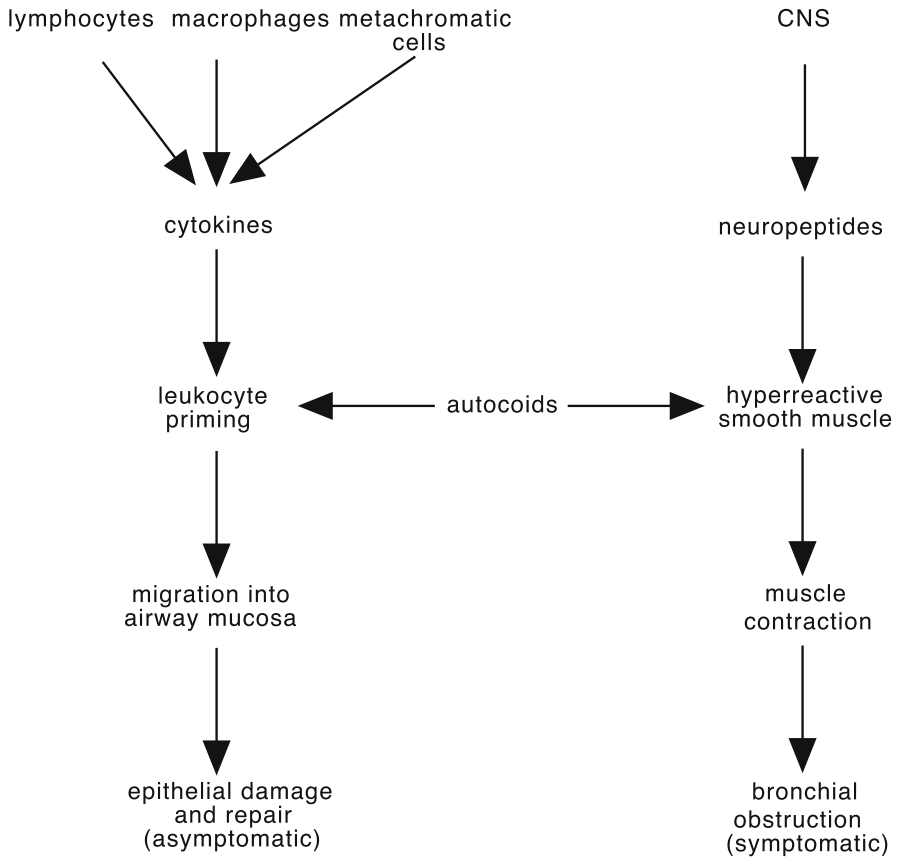


Fig. 11.34. Pathological processes linking inflammatory events to airway hyperreactivity without a causal interrelationship. The autocoids provide the second signal to start both processes, either separately or more frequently conjointly, so that their signal becomes effective. (Modified from [418])

cigarette smoke [315, 337] has recently been defined. Furthermore, the afferent sensitive fibers, even terminating normally in the brain, can branch out and, by antidromic stimulation, be induced to release tachykinins and other mediators in peripheral areas via an axon reflex [426]. The final result therefore is that in the asthmatic, unlike in a healthy individual, *the balance tends toward bronchoconstriction effects* [90]. Neuropeptide action can be linked to that of ILs, with a point of unification represented by the joint action of autacoids, substances physiologically active such as histamine, serotonin, PG, etc. Thus, on the one hand ILs contribute to asymptomatic epithelial damage, while, on the other, neurotransmitters contribute to bronchoconstriction (Fig. 11.34) [418].

Other ANS Dysfunctions

Another factor can be represented by dysfunctions in the stimuli–response relationship. As is known, Szentivanyi [636] advanced the theory that a β-receptor anomaly was at the basis of BHR and other related disturbances. However, there is an interesting paradox worth mentioning: β-adrenergic receptors are quantitatively normal, but cell response to β-adrenergic stimulation is reduced, as can be noted in atopic patients who have not received treatment. Further, administering β-adrenergic drugs causes a rapid receptor desensitization [318]: what conclusion can finally be deduced from this? β-adrenergic receptors are made up of peptides from the cellular membrane which bind either to epinephrine or to norepinephrine. The link to one of these hormones activates Gs – a G-protein – which in turn stimulates adenylyl cyclase to produce cyclic adenosine monophosphate (cAMP), while the Gi inhibit this process. A chain of Gs is deactivated by pertussis toxin. This activity was studied in the murine model in which it reproduces both the immunological anomalies of atopy and hyperreactivity in diverse organs [516]. An experimental model of this type has made possible the definitive demonstration that *G-protein deficit is at the basis of signal transduction anomalies* [318], or of *stimulus–response relationship common to both AD and asthma.*

Anomalies of Bronchial Smooth Muscles

Hyperplasia and hypertrophy of bronchial smooth muscles have been observed, though insufficiently to provide an explanation of BHR pathogenesis, since it is improbable that they are already present in asthmatic children. The reduction of bronchial caliber, especially of bronchioles, which are unique in being surrounded by smooth muscle [255], caused by the joint action of

mucosal edema, smooth muscle constriction and intraluminal secretion increase, cannot, in itself, constitute the *primum movens* of the pathology under examination [722]. Mast cells and eosinophils are both able to contribute in a more significant manner – by means of tryptase action on smooth muscle tone [154]. In the asthmatic, smooth muscles could evolve under the effect of certain stimuli from a multiple-unit to a single-unit system. These changes in the contractile properties of smooth muscle in human lungs may be associated with changes in myosin H chain isoforms, thus contributing to BHR [138].

It has also been suggested that in asthmatics there is a reduction of an epithelium-derived inhibitory factor (EDIF) of the muscle contractility, or of NEP, a factor with a relaxing effect on smooth muscle [675], where it is located. In any event, the result is the loss of the barrier mechanism and therefore of bronchoconstrictor control. A noteworthy impulse has also been given to cAMP study, which that is entrusted with muscle relaxant effects and inhibits mast cell degranulation and consequently histamine discharge, unlike cGMP (cyclic guanosine monophosphate). Normally there is a balance between cAMP and cGMP, which can be broken in either one direction or the other. Maintaining the cAMP level within normal limits is useful to bring adrenergic receptors back to a functional stage in which they can again be activated. Recent research shows that cAMP production induced by adrenergic β receptors is not deficient in the PBMCs of asthmatics compared to healthy subjects [155], as was suggested previously. However, PBMCs of patients even with stable asthma do not have the ability to make adenylyl cyclase more powerful [155], so the lack in increase of cAMP concentrations is correlated with a transduction defect. There are two possible pathogenic mechanisms: the existence of a generalized deficit involving bronchial smooth muscles, no longer relaxed under the effect of cAMP metabolism, and therefore the impossibility of potentiating cAMP when facing a bronchoconstriction stimulus, which could result in an equilibrium upset between cAMP and cGMP; or, alternatively, the adenylyl cyclase deficit could reflect negatively on IL production by PBMCs, the tissue inflammation being at the basis of an increased BHR [155]. In vitro, in biopsy specimens of airway smooth muscle of asthmat sufferers, there is a correlation between muscle functions and BHR level, indicating that BHR, which is observed in the presence of a great variety of stimuli, is a demonstration of the noteworthy contribution given to bronchoconstriction by the bronchial smooth muscle spasm. The differences between asthmatics and nonasthmatics have not yet been fully elucidated, nor have the variations induced by different types of stimuli. It is believed that there are other exogenous factors at the basis of this anomaly. Probably, the airway infiltration of inflammatory cells contributes to smooth muscle tone by the action, on a local level, of various mediators such as histamine, PAF and eicosanoids [138].

Anomalies in Airway Epithelium

The effect on the mucosal epithelium by toxic products of eosinophil origin has been analyzed, together with changes that range from loss of ciliated cells to complete epithelial denudation:

- As previously said, the *epithelial barrier permeability* is increased by histamine, LTs, BKs, CPS, SP, TNF-α, reactive oxygen metabolites, viruses, etc. Consequently, access to the mucosa and submucosa by inhaled molecules such as allergens, microorganisms, dusts, environmental pollutants and inflammatory mediators is made easier. Among those last named, superoxide anion has a particularly harmful effect on the airways with its highly destructive radicals [726]. The epithelial damage exposes intraepithelial mast cells, afferent nerve endings and, in particular, irritating vagal receptors to the combined action of various stimuli. These lie in the upper and lower tract mucosa immediately below the epithelial tight junctions [722], which, though physiologically impermeable, allow a greater absorption of allergenic and irritating substances to take place if altered. In addition, the mucociliary clearance reduces its filtering action of inflammatory substances and airway secretions.
- Experimental studies have highlighted the role played by epithelial denudation, which could lead to NEP loss, usually found not only on smooth muscle, but also on target cell membranes such as epithelial, endothelial and alveolar type II cells, submucosal glands, postcapillary venules and nerves, all of which are sites of tachykinin activity [126]. NEP selectivity makes it possible to degrade SP and NKA, blocking their bronchoconstriction activity while the inhibition, especially if associated with O_3-induced BHR [406], potentiates NKA [126] and SP effects [421]. Exfoliation of epithelial cells and other serious cytopathic effects – caused by viruses harmful to cells [20, 72, 130], chemical substances, pollutants, etc. – points first to a NEP deficiency, thereby preventing both the destruction of bronchoconstriction neuropeptides as well as the synthesis of those with bronchodilator action, also amplifying the inflammatory action of tachykinins and reflex bronchoconstriction [18]. If, as a result of epithelial damage, the sensitive nerve endings of bronchial mucosa remain exposed to the bronchial lumen, having a much lower threshold of stimulation, they are stimulated continually by inflammatory metabolic products [21]. In this way, as a result of antidromic stimulation of these nerve endings, an exaggerated neuropeptide release with a highly rapid and diffuse action on bronchial airway occurs [21]. BK could play a part in allergic asthma since, administered either inhaled or IV, it provokes bronchospasm in asthmatics, but not in healthy subjects. It works indirectly, probably via the stimulation of sensitive nerve terminals, with consequent retrograde release of SP no longer degraded by NEP, NKs and CGRP by an axon reflex [421, 429]. BK antagonist therapy is based on these premises [18]. Following these phenomena of

neurogenic inflammation affecting target cells, cholinergic reflex bronchoconstriction, hyperemia, edema and mucus hypersecretion, with an increase in vasal permeability will follow. This axon reflex, amplifying the immune inflammation, could therefore represent an important pathogenic element in provoking asthma and BHR [21].

- Moreover, there is *a receptor loss:* if they belong to the β type, there are no disturbances of note; if they are H_2, production of PGE_2, which acts by inhibiting, at least in part, the smooth muscle responses to histamine and NKA ceases. The receptor loss by numerous epithelial ILs involves mainly IL_1, with a possible amplification of the inflammatory response and consequential jeopardizing of lesion repair [22].
- Other studies have shown the *loss of NO production,* catalyzed by iNOS (inducible NO synthase) and induced into the epithelium by TNF-α, $IL_{1β}$ and IFN-γ, also compromising both vascular muscle regulation and harmful chemical substance clearance [10].
- Another pathogenic hypothesis cites the *inhalation of endotoxins* present in Gram-negative germs, identified with the somatic antigen, which are located in the cell walls as LPS as a possible culprit. Such toxins, brought into homes by environmental germs, could provoke bronchoconstriction in asthmatics in doses of 20–40 μg, and in healthy subjects as well in doses of 200 μg [433].
- BHR persistence, also produced by MBP-induced discharge of factors of epithelial origin stimulating muscarinic reactivity [57], could be explained by the thickening of the *lamina reticularis* below the BM [600]. A crucial point is played by *myofibroblasts,* capable of producing subepithelial fibrosis, whereas in asthmatics an excess in their numbers below the bronchial epithelium, with a close correlation between the number of myofibroblasts and the collagen layer thickness, has been reported. It is therefore evident that fibrosis jeopardizes the respiratory function and that a network of contractile myofibroblasts underneath the BM markedly contributes to BHR persistence. Moreover, activated eosinophils present in these locations, as well as deposits of ECP, stimulate the synthesis of hyaluronate and proteoglycans in human fibroblasts [600].

Alterations of Biochemical Homeostasis

The main cause of asthma inflammation, which, apart from smooth muscls spasms, also depends on mucosal edema and on secretion changes, with formation of mucus plugs in the smaller bronchi.

Bronchial Edema

Bronchial edema is attributable to the increase in capillary permeability with exocytosis of serum proteins in interstitial areas with an array of mediators among which are histamine, BKs, PAF, PGE and LTs, potentiating each other in turn. For example, PAF-produced edema resulting from the harmful action exercised on microvasculature is increased by the coincident action of LTC_4 [283]. There are several other edema factors such as cigarette smoke, viral infection, pollutants, neuropeptides and proteases, all capable of altering epithelial integrity and mucosal permeability: thus allergens may penetrate *across the junctions that are no longer tight,* for their encounter with APCs [619]. Even if the pathophysiological contribution of this process is little known, it has been seen that following an airway effect of local BPT, an extensive edema is formed and that both edema and inflammation increase the thickness of airway walls, thus contributing to bronchospasm in children [619]. Therefore, pharmacological prevention of the increase in vascular permeability requires the inhibition of histamine receptors and of arachidonic acid metabolism through cyclooxygenase and lipoxygenase, with the aim of simultaneously blocking PGs and LTs, respectively. The role of bronchial edema has been the object of few studies and merits a closer examination [283].

Mucus Hypersecretion with Formation of Plugs in the Smaller Bronchi

The formation of tenacious mucus plugs in the airways is one of the characteristics of asthma [255]. Mucus hypersecretion, a possible consequence of hyperplasia and metaplasia of submucosal glands and goblet cells that cover the respiratory tree, contributes to an exaggerated insufflation and focal atelectasis. The pathogenesis is complex: various mediators and cells of inflammation are able to contribute – along with C-fiber activation also stimulated by BK and inhalants – leading to mucus-producing cell quantitative increase and exocytosis, as well as to myoepithelial cell contraction [362]. The mediators (Tables 11.4, 11.5) that are principally responsible are, in order: LTD_4 >LTC_4 (active in picomolar concentrations) >HETE (nanomolar) >PGF_{2a} = PGD_2 = PGI_2 = PGE_1 = PGA_2 >histamine H_2 (μmolar) [283]. Mucus hypersecretion may be produced by IL_{13} even in the absence of inflammation [321].

Increase in Permeability

We summarize the numerous mechanisms that lead to vasodilation and hyperpermeability evidenced so far, with consequent bronchoconstriction amplification. Plasma proteins release from tracheobronchial microvasculature and the forcible fluid passage deriving from it are important inflammation components and, consequently, a basic characteristic of pathogenesis [177]. They occur gradually within 10–20 min after an allergenic BPT in the airways: the stronger the stimulus, the greater the exudation [490] (Fig. 11.32). The continuous

vascular extravasation can give rise to the production of mediators of inflammation facilitating the intraluminal transmigration of inflammatory proteins. Since the absorption of solutes is directed above all by mediators, the mechanisms that reduce or abolish their activity cause the regression of protein accumulation in the lumen, an effect which can be achieved following treatment with CSs in moderate doses (400 µg/day) [669]. Nonetheless, measurements in controlled asthma have shown little or no increase in airway permeability and no correlation with BHR degree [722].

Surfactant Dysfunctions

The loss of pulmonary tissue elastic properties can cause bronchoconstriction. Testing immunized animals to BPT with ovalbumin, the resulting protein transudation in the airways inhibits the surfactant, with a consequent Raw increase [356].

Effects of NO

Until 10 years ago, NO was known only as one of the components of the harmful gases discharged by vehicles and cigarette smoke, also held to be jointly responsible for acid rain and the O_3 hole. Recently NO equal to EDRF has been localized at the airway epithelium, where it could act as a mediator of bronchodilation under the nervous control to counterbalance the bronchoconstriction caused by mast cell degranulation (or by ET). Moreover, NO, produced in large quantities, provokes an increase in vascular permeability and cytotoxic effects, contributing to epithelial denudation [10]. In this cytotoxic activity, it also mediates O_3 effects – a significant fact given the increased incidence/prevalence of asthma [759]. From a pathogenetic point of view, NO deriving from airway epithelium (as well as from macrophages, mast cells and Th1 cells) could play an important role in amplifying and perpetuating the Th2-mediated inflammation (Chap. 4). iNOS can be induced in the epithelium by pro-inflammatory ILs such as TNF-α and $IL_{1β}$, secreted by macrophages, and IFN-ɣ derived from Th1 cells [759]. It is also feasible that even viral infections induce iNOS production by the epithelium, thus increasing NO secretion during asthmatic attacks [443]. Elevated concentrations of NO thus generated in the airways carry out a suppressive action on Th1 cells and a reductive action on IFN-ɣ, resulting in a net proliferation of Th2 cells. CSs inhibit iNOS both directly and by blocking pertinent IL synthesis by macrophages [443]: in fact, in asthmatics treated with oral or inhaled CS (ICS), NO levels are reduced, in contrast to untreated patients [293]. Exhaled NO levels are increased in atopic non-asthmatic children and, all the more, in atopic asthmatic children, but were reduced by 53% by ICS treatment [587]. Therefore, ICSs inhibit NO production by epithelial cells, stimulating the proliferation of Th1 at the expense of Th2 cells [443]. Further improvements in techniques will permit monitoring inflammatory events, by measuring exhaled NO (eNO) levels and providing a noninvasive marker for the early diagnosis (Chap. 6) and a more precisely aimed treatment of pediatric asthma [759].

Main Exogenous Factors [333]

- Pharmacological stimuli, histamine, methacholine, acetylcholine, hypotonic aerosols [154], all medications may provoke adverse responses (see "Treatment"). In a cross-sectional study on 1,881 children aged 6–7 years, the use of antibiotics during the 1st year of life was significantly associated with wheezing. This increased risk was a prerogative of children genetically predisposed to atopic immune responses [156].
- Physical stimuli: fresh and dry air [206], ultrasonic mist, smog, inert dust, atmospheric pollutants, physical exercise [606].
- Chemical and pollutant stimuli: oxidant, harmful and irritating gases, tobacco smoke [167, 195, 621] (Table 2.26)
- Allergens, especially aeroallergens
- Viral infections [167]

Predisposing Factors

Anatomical and Physiological Predisposing Factors

Anatomical and physiological factors, specific to the pediatric age, can predispose to the airway obstruction, as can extrinsic factors more readily predisposing, some of which are even etiological factors.

Strictly Anatomical Dynamic Factors

Anatomical dynamic factors are represented by an evident reduction, more or less to scale, according to age, of anatomical parameters.

The following [174, 204, 274, 589, 648] show that the child's airways are not to be viewed as a miniature replica of the adult's airways:

- At birth, the lungs weigh ≈48 g; at 1 year 130 g; at 12 years 390 g; in the adult ≈1,200 g.
- The estimated gas-exchange area at birth is almost 27-fold lower than that of an adult: 2.8 m^2, 32 m^2 at 8 years and 75 m^2 in the adult.
- The total pulmonary capacity of a newborn child is 180 ml; at the age of 16 it is 5,100 ml.
- Bronchi and bronchioles have reduced caliber and length. The diameter increases by 200%–300% from birth to adulthood.

- The alveoli are reduced in number. The number of alveolar structures increases exponentially from 30–32 weeks of intrauterine life until birth, the newborn has only 8%–11% of the alveoli of an adult and with a less differentiated structure, and by the age of 8 the alveoli increase tenfold, and proceeding to adulthood, the airways triple in diameter.
- The cartilaginous tissue, the submucosal glands and the smooth muscle of lower airways complete their development at 8 months; the smooth muscle of proximal airways in adulthood.
- Formed *cilia* and mucus are found in the airways at 13 weeks of life.
- The thorax is extraordinarily flexible in infancy and it stiffens with age.

Within this anatomical framework, the neonatal epithelium develops structurally and provides important functions in the normal airway development.

Anatomical and Physiological Factors Predisposing Children to Airway Obstruction [584]

The younger a child, the more evident the anatomical details facilitating the onset of bronchospasm, therefore provoked by trivial causes such as a mucosal edema and/or a catarrhal secretion. The relevant infantile characteristic of the respiratory mechanism in this context is the resistance to air flow. A reduction in bronchioles and/or bronchi caliber translates into a hindrance of air flow so that, in young babies and children, an obstruction, even a limited one, requires great exhaling pressure during exhalation, from which derives a dynamic compression of the peripheral tracts which worsens the obstruction itself. Among the principle causes of this, some of which have been better defined by new methods of investigation (Chap. 6), are the following.

Reduction of the Caliber of the Peripheral Airways

The relationship between the caliber and upper and lower airways is physiologically reduced as compared to that observed in adults. This condition exposes an infant, often male, to severe bronchoconstriction because the edema, the secretions and the cellular debris cause stenosis more easily in bronchioles of a smaller diameter. We must also consider that Raw varies inversely with the fourth power of the radius of bronchioles. Therefore, it is sufficient that the radius is halved, for example caused by a viral infection, for the Raw to be increased 16-fold. An increase in caliber occurs only towards 5–6 years of age.

Decreased Lung Elastic Recoil

The infant chest wall is less rigid, so that the thoracic wall and airways have a greater propensity to deformability. The decreased static elastic recoil properties predispose to an early airway closure even during tidal breathing. On the other hand, greater elasticity means that the airways of babies are more easily susceptible to vibratory movements, which are at the basis of wheezing. The cartilaginous substances of the trachea and segmental bronchi also are less rigid, so that airway collapse is facilitated during expiration. The early airway closure at elevated pulmonary volumes determines an alteration of the ventilation/perfusion rate, potentially complicated by hypoxemia.

Decreased Smooth Muscle in Peripheral Airways

Decreased smooth muscle in peripheral airways facilitates obstruction especially following an edema, mucous hypersecretion and infiltrations by inflammatory cells. Infants and children are therefore more vulnerable to small airway pathologies. Smooth muscle reduction compared to that of an adult also contributes in many cases to a poorer response to bronchodilators (the younger the baby, the more this is the case).

Mucous Gland Hyperplasia

Compared to an adult, an increased percentage of mucosal glands in walls of the larger bronchi contributes to intraluminal mucous secretions and in turn to airway obstruction.

Diaphragmatic Disadvantage

Another anatomical disadvantage from a mechanical point of view is the position of the diaphragm since the angle of insertion on the ribs is horizontal, in contrast to an oblique insertion in adults, which, combined with the compliance of the bronchial wall devoid of cartilaginous support and that of the thorax with low skeletal or muscular support, cause retraction of the rib cage during inhalation, therefore requiring greater effort, whereas in adults the diaphragm tends to elevate the rib cage, thus increasing its diameter.

Functional Insufficiency of the Diaphragm

Functional insufficiency of the diaphragm occurs because the increased effort cannot be sustained efficiently, caused by relative reduction of muscle fibers. The diaphragm must ensure >70% of respiratory excursions, given that a child up to the age of 6–7 years con-

tributes only in part to thoracic cage expansion, either because of increased compliance (see "Decreased Lung Elastic Recoil"), or because the movements of lifting and lowering the rib cage are conditioned by their almost horizontal position, and by the almost cylindrical form of the thorax, so that diaphragmatic contraction during inhalation can cause subcostal retraction rather than rib elevation.

Decreased Alveolar Pores and Decreased Collateral Ventilation

The alveolar openings that permit ventilation between alveoli (pores of Khon) and the bronchoalveolar communications (canals of Lambert) are decreased in number and size in the infant lung, thus impeding a normal collateral ventilation. For this reason, young babies and young children are more easily susceptible to atelectasis distal to obstructed airways.

Role of Pulmonary Volume

The reduced caliber of peripheral airways can be a risk factor for relapsing wheezing. The functional residual capacity (FRC), airway conductance (Gaw) and V_{max} were found to be significantly lower in young babies suffering from bronchiolitis before the age of 1 year and related relapses [375].

In conclusion, the fact that the airways of the very young face obstructions more easily and more rapidly than older children is not surprising. Therefore the lung is more vulnerable to inflammation in this early age and this may result in *persistent airflow limitation* [274]. We have so far outlined some of the principal causes for which the particular predisposition needed to develop not only *reversible wheezing* in the very first period of life, but also bronchoconstriction and respiratory insufficiency, which is at the basis of the high number of hospital admissions and assisted breathing that is recorded in this age group.

Predisposing or Etiological Factors

Several factors can modulate the onset and severity of asthmatic clinical symptoms in a child [174]. With advancements in research, knowledge regarding the way in which these factors provoke alterations at the airway has been perfected, even if still far from achieving a univocal pathogenetic mechanism. It is certain, however, that asthmatic children display BHR, an abnormal marked sensitivity to most varied stimuli, which in healthy subjects usually do not induce responses of any particular clinical relevance. In asthmatic children, these and other stimuli can provoke smooth muscle spasms of the lower airways, bronchial edema, dense mucous secretions and respiratory dynamic alterations as well [307].

Aeroallergens

Sensitization to inhalants is rare in very early infancy, but clearly prevalent from 3 years onward (Fig. 9.39), with high successive sensitization to pollens and to Der p 1 (Fig. 5.22), and an unusual and elevated prevalence of positive SPTs to cat derivatives in children >9 years (Fig. 7.17) and of sIgE in 17-month-old infants (Fig. 5.21). Multiple sensitization to inhalants (Fig. 5.20, Tables 5.18, 5.19) may be very prevalent among children suffering from multiple FA (Chap. 9).

Other Atopic Disease

Of children affected with AD, 44%–53% are at risk of developing other atopic disease, namely RA or asthma (Table 5.8), and 47% of these children have asthma with or without BHR (Table 7.10). Additionally, some foods can be responsible for asthma in allergic children, more frequently than in adults (Tables 9.18, 9.19). M cells transport allergens to subepithelium where they facilitate allergen access to lymphoid tissues, then interacting with allergen-specific T cells or with B_{IgE} lymphocytes, finally reaching the airways via blood vessels and causing BHR and asthma [537] even in children <2 years of age (Fig. 11.35) [450]. In our division, it is not infrequent that children react positively to food provocation testing manifesting wheezing.

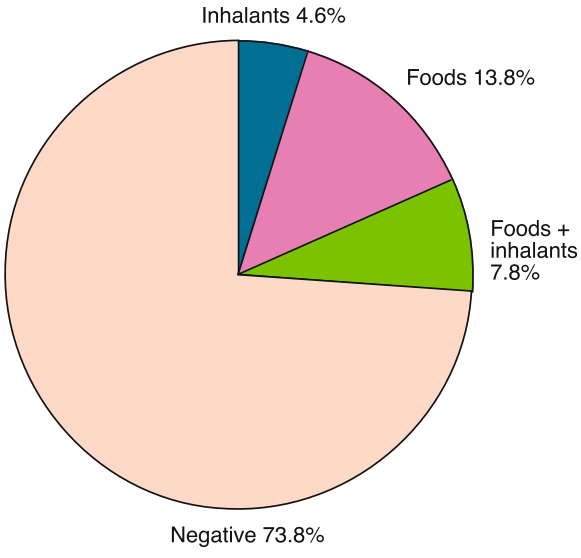


Fig. 11.35. PTC results in 65 <2-years old asthmatic children. (Data from [450])

Physical Exercise

Aretaeus reported cases of *exercise-induced asthma* (EIA) at the first Olympic games recorded in history [13]. Twenty-four centuries later, approximately 3%–10% of athletes taking part in international competitions are affected by asthma. Physical activity regularly leads to a decline in PFT in children and adolescents with asthma. This decline is a consequence of what is known as EIA, a theme of much research in recent years [542]. EIA is found in up to 63% of asthmatic children (Table 5.11), in 30%–40% with RA [307, 584], in nearly 3%–8% of schoolchildren and in 13% of child athletes [542]. The main cause is BHR that typically appears after 3–8 h of vigorous exercise, so that in diagnosing EIA, the subject is required to run on an equipped treadmill under controlled environmental conditions [39]. After 5–15 min, bronchoconstriction, ranging from moderate to severe and lasting 15–30 min can occur, followed by a refractory period of 2 h during which no exertion can provoke EIA. Delayed reactions can occur even 8–12 h later, and this two-phase reaction is not uncommon [39].

Free-running tests and treadmill tests (Fig. 6.24) induce EIA [542] more easily. Objectively, arduous exercise – for example: running, bicycle riding or cross-country skiing, but not long-distance skiing [335] – provokes bronchoconstriction in at least 70%–80% of asthmatics, which is neither intense nor prolonged [394]. Running provokes EIA more easily than jogging and this, in turn, more frequently than walking. In evaluating the triggering factors, a variety of issues come into play including cold, dry air, environmental pollutants, a recent allergic exposure or inflammatory mediators. The pathophysiology and cause of EIA remain controversial, but two theories have been offered: 1) the hyperosmolar theory and 2) the airway rewarming theory. Warm air and humidity can accelerate bronchospasm, exacerbated by cold air inhaled during exercise, especially if open-mouth breathing results from increased need for O_2 [606]. Hyperpnea induced by physical exertion causes water release via evaporation of mucosal periciliary fluid with consequent local hyperosmolarity [606], which could provoke bronchospasm via direct vagal action, or by induction of mast and other cells to produce mediators, thus activating the successive cascade of immunological events [464]. Other authors, however, place emphasis on heat loss caused by hyperpnea, which could have consequences on subsequent rebreathing, with vasodilation of peribronchial vascular plexus, hyperemia, edema and, finally, bronchoconstriction [66]. Therefore, thermal shock could occur [394]. Since asthmatics have a hyperplastic vascular bed possessing greater permeability, the initial cooling down and subsequent warming up could cause bronchospasm via a true vascular blockage, with the onset of reactive hyperemia and edema involving the bronchial walls [394]. At the moment, however, there is insufficient data to clarify which of the two theories (warming up and hyperosmolarity) is pre-eminent. In studying BALF before and after exercise, no significant differences were found in histamine and tryptase levels. This could make doubtful a possible intervention by the airway mast cells accompanied by liberation of histamine and other mediators [278]. However, Kikawa et al have shown that in children with severe asthma, following physical exercise, LTE_4 urinary levels were clearly higher than those of controls, thus concluding that airway reactivity seems to be a salient factor in EIA development [294, 295]. The analyses of histaminemia have revealed elevated levels of histamine 10 min after BPT, returning to normal after 20 min and significant subsequent reduction of T lymphocytes 2 and 4 h after the test [66].

Drugs and Additives

Certain drugs such as ASA and food additives can induce bronchospasm in asthmatic and nonasthmatic subjects, by means of a nonallergic mechanism probably linked to the interference on mediator regulation (Chap. 10).

Irritant Factors

Irritant factors [584] like smoke, gases, dusts, vapors and immunotoxic substances, in addition to being harmful to the cells, have a more negative clinical impact on asthmatics compared to the general population, being able to provoke or aggravate asthma in subjects with BHR. Irritants, formaldehyde, fine wood dusts, etc. must be avoided in the home of asthmatic children (Chap. 24). Other factors involve meteorological, hygrometric and/or barometric variations. *Fog,* in addition to its specific effect (larger drops deposit in smaller bronchi and trigger bronchoconstriction, smaller drops stratify on bronchial walls increasing mucous fluidity), can also transport harmful dusts and fungi.

Environmental Pollutants

Tables 4.15–4.17 and Fig. 4.22 illustrate the agents that compromise the immune system in test animals and, prospectively, in human beings. The *immunotoxic substances* include: exhaust gas, atmospheric dust, industrial smog – notably SO_2, O_3, NO, NO_2 – as well as anion superoxide, from which H_2O_2, OH^-, O_2 "singlet" ($^1O_2$) and other O_2 radicals are derived, with directly damaging effects on human cells [262]. Small doses induce BHR and high and/or persistent doses induce lasting toxic effects [262]. TNF-α and $IL_{1\beta}$ are able to elicit a rapid and temporary increase in ciliary beat frequency following release of NO and, consequently, of iNOS [648]. Experimental data show how in children the in-

crease of respiratory symptoms and asthma are proportional to that of pollutants [694], *even acting in newborn babies* (Table 2.26). As yet, no studies have quantified the relationship between atmospheric pollution and BHR [694]. In communities with high O_3 concentrations, the RR (relative risk) of developing asthma in children playing three or more sports was 3.3 (95% CI, 1.9–5.8), compared with children playing no sports; thus, air pollution and outdoor exercise could contribute to pediatric asthma development [390]. Patients exposed to particulate air pollution and gaseous pollutants such as NO_2 concentrations can present with an increased prevalence of asthma symptoms and medication use [687]. Children are more at risk both because of the *smaller caliber of their airways,* and because of the increased RR with a resulting increase in inhaled pollutants per kilogram of body weight [521]; therefore an irritation provoked in an adult may turn into a significant bronchoconstriction in a very young child.

Cigarette Smoke

Passive smoke could constitute the most important environmental factor in the etiology of early infantile asthma (Tables 4.21–4.23), being involved in 38%–65% of cases (Fig. 4.23 and Table 5.11).

Viral Respiratory Infections

Viral respiratory infections (VRIs) have been related to the onset of recurrent wheezing illness and asthma in infants and are probably the most frequent cause of exacerbations of established disease in children. Studies discussed in Chap. 4 have shown that children with siblings have a lower prevalence of allergies and asthma than do children without siblings, an association attributed to a preventive effect of early infections, according to which early-life infections up-regulate Th1 lymphocytes that may inhibit the expansion of allergen-specific Th2 lymphocytes, thus limiting the development of allergic diseases. The action of respiratory viruses on the airways is modulated by several factors, including atopy, male sex, age, type of virus, possible BHR and development of correlated symptoms [545]. Infection by respiratory viruses can induce in children (even nonatopic children) [72] airway obstruction even though limited to the upper tract, BHR, functional changes in the airways, and PFT changes, which can persist for weeks after recovery (Table 11.9) [20, 72, 130].

Infections, especially if contracted in the early stages of life, can damage the mechanisms underlying the mucosal barrier, thereby allowing a massive allergen penetration. As seen in Table 11.9, the necrotizing action that various influenza and parainfluenza viruses can have on the bronchial mucosa has been demonstrated: *denuding it of its epithelial covering, they allow a greater penetration of inhaled viral allergens* and their absorption [20]. Clinical studies confirm that VRIs markedly aggravate or even directly trigger asthmatic symptoms in a high rate of cases, most especially *in very young children* [20, 167, 611] (Table 6.1). A re-examination of studies done in pediatric cohorts (0–>17 years old), with respiratory infections of both upper and lower airways and asthmatic symptoms, found a link with viruses in 25.2% of general cases and in 35.6% of asthmatics [69]. In the follow-up of 11- to 17-year-old students with asymptomatic BHR, 45% developed a symptomatic form and 80% had suffered from past respiratory infections [753]. More recently, early respiratory infections indicated an increased, rather than a decreased, risk of developing bronchial obstruction during the first 2 years of life and of having asthma at 4 years of age [427]. Two cohort studies indicate that RSV lower respiratory tract infections (LRTIs) during infancy are associated with increased risk of asthma in the following years [585, 597]. Respiratory inflammation (RSV) during the 1st year of life seems to predispose, possibly via IL_{13}-mediated mechanisms, to augmented allergic airway responses [361]. Moreover, both pneumonia and RSV LRTI during the first 3 years of life are associated with an increased risk of asthma or asthma-like symptoms up to 11 years of age [88, 616], or up to 5 years in Gambian children [670].

In infants and toddlers, RSV plays an especially important role and can induce respiratory disturbances with variable degrees of severity, culminating in hypoxia [714] and even death [271]. In particular, the course of bronchiolitis in a baby can prove very severe, sufficient to warrant RSV classification as *the greatest agent of morbidity and morbility in the 1st year of life* [477]. As with other respiratory viruses, RSV causes an increase in IgE [428], altering their immune regulation, and of eosinophils, which show a greater ability to participate in the immune inflammation [130]. It is highly probable that during infections, virus-specific IgE linked to mast cells react with an RSV antigen, by releasing vasoactive and inflammatory mediators, and start the whole cascade of events leading to BHR already described [72]. The increased airway permeability opens the door to allergens triggering allergic asthma, with an increase in cholinergic responsiveness and a decrease in the β-adrenergic responsiveness, thereby increasing BHR and airway inflammation.

RSV has an additional specific influence on PMNs producing $O_2^{\cdot-}$ and TXB_2, with a significant chemiluminescence when incubated with RSV, and cytotoxicity for target cells [298]. They are the dominant inflammatory cells in the nasal secretions of babies with RSV-induced bronchiolitis, and in BALF of infants who have undergone ventilation for severe VRI [182]. It appears that PMN-deficient motility (Table 2.10) protects very young babies from RSV. RSV pathogenicity is shown by its ability to activate eosinophils notably producing cationic proteins in greater quantities, and obviously causing persistent BHR and damaging the respiratory

Table 11.9. Mechanisms of virus-induced wheezing and/or asthma exacerbation

Direct effect on the airway epithelium
A. Damage to the airway epithelium
Epithelial denudation
Disrupted epithelial integrity
Enhanced allergen penetration
Reduced mucociliary clearance
Loss of neutral endopeptidase, cyclooxygenase
Enhanced contractility to substance P
Up-regulation of adhesion molecule expression
Loss of protective mediators, including the endothelium-derived relaxing factor (EDRF)
Altered airway surface fluid osmolarity
Disruption of tight epithelial junctions
Exposure to irritant and cough receptors
B. Effect on vascular endothelium
Increased permeability
Fluid and plasma protein efflux
Production of inflammatory mediators
C. Increased mediator release
Histamine release
Increased arachidonic acid-derived lipoxins
Kinin generation
Enhanced activity of lipid mediators and superoxide
D. Enhanced leukocyte inflammatory function
Activation of basophils, mast cells, macrophages, eosinophils and neutrophils
T-lymphocyte-mediated cytokine release
Activation of cell-mediated immunity
E. Altered neural system function
β-adrenergic blockade
Cholinergic stimulation
Enhanced neuropeptide release
Defective NANC responses
F. Antibody-dependent enhancement of inflammation and cytotoxicity
G. Generation of virus-specific IgE antibody
Allergic sensitization
Mediator release
H. Effect on metachromatic cells
Direct activation
Activation via virus-specific IgE antibodies
Anaphylotoxin activation of complement cascade
Chemokine-mediated activation
IFN-γ amplified activation
I. Effect on lymphocytes
Stimulation of IFN-γ production
Activation of cell-mediated immunity
$CD8^+$ deficiency
L. Effect on cellular adhesion molecules
Stimulation of interleukin generation
Up-regulation of adhesion molecule expression
Increased eosinophil and neutrophil migration and adhesion
M. Neutrophil activation
Neutrophil respiratory burst up-regulation
Consequences
1. Mouth breathing
Reduced nasal filtering of inhaled allergens → increased penetration to lower airway
Reduced conditioning of inspired air → low temperature/humidity → bronchospasm
Reduced lower airway temperature → increase of viral replication
2. Increased circulating mediators and/or cytokines
Enhanced bone marrow production and subsequent activation of inflammatory cells
3. Increased serum IgE levels
In conclusion:
a) Epithelial changes and mucociliary clearance alteration favor a deeper penetration of viral inhaled allergens
b) Increased histamine release and IFN-γ-induced greater mucosal permeability may prime allergen absorption
c) Respiratory virus produce airway inflammation, and by increasing cholinergic receptor sensitivity promote a vagus-mediated reflex bronchoconstriction
d) Respiratory virus, directly or via metabolites produced by infected cells (including IFN-γ), impair airway β-adrenergic tone, thus perpetuating the inflammatory changes
e) Respiratory viruses, alter small airway geometry thus leading to bronchiolar wall narrowing and plugging with mucus, a mechanism of virus-induced airway obstruction
f) Virus-specific IgE antibodies trigger mast cells and basophils with subsequent mediator release, which may contribute to airway sensitization

These studies, as a whole, disprove that respiratory infections may be protective toward asthma.
Data from [20, 72, 130].

Table 11.10. Potential capacity of Rhinovirus to cause asthma in sensitized subjects

Pathophysiology						
Sublminal exposure in sensitized subjects	→	CD54 induction on respiratory epithelium Peristence of inflammation	→ →	Increases susceptibility to Rhinovirus infection Rhinovirus infection	→	**Asthmatic attack**
Clinical manifestations						
1. Clinical latency		2. Symptoms		3. Bronchospasm		

Modified from [81].

epithelium with subsequent airway exposure of vagal receptors [298]. Studies [201, 298] indicate that *RSV triggers eosinophil degranulation by directly activating them* [299], a plausible and stimulating link between VRI and asthma exacerbations. Attention has been drawn to the role of alveolar macrophages in RSV infections: once infected, they acquire the ability to replicate RSV, also expressing HLA-DR and immunoregulatory ILs such as $IL_{1\beta}$ and TNF-α, capable of stimulating phagocyte cytotoxic responses. Consequently macrophages, by regulating local immune responses, perform an antiviral activity conditioning both viral replication and disease severity [401]. Lymphocyte studies have shown that the CD4/CD8 ratio in the lower tract is 22.5:1, and in the upper tract 15:1: thus diminishing the CTL importance, residing, however, in intraparenchymal locations, not intraluminal or bronchiolar [182].

Even influenza and parainfluenza viruses, *Rhinovirus*, *Mycoplasma* and *Adenovirus* often trigger respiratory infections, which can be found in very young babies as copathogenic factors [514], becoming recurrent in older children (Chap. 22). In 70% of nasal lavages in children <2 years with respiratory wheezing, RSV has been identified in 35% and *Rhinovirus* in 15% of cases [161], whereas in the >2-year-age group, only 31% of the cultures proved positive, with *Rhinovirus* predominant and an almost total lack of RSV [161]. Therefore, in older children, *Rhinovirus* influenza virus, *Mycoplasma pneumoniae*, etc. are the most frequent cause of wheezing. In a group of children aged 1–6 years, the viral origin of 45% out of 115 infection episodes of the airways has been ascertained, underlining, in the following order, the causative role of: *Coronavirus*, *Rhinovirus*, influenza virus A, and parainfluenza 1–3 viruses, *Adenovirus,* and *Mycoplasma*. The study revealed an RSV absence [400] and a total agreement with the identification process carried out in three other pediatric cohorts [477]. As well as *Chlamydia trachomatis* active in bronchiolitis, *Chlamydia pneumoniae* can induce in asthmatic children, especially <4 years [428], asthmatic crises whose onset is linked to sIgE production with consequent triggering of symptoms, which can wholly overlap what is known properly asthma. Indeed, the more protracted and pronounced sIgE production, the longer the disease duration [175].

Infection by *Rhinovirus* is by far the most common cause of wheezing disease in older children [280, 547] (Table 11.10) [81], in that it significantly stimulates mast cell mediator release, contributing to BHR development and possible late reactions [341]. In ten patients with AR and BHR, their responses to BPT to both histamine and allergen (*ragweed*) were evaluated during an experimental infection from *Rhinovirus.* Four weeks later, BHR had increased in both tests; specifically, only one patient reacted prior to infection with a late response to the allergen, while 8/10 manifested it during observation, 5/7 persistently (Fisher, 0.0027) [341]. *Rhinoviruses* are able to increase BHR and promote the appearance of *late reactions* to the specific allergen. Even if the virus does not usually have a direct effect on the airways, the infection heightens the eosinophil response to challenge [341]. The major risk of causing asthma in children aged >2 years was that of the association of *Rhinovirus* infection with the sensitization to domestic allergens and exposure to passive smoke [161]. In asthmatic children the triggering element of the exacerbations was represented in 66% of cases by *Rhinovirus,* compared to 13% in controls. Furthermore, the relapses and PEF reductions were numerically equal to hospital admission [281]. It is hypothesized that even a subliminal inflammation induces in endothelial cells CD54 expression, CD11a/CD18 receptor, which plays the role of the linking site for the virus. Linking to CD54, the virus can interfere with CD11a/CD18 and the host's natural defenses, thus inflammatory reactions to viral infection can stimulate *Rhinovirus* receptors to induce new ones, thereby spreading viral infection [280].

To summarize these reports, a unifying mechanism has been proposed [72], suggesting that *virus action has a major implication in triggering asthma.* From Table 11.10, it can be deduced that the epithelial damage and persistent inflammation that follows have an effect on sensitive nerve endings, provoking the onset of bronchoconstriction reflexes. Above all, respiratory viruses, in addition to provoking airway inflammation and prolonging its effects – either directly or through cellular products infected by them (such as IFN-γ) – promote the formation of virus-specific IgE and the release of mediators.

Emotional Factors

The influence of emotions on asthmatic attacks has been widely documented; therefore any emotional conflict being experienced by the child must always be taken into consideration (Table 5.11), even if for parents such occurrences are rare (Table 6.1). It is known that in 12%–13% of wheezing increase can be triggered by worries regarding school [307], a forthcoming trip, the birth of a sibling, etc. – all events that carry great emotional weight. *Often the child uses the disease* as a means of attracting parental attention, or to obtain privileges which might not otherwise be granted, or to divert existing tensions within the family nucleus, thereby avoiding subsequent conflicts. Studies carried out using BPT have confirmed the asthmogenic role of physiological factors: it has been noted that blind placebo (BP) inhalation prior to the test significantly reduced the onset of asthma, while open BPT with placebo, which the children believed to be the specific allergen, provoked an obvious bronchospasm [363].

Crying and Laughter

Sometimes, a child has bronchospasm following laughter or crying, in the latter case with a frequency of 40% [339]: this can be explained by the mechanical airway irritations caused by intense and rapid exhalations, as well as by hyperpnea, which, as previously mentioned, plays its part in EIA and can trigger bronchospasm even in healthy subjects. We have observed numerous cases of parents of asthmatic children who literally *forbade them to laugh,* for fear of setting off an asthmatic attack, and, conversely, other *children tried blackmail* by threatening to cry [68]. Pediatricians should clarify all these issues both to parents and children, especially explaining that these forms of attack recede spontaneously within a few minutes and should not be viewed as a cause for alarm; but should not be underestimated [68]: a child belonging to a small group of asthmatics under observation over a period of 10 years *died following a fit of laughter* [339].

Collateral Pathologies

AR, frequently complicated by sinusitis, can aggravate asthma (Table 11.1). The presence of such conditions should therefore be ascertained in all asthmatic children and, especially, in those affected with chronic asthma who also presented Rx evidence of sinusitis [179].

GER (gastroesophageal reflux) may be a severe disease and is often neglected in the etiopathogenesis of asthma [702]. GER is a pathology more commonly found in younger infants who have not yet learned to sit up and is determined by functional immaturity of the lower esophageal sphincter. GER is caused by the retrograde movement of gastric contents across this sphincter into the esophagus [702]. The estimated incidence of GER in asthmatic children reaches 50%–60% and can be an aggravating factor of the disease, higher than in the general population [702]. The reflux of acid material provokes bronchospasm, very probably through a vagal reflex. Exposed receptors could be activated with stimulation of the X afferent fibers situated in the esophageal mucosa. This could provoke a vagus-mediated increase of BHR with amplification of the bronchoconstriction effects of other exogenous stimuli [221]. The activation of this reflex between the esophagus and the airways is also related to the fact that embryologically both derive from the anterior archenteron and that they have a common autonomous innervation (Chap. 9). Based on this pathogenetic mechanism, the cough-producing clinical symptoms that have no other etiologies [170] such as in the child with asthma who is difficult to control, or who shows symptoms 1–2 h after having gone to bed, etc. [584], can be assessed. In 40 children aged a median of 14 months with persistent respiratory symptoms, a significant decrease was noticed in the number of further GER-related episodes of recurrent bronchopneumonia, reactive airway disease, nocturnal symptoms and in their nutritional status after starting antireflux therapy [277]. An esophageal scintiscanning was used to detect GER in 126 asthmatic children with a mean age of 2.31 years who were refractory to routine antiasthmatic medication [647].

Drugs to Be Used and Routes of Administration

The treatment of the asthmatic child, summarized in Table 11.11 [26, 360, 476, 508], is determined according to age and according to the severity of the symptoms.

Routes of Administration

There are four routes of administration:

The *parenteral route* is recommended for more severe cases, to achieve elevated concentrations of a drug in a short period and to obtain a good bronchodilatory effect. This method does not produce selective effects in that the drug spreads more or less at the same rate to all areas; it is most often used in EDs or in intensive care units (ICU) and, in any event, under medical supervision for *asthmatics in severe conditions* (in a state of unconsciousness, requiring intubation).

The *oral route,* having a latency time of 1–2 h linked to the rate of gastric absorption, has neither rapid nor selective effects, but certainly finds higher compliance. Higher dosages are needed than for the inhalation route to reach effective levels in the bronchial area, possibly with a higher rate of incidence of undesirable effects as a result of β_2 and β_1 receptor stimulation in extrapul-

Table 11.11. Features of four delivery systems to be used with children

MDI	MDI + spacer and face mask	Dry-powder inhalers	Nebulizers
Requirements			
Shake the canister thoroughly	Rinse the mouth	Elevated inspiratory flow after 6 years of age	Selection and maintenance of a well-functioning device
Hold the canister upright	Shake the canister + spacer	Both Turbohaler and Rotohaler should be held vertically	Preparation of drug solutions to be nebulized in the correct proportions
Place the inhaler between the lips	Actuate the MDI and fill the large volume spacer	Inhale as quickly as possible	Rinse the mouth after each inhalation
Breathe out steadily	Inhale from the spacer by five tidal breaths		When a close fitting mask is used, babies should breathe by their mouth and with no gap between mask and face
Release the dose and take a slow, deep breath	Synchronize dose release with inspiration	Gargle/mouth rinse after steroid administration	
Assessment of correct dose (delivered to the lungs)			
High (80%)	**Very low (<5%)**	**High (60%)[a]**	**Very low (5%)**
Advantages (rate of inhaled drug)			
Delivery efficiency	Rapid onset of action	Delivery efficiency	Adaptable to doses
Rapid onset of action	Rapid onset of action	Minimal child coordination required	
	High effectiveness and low adverse effects	Absence of dispersing propellants	Utilizable in noncollaborating babies
(7%–14%)	(15%–25%)	(30%)[a]	(10%–15%)
Mean age (years)			
6 or more	**1–5**	**5–6**	**1–3**
Therapeutic issues			
None	In acute and chronic asthma these are effective, cheaper, less time-consuming	Only with medications with no adverse effects	Severe bronchoconstriction In absolute absence of cooperation

Face mask devices should be applied firmly to the face, especially if a valved spacer is used; with nebulizers the mask should be held as close to the face as practicable without undue disturbance. Any gap reduces the dose dramatically.
Data from [26, 359, 476, 508].
MDI metered-dose inhaler.
[a] By Turbohaler.

monary sites. In any event, it is recommended for the *very young who are not able to tolerate aerosols* and in cases of nonsevere asthma [79].

Inhalation is the preferred route, in that it reaches the tracheobronchial tree immediately and requires lower doses, with a parallel decrease in adverse effects compared to other routes of administration.

The *subcutaneous (SC) route* is reserved for epinephrine and terbutaline.

To obtain the desired therapeutic effects using inhalatory routes of administration, the following must be considered:

- The size of the inhaled particles
- The appropriateness of the apparatus used
- The child's compliance
- The child's age, strictly linked to the above (Table 11.11), because MDIs (metered-dose inhales) developed for use in adults may be difficult for young children to

use, because they may have difficulty coordinating actuation with inhalation [28].

Regarding the first point, only particles with a diameter of 1–5 µm efficiently reach the lower airways. If they are larger, they will stop in the upper airways and, if smaller, they will reach the alveoli, or be exhaled.

In asthma, there are 4 routes to administer drugs by inhalators:

1. Predosed MDI or pressurized MDI spray can (with spacer)
2. Dry-powder inhaler (DPI)
3. Pneumatic nebulizer with compressor
4. Traditional aerosols

Predosed Pressurized Sprays

These are predosed spray cans or MDIs that manually release fixed predosed drug doses (puffs). These MDIs, if not used properly, deposit up to 90% of the drug in the oropharynx with the same effect of a drug administered *per os;* moreover, steroids may cause dysphonia and predispose the child to oropharyngeal candidiasis [517]. Therefore, the instructions for use should be carefully followed [78]:

- Shake the inhaler.
- Hold it upright and exhale deeply.
- Close the lips around the mouthpiece.
- Press the inhaler while inhaling slowly and as deeply as possible.
- Hold the breath for at least 10 s (in such way that the drops are deposited on the airways as a result of gravity and not of impact).
- Exhale slowly and wait 1–2 min, if the dose is to be repeated.

MDIs are suitable for children at 10 years (Table 11.11) and 7–8 years of age if properly instructed [273]. The main difficulty arises as a result of poor coordination at the moment of discharge, mostly due to:

- Rapid inhalation
- Blocking the inhalation at the moment when the aerosol discharges its content
- Breathing though the nose
- Premature or delayed breathing (lack of hand-lung coordination) [78]

The major problems can be obviated by keeping the mouthpiece 3–5 cm away from the lips, an expedient that overcomes the instinctive defense of small children using their tongue to impede any further drug entry to the lower airways: the effect becomes noticeably increased, also allowing for confirmation of its proper use, but it is not always a recommended method for this age group [78]. It should be noted that MDIs contain chlorofluorocarbons (CFCs) 11, 12 and 114, which provoke cough and reduce ventilatory function in one-third of patients [128]. New hydrofluorocarbon inhalers (HFIs) deliver albuterol, BDP, and various other medications.

Spacers or Holding Chambers

To overcome the difficulty caused by the lack of synchronization between discharge and act of inhaling, *spacer devices* are available. These are adapters consisting of an expansion chamber placed between the MDI and the patient's mouth, facilitating use in preschool children [273]. It consists of a container in which drug particles are held in suspension, that is held inside the apparatus until the moment of inhalation, discharging the required amount even in situations of noncompliance. Consequently, *a minimum deposit in the oropharynx* is assured and *a higher percentage of inhaled drug, as well as a decreased incidence of side effects* resulting from systemic absorption [25, 220, 359]. After the puff, the majority of finer small drops remain suspended inside the spacer for at least 30 s, thereby allowing even *the youngest children* to undergo treatment. The larger aerosolized particles, on the other hand, are withheld, thus obtaining a more rapid administration of the drug and a more efficient supply and an unquestionably adequate cost-effectiveness ratio [436]. In addition to being helpful to children unable to use MDIs correctly, such adapters become indispensable for those undergoing an ICS treatment, to reduce the pharyngeal drug impact and prevent the side effects listed above [80] (Fig. 11.36).

Another innovation has been the introduction of a valved holding chamber (VHC) at the mouthpiece of large-volume spacers to aid the inhalation by very young babies. The principal MDI models with valve spacers are as follows: with the Aerochamber (150 ml volume), also available with a mask for infants and young children, the rate of swallowed medication is barely 2%–3% compared to 60% and 80%, respectively, with the spray or MDI; inhalation is also correct [357], while the rate reaching the airways is 17% compared to 14% of an adequate MDI inhalation [357]. Also available are Babyhaler (Fig. 11.37) and Volumatic, more suited for older children, with volumes of 350 ml and 750 ml, respectively, Fisonair, Nebuhaler and Turbohaler for DPI budesonide (BUD) (Oxis) Turbuhaler for formoterol and Diskus for salmeterol. In addition, there is the pocket-size spacer-discharger of predosed aerosol (Jet) for BDP (Fig. 11.38), which avoids the synchronization problem mentioned and has an inhalation quota of 30% and a swallowed one of 10% [359]; and the *breath-actuated* Autohaler, a dispenser of albuterol. Therefore these last two are useful in acute attacks and for children who have difficulty in using MDIs [128, 485] – not for everyone. Patients aged 5 years have difficulty with the Autohaler since it requires quick inhalation, as with MDIs [128].

For nurslings and very young children, a well-designed mask must be placed on the dispenser (Fig. 11.37), or alternatively, an Aerochamber can be used for nurslings with a closely fitting anatomically designed mask [180], to avoid an empty space between the mask and the face,

Fig. 11.36. A child about to use a pressurized MDI with incorporated PEF meter

Fig. 11.37. A girl using a valved large-volume spacer with face mask

Fig. 11.38. A girl using a pocket MDI with spacer

eventually changing the type of mask if necessary. The choice between large or small chambers has been resolved in favor of the latter, which are easier to manage if the child rejects the treatment, and are also less frightening. They release at least the same amount of medication as the larger ones, if not more, at a low tidal volume (TV) rate [180]. If the spacers are not equipped with valves suitable for nurslings, during inhalation the air can be contained within a nonanatomical mask, thus reducing the dosage inhaled [44]. For children unable to tolerate a face mask, a MDI with a polystyrene cup can be used, especially in case of emergency [508]. In conclusion, face mask devices should be applied firmly to the face, especially if a valved spacer is used. With nebulizers the mask should be held as close to the face as practicable without undue disturbance. Any gap reduces the dose dramatically; therefore in very young children high doses of ICS are not effective because a lot of the spray is lost and does not reach the lesser airways, because of the small TV in these babies.

Another characteristic of critical importance is the volume. The right size is a matter of balance. Using a small, low-resistance, two-way chamber with nurslings and children, 38% of the dose is received, whereas with larger chambers, only 20% is received [44], obviously depending on details: it is larger, more cumbersome and inconvenient to use.

An alternative to the MDI is the small-volume nebulizer (SVN). Advantages to SVNs include use at any age, or administration while asleep [102]. A recent meta-analysis has found the efficacy of MDI-VHC superior to that of SVNs, particularly in regard to onset of action and reduction of hospitalization [86]. In 123 children <2 years of age with moderate-severe exacerbations of wheezing seen in the ED, the response to salbutamol delivered by MDI-VHC and facial mask was faster than to salbutamol delivered by SVN [540].

Dry-Powder Inhalers

DPIs are ecological since they do not contain CFCs: the micronized powder is contained in capsules, is inhaled by special dosers such as Spinhaler for DSCG (cromolyn); Diskhaler, Rotahaler, and Easyhaler for albuterol, and Turbohaler for BUD and formoterol. They do not require coordination of inhalation techniques since the patient inhales directly from the mouthpiece, the inhalator being breath-actuated. Multi-dose apparatuses, in addition to their convenience, ensure greater compliance, especially from preschool children [232]. Even with this system, difficulties arise. The very young should be taught to rapidly inhale without holding his (her) breath and told not to lean their head backwards [508]. In fact, the micronized particles of the medicine tend to agglutinate in clumps of excessive size. For effective use, children should inhale rapidly with high peaks of inspiratory flow (PIF) of at least 30–60 l/min, to create

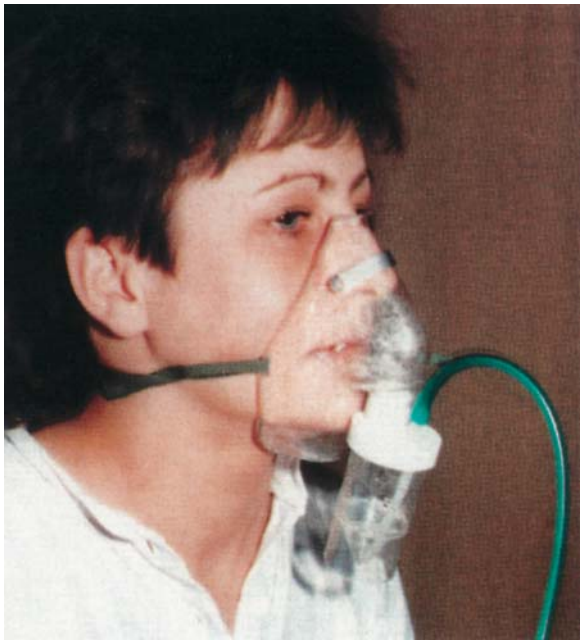


Fig. 11.39. Nebulizer system

Fig. 11.40. Nebulized β_2-agonist therapy is primary in the management of severe asthma also with toys

sufficient turbulence to break them up: at a lower rate the functionality, for example of the Rotohaler, drops to 10% [128]. Since large amounts of medication may remain in the mouth, it is advisable to have the child rinse it thoroughly after inhalation [508]. These models are not suited for children suffering from acute asthmatic attacks [44]; they are advisable for use with children aged 5–6 who can use them [44].

Nebulizers

Jet nebulizers can be used with a mask or directly from the mouthpiece, to avoid the frequent problem of small infants and young children unable to use MDIs, or suffering from acute attacks or persistent bronchoconstriction (Fig. 11.39). The advantages are obvious: even superficial breathing over several minutes can effectively deliver the medication deep into the airways. Compared to traditional MDIs, they produce a larger number of particles in a given time and particles that are also smaller in size (0.5–5 µm instead of >5 µm), thereby distributing the drug more quickly. However, if children are not cooperating during the brief time of discharge, this technique will be inadequate. During nebulization, if the mask is distanced even 1 or 2 cm from the face, the delivered medication is reduced by 85%. Given that even the most tranquil child cannot stay still for more than 5 min, a means of reducing this time frame could be studied [181]. Ultrasonic nebulizers (for example, Fisoneb) ensure rapid nebulization, but are commonly less efficient for drug delivery, especially with suspensions, have a poor cost-benefit ratio and are difficult to handle [26]. Some nebulizers (for example, Nebula and Soffio) can also be used at home (Fig. 11.40). With nebulizers equipped with electric compressors, the need for good coordination from the moment of discharge and the act of inhalation is eliminated, thereby facilitating a sustained administration [476]. Newer types of hand-held nebulizers are small enough to be safely carried in a briefcase, a purse, or a backpack.

Even with nebulizers some rules should be followed:

- Regular checks of the proper functioning of the machine.
- The drug must be diluted in saline, 4 ml.
- Time of nebulization about 10 min.
- Careful rinsing of the oral cavity, especially after using CSs.
- Washing under running water of the flask, mouthpiece and mask after use.

Traditional Aerosols

The traditional models are suitable for all children who will accept them. Usually, they produce too large particles (>5 µm) to reach the airways effectively but, unlike cromolyn and BDP, they are suited to β_2-adrenergics, which are characteristically effective even in low doses, since they are easily absorbed by the oral/nasal mucosa. Even in this case, wheezy infants or toddlers should inhale with an open mouth, otherwise 75% of the aerosol is blocked, with the nose acting as a filter [549]; nor should the mask be distanced from their mouth [192].

Table 11.12. Starting and subsequent drug doses recommended in children <5 years in severe conditions (maximum doses and minimum do intervals for domiciliary use in parentheses)

Age (years)	Device	Symptomatic	Prevention
0–2	MDI spacer and face mask[a]	Albuterol 200 µg q 6 h (1 mg q 3 h) Terbutaline 250 µg q 6 h (2.5 mg q 3 h) Ipratropium bromide (IB) 1.25 µg q 6 h (250 µg q 6 h)[b]	Cromolyn 5–10 mg q 6–8 h BDP 50–200 µg (600 µg) q 12 h FP 25–100 µg (250 µg) q 12 h BUD 50–200 µg (600 µg) q 12 h
	Nebulizer	Albuterol 2.5 mg q 6 h (q 3 h) Terbutaline 5 mg q 6 h (q 3 h)	Cromolyn 20 mg q 6–8 h[c] BUD up to 800 µg q 12 h
>2–5	MDI+spacer	Albuterol 200 µg q 6 h (1 mg q 3 h) Terbutaline 500 µg q 6 h (2.5 mg q 3 h)	Cromolyn 10 mg q 6–8 h BDP 50–200 µg (600 µg) q 12 h FP 25–100 µg (250 µg) q 12 h BUD 50–200 µg (600 µg) q 12 h
	Nebulizer	Albuterol 2.5–5 mg q 6 h (q 3 h) Terbutaline 5–10 mg q 6 h (q 3 h)	BUD up to 800 µg q 12 h

[a] Doses of inhaled corticosteroids are recommended for children in such severe condition that they are no longer controlled by nonsteroidal anti-inflammatory drugs. However, higher doses and concentrations may be required for younger children because of the relative inefficiency of delivery devices (see Table 11.13).

[b] IB should not be administered at intervals less than 6 h to avoid atropine-like toxicity.

[c] Cromolyn is more effective if administered at age 9 months by nebulizer [479].

When prescribing steroids, pediatricians should always prescribe the lowest dose required for symptom control; when symptoms remit, steroids may be stepped down and started up again when symptoms return.

Data from [56, 604].

BDP beclomethasone dipropionate, *BUD* budesonide, cromolyn, *FP* fluticasone propionate.

Age Ranges for Inhalant Therapy

To summarize [476, 508, 699] (Tables 11.10, 11.12) [56, 604]:

- *Children 1–2 years of age*

Nebulizers are useful for acute attacks not requiring the child's total cooperation [272]. The more economical MDIs with valve expanders are also recommended. Both are provided with face masks suitable for use even with nurslings and toddlers if the problem of keeping the appliance in position on the face can be overcome, adapting the expansion chamber volume to the specific needs listed in the previous section [180, 181]. Only 0.3%–1.5% of the nominal dose inhaled by children, even 9–16 months old, reaches the lungs, significantly more if nebulized [549]. A recent trial has established that MDIs with spacers may be as efficacious as nebulizers for the ED treatment of wheezing in children aged ≤2 years [143]. For decades we have used in children aged 6 months and older with wheezing a BDP or flunisolide suspension for nebulization dosed in drops (1 drop/kg) plus albuterol solution for inhalations (half dose), 3–4 doses/day as needed. The symptoms are improved in 90%–95% of cases.

- *Children 3–5 years of age*

MDIs with VHC spacer plus face mask [44, 56] are preferred; however, many children probably use nebulizers because that is the route they began [78]. New cases should first try MDIs with spacers [56]. Devices have been created that allow the child to breathe while receiving the medication, and placebo spray cans produce excellent preventive training. Breath-actuated MDIs are not recommended for this age group [57].

- *Children 6–8 years of age*

At this age children spend a great deal of time outdoors, and they need a portable model that is easy to use and efficient. The aerochamber fulfills all these requirements well. The DPI is useful for subjects who have not yet become accustomed to MDIs. Both systems avoid the problems associated with CFCs.

- *Children aged >10 and adolescents*

Young people of this age are able to use MDIs, which are economical and fulfill the requirements needed [476].

The instructions provided with the device are unsuitable for younger children (Table 11.13) [476, 486], and even adults have found difficulty in using the equipment [103, 350], to the point that manufacturers were invited to change the mouthpiece [350]. Additionally, there are problems related to the dosage absorbed and to be delivered [112]. In the first instance, a pharmacokinetic analysis revealed that nurslings and toddlers absorb only 0.13%–0.33% of the dose being delivered by MDI or nebulizers, respectively [549], and on average only 14% of nebulized medication is deposited in the airways, increasing from 9% to 19% in older children [357]. Reports have shown nebulizer reactions including tachyphylaxis or increased BHR due to β-agonist overuse

Table 11.13. Frequency of the most common problems encountered by 217 children using an inhaler (%)

Problems	MDI inhaler (no. 132)	Tube spacer (no. 85)
Forgetting to shake the canister	49	34
Forgetting to exhale before inhaling	45	51
Neck flexed during inhalation	12	14
Coordination problems	55	17
Too rapid inhalation	67	28
Breath holding for 7 s	42	39
Stopping inspiration when firing aerosol	38	6
Nasal inspiration	24	32
Submaximal inspiration	23	19
Help from the parents	5	6
Possible explanations of the poor compliance:		
Misunderstanding		
Poor reliance on drug effectiveness		
Fear of adverse effects		
Rebellion		
Coordination problems with administration technique		
Coordination problems with administration timing		

Data from [475, 486].

or allergic reactions to the medication or the excipient. Recently two asthmatic children experienced a life-threatening exacerbation of their symptoms after nebulizer use. Cockroaches were detected in the reservoir of the nebulizers used [48].

Dosages for the Very Young

The point of departure is the anatomical differences found in the very young. Nurslings inhale with a relatively rapid TV and in so doing retain the therapeutically useful particles for a shorter time [436]. Nonetheless it should be noted that TV and PIF are reduced, compared to older babies and preschool children, so that infants <6 months inhale the aerosol directly, thereby receiving a greater dosage of the undiluted medication, whereas an older infant or preschool child inhaling with normal TV, but at ambient air, receives a comparatively smaller dose, underscoring the dosage/kg problem [112]. Taking into consideration that the reduction in caliber of the lower airway tracts reduces the penetration drug in the peripheral areas [584], and that newborn infants tend to breathe through their nose, in the best hypothesis the amount of the drug that is deposited in the airways is only 33%–50% of that inhaled *per os* [344]. Also, the correlation between the drug concentration in the aerosolized or nebulized solution (Cs) and that per inhaled volume unit of gas (Ci) appears to be inconstant, since for a given Cs the Ci can be five times greater in a young infant compared to an older child, in whom the inhaled dosage is independent of weight [112]. Therefore, doses for children aged 6 months to 5 years have to be regulated according to weight in kg, to balance the discrepancies that arise from a dosage based on a single measure for all [112]. If no correction is made, children aged 1–5 might receive a larger dosage than those aged 5–10 [344, 488]. However, based on studies conducted related to the plasmatic concentrations of albuterol, it has been noted that doses calculated on the basis of weight can prove to be less in children aged 6 months to 5 years. Therefore in cases of severe asthma, the dose can be increased – even up to 25%–50% [488] or more – with no risks, whether it be the result of greater drug clearance in the airways or of an insufficient inhalation coordination between the method of discharge and the technique [488]; and finally – we should add – whether it also be because of the great differences among individuals. One could settle for a fixed dose for all patients, given that the results produced are similar [459]. Further research will ascertain which dosage is *the most suitable in early infancy,* based also on pharmacokinetic studies of each drug.

Drugs to Be Used

The drugs to be used are the following:
- Adrenergics
- Anticholinergics
- Theophylline
- Corticosteroids

Table 11.14 [124, 617, 634, 635] sets forth the action mechanisms whereby drugs that can be used to treat acute asthma work; Table 11.15 [174] those of β_2-adrenergics; Table 11.16 [35, 56, 406, 437, 552, 582] specifies the dosage for each method of administration.

Adrenergic Drugs

Adrenergic or sympathomimetic agonists can be classified, according to their respective actions on the receptors, as follows:
Drugs acting on receptors α, β_1 and β_2: epinephrine
Drugs acting on receptors β_1 and β_2: isoprenaline, orciprenaline
Drugs with selective action on receptors β_2 (β_2-adrenergics, β_2-agonists), further divided into:
- Catecholics: isoprenaline
- Resorcinolic: fenoterol, orciprenaline, reproterol, terbutaline

Table 11.14. Mechanism of action of asthma medications: comparative effects

	Bronchodilation	Protection		Improvement[b]
		Allergens[a]	Histamine	
Nonbronchodilators				
Cromolyn	–	I, L, BHR	–	++[c]
Nedocromil	–	I, L, BHR	–	+++
Inhaled steroids	–	L, BHR	–	+++
Antileukotrienes[d]	++	I, L	–	+++
Zileuton	++	I, L	ND	ND
Zafirlukast	±	I, L	ND	ND
Montelukast	++	I, L	++	ND
Bronchodilators				
β-Agonists				
Short-acting[e]	+++	I	+++	–
Salmeterol	+++	I, L	+++	–
Theophylline	++	I, L	+	+
Anticholinergic agents	+	–	ND	ND

Data from [124, 617, 634, 635].
ND not done.
[a] Blocks immediate (*I*) and/or late-phase (*L*) reactions and the consequent bronchial hyperreactivity.
[b] BHR reduction by chronic therapy.
[c] Full-dose therapy.
[d] Montelukast, personal data.
[e] Salbutamol.

Table 11.15. Role of β_2-adrenergic function in asthma

1. Bronchodilator effect acts on bronchial smooth muscles
2. Functional antagonism opposes bronchoconstrictor agents on bronchial smooth muscles
3. Inhibition of microvascular permeability, especially of tight junctions
4. Inhibition of mast cell degranulation and mediator release
5. Direct action on irritative receptors
6. Stimulation of mucociliary clearance
7. Increased water flow to the secretions
8. Inhibition of leukocyte lysosomal enzyme release

Modified from [174].

- Saligenins: albuterol (salbutamol), salmeterol, carbuterol
- Others: clenbuterol, procaterol, formoterol, bambuterol

Epinephrine (adrenaline) is a catecholamine that becomes active within 5–6 s and has an effect that lasts 30 min. For SC administration of terbutaline, 0.5 mg vials are available. Symptoms of epinephrine overdose (Chap. 20) may be frequent (47%) in children treated with SC epinephrine compared to those given terbutaline inhalation (11%). In Chap. 20, b/w based doses of inhaled epinephrine are detailed. In case of a severe attack, epinephrine remains the preferred drug [352].

β_2-Adrenergics for predosed MDIs or nebulizers (Fig. 11.40) offer the best results because of rapidity of action, good efficacy and limited adverse effects on the cardiovascular apparatus. Given the recognized benefits of short-acting β_2-adrenergics, attention was directed to extending the duration of effect of β_2-adrenergic agonists. As a result, long-acting β_2-adrenergics with a 12-h duration of action were introduced: salmeterol and formoterol. For an immediate effect, lasting 4–6 h, albuterol is recommended: to limit the adverse effects of short-acting β-agonists, the stereoisomer of albuterol, levalbuterol, compared to racemic albuterol significantly reduced the number of hospitalizations in the ED management of acute asthma in children aged 1–18, but the hospitalization length of stay was not significantly shorter between the two groups [84]. For an effect with 5 min of latency, but with long-term effect (12 h), salmeterol 25 µg is recommended. A dose of 50 µg has induced a significant HR increase in the first 5 min [19] in children of 13.4±2.5 years. Formoterol has the same latency and lasts 12 h [441], while systemic administration of formoterol (via the oral route) displays a short duration of action (4 h). Salmeterol is a long-acting β_2-agonist with a long side chain, diffuses quickly from

Table 11.16. Pediatric doses and routes of administration of epinephrine and main β_2-adrenergic (quick-relief) medications

Medication	Route	Dosage form	Dose
Adrenaline (epinephrine)	SC	1 ml vial of a 1:1,000 dilution	0.01 ml/kg repeated q 15 min for 3–4 doses[a] as needed. Up to a maximum of 0.3–0.5 ml per dose or inhaled (Chap. 20)
Racemic	Nebulizer solution (2.25%)		0.1 ml/kg diluted to a total of 2 ml in 0.9% NaCl, or ″ 2 years: 0.3 ml in 1.5 ml of saline <2 years: 0.5 ml in 2.0 ml of saline
Albuterol (salbutamol)	Oral	Tablets 2–4 mg Syrup (10 ml = 4 mg)	Children <6 years 0.1–0.2 mg/kg × 3–4 daily Children 6–12 years 2 mg/kg × 3–4 daily Children >12 years 2–4 mg × 3–4 daily
	Rotocap DPI	(200 µg/capsule)	200 µg × 3–4 daily prn or q 4–6 h
	Nebulizer solution (0.6%)		2.6 mg (0–2 years) to 5 mg (2–5 years) prn or q 4–6 h
	MDI	(100 µg/puff)	100–200 µg (1–2 puffs prn or 3–4 puffs daily)
	IM/IV vials	100/500 µg	2–6 µg/kg × 3–4 daily
	Levalbuterol	Unit dose vials of 0.31 mg, 0.63 mg, 1.25 mg	Pediatric 1.25 mg qid, or q 20 min (max 6 doses)
	Racemic	Solution of 5 mg/ml or unit dose vials	Pediatric 2.5 mg qid, or q 20 min (max 6 doses)
Bambuterol	Oral	Solution in 2- to 5-year-old children	10 mg in the evening
		Tablets in 6- to 12-year-old children	10–20 mg in the evening
Bitolterol	MDI solution 0.2%	(1 puff = 0.5 mg)	Children 4–12 years 1–2 puff q 4–6 h prn
	Nebulizer solution (0.1%)		1 mg (range 0.5–1.5 mg) q 4–6 h prn
Fenoterol[b]	Oral	Syrup 0.05% (10 ml = 5 mg)	0.1–0.2 mg/kg × 3–4 daily
	Nebulizer solution 0.1%	(20 drops = 1 mg)	Children <6 years 50 µg/kg Children >6 years 100 µg/kg × 1–3 daily
	MDI	(100 µg/puff)	100–200 µg (1–2 puffs) x 3–4 daily
Formoterol	MDI	(9–12 µg/puff)	Children >6 years 1–2 puffs × 2 daily or × 3–4 daily prn
	DPI Turbohaler	(9–12 µg/capsule)	Children 5–12 years 12–24 µg × 2 daily
Pirbuterol	MDI 250 µg/dose		>12 years 2 puffs × 3–4 daily or prn
Procaterol	Oral	Tablets 5 µg	1 µg/kg × 2 daily
		Syrup 5 µg/ml (10 ml = 50 µg)	1 µg/kg × 2 daily
	MDI	(10–25 µg/puff)	10–25 µg (1–2 puffs) × 2 daily
Salmeterol	MDI	(25 µg/puff)	25–50 µg (1–2 puffs) × 2 daily[c]
Terbutaline	Oral	Tablets 2.5–5 mg	Children <12 years 0.05–0.1 mg/kg (max 5 mg) q 6–8 h, Children ≥12 years 2.5–5 mg q 6–8 h
		Solution (0.1%)	0.01 ml/kg up to 0.3 ml SC
	1 mg/ml		q 2–6 h prn
	MDI	(250 µg/puff)	0.25 mg dose (1 puff) q 6–8 h prn

Two dosages (10–25 µg/puff) means that two different preparations are available, or that two different doses are suggested. The rate of inhaled drug may be lower than 30% (Table 11.11). In small children, spacers with a mask are imperative. Epinephrine is suggested for babies with bronchiolitis and children with status asthmaticus.
MDI metered-dose inhaler, *DPI* dry-powder inhaler.
Data from [35, 56, 158, 406, 437, 552, 583, C Kercsmar, pers. comm. Nov 30, 2005] and Aucoin RG. Respiratory pharmacotherapy (Accessed at http://search.yahoo.com/search?fr=fp-pull-web-t&p; http://picuBOOK.net/1999/04-001(e1).htm).

[a] Acts within 5–6 s, duration is of 30 min; more data on epinephrine and terbutaline are in Chap. 20.
[b] Decreased doses have been suggested, see text for details.
[c] Adult dosage, the 25- to 50-µg dose should be the highest in children aged 6–12 years [582].

the aqueous phase into cell membranes of the lung with maximal bronchodilation ≈1 h after administration. Formoterol has a medium-sized side chain and diffuses both into the aqueous phase and cell membranes of the lung with an onset of action within minutes. Two recent articles have reported that formoterol provided superior bronchodilator efficacy over 12 h compared with salmeterol [501], or at least as effective as salmeterol [183]. So once daily oral bambuterol may be an interesting and less expensive alternative to twice-daily inhaled formoterol and salmeterol [158]. The increased efficacy of the first (single) dose compared to regular follow-up treatment should be kept in mind, being equal to a PD_{20} improvement of 1.7 or 0.7 [680]. Therefore, with the *new, long-acting β_2-adrenergics* the late reaction is inhibited and adverse effects are reduced. Nevertheless, some undesirable side effects can result, especially with continued and/or high-dose use:

- Muscular effects: muscle tremors (as a result of action on β_2 muscle receptors)
- Metabolic effects: hyperglycemia (for glycogenolysis) with hyperinsulinism, hyperkalemia and therefore hypokalemia as a result of K^+ deposits at the cellular level
- CNS effects: agitation, insomnia
- Cardiovascular effects (in cases of overdose): tachycardia, extrasystole, hypertension

Possible effects resulting from prolonged usage include:

- Tachyphylaxis (reduction in therapeutic responsiveness)
- Difficult management of the disease: asthma worsening, BHR increase [571] due to tolerance increase [456]

An Assessment of β_2-Adrenergics

We draw attention to the results of certain studies focusing on β_2-adrenergic use, especially when used over a prolonged period and/or in high dosages [697].

1. Sears et al [571] obtained better results when β_2-adrenergic drugs were used only as needed. Control of the disease is reduced in patients who treat asthma with bronchodilators continually, for a long time and regularly, patients becoming dependent, and thus inhaling ever higher doses: in this way desensitization of β-adrenergic receptors occurs, that is repeated administration reduces the bronchodilator effect.
2. According to Page [467], this is probably caused by a disrupted equilibrium between inflammatory and anti-inflammatory factors, as a result of reduced anti-inflammatory release, caused by the powerful antidegranulation accomplished by β_2-adrenergics on mast cells. In time, this leads to the deactivation of natural defense mechanisms, allowing the inhaled allergens to penetrate in the bronchial tree more deeply [467], with loss of the action inhibiting BM thickening and smooth muscle hypertrophy, deleterious aspects for asthmatics [516] likely leading to an irreversible chronicity [467].
3. According to Spitzer et al [608], these drugs aggravate asthma, rendering the airways more susceptible to stimuli. In effect, though reducing the acute episode, they promote delayed bronchial inflammation, which is at the basis of chronic asthma.
4. Burrows and Lebowitz [62] agree with these findings and advise against regular, high dosages of β_2-adrenergics.
5. Prospective studies in children have shown that prolonged usage of β_2-adrenergics does not reduce BHR [571].
6. In patients treated with terbutaline, a rebound type of BHR can occur when treatment is stopped: conversely, in children 1.5–11 years admitted to an ICU, the same drug was shown to be safe and effective [288].
7. It should be noted that for β_2-adrenergics, dosages that double the usual ones, even if only for 7 days, amplify airway reactivity even in the late reaction [110]. Chronic use of high doses results in hypokalemia, because ATP stimulation causes K^+ passage within the skeletal muscles, an effect that is strengthened by hypercapnia, hypoxia and hypokalemia, characteristics of acute asthma-status asthmaticus [310, 697]; even theophylline can provoke hypokalemia, and together with CSs, hyperkaluria [310].
8. Few and contradictory results have been obtained with these drugs in bronchiolitis: epinephrine was more efficacious than salbutamol [401]. Probably, the negative results depend on having measured the Raw index of seriously ill infants with widespread edema and mucous secretions, thereby reducing the dose of the aerosolized drug [112]. Moreover, the total resistance of upper airways can be reduced by 50% at this age, making measurements unreliable [206]. This can also be related to a reduced response to β-adrenergic stimuli effected by respiratory viruses and therefore to that of β-adrenergic drugs [73].
9. In conclusion, there is no clear benefit of using β_2-adrenergics in the management of recurrent wheeze in the first 2 years of life, although there is conflicting evidence [140].

Anticholinergic Drugs

These drugs stimulate the muscarinic receptors with reduction in intracellular cGMP by competitively inhibiting α-adenylcyclase [77]. The bronchodilator effect works on medium- or large-caliber bronchi; it is less rapid (15 min after administration, maximum effect after 30 min to 2 h), but longer-lasting than short-acting β_2-adrenergics (exhausted after 3–5 h). They should be used in the intercritical periods, or in association with inhaled β2-adrenergics, which allows the dose of the latter to be reduced. This also reduces the risk of side effects as a consequence of the therapeutic margin of these drugs [79].

IB has an anticholinergic activity that is similar to that of atropine, but also a reduced systemic absorption. IB is most often used with other drugs, providing good bronchodilator action and few side effects (mucosa dryness) [371, 663] when utilizing a DPI – preferred by young children rather than an MDI [663] – or a spray with spacer [371].

Theophylline Drugs

Theophylline drugs are enjoying a renaoissance mainly because of their rediscovered anti-inflammatory properties [274]. Theophylline is a methylxanthine similar to theobromine and caffeine. The mechanisms of its action [124, 635, 740] and the anti-inflammatory effects [101, 114, 461, 658] are summarized in Tables 11.17 and 11.18 [101, 114, 124, 461, 635, 658, 736], from which the induction of apoptosis in eosinophils clearly emerges (Fig. 11.41). Bronchodilation is caused by PDE inhibition, which causes an increase in cAMP levels. It also displays considerable anti-inflammatory properties (Table 11.14) and promotes the production of anti-inflammatory IL, IL_{10}, deficient in asthmatic airways, depressing instead that of IFN-γ [513]. Significantly, in young asthmatics, abatement of IgE levels occurs with slow-release theophylline, but not with cromolyn or BPD [658].

In examining the pharmacological aspects, it is useful to review a few basic facts [404, 706]:

- *The therapeutic range of theophylline* is between 10 and 20 µg/ml (56–111 µmol/l), even though it is known that bronchodilation can take place with therapeutic levels of 5–15 µg/ml. Below the minimum level the effects are few and side effects – even severe – can occur when the theophylline level exceeds 15–20 µg/ml. Thus the therapeutic range is extremely close to the toxic range.

Table 11.17. Anti-inflammatory action of theophylline

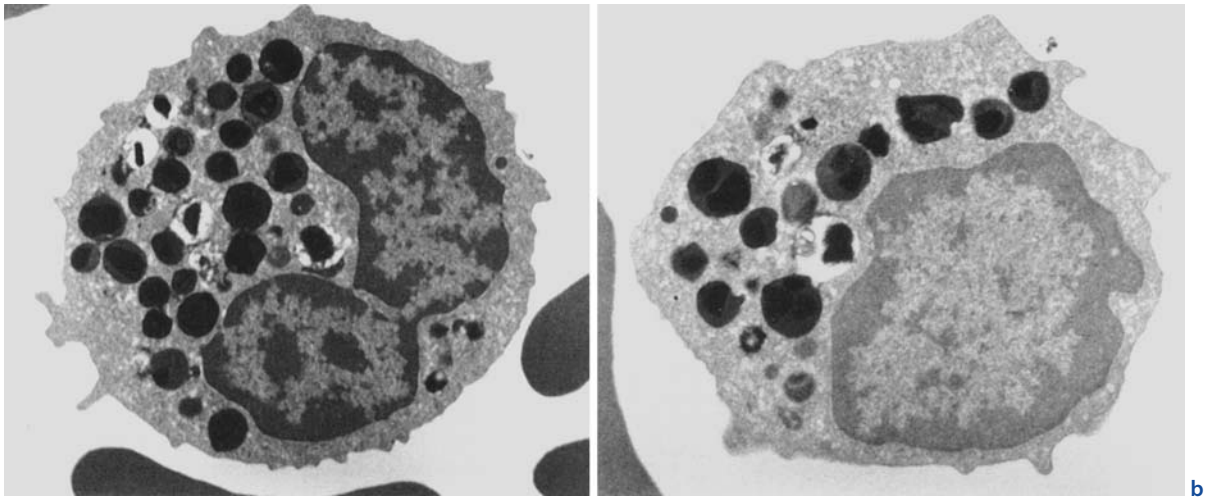
Increases mucociliary transport
Increases diaphragmatic contractility
Decreases muscle work
Bronchodilation
Inhibits phosphodiesterase
Suppresses histamine release by mast cells and basophils
Inhibits vascular permeability
Inhibits PG and LT
Increases cAMP intracellular concentrations
Inhibits immediate and delayed asthmatic reactions, a change not observed in any other group
Inhibits airway responsiveness

Data from [124, 635, 735].

Table 11.18. Immunomodulatory effects of theophylline

Inhibits neutrophil and mononuclear cell chemotaxis
Suppresses IgM, IgG and IgA synthesis
Reduces serum IgE levels in 4- to 6-year-old asthmatics
Inhibits TNF-α and $IL_{1β}$ expression and release
Reduces CD3, CD4, CD8, CD15, and CD25 cell counts
Enhances trafficking of peripheral blood-activated T cells
Inhibits eosinophil influx after allergen challenge and cationic protein production
Enhances eosinophil apoptosis

Data from [104, 114, 658].



a b

Fig. 11.41 a,b. Morphological changes of eosinophils by adding theophylline to culture medium with IL_5. **a** The eosinophils, without additions, are "normal" in shape. **b** By adding theophylline the cells show apoptotic changes including aggregated chromatin, smooth cell surface and cytoplasmic vacuoles

• *Theophylline metabolism* results in 85% of theophylline being metabolized by the enzymatic system of hepatic cytochrome P450, so all factors or drugs that interfere with this system can alter its metabolism (Table 11.19) [244], increasing or reducing theophylline clearance, with reduction or, respectively, increase of its concentrations [706].
• *Theophylline clearance* (distribution volume × unchanging level of elimination) varies according to age [404]:
– From birth to 7–9 months, it is reduced.
– Between 7–9 months and 9 years of age it increases (1.44 ml/min/kg); therefore, proportionately higher doses are required.
– From 9 to 16 years the clearance is reduced again (9–12 years=1.26; 12–16 years=0.9 ml/min/kg).

Such levels are appropriate for good theophylline distribution and function. Other factors that must be borne in mind are [244]:
• Individual variations in the rate of absorption.
• Differences of half-life in children are half that of adults (≈3.5 h instead of 7 h).
• If the patient is obese, the correct dosage must be prescribed according to ideal standard weight, and not to the actual weight.

Theophylline absorption also depends on *circadian rhythms*. The indispensable interval of time to reach peak concentration of the medication is significantly shorter (and absorption increased) when oral doses are taken in the morning, compared to evening effects. Analogously, clearance is influenced: the elimination rate is greater with morning doses compared to evening doses in children treated with slow-release or IV medications, so a standard dosage with equal doses at regular intervals will provoke an increase in nocturnal serum levels compared to daytime levels, to the advantage of nocturnal asthma sufferers [32]. All this is of great importance in establishing dosages which must be *personalized* whenever constant monitoring for an entire 24 h period become necessary. Similarly, for the study of theophylline pharmacokinetics, the hours at which both doses and blood samples were taken must be recorded [244].

Indications

• Children with acute asthma who can benefit from theophylline, but reducing the respective doses when associated with β_2-adrenergic.
• Mild or moderate forms of acute attacks, if there has been no response to the β_2-adrenergics. Eventually it can be associated with them, again reducing the respective doses.
• Moderate to severe forms of chronic asthma resistant to other medications [707].

The scientific rationale behind theophylline lies in its anti-inflammatory quality, not shared by other medica-

Table 11.19. Factors and drugs affecting theophylline serum concentrations

Increased concentrations (decreased clearance)	(%)
Physiological alterations	
Bronchopulmonary dysplasia	Variable, notable
Caffeine (tea, cacao, Coca-cola)	?
Cardiac and/or hepatic insufficiency	Variable, notable
Febrile illness	?
High-carbohydrate and hypoproteic diets	?
Hypothyroidism	50
Influenza A vaccine	?
Obesity	?
Premature and newborn babies	?
Prolonged fever (>24 h)	50
Undernourishment	?
Drugs	
Allopurinol (high doses)	25
Cimetidine	50
Ciprofloxacin	30
Erythromycin	25
Methotrexate	20
Oral contraceptives	30
Propanolol	20
Troleandomycin	50
Low dose	25
Decreased concentrations (increased clearance)	**(%)**
Physiological alterations	
Cystic fibrosis	?
Hyperthyroidism	20
Ingestion of meat cooked on coal-stoves	?
Low-carbohydrate and hyperproteic diets	?
Tobacco smoking	50
Young age (1–16 years)	?
Drugs	
Barbiturates	?
Carbamazepine	60
Isoproterenol (IV)	20
Phenytoin	75
Rifampicin	80

Data from [244].
? Unknown rate.

tions, which we have always taken into consideration. Logically it holds an undisputed and, to this day, unique place ensuring the compliance of young and very young children who are not used to taking inhalation drugs. According to Szefler [634], the clinical effect of chronic therapy with theophylline, cromones, and anti-LT on symptom scores, PEFR, and acute exacerbations (morning, or evening) is comparable.

Dosages and Methods of Administration

1. IV in severe asthma attacks to achieve a more rapid therapeutic effect.
2. *Per os* the absorption is slower; depending on the nature of the case the following can be used:
- Fast-acting theophylline.
- Long-acting theophylline, which, having a greater serum half-life, is preferable for long-term treatment. Also, it is suitable for the very young [77].

Dosages advised for fast-acting theophylline are as follows, four doses every 6 h [244, 707]:
1. Initial dosage for children 6 months old: about 10 mg/kg/day up to a maximum of 300 mg/day. *After 3 days, if the dose is tolerated:*
- First increment: about 13 mg/kg/day up to a maximum of 450 mg/day. *After a further 3 days, if the dose is tolerated:*
- Second increment: about 16 mg/kg/day up to a maximum of 600 mg/day.

In view of the rapid metabolism that the drug undergoes in the child, an early and periodic monitoring of theophylline to verify that the levels are not subtherapeutic (5.6 µg/ml) is indispensable. We suggest measuring theophylline levels after at least 3 days to coincide with the administration of the maximum dose tolerated, *4 or 8 h after the morning dose,* depending on the medication being used. Once theophylline levels have been monitored, the dosage should be adjusted based on serum concentrations in µg/ml [244]:

<7.5	Increase dose about 25% (check serum theophylline levels for guidance in further dose adjustment).
<7.6–10	If tolerated increase dose about 25%.
10–15	If tolerated maintain dose (recheck serum theophylline levels at 6- to 12-month intervals).
15.1–19.9	Consider a 10% dose reduction (to provide greater margin of safety.
20–25	Skip next dose and resume treatment based on the last increase tolerated.
25	Skip next two doses and resume the initial dosage, or that of the last increase.

Whenever side effects occur, dosage for all children should be reduced to the minimum dose previously tolerated.

2. Dosages for infants <1 year of age [244]:
- Initial dosage in mg/kg/day: (0.2) (age in weeks) +5.0; this equation results in levels of theophylline between 5 and 10 mg/l [244]. Serum theophylline concentrations should be monitored within 6–12 h after start of treatment [256].
- Subsequent dosages should be based on serum theophylline levels measured at least 3 days after start of treatment.

The average dosage of long-acting theophylline is 7–10 mg/kg every 12 h [83]. This formula, having a greater half-life, is recommended for treating nocturnal asthma, among other reasons because of its protracted bronchodilator effect. Additionally, capsules that can be emptied and mixed with foods minimize the child's opposition [83]. For cases using long-acting theophylline, a noninvasive, reliable, and convenient method for measuring theophylline levels in saliva has been proposed [640]. Three different controls are recommended, to characterize around-the-clock theophylline profiles:
- Good symptom control, no side effects: measurement 4 h after morning dose, every 3–6 months
- Nonoptimal control: as above with the addition of a further measurement just before evening doses
- Poor or no clinical response: 24 h profile, even at home [640]

In conclusion, the use of theophylline has but one disadvantage: for safe treatment we recommend measuring theophylline levels to reach and maintain levels encompassed within the therapeutic range [706]. This is also valid to avoid undesired side effects [244].

Notwithstanding what has been said, *theophylline is completely safe*. A study of 35,000 patients who filled 222,000 prescriptions for 9 years registered only one case of seizures in a child (without specifying the cause), equal to 0.003% of cases and to 0.00045% of the prescriptions [404].

Theophylline Assessment

From time to time, North American and North European authors have underlined the negative effects of theophylline with the result that experts tend to relegate this drug to a secondary role among the forms of treatment [272, 435, 504], thereby contributing to an unjustified decline in its use [536, 635]. In particular, three recent studies [89, 148, 622] excluded its use in hospital treatments of children aged 2–18 years with moderate to severe asthma and, specifically, in association with MDI-delivered albuterol and IV-administered methylprednisolone, even though no undesired side effects were found to justify the exclusion [622]. Nevertheless, the studies *did not include patients in severe conditions*, a limitation that prevents the evaluation of theophylline ability to preclude the necessity of ICU admission and/or assisted ventilation. Furthermore, in our opinion, the above-mentioned associations have not been

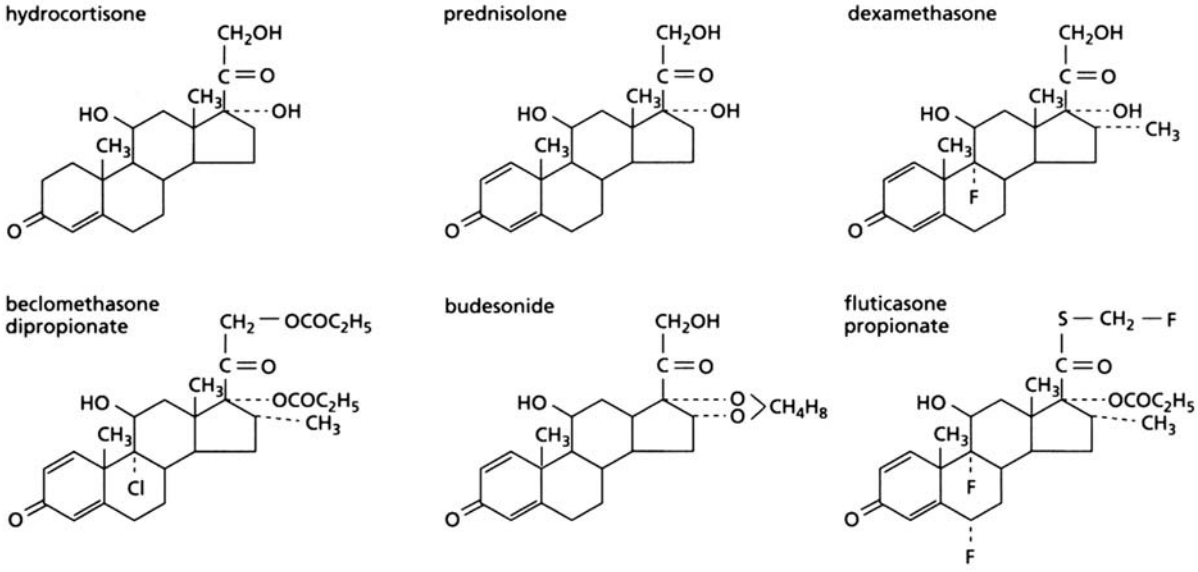


Fig. 11.42. Structural formulas of common corticosteroids.

properly evaluated since children had already reached maximum bronchodilation with "liberal and elevated" doses of nebulized albuterol [89]. Even in children or teenagers with nonsevere asthma treated with albuterol and IV-administered methylprednisolone, DiGiulio et al [148] did not find clinical differences between theophylline and placebo, although at the cost of possible type II errors. Although concern was expressed about the dangers of improper use of theophylline [89], no related adverse effects were reported in either study: nausea was the most toxic symptom encountered [622]. On the other hand, several studies have shown theophylline effectiveness (ref in 615). In patients admitted to ED and treated with theophylline, discharges were more frequent than admittance to hospital (6%), which differs from patients treated with metaproterenol and cortisone (21%), and with an overall cost fivefold lower [740]. Mechanical reasons, especially in life-threatening forms of infantile asthma, can impede β_2-adrenergic passage. This is theophylline's strong point: administered IV in these forms it produces an almost immediate clinically detectable, objective bronchodilation [664]. In 94 children aged 1–17 in severe status asthmaticus admitted to the PICU theophylline safely hastened the recovery of these children who were also receiving albuterol, IB, and CS therapies [49, 515].

On a therapeutic level, other side effects were noted [706] such as learning and behavioral problems. Re-examined by various researchers in ten studies, they were unable to definitively verify this correlation, partly due to methodological errors [123], or because the former were completely absent, whereas the rest normalized, or improved, within 2 weeks [613]. No school-related differences were found between asthmatics (whether theophylline-treated or not) and controls [353]. Parents were unable to decide to which treatment (theophylline or placebo) to attribute the improvement in memory and attention [34]. Other researchers, not finding any side effect, suggest that the potential vulnerability only concerns children already suffering from similar problems [562]. In a systematic review of 12 pediatric studies, no behavioral or cognitive adverse effects of theophylline were identified [597a].

Corticosteroids

The CS action mechanism (Tables 11.20 [23, 249, 284], 11.21 [120], 11.22 [23, 203, 284, 633] and 11.23 [56, 117, 437, 581], Fig. 11.42 [105]) is performed on adenylyl cyclase by increasing cAMP levels, and on the inflammation, reducing the edema and inhibiting histamine release [703]. In particular, cAMP level maintenance within a normal range is useful for bringing the adrenergic receptors back to a functional state in which they can again become activated [703]. CSs inhibit the metachromatic cell mediators and basophil releasability, and reduce circulating eosinophil number and presumably also those in the tissues, but not of macrophages [212]. These effects are due to IL inhibition that activates these cells and stimulates them into reaching inflammatory sites [570]. In asthmatic children (80% atopic), serum concentrations of IL_5, CD25, and ECP remained significantly higher than controls, even after treatment with oral CSs. Thus, T cell-mediated inflammation may persist in childhood asthma despite apparent clinical remission associated with conventional treatment [172]. The most powerful action of endogenous CSs is directed against inflammation, markedly exacerbated if the adrenal gland is removed [570]. In this process, the main activity is aimed at suppressing the transcription of genes involved in inflammation, and, to this end, their re-

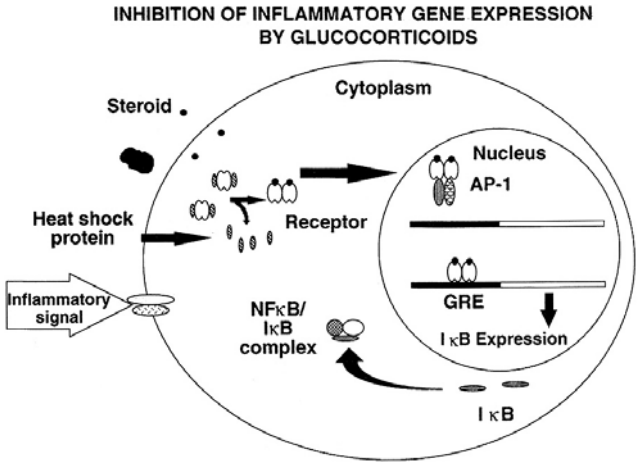


Fig. 11.43. Inhibition of inflammatory gene expression by CSs. *AP-1* apolipoprotein 1, *IκB* inhibitor of NF-κB, *NF-κB* nuclear factor kB

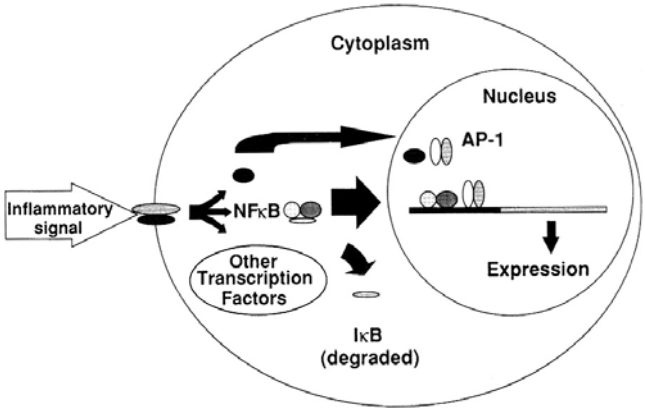


Fig. 11.44. Activation of inflammatory gene expression. *AP-1* apolipoprotein 1, *IκB* inhibitor of NF-κB

ceptors interact with AP-1 (activator protein-1) and NF-κB (nuclear factor of chain κ of Bs) (Fig. 11.43) [568] or, diversely, render more potent the inhibitor of the chain κ of B, type α (κB-α), inhibitor of NF-κB (Fig. 11.44) [568]. However, the bronchial smooth cells lack the CCAAT/enhancer binding protein α (C/EBPα) required to form a complex with the CS receptor, therefore CSs cannot contrast the ADAM33 accelerated proliferation of bronchial smooth-muscle cells and the resulting BHR [539]. From a clinical point of view, the latency time is lengthened (6–12 h) compared to other drugs [683], and a maximum bronchoprotecting effect can be attained after many months of continuous treatment [570]. Consequently, although CSs are an effective treatment on ameliorating asthma symptoms and represent the most effective anti-inflammatory therapy of asthma, *this therapy is symptomatic since the disease flares when treatment is stopped* [517, 645, 671, 680]. If ICSs are discontinued, the asthmatic children return to

Table 11.20. Molecular mechanisms of corticosteroid (*CS*) action in asthma

A. Specific effects of CSs on transcription of genes relevant to asthma
Increased transcription
a. Lipocortin-1
b. β_2-Adrenoreceptor
c. Endonuclease
Inhibition of transcription
a. Leukocyte protease
b. Cytokines
Decreased transcription
a. Cytokines (IL_1–IL_6, IL_8, IL_{11}–IL_{13}, IL_{16}, TNF-α, GM-CSF)
b. Chemokines (eotaxin, MIP-1α, RANTES, SCF)
c. iNOS
d. Inducible cyclooxygenase
e. Inducible PLA_2
f. ET-1
g. CD54
h. $N\kappa_1$ receptors
B. Effect on IgE synthesis
a. Reduction
b. Probable enhancement[a]
C. Effects on mast cells, basophils and eosinophils
a. Reduction in mast cell number
b. Inhibition of mast cell proliferation
c. Inhibition of mediator release
d. Inhibition of histamine synthesis
e. Inhibition of eicosanoid release
D. Effects on lymphocytes
E. Reduction in lymphocytes with activation markers (CD3, HLA-DR)
F. Reduction in cellular traffic and functions
G. Effect on specific processes
a. Inhibition of late reactions
b. Reduction in vascular permeability
c. Inhibition of mucosal secretion
d. Induction of vasoconstriction
H. Potential relevant mechanisms
a. Anti-inflammatory action
b. Increase in number and sensitivity of β-adrenergic receptors
c. Adenylate cyclase activation
d. Increased cAMP
e. Eosinophilopenia, basophilopenia, monocytopenia

The IL-13 inhibition by steroids may, at least in part, account for their therapeutic effects [150].
See Tables 7.17 and 7.18 for side effects of CSs employed topically in atopic dermatitis.
Data from [23, 249, 284].
[a] See text.

Table 11.21. Adverse effects of inhaled CSs in 163 children (%)

1. Hypertension	88
2. Cushingoid features	66
3. HPA-axis suppression	56
4. Myopathy	50
5. Osteopenia	46
6. Growth suppression	39
7. Obesity	30
8. Hypercholesterolemia	30
9. Posterior subcapsular cataracts	14

Osteopenia was strongly associated with growth suppression (odds ratio, 5.6). Note: 50% of children required chronically administered oral CS therapy.
Data from [120].

their baseline PFT and indices of airway inflammation *after 2 weeks* [593]. CSs do not relieve symptoms promptly and children may not improve within a few days [517]. So CS efficacy is significant in late asthmatic responses, chronic asthma and, more generally, in suppression of chronic inflammation [570]. This effective anti-inflammatory action on mast cells and eosinophils occurs in the epithelium (each 2.5-fold) and in submucosa (from 2- to 10-fold, respectively) and, on clinical grounds, in BHR improvement [258] (Table 11.14). Basically, there are no definitive effects, since *the underlying inflammation is still active in childhood asthma* despite apparent clinical remission even after years on ICSs [476]. Thus, any beneficial CS effect on BHR is not due to the prevention or resolution of remodeling of the airway wall [645]. The reason is that CSs have marked effects in inhibiting T-cell activation, but these effects are not reflected in changes of overall cell numbers in the circulation. So there is neither a discernible effect on the underlying mechanisms of inflammation [208], nor on methacholine responsiveness [645]. In addition, during the period of alveolar development, CS administration may result in decreased lung-cell mass and in the presence of too few abnormally large alveoli [727]. Also, for this reason it may be prudent to avoid CS use in the very young, because they appear not to be very effective, as is also demonstrated by their lack of impact on PFT [620]. FP, mometasone furoate (MF) and, to a lesser extent, BDP are thought to be second-generation ICSs in that they display both increased anti-inflammatory potency and systemic bioavailability [434].

An Assessment of Corticosteroids

In addition to dysphonia and oral candidiasis, several undesirable effects of inhaled CS (including osteoporosis) occurred in 163 children aged 14.4±2.1 years with

Table 11.22. Mechanisms possibly explaining a poor response to CSs in asthma

1. Additional respiratory disorders (cystic fibrosis, vocal cord dysfunction, etc.)
2. Overwhelming allergen exposure
3. Irreversible airway hyperreactivity
4. Poor compliance (children, parents, etc.)
5. Psychological problems
6. Inadequate dose for severity of asthma
7. Pharmacokinetic motives
a. Rapid metabolism
b. Poor distribution at the site of action
c. Poor/partial absorption from oral administration
8. Immunological resistance
A. Monocytes
a. Unable to decrease MCR and CRI expression
b. Unable to decrease PHA-induced proliferation
c. Unable to decrease cytokine production (TNF)
d. Quantitative and functional defect of intracytoplasmic receptors
B. Lymphocytes
a. Unable to decrease PHA-induced proliferation
b. Unable to decrease cytokine production (IL_2, IFN-γ)
c. Unable to inhibit activation (CD25, HLA-DR)
C. Abnormal receptor or postreceptor down-regulation

The use of high doses of inhaled β_2-agonists in acute asthma exacerbations may result in resistance to high-dose intravenous CSs in the treatment of these exacerbations [23].
Data from [23, 203, 284, 633].
cAMP cyclic adenosine monophosphate, *CRE* complement receptor increase, *HPA* hypothalamic-pituitary-adrenal, *MCR* monocyte complement receptor.

severe asthma receiving high-dose inhaled CS therapy (1675±94 µg/d), 50% of whom required chronic oral CS therapy [120] (Tables 11.20–11.22) [21, 120, 203, 249, 284, 633]. Pharmacokinetics studies suggest that the fraction deposited in the airways is absorbed in an active form in variable concentrations, and passes, in turn, into the circulation. In fact 80% of the amount deposited in the oropharynx is absorbed by the intestine, passing through the liver where most of it is metabolized. Part of it reaches the systemic circulation where it joins with the amount originated in the airways. These two rates are aggregated, but are continually reduced as a result of recirculation and hepatic deactivation, as is evident from Fig. 11.45 [703]. Nonetheless, there is a different *bioavailability* (the drug rate bypassing the hepatic filter) and a rapid CS metabolic clearance CS reaching the circulation [22, 359]. Substandial differences can

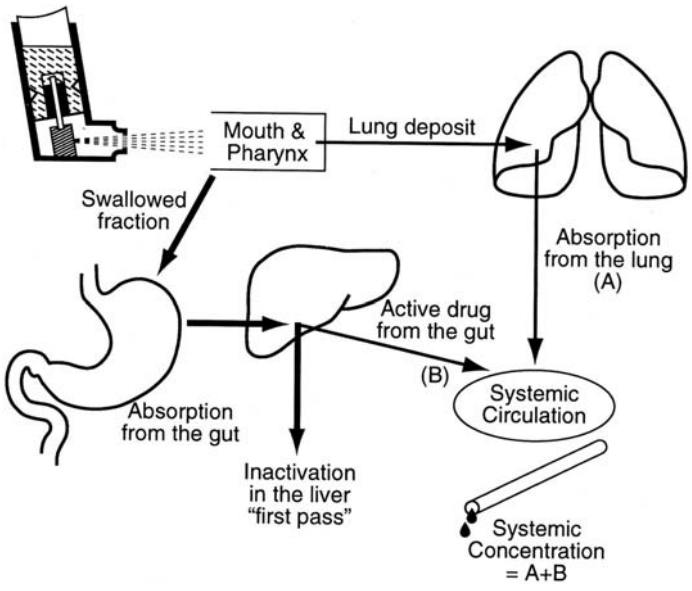


Fig. 11.45. The fate of inhaled CS.The amount of an inhaled CS that reaches the systemic circulation is the sum of pulmonary and orally bioavailable fractions. The fraction deposited in the oral cavity will be swallowed and systemic availability will be determined by absorption from the gastrointestinal tract and the degree of liver inactivation. The systemic concentration will be reduced by continuous recirculation and liver inactivation

Table 11.23. Usual dosage for corticosteroids in pediatric asthma

Medication	Route	Dosage form	Dose
Beclomethasone Dipropionate	Aerosol	Metered-dose inhaler	42 µg inhalation 2–4 puffs × 2–4 daily
	Inhalation	Dry-powder inhaler	100 µg 4 inhalations daily
Betamethasone	Oral	Tablets, 0.6 mg Solution, 0.6 mg/ml	0.1–0.2 mg/kg daily
Budesonide[a]	Aerosol	Metered-dose inhaler	200 µg inhalation 1 inhalation × 2 daily
Deflazacort	Oral	Tablets, 6–30 mg	1.2–2.4 mg/kg daily
	Oral	Drops, 1 drop/mg	1–2 mg/kg daily
Dexamethasone	Oral	Tablets	0.1–0.2 mg/kg daily 0.5–0.75 mg
Flunisolide	Aerosol	Solution 0.1 % (1 mg/ml) Metered-dose inhaler	0.5–1 mg × 2 daily 250 µg × 2–3 puffs daily
Fluticasone propionate	Aerosol	Metered-dose inhaler DPI 30, 100, 250 µg/dose	44 µg × 2–4 Puffs 50 µg 2–4 inhalations
Methylprednisolone	Oral	2-, 4-, 8-, 16-, 32-mg tablets	0.25–2 mg/kg daily as needed
Mometasone furoate	Inhalation	Dry-powder inhaler	440 µg once-daily
Prednisone	Oral	1-, 2.5-, 10-, 20-, 25-mg tablets	1–2 mg/kg daily
Prednisolone	Oral	2.5- to 5-mg tablets	0.5–2 mg/kg daily
Triamcinolone acetonide	Aerosol	Metered-dose inhaler	100 µg inhalation 4–8 puffs daily

Data from [56, 117, 437, 495, 581].
[a] by Turbohaler.

also derive from the metabolism of single molecules. BDP is metabolized in monopropionate in various tissues, including the airways, but it is not clear which rates are absorbed or metabolized in humans. FP (fluticasone propionate) and BUD, among the commercialized molecules, appear to be equipped with the lowest oral bioavailability as a result of a very high liver metabolism of first passage, so that 99 % of FP and 90 % of BUD are

deactivated by the liver, and only a limited amount, equal to 1% and 10% [23], reaches the circulation. Side effects are few with preparations *active by inhalation and with reduced systemic absorption*, which, undoubtedly, have resulted in therapeutic progress – among these preparations are BDP, BUD, deflazacort, flunisolide [26], even if giving rise to controversy [668]. FP, because of its more favorable risk–benefit ratio, is preferable to BDP for the long-term treatment of children with asthma, especially if moderate doses are required [135]. It should, however, be clarified that with inhaled doses of 400 µg/day and using a valve spacer, the systemic absorption is reduced to zero, given that all of the drug ingested remains in the spacer, thereby eliminating any difference in the bioavailability of different medications [359]. Both BPD used with the Volumatic or the Jet as well as BUD taken with Nebuhaler have a lesser systemic activity compared to the same dose inhaled with an MDI or a DPI, even if rinsing the mouth after use reduces or abates ingestion, as previously mentioned [26]. Additionally, drop formulations (deflazacort is prescribed in doses of 1–2 mg = 1–2 drops/kg) favor personalized prescriptions and ensure compliance in younger children.

Suppression of the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Other adverse phenomena, such as HPA axis suppression, growth retardation [120, 285], and are, in our opinion, provoked by prolonged treatment and by administering high dosages, chiefley orally [120], which can lead to a potential axis inhibition, with reduced ACTH secretion due to negative feedback mechanisms and the adrenal gland inability to respond to adrenocorticotropic stimulation, giving way to severe risks in patients prone to respiratory arrest during severe acute attacks [517]. A complete functional restoration may require up to 1 year, whereas treatment limited to 1 week is usually devoid of negative consequences: 8–10 days after stopping treatment, HPA function returns to normal [560]. In adults, a possible axis deficiency can be revealed only through metapyrone or insulin tolerance tests, or through a CRH test [560]. Recent data suggest that CRH and its CS regulation may be essential for the control of airway inflammation and the maintenance of lung homeostasis in asthma [588]. Several studies have not found such anomalies [487, 684], or a small but significant degree of growth impairment in children receiving only 400 µg/day of BDP [157]. Regarding the dosage factor, important undesired systemic effects of chronically and systemically administered medications (Tables 11.20–11.22) are found in children 7–10 years old with severe asthma, treated with doses of FP increased from 800–1500 µg up to 2,250 µg/day, leading to an increased risk of systemic side effects such as undetectable cortisol levels [650]. Acute adrenal crises have been reported by several studies, all in children treated with inhaled FP [630, 650–652, 736, 757] (see "Linear Growth"). Similarly, 22 children aged 3.3–10 years (with an incidence of 3.1% of total cases) treated with inhaled FP (91%), with doses ranging from 500 to 2,000 µg/day presented with acute adrenal crisis, acute hypoglycemia, and with decreased levels of consciousness, coma, or coma and convulsions. The remaining six children presented mainly with lassitude, weakness, nausea, and dizziness [652]. Severe growth retardation and adrenal suppression were described in children taking FP doses >1,000 µg/day [715], and acute adrenal crisis associated with inhaled FP in three children presenting with hypoglycemic coma and convulsions [651]. A probable explanation for these findings is that FP taken twice a day accumulates in the blood, which has a half-life of 7–8 h and a distribution 4 to 5-fold greater than other drugs, leading to an increased risk of systemic side effects such as growth retardation and adrenal insufficiency [61]. HPA was also reported in 4 children aged 4.2–4.8 years receiving FP 500 µg/day, in 1 child aged 7.2 treated with FP 1,000 µg/day, in 3 children aged 4.8–6.1 treated with BUD 400 µg/day and a 9-year-old treated with BDP 600 µg/daily [475]. Adrenal suppression was found in 9–18 children aged 7–17 receiving FP 477 µg/m^2/day for 5–16 weeks [368]. Given to asthmatic children in high dosages, flunisolide has provoked no negative effects on the axis, nor on glycolipid metabolism [215, 494]. These results have been confirmed with BUD, administered for 3–5 years in doses of 200 µg/day [685], with measurement of 24-h plasma and urine cortisol [684, 685], 24-h urinary cortisol alone [157, 487]. Yet, other studies found no HPA deficiency, nor growth deficiency in children [541] or in schoolchildren undergoing treatment with BUD [646]. In asthmatic children under treatment for 3 months with inhaled BDP in doses of 400 µg/day, a 63% decrease of plasma cortisol after evening doses and a 29% decrease after morning doses [637] was observed. Such data were subsequently confirmed by reductions of serum cortisol [493], urinary cortisol [505], or both [439], after treatment with BDP and BUD in doses of 400 µg [445], or with FP in doses of 250 µg [757], and with an MDI without a spacer [220]. Among the possible causes could be *the use of MDI spray cans without spacers,* given that they increase oropharyngeal deposits [220].

Bone Density

Another question being debated is whether inhaled medications, properly used, have a negative effect on bone density, an effect which, however, is independent of HPA-axis suppression, possibly related to the inhibition of insulin-like growth factors [517]. Using the equivalent of 8 µg/kg of BDP [649], a child should be considered at risk of HPA-axis suppression or of delayed growth [198]. The effects on the skeletal system have been evaluated by dosing two markers correlated with osteosynthesis and growth rate, osteocalcin and the carboxy terminal propeptide of type I procollagen (PCIP), showing a growth decrease after 1 and 5 months of

treatment with BUD, both in full doses of 800 µg/m^2/day and half doses, with no variations in growth measured by conventional parameters [603]. The combined examination of PCIP and amino terminal propeptide of type III procollagen (PCIIINP) has shown the suppression of both bone turnover and collagen after twice-weekly treatment with DPI BUD and BDP(800 µg/day), more evident in the latter case [42]. The effects on PCIIINP [734] and on osteocalcin were subsequently confirmed by other studies [655, 733]. Bone density was also measured using a densitometer and an absorptiometer, without noting any significant differences between asthmatic children and controls. Vice-versa, osteocalcin was shown to be reduced in the densitometer compared to the absorptiometer, a result that can be ascribed to the disease and not to BDP, according to the authors [312]. Some maintain that osteocalcin is not a marker sensitive to bone turnover, in that asthma *per se* can decrease it tangibly, making it of little use in evaluating the effects [312]. Others have measured bone density without finding any significant difference [17, 300].

Linear Growth

Concern has been raised that the use of ICSs in children may be associated with growth suppression. Recent evidence presents conflicting results regarding reduced [157, 593, 649, 690] and normal growth [444, 684, 718], including a wide and well-documented study [8] that meta-analyzed 21 pediatric studies, based on statistical analysis and excluding the studies employing different methods of evaluation [729]. A recent meta-analysis [583] has concluded that moderate doses of the inhaled steroid BDP in children with mild to moderate asthma has been shown to significantly affect linear growth. Also, for the effect of moderate doses of inhaled fluticasone, a statistically significant difference was revealed. However, two recent long-term studies have been reassuring [6, 645]. The growth of a cohort of 142 children with mild to moderate asthma was followed until they reached adult height; the subjects received a mean dose of BUD of 412 µg/day for a mean of 9.2 years. The adult height of this cohort on long-term inhaled BUD therapy was 0.3 cm greater than expected. Its sole weakness was a high rate (72%) of drop-outs in the control group [6], as in other conventionally treated children [568]. In the Childhood Asthma Management Program (CAMP) study, a randomized trial for more than 5 years in >1,000 children with mild to moderate asthma, neither inhaled BUD nor nedocromil was better than placebo in terms of PFT, but BUD provided substantial clinical improvement as compared with nedocromil or placebo [645]. Significantly, both studies reported a transient reduction in growth velocity *only in the 1st year of treatment* [6, 645]. In longer clinical trials, despite a strict selection process, compliance problems can be noted. Problems increase with duration of participation, increasing child age, and the presence of less family cohesion or attention problems in children [625]. Height may diminish with an increase in dose and return to normal when treatment is reduced or stopped [646]. The attitude of inhaled CS overdosing in children was recently clearly documented. In 8,913 children, the CS inhaled at daily dose ranges equivalent to BDP were 350–*2400* µg, 265–*3400* µg, and 300–*4800* µg for the 0–4, 5–11, and 12- to 16-year-olds [171]. We believe that many results not associated with HPA-axis suppression may depend on the use of safe doses of 100–200 µg/day, which should proportionally be reduced as compared to adult doses, better in µg/kg, as has previously been pointed out. Although inhaled FP was found to reduce morning serum cortisol concentrations by 17%–43%, even at low doses of 176 µg/day [169] or of 200 µg/day; prepubescent children treated with FP 100 µg and 200 µg daily for 1 year showed no statistically significant differences in growth rates, similarly to placebo-treated control subjects [7]. FP DPI 100–200 µg administered daily to 437 children (4–11 years old) with persistent asthma for 12 weeks in a randomized, DB, parallel-group, multicenter trial did not affect 24-h urinary free-cortisol excretion, and improved PFT in children even as young as 4 and 5 years old [484]. Often, good results are obtained in children with *low doses* [205, 438, 487, 684, 729], and even in adults, doses can be reduced with the same results [231, 285]. A 12-month 100-µg daily treatment of FP in 625 children aged 1–3 years with mild to moderate recurrent wheeze resulted in improved symptom control with no effect on growth rate, and a serum and urinary cortisol concentration suppression of 10% and 14%, respectively [45]. A recent study has set up a milestone in the treatment of pediatric asthma, having demonstrated that repeated bursts of oral CS at 1–2 mg/kg/day (maximum=50 mg) for a 5-day period are not only effective, but above all are *safe in children*, since they are not associated with any lasting perturbation in bone metabolism, bone mineralization or adrenal function [160]. In addition, low cortisol levels normalized after discontinuing inhaled FP [757]. Others have noted that growth decline is reduced after the first 6 weeks or at most within 18 weeks [159]. A 6-month treatment with inhaled BUD and FP did not induce body fat accumulation in 21 of 26 asthmatic children; however, in five children aged from 4.3 to 5.3 years, the treatment was associated with growth velocity below the third percentile [550]. The magnitude of these changes in linear growth has varied between other studies using different ICS preparations, indicating on the one hand that either study design or specific steroid preparation/dose may be important considerations [434], and on the other the lack of reliable data on compliance may seriously confound the study of long-term growth effects of ICSs [732].

DB, randomized placebo-controlled tests have also been carried out [729–731, 733], employing knemometry (from κνημη, leg), which ensures reproducible findings (Figs. 11.46–11.48) [365], to evaluate ICS effects on lower limb linear growth, which always proved to be re-

Fig. 11.46. A knemometer. The child is positioned so that the knee is positioned at a right angle and the feet are comfortably placed. At the first visit a template is made by drawing around the child's feet, so that at the subsequent visit positioning is identical.

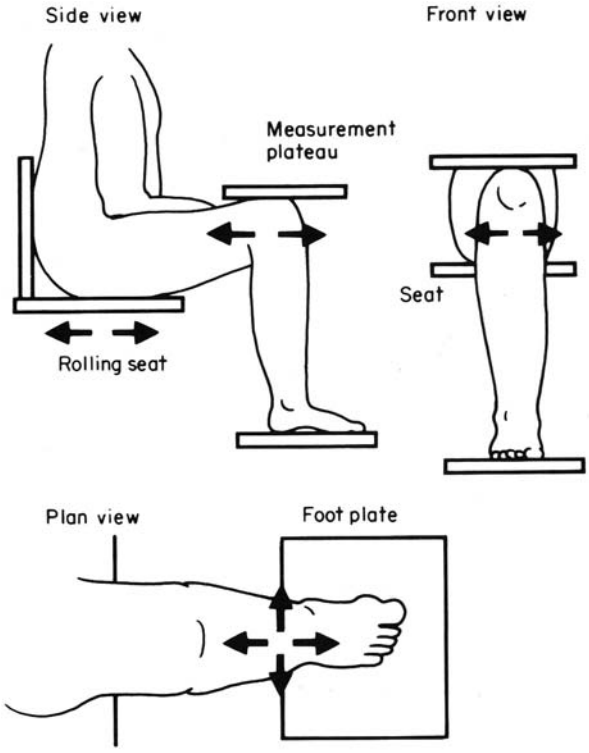
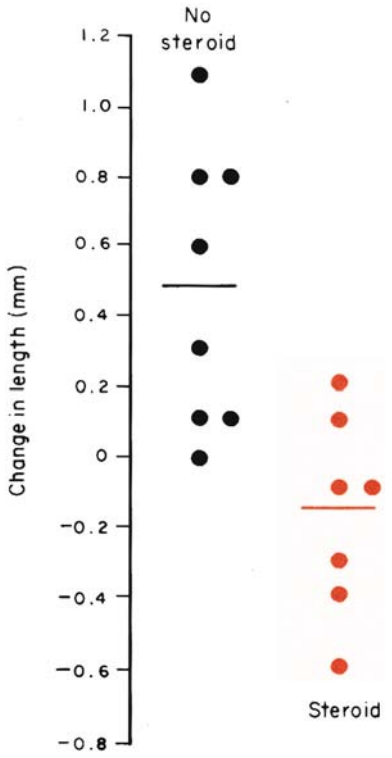


Fig. 11.47. Diagram showing knemometer and manipulation of the lower leg. The lower leg is moved around in order to gain maximum reading of the digital ruler. Alternatively, knemometry may be measured by a hand-held knemometer, provided with an electronic caliber, which measures the length of the lower leg from above the knee to below the heel. Measurements are taken with the child in a supine position with a 90° flexion in hip, knee, and ankle joints. A fixed cap is held against the knee and the other, adjustable cap is placed under the heel. Both caps are parallel, and the distance is ongoing measured electronically with a resolution of 10 µm. An investigator stabilizes the knee cap with one hand, while slowly compressing the heel cap up to a predefined pressure of 80 g with the other hand, as determined by an interposed spring. At this pressure, a microswitch is activated and the reading is recorded on a computer

Fig. 11.48. Changes in total length on alternate day steroid dose



duced, though not with FP [731]. The results on PCIIINP of the same group [734], completed by measuring cortisol and insulin-like growth factor levels [735], and additional data have confirmed these studies [365]. In children aged 0–3 years, within a 3-week period the increases in the lower-leg length during placebo, BUD 400, and FP 400 treatments (200 µg inhaled twice daily) were 85, 45, and 34 µm/d, respectively [11]. Nevertheless, some aspects of the *assessment of safety are unique to younger children,* including the rapid growth velocity and their different metabolism. The rapid growth in the first 2–3 years of life may make the child more vulnerable to the adverse effects of drugs and/or disease. More seriously, the safety findings in adults and schoolchildren *cannot be extrapolated uncritically to younger children* [11]. We deem that the concern about potential dysfunc-

tions is valid in cases of oral and/or high doses as an intercurrent necessity [347]. A meta-analysis suggests that moderate doses of inhaled BDP in four studies and 450 children and FP in one study and 183 children with mild to moderate asthma cause a decrease in linear growth velocity of 1.51 cm/year and 0.43 cm/year, respectively [583]. However, the increase in height of 277 children aged 4–11 years with chronic asthma was greater with FP than with BDP, 5.01 vs 4.10 cm/year, respectively [151]. These findings should not limit ICS administration in children with moderate/severe asthma [28, 282]. A possible choice is flunisolide, which is freer of adverse effects [215, 228, 494], provides better penetration and a more homogeneous distribution in the distal airways, also parenterally administered if asthma is more severe [706]. In moderate to severe asthma, the benefits may outweigh the risks associated with continuous use [703]. Various immune parameters are normal in long-term ICS-treated children [347], but eosinophils are reduced only in patients taking monodoses [497]. In conclusion, *short-term and/or alternate-day treatments are safer* [438], with a return to normal basal parameters in a short time, as already detailed [285, 757]. To further reduce potential damage, preparations should be chosen with a longer duration of action and to begin treatment between 3:00 and 5:00 PM (monodose), as there are no differences with qid therapy, nor does it influence 24-h plasma or urinary cortisol levels [497]. It will be sufficient to monitor the effects of treatment in individual patients, while in other cases the risk–benefit analysis is strongly in favor of preventive drugs and of theophylline [285]. However, refer to the comparison of SIT/steroids in Chap. 13. We deem, however, that periodically measuring the growth of asthmatic children on long-term treatment with ICS is a useful practice [728]. An FDA mandate (November 9, 1998) requires labels on inhaled and intranasal CS warning of a potential reduction in linear growth in children demonstrates this concern (accessed from: http://www.fda.gov/cder/news/cs-label.htm).

Table 11.21 enumerates CS side effects and the consequent measures required, and indicates a few possible causes of treatment failure with these drugs. No posterior subcapsular cataracts have been observed in children treated with ICS [592], which were seen, however, in 11.3% of 274 children [718], and in 15%–21% of 163 children [120], most probably related to *chronic oral treatment.* Synthetic CS prime IgE synthesis in vitro [304, 452] and the increase in serum IgE levels after 7 days of steroid treatment – viewed as being caused by steroid immunomodulator effects on T lymphocytes – which is also associated with an FEV_1 reduction [754]. Such effects were not seen *ex vivo* even after 1 week of steroid treatment [304]. It is not excluded, however, that with larger doses and/or long-term treatments, significant increases in IgE can be seen [318]; such reports require further evaluations; however, in this respect ICS may not be suitable in children. An in vitro study has demonstrated that hydrocortisone enhances total and allergen-specific IgE production by PBMCs from atopic subjects in vitro. The induction of Der p-specific IgE synthesis in subjects with high serum allergen-specific IgE levels was even greater than that of IL_4. This is the first report to show that hydrocortisone enhances Der p-specific IgE production from circulating B cells in atopic asthma [101]. The immediate in vivo interpretation of these findings would be that CSs promote allergy, which in theory would mean that the most efficacious treatment for allergic diseases is detrimental regarding disease progress. However, the underlying mechanism by which CSs enhance IgE production in vivo is unknown [101]. High dosage can cause an increase in anxiety, depression, memory reduction, and psychological vulnerability, with a potentially negative impact on asthma severity and on the child's own ability to cope with the disease and its treatment. If, on the other hand, CSs are used in brief cycles, school performance, memory and behavior remain unchanged [432]. In conclusion, CS-induced adverse effects in 163 children with severe asthma are inadmissibly high. Given the frequency with which they occur, evaluation and close monitoring of potential adverse effects is clearly warranted, even in those patients who are not CS-dependent but require high-dose ICS and frequent rescue oral CS therapy [120].

Comparison of the Various Forms of Drugs

Some data will be useful in making a more prudent choice regarding medications to be used, especially in cases of severe asthma (Table 11.14) (in parentheses the variable effects) [284]:

- Muscle relaxant: β_2-adrenergics, theophylline
- Antiedematous: β_2-adrenergics, theophylline
- Anti-mucus secretion: CS, (β_2-adrenergics, theophylline)
- Prevention of late reaction: CS
- Inhibition of mast cell degranulation (β_2-adrenergics, theophylline)
- Attenuation or resolution of hyperreactivity: CS

β_2-Adrenergics, compared to other drugs, are characterized by:

- Greater selectivity
- Quick relief (greater effect on early asthmatic response)
- Very brief latency time by inhalation (a few minutes)
- Lower incidence of side effects

Duration of effectiveness: catecholics 2–4 h; resorcinol and salagen 4–6 h; clenbuterol and procaterol >6 h; fenoterol and salmeterol 12 h, and oral bambuterol 24 h [158].

Anticholinergics, compared to β_2-adrenergics, induce a lesser degree of bronchodilation, are more delayed but more prolonged in duration (compared to most β_2-adrenergics. IB is to be preferred because of the lesser

impact of side effects and protracted duration of its action: 4–5 h. Nevertheless, other controlled studies are needed before they can be used effectively and safely in maintenance therapy.

Theophylline drugs, compared to β_2-adrenergics, have a more gradual action with more frequent and more significant side effects. *Long-acting theophylline* [83] has proved useful in young children who do not readily accept inhaled procedures, in children with asthma or wheezing in the morning as the only symptom, in cases of chronic asthma to avoid waking the child during the night, and in adolescents with severe symptoms before adding CS to their maintenance treatment [706], or to reduce doses when possible [566].

CSs compared to β_2-adrenergics and theophylline drugs have a greater latency time and are the preferred choice for use in severe crises for short periods. In prolonged treatments, the wide spectrum of side effects makes it preferable to use hydroxylase compounds in acute phases because of their short plasma half-life and the faster bioavailability of their active principle [633]. Preparations that interfere less with the metabolic processes are preferred for chronic forms [633].

β_2-Adrenergics can be combined – depending on the case – with anticholinergics, theophylline and CS:

- In the first case *a functional synergy* is obtained without causing an amalgamation of the side effects of the two drugs considered individually.
- With the combination of β_2-adrenergics and theophylline, *an increased bronchodilation* in cases of severe acute asthma and a better control of the symptoms in the several forms with a chronic trend could be achieved, but there are few recent studies that could confirm the usefulness of this association, when carried out on a regular basis, whereas if doses and the frequency of administration of inhaled β_2-adrenergics are reduced, the association with theophylline is effective on bronchodilation.
- CSs together with β_2-adrenergics *enhance their therapeutic efficiency* by acting on cAMP; cAMP levels are maintained within normal limits, with the above-mentioned effects, also improving oxygenation [545]. Furthermore, β_2-adrenergics with long-term half-life provide better control of asthma when symptoms persist, notwithstanding treatment with CS, and considering that this association can be useful in covering the CS latency period [359].

Assessment of Guidelines and Pediatric Compliance

One major problem is the treatment guidelines, an issue frequently quoted: written guidelines are often difficult to read. In the US, the mean grade levels obtained for the leading guidelines ranged from 4.9 to 9.2 [191]. Only 2.1±1.0% of the directors from 376 sampled EDs reported the use of written protocols or guidelines [121]. These guidelines are likely adopted by 5.7%–7.9% of readers of NIH guidelines [121]. Even within private practice, patients' race and ethnicity are associated with clinician nonadherence to national guidelines [463]. In a recent study of children who presented to an ED, 71% of children (mean age >8 years) did not have a written plan. Only 7% of those with a plan consulted it at the onset of wheezing and 4% consulted it before going to the ED. Also, 48% did not use a holding chamber with their MDIs and 66% did not use their PEF meters. Regarding exacerbation response, 71% did not have a written action plan, and 89% did not maintain a symptom diary [557]. In 1114 children aged 6 (mean) evidence-based guidelines for pediatric asthma demonstrated an improvement of asthma management plans, but failed to influence patient outcome [385]. Moreover, US guidelines are structured "for children 5 years of age and older" [435–437], while European consensus guidelines [698–700] consider children aged 0–5 years as well. Guidelines do not master factors associated with lower adherence to the medical regimen in young children, such as *medication taste,* and the *congestion of too many medications with multiple dosing intervals* [27]. Children with many risk factors experienced 0.80 more days of wheezing and 1 more day of activity restriction as a result of asthma compared with children with few risks for nonadherence [27]. For *children younger than 5 years,* expert recommendations are based on *extrapolations of studies in older children* [434]. The issues related to minorities in the populations are pertinent: black and Latino children had worse asthma status and less use of preventive asthma medications than white children within the same managed Medicaid populations [351].

The parents may not always be aware of the severity of the disease: in one trial, 36% of parents stated that they would administer a β_2-agonist, but 57% would go to the ED without giving a β_2-agonist first. Also, fewer than 5% would call the physician and use a PFM [696]. In a separate sample, the figures were 13% and 1%, respectively [338]. One-third or fewer parents followed other NHLBI recommended steps, including using a PFM, calling or going to see the doctor, or going to the ED [557].

Bronchiolitis

Definition

Bronchiolitis is an acute respiratory disease that affects infants, generally between 2 and 10 months of age [499] and involves chiefly the small arteries, is characterized by an acute onset of wheezing, expiratory dyspnea, tachypnea, fever, with or without diffuse fine crepitations of rhonchi and rales on auscultation, and with radiological appearance of emphysema [69]. This definition prevails in Europe and Australia, and by American authors all first-time wheezing associated with a respi-

ratory tract infection in infants is included [674] as wheezing-associated respiratory infection (WARI). It probably represents the first episode of asthma disease. Episodes of lower airway illness labeled bronchiolitis in the 2nd year of life is more likely to be asthma [747].

Prevalence

Bronchiolitis is highest in infants (50%–60% of cases), but can also be seen in the 2nd year of life, even if with a much lower prevalence. It is more frequent in winter months and usually affects males, as does asthma. Since 1980, the rate of hospitalization for children with bronchiolitis has increased from 5.4% to 16.4%, whereas mortality rates for the disease have remained constant [372]. In the Netherlands, the national number of bronchiolitis hospitalizations significantly increased from 1991 to 1999 in children aged 0–4 years [674].

Predisposing Factors

The host's characteristics stand out in the clinical manifestation of the disease [226, 271, 391, 417, 512, 611, 711]:

- Age (<1 year) holds first place, given the reduced caliber of nursling bronchioles. Our studies indicate that the onset age <6 months is even more significant (Tables 11.24, 11.25) [69].
- Genetic predisposition.
- Patient's immune state.
- Prematurity.
- Lungs more or less immature (additional effects from smoking).
- Male sex.
- Bottle feeding.
- Presence of cardiopathies, pneumopathies, polymalformative syndromes.
- Poor socioeconomic status and/or living conditions.
- Exposure to allergens and/or pollutants.
- Passive smoke, particularly maternal.

Breast feeding plays a key role, since maternal milk contains *anti-RSV sIgA*. Breast feeding for at least 1 month and, negatively, parental smoke equal to >20 cigarettes are highly significant compared to controls [391]. Assay of cotinine levels emphasizes a significant relationship between admissions to hospital and mother's and parents's secondhand smoke ($p<0.0005$), compared to a similar group not affected by bronchiolitis ($p=0.0181$) [519]. In the cohort of 240 infants, 29% had a smoking mother [747]. The impact of daycare attendance on the incidence of infantile LRTI amounts to a doubled increase in the risk of such infections. In a cohort of children prospectively studied in the first 3–6 months of life by a polyfactorial analysis, the factors increasing the risk of a care setting different from the child home have been defined. They include repeated transfers from one place to the other, twice-daily changes of the environment (both heralding potential infections), worries, probably lesser care by persons replacing the mother, and higher prevalence of infections (8.6%) [257]. Compared to the age of 4 months to 3 years, the presence of >3 children of the same age, and/or that of smoking persons in daycare settings (personal experience) exposes the child to the risk of acquiring respiratory infections, but not at home, even with > three siblings, and persons caring for the child or sharing the bedroom, etc. [257].

Etiology

In 87% of cases, bronchiolitis is caused by *respiratory viruses* such as RSV (see Chap. 4) and the remaining rate to parainfluenza 3 and 1 viruses, adenovirus, etc. About 25%–50% of young babies show seroconversion for RSV or parainfluenza virus in the 1st year of life, though only a certain number of babies manifest signs of disease. RSV provokes, in most subjects, nonsevere or imperceptible clinical features, while in infants it can cause bronchiolitis with all ensuing symptoms, showing its greater impact on young infants. With passing time, the number and severity of the episodes decrease [323]. Regarding the frequency of reinfections, it has been reported that a second infection recurs in 75% of cases and a third in 65% of cases, thus being the major cause of lower airway disease in infancy. As with all infections sustained by respiratory viruses, bronchiolitis can occur in epidemic forms, more severe in some years and less so in others, and more than once in the same child during the 1st year of life [546].

Genetic factors such as FHA [271] (71%, [499]) or allergic sensitization [586] and family predisposition to asthma (43%, [499]) are crucial elements that condition the onset of bronchiolitis, as well as the persistence of bronchoconstriction. It is important to emphasize that one atopic or asthmatic parent is a highly predictive factor relating to the insurgence of asthma in the child [271] (Tables 11.24, 11.25). In a prospective study of 47 infants, it is interesting that among children with RSV, 6/11 (54.5%) children with FHA developed asthma compared with 5/36 (13.8%) matched controls without FHA. In these children at age 7 $^1/_2$ asthma was found in

Table 11.24. Family history and asthma development in 70 children with bronchiolitis (Follow-up, 6 years)

	No. of cases 70	Family history	
		+	–
Asthma +	28	16	12
Asthma –	42	4	38

Fisher = 0.0000.
Data from [69].

Table 11.25. Onset age of the first episode of bronchiolitis and asthma development in 70 children with bronchiolitis (follow-up, 6 years)

	No. of cases 70	Age at the first episode	
		<6 months	6 months
Asthma +	28	16	12
Asthma –	42	10	32

Fisher = 0.0050.
Data from [69].

11/47 (23%) of the RSV group and in 2/93 (2%) of the controls, and in 7/47 (14.9%) of the children with FHA and 1/93 (1%) of the matched controls without FHA (RR = 13.88). A positive test of IgE to inhalants was found in 14/44 (32%) of the RSV group and in 12/87 (14%) of the controls (RR = 2.31) [586]. Moreover, a positive test for IgE was found in 14/44 (31.8%) RSV children and in 8/92 (8.7%) control children [587]. In a cohort of 240 2- to 10-week-old infants, 61% had a FHA and 40% a history of asthma [747].

Pathogenesis

If we consider the infant's still immature immune system, already Th2-controlled at birth, it is evident how, with the decrease of passively acquired antibodies and with the addition of the aggravating factor of low IFN-γ generation and progressive decrease in anti-RSV sIgA in maternal milk, the infection can progress. The functional characteristics of young infants are dominated by an insufficient development of bronchial cartilages, smooth muscles and the number of alveoli, and by reduced elastic recoil [512]. RSV has a certain tropism for small airway epithelial cells, whereas, for example, the parainfluenza virus prefers the subglottic epithelial cells [125]. The viruses have immunosuppressive and cytopathic effects on monocytes, macrophages and T lymphocytes, subsequently interfering on the normal process of macrophage-induced lymphocyte activation [503], as previously seen (Table 11.9). It can be assumed that in a selected number of children, either affected previously, or at the time of primary RSV infection, there is an alteration of T-cell regulatory mechanisms [523]. Therefore, bronchiolitis can be regarded as an immune system disorganization related to host immaturity, in which T-cell hyperactivity is unraveled, in parallel with persistent and elevated IgE-mediated responses, which, via increased histamine and other mediator release, can, in future, predispose to asthma and BHR [611]. Infants affected by bronchiolitis have a markedly lower number of $CD8^+$ lymphocytes (inversely proportional to IgE maximum titers) than infants suffering from other types of RSV infection [715] during convalescence. Similarly, CD8 T cells are reduced during bronchiolitis [523] and in infants manifesting >3 episodes of wheezing during their 1st year of life [546].

From the fundamental studies of Welliver et al [714–716], the presence of anti-RSV sIgE in the nasopharyngeal secretions of children after an episode of bronchiolitis or of pneumonia with wheezing provoked by RSV has been noted, but not in controls also affected by RSV-induced respiratory infections and without bronchoconstriction. Also known is the longer persistence of anti-RSV sIgE in the study babies compared to controls (Table 11.26) [714]. At the same time, an elevated histaminemia and a clear correlation of anti-RSV sIgE titers with hypoxia as the objective measure of disease severity has been displayed, results that are totally overlapping those following infection by parainfluenza viruses [716]. Furthermore, the higher the levels of anti-RSV IgE in children suffering from bronchiolitis, the more easily the wheezing relapses (70% compared to 20% of those whose levels are not measurable) [715]. IgE produced by RSV therefore has immediate implications on atopic asthma, resulting in the IgE-mast cells linking and mediator and IL release. A subsequent direct mast cell intervention could be inferred from tryptase levels present in BALF of 91.8% of infants, but not so elevated as compared to the levels found in controls, to imply a confirmation [184].

Table 11.26. Mean RSV-IgE responses in nasopharyngeal secretions according to the illness group and related to respiratory symptoms

Symptoms	No. of children with positive RSV IgE titers/number of babies tested	
	Acute	Convalescent
I. Upper-respiratory-tract disease only	0/9	0/4
II. Pneumonia without wheezing	0/9	1/7
III. Pneumonia with wheezing	3/10	6/10
IV. Bronchiolitis	21/43	17/25

Statistically significant differences between groups III and IV and groups I and II (combined) and between groups III and IV (combined) and groups I and II (combined).
Data from [715].

By an aspecific phagocytosis of RSV by the cells or an interaction with a receptor on eosinophils, RSV prime the eosinophils to start the chain of $O_2^{\cdot-}$ generation and activation and to release mediators in greater quantities, confirming the hypothesis that some inflammatory signs seen in the airways of these children are *eosinophil-induced* [298]. Such RSV-induced effects on inflammatory cells in bronchiolitis may be much more pathogenic than was formerly believed, as demonstrated by their relationship, which has come to light in recent years, with *eosinophils,* whose activation appears pathogenically to be more important than that of neutrophils/macrophages and is characteristic of bronchiolitis but not of other respiratory disease [298]. Significantly higher ECP levels have been reported in infants with RSV-induced bronchiolitis than in controls suffering from URTIs and LRTIs that were also RSV-induced. High ECP levels in the nasopharyngeal secretions are predictive of the development of bronchiolitis at the time of RSV infection, and in parallel of clinical severity [201]. Especially in males, ECP levels are associated with the disease and its severity more than with peripheral eosinophilia, presenting as markers of disease progression [202]. The same difference was observed in infants with other RSV infections, but in females rather than males [751]. ECP was not predictive of asthma development in 6/34 children 2 years old after hospital admission at the age of 3 months [585].

There is, however, a correlation between LTC_4 and RSV-specific IgE levels in the nasopharyngeal mucosa [686, 714] of 67% of children with RSV bronchiolitis, vs 33% of controls with URTIs, also caused by RSV ($p<0.001$), who, moreover, showed LTC_4 concentrations fivefold lower than the study children ($p<0.02$) [686]. PaO_2 titers were lower in children with detectable LTB_4 than in those with undetectable LTB_4, and LTB_4 titers were inversely correlated with initial PaO_2,values which suggests a connection with disease severity [203]. Therefore, the *damage produced by RSV at the bronchial epithelium,* associated with toxic effects of basic proteins, and edema and bronchospasm triggered by LTs, could induce an *airway obstruction,* followed by a postinfection persistent BHR [323]. Other than BHR, bronchiolitis is dominated by activation of cellular immunity with production of Th2-like T cell ILs, clearly indicating an immune response to RSV and predictive of the development of asthma. An increased rate of $CD4^+$, $CD25^+$, and $CD23^+$ lymphocytes was found in infants at 5 months compared with the time of bronchiolitis and with healthy subjects of the same age. Moreover, the $CD4^+$ increase is not associated with CD8 increase, which remains low, whereas IL_4 increased in both groups. Eosinophils also increase significantly and are related to the number of days of wheezing – therefore, a classic Th2 response [523] manifested by RSV-induced IL_{13} production [670]. It is unclear whether genetic factors condition the phenotypic expression of RSV-induced bronchiolitis and the development of asthma, or RSV predisposes infants to asthma.

Even more pathogenetic is soluble CD14 (sCD14), a predictor of subsequent wheezing in children aged 2–14 months with RSV-induced brochiolitis, although not influenced by FHA+, sex, or breast-feeding [601].

Anatomopathological study shows that RSV replication in the airways has cytolytic effects on the epithelium. Significant changes in airway morphology are seen in animals with acute viral respiratory infection: the airways become edematous and infiltrated with inflammatory cells. The lower airways show marked bronchial narrowing and collateral ventilation and elastic recoil reduction. The reduction in caliber of distal airways makes the small bronchi and bronchioles obstructed by cellular debris from virus-specific epithelial necrosis, increased mucus secretion, intraluminal secretions of relatively dense and viscid exudate, bronchiolar inflammatory infiltrates and edema of both submucosa and adventitia. The lesions are aggravated by replacement of necrotic cells by cuboid nonciliated cells, thus impairing mucus clearance, which condenses in obstructive and potentially occlusive plugs [611].

From a functional viewpoint bronchospasm can be absent. Vice versa, alterations of the respiratory mechanism are present, denoted by a 50% increase in FRC compared to normal, reduced pulmonary compliance, increased resistance to air passage, especially during exhalation, since during exhalation – especially if forced – the airway caliber is reduced as a result of bronchiolar obstruction. The work of breathing is aggravated by air trapping with pulmonary hyperdistention or atelectasis, which are at the basis of hypoxemia – warning signs of ventilation-perfusion imbalance [271].

Clinical Presentation

Bronchiolitis is an acute disease with a sudden onset and a steady worsening in the first 24 h. *It presents a severe clinical situation in young infants* caused by respiratory changes, with prolonged exhalation, cough, sustained resting polypnea (RR ≥70–80/min), and obstructive type dyspnea, sometimes cyanosis and/or lethargy [474]. Moreover, symptoms can include coughing (100%), nasal congestion (94%), wheezing (89%), difficulty breathing (87%), poor feeding (70%), poor sleeping (69%), irritability (67%), fever (59%), vomiting (51%), and choking (41%) [499]. If the disease worsens further, RR decreases (Appendix 11.1) [302, 544] and signs of hypoxia and difficult breathing occur 22% to 46% of children so affected are admitted to hospital, especially if RR is >70 [499] or are admitted to the PICU at a median age of 1.7–2.27 months [506] and requiring mechanical ventilation in 31.4%–34.1% of cases. Tachypnea makes breast-feeding difficult, not leaving a sufficient interval of time for sucking and swallowing.

The child can become dehydrated as a result of an increased *perspiratio insensibilis* and of shock. Fever if present, in most cases does not rise beyond 39 °C [270].

Objectively, nasal flaring, use of accessory respiratory muscles due to intercostal retractions can be noted. During auscultation, a prolonged expiratory phase can be heard, together with expiratory wheezing and fine diffused rales or rhonchi. The rales indicate an obstruction of the main bronchi, and the rhonchi an alveolar hypoventilation. High-pitched expiratory wheezes in all lung fields may be audible. *Hypoxemia and hypercapnia* deriving from the alteration of the ventilation-perfusion balance and of pH and $PaCO_2$ are present. Aspecific symptoms can also be noted in young infants such as lethargy and irritability or other neurological symptoms of hypoxemia. Apnea and/or cyanosis can be the only symptom [512, 594].

Diagnosis

Diagnosis is above all clinical. SaO_2 monitoring is determinant to ascertain the degree of airway obstruction, and in cases of severe respiratory distress it should be integrated with the acid-base balance test; arterial samples may verify $PaCO_2$ levels. Pulsus paradoxus is a clinical correlate of cardiopulmonary interaction during asthma and correlates with the severity of the asthma attack. The degree of pulsus paradoxus can be measured directly with a hand-operated BP cuff or estimated from pulse oximetry [372, 717], a noninvasive method for detecting severe cases and for measuring O_2 saturation (SaO_2), but not always reliable [512] because it can be affected by the child's movement and by peripheral vasoconstriction, which occurs in the more severe cases [509]. This pulse may be related to RR in young children [372], but it cannot always be utilized because of HR increase; nor is there a clear correlation between peripheral pulse increase and asthma aggravation [738]. If it is >20 Torr, a moderate to severe obstruction is present [174]. In a survey of 519 physicians, the decision to transfer children to an appropriate emergency setting was significantly influenced by the 2% difference between SaO_2 values of 94% and 92%. This finding may help to explain the increased numbers of admissions for bronchiolitis since the popularization of pulse oximetry [372]. However, in 67% of cases oximetry is done in pediatric ED as part of the initial procedure and to document improvements after treatment [121]. Full blood counts are normal [125] in 80% of children [512]. Chest X-rays show typical airway emphysema resulting from air trapping, chest hyperinflation, diaphragmatic flattening and accentuation of the bronchial network; multiple areas of atelectasis can mimic areas of thickening. No correlation between these findings and severity of bronchiolitis is found [512].

Cytological examinations of nasopharyngeal aspirates and titers of specific antibodies may facilitate the etiological agent identification. Confirmation is obtained by using the indirect immunofluorescence technique and by ELISA. In any event, these are roundabout methods and can take more than 10 days to carry out [125]. Even if in 3%–10% of cases *Chlamydia trachomatis* and *Mycoplasma pneumoniae* [514] are isolated, both illness severity and prognosis are parallel to cases in which only RSV has been found.

Differential diagnosis is summarized in Tables 11.27 and 11.28 [594]. It is made difficult in babies by the frequent analogies between the clinical picture, the objectivity and the X-ray results.

Bronchiolitis has characteristics other than those of asthma [125]:

- It manifests itself in the cold seasons.
- It occurs in epidemic waves.
- Episodes are frequently preceded by rhinorrhea and fever.
- It is particularly common in daycare settings.
- In the same family other members suffer from influenza.
- It is primarily a disease affecting babies in the 1st year of life, which is why diagnosis can be limited [747], whereas asthma normally occurs after this age (Figs. 4.14 and 5.3).

However, pediatricians generally do not need to differentiate virus and nonvirus-induced severe wheezing, since treatment of airway obstruction is unaffected, apart from an epidemiological interest in etiology [594].

Treatment

Treatment is symptomatic. If breathing difficulties are severe, there is need to humidify the air and to hydrate the baby adequately (not >1,500 ml/m^2, correcting the acidosis), also to choose the diet and to control hydrosaline balance: *in young infants rehydration is cardinal* even though cases of severe dehydration are rare [26, 222]. Electrolyte concentrations should be carefully monitored in all infants with acute bronchiolitis [512]. If cyanosis is present and SaO_2 is ≥95% in room air, warm and humidified O_2 can be delivered into a tent or headbox, adjusting O_2 saturation. If necessary, give a dose of epinephrine 1:1,000 (Table 11.16), with a vasoconstriction action and, therefore, an antiedematous action, which can, if required, be repeated after 15–20 min, since it is rapidly metabolized. Inhaled epinephrine is discussed in Chap. 20. *Racemic epinephrine* [551] (not registered everywhere) has proved most effective, as shown by concrete improvement and few or no side effects. The effect of the racemic form derives from its action on α-adrenergic receptors, capable of reducing microvascular leakage by causing constriction of precapillary bronchial arterioles and hence bronchial mucosal edema [551]. The drug is administered through a nebulizer in doses of 0.1 ml/kg [551]. In severe cases, assisted breathing is needed [271]. Racemic epinephrine proves

Table 11.27. Pediatric differential diagnosis of bronchiolitis based on clinical features

Symptoms/signs	Diseases associated with wheezing	
	In infants	In older children
Association with positional changes	GER, anomalies of great vessels	GER
Failure to thrive	Cystic fibrosis, tracheoesophageal fistula, bronchopulmonary dysplasia	Cystic fibrosis, chronic hypersensitivity pneumonitis, α_1 antitrypsin deficiency, bronchiectasis
Association with feeding	Tracheoesophageal fistula, GER	GER
Environmental factors	Allergic asthma	Allergic asthma, acute hypersensitivity pneumonia
Sudden onset	Allergic asthma, croup	Allergic asthma, foreign body aspiration, croup, acute hypersensitivity pneumonia
Fever	Bronchiolitis, pneumonia	Acute hypersensitivity pneumonia, croup
Rhinorrhea	Bronchiolitis, pneumonia	Allergic asthma, croup
Concomitant stridor	Tracheal or bronchial stenosis, anomalies of great vessels, croup	Foreign body aspiration, croup
Finger clubbing		Cystic fibrosis, bronchiectasis chronic lung disease (CLD), allergic asthma

Table 11.28. Pediatric differential diagnosis of bronchiolitis and asthma

Parameters	Asthma	Bronchiolitis
Positive family history	Frequent	May be frequent (Table 11.22)
Etiology	Allergens, viruses, etc.	RSV
Age at onset	50% by 2 years of age 80% by 5 years of age	<2 years, frequently at <1 year
Recurrent wheezing	Characteristic	70% (≤2 episodes) 30% → asthma (≤3 episodes)
Onset of wheezing	Acute if allergic or exercise-induced	Insidious
Association with allergic disease	If allergic asthma, allergic rhinitis, atopic dermatitis	Commonly absent
Concomitant symptoms of RRI	Infrequent	Yes
Chest auscultation	High-pitched expiratory rales	Fine, sibilant rales, coarse inspiratory and expiratory wheezes
PRIST	May be elevated	Usually normal
Nasal eosinophilia	With allergic rhinitis	Absent
Response to bronchodilators	Characteristic	Scarce or wholly absent

Updated from [594].
GER gastroesophageal reflux, *RRI* recurrent respiratory infections, *RSV* respiratory syncytial virus.

to be better, as has been confirmed in the US [551] where a shorter length of hospitalization of children compared to a group treated with albuterol has been documented [399]. If the mean percent SaO_2 at 60 min was significantly higher in the epinephrine group [399], either the acidity of the solution (pH 3.2), or the content of

metabisulfites, present in levo-epinephrine preparations as a preservative [543], or in epinephrine acid tartrate as a vehicle [689] or both, might have been responsible [543]. These causes may explain why no improvement was shown in infants with acute bronchiolitis when compared with placebo [2, 226, 689].

Four recent randomized, DB studies have evaluated *nebulized epinephrine* in the treatment of infants with bronchiolitis. The delivered types of epinephrine were L-epinephrine [2], racemic epinephrine [237], epinephrine diluted in normal saline solution [373], or epinephrine acid tartrate, 1% [689]. Nebulizations were administered using a nebulizer and face mask, and O_2 was given as needed as above. No significant overall differences were found between the groups [689] or between treated and placebo groups [2, 237]. A decrease in symptoms and length of hospitalization was reported in 54 infants [373]. When epinephrine diluted in normal saline was nebulized to these infants with viral bronchiolitis, the in-hospital stay was reduced by 25%, from 4 days in the 0.9% saline solution group (group 1) to 3 days in the 3% saline solution group (group 2) [373]. This outcome could bear an important economic and clinical impact worldwide; in the US, $>10^5$ children are hospitalized annually at a cost of US $300 million. Decreasing this burden by almost 25% could theoretically save nearly US $75 million annually in the US alone [373]. A Cochrane review concludes that there is insufficient evidence to support the use of epinephrine for the treatment of bronchiolitis among inpatients *aged ≤2 years* except a significant change in clinical score at 60 min post-treatment [239]. Usually, severely ill, hospitalized infants are aged a few days [2, 470] or 3–6 months [69, 237, 372, 373, 470, 474, 499]. We analyze the differences in epinephrine preparations in Chapter 20.

As has been discussed, the use of bronchodilators is controversial, since usually they increase the state of agitation of infants in whom bronchiolar smooth muscles are barely developed before the 18th month, even if muscular tissue appears at the 23rd week of intrauterine life and by the 25th week is uniformly distributed at all levels of the bronchial tree [632]. As stated, bronchospasm is not a main component of bronchiolitis, and in acutely ill infants the production of mucosal edema and increased mucus secretion may impair medication effects [206]. In some cases, β_2-receptors in healthy infants of 5.6 months [206] have been found, even in reduced numbers, and the efficacy of nebulized albuterol has been reported in infants 1–24 months old [565]. A causative factor, as noted, is the β-adrenergic receptors' desensitization caused by RSV. The safety of β_2-adrenergics for use in infants of 7.5 months [36, 370, 371] has been ascertained, whereas in infants <1–6 months [199, 250] β_2-adrenergics induced a significant HR increase and a SaO_2 reduction. Even if tachycardia can be viewed as an index of effectiveness [199], we believe that β_2-adrenergic may be useful in older children who can no longer be diagnosed as having bronchiolitis. This could account for the controversies summarized elsewhere [323]. As an alternative, consideration should also be given to forced nasal respiration of the infant and to the greater aerosol residual in the oropharynx compared to an adult [549]. In the survey from 519 physicians, most respondents recommended use of bronchodilators (96%), and few recommended steroids (8%), or antibiotics (2%). Inhaled albuterol was the most common drug specified (84%). Inhaled epinephrine (57%) followed by inhaled albuterol (35%) were the most commonly second recommended treatment [372]. Epinephrine was given to 58% of 237 infants in pediatric EDs in Canada [499]. In 149 infants hospitalized with bronchiolitis, there were no group differences in the effectiveness of therapy of either nebulized epinephrine or albuterol every 1–6 h. This is probably because by the time infants present to medical care, the amount of virus-induced necrosis may already be substantial [474].

Much debated is CS use in 5% of cases [499]. The rationale for their use is in direct relation to the anti-inflammatory power carried out on the bronchiolar walls and the restoration of β_2-receptors; but the small airways of these infants are, for the most part, unresponsive [711]. Furthermore, since they retain fluids in the tissues, CSs should be used with caution [594] in view of the inappropriate secretion of ADH (antidiuretic hormone), potentially present in these babies along with hyperreninemia [222]. Five percent of infants received CSs while in the hospital and 4% on discharge [499]. Antibiotics have no therapeutic value in a viral disease; unless a bacterial association is suspected (in 2% of cases), or confirmed by clinical or laboratory evidence, and severe, clinical signs, such as persistent febrile condition and/or an increase in the indexes of inflammation [512]. However, there are concerns about risks of adverse reactions, and the effect of antibiotics on bacterial resistance (Chap. 19).

Good results have been achieved with *ribavirin,* a synthetic nucleotide with virostatic properties on many viruses including RSV, interfering with the expression of mRNA of RSV itself, the effects of which on clinical parameters are noticeable 24–48 h after therapy [599]. The drug is administered by a small-particle aerosol generator and is delivered to a head box, face mask or O_2 tent in a solution containing 20 mg/ml of water, for 12–18 h consecutively per day for 3–7 days, depending on the disease progression [599]. Given the potential for environmental contamination during treatment, it is preferable to administer it in high doses by means of an O_2 hood at 6 g/dl water for 2 h instead of 18 h, achieving the same effects [176]. After the initial positive results, reservations were expressed, due also to the unfavorable cost-benefit ratio [712], its limited strength and the drug's possible toxicity and environmental contamination [176], which is why it is recommended as an optional choice [235].

Prevention

Immunoglobulins with high titers of anti-RSV antibodies [227], IV administered (RSV-IVIg), in a prospective blinded randomized, multicenter study over 3 years, with high (750) or low (150 mg/kg) monthly infusions can be used for prevention. Compared to controls, *high-dose RSV-IVIg* reduced the incidence of LRTI, RSV-associated hospitalizations, ICU days, and ribavirin doses (p=0.01–0.05) with 3.3% adverse reactions. The protection was extended to the follow-up with a clear reduction in severe consequences, even among subjects who were at greater risk [227]. In premature babies, the efficacy is the same, compared to controls, with a reduction in the number of days spent in hospital or ICU associated with RSV infection [226]. Even though offering a valid defense, it is not preventive in 100% of cases; nor has it lowered the mortality rate [9]. It is administered IV (750 mg/kg/2 h, once a month, during the RSV season: November to March-April): it causes overhydration, requires the same monthly controls and has an elevated cost, as does ribavirin [235]. In 1996, the FDA approved the use of RSV-IVIg as a prevention against RSV infections, excluding, however, children with congenital cardiopathy. Other DB, randomized studies are further testing the advantages and limitations of RSV-IVIg [9].

Another molecule, *palivizumab,* a monoclonal antibody directed against RSV, is now marketed for preventing respiratory tract infection by RSV in infants. The results of six trials suggest that the optimal dose is 15 mg/kg palivizumab by monthly injection throughout the seasonal epidemic period. The AAP has restricted its use to infants with CLD and congenital heart disease (CHD) [9]. Two recent studies [225, 515] have shown that the hospitalization rate for RSV bronchiolitis decreased significantly (46.2% vs 11.8 and 3.8%; $p < 0.01$) in premature infants with a gestational age ≤32 weeks and with CLD [225]. The emerging problem is that 83% of the children needing PICU admission for mechanical ventilation for the RSV bronchiolitis treatment from 2000 to 2002 born at term did not have CLD and were not candidates for RSV prophylaxis according to the current recommendations [9, 515]. Certainly RSV prophylaxis would increase the net cost of care if palivizumab were administered to the population of infants with bronchiolitis.

Prognosis

Generally speaking the prognosis is favorable. The mortality rate in hospitalized infants is 1%, which rises to 3.5% in premature infants and babies born with heart diseases, chronic respiratory disease, primary and secondary IDs, etc. [323].

Long-term prognosis can be complicated by asthma. Many studies have examined why children with genetic predisposition to atopy develop bronchiolitis more frequently or, afterwards, an asthmatic condition, especially if with IgE elevated levels. The influence of predisposing and confounding factors is well known: passive smoke – especially maternal – environmental pollutants, etc. Nevertheless, RSV infection remains the main factor that facilitates the insurgence of asthma. In this context, the report that more than one-third of children who have asthma during childhood have suffered from RSV-induced bronchiolitis (Tables 11.24, 11.25) seems particularly relevant. Consequently, PRIST is useful for identifying children with persistent wheezing who will continue to suffer from asthma [69], in many of whom the IgE increase is followed by sIgE development [70]. Several studies have indicated the predictive nature of total IgE [300] and/or of CBIgE [368]; others underline SPT effectiveness as a screening method (Chap. 6).

What is the role of atopy? Several authors have expressed controversial opinions [69, 70, 184, 383, 391, 453]. Often the studies have proved inconclusive, some linking the unfavorable prognosis to atopy and maternal passive smoke [391]. Others do not confirm FHA+ with atopy, emphasizing instead the association with parental smoke and sibling presence [400]. The results of Rylander et al [545] appear to be very eloquent: re-examining 79 children 4 years after their admission to hospital, they observed in only 22 of those with recurring wheezing a statistically relevant association with FH and an equally significant PEF and MEF_{25} reduction. In 83 children followed-up to the age of 8 [313, 314, 320], the risk of atopic children developing asthma or BHR during their school age was significantly related to recurring wheezing that appeared in the 1st year of life and to a premature IgE increase [314] compared to controls. At age 19 wheezing in early childhood was a significant predictor of asthma, and also seemed to predict PFT abnormalities in early adulthood. Thus, although the outcome of children with early wheezing is good at school age they may become symptomatic again as adults [495]. In other entrants, at the age of 4 years, asthma was present in 62.5% of ex-bronchiolitis sufferers vs 6.3% of controls and in 88.5% of children with high IgE titers compared to 32% of those with normal IgE levels [555]. In children of atopics, at the age of 11 atopy and wheezing were closely related [609].

Many different lines of research agree that *RSV-induced bronchiolitis is responsible for a subsequent asthmatic syndrome*, persistent for many years after the primary infection. In particular, it is hypothesized that anti-RSV IgE production can constitute a marker for the predisposition to develop specific response to VRI of early and late infancy, able to trigger recurrent asthmatic episodes [453] (Table 11.29) [4, 117, 161, 178, 214, 241, 246, 302, 381, 375, 423, 447, 454, 470, 495, 587, 616, 637, 644, 713, 726, 745]. Studies fail to clarify the pathogenesis of this higher prevalence of asthma in these subjects [585, 587]. It can be speculated either that the atopic risk preceded and/or provoked RSV-induced bronchiolitis and wheezing episodes, or that, at the origin, a reduced

Table 11.29. Bronchiolitis, IgE antibodies, atopy and asthma development: a meta-analysis

Relationships between bronchiolitis and IgE antibodies
Viral infections do not provoke an IgE antibody expansion and a higher atopic risk in children aged <2 years, frequent at 2–4 years and more frequent after 4 years [161]
Development of specific IgE in 44% of asthmatic children and in 17% of nonasthmatic children up to 2 years [587]
Significant association between FHA/asthma and RSV infection at 3 years, with similar differences in specific IgE titers between study children and controls [587]
Atopy in 67% of ex-bronchiolitics (aged 19) and in 50% of controls [495]
Children who were current wheezers at ages 7–8 had detectable RSV-specific IgE during their initial episodes but no relation to alteration of spirometry tests at age 7–8 years [712].
Relationships between bronchiolitis and asthma/recurrent wheezing
Recurrent viral infections unrelated to BHR at 1 year [644], 3–6 [4, 725], 7–8 [724], or at 13 years [616]
Aspecific BHR at methacholine inhalation challenge (MIC) in 80% of children of 12 years hospitalized in the 1st year of life due to severe bronchiolitis, vs 15% of controls [178]
BHR to MIC was present in 48% of ex-bronchiolitics and in 32% of controls [495]
Recurrent bronchiolitis not followed by asthma in 85% of cases within the 10th year of life (despite numerous episodes experienced during the 1st year of life), or significantly predictive of spirometry value reduction, also in RAST-negative children, moreover in 45.2% of cases methacholine challenge was correlated with dust mite-positive RAST [246]
Among 6-year-old children who were hospitalized with RSV bronchiolitis as infants, there was a ≈ 3-fold increase in wheezing [423]; at 9–10 years of age, 33% of these children with RSV bronchiolitis in infancy required bronchodilator therapy, compared with 3% of the control group [447]
Infants with reduced conductance have a fourfold greater (in males tenfold) virus-induced risk of wheezing within the 1st year of life; in females the risk is 16-fold greater in case of reduced FRC before the illness [382]
Elevated Raw and reduced FRC are predisposing factors for long-term wheezing respiratory illness in infants [381]
Children with wheezing in the 1st year of life and at least one episode in 3 years of follow-up, experience at birth, compared to controls, a 22%–25% PFT reduction [382]
In children with chronic cough and previous LRTI, the intercellular spaces are 17-fold increased with notable edema and the inflammatory cells increased sevenfold (91% of lymphocytes, 9% of mast cells and eosinophils), whereas ciliated cells are reduced threefold [241]
In prematurely born babies with gestational age of 29 weeks, a high Raw is significantly associated with respiratory manifestations at 2 and 3 years of life [214]
Prematurely born babies with birth weight of 0.5–1.5 kg who suffered from >1 wheezing illness in the first 2 years of life develop asthma at 5 years in 18.1% and at 8 years in 21.3% of cases vs 10% of controls [302]
BHR is observed at birth in healthy neonates [744] or at 8 months, 3.6 months after having suffered from bronchiolitis [644]
In conclusion:
A high proportion of infants hospitalized with bronchiolitis go on to develop asthma-like symptoms [712]; such infants are more likely to have reduced PFT at 1 month of age [637], thus raising the chance that bronchiolitis may identify the infants with poorly developed airways and therefore at an increased risk of developing asthma-like symptoms in response to respiratory infections
Viral bronchiolitis in infancy enhances the risk of asthma and recurrent wheezing in later wheezing by increasing the likelihood of Th2 sensitization to subsequent respiratory infections and to aeroallergens [470]
25%–56% of bronchiolitic subjects are eventually diagnosed with asthma, more commonly with a personal or family history of asthma [117]
PFT abnormalities may persist in asthmatic children up to 17 years of age and recur in the adult age [454]
Decreased indices of small airway obstruction (PEFR, FEV_1, FEF_{25-75}, FEV_1/FVC) and increased Raw have been found in children following bronchiolitis [117]

BHR bronchial hyperreactivity, *FRC* functional residual capacity, *PFT* pulmonary function testing.

PFT is already present in the first weeks of life [747]. In fact, these alterations, years after an episode of bronchiolitis, can reflect pre-existing damage in the pulmonary system and/or in airway's mechanical properties, already present at birth [381, 638, 747]. A meta-analysis [453] did not find a causative connection between the initial type of infection and subsequent respiratory changes [611]; therefore bronchiolitis during infancy might not cause a greater morbility for respiratory disease in older children [747], or the long-term prognosis

is open to all types of complications. It is likely that a percentage of children will suffer from asthma (15%–63%) [130, 320, 417, 453, 555], especially with FH+ and/or high titers of total and/or specific IgE and PC_{20}-histamine >2 mg/dl, $FEF_{25–75}$ over 70%, and FEV_1/FVC over 70%) of predicted status asthmaticus [453], or independent of FH [523, 616], but with Th2 producers of IL_4, virtual absence of CD8 T cells and almost undetectable IFN-γ [523]. In 7- to 8-year-old ex-sufferers of severe bronchiolitis, RSV infection primes memory T cells that make strong IFN-γ responses. However, IL_4-producing T cells responding to RSV and cat antigens are significantly more frequent in ex-bronchiolitics, thus increasing the risk of allergic sensitization by providing a local IL_4-rich environment in which aeroallergens are first encountered [470].

The other important question remaining to be clarified is that together with children in whom wheezing associated with atopy and BHR (development that is a prelude to asthma) began after the age of 2 [423], there are others with early onset of wheezing (<2 years), which ceases, more or less without consequences, at about 10–11 years of age [726], in relation with reduced airway development in this period, and with the particular vulnerability to VRIs [725]. Similarly, the age which should be taken as a reference has proved to be a relative factor [723]. To this end, let us stress that 49% of children labeled as asthmatic at the age of 5 years were asymptomatic at 10 years [471]. A more valid criterion is allergen sensitization, which is not only linked to the diagnosis of asthma, but is also predictive of asthma in children with wheezing [142]. The differential diagnosis between the two forms is summarized in Table 11.30 [383, 723], and that of asthma and bronchiolitis in Tables 11.27 and 11.28.

For the prevalence of wheezing, see Tables 5.10 and 5.12. Wjst et al show that it is on the increase from 10.9% to 17.6% in the 1- to 3-year age group, then decreasing from 16.4% to 9.7% from 4 to 9 years [726], while others report figures of 60% [638] or 50% [383]. In this study, the children examined at birth and re-examined at the age of 3 or 6 based on the time of onset and pattern of wheezing symptoms were divided into three wheezing phenotypes:

- 19.9% with transient wheezing only in the first 3 years of life
- 15% with late-onset wheezing, not present in the first 3 years, (but with symptoms beginning between 3 and 6 years)
- 13.7% with persistent wheezing in the first 6 years of life.

Children with transient wheezing showed significantly altered PFTs, to a greater degree than the other groups, and also with a smoking mother (only the 2nd and 3rd group had asthmatic mothers), IgE titers significantly high (higher in the 3rd group), SPT+ and normal PFTs at 1 year of age and altered PFTs at age 6: this is the minority with a predisposition to asthma [383]. Persistent wheezing is more commonly seen in children with asthmatic parents who have significant LRTIs with RSV [616]. The three groups can be further reduced to two: one with a viral infection and one with an atopic disease, probably aggravated by the infection [547]. Measuring specific Raw (sRaw), 200 children who wheezed at least once during first 3 years of life had significantly higher sRaw than the 303 who had never wheezed in the first 3 years of life, who had significantly higher sRaw if they were atopic or were at high genetic risk of atopy. These high-risk children, even if they had never wheezed, had a higher sRaw than children at medium and low risk. Atopic children had significantly higher sRaw than those who were not atopic, but nonatopic children at high risk had higher sRaw than those at medium risk [351]. Therefore, higher sRaw predominates in atopic or high-risk children independently of the number of wheezing episodes. Persistent symptoms after 3 years of age are associated with the concrete risk of developing asthma [152]. At the age of 11, 17 of 21 children still had wheezing and 12 of 21 had BHR [609].

Table 11.30. Differential diagnosis between wheezing and wheezing/asthma

Characteristics	Wheezing	Wheezing/ asthma
Aspect	Episodic	Alternating/ episodic symptoms
Age	0.5–3 years	>3 years
Etiology	Viral, passive smoking mostly maternal	Atopy (+ viral infections)
IgE	±	+ +
BHR	Normal	Increased
Prognosis	Transient, up to school age	Persistent

Data from [383, 723].

Asthma

When asthmatic children encounter the allergen, an acute asthmatic episode is triggered that can evolve in three stages. The first begins 20–30 min after allergen exposure and essentially consists of a bronchospasm, a clinical consequence of metachromatic cell degranulation; the airway caliber generally returns to normal within 2 h. The second stage reaches its maximum intensity about 6 h after the provoking event, coincident with the eosinophil inflammatory response in the airways. The third stage, which can last up to 3 weeks, is sustained by the intense inflammatory response induced by mediator release and progressive recruiting of

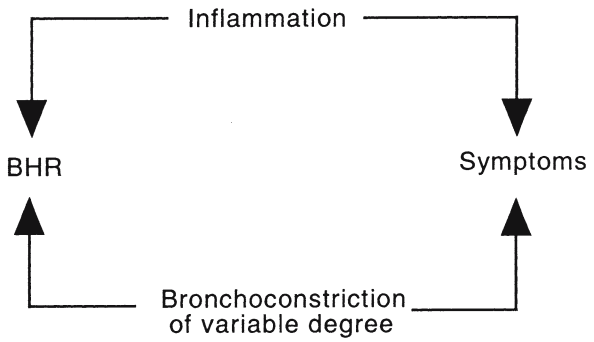


Fig. 11.49. Inflammation contributes to clinical manifestations. (Modified from [454])

Table 11.31. Incidence of prodromal signs of bronchial obstruction (%)

Prodromal signs	<6 Years	>6 Years
Cough	89	86
Rhinitis[a]	55	71
Sleep disorders	50	48
Asthenia	28	37
Nervousness, irritability	39	33
Orbital darkening	44	30
Loss of appetite	44	24
Fever[a]	28	26
Pruritus	0	15
Abdominal pain	17	8
Headache	0	3
Additional symptoms	11	8

Data from [25].
[a] Early symptoms.

inflammatory cells: in this stage the airways react acutely to contact irritating substances. All these events lead to the noted effects of inflammation and bronchoconstriction, which, as shown in Fig. 11.49 [454], are expressed by clinical features.

What is defined as asthma is therefore structured on two levels: the asthmatic attack and the intercritical period. The first is the final link in a chain of events and reactions whose immediate causes do not represent the core of the issue. It could be better to analyze the predisposing factors carefully, the latent anomalies, the causes predominating at the beginning of the process and the pathogenic mechanisms at the basis of the re-exacerbations. Clinical features do not present clearly defined aspects and are *extremely variable from an almost normal state to one of extreme severity*. The onset can be gradual and often the diagnosis is not clear. The early recognition of premonitory signs (Table 11.31) [25] before wheezing is perceptible can be finalized by an often resolutive immediate therapy. The hallmark is *wheezing* during expiration provoked by air rushing through larger but narrowed airways in sufficient force to generate air vibration, heard as a whistling sound associated with breathing. Additional symptoms are a feeling of a tight chest and a hacking, recurrent cough, especially at night, which can also be the only symptom. After the age of 1 year, the bronchospastic component predominates in acute attacks, which are rapidly reversible. The hypersecretion component dominates in chronic asthma and in more prolonged, severe attacks that are less responsive to therapy with bronchodilators (*status asthmaticus*). In addition to respiratory dyspnea, children often complain of abdominal pain, or headache, or a general sense of feeling unwell as the first subjective signs. Abdominal pain, particularly in younger children, is secondary to the use of abdominal muscles and of diaphragm. The dry, irritable, nonproductive cough of the first stages is often accompanied by a feeling of anxiety and is found together with tachypnea and tachycardia. The child is almost recumbent in bed, has difficulty in walking and talking, generally adopts particular postures to facilitate breathing, for example, sitting in a rigid position or leaning forward, to better use the auxiliary respiratory muscles. Wheezing may be a late symptom. It results from air being exchanged through partially obstructed airways and occurs in the larger airways where airflow is turbulent. The small airways do not produce wheezing since the airflow is laminar rather than turbulent. Consequently, marked small airway obstruction may be unrecognized on auscultation [481]. This fact, objectively important, does not always reveal the obstruction and precisely defines its evolutive stages. These limitations can be overcome by spirometry.

Classification

Infantile asthma is classified as acute and chronic; however, rigidly following structured frameworks can lead to imprecise evaluations. Table 11.32 [481] illustrates the assessment of varying factors that trigger the clinical features and the differential prevalence according to age. *Classifications of asthma based on clinical presentation* (Tables 11.33–11.35) [1, 435–437, 494, 592] are also useful in defining the several phases of treatment [437].

Aas staging [1] is often requested for the purpose of publication in international journals. Table 11.36 [186, 279] presents a summary of the causes of persistent cough. Figure 11.50 [694, 695] presents an indicative diagram on the diagnosis of asthma, which, in most cases, can be made on the basis of history, objective examination and instrumental proofs [102].

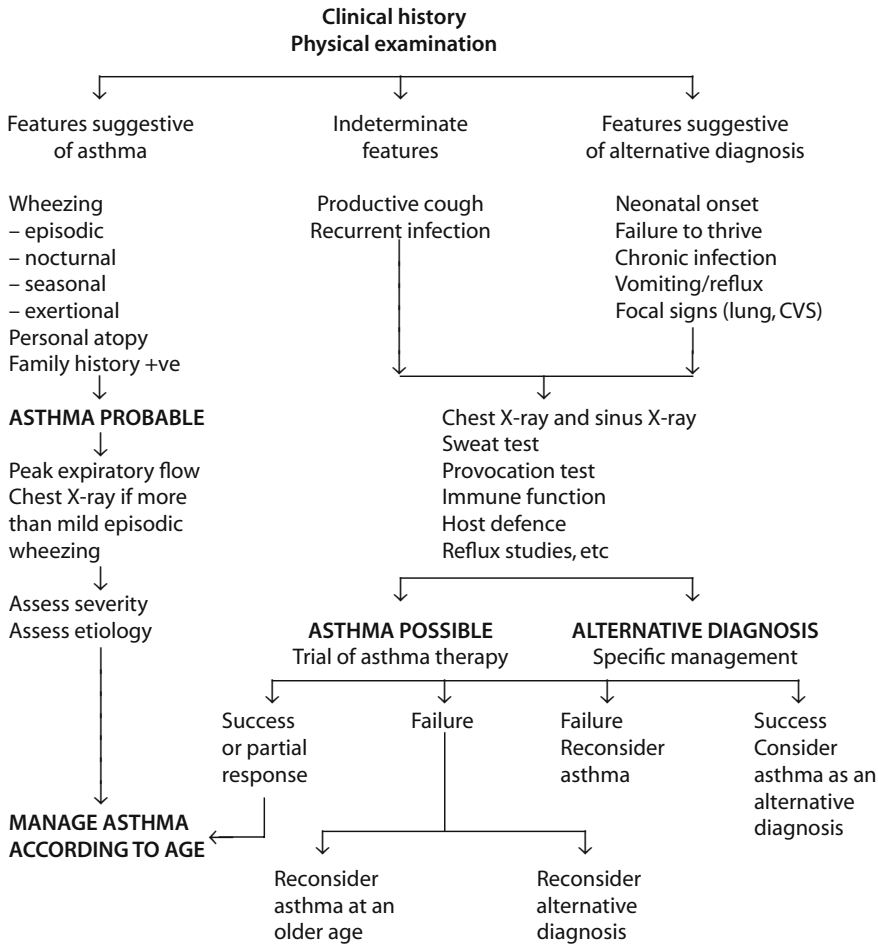


Fig. 11.50. Algorithm for the diagnosis of asthma in children unable to perform lung function tests. *CVS* cardiovascular system. (Modified from [698, 699])

Table 11.32. Assessment of the parameters underlying the clinical manifestations and the higher age-related incidence

Parameters	Age			Relative weight (%)
	<2 years	2–5 years	5–12 years	
Foods	++	+	±	
Exercise	+	++	+++	70
Emotional factors	±	±	++	
Irritant factors	+	++	+++	100
Environmental inhalants	+±	+++	+++	
Viral infections	++++	+++	+±	90
Pollens	+	++	+++	
Allergens on the whole				80

Modified from [481].

Table 11.33. Stepwise approach for managing infantile asthma based on symptom frequency

Type	Clinical manifestations
Episodic	75% of cases; an exacerbation every 4–6 weeks with infrequent symptoms (<5 symptomatic days every month); long periods of well-being, moderate wheezing after prolonged effort; no urgent therapy, normal PFT between exacerbations
Frequent	20% of cases; recurrent symptoms (<once a week), asymptomatic periods interrupted by more frequent attacks and wheezing after modest efforts; urgent therapy: from never to often; rare hospitalization; PFT variability 20%–30%
Chronic	4% of cases; frequent symptoms with no periods of well-being (symptoms >5 days/month, over >3 months and 50% of days of each 1st month of disease); wheezing after mild effort; urgent therapy: rarely/less than once/month, variable PFT
Seasonal	Symptom development similar to the chronic type, but restricted to seasonal periods due to exposure to inhalant allergens

Data from [434–437, 494, 592].

Clinical Presentation

Recognition of the different patterns is useful to evaluate the symptoms based on age (Table 11.32), frequency and severity of the symptoms (Tables 11.33–11.35), and risk factors (Table 11.37) [310, 336, 481, 485, 623]. The procedure will distinguish between noncomplicated/nonsevere cases or a severe asthmatic attack reflecting status asthmaticus.

Noncomplicated/Nonsevere Cases

History

The guidelines indicated in Chap. 6 are followed, examining the content of Table 11.38 [164, 584]. If it is ascertained that the child has already manifested recurring episodes of cough and wheezing, likely linked to EIA, diagnosis is almost achieved. Also, it is possible that the child under observation belongs to a group of young patients with chronic and nonproductive nocturnal cough, or with laborious breathing after running, etc., but never wheezes (Table 11.36).

Physical Findings

The physical examination includes thoracic examination as well as verifying:

- The general condition of the subject
- The state of nutrition, including weight and height
- The possible presence of effort-induced dyspnea

Diffuse hyperphonesis, unequal reduction of breathing sounds according to the conditions, wheezing, expiratory whistling, prolonged expiration, and rales of varying loudness (sometimes only rales can be heard) reflecting airflow limitation are ascertained; liver and spleen are often palpable as a result of diaphragm depression, following marked lung hyperinflation. Wheezing is *the tip of the iceberg* as most of the iceberg is not noticeable, airway obstruction begins well before the tip is evident, and wheezing is heard, therefore its presence can be measured by spirometry before clinical objectivity reveals wheezy breathing [481]. When symptoms occur gradually, it is surprising how children are able to get used to the reduced respiratory function, considering it a normal condition and even expressing a mild sense of well-being so that a <50% fall in PEF, and the related reduction of dynamic parameters can be underestimated for a long time. The symptoms that should arouse suspicion in these children are asthenia, a lessened ability to concentrate and persistent sinusitis.

Diagnosis

Laboratory Examinations

- Eosinophilia is often higher than 250–400 cells/mm^3. The count can be repeated in the expectoration. In children this could be unrelated to ECP levels; however, 27% of 92 children aged <2 years had serum ECP ≥8 µg/l, 76% of this group developed physician-diagnosed wheezing, and 48% had hospital admissions for wheezing.
- PRIST is often elevated. SPTs and RAST can be carried out to spot allergic asthma.
- Spirometry can provide useful information with collaborating children [429].
- If necessary, PFTs and/or BPTs (Chap. 6) can exclude other causes of wheezing (Tables 6.2, 6.3, 6.11). Even if BPT has proved useful in excluding the diagnosis of asthma, especially in adolescents, BPT with histamine is not a substitute for traditional medical diagnostic ability; children aged 2 years may be able to perform BPT (Fig. 6.25b).
- Other research has proved futile or misleading. For example, leukocytosis >15,000/mm^3 may be related to child age, the effect of stress and of adrenergic drugs [41].

Table 11.34. Stepwise approach for managing infantile asthma based on symptom severity

Classification	Mild intermittent	Mild persistent	Moderate persistent	Severe persistent
Clinical manifestations				
Symptom frequency	Intermittent ≤2 times a week	Intermittent >2 times/week, but <once a day	Daily Daily symptoms	Continual Frequent severe symptoms
Exacerbations	From a few hours to a few days	May affect normal activity/sleeping	Affects normal activity/sleeping ≥2 times a week	Frequent
Between exacerbations cough/wheeze	Asymptomatic	Asymptomatic	Often cough/wheeze	Continual
Nocturnal asthma	≤2 times a month	≥2 times a month	≥Once a week	Frequent
Exercise tolerance	Not reduced	Reduced by vigorous effort	Reduced	Very reduced
School attendance	Regular	Regular	May be affected	Frequent absences
Lung function				
FEV_1 or PEF % of predicted	≥80%	≥80%	>60–<80%	≥60%
Daily variability	<20%	20%–30%	>30%	>30%
Spirometry				
Signs of obstruction	Absent	Absent or minimal	Modest	Severe
Response to bronchodilators	Normal	>15%, normal values or modest increase	Incomplete normalization	Incomplete/ absent normalization
BPT to methacholine (PC_{20})		>20 mg/ml	2–20 mg/ml	<2 mg/ml

Data from [435, 437, 494, 592].

Table 11.35. Grading of clinical severity

Grading	Clinical criteria
1	<5 episodes per year with >7 days duration of symptoms and functional restriction each time, and long symptom-free intervals with apparently normal PFT[a]
2	5–10 episodes per year with >7 days duration of symptoms and functional restriction each time, and long symptom-free intervals with apparently normal PFT[a]
3	More than 10 episodes per year with <7 days duration of symptoms and functional restriction each time, and long symptom-free intervals with apparently normal PFT, or more prolonged periods (totaling 12 weeks or more per year) with symptomatic bronchial obstruction or apparently impaired PFT[a]
4	More than 5 episodes per year with prolonged obstruction (totalling ≥6 months per year) following most episodes, or chronic symptomatic obstruction with restriction of function. Bronchial asthma in need of institutional treatment and/or continuous use of corticosteroid medication (any route) to classify for grade III or better[a]
5	Chronic, incapacitating asthma with severe, acute exacerbations despite continuous medication following appropriate and safe dosage regimen

The subgroups are formed according to those in Table 11.32 (episodic, frequent, chronic) or Table 11.33 (mild intermittent, mild persistent, moderate persistent, severe persistent) and to FEV_1 values between exacerbations.
Modified from [1].
PFT Pulmonary function testing.
[a] If children have symptoms and signs of more prolonged bronchial obstruction (including subclinical obstruction) the next higher grade is given; simple exercise-induced asthma is not taken into account provided that recovery is complete with rest and/or a single dose of bronchodilator.

Table 11.36. Differential diagnosis of persistent cough

System	Causes
Central nervous system	Psychogenic
Upper airways	Irritation: foreign bodies, cigarette smoke, dust
	Inflammation: typical asthma, tracheitis, bronchitis, pertussis, recurrent infections by virus and *Chlamydia* and *Mycoplasma*
	Tumors: benign, malignant
	Extrinsic compression: lymphadenopathy, tumors
Pulmonary parenchyma	Inflammation: bronchiolitis, alveolitis, pneumonia
	Vascular: pulmonary emboli, cardiac insufficiency
	Respiratory disorders: cystic fibrosis, measles, bronchiectasis, bronchomalacia, tuberculosis
Extrapulmonary	Stimulation of vagal auricular branches
	Pleural, diaphragmatic or pericardial irritation
	Sinusitis
	Esophageal disease: tracheal fistula, foreign body aspiration, gastroesophageal reflux
	Humoral immune deficiencies
Others to be specified	
Medications	β-Agonists, inhibitor of angiotensin converting enzyme

Data from [186, 279].

Table 11.37. Risk factors for status asthmaticus

Asthma since infancy
Age <6 years especially if <3 years
Males with severe chronic asthma
Barrel chest
Small height
Weight lower than normal
Recent need for oral steroids
Poor compliance
Poor family support
Previous severe asthma attacks without warning
Poor response to an appropriate treatment
Discontinuity of medical care
Subjects at high risk
Younger children:
a. Diagnostic doubts
b. Develop respiratory failure more rapidly
Older children:
a. Weaning of oral steroids after hospitalization
b. Hospitalization for asthma in past year
c. History of prior severe attacks
d. Poor compliance
e. Psychosocial problems
Children and adolescents
a. Prior admissions to pediatric emergency department
b. ≥2 admissions in past year
c. ≥3 admissions in past months

Data from [310, 336, 481, 485, 623].

- Chest X-ray and ECG are not necessary in cases of uncomplicated asthma.

Chest X-ray shows hyperinflated lungs, depressed and not very mobile diaphragm, increased thoracic anterior-posterior diameter, peribronchial interstitial infiltrates, and sometimes parenchymal opacities often leading to atelectasis are found.

In all cases of recurring bronchoconstriction especially with increased susceptibility to infections (Chap. 22), it could be necessary to evaluate immunological parameters such as quantitative serum (Table 1.15) and secretory Igs, and lymphocytes and subpopulations levels (Tables 1.34–1.41, with BALF data). Determination of IgG subclasses is superfluous in the asthmatic child, as there are no differences compared to healthy subjects, with the exception of a few specific cases [468].

If a differential diagnosis with other conditions that should be differentiated from asthma is indicated, specific tests can be asked for such as PFT, BPT, as above, bronchoscopy, diagnostic imagining, bronchography, CT (computerized tomography), scintigraphy, etc., functional and cytological analysis of ciliary structures, and the sweat test.

Differential Diagnosis

Differential diagnosis includes the most common clinical conditions that mimic asthma at various age levels: Table 11.39 [481] shows the relative prevalences. Table 11.40 [186, 481, 584] shows the most common precipitants of asthmatic symptoms and Table 11.36 those of persistent cough. A brief description of the condi-

tions that most frequently present difficulties [186, 469] follows, also based on Appendix 11.2 [186, 469]:

- *Aspiration of a foreign body:* 40% of cases occur in children ≈2 years old; if not recognized and treated promptly, it can cause disease and even death. Usually it begins abruptly in otherwise normal subjects. Limited wheezing reduced to a single hemithorax is characteristic; it can be confused with asthma. Nevertheless, it does not respond equally promptly to bronchodilators, potentially implying that inappropriate treatments were instituted. Smaller bodies may induce progressive symptoms. Chest X-rays are not always discriminatory, since many foreign bodies are radiopaque.
- *Vocal cord dysfunction* is a functional disturbance that mimics asthma with paroxysmal attacks and severe dyspnea and is unresponsive to any treatment. Sounds can be heard on auscultation during inspiration and expiration that is otherwise normal. Instrumental analysis gives consistently negative results.
- *Hyperventilation syndrome* and panic attacks can coexist with the basic disease. The patient complains of being breathless. There are asthmatic symptoms that contrast with negative objective findings, as do most other tests.
- *Bronchiolitis:* see Tables 11.27, 11.28, 11.30 and 11.32.
- *Pertussis* can lead to a mistaken diagnosis that should be avoided through a lymphocyte count and nasopharyngeal cultures.
- *Cystic fibrosis:* there can be initial asthma-like symptoms and BHR. The sweat test is a decisive means of providing a definitive answer, also necessary, especially considering the marked frequency in Caucasians [469].
- *Bronchiectasis:* wheezing and BHR are found. CT enables differentiation from asthma.
- *Ciliary dyskinesia* is suspected when a chronic obstructive respiratory disease is seen, accompanied by rhinosinusitis, otitis and X-rays showing emphysema [469].

Table 11.38. History of asthma in infants and children

Family history of atopy
Atopy of parents, brothers and sisters, and relatives
Sex
Genetic susceptibility to asthma
Asthma exacerbations
Mild/moderate/severe manifestations
Age of onset
Early onset, frequency and duration
Course and intensity
Free intervals
Nocturnal asthma
Past treatment (type and effectiveness)
Administration route
Admission (hospital, emergency center)
Prodromal signs (rhinorrhea, cough, etc.)
Past clinical tests
Precursor signs of exacerbations
Recurrent rhinopharyngitis and otitis
Bronchiolitis, laryngitis
Other recurrent lung disease
Persistent cough
Atopic dermatitis
Specific triggers of asthma
Allergens
Exercise-induced asthma
Changes in temperature and/or barometric pressure
Moving
School, vacations
Emotional factors, conflict
Pollutants (passive smoke)
Foods, additives, drugs
Early feeding
Viral respiratory infections
Specific factors of the individual child
Consequences of the disease
Child
Limitation of exercise
School absenteeism
Thoracic deformities
Growth retardation
Psychosocial factors
Quality of life
Family
Anxiety, disruption of family functions
Cooperation with the child and his/her family
Compliance
Fulfillment of medical prescriptions
Fulfillment of allergen avoidance
Socioeconomic factors

Data from [164, 584].

Severe Asthmatic Attack – Status Asthmaticus

Definition

Status asthmaticus is an attack that lasts more than 1 or 2 days, in whom conventional forms of therapy have failed, and may require admittance to hospital, with the child often in progressive respiratory failure. It is a medical emergency in which the child with acute asthma fails to improve following appropriate aggressive treatment in an ED or outpatient setting [717]. However, children 3–5 years old are significantly more likely to have an ED visit (OR – odds ratio 1.6; 95% CI, 1.3–2.0; $p < 0.0001$) or a hospitalization (OR 2.9; 95% CI, 2.0, 4.3; $p < 0.0001$) than older children [5].

Risk factors of status asthmaticus are summarized in Table 11.36. The risk of hospitalization is very high with on household smoking (Table 4.25). The risk is greater in children aged <2–4 years as a result of the above-mentioned physiological particularities. Bronchial smooth muscles are particularly reduced in children

Table 11.39. Relative incidence of most common clinical patterns entering the differential diagnosis of asthma in different age groups

Disorder	Infants	Schoolchildren	Adolescents
Acute laryngotracheobronchitis	++	++	
Aspiration bronchopneumopathy	+++	±	±
Bronchiectasis	+	+	+
Bronchiolitis	+++	+	
Chronic viral infections	+++	++	
Congenital anomalies	+++	+	
Cystic fibrosis	+++	+	±
Epiglottic laryngitis	+++	++	
Foreign body	++	+++	±
Hyperventilation syndrome		+	++
Hypoglottic laryngitis	++	+	
Laryngotracheobronchomalacia	++	±	
Mitral valve prolapse			+
Pertussis	+++	+	

Modified from [481].

Table 11.40. Relative incidence of most common precipitants of wheezing in different age groups

Disorder	Infants	Schoolchildren	Adolescents
Aspirin	?	?	?
Exercise	+	++	+++
Food allergens	++	+	?
Inhalant allergens (perennial)	+	+++	+++
Inhalant allergens (seasonal)	?	++	+++
Irritants (ozone, cigarette smoking)	+	++	+++
Viral infections	++++	+++	++

Data from [186, 481, 584].

aged <3 years, in whom the obstruction is more a result of edema than of bronchospasm. On the other hand, mucosal glands are numerous, with a consequent increase in the Reid index (mucosal glands/thoracic wall). Furthermore, the relative scarcity of diaphragmatic fibers, which contributes to a reduced muscle resistance to the work required of them, the increased pulmonary peripheral resistance and reduced alveolar surface should not be forgotten. Pulmonary mechanics and volumes are markedly altered in status asthmaticus. Caused by severe lower airway airflow limitation, premature airway closure leads to increases in closing capacity and FRC. Inspiratory muscle activity persists throughout expiration, attempting to counteract expiratory airway closure by increasing the forces holding the airway open. Hyperinflation results [717]. Nonhomogeneous distribution of areas of premature airway closure and obstruction causes ventilation/perfusion mismatching and hypoxemia results. Increased work of breathing under hypoxic conditions and some degree of dehydration combine to cause accumulation of inorganic acids. This acidosis is initially offset by respiratory alkalosis, but once respiratory failure ensues, a rapid and often profound decrease in pH will occur [717]. The combination of these factors explains the increased severity of asthma, the higher incidence of hospitalization, and the relatively scant response to bronchodilators in the pediatric population aged <5 years, and particularly <3 years.

History

It is of major importance to ascertain [509]:

- Chronology of the episode underway
- Apparent cause
- Severity of the symptoms
- Oral solid and fluid intake in the last 12 h
- The performance – or the contrary – of normal activities
- Type and duration of sleep
- Moods
- Name, dosage, and administration time of any medication ingested during the last 24 h
- Effect of the treatment
- Outcome of the preceding episodes
- Possible admittance to hospital or ICU
- Family ability to carry out necessary therapy

Objective Examination

Objective examination (Table 11.41) [273, 435–437, 485, 623] requires that on inspection, the general conditions and the presence or lack of be observed:

- Possible signs of risk affecting the psyche (anxiety, agitation, apathy, drowsiness)
- Inhalatory retractions and use of the accessory muscle
- Dehydration
- Polypnea
- Forced posture
- Cyanosis
- Sweating
- Tremors
- Breathlessness

After the evaluation of the general conditions, hydration, decubitus, etc., and of thoracic objectivity, several other parameters can be considered to make a diagnosis as quickly as possible and initiate treatment. Table 11.41 provides a general assessment; therefore it is not necessary for all parameters under consideration to be present. However, *the greatest risk in respiratory failure during episodes of severe asthma regards young children* (Table 11.36), in whom PEFR measurement is not always easy; therefore an evaluation of these parameters can permit a strict control of the child's condition [436].

Vigilance is the participation in the environment and can help in evaluating the child's fatigue.

Dyspnea is the parameter most noted by parents and doctors and can be helpful in evaluating the level of airway obstruction. It can be evaluated semiquantitatively by asking the child to repeat a phrase or count to ten within a single breath. The condition improves if the length of the phrase or the numbers counted increase.

The *use of auxiliary muscles* is an indication of bronchoconstriction. Sternocleidomastoid use is linked to PEFR or FEV_1 <50 % of predicted value. *Flaring of nasal wings* (Fig. 11.51) is a visible sign of dyspnea and shows the involvement of auxiliary muscles in respiration. Diaphragmatic depressions can be noted and inspiratory retractions, especially intercostal [481] (Figs. 11.51, 11.52).

Status asthmaticus is an ingravescent asthma, resistant to therapy, that progresses to a state of emergency [310]. The symptoms given in Table 11.41 can assist in its diagnosis. It eventually becomes hypercapnic, caused by CO_2 accumulation with ensuing development of respiratory acidosis.

On *auscultation,* prolonged expiration, wheezing, due to obstruction worsening, is audible during both inspiration and expiration, while respiratory sounds are re-

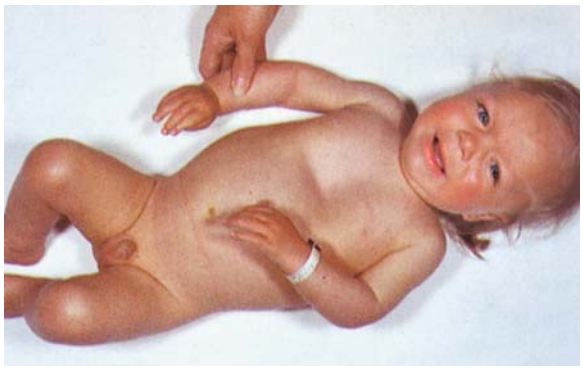


Fig. 11.51. A 13-month-old baby with acute asthma: note the marked indrawing of lower sternum

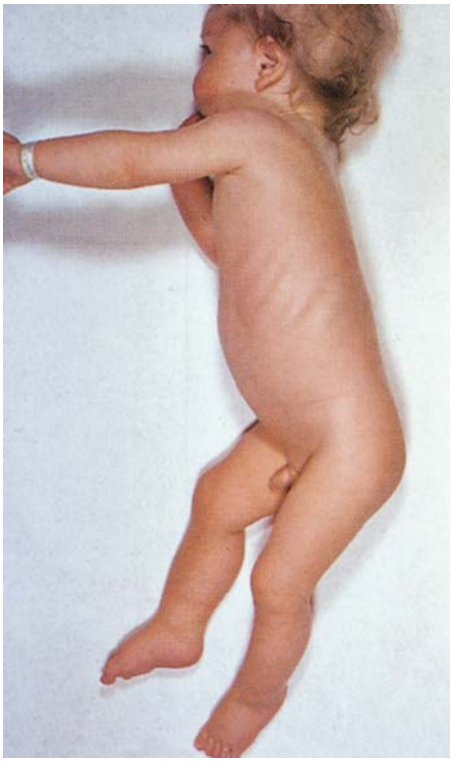


Fig. 11.52. A 1-year-old baby during an acute asthma attack: note the marked indrawing of intercostal spaces

Table 11.41. General assessment of a severe asthmatic attack in children

Symptoms	Mild	Moderate	Severe	Resp. arrest imminent
Dyspnea	Absent/mild	Moderate	Severe	
Older child	Walks, plays	Walks, speaks	Resting, poor speaking	
Infant	Softer, shorter cry	Difficulty feeding	Stops feeding/suckling	
Decubitus	Can be down	Prefers sitting	Sits upright	
Talks in:	Normal sentences	Short phrases	Words or single letters	
Alertness	May be agitated	Usually agitated	Always agitated	Drowsy or confused
Color	Normal/reduced	Pallor	Cyanosis ±	
Accessory muscles	Absent/mild retractions	Modest retractions, use of sternocleido-mastoid muscles	Marked retractions, nasal flaring during inspiration	Paradoxical breathing
Wheeze	Moderate, end-expiratory	Loud, expiratory inspiratory	Reduced air penetration →	Silent chest
RR by age [485]				
<3 months	<60/min	60–70/min	>70/min	
3–12 months	<50/min	50–60/min	>60/min	
1–6 years	<40/min	40–50/min	>50/min	
>6 years	<30/min	30–40/min	>40/min	
HR by age [485]				
<1 year	<150	150–170	>170	
1–2 years	<120	120–140	>140	
>2 years	<110	110–130	>130	Bradycardia
Pulsus paradoxus[a]	Absent <10 mmHg	Present ± 10–20 mmHg	Often present 20–40 mmHg	Absent[b]
PEFR (% of predicted or personal best) (pretreatment) see Figs. 6.26 to 6.28)	>80%	50%–80%	<50%, life-threatening	<33%
SaO_2	>95%	91%–95%	<91%	
$PaCO_2$	<35 mmHg	40 mmHg	>40 mmHg, possible cyanosis	
PaO_2 (room air)	90–100 mmHg	60–90 mmHg	<60 mmHg, possible respiratory insufficiency	

Children exhibiting moderate symptoms should be considered for admission. Normal age-related RR values are in Appendix 11.1

Data from [273, 435–437, 485, 623].

SaO_2 O_2 saturation, *PaO_2* partial pressure of O_2 in arterial blood, *$PaCO_2$* partial pressure of CO_2 in arterial blood, *RR* respiratory rate.

[a] It is more reliable when >20 mmHg (see text).

[b] Suggests respiratory muscle fatigue.

duced. In children with signs of respiratory distress, wheezing is absent because of airway obstruction.

The severity can also be measured by means of a respiratory score (Table 11.42) [174, 476], especially for children aged <6 who have little experience with PFM, or children in significant respiratory distress, in whom it is difficult to obtain an accurate PFM measurement [102].

Laboratory Evaluation

Blood gas analysis: measurement of $PaCO_2$ is the parameter most indicative of severity [485].

Appendices 6.6–6.12 indicate PEF values according to sex and age. No response to a β_2-adrenergic, whether evaluated clinically or instrumentally, is a sign of severe obstruction related to the degree of asthma severity.

Table 11.42. Pediatric clinical score to estimate the severity of an acute exacerbation of asthma: clinical score

	0	1	2	3
Wheezing	Absent	Expiratory	Expiratory and inspiratory	Silent chest (presence of severe obstruction)
RR increase	Normal	Normal to 30%	30%–50%	Over 50%[a]
HR	<120–140	≈	≈	>120–140[a]
Accessory muscle use	Normal	Mild retractions (negligible)	Moderate (intercostal retractions)	Severe (marked tracheosternal and intercostal retractions)
Muscle retractions	–	+	++	+++
Orthopnea	Normal		Inconstant	Constant
Activity	Normal	Limitations on vigorous exercise	Very reduced, nightly disturbance, anxiety	Stops feeding, stops sleeping, agitation and/or prostration
Duration	<1 h	1–4 h	4–8 h	>8 h

Severity: mild exacerbation <7 points, mean exacerbation 7–12, severe exacerbation >12.
Data from [174, 476].
[a] With other parameters reaching a score value of 2–3.

Table 11.43. Pediatric clinical score to estimate the severity of an acute exacerbation of asthma: respiratory score

	0	1	2
$PaCO_2$	<36	40	>40
PaO_2 (mmHg)	90–100	60–90 (room air)	<60 (in 40% O_2)
SaO_2	>95%	91%–95%	<91%
Cyanosis	Normal	Room air	In 40% O_2
Pulsus paradoxus (mmHg)	<10	10–20	20–40
Accessory muscle use	Normal	Moderate	Marked
Auscultation (wheeze)	End-expiratory	Inspiratory and expiratory	Loud or absent
Alertness	Normal	Normal/decreased	Decreased

To each parameter is assigned a score from 0 to 3. Summing up the single scores, the severity of asthma exacerbations can be estimated according to the following scale: 0–4, no immediate danger; 5–6, impending respiratory insufficiency; 7 or more =, ongoing respiratory insufficiency.
Data from [174].
SaO_2 O_2 saturation, *PaO_2* partial pressure of O_2 in arterial blood, *$PaCO_2$* partial pressure of CO_2 in arterial blood.

The respiratory score (Table 11.43) evaluates the need for hospitalization and monitoring the outcome of clinical symptoms in a hospital setting.

Particularities of Small Children

- Inspiratory retractions at rest and nasal flaring (in infants rhythmic head flexion during inhalation and extension during expiration).
- HR and RR: RR can vary from 20%–30%, depending on whether the infant is awake or asleep. It is advisable to measure it also during sleep.
- SaO_2: toddlers tend to develop hypoxemia earlier than adults [435].

Treatment

Treatment of Acute Asthma Attack

An acute attack or asthma exacerbation always requires the greatest medical care [559]. The aims of treatment are summarized in Table 11.44 [79, 604]. On the basis of the flow chart in Fig. 11.53 [174, 435, 436] and the scores in Tables 11.42, 11.43, it is possible to establish the most

Table 11.44. Targets of antiasthmatic treatment in children

Maximum clinical improvement by minimal use of medications
Reduction of both frequency and severity of acute attacks
Efforts must be made to reduce visits to emergency wards and hospital admissions
Maximum improvement of lung function tests
Patient education to asthma and its treatment
Normal night rest
No symptoms at awakening
No missed day from school
Participation in physical, sport and social activities with no restrictions
Improve their quality of life
Minimize potential adverse effects of asthma medications
Normal growth

Data from [79, 604].

appropriate therapeutic approach, but there are others that are equally valid. Given the variability of the clinical picture, however, they cannot constitute a rigid guide, but should be interpreted on the basis of clinical evidence [268] in which history and progression of clinical symptoms are important [485]. To evaluate the severity of an acute asthma attack in the child, follow Table 11.41. Immediate management in the hospital receiving room can reduce hospital admissions for acute asthma, allowing more children to be safely managed in the community [115]. As a consequence, >70% of asthma hospitalizations could be cared for in alternative settings with supplemental O_2, nebulized medication treatments, and close nursing observation [392]. Depending on symptom relevance (mild, moderate or severe), various drugs or combinations of drugs in sequence can be used. Diagnostic tests, if convenient, with the aid of a respiratory score, can evaluate the necessity for recovery: a score of 0–4 is reassuring, in that an immediate danger of respiratory failure can be excluded; it must, however, be monitored at regular intervals. With scores >5, admittance to hospital could be unavoidable [174]. Because of RR variability, it is recommended that it be measured as described below, as well as pulsus paradoxus: if the score is >20%, a serious airway obstruction is present.

If the child is old enough or can collaborate, the reduction in pulmonary volumes and flows can be measured (spirometry, PEFR) [437]. These findings, if possible, should be obtained at once, then at 30- to 60-min intervals, which must be reduced to 15–30 min if the child does not respond to treatment [268]. If spirometry cannot be carried out, even because of the severity of the child's conditions, ascertain PaO_2 (Table 6.25) and $PaCO_2$ by measuring arterial blood gas level.

Figure 11.54 [140, 174, 435–437, 559, 699] indicates various treatment plans, either at home or in an ED. Therapeutic interventions should be integrated, according to the case, with good hydration, correction of acidosis, and *O_2 therapy* when necessary (see below).

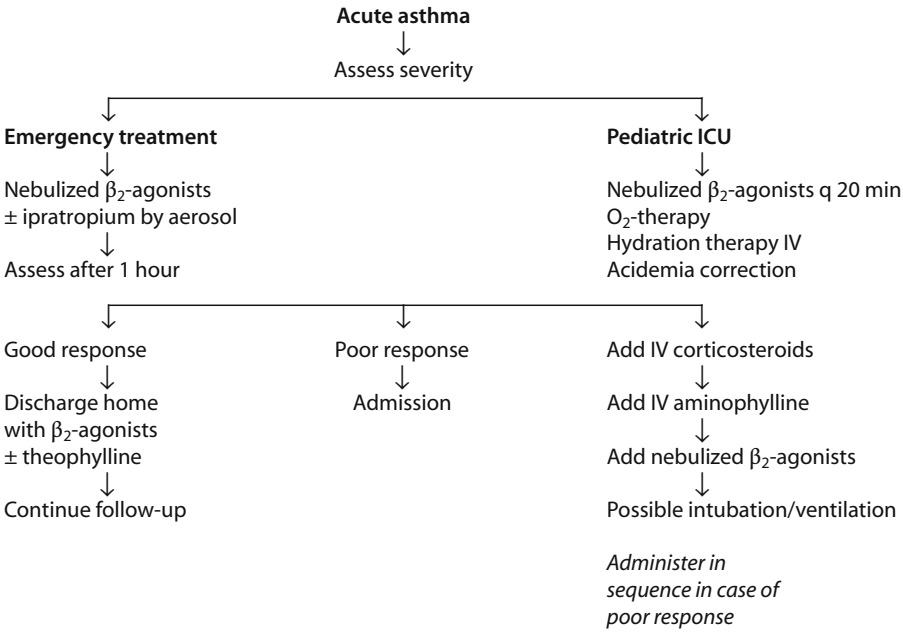


Fig. 11.53. Flow chart for severe pediatric asthma management in a pediatric emergency department or pediatric intensive care unit. (Data from [174, 434, 436])

We repeat that *in early infancy rehydration* is cardinal, since dehydration can easily occur as a result of RR increase and the reduced supply of nutrients and water: a 5% dehydration should be corrected when necessary.

Children experiencing an asthmatic attack, even if not severe, become hypoxemic more easily than older children [605] as a result of perfusion-ventilation [554]. The degree is related to respiratory obstruction and, above all, to PaO_2 and FEV_1 [267, 605].

$PaCO_2$ increase is an index of severity. The respiratory silence associated with hypoxemia (PaO_2 <50 torr) and/or hypercapnia ($PaCO_2$ >45–50 torr), or SaO_2 <91%, is also an index of severe respiratory insufficiency, which makes recovery in an ICU urgent [406].

The AAP recommends, as the first treatment in a child whose condition may be defined as severe, the use of SC epinephrine in standard dosages [509]. Moreover, the α-stimulating action makes epinephrine the drug of choice [485]. If a moderate attack occurs, which can be treated at home, the first treatment to be done at once should be with epinephrine, which has specific indications in cases of severe asthma, particularly suitable for use with children who, by definition, do not suffer from cardiovascular disturbances, apart from effects due to overdose. For the inhaled racemic form see above and Table 11.16; otherwise it can be substituted with inhaled β_2-adrenergics, for example albuterol: unit dose 100–200 µg with dosed MDI, or else 150 µg/kg diluted in 3 ml of saline to be nebulized in 10 min by facemask or spacer + VHC, measuring periodically the above parameters. All β_2-adrenergics are equivalent, but because of its wide international use, albuterol remains the drug used as a reference point [406].

If, after the use of epinephrine/β_2-adrenergics, an efficacious clinical response is obtained, a home maintenance treatment can be suggested, without disregarding subsequent treatment plans (Fig. 11.54). If no obvious improvement is observed, IB can be added to nebulized solution (150–250 µg according to the age, to be repeated every 6 h). ICS can be added to the treatment [406]. Interestingly, in children with acute asthma a single inhaled dose of BUD decreases ENO and is correlated to a PEFR increase [659], and two doses of dexamethasone may provide similar efficacy with improved compliance and fewer side effects than five doses of prednisone [510]. In two studies in ED children aged <5 [149, 556], the treatments compared with prednisone were nebulized dexamethasone 1.5 mg/kg and nebulized BUS 800 µg three doses at 30-min intervals, there were no significant differences in admission rates, with a nonsignificant trend favoring ICS (OR 0.49; 95% CI, 0.22–1.07); thus oral CS appear to be at least as effective as ICS in children with exacerbations of asthma. Another ED trial specifically including 100 children aged >5 with the most severe asthma on presentation (FEV_1 <60% predicted) found prednisone better than 2 mg FP by MDI and spacer at 4 h (FEV_1 45% predicted at trial entry, admission rate 31% on FP vs 10% on prednisone) [567]. Using much higher doses, the bulk of evidence suggests that ICS may be as effective as oral CS in all but the most severe episodes. One trial compared nebulized BUD (2 mg/8 h) with two doses of prednisolone 2 mg/kg on admission and at 24 h in 46 children admitted to hospital with severe asthma. Life-threatening episodes were excluded. Outcomes were comparable at 24 h and at 3- and 24-day follow-up [386].

Predictors of hospitalization in children with acute asthma are five variables: previous ICU admission, baseline SaO_2 ≤92%, 4-h SaO_2 92% or less, 4- h clinical asthma score 6/9 or higher, and hourly albuterol dosing intervals, associated with long vs short therapy, all with high odds ratio (OR). Probability of long therapy was 91.5%–99% for ≤3 predictors, but only 40.6%–61.8% for individual variables [291].

Otherwise, follow what is indicated in the treatment of status asthmaticus, or take the child immediately to a pediatric ED, especially if *there is no response to the β_2-adrenergic* administered by MDI of parenterally [41]. *Persisting with treatment at home in a child with little response to the bronchodilators leads only to a worsening of the episode*, which becomes even more refractory to drugs [267].

Treatment of Status Asthmaticus

Ingravescent asthma *unresponsive to treatment* is a life-threatening condition usually requiring admittance to ICU. Table 11.45 [434, 435, 485] indicates the dosages of the drugs to be used in an acute severe attack of status asthmaticus. The clinical characteristics, to be evaluated together with the clinical asthma score in Tables 11.42 and 11.43, are summarized in Table 11.46 [56, 336, 476, 485]. Monitoring of clinical parameters is integrated with laboratory parameters only under a regimen of hospitalization and observation. In emergencies, it is better to treat the child rather than waste time in consulting textbooks or prescribing tests [485]. It should be underlined that because of potential hypoxemia, SaO_2 (which must always be >93%) should be measured regularly. Nevertheless SaO_2 decrease is often an early sign of moderate or severe obstruction. If it is found to be <91%, it is an indication that hospitalization is required [663]. Therapy is summed up in the following points [267, 268, 435, 485, 564, 683, 699, 717] (Fig. 11.54, Table 11.45).

An urgent strategy in a child with status asthmaticus is a controlled ventilation. Over 11 years, 11.3% of 290 PICU admissions for status asthmaticus required ventilation: 13 children (aged 2–18) presented with rapid respiratory failure en route, or within 30 min of arriving to the ED [366]. Pressure-controlled ventilation (PCV) in 40 children with severe respiratory failure was an effective strategy. Four hours after starting PCV, median pH increased to 7.31 (6.98–7.45, p<.005), and Pco_2 decreased to 41 torr (21–121 torr, p<.005), which were

below 7.21 (range, 6.65–7.39) and 65 torr (29–264 torr), respectively. For children with respiratory acidosis (Pco_2) (>45 torr) within 1 h of starting PCV, the median length of time until Pco_2 decreased to <45 torr was 5 h (1–51 h). SaO_2 was maintained >95% in all patients. Median duration of PCV was 29 h (4–107 h), PICU stay was 56 h (17–183 h), and hospitalization was 5 days (2–20 days). Therefore, PCV represents a therapeutic option in the management of such children [554]. Children with rapid respiratory failure had greater improvements in ventilation (significantly shorter) and oxygenation than those with progressive respiratory failure [366].

Status asthmaticus must be treated with IV therapy. Many children are dehydrated due to excessive insensible loss from respiratory effort, hyperdiuresis, and vomiting. Initial therapy must include intravascular volume repletion with normal saline, correction of electrolyte imbalance, and fluid and electrolyte maintenance.

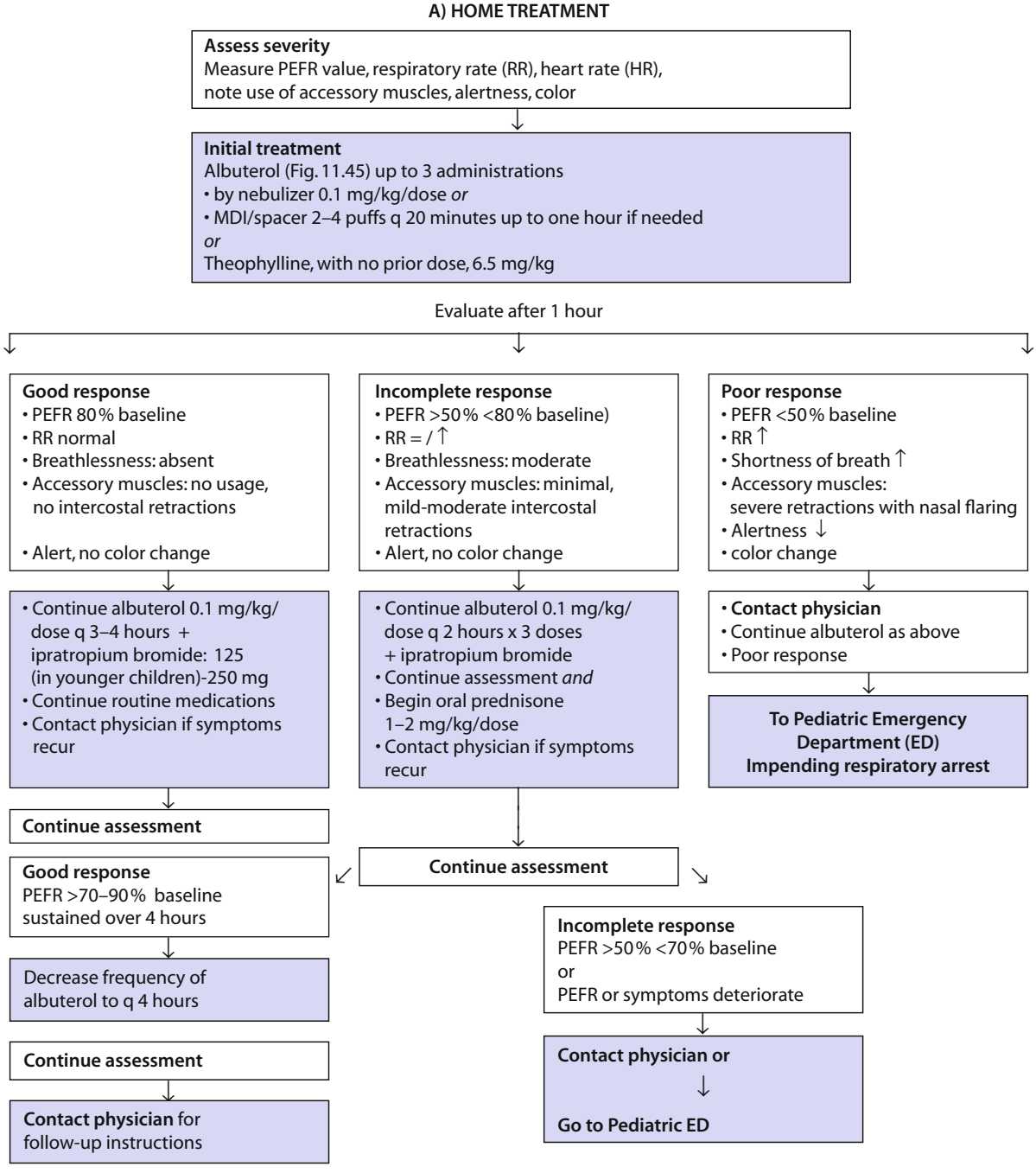
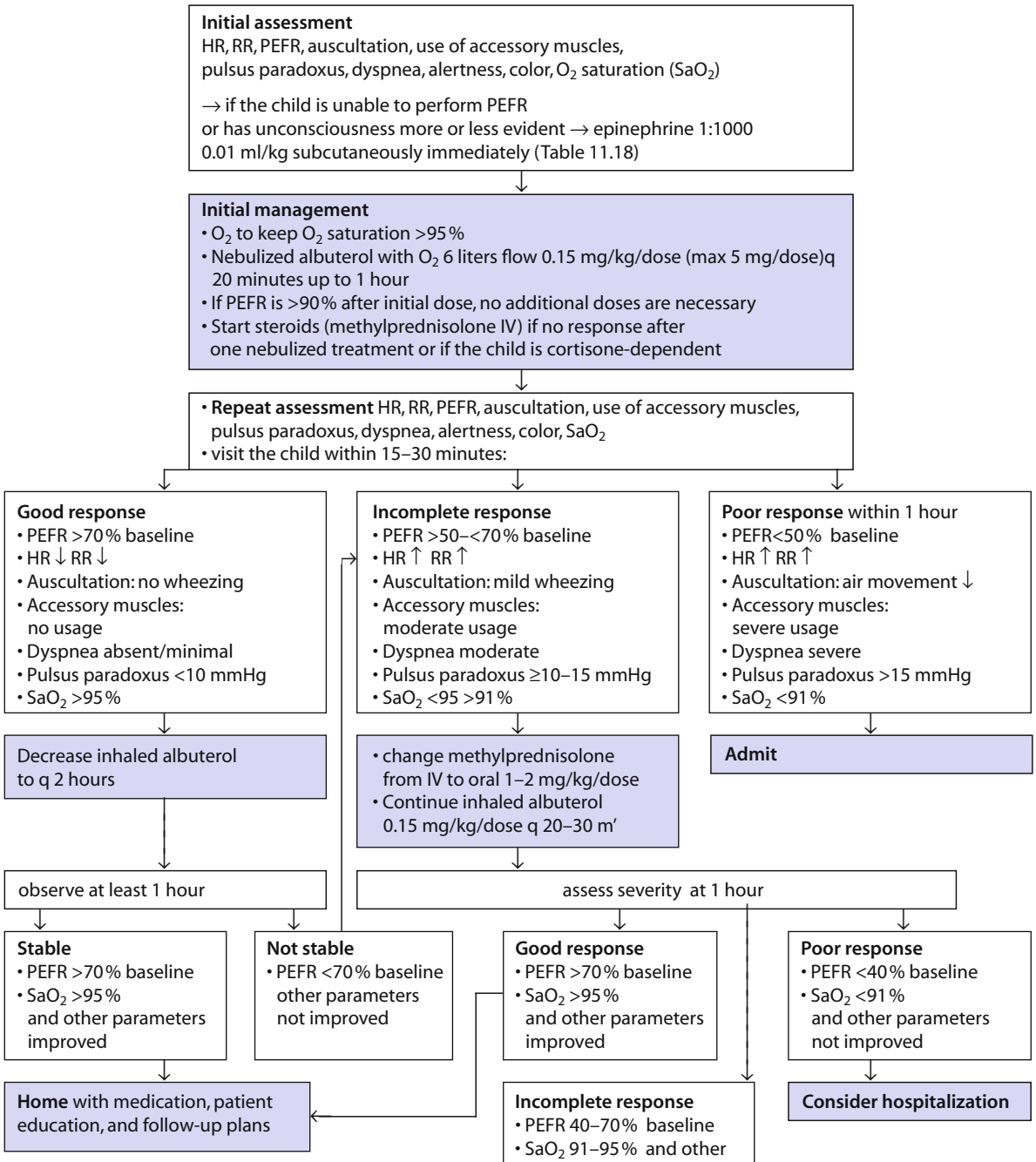


Fig. 11.54. Flow chart for the treatment of pediatric acute asthma exacerbations. **A** Home treatment.

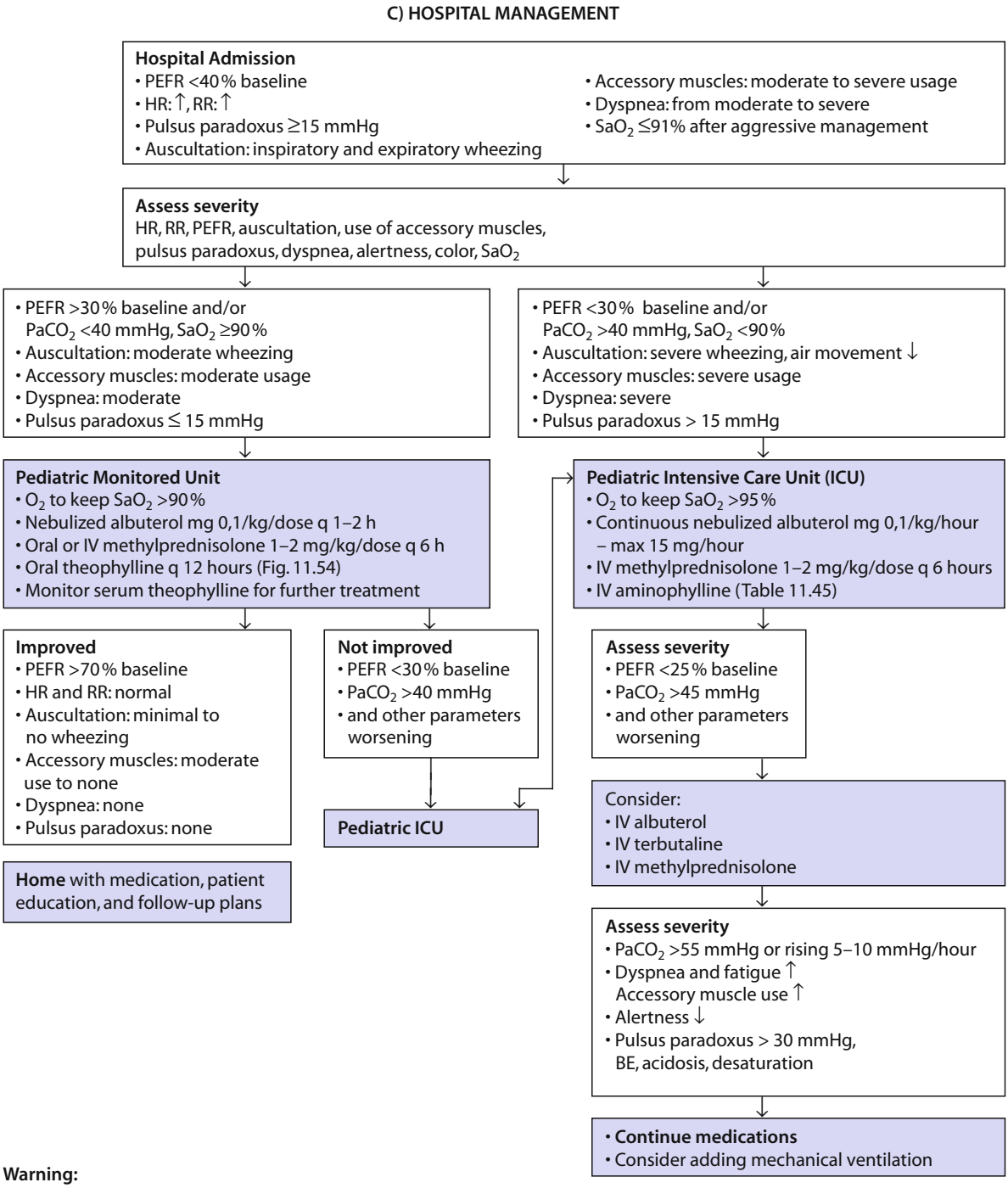
B) PEDIATRIC ED



Factors suggesting hospitalization:

1) Inadequate response to treatment within 1–2 hours from start of treatment
2) PEFR < 40% of predicted or personal best
3) Past history of acute asthma exacerbations, especially if hospitalization was required
4) Presence of high-risk factors (Table 11.40)
5) Prolonged increase in asthma symptoms before the emergency department visit
6) Inadequate access at home to medications and medical care
7) Difficult home conditions, without sufficient monitoring facilities and nursing care
8) Discontinuity of medical care and failure to attend a general practitioner between attacks

Fig. 11.54 (continued). Flow chart for the treatment of pediatric acute asthma exacerbations. **B** Treatment in the ED.



Warning:
In any moment the child presents:
- excessive fatigue /weakening
- confusion or drowsiness
- PEFR is <50% and/or hypercapnia and hypoxia persist or worsen admit the child to ICU

Note: in most cases holding chambers could be substituted for nebulizers to deliver β_2-agonists in acute asthma in the ED [140].
Data from [174, 435–437, 559, 699]

Fig. 11.54 (continued). Flow chart for the treatment of pediatric acute asthma exacerbations. **C** Hospital Management.

Table 11.45. Medication and dosages for pediatric status asthmaticus

Medication	Preparation	Doses
A. Inhaled β_2-agonists		
Albuterol		
Metered-dose inhaler	90 µg/puff	4–8 puffs q 20 min × 3, then 4–8 puffs q 1–4 h
Nebulized solution	0.5 % (5 mg/ml)	0.10–0.15 mg/kg (minimum dose 2.5 mg, maximum 5 mg) in 2- to 2.5-ml saline q 20 min × 3
		Or 0.15–0.3 mg/kg q 1–4 h
		Or 10–20 mg/h in saline nebulized continuously
Terbutaline		
Metered-dose inhaler	200 µg/puff	2 puffs q 5 min up to a total of 12 puffs
B. Systemic β_2-agonists		
Epinephrine	1:1,000 (1 mg/ml)	Tables 11.16, 11.17
Terbutaline	Solution (0.1 %) 1 mg/ml	0.01 ml/kg up to 0.3 ml SC q 2–6 h prn
		Or IV: load 10 µg/kg over 10 min maintenance dose: 0.4 µg/kg/min; increase by 0.1–0.2 µg/kg/min every 15–20 min
C. Inhaled anticholinergic		
Ipratropium bromide		
Metered-dose inhaler	18 µg/puff	4–8 puffs q 2–4 h
Nebulized solution	500 µg/ml	<40 kg: 250 µg (0.5 ml)/dose =40 kg: 500 µg (1.0 ml)/dose q 20 min × 3 doses, then q 2–4 h
D. Methylxanthines		
Theophylline		IV: initial dose (load): in 20–30 ml saline in 20–30 min
		If theophylline serum levels are known :1 mg/kg/IV for each increase of 2 µg/ml of theophylline levels
		If theophylline levels are unknown:
		If the child in the past 24 h *has not taken oral theophylline,* 5 mg/kg of aminophylline IV
		If the child has taken oral theophylline, 3 mg/kg of aminophylline IV
		Maintenance IV doses (5–6 doses up to clinical improvement = stable concentration of 10–12 mg/ml)[a] or: 1–6 months 0.5 mg/kg/h[b] 6 months – 1 year 0.7 mg/kg/h 1–9 years 1.0 mg/kg/h 9–12 years 0.8 mg/kg/h 12–16 years 0.7 mg/kg/h 16 years 0.6 mg/kg/h
E. Corticosteroids		
Outpatient children	Prednisone, prednisolone, methylprednisolone	1–2 mg/kg/day (single or divided doses)
Hospitalized children	Prednisolone	Loading dose 1–2 mg/kg/dose (max 60 µg), then 2 mg/kg per 24 h divided into two doses
	Methylprednisolone	Loading dose: 1–2 mg/kg/dose q 6 h per 24 h, then 1–2 mg/kg/day in divided doses q 8–12 h

See Figs. 11.50 and 11.53.
Data from [434, 435, 485].
[a] Theophylline is indicated in children who have responded to theophylline or who are taking theophylline, have low levels, and are not improving.
[b] Monitor theophylline serum levels (see text).

- *Rehydration:* in general not >1–1.5-fold the normal daily needs, monitoring fluid and electrolyte balance because of potential hypersecretion of ADH and osmolality [336]. Do not exceed the dosage because the high negative pleural pressures during inhalation encourage fluid accumulation in interstitial spaces and therefore the insurgence of pulmonary edema [174]. Provide adequate K^+ chloride (25–40 mEq/l) also because β_2-adrenergics may produce hypokalemia, and fluid supplementation, 300–400 ml/m^2 body surface in the first hour, with 24 h continuation of 200–300 ml/m^2.
- *O_2 treatment* is O_2 humidified to 30%–40% via a face mask or nasal cannula or via a partial or nonrebreather mask at a flow rate of 4–6 l/min not longer than 30–60 min, to keep PaO_2 >85 torr. It must not be excessive to avoid increased ventilation.
- *Inhaled β_2-agonist* with albuterol at 0.15 mg/kg (maximum, 5 mg) per dose should be started every 15–20 min for at least 1 h, even if some improvement occurs after the first inhalation; inhaled albuterol should be continued at the above doses at 30 min intervals over an additional 1–2 h, then at hourly intervals for another 2–3 h, and at decreasing intervals, by nebulization with mask in the very young and with MDI-spacer in older children.
- If *children fail to respond* within 2 h to β_2-adrenergics, start IB via MDI in the doses indicated; it could be added to IV β_2-adrenergics.
- *Continuous infusions of aminophylline* in children who do not respond satisfactorily to β_2-adrenergics only. A reasonable starting point is a bolus of 3–6 mg/kg over 10–20 min, followed by 1 mg/kg/h depending on whether the theophylline level is known or not or the child is following an oral therapy (Table 11.45). Measure the theophylline level as soon as possible; it must be within 10 and 20 µg/ml, considering that 1 mg/kg of aminophylline increases by 2 µg/l the serum concentration [707].
- *IV CSc.* It has been shown the best results by administering a single IV dose: hydrocortisone 4–6 mg/kg/dose or methylprednisolone 1–2 mg/kg/dose, both every 6 h. Continue with inhaled doses. If ICSs are not available the oral type can be taken, but it requires 6 h to reach peak levels. It is recommended that the first dose be administered as soon as bronchoconstriction worsens [683].
- *Measure pH* and *base excess* (BE) to correct acidosis with $NaHCO_3$ = mEq required to be calculated with the formula: BE × 0.3 × kg in weight.
- The use of sedatives to reduce the state of agitation should be employed with great caution because they depress the respiratory centers [272] (see "Death by Asthma").

If monitoring of the above parameters shows a continuous and significant clinical improvement for at least 4 consecutive h, drug doses can be gradually reduced, but monitoring the drugs necessary in case intensive use be made of β_2-adrenergics. Some children can worsen

Table 11.46. Immediate treatment of status asthmaticus

Severe symptoms
Dyspnea associated with severe functional limitation ongoing from >8 h
Response to β_2-agonists poor or of short duration requiring frequent administration <2–3 h).
Child stops sleeping, must sit upright.
Agitation or confusion.
Too breathless to speak: children talk in single words.
Visible accessory muscle retractions.
Pulsus paradoxus: if >20% a severe bronchoconstriction is impending.
PEFR (if available) <50% of best
Life-threatening features requiring immediate treatment without carrying out labor test
Cyanosis, sweating
Paradoxical thoracoabdominal movement
No expiratory rales (silent chest)
Fatigue/exhaustion/drowsiness
Agitation, or reduced level of consciousness
Bradycardia and/or severe RR alteration (<50%)
Young children develop respiratory failure more readily than can be assessed
PaO_2 ≤91%
PEFR (if available) <33% of best (in collaborating children >6 year)

The presence of any of these life-threatening features should alert the doctor.
Data from [56, 336, 476, 485].

Table 11.47. Indications for hospitalization

1. Deteriorating alertness
2. Suprasternal retractions
3. Retractions of sternocleidomastoid and diaphragmatic muscles
4. Pulsus paradoxus >20%
5. PEFR <50% of best
6. SaO_2 <91%
7. $PaCO_2$ >40 mm Hg
8. PaO_2 <60 mm Hg
9. Poor response to treatment in the 1st h
10. Fatigue or exhaustion
11. Insufficient care at home
12. Poor access to medical care

Data from [56, 485, 509].

Table 11.48. Routine emergency treatment of childhood asthma: United States

Intervention	Emergency interventions in 118 and 137 pediatric departments (%)	
	1988	1994
Use of clinical scoring system	20	19
Use of pulmonary function testing	56	73
Chest Rx during the first episode of wheezing	59	50
Oxygen given to all wheezing children	33	40
First medication given		
β_2-Agonists by injection	72	1
β_2-Agonists by inhalation	17	136
Either (no preference)	11	0
Injected agonist of first choice		
Epinephrine	81	63
Terbutaline	17	19
Albuterol	2	2
None	3	12
Inhaled agonist of first choice		
Albuterol	47	95
Metaproterenol	39	3
Isoetharine	12	1
Terbutaline	9	3
Isoproterenol	2	0
Maximum number of doses (injected and inhaled)		
1	1	0
2	5	0
3	58	40
4	16	18
5 or more	20	39
Corticosteroids usually employed		
Early	21	82
At time of disposition	47	11
Variable	29	7
Not at all	3	0
Inhaled anticholinergic agents		
Frequent	2	12
Rarely	50	63
Never	48	23
Variable	0	2

Number exceeds 100 since some hospitals utilized more than one agent.
Data from [332, 536].

Table 11.49. Routine emergency treatment of childhood asthma: Canada

Drug (%)	ICU	At home (oral/inhaled)
Nebulized β_2-agonists	100	99
IV steroids	94	42
IV albuterol	38	
Nebulized ipratropium bromide	10	
IV isoproterenol	10	
Cromolyn		25

Data from [615].

however, with progressive respiratory failure. Therefore, dependent on their condition, they should be *transferred to the ICU.* Table 11.47 [56, 485, 509] lists data evaluating the relevance and *urgency of hospitalization.* The first item on the list of decisive factors is if the equipment for blood gas level tests or drugs necessary to continue treatment are unavailable.

We recommend that O_2 be administered as needed in a hospital environment and, during treatment and that HR, RR, PA, blood gas level tests and/or SaO_2 and electrolyte levels be monitored [406]. In life-threatening cases, to avoid the need of assisted ventilation [47], terbutaline and albuterol have been injected IV. The first by a bolus of 10 µg/kg administered over 30 min and repeated after a further 30 min, followed by maintenance dose of 0.5 µg/kg/min over 44 h [146], the second by a bolus of 10 µg/kg over 10 min, followed by a gradually increased maintenance dose up to an average of 1.7 µg/kg/min over 36 h [47]. Kelly et al administered terbutaline to children 4.5–14 years old, nebulized continuously in doses of 0.4 mg/kg for an average of 9.4 h, for up to 24 h [288]. Current guidelines [434–436] suggest starting with an albuterol dose of 0.15 mg/kg (minimum of 2.5 mg, maximum of 5 mg) per nebulizer treatment for all age groups. For infants <6 months, half the dose (1.25) can be used if 2.5 mg provoke unacceptable tachycardia. During the first hour of wheezing, 3–4 *albuterol* nebulizer treatments are given every 15–20 min. If there is an inadequate response to treatment, the child is placed on continuous nebulization. Others have shown that doses of 0.15 mg/kg of albuterol repeated every 20–30 min are better than doses of 0.05 mg/kg and both have no side effects, and that children (under careful surveillance) respond more promptly to higher doses [564]. High doses of albuterol, especially in very young infants, are devoid of side effects and can be justified by above-mentioned studies [488]. Therefore, aggressive doses of albuterol (every 30 min) are more effective than those administered every 1–4 h [116]. To achieve positive results, higher doses of albuterol, which are needed because the decreased airway caliber decreases the aerosol penetrance, and the quantity of drug reaching the airways decreases as the child pattern of respiration is altered [607], provided that β_2-adrenergics are nebulized with an appropriate face mask, thus ensuring a more rapid improvement, which either makes it unnecessary to send children to an ICU, or shortens the period of hospitalization [683].

A note to explain the rational behind the use of *theophylline* in severe, life-threatening cases, above all in children [615] who, for anatomical reasons, experience bronchoconstriction more than bronchial obstruction. *Inhaled β_2-adrenergics* may not reach the inflamed bronchus because of severe bronchospasm, not because of bronchus failure to dilate. On the contrary, the theophylline reaches it systemically, performing anti-inflammatory effects (Tables 11.17, 11.18) and producing a diffuse bronchodilation that reaches the bronchi obstructed by edema and mucus [393]. At this point, β_2-adrenergics, inhaled or nebulized, can take advantage of the opening and reach the terminal airways to the point of inducing a therapeutic effect. Table 11.48 [332, 536] compares the use made in 1988 of various drugs in 118 pediatric EDs in the US, compared to data 6 years later [332], to evaluate the effect of 1991 guidelines [435]. It should be noted that the great use made in the US of epinephrine and the marked increase in albuterol (first choice in 95% of cases) and CS use, whose route of administration is not specified. In a survey of 376 directors of ED settings, 80% reported the use of inhaled β-agonists as the initial treatment. Only 44.7% ±2.9% reported the use of steroids if there was a poor response to the initial treatment [121]. Also, in 125 children nebulized β_2-adrenergics in ICU and at home were used in 100% of the cases (Table 11.49) [615]. A subsequent survey of 348 ED directors confirmed the high preference for inhaled β_2-adrenergics (96.5%), significantly more public (24.6%) and community hospitals (17.1%) than pediatric EDs (3.5%) reported the use of SC epinephrine as the first medication, compared to steroids (18.1%) as a routine part of the treatment [121]. However, North American authors have shown that *epinephrine is comparable to inhaled β_2-adrenergics* for its rapidity of action, effectiveness and duration of effect [537]. Also notable is how much easier and more practical it is to use epinephrine in the doctor's office, at the child's home or in a busy ED [536, 750], especially if auto-injectable (Chap. 20). It has been noted that if a visit to the ED is followed by treatment with albuterol every 30 min for 4 h, and by giving prescriptions to continue treatment at home, after 4 h 43% of children are released, rising to 61% if *a dose of oral prednisolone* is added [116]. Also in asthmatics with acute forms and admitted to ED and hospitalized, relapses are reduced by 50% and nocturnal disturbance by 75%, if referral to an asthma specialist is facilitated and treatment continued thereafter, compared to patients treated by a family doctor [750].

The effectiveness of treatment in subjects with status asthmaticus is such that on the 3rd day of hospital treat-

ment there is a significant reduction – correlated with amelioration of bronchoconstriction – of T, IL_2R, HLA-DR cell levels, present in higher concentrations than in normal controls [119]. An intubated child with status asthmaticus was treated with two doses of intratracheal recombinant human DNase (*rhDNase*) therapy, a mucolytic agent used to relieve peripheral airway obstruction. The child was *extubated 26 h after receiving* the rhDNase treatment with no adverse effects [163]. A dramatic sustained improvement followed this treatment in a 3-year-old boy with acute life-threatening asthma in whom 48 h of aggressive therapy had failed [473].

Comprehensive Post-Attack Care

If symptoms evolve towards a concrete improvement, it is suggested that children remain under observation for 12–24 h, monitoring both symptoms and parameters, weaning the child to a drug regimen that includes bronchodilators and steroids, either inhaled or orally, then discharging the child, prescribing therapy for 3–5 days and ensuring that the child is able to follow the inhalatory technique correctly, with a follow-up with the family pediatrician about 1 week after discharge [272, 406, 437], which was positive in 59.8%–74.% of cases [121]. Drugs prescribed at discharge were bronchodilators (95.3%), CSs increasing with age group, and theophylline (21%–34%) [121]. Having achieved remission, the therapeutic strategy continues the drug regimen for at least 3–4 weeks, during which the family pediatrician will check the clinical progress by means of twice daily measurements of PEFR and sending the child to a center for infantile respiratory physiopathology for necessary PFT follow-up [164]. The month-long interval can be usefully spent establishing how the problems related to environmental and pharmaceutical prophylaxis will be managed, and in informing both the child and family how to handle the situation [434], also to work on a plan of action for frequent asthma, which is dealt with in the next section. We underline that in children aged <5, the necessary compliance for PEFR measurement is missing, while the child is at greater risk of a symptom worsening. Therefore attention paid to the clinical parameters (Tables 11.31, 11.34, 11.38 and 11.41) can prove equally valid for defining a post-attack strategy [436]. Given that BHR and/or PFT changes tend to persist for an indeterminate time in the form of chronic coughing, nocturnal asthma, or as a result of physical activity [359], therapy must overcome all of these, before a possible treatment discontinuation can be considered (though not in <2 months), also and above all to ensure the *normal quality of life* [164] recently ensured by omalizumab [342] and formoterol [183] treatment. The family and older children should understand that if asthma is not dealt with decisively and controlled effectively it will eventually become a disease that will render the sufferer an invalid in adulthood [218].

Treatment of Episodic, Frequent, Chronic and Other Forms of Asthma

Treatment of asthma must be personalized according to the frequency and severity of symptoms (Table 11.32). The main points for consideration are [571, 700]:

- Deciding which drugs to use, whether continuously or prn (if required)
- Determining the most suitable means of administering treatment
- Using an MDI suited to the child's age, if a drug should be inhaled
- Making sure that the medicine is working
- Regularly verifying the effectiveness of treatment
- Discussing with children and their family the general lines of treatment
- Listening to the problems that asthma or its management provoke in children and their family
- Keeping a clinical diary in which daily variations are noted

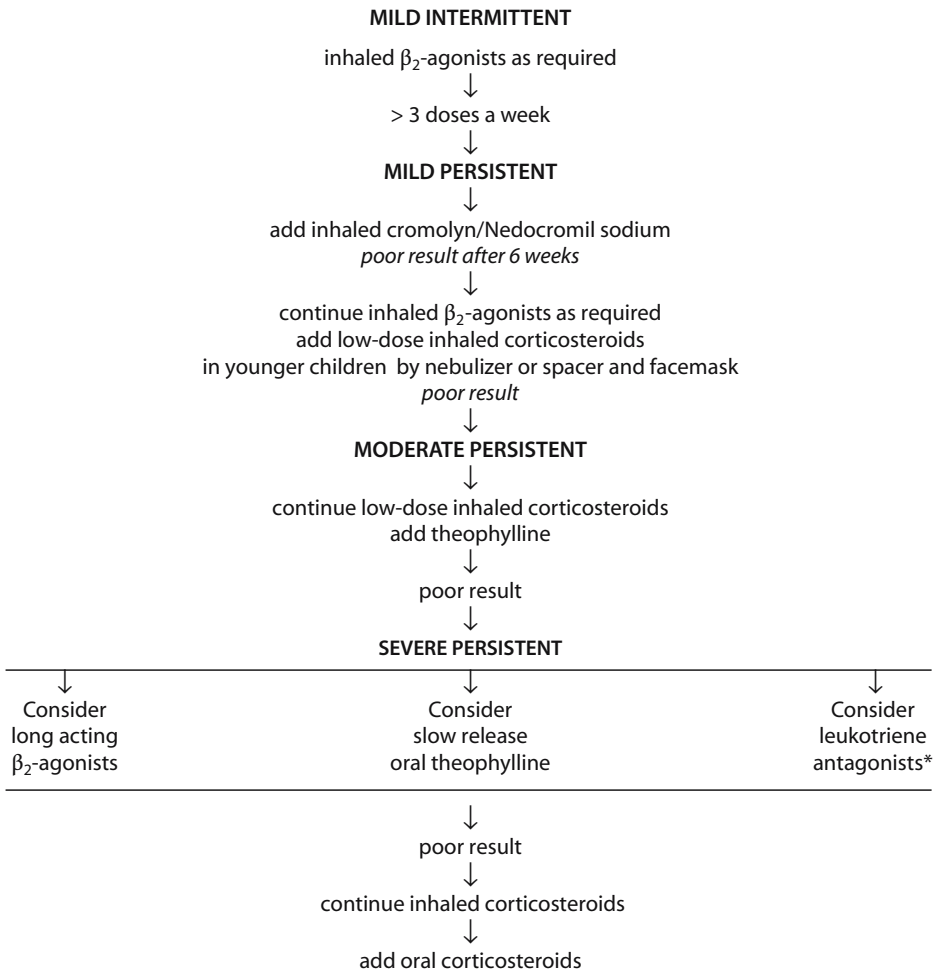
Below, in order of severity are listed the different types of asthma:

1. Episodic asthma
2. Mild intermittent asthma
3. Chronic asthma (mild, moderate, severe) (Figs. 11.55 to 11.58)
4. Administration of drugs at various age levels
5. Specific forms (asthma associated with VRI, EIA, nocturnal asthma, cough variant asthma)
6. Unresponsive asthma
7. Collateral pathologies
8. Complications

Differentiating among these types of asthma is essentially didactic, to better understand the various therapeutic particularities. The possibility of finding the therapy suitable for the control of the symptoms will enable asthmatic children to experience a quality of life that as much as possible resembles that of their healthy companions. From a therapeutic point of view, many treatment alternatives exist, proposed by several authors of proven experience, all of which are usually effective. The problems most frequently met with are represented by asthmatic attacks, by severe forms and by *the day-by-day or maintenance treatment*. We will try to give precedence to the aspects correlated to symptom severity *based on the child's age*. Evaluating these factors separately, even if in practice a certain overlapping between frequent, more demanding forms and moderate, chronic forms is possible, it seems to us useful for ensuring greater clarity.

Episodic Asthma

This is a mild and infrequent form; ≈75% of asthmatic children experience mild episodes of coughing and wheezing every 2 months on average (in relation to season and stimuli), then remaining asymptomatic for



* see Fig. 11.56. Data from [435–437, 699, 700].

Fig. 11.55. Therapeutic algorithm for pediatric mild intermittent asthma: a stepwise approach (step 1)

relatively long periods. They are therefore able to play, attend school and take part in sporting activities without any problems, and usually their sleep is not interrupted by coughing. Prophylactic treatment should be sufficient in most cases. Measurements of PEF in 75% of these asthmatics is usually of no use [700].

Mild Intermittent Asthma (Step 1)

No daily medication is usually required. It is characterized by frequent or infrequent attacks, but of moderate severity, use of bronchodilators prn, persistent airway obstruction not correlated with clinical symptoms, reduction of physical activity, night awakenings by asthma 1–2 nights a month, routine reduction in school attendance. A course of treatment is illustrated in Fig. 11.55 [434–437, 699, 700]. Recommendations for treatment of infants and young children with moderate or severe cases of asthma are based on extrapolations from studies in older children and adults [434]. As can be seen, the first choice of drugs is always for inhaled or nebulized β_2-adrenergics. If results are good, step-down treatment with cromones (see "Prevention"); if insufficient, consider stepping in with ICSs and anti-LT or theophylline [434], associating flunisolide with albuterol inhalation [228]. However, albuterol bronchoprotection lasts <4 h, formoterol DPI at least 8 h in 2-to 5-year-old asthmatic children [441] and bambuterol (once daily dose) [158] (see Tables 11.12, 11.16 and 11.23 for doses). This therapy is recommended in children with persistent cough or asthma. In moderately severe cases, one or two doses should be sufficient. Furthermore, BUD, DPI or BPD by spacers + VHCs as indicated can be used as a good alternative. In children with almost continuous relapses or suffering from chronic asthma and steroid-dependence who are undergoing systematic treatments, we advise ICS once the acute phase of the crisis has been overcome. Given that steroid maximal effects are usually delayed 6 h, it is necessary that children be under control with antileukotrienes, active on immediate and late reactions.

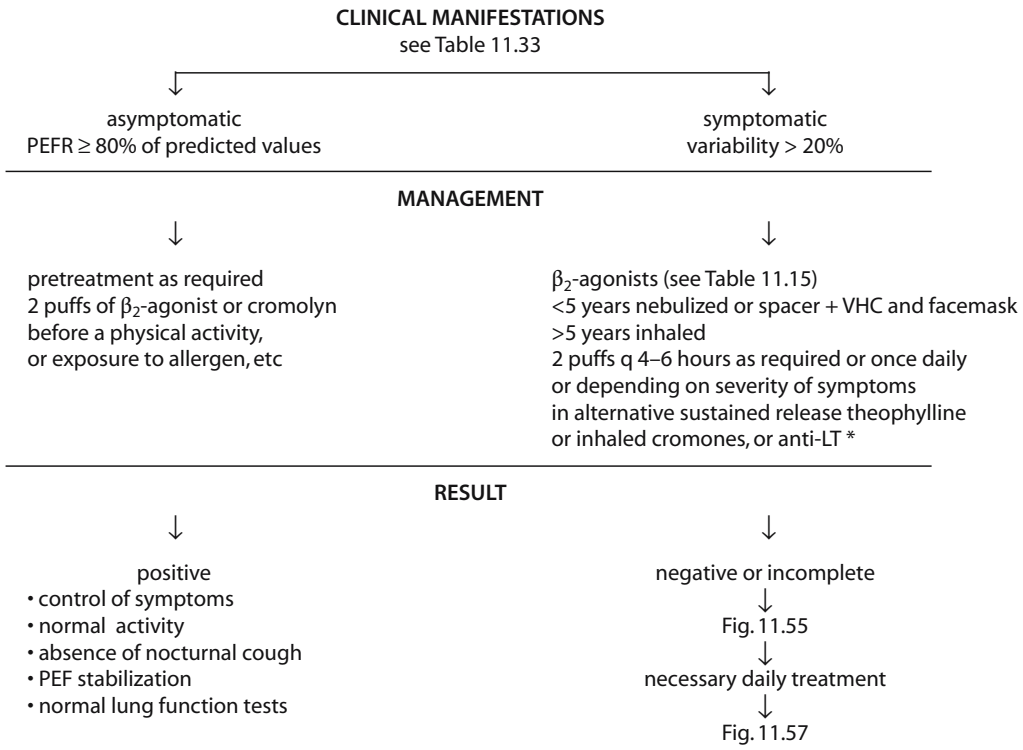
Chronic Asthma

As we have already stated, asthma is an inflammatory process that, beyond the immediate episodes, continues in chronic form as a result of the persistence of inflammatory cells. Usually, chronic asthma is related to children affected with asthma in the first few years of life, but the disease assumes its maximal severity after the age of 5 years. At recruitment the children with severe asthma totaled 8 (11%), but at age 42 the people with severe asthma totaled 33 (47%) with reduced lung function [264]. Generally, the symptoms appear early without an allergic nature being promptly identified, nor proper treatment started. Often, a careful study leads to the discovery of a variety of allergic diseases, for example AR, frequently complicated by *sinusitis*. It is therefore necessary to ascertain its presence, especially in children affected with chronic asthma. Usually frequent symptoms without perceptible periods of well-being are observed, given that bronchoconstriction and effort-induced dyspnea are constantly present, in practice children learn to live with asthma. This form, whose prevalence is 4%–5%, often induces invalidating symptoms, which can be summarized as follows: persistent bronchoconstriction, almost daily symptoms, frequent night-time coughing and limited physical activity [476]. In 37 randomly recruited children with chronic asthma compared to 37 controls with episodic asthma, we noted significant early onset of symptoms, delayed diagnosis, and poorer spirometry results, in addition to a positive personal or FHA, as always a potent determinant of atopy. Especially significant was the greater number of children sensitized to multiple aeroallergens. A strong influence of environmental factors on the development of severe asthma is demonstrated by the significant prevalence of maternal smoke during pregnancy, parental smoke, damp houses, and viral infections. Among the drugs available, we have four possible options: cromones, β_2-adrenergics (even with a long half-life), ICSs, and theophylline in drops or the long-acting type from 2–3 years of age onwards.

Chronic asthma is subdivided into three types, according to whether symptoms are mildly persistent, moderately persistent or severely persistent, each subclass being present in 60, 30 and <10% of cases, respectively [476]. The intermittent form [436], which could be defined as the transitory form between acute and chronic asthma, is considered to belong to the mild forms, to be treated only as needed [437], with four stages: *mild intermittent, mild persistent, moderate persistent and severe* [437]. It should be noted that NIH guidelines (Expert Panel Report 2) tabulate treatment as follows: first-line therapy may begin first with ICSs (low dose), then with cromolyn, or nedocromil as preferred therapy, or alternatively with sustained-release theophylline, or an anti-LT [437], or GINA (global initiative for asthma) guidelines introduce a daily base treatment with CSs, adding cromones only for mild forms and using β_2-adrenergics as symptomatic (rescue) drugs [436]. The Expert Panel Report 2002 believes that a *diagnostic trial* of inhaled bronchodilators and anti-inflammatory medications may be helpful; however, infants consistently requiring symptomatic treatment more than twice a week should be given daily long-term-control therapy, and ICSs should be the preferred treatment [434]. These guidelines have not been confirmed by three longitudinal studies [210, 465, 575], and the Melbourne 37-year longitudinal study *in asthmatic children followed up to adulthood* show no differences between those who did and those who did not take steroids [210], nor has the proposal to introduce CS use from the onset of the infantile asthma been validated, *not even in the mild forms* [465]. The PFT impairment in the group with persistent asthma was greater in those with persistent BHR and in those treated with ICSs [575]. *Cromones and/or theophylline* are important for reducing CS doses [437, 566]. Nor are GINA guidelines productive, since a retrospective study of 175 children followed on average for 8.4 years concluded that, contrary to what the guidelines suggested, starting treatment with cromones and not with CSs improves the outcome, while in the mild-moderate forms positive effects are obtained with bronchodilators [311]. Without doubt, further prospective studies are needed. For our part, we have seen the *positive results of early treatment with cromones,* in agreement with the 3rd Consensus for infantile asthma [700]. One of the tasks of a pediatrician is to strive for a better compliance and quality of life for asthmatic children. There are often reasons for noncompliance to long-term medications when these are numerous, including in adolescence the fear of CS side-effects, a poor understanding of treatment, and a wish to be like one's peers [158]. To our knowledge, one proposition consists of simplifying the treatment program by reducing the frequency of drug administration from twice daily, as usually recommended, to once daily. However, if efficacy is preserved, can prophylactic asthma treatment be prescribed once daily for each drug and for each child, whatever the age, the device and the asthma severity [158]?

Mild Persistent Asthma (Step 2)

Normally, PFT basic anomalies are absent in mild persistent asthma (Fig. 11.56) [435–437, 699, 700]. There are asymptomatic periods between exacerbations, symptoms often follow physical activity, exposure to environmental allergens or respiratory infections, with a decrease in FEV_1 of 20% or less [198]. PEF offers adequate indications, even if in children of 3–5 years the results may be not significant. Symptoms must be carefully checked: coughing, wheezing, difficulty in daily activities or physical exercise, nocturnal disturbance, even if intermittent [589]. Treatment is begun at the first signs of the illness or if PEF measurement is underway, when



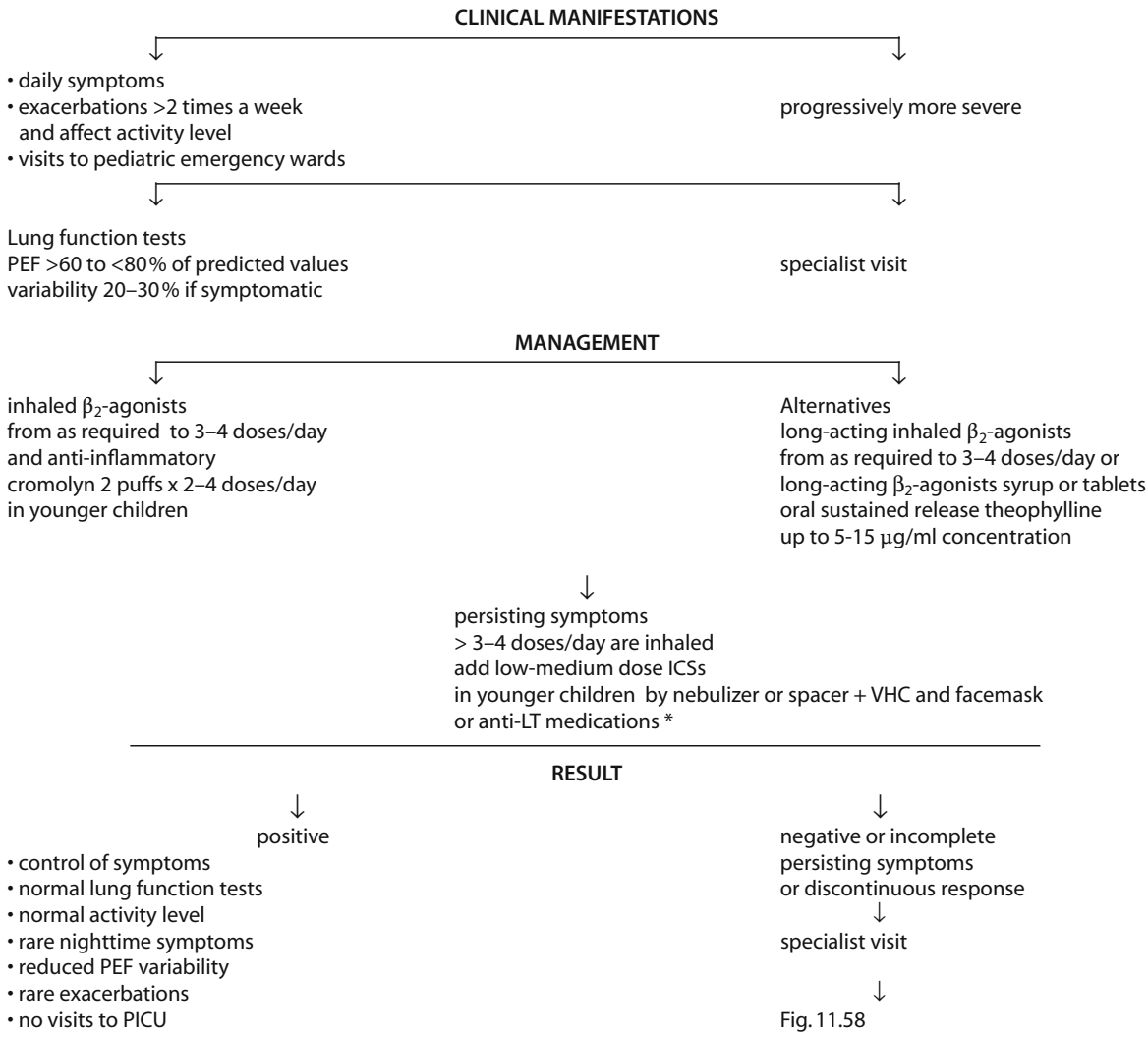
* anti-LT oral administration is preferred by children: montelukast for children aged ≥2, zafirlukast for children aged ≥7, zileuton for children aged ≥12.
Data from [435–437, 699, 700].

Fig. 11.56. Therapeutic algorithm for pediatric mild persistent asthma: a stepwise approach (step 2)

it decreases by 10%–20% [273]. During symptomatic periods, children should be treated with nebulized β_2-adrenergics (Table 11.16), every 4–6 h prn for the duration of the episode, until PEF is stabilized or there is a clear and constant improvement [566]. In children <5 years of age, PEF measurements can be substituted by clinical parameters, such as coughing and dyspnea, while in children >5 years of age, starting a continuous PEF monitoring period at home to evaluate the severity of asthma is suggested [699]. The choice of drugs is less difficult, since response to β_2-agonists is often variable *in young infants*, provided they are used correctly [26]. Inhaled β_2-adrenergics can be used prn if control of symptoms can be coupled with a normal level of activity, absence of nocturnal coughing and PEF stabilization, [697]. If, on the other hand, the condition progressively worsens and β_2-agonists are taken more than twice a week, reaching the point of daily intake dosages or 3–4 times/day, long-acting theophylline or cromones can be introduced. In children ≥2 years we suggest that anti-LT can be considered when inhaled medication delivery is suboptimal because of poor technique or adherence, or BUD which fared well at a dose of 0.25, 0.5, or 1 mg once daily in 359 children aged 6 month to 8 years [290]. If control is missing or incomplete, Fig. 11.57 will be useful to reach the most appropriate decisions.

Moderate Persistent Asthma (Step 3)

Moderate persistent asthma (Fig. 11.57) [435–437, 699, 700] is characterized by two or more weekly acute asthma exacerbations with a 60%–80% PEF decrease and with PFT values up to 20% below existing readings or personal best [198]. For treatment, cromones are considered, which, in addition to their anti-inflammatory action, have the advantage of not being absorbed throughout the system, which renders them preferable to theophylline, remembering that for some cases a long-term prophylactic treatment might be required. If cromones have no effect within 4–8 weeks, the use of ICSs, which have a good anti-inflammatory action, can be started. We have treated with inhaled BUD (200 µg/day) 74 children aged 3.5–5.9 years (mean 4.2 years) and affected by moderate to severe asthma, despite conventional therapy, compared to 71 controls of similar age range and treated with antihistamines. The primary outcome measure was the change in asthma severity, as measured by the mean asthma score during the last 2 weeks of a baseline period and the last 2 weeks of each treatment. The mean difference in asthma score between BUD and antihistamines was: –7.5; 95% CI, –11.70 to –3.29 (p<0.0001). Spirometric data demonstrated a significant improvement. ICSs are suitable for



* for anti-LT medications see Fig. 11.56.
Data from [435–437, 699, 700].

Fig. 11.57. Therapeutic algorithm for pediatric moderate persistent asthma: a stepwise approach (step 3)

children >5 years who take cromones, but who also need β_2-adrenergics 3–4 times a day, or who have nocturnal asthma [476]. Recently, with once daily morning treatment, *monometasone furoate* DPI 440 µg significantly changed FEV_1 compared with BUD DPI 440 µg [117]. β_2-adrenergics, especially those with a long half-life and limited to 1–2 doses a day, are useful together with IB [566]. Long-acting theophylline has the advantage of oral administration. Regularly monitoring its serum concentrations and recording any adverse symptom [198], it proves to be very effective in this form of asthma, as in mild forms [649]. With regular use of these drugs, β_2-adrenergics can be stopped, or retained as part of the treatment only as needed [435]. When prescribing long-term treatment, parents should be informed of the latency time of the drugs being used.

Severe Persistent Asthma (Step 4)

Severe asthma increases the risk of the airways progressing into a persistent and unresponsive airflow narrowing [274]. Children with severe asthma (Fig. 11.58) [435–437, 699, 700], despite appropriate treatment, often display PEF variations >30%–50% and PFT <60% of predicted or personal best, which can worsen during most severe exacerbations [198]. For daily therapy, bronchodilators are prescribed, namely long-acting theophylline and β_2-adrenergics prn, up to 3–4 times a day, in association with CSs [559]. In children 16 months and older [136], ICSs are suitable for anti-inflammatory effects, recurring to oral preparations if problems of compliance or financial difficulties arise. Infants use the more economical formulations *per os* [435, 476] more readily and willingly. Steroids with short half-life are

CLINICAL MANIFESTATIONS
- continual symptoms
- frequent exacerbations
- limited physical activity
- frequent nighttime symptoms
- visits to pediatric emergency department and hospitalizations

EVALUATION OF LUNG FUNCTION
PEF < 60% of predicted values
Variability of 20–30%
with a routine treatment

DAILY MANAGEMENT
short-acting inhaled β_2-agonists
from as required to 3–4 doses/day *
< 5 years by nebulizer or MDI + facemask
> 5 years inhaled:
2 puffs x 4-6 doses/day
in case of need with spacer + VHC
and
antiinflammatory
- low-dose ICSs
  2–4 puffs x 2–4 doses/day
- long-acting inhaled β_2-agonist;
  adding or not in case of need
- cromolyn 2 puffs x 2–4 doses/day
  to minimize ICS dose
  adding or not in case of need
- sustained release theophylline
  (especially for nocturnal asthma)
  up to a concentration of 5–15 µg/ml
  to minimize ICS dose

PEF variability > 20–30%
during severe exacerbations
↓
Consider oral corticosteroids
- < 5 years 5–10 mg tapered to the lowest alternate morning regimen that provides control of symptoms and PEF
- > 5 years the lowest AM schedule is tapered over several days to a week depending on symptoms and PEF

RESULTS
- PFT improvement
- reduced PEF variability
- almost normal activity level
- rare nocturnal symptoms
- reduced incidence of relapses
- little need for relievers
- little need for ICSs
- little need for visits to PICUs
- normal growth and development

* if control is not achieved with 3-4 doses/day consider step up or review the therapy plan: eg adding anti-LT medications: see Fig. 11.56.
Data from [435–437, 699, 700].

Fig. 11.58. Therapeutic algorithm for pediatric severe persistent asthma: a stepwise approach (step 4)

preferred, such as flunisolide = h 1.6±0.35 and BUD = h 2.8±1.1, compared to the 15 h of BDP [633], tapered to the lowest effective single regimen (which provides the same results as that of dividing the administration into four doses) [497] taken on alternate days in the early afternoon (3:00–5:30 PM) [378, 497] to wean to the least undesirable effects, chief among which is that of inhibiting the HPA axis [198, 633]. The ICS dose should be gradually stepped down to the lowest possible doses of medication required to maintain asthma control and perhaps discontinued if a child remains asymptomatic for more than 1–2 months [198, 290, 418, 566, 634]. By following this guide, danger of the baby or child encountering any sort of problem can be avoided; most of the symptomatic benefit obtainable from ICSs occurs by reducing doses of BUD to 100–200 µg/day, with little effect from dose increments [487]. Such lines have been confirmed in a 4-month pediatric trial, which evaluated many varied parameters: BDP achieved the best clinical results, but associated with albuterol and theophylline proved to be most effective in reducing asthma attacks, and in 10 out of 16 parameters that evaluated the most frequent adverse reactions, it registered the smallest rate (albuterol + theophylline the greatest), including the critical points such as asthma attacks and symptoms [396].

All CS-treated children should receive specific medical advice regarding *calcium intake and vitamin D supplementation.* Review treatment every 1–6 months; a gradual stepping down in treatment may be possible and is needed to identify the minimum therapy required to maintain control. If control is not maintained, consider stepping up after reviewing patient medication technique, adherence, and environmental control (M•Plan Asthma Expert Panel).

In conclusion, being the cause of acute and chronic symptoms, infantile chronic asthma should be viewed *a multiform pathology,* whose therapeutic strategy should be based on multiple grounds, that is treating other allergies and eliminating food, environmental and infectious triggering factors, as well as on SIT, which recorded excellent results in asthmatic and rhinitis-suffering children (Table 13.2). The pharmacological choices summarized in Table 11.50 [335, 698, 699] can be recommended, according to age and symptoms. Table 11.51 focuses on the difficulties that can be encountered in the treatment of the very young [472].

Table 11.50. Stepwise and age-related approach for managing children with chronic asthma symptoms

Type of chronic asthma	Younger children[a]	Older children
Mild persistent	Cromolyn/nedocromil sodium or inhaled β_2-agonists with MDI and face mask	Inhaled β_2-agonists as required for symptoms or cromolyn/ nedocromil sodium
Moderate persistent	Cromolyn/nedocromil sodium Inhaled β_2-agonists	Cromolyn/nedocromil sodium Low-dose inhaled corticosteroids Long-acting theophylline
Severe persistent	Inhaled β_2-agonists up to 3 times a day Oral/inhaled corticosteroids	Medium-dose inhaled corticosteroids Inhaled β_2-agonists up to 3 times a day Antileukotriene medications and/or cromones

Data from [335, 698, 699].
[a] Inhaled medications by nebulizer or spacer and face mask.

Table 11.51. Issues in the treatment of asthma in the very young

Recurring wheeze and cough have a typical onset with viral respiratory infections, often without positivity of family history
The diagnosis relies almost wholly on clinical symptoms that may be variable, without the objectivity of pulmonary function tests
Treating young babies with inhaled therapy presents unique challenges due to inappropriate devices prescribed for age and capacity of the child, or inadequate training given to enable the child to use spacing/holding chambers effectively
There are very few controlled studies on asthma therapy and they are often related to older children
The response to bronchodilators is variable, at the first place remains epinephrine in case of need
The younger the child is, the more conditions there are that may masquerade as asthma

Modified from [472].

Administration of Drugs at Various Age Levels [485, 566, 698–700]

- In *babies and infants up to 1 year of age* (Fig. 11.59), it is unnecessary to treat mild and infrequent symptoms, especially if there is no interference with daily life, night-time sleep and behavior. Often the infant whistles as a result of the greater elasticity of the bronchial tree or for intercurrent VRIs, thus rendering CS use difficult [620]. If the child is well and growing normally, only follow-ups are necessary. If symptoms intensify, even if infants show little therapeutic response to β_2-adrenergics, treatment with spacers/nebulizer and face mask is called for, with recourse to cromones or to theophylline if the desired effect is not achieved. If the condition deteriorates, intervention with IB and/or CS by nebulizers and/or with epinephrine, and O_2 therapy in emergencies is suggested.
- In *children 1–3 years of age* (Fig. 11.60), inhaled drugs must be administered by nebulizer and face mask. If symptoms are mild, satisfactory results can be obtained with β_2-adrenergic and/or theophylline. If attacks should become of moderate intensity, with persistent wheezing and coughing, the use of associated cromolyn/nedocromil sodium is advised, making use of CSs in the case of a further deterioration of the symptoms, always by nebulizer and face mask and orally in the minimum effective dose, on alternating days in single-doses.
- In *children 3–5 years of age* (Fig. 11.61) and with mild asthma, β_2-adrenergics may be useful. If coughing and wheezing persist and inhaled bronchodilators fail to elicit a significant improvement, prophylactic treatment with cromolyn/nedocromil sodium or, alternatively, theophylline is called for. The combined use of β_2-adrenergics and MDI + spacer CSs should be reserved for when symptoms become acute again. A further worsening will require a CS treatment in the forms indicated, such as inhaled BUD at a total dose of 800 µg daily, which significantly improved symptom scores, asthma exacerbation rates, PFTs, and BHR in asthmatic children aged 2–5 years [440]. Anti-LT medications should be introduced as symptoms step up.
- In the *age range from 5 to 18 years* (Fig. 11.62), the differences separating cases of mild and medium/moderate asthma should be observed, alternating or combining the drugs and modifying the routes of administration. As can be seen in the figure, if asthma is severe and persistent, recourse to CS use is necessary. Asthma improves only in 41.3%–47.5% of cases (Table 5.15) (the earlier the age of onset, the greater the risk of relapses) [575], so in most adolescents who are not SIT-treated, delivery of medical care may also be challenging [476]. Anti-LT medications should be introduced as symptoms step up.

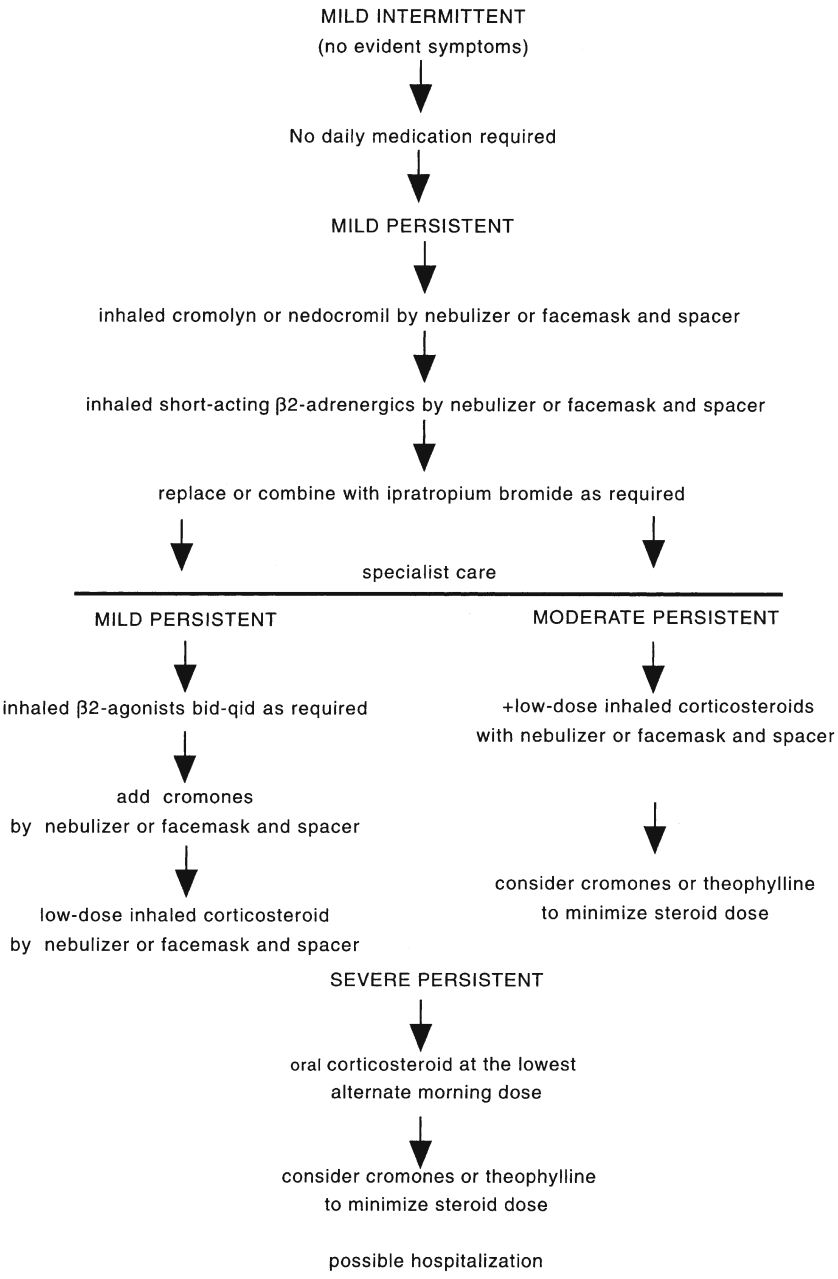


Fig. 11.59. *Algorithm for management of asthma in children aged 0–1 years:* a stepwise approach. Inhaled medications are delivered by pMDI/with spacer + VHC with face mask or nebulizer. Face mask devices should be close fitting, especially if a valved spacer is used, with nebulizers the mask should be held as close fitting as practicable without undue disturbance. Any gap reduces the dose dramatically. (Data from [485, 566, 698–700])

In conclusion, the necessity for a targeted use of the diverse drugs should be recognized in relation to the characteristics of age-related clinical symptoms. Cromones are effective even in the very young, and advantage can be taken of their preventive abilities and of their use in combination with albuterol + flunisolide, all inhaled. Anti LT-medications should be introduced as symptoms step up in alternative with SIT. A three-part *respiratory diary* should be kept, divided into symptoms (asthma, EIA, malaise, cephalea, abdominal symptoms, insomnia, nervousness, etc.), prevention and therapy, noting in addition PEFR and school absences. Symptoms are classified on a graded scale as follows: 1 intermittent, 2 mild, 3 moderate, 4 severe.

Specific Forms of Asthma

Asthma Associated with Viral Respiratory Infections

URTIs make patients vulnerable to asthma development or recurrence and potentially to the establishment of chronic forms. The considerable impact on the asthmatic child who experiences irritations more frequently, often more coincident with VRIs than with the numerical increase of mites in the air or in mattresses, should not be forgotten. For this reason, as virus trigger relapses, CS preventive administration caused a significant decrease in the number of wheezing days, attacks, ED visits, and hospitalizations [60].

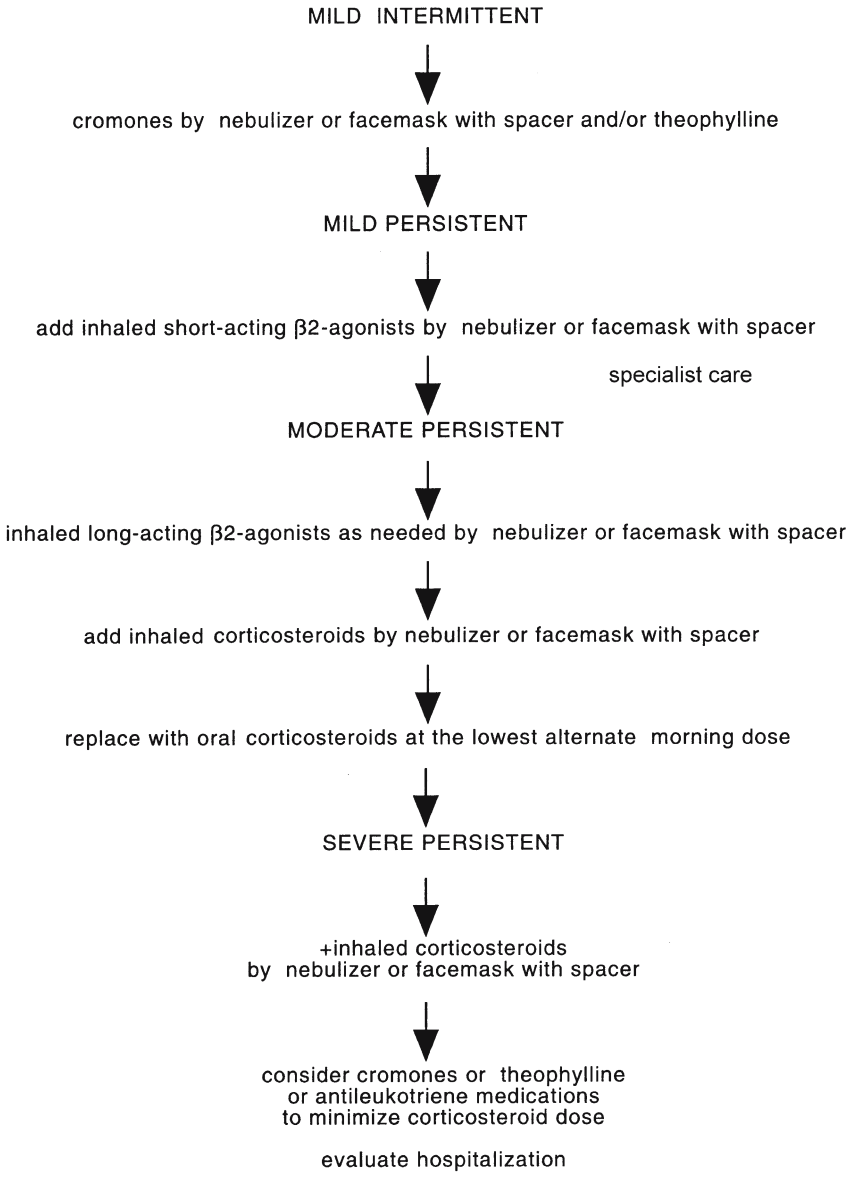


Fig. 11.60. *Algorithm for management of asthma in children aged 1–3 years:* a stepwise approach. Inhaled medications are delivered by pMDI/with spacer + VHC with face mask or nebulizer. Face mask devices should be close fitting to the face, especially if a valved spacer is used; with nebulizers the mask should be held as close fitting as practicable without undue disturbance. Any gap reduces the dose dramatically. (Data from ([485, 595, 698–700])

Exercise-Induced Asthma

The fact that parents often make no mention of their children's EIA seems more closely related to the objective difficulties of not being able to recognize EIA than to its absence; but its occurrence in 63 % of 273 subjects is relevant, the greater part of whom asthma had not been diagnosed [446]. Whereas EIA diagnosis should be made with urgency, given that it can be prevented, thus allowing the child to enjoy a *normal social life*. Children depend on taking part in sporting activities for correct mental, social and physical development. Parents, often easily troubled by the parallel between exercise and the onset of an attack, often constrain their children to limit, or even stop, these activities, forcing them to adopt a sedentary and solitary life, with negative repercussions. It is therefore indispensable that parents be instructed on the significance of and how EIA can occur, since EIA worsens the prognosis of asthma [214]. The cornerstone on which diagnosis relies is history. Typical EIA appears in asthmatic children, but it may also become clinically manifest with exercise in children experiencing a subclinical degree of obstruction [214]. EIA diagnosis was possible in 88 children of 12.4±3.2 years by employing a treadmill test in which the speed was gradually increased, with positive results in 73 %; 35 of 36 boys complained of pain and dyspnea, but not of wheezing, entirely reduced after albuterol inhalation [720]. Significantly, without these tests, 64 % of those affected [542] and, often, also 40 % of children complaining of thoracic pains [720] remain undiagnosed. Correct identification therefore is essential. Sporting activity must represent in an asthmatic infant or child – as it does in a healthy one – an appreciable part of life, and the child should be made aware of his or her *virtual competitiveness even with healthy subjects* [214]. EIA can be effectively pre-

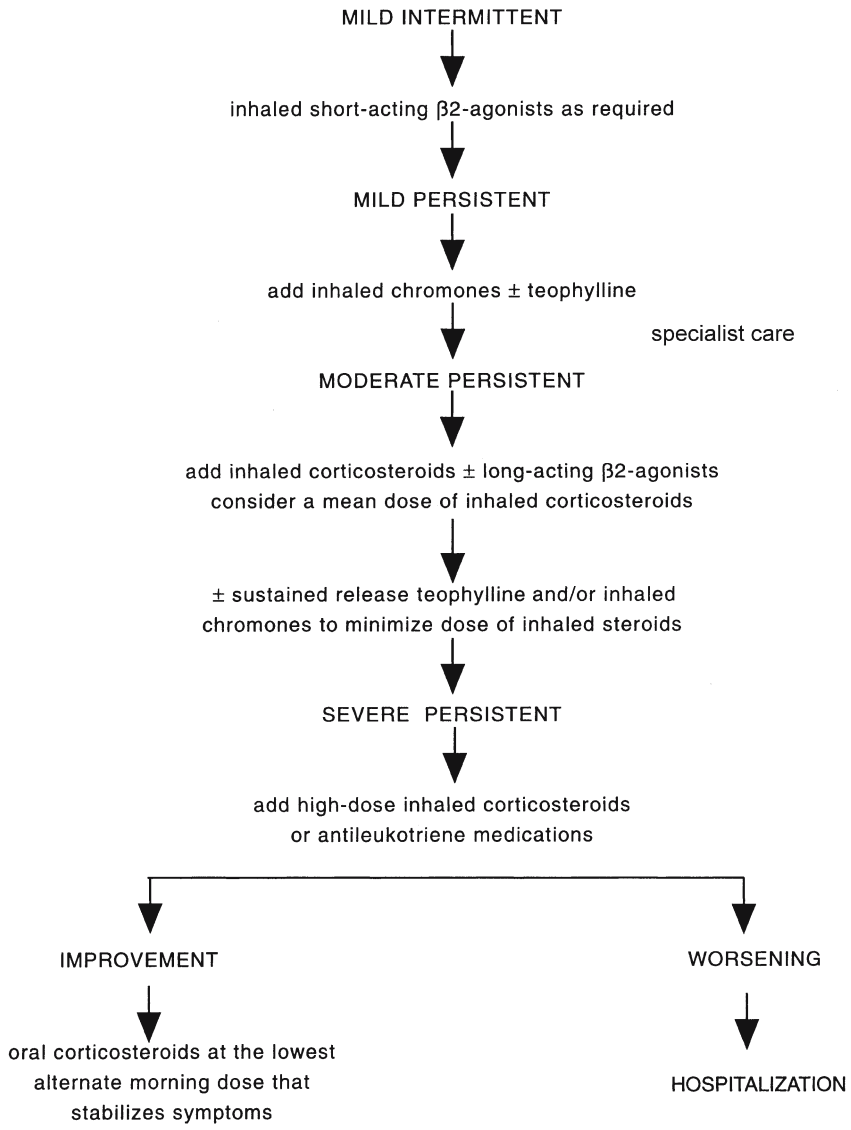


Fig. 11.61. *Algorithm for management of asthma in children aged 3–5 years:* a stepwise approach. Inhaled medications are delivered by pMDI/with spacer + VHC with face mask or nebulizer. Face mask devices should be close fitting to the face, especially if a valved spacer is used; with nebulizers the mask should be held as close fitting as practicable without undue disturbance. Any gap reduces the dose dramatically. (Data from [566, 698–700])

vented both by warming up, as well as by inhalations of β_2-adrenergics about 5 min, or cromones about 15–20 min before beginning the activity [476]. This has proved effective in 70 % of cases [39]. Studies in children have yielded significant results: after administration of *cromolyn/nedocromil sodium,* 10 or 4 mg, respectively, with MDI [113, 134, 451], there was a significant lowering of FEV_1 compared to basic tests [134, 451]. Cromolyn remained effective for up to 4 h and procaterol up to 8 h after the test, though with reduced effect [419, 450]. *β_2-adrenergics* are preferred due to the better clinical results obtained as well as to their duration of action. Despite recognized shortcomings in the treatment of moderate to severe asthma, cromones are preferred for limited or zero incidence of adverse effects [603]. In more resistant cases, β_2-adrenergics can be associated with cromones: both offer equal protection to children after only 30 min. Formoterol in doses of 9–12 µg has an action lasting 6.5 h in reducing 50 % of symptoms, while albuterol in doses of 200 µg lasts for 1.5 h. Using halved doses, albuterol was shown to be equal to formoterol [247]; therefore effectiveness is linked to a dose of 100 µg/puff. Salmeterol has a lasting action of 12 h [343] and bambuterol even more [159]. *Long-acting theophylline* administered to 12-year-old asthmatics 2 h before the challenge prevented both IAR and LAR (8 h), thereby covering an ample time period [270]. Among *CSs*, BUD is indicated in modest doses; 53 % of the maximal effect is reported in children with doses of 200 µg/day, and 83 % with doses of 400 µg/day, registering significant differences with 100 µg/day [487], and parallel results with doses of 400 µg/day [216]. Often, children forget preventative medical measures or are reluctant to be seen while they are taking the medication. In such cases, oral bambuterol [158], salmeterol [343], formoterol [441] and theophylline [270], anti-LTs [482],

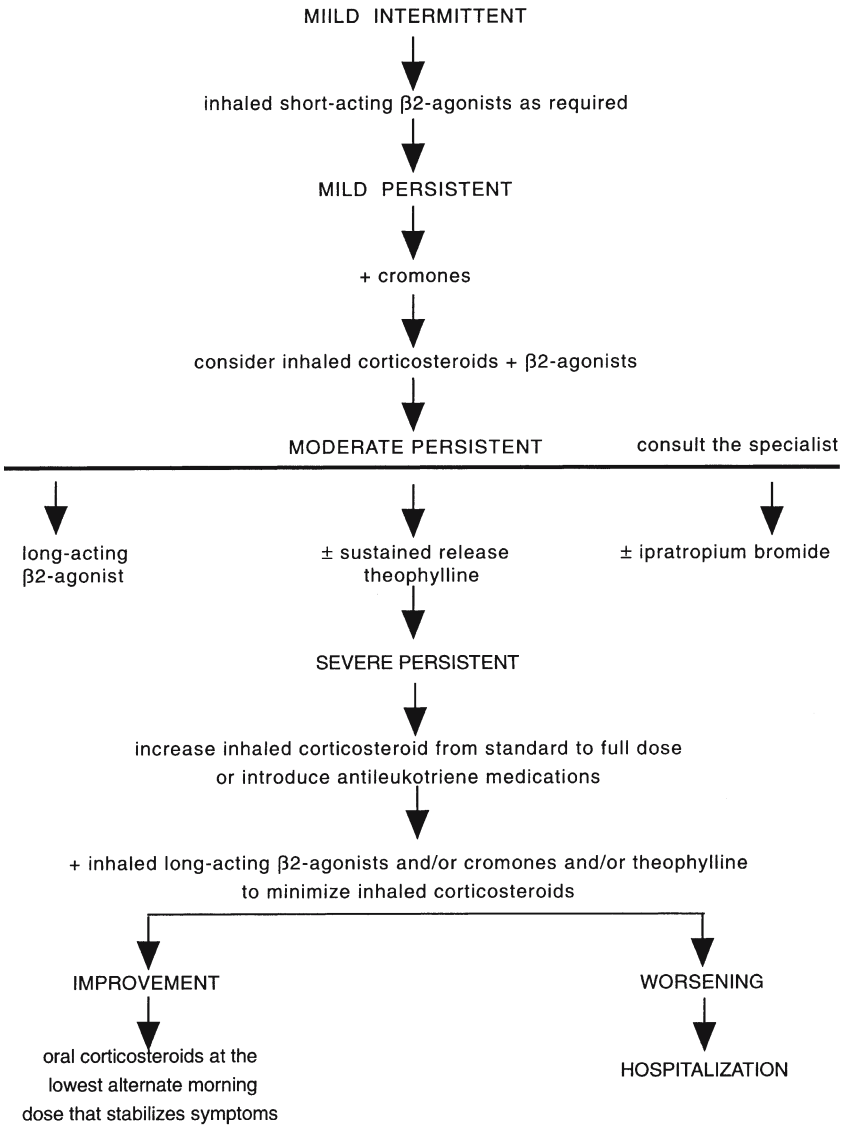


Fig. 11.62. *Algorithm for management of asthma in children aged 5–18 years:* a stepwise approach. (Data from [566, 698–700])

levo-cetirizine, and oral bambuterol [158] are useful. As an alternative, the following measures are suggested:

- *Before beginning* whatever form of activity, choose a warm, humid environment: free running in cold and dry ambient air causes much greater bronchoconstriction than swimming [214].
- *Nasal respiration* is preferred because, as noted, it humidifies the inhaled air and reduces the cooling effect on the airways.
- Do a *gradual warming up* exercise almost to the point of provoking a bronchospasm, thus delaying an asthmatic attack about 2–3 h.
- Alternatively, wear *a mask with a filter* that retains heat and humidity and that has proved to be effective [618].

Usually, the pharmacological pretreatment is followed by a warm-up period of 10 min requiring light effort. This is followed by intermittent training, which alternates more demanding exercises with lighter ones; and finally a training period (for 6–8 weeks, 2- to 3-fold a week for 45–60 min) with a submaximal work-load in such a way that HR does not go beyond 160–170 beats/min in prepuberal children and 180 in the younger ones [214].

Another frequently encountered problem is the child doing sports. Commonly, asthmatic children seem to be unwilling to take part in sports, but this presumed inaction toward sports in general is often caused by the limitations imposed by the EIA of which they suffer [617]. As for athletes in all branches from sport, including those taking part in athletic competitions, EIA is not a contraindication for children. Not all children are natural sportsmen, but all children should have a chance to share sporting with companions. Usually limitations imposed by asthma on daily childhood activities are not recognized until the child begins to participate, and then gains interest in physical activity [617]. In addition to pretreatment, children should avoid activities in

places with excessive air pollution and/or high concentrations of allergens; if all conditions are optimal, warm-up exercises can begin [419]. Swimming, ball games, relay races and dancing are examples of useful activities in the training and rehabilitation of children and adolescents with EIA. We suggest that asthmatic children and adolescents be helped to participate with others in the most suitable sports (in asthmogenic ascending order): swimming, water polo, canoeing, long-distance skiing, volleyball, free dance, speed walking, baseball, basketball, etc. When treatment does not control the problem, then further diagnostic evaluation should be done to rule out conditions other than EIA, and all children with cough, shortness of breath, or history of recurrent bronchitis should be followed to make sure that the correct diagnosis is made and to make sure that treatment is effective [482].

Nocturnal Asthma

Cough is the distinctive characteristic of asthma and nocturnal symptoms may be so dominant and bothersome as to disrupt the sleep of both the child and family. Much has been written on the condition, but a significant trend was found with more nights awakened in the past 4 weeks and with reduction in the quantity and quality of sleep and daytime sequelae of nocturnal asthma, including missing school, educational problems, and parents missing work or other usual activities [147]. Nocturnal cough generally reflects PEFR diurnal variations. Usually, examining at what time the episodes occurred, it can be seen that they do not always coincide with PEFR minimum level. Cough is more frequent about 2 h after the child goes to bed, and then again before awakening in the morning, and not between 3:00 and 5:00 AM when PEFR is lowest, reaching its nadir around 4:00 AM [32]. BALF studies in adults with nocturnal asthma have shown an extraordinary correlation: at 4:00 AM, BALF was found to contain a significantly greater number of lymphocytes, eosinophils, neutrophils and epithelial cell than at 4:00 PM [379]. This data shows that BHR is greatest at 4:00 AM compared to the corresponding afternoon hour, in correlation with FEV_1 and PC_{20} equivalent variations [380]. Such are the effects of *circadian rhythms*, linked to the hypothalamic clock, which is linked in turn to the solar clock, with night and day alternation, which is why night-shift workers register the best test scores during nocturnal hours [32]. Another potential factor is the *nocturnal body cooling* independent of ambient T, correlated with clinical evidence that small reductions of body T provoke cold-induced bronchoconstriction [378]. Relative to allergen influx on circadian rhythms, it has been noted that exposing mild asthmatics without nocturnal asthma to an allergenic burden, they all responded with IAR both in the daytime and night-time, but also showed LAR after the evening test [364]. The connection existing between nocturnal asthma and circadian reductions in histamine concentrations is cited as evidence of a so-called permissive effect on mast cell histamine release, a mechanism that could explain the asthmatic attacks recurring over several consecutive nights following a single exposure to provoking allergens [32].

The relationship between nocturnal asthma severity and PFT during the daytime is still the subject of study, in that as yet no conclusive results are available. A controlled study in children has confirmed that nocturnal asthma is not dependent on an increase in bronchoconstriction [665]. Concerning treatment, the drugs used for daytime treatments, especially inhaled bronchodilators, do not last long enough to cover the night-time as well [364]. In these patients, a dose of long-acting theophylline at the time of sleeping is generally effective [83, 760]. On the basis of previous analysis on theophylline and circadian rhythms, a treatment with equal doses at regular intervals will be able to increase theophylline serum levels at night compared to daytime levels [760]. The persistence of nocturnal symptoms may indicate the necessity of including an anti-inflammatory treatment [379]. This option generally seems to be unnecessary since at precisely 4:00 AM the drug blocks the flow of inflammatory cells in BALF, an effect mediated by LTB_4 [316]. If theophylline, and inhaled β_2-adrenergics and steroids taken as late as possible do not control night-time symptoms, it will be necessary to resort to the usual therapy for day-time asthma. In children, slow-release terbutoline is more effective than inhaled β_2-agonists in preventing nocturnal asthma [398], while ICSs are more effective and less harmful if taken between 3:00 and 5:30 PM, as noted above. A pre-eminent position might be held by formoterol [183], salmeterol, bambuterol [159], and long-acting antihistamines may ensure overall night-time symptom reduction, which of course do not offer the anti-inflammatory potency of theophylline [760].

Differential Diagnosis. Nocturnal asthma might identify those children at risk for severe exacerbations caused by lability of airway function, total IgE levels, changes in clinical symptoms and need for albuterol, which are more likely to predict nocturnal awakening than a decrease in PEF [626].

Maximum environmental prevention is essential against dust mites and pet epidermal derivatives, particularly in children allergic to dog or cat when there is a large amount of dog or cat allergen in the environment also rich in Der p 1 [626]. The bulk of evidence stresses the time the child spends at home; the largest part of which is sleeping, commonly spent for the most part in this environment (see Chap. 24). The influence of environmental factors also relies on the threshold of bronchial reactivity in hyperreactive subjects that is further reduced at night, not related to airway obstructions, but to fluctuations of circadian rhythms [380]. In some cases, the possible association with GER, often occurring during night-time because of the lack of opposition

by gravitational forces and, by means of a reflexive obstructive mechanism stimulated by gastric juices, could also be critical [221]. Furthermore, GER could increase bronchoconstriction by activating a vagal reflex (see "Collateral Pathologies"), but the links with nocturnal asthma are not yet clear [398], also because of a lack of precise correlation between esophagus acidity and altered respiratory function [170].

Cough Variant Asthma

A general summary of the many causes of persistent coughing is given in Table 11.36. This is considered to be a mild form of asthma that is frequently unrecognized, resulting in inadequate treatment. Persistent coughing is a form of asthma that is not always well defined, which, according to recent data, is commonly found in all age groups as a variant of clinical asthma and presents symptoms that frequently result in asthma escaping detection and correct classification. At least one-third of asthmatics suffer from chronic cough [741] and among 10,063 asthmatic children, 785 (7.8%) had cough variant asthma [100]. Several groups of children with and without wheezing [306, 472, 741] and aged <18 months [279] were kept under observation. With the meta-analysis of the data collected from these studies [151, 306, 455, 472, 653], we have ascertained that the risk of developing asthma is statistically very significant (p=0.0001) and we agree with those authors who have defined it as *hidden asthma*.

Regarding the pathogenesis, the cough depends on the following [151, 306, 455, 472, 653]:

1. Upper airway obstruction where cough receptors are more numerous
2. Respiratory difficulty due to peripheral airway obstruction where receptors are scarce
3. Use of anti-cough medications, potentially in relation to cough receptor hyperresponsiveness
4. Possibly a higher wheezing threshold, apparently with no difference in BHR.

In children with classic asthma as well as in those with the variant form, a marked FEV_1 reduction has been noted, as though there was an increased bronchoconstriction.

Symptoms may be summed up in a few points [741]: symptoms reminiscent of a mild form of asthma with chronic, persistent, nonproductive cough that causes interference with sleep, vomiting, and interrupted school attendance, exacerbated by airborne viral infections, physical exercise and inhaled cold air [279].

For diagnostic purposes, the algorithm of Fig. 11.63 [279, 472] can be followed. Moreover, chest objectivity is scarce; PFT and BPT can be normal and the only diagnostic confirmation is the positive response to bronchodilators [741]. However, since the prevalence of risk factors (atopy, FHA, and allergy) are similar to classic asthma, it is not easy to diagnose those children who, subsequently, have wheezing [472, 741]. Among the 785 children, only 1/3 had a correct diagnosis [100]. Above all, differential diagnosis is needed (Table 11.36), and atopy may distinguish groups of coughers from groups of wheezers [395]. The natural history is highly variable: 9%–75% of children (on average 40%–50%) develop full-blown asthma over 6–96 months [279], while a number of cases not easily quantified (about 50%) evolves toward a disappearance of clinical symptoms. In two groups of children aged 5.7 (mean) [653] or 7–15 years [306] to 54% [653] to 55% [306] developed classic asthma. Interestingly, asthma-positive children developed cough variant asthma at a young age [653]. For example, GER may be the cause of chronic cough and BHR [170]. In children with clinical wheezing the methacholine PD_{20} test significantly decreased as these children developed wheezing [306]. On the other hand, 83% of a group who were SPT+ to inhalants and asthma-like night cough and worsened by exercise, was found to have improved at follow-up 2 years later, while 25% developed recurrent wheezing [348]. In 93% of cases, these children enjoyed the benefits of antiasthmatic therapy [151, 279]. To achieve satisfactory control of the disease, it is also necessary to eliminate the triggering mechanisms.

Unresponsive Asthma

Some infants, children or adolescents continue to suffer from asthma that is either greater or lesser in severity, despite the apparently appropriate treatment they are undergoing. This could be caused by various factors (Table 11.52) [476]: inappropriate doses of medication, poor child compliance, and inability to regularly follow the treatment. A more uncommon cause is a variety of atypical outdoor antigens to be considered in all children with nonresolving chest disease or unresponsive asthma [137]. It could also be the result of objective elements related to asthma severity requiring the continuous use of steroids, especially if elevated doses are needed [633], as well as GER [179]. From a pathogenic point of view, this could be a result of anomalies of the CS receptors (CR) (Table 11.22), whether for an IL-induced reduced linking ability [23] due to a receptor irreversible reduction or because different T subpopulations are active, which, in resistant subjects, are more activated as they have an increase in CD25 and HLA-DR [131] or because of different CR links to DNA [23].

A cohort of 103 entrants aged 9–17 suffering for years from poorly controlled severe asthma and admitted several times, were hospitalized on average for 75 days to rationalize their treatment [624]. After a year, follow-up visits, admittance to hospital as well as days requiring hospitalization, visits to EDs or doctors' offices for acute asthma were found to be significantly reduced, with an obvious amelioration in 82% of the hospitalized chil-

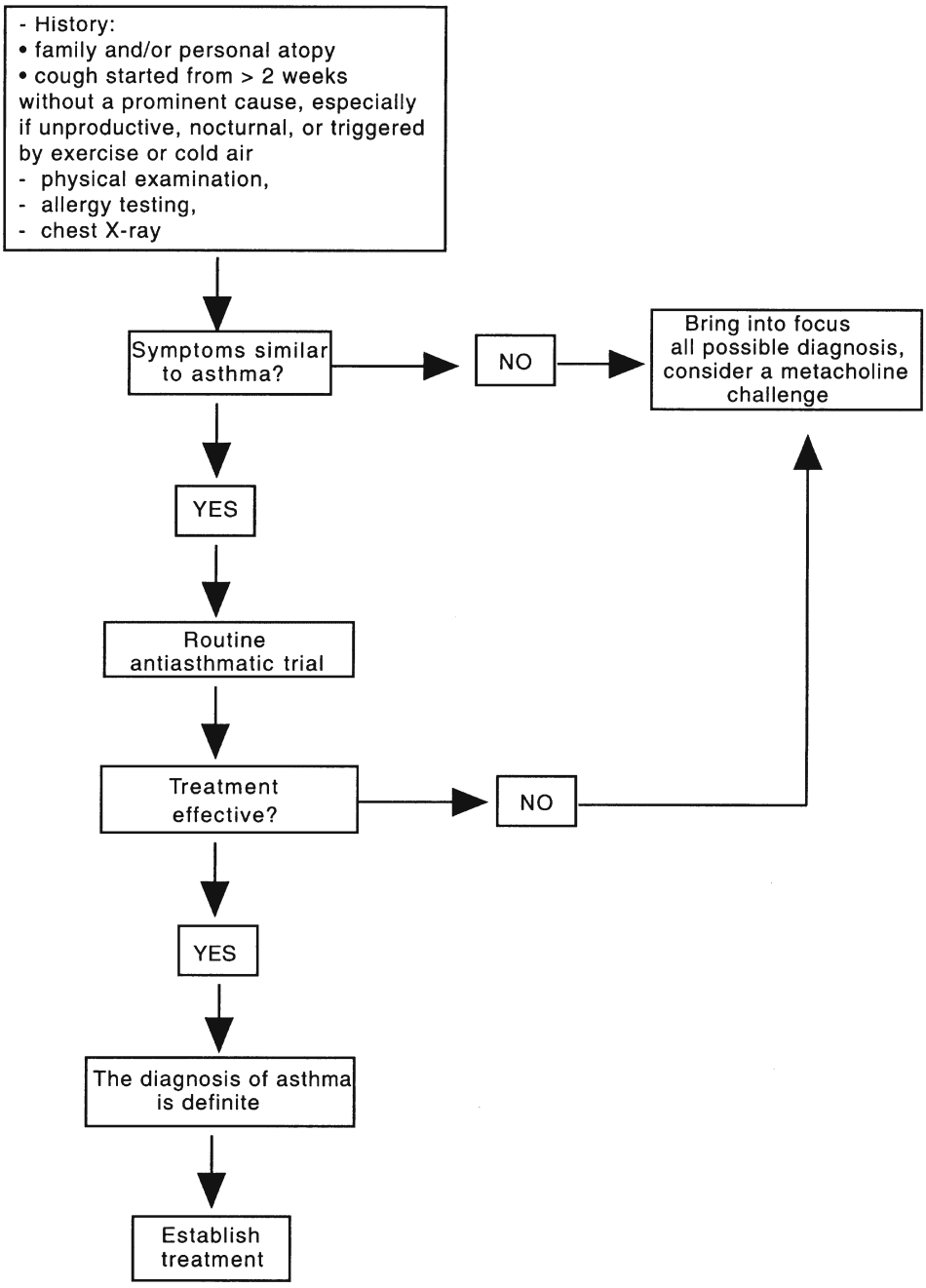


Fig. 11.63. Algorithm for the diagnosis of cough variant asthma. (Data from [279, 472])

dren [624]. On the other hand, children with similar clinical characteristics did not gain any benefits from treatment at a hypoallergenic center [489]. New horizons could be opened by high-dosage IVIg treatment (2 g/kg/month) on steroid-dependent children. After 6 months, doses of oral cortisone for the maintenance and control of asthma exacerbations were reduced 3.2-fold, with an equal reduction in PEFR, clinical score and SPT results [388]. The only doubt is that the sample of this study was not very representative: IVIg treatment could be appropriate since in severe chronic asthma there is a deficiency of one or more IgG subclasses, which respond favorably to IVIg treatment [468]. The effect could also be due to the correction of steroid-induced hypogammaglobulinemia [253]. A trial with recombinant IFN (rIFN) in a group of children with analogous dependency did not prove effective [46], although rIFN reaches the airway epithelium [276]. In adolescents with unresponsive asthma, only theophylline proved to be effective [707]. Use of methotrex-

Table 11.52. Apparent failure of therapy: possible causes

1. Inefficient inhalation technique
2. Lack of confidence in efficacy of the drug
3. Difficulty with the method of administration
4. Poor child compliance
5. Introduction of prophylactic drugs without a clear-cut indication
6. Difficulty with the timing of administration
7. Psychosocial factors
8. Complications (aspirin intolerance, gastroesophageal reflux, etc.)
9. Inappropriate therapeutic intervention (no SIT prescription, cough medicines, antibiotics, etc.)

Modified from [476].

ate and cyclosporine in the pediatric age raises motivated doubts [365], as they provoke toxicity at the pulmonary and hepatic level and blood alterations as well as nephrotoxicity and hypertension [131].

The collaboration of both children and parents is of primary importance to make a treatment successful. In recent years several *educational programs* have been proposed. We have mentioned the relevance of psychotherapeutic and psychosocial factors in the whole spectrum of treatment for pediatric asthma. We believe the intervention of a specialist in this area to be particularly appropriate and productive. On the other hand, a group of pediatricians has devised a program aimed at improving the quality of life of children, with particular *emphasis on educating the family,* who received basic physiopathological information and were followed by a specialized team who tended to the cleaning of the environment, in addition to participating in meetings aimed at ensuring maximum compliance with the prescribed therapy. At the end of the follow-up, an overall improvement was noted in all clinical and psychological parameters [708]. An analogous program should be centered on the following points: an informative meeting, distribution of preprinted forms to assist in the recognition and treatment of acute attacks and *follow-up visits every 2 months.* At the end of the program, the disappearance of ED visits, the better understanding by parents of the measures to be taken during acute episodes and a greater confidence in their own ability to effectively handle their child's disease are necessary.

Collateral Pathologies

GER may be one of the possible contributing factors in any child with recurrent and persistent respiratory complaints, so evaluation for GER should be considered in infants with persistent wheezing [170]. Children with GER-associated cough often show signs of BHR, and therefore the presence of GER should be suspected when faced with persistent wheezing. Symptomatic or functional GER therapy also appears to be necessary for the possible complications related to nocturnal asthma. The basic principles are:

1. Small meals, but more frequently, with food-thickening agents for bottle-fed babies
2. Avoid eating and drinking between meals and 2 h prior to reclining
3. Avoid excessive intake of lipids
4. Keep the head elevated, in a prone position on a surface inclined 30° adopting an anti-Trendelenburg position. To this end we suggest placing pillows (10–15 cm depending on the child's age) to raise head of the bed and keeping the child upright by lifting the upper part of the body with foam rubber supports or air cushions

Especially in young children, early diagnosis and antireflux therapy in cases with GER-related respiratory complaints can result in significant improvement in symptoms. Drugs heightening lower esophageal sphincter pressure are used, for example domperidone 0.1–0.2 mg/kg/ 3- to 4-fold a day 20 min before meals or metoclopramide 0.1 mg/kg qid by monitoring side effects. If esophagitis is present, antacids, H_2-receptors, or in severe cases, omeprazole or lansoprazole are indicated. During follow-up visits to the age of 4.5 years, in 25% of infants (age, 4–11 months) asthma treatment was necessary [170]. Omeprazole (0.3–1.0 mg/kg daily) was effective in 78 children. After 8 weeks of therapy, rebounding was reduced by 66%, heart burn by 56%, vomiting by 33%, abdominal pain by 64% [132]. The use of H_2 antagonists (to impede the production of gastric acid) such as ranitidine 5–10 mg/kg/day divided into two doses for 8 weeks as an attack therapy, to be subsequently halved during the maintenance phase (in the evenings for 6 months), is justified in the presence of GER-induced disease, documented by endoscopy. We suggest making PFT control before and after antireflux therapy [170]. The outcome over 1–4.5 years of follow-up was excellent; only one patient required further asthma medications [170].

Finally, asthmatic symptoms present in children with FA (Table 9.12), the numerous triggering foods (Tables 9.18, 9.19) and pseudoallergy from additives (Chap. 10) should not be neglected.

Complications

The clinical situations which can complicate asthma are pulmonary and extrapulmonary [41].

Pulmonary Complications. Atelectasis can be either segmental or subsegmental and often involves the middle lobe. In the absence of immunological deficiencies or of aspiration of foreign bodies and with negative results to the sweat test, even though having undergone regular treatment, hypoxemic children affected with

middle-lobe syndrome can have frequent relapses, or cough, wheezy breathing or dyspnea persistency, etc. [336]. Using fiberoptic bronchoscopy and a count of BALF cells, a study on 3.3-year-old asthmatic children showed the presence of pathogenic bacteria in 47.6% of cases, re-evaluating the role of infections [610], often favored by malformations and, in 11.5% of cases, by GER [336]. In these children, the possibility of underlying asthma must be suspected, especially if faced with a documented parenchymal thickening [481]. In a group of 56 asthmatic children, only 5 of 63 episodes had physical symptoms of middle-lobe syndrome [577]. This was the case of a 7-year-old boy with intubated status asthmaticus complicated by refractory mucus plugging and atelectasis [163]. Atopy is present in 35% [577] to 38% of cases [336]. The right middle lobe is most commonly involved due to anatomical factors, which facilitate its obstruction, further aided by the combined action of bronchospasm, edema, mucosal thickening, and mucus plugging, with the possible complication of bacterial infection. It follows that the bronchus tends to twist with hyperinflation, resulting in a partial occlusion with repercussions on the middle lobe [41]. Atelectasis and hypoventilation are also sustained by the particular relationships of this lobe with the others, which are not ideal for ensuring a collateral circulation of alveolar gases [41]. It is not clear if the occlusion of the right middle lobe is more prevalent among females [41, 481, 577] or males [163, 610]. The boy was successfully treated with two 10-mg doses of intratracheal rhDNase (see "Treatment of Status Asthmaticus") [163].

Pneumomediastinum presents itself as a sudden complication in 5% of children with status asthmaticus [554]. The most common cause in children is asthma [93], but it is not a characteristic of severe asthma and rarely is manifest before the age of 2 [336]. The only exception is an 18-month-old girl who also had subcutaneous emphysema [211]. Paroxysmal cough and bronchospasm superimposed on hyperinflation, or related to atelectasis, pneumonia, structural weakness, intermittent positive pressure breathing (IPPB), cause air to rupture alveolar bases and spread along vascular sheaths, consequently causing *pulmonary interstitial emphysema*. This may lead to severe cardiovascular insufficiency with reduced venous return, cardiac output and blood pressure [41]. If the air makes its way to the mediastinum and reaches the pericardium, one has auscultatory findings of a crackling sound synchronized with heart beats (Hamman's sign), pathognomonic of pneumopericardium. In other cases, air, through the fascial planes, may escape into the subcutaneous tissue of neck, shoulders and axillae, releasing the mediastinal pressure [41]. Diagnosis is made following tachycardia, retrosternal pains that spread to the arms and neck aggravated by breathing and sometimes by swallowing [336]. However, the association with severe hypoxia, tachycardia, metabolic acidosis, and high ventilation pressures indicates clinically significant tension in the mediastinum [93]. Pneumomediastinum generally resolves spontaneously within a few days, 6 in the girl or 1–5 days in 5 asthmatic children [211], meaning that ambulatory treatment is usually appropriate. Management consists of treating the underlying cause, rest, analgesics, and simple clinical monitoring [93].

Pneumothorax is the rarest complication of acute infantile asthma, which can, for example, also become present following paroxysmal coughing or as a result of IPPB. It should be suspected in cases when the child suddenly worsens together with signs of hyperphonesis and reduction of the murmur of the affected side [41]. If it is not widespread, is self-limiting, but in case of pneumothorax tension, it provokes respiratory breathing difficulties and continuous aspiration is necessary [313]. Pulmonary infiltrations are frequent radiological findings, but they do not require urgent interventions. Conversely, total pulmonary collapse can be provoked both by the severity of asthma as well as external incidental factors [41].

Extrapulmonary Complications. Generally, extrapulmonary complications are rhinitis, sinusitis and secretory otitis media that interact with asthma, but see Chaps. 12 and 15 for the appropriate treatment. Nonetheless, *sinusitis* (whether acute or chronic), common among allergic children, can accompany the worsening of asthma or forms of difficult asthma [334], whereas suitable therapy reflects positively on asthma (Chap. 15).

Other Complications

Other complications are more or less frequent and diverse according to whether the asthma is acute or chronic (Table 11.53) [584]. The most frequent compli-

Table 11.53. Selected complications

In acute asthma
Bronchiectasis
Emphysema
Pneumothorax[a]
Pneumomediastinum[a]
Viral and bacterial infections
Bronchitis
Acute otitis
Pneumonia
Sinusitis
In chronic asthma
Adverse effects of drugs
Chest deformity (barrel chest)
Clubbing
Emphysema
Growth retardation
Psychological/emotional problems

Modified from [584].
[a] Associated with status asthmaticus.

cations are bacterial infections, especially in asthmatics <5 years, acute and chronic asthma and viral infections, to which asthmatics are particularly susceptible. Another possible complication is vasopressin excessive production (*inappropriate ADH secretion*): such levels are high in patients with severe asthma, independent of natremia, probably as a result of the effect of severe asthma on pulmonary circulation. Vasopressin levels fall parallel to patient improvement. Since during an asthma attack hyponatremia can occur, in case of hospital admission BE must be carefully monitored as well as electrolyte levels, and the subsequent parenteral fluid administration must be handled accordingly [41].

Death by Asthma

For a long time asthma mortality was low: only during the first half of the 20th century was it observed that it could be fatal in children [623]. Starting in the 1960s, a few English authors noted a sudden increase in the mortality rate among asthmatics, attributing the cause to the β-adrenergic isoproterenol, the use of which was discontinued though the doubts had not been clearly proved [479]. The pharmaceutical industry introduced new β_2-adrenergics on the market, but in the 1970s, other scientists from New Zealand spoke out against the most widely diffused of these: fenoterol. These authors said that the problem was not due to the drug's direct toxic effect, but rather to its abuse because it provided immediate relief [326]. Both isoproterenol and fenoterol are relatively nonselective potent full agonists with both greater long-term and immediate adverse effects than other β-agonists. Likely the regular use of these agonists led to worsening asthma control and their overuse to treat life-threatening asthma attacks caused an increased risk of death resulting from adverse cardiac effects in the presence of severe hypoxia [569]. In the US, high-dose fenoterol and isoproterenol were not approved for use. In effect, after the ban on the drug, the mortality rate among 5- to 34-year-olds fell from four to <1 case/10^6 [480]. Consequently, albuterol was given preference as it was less dangerous according to New Zealand scientists [738] since fenoterol prescription was associated with an increased risk of death compared to albuterol [608]. We have prescribed albuterol without noting adverse effects and a case–control study recognizes that such alarm was unjustified [738]. Recent studies have instead documented that mortality rates decreased, while sales of ICSs increased [628, 630], as well as of β_2-adrenergics [331]. Some ecological studies conducted in various countries state that the introduction and sharp increases in the use of ICSs correlate to important reductions in the asthma death rates, whereas other cohort or case–control studies indicate that ICSs might not prevent asthma death [628]. An analysis of 96,258 UK asthmatic patients has confirmed in vivo that regular use of ICSs is associated with a decreased risk of asthma death [331].

Increase or Decrease in the Mortality Rate Due to Asthma

Despite the progress made in the diagnosis and therapy of asthma, since 1980 the mortality rate is again on the increase at the rate of 6.2 ± 1.2% a year, especially among 5- to 14-year-old children [710]. As can be seen in Fig. 5.19, the increase in the world population (1985–1987) places Israel in the first position, followed by Finland and Denmark, but if the rates in the 5- to 34-year-olds in 1987, compared to the level in 1980, is calculated, Australia ranks first, followed by Singapore and New Zealand, whereas Israel is in ninth place and Finland and Denmark are absent [569] or in the last places (Table 5.17). These rates in patients with severe asthma are markedly greater than mortality rates in asthmatic patients in the general US population, which can be estimated at 0.02% if the death rate is 1.5×10^5 and asthma prevalence is 3%–5% [595]. After a gradual decline in asthma mortality rates in the 1960s and 1970s, rates have increased progressively in the US during the past 2 decades. In most other Western countries, the rate of asthma mortality decreased during the 1990s after progressive increase through the 1980s (0.36×10^5 in some European countries vs 0.47×10^5 in the US. The underlying reasons, including the role of management, will require further investigation if strategies are to be implemented successfully to reduce asthma mortality rates [29]. In a PICU, a 6-year study resulted in an 8.9% incidence of acute severe asthma, while all pediatric admission totalled 1.9% [464]. However, the marked underestimation, varying between 36% and 127% for 5- to 34-year-olds, should be noted, results from a position common to other European countries with the highest levels [375]. *Contrary to the statistical findings, beginning from the 5th year*, an epidemiological study has established a mortality rate among 11- to 14-year-olds of 0.34×10^5 (Tables 11.54, 11.55) [190, 192]. The inclusion of 45.5% of 13- to 16-year-olds and of *32% of 1- to 4-year-olds* is very significant [190] (p=0.0006). In another cohort [192], the death rate among 1- to

Table 11.54. Increase in mortality rate for asthma

Age (years)	%	Female prevalence %
1–4	32	
5–12	23	
13–16	45	
Up to 12 years		75
Total		59

Data from [190].

Table 11.55. Variations in mortality rate for asthma

Age (years)	Periods studied	
	1952–72	1973–88
1–4	10.4%	6.2% ($p = 0.0396$)
1–19	–34.3%	
15–24	+50%	

Data from [192].

19-year-olds dropped and among 15- to 24-year-olds doubled, with a male prevalence in the former (55% of cases) and of females in the second group (59%) [190]. Underdiagnosis is more common among females; therefore the cases of death seem to be more frequent precisely in the groups that statistically appear to be least at risk, that is the 15- to 34-year-olds, 5- to 14-year-old females [14] and children 1–4 years of age in general [190].

Examination of Possible Causes

Tables 11.56 [80, 145] and 11.57 [67, 403, 579, 623] indicate the possible causes of a worsening of the prognosis, including so-called self-management and possible undertreatment [623]. This assumption is based on the fact that asthma is a very common disease, implies a substantial impairment in children's quality of life and requires challenging medical interventions and treatments often accepted by children reluctantly [375]. Moreover, the deeper understanding of asthma by patients, along with the undeniable positive aspects can, in some cases, give way to *a loss of the doctor–patient relationship*, which on the contrary is indispensable [67]. Among other frequent disadvantages of *self-management* is the improper use of some drugs, particularly β_2-adrenergics [569]. Continuous drug dependency occurs, which is reflected negatively on the quality of life and involves a condition of *undertreatment*, since the patient does not use, or misuses, those drugs that are needed in his or her case [478]. Chronic underuse is also attributable to a false feeling of safety occasioned by masking the effects that systematic use of medications has on symptoms [109]. Thus, on the one hand a masking or an underestimation of the underlying inflammatory process can occur, so that when a sudden emergency materializes, the use of ICSs and/or cromones is delayed [67, 375]; on the other hand, the rapid beneficial effects on clinical features prevents the patient from realizing that *hypoxia worsens* [296], dangerously postponing a doctor's visit. Undertreatment increases death cases, since the moderate reduction in mortality is related to the progressive increase of ICS sales [596, 628, 630]. It is recommended to closely follow subjects who have experienced fatal crises or near-fatal attacks [326].

Table 11.56. Features of children at risk for asthma death

Early onset of asthma, especially in the 1st year of life
Severe episodes:
Asthmatic episodes frequently requiring hospitalization
Increasingly severe airway obstruction persistent all day long
Respiratory insufficiency requiring mechanical ventilation
Hypoxic seizures associated with asthma attacks
Nighttime asthma in rapid progression
Attacks precipitated by foods
Unperceived severity of attacks
Weaning medications, especially oral CSs after exacerbations
Excessive β_2-agonist use neglecting CSs during acute episodes
Steroid dependence with an increase in oral or inhaled CS doses
Inadequate medical and asthma care during hospitalization
Psychological disturbances, overt depression, self-treatment of asthma

Data from references [80, 145].

For example, in Australia, the government has been engaged in distributing guidelines among doctors for treating asthma [598]. A correlation between the increase in both death and asthma prevalence has also been hypothesized; nevertheless, in some countries the mortality rate has diminished, despite the increase in prevalence [598].

Subjects at Risk

Epidemiological studies have shown that in addition to the age factor, the following categories of pediatric patients are particularly at risk (Table 11.57):

- Children in a home where *family interference is dominant.* A paradigmatic case of late referral is that of a 5-year-old male whose parents had objected to steroid treatment and had even hesitated 5 days in taking him to hospital [464].
- Adolescents suffering from chronic asthma and from a recent episode of acute asthma, who frequently *do not regularly follow treatment* for a variety of reasons [478], whose family rarely looks after him or her or on the contrary whose supervision has been rejected [326].
- *Adolescents who do not go to their doctor* and do not follow their therapy, with periodical relapses of status asthmaticus as a result of *undertreatment* [595], only occasionally undergoing therapy with insufficient doses

Table 11.57. Patterns of asthma death in children and adolescents

Risk factors

A. Onset of asthma before 3–4 years of age, especially in the 1st year of life

B. Age between 10 and 20 years of age

C. Generally severe asthma or near death episodes:
   a. Past history of severe asthmatic attacks
   b. Frequent admissions to hospital or emergency wards in the past year or
   c. One or more emergency department visits for asthma in the past year with probable intubation and/or mechanical ventilation

D. Insufficient patient education from the physician

E. Poor patient collaboration

F. Problems arising from self-treatment:
   a. Improper perception of airway obstruction
   b. Decreased use of prescribed medications
   c. Lack of adherence to asthma therapy
   d. Blunted perception of asthma

G. Poor family support for ongoing and acute care

H. Family history of atopy

I. Increasing use of short-acting β_2-agonists

J. Use of three or more antiasthmatic medications

K. Active and passive smoking

L. Respiratory infections

Contributing factors

1. Telephone prescriptions
2. Poor compliance with long-term treatment
3. Discontinuity of treatment or medical care
4. Psychosocial factors
5. Failure of family (and physician) to recognize severity of the attack
6. Delays in hospital admission
7. Undertreatment during the last attack: delays in instituting an appropriate treatment, no use or inappropriate use of CSs

Fatal attack

a. Delay in seeking care
b. Failure to recognize severity of deterioration
c. Excessive reliance on bronchodilators
d. Insufficient use of systemic steroids
e. Unclear criteria for initiating treatment of exacerbations

Negative effects of sedatives during acute asthma

1. Sedatives mask the agitation that is usually a sign of hypoxemia and delay an adequate clinical evaluation of progressive bronchial obstruction or of response to therapy
2. Sedatives suppress both ventilation and cough

Prevention strategy

A. Physicians should emphasize to parents and/or children the necessity of initiating medication to be taken at the onset of symptoms and of prescribing rapidly effective medications

B. A maintenance therapy should be followed on a regular basis, with peak flow readings test and PFTs checked by frequent visits to determine the first symptoms of airway obstruction

C. An inadequate response to bronchodilators should advise to initiate prednisone therapy

D. Parent and physician nonrecognition of asthma severity is often the cause of delays in care during the attacks and in planning admission to an ED

E. Physicians should ensure patients and/or parents of his (her) fully availability

F. Such patients should have home epinephrine for rapid use and require Medic-Alert bracelets

Data from [67, 403, 579, 623].

[377], to the point that the prescribed drug serum levels are between 0 and subtherapeutic levels [43]. This is especially alarming when these drugs are ICSs: their regular, uninterrupted use significantly reduces the risk of death from asthma by at least 50%. Instead, treatment interruption is associated with an almost fivefold increase in asthma deaths [630], likely because the *CS effect disappears after 2 weeks* [593].

- Young patients under proper observation, who are faced with *sudden attacks, progressively becoming worse* to the point of respiratory insufficiency and death within 20 min to 3 h [190], probably because of the effect of a heavy allergenic load or the intervention of other highly negative factors, but who did not avail of help quickly enough [409].

Risk Factors

It happens, however, that children and/or adolescents do not fall into this classification and die as a result of insufficient management of asthma [623], or for excessive delays in the final moments [190]; 80% of the cases studied were preventable. They can be divided as follows:

- A long period of *undertreatment,* or of scant medical care (64%)
- *Inadequate care* in the final stage (45%) or delays in asking for help, or insufficient physician knowledge of emergency treatments

Some families hesitate to call a doctor during weekends or at night [190]; In fact 75% of deaths occur between 7:00 PM and 6:00 AM [375] and life-threatening attacks and deaths show a pattern of occurring on Sunday [527]. Two studies showed that 68% of Swedish children and 35% of English children died within 3 h of the onset of the attack [190, 192]. Therefore, there is an impact of unpredictability that could also be due to *overlooked nonsevere cases* [192], whereas in dispatching an ambulance with a doctor on board (the person making the request had indicated asthma to the hospital operator), the arrival of help was shortened by 64% and the mortality rate decreased sixfold [595]. Survival in the period after the attack is made difficult by mistakes, for example, inadequate monitoring [377] or inadequate actions on the part of patients, especially those at high risk. Some consented to regular check-up requesting a medical visit after 7 h, others refused and died, having requested the medical visit only after 3 days [410].

How Patients Die of Asthma

The cause of death in cases of severe asthma can vary [76, 578]:

1. Some patients, who *died suddenly of a heart attack* preceded by tachycardia and extrasystoles, had in all likelihood taken drugs in excessive doses. To this end an abnormal pharmacological mechanism could be regarded as being the cause (possibly prostaglandins), triggered by an exogenous agent [578]; such cases habitually occur at home [190]. However, postmortem examinations carried out on some children have shown equal amounts of specific cardiac lesions among those who had abused β_2-adrenergic drugs and those treated with other drugs [595], although probably the severity of the complications in these patients was due more to hypoxia than to cardiac factors [409].
2. Often the *abuse of inhaled drugs,* such as β_2-adrenergics [375, 569, 578] has been stressed, both because they are very handy to use as they are supplied with dispensers, and because some have a longer and more intense action and an MDI dosed at 200 µg/puff [479], whereas, for example, the dosage of albuterol is reduced by half.
3. The theory that the *abuse of β_2-adrenergics* can lead to a masking of worsened underlying disease, whereas CS treatment could be needed [571], is, however, contradicted by the increase in sales for both classes of these drugs [480].
4. Another probability is that in *status asthmaticus* the lack of use or underuse of CSs, if taken in a timely manner, prevents the formation of obstructive plugs [76]. In fact, postmortem findings are often marked by highly thickened bronchial walls, with mucus plugs blocking the bronchial lumen and diffused throughout the airways [578].

Death by asthma can be sudden or progressive: in the first instance, the preponderant role of an intense bronchospasm is suspected, as it has been ascertained that recovery from a near-fatal attack occurs more quickly in these forms than in those that evolve more slowly, characterized by respiratory insufficiency following generalized bronchoconstriction and long-term inflammatory phenomena [578] similar to that found in status asthmaticus. Two potentially contributing causes should not be underestimated: *drug cardiotoxicity,* especially if hypoxemia is present, and the *onset of respiratory insufficiency,* rapidly aggravated by severe hypoxia [409]. Thus, the underlying mechanism can be represented by a reduced perception of dyspnea and by a very low chemosensitivity to hypoxia [296].

Controversies Surrounding the Risks Linked to Drugs

Sears et al returned to the risks associated with continuous therapy (in adults) with high doses of fenoterol, including a worsening of asthma and an increase in aspecific BHR [569, 571]. At the end of a retrospective study on 12,301 Canadian asthmatic adults, Spitzer et al [608] declared there was a danger related to the use of bronchodilators in MDI spray cans, maintaining that *all β-adrenergics, including albuterol, but also theophylline and oral steroids, worsen asthma and can be related to fatal cases,* without in effect providing clear evidence related to a cause–effect relationship [109]. Cromones as well as ICSs are totally excluded from a careful analysis in this data, as is albuterol if not taken in combination with fenoterol, which was the most common drug used among the deceased [608]. Following the publication of the preliminary results, the North American press underlined the serious dangers deriving from the abuse of β_2-adrenergics and the American College of Allergy and Immunology sent photocopies of the articles to all its associates as well as its Position Statement on the matter [185]. The dissertation by Spitzer et al [608] brought in a large number of controversial letters. The New Zealand authors exclude that in their country the use of albuterol had increased the cases of death between 1969 and 1976 [738], which occurred when the less selective fenoterol was commercialized [571], and therefore it is *the prolonged use at high doses* that is correlated with the risk of death by asthma or near-fatal asthmatic attacks [331]. The same group [629] subsequently quantified the risk in 10^5 asthmatics/year. The statistics are 42.8% for nebulized β_2-adrenergics and 19.2% for oral β_2-adrenergics, 44.6% for oral CSs; in direct comparison, fenoterol alone vs albuterol alone was 60.2% vs 7.4%, so the conclusion is that *albuterol is safe if inhaled alone*

with MDI, as are inhaled cromones and CSs [629]. Abuse is dangerous because β_2-adrenergics exacerbate BHR. Studies [110, 456] have reconfirmed that β_2-adrenergics show tolerance with continued exposure with a loss of clinical effect that can worsen asthma. Abuse and high doses during acute attacks lead to hypoxemia and/or hypokalemia as well as a delay in seeking medical assistance [409, 410, 479].

Therefore the use of bronchodilators is not intrinsically dangerous, but the substantial irrationality of treatment based only or mainly on β_2-adrenergics should be pointed out; its use should be associated with regular anti-inflammatory and preventive therapy [62, 68]. The main risk in the use of fenoterol is a dose-effect ratio between mortality and prescription [30], potentially caused by excessive basic dosages, as mentioned above. In line with such reports, on the one hand there is a recourse, as urged by some authors, *to halve the dosages* [612]; on the other hand the drug has been shown to be effective in moderate forms even in dosages between 10 and 50 µg, even reduced up to 1/20 of the recommended dosage [196]. However, the adverse effects of fenoterol became known when it was compared to placebo for *6 months* [571]. What criteria is used to freely commercialize β-adrenergics controlled by studies lasting barely *4 weeks* [133]? In a large sample of 16,787 patients treated for 16 weeks with salmeterol, the number of cases of death by asthma were 12 [91], among which only *one boy 14 years of age* (0.006%) [197]. There were only two deaths in the group treated with albuterol, one of whom was 20 years old [197]. Therefore we do not agree with the concerns raised by the potential danger of salmeterol in young people, provided that – as with formoterol – it is not used to treat acute asthmatic attacks since it has no effect in a short time, a specification included in illustrated leaflets only in 1994. Therefore it should not be taken too often because of the risk of accumulation causing adverse effects. As a consequence, it is necessary to start preventive maintenance therapy [76] with cromolyn, nedocromil sodium, ICS or long-acting theophylline, resorting to inhaled β_2-adrenergics prn [467], while taking care to eliminate the environmental allergenic load [76]. Theophylline (RR, 1.0), cromolyn and steroids (RR, <1.0) were associated with decreased mortality [331].

It has been highlighted that exposure to high titers of *Alternaria alternata* spores (Fig. 1.79) can trigger severe asthma exacerbations and is a risk factor for respiratory arrest in asthmatic children and young adults [457], with mortality cases significantly correlated with spore counts >1,000/m^3 [641]. In a total of 6,840 children with respiratory allergy, we have evaluated *Alternaria* prevalence. Only 89 of 6,840 children (1.3%) had monosensitization to this allergen, and all were asthmatic. Of these, 29 had perennial manifestations, 18 seasonal prevalence, which was in autumn-winter in 13 of these 18, and in spring-summer in 5. Thus, this allergy should not underevaluated, due to its protean manifestations with possible life-threatening reactions [84]. It is therefore necessary for infants and children allergic to this aeroallergen to wear an *identification bracelet* so that they can be quickly identified in case of need, as well as asthmatic *adolescents or children also suffering from FA*. Of children who died or suffered from severe shock (see Chap. 20), 92.3% were in fact asthmatic.

It is assumed that some fatal episodes have occurred in countries lacking a widely available National Health Service, or that such fatalities should be related to an absence of specialized medical assistance as well as to a lack of proper facilities to deal with such emergencies in schools [596].

Prevention

Prevention comprises a series of articulated measures aimed at reducing the occurrences or severity of re-exacerbations, to possibly enable children to enjoy a normal life similar to that of their healthy peers. In 1881, it was clear that "to prevent a return of new attacks one must advise the patient to avoid all harmful influences... to all asthmatics one must recommend *to live in pure, dry air, to avoid places exposed to dust, smoke and wind...*" [82]. As will be seen in Chap. 24, numerous factors have contributed to the increased prevalence of allergic disease. Proof of the negative influences of these factors in children with FH+ is provided by the double reactivity in 74% of children to Der p 1 and to pets and in 84% to pollens and molds [483]. Given the severity of infantile asthma, it can be understood how important it is that interventions for allergen avoidance are scrupulously carried out, which, we note incidentally, are overlooked in several guidelines where emphasis is placed above all on drug therapy [272, 435, 504, 545, 698–700]. Some *environmental measures* are truly effective. It is known that asthmatics improve markedly in aseptic environments, residence in dust-free places can have positive effects on clinical features, children improve when transferring to houses so meticulously cleaned of allergenic factors as to be comparable to a hospital room, with 50-fold reduced Der p levels [624].

Preventive Therapy

Preventive therapy is required once allergic asthma has been recorded to adopt appropriate measures to prevent recurrences. It is carried out using cromolyn, nedocromil sodium (Figs. 11.64, 11.65), ketotifen and other preventive medications [105]. The indications for prophylactic therapy, especially for a long-term therapy, are summarized in Table 11.58 [476]. These medications have no particular role in international [272] and US guidelines [434–437]. It was proposed to remove cromolyn from international guidelines recommending it as a first choice in prophylactic asthma treatment [642]; however,

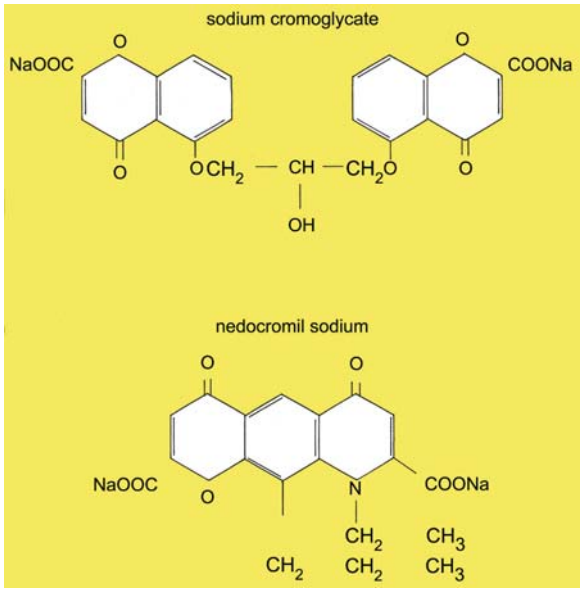


Fig. 11.64. Structural formula of sodium cromoglycate and nedocromil sodium

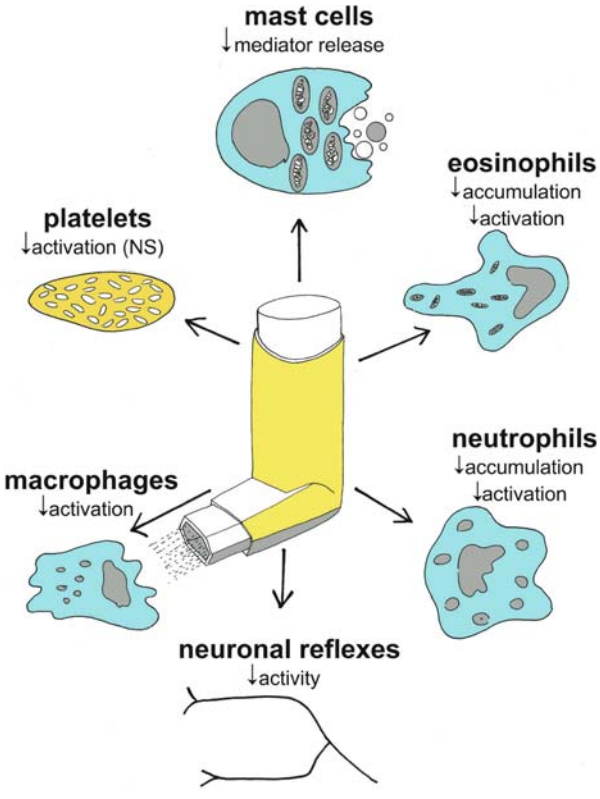


Fig. 11.65. Target cells and tissues for sodium cromoglycate and nedocromil sodium. Cromolyn sodium cromoglycate, *NS* nedocromil sodium

Table 11.58. Targets for prophylaxis in childhood asthma

Cough and wheezing more than 1–2 times a week or more than 1–2 nights a week
Necessity of bronchodilator use more than 1–2 times a day
Persistent PEFR alterations, an index of airway obstruction
Frequent exacerbations (>1 every 4–6 weeks) and of >24 h duration
Frequent hospital admissions

Modified from [476].

the Expert Panel's opinion is that cromolyn for children of all ages and nedocromil for children >5 years of age could be considered in the treatment of persistent asthma, but they *are not preferred therapies* [434].

Cromolyn is a widely used preventive drug that, by blocking mast cells mediator release, inhibits both early and late bronchospastic reactions induced by allergen inhalation, cold or nebulized air, fog and various irritating chemical substances. Thanks to these characteristics, it is currently used in the prevention of asthma and EIA symptoms in childhood [134, 419, 458, 476], starting from 13 months of age [107], having the advantage of inhibiting the response to BPT with allergens both in the early and late phases, and that of reducing aspecific BHR [251]. The numerous and extensive long-term studies that have been referred to so far in this book have confirmed both its clinical safety and its therapeutic efficacy. A recent meta-analysis on 24 trials out of 251 selected and mostly on hospital-based populations of children [642] demonstrates no improvement in the outcome of 1,000 children aged 1–4 years with mild asthma given inhaled cromolyn at a dose of 10 mg tid. However, cromolyn had a statistically significant effect on cough and wheeze compared to placebo (95% CI) and an additional decrease in cough score compared to placebo (0.13 to 0.27) and in wheeze score (0.11 to 0.26) [166, 642]. Usually, the relatively low dose of cromolyn used and the inclusion of only children with milder symptoms makes it difficult to draw conclusions about the efficacy of cromolyn in children who are given higher doses for moderate asthma [282]. Several letters commented negatively on the paper, one very well documented [166]. A meta-analysis followed reaching the same conclusions [643]. In our clinic and personally, it has always been prescribed *because of its preventive capacity,* which has been recognized by two International Consensus papers on the treatment of pediatric asthma [698, 700], which places it in the pole position in the prophylaxis of asthma.

Its effects can be summarized as follows [86, 105, 251, 258, 545]:

- Stabilization of mast cell membrane, which can be explained by:

– Closure of Ca channels, with a subsequent inhibition of mediator release
– Inhibition of phosphodiesterase, with an increase of cAMP intra-mast cell levels
– Blockage of oxidative phosphorylation that prevents mediator release
• Inhibition of the activation of leukocytes, neutrophils, eosinophils and blood platelets
• Inhibition of PAF action
• Attenuation of contractile response to acetylcholine, histamine, serotonin, bradykinin and $PGF_{2\alpha}$
• Up-regulation of PGE_2 and β_2-adrenergic-mediated bronchodilation
• Prevention of mucociliary clearance alterations
• Inhibition of macrophage degranulation via the following mechanisms of action:
– Inhibition of macrophage lysosomal enzymes (for example β-glycuronidase)
– Inhibition of chemotactic factor release (for instance LTC_4)
– Inhibition of O_2 metabolite production
• Additional properties inhibiting CD4, CD8, CD19 (B cells) and PBMCs expressing sCD23, demonstrated at the skin level (Chap. 7), making cromolyn potentially useful for treating asthma
• In vitro inhibition of IgE synthesis by B lymphocytes [297], probably by blocking their IL_4-primed isotypic switching [358], suggesting an early prophylactic effect
• Enhancement of IgG production by B cells [297], also with tachykinin antagonizing properties (Chap. 7).

Based on what has been stated, cromolyn's use in acute forms is not advisable.

Cromolyn is administered:

• By DPI, in capsules each containing 20 mg, with a predosed dispenser (Spinhaler, see "Predosed Pressurized Sprays"), with a dose of 60–80 mg/day, in 3 fractionated doses
• By MDI (20 mg/2 ml of solution),
• By pressurized MDI, with 5 mg release (average dose of 2 puffs qid).

In the US an MDI formulation of 1 mg/puff is available, which is less able to decrease BHR compared to the MDI formulation of 5 mg/puff which is available elsewhere, thus possibly delivering less medication to the lower airways [252]. Compared with the convenience of 1–2 daily doses of BUD inhalation, the dosing of cromolyn bid–qid likely increased caregiver burden [422].

Several weeks of regular use are required before it is able to carry out its effect on LAR. However, the delivery system may by a limiting factor in the efficient cromolyn delivery to young children [549].

Cromolyn is indicated:

• In *short-term prophylaxis:* 15–30 min preceding the allergen encounter, or before intense physical exercise
• In *long-term prophylaxis:* from 4–6 weeks preceding the critical period until the end of exposure
• If *a small airway obstruction* remains after bronchodilator administration

Cromolyn associated with CSs confers a *significant protection* against asthma exacerbations, asthma drug therapy, inhaled anti-inflammatory agents, *hospitalization, and ED visits* [5].

All asthmatic children can derive benefit from cromolyn therapy [436], which is virtually devoid of any side effects [86].

Nedocromil Sodium

Nedocromil, disodium salt of pyrano-quinoline-dycarboxilic acid, is an antiallergic and anti-inflammatory drug endowed with the following properties [397, 407, 424, 507, 578, 659, 704]:

• *In vivo:*
– It *inhibits mediator release* by sensitized mast cells, induced by the specific antigen and anti-IgE antibodies.
– It *reduces the amount of histamine and* PGD_2 released by sensitized mast cells, both spontaneously and following aspecific stimuli.
– It *suppresses IL-dependent IgE* production.
– It *inhibits chemotactic responses* by eosinophils (to FMLP and NAP-1/IL_8) that are stimulated by cytokines (GM-CSF and IL_3).
– It *inhibits neutrophil chemotactic* action.
– It *inhibits BHR induced by* IL_3*R*-stimulated PAF.
– It may inhibit the activity and functionality of T cells.
• *In vitro:*
– Even in very low doses, it *inhibits* chemotactic factor-induced *activation of neutrophils and eosinophils,* probably acting on proteinkinase C.
– It inhibits the *PMN-mediated mechanisms* that lead to histamine release.
– It inhibits the *release of mediators* by neutrophils and eosinophils and the release of LCB_4 and 5-HETE by alveolar macrophages.
– It prevents the *IgE-mediated monocyte* and blood platelet activation and, in high doses, activation of complement-induced proteins associated with eosinophil granules.
– It reduces *the release by the bronchial epithelial cells* exposed to asthmogenic stimuli of arachidonic acid metabolite able to induce mast cell degranulation and mucus hypersecretion.
– It inhibits the *variation in density of eosinophils* and LTC_4 production.
– As opposed to cromolyn, it also *acts on MT mast cells* and on *basophils*.

Moreover, nedocromil mitigates or halts MBP-induced harmful mucociliary dysfunction [648].

Several clinical studies have documented its efficacy in adults, also noting that it is less so in children [71, 113, 134]. In children, it significantly reduced urgent case visits and courses of oral prednisone as compared with placebo [645]. In our experimental double-blind placebo-controlled (DBPC) study [71] in children affected by grass-induced bronchial asthma, the results showed an

overall improvement both in asthmatic symptoms and in PFT compared to controls. The different opinions expressed by both physicians and parents were statistically highly significant.

The *dosage i*s two puffs (2 mg) qid.

Table 11.14 details the cromone's action mechanism.

Ketotifen has a mechanism partially analogous to that of cromolyn and is capable of the following: [105, 462, 528, 545]:

- It *inhibits mast cell mediator release,* in particular of PGD_2 [462].
- It interferes with the action carried out by some of these mediators.
- It carries out *antihistamine activity* at the level of H_1 receptors.
- It is active on the two phases of the asthmatic response.
- It inhibits *blood platelet migration and PAF release,* so that anti-PAF activity can explain many positive effects [528].

Additionally, it prevents β_2-adrenergic hyporesponsiveness and restores their responsiveness, thus improving in asthmatics β_2-adrenoceptor function and β_2-adrenergic bronchodilation [528].

In five DBPC pediatric studies [74, 511, 545, 589, 667], the following effects were documented:

- Reduction of asthmatic attacks and auscultatory findings of long-term wheezing
- Reduction in the number of days of disease
- Statistical reduction in the use of other drugs [511, 545]
- Anti-asthma prevention in 91% of cases after 3 years of therapy [74]

The effects in other pediatric studies were controversial [589, 667]. In one DBPC study, ketotifen proved less effective than cromolyn, possibly because one dose of syrup 4 ml/day was used on subjects weighing 14–18 kg; no data regarding compliance was noted [127].

Dosages. For dosages, see Table 7.19. Younger children find ketotifen particularly pleasant since it is also available in syrup form, has a pleasant taste and is easily administered. Drowsiness is its only side effect [406], which, however, we noted disappears after a few days of use. The prescribing physician should inform parents of this effect. In older children, the capsule form can be taken, preferably at night.

Antihistamines

For some years *second-generation antihistamine drugs* have been utilized and experimented in pediatric treatment of asthma [259] (Fig. 11.66). It should be noted, however, that experimental studies have yielded no consensus since there are noticeable variations regarding the type of molecule or the dosages [105]. While azelastine can reduce IAR, several other drugs seem to express their effect on LAR, an important result for the known correlation between these reactions and symptom severity [139, 303]. Table 11.59 [105, 139, 303, 528] summarizes the principal effects that are attributed to these

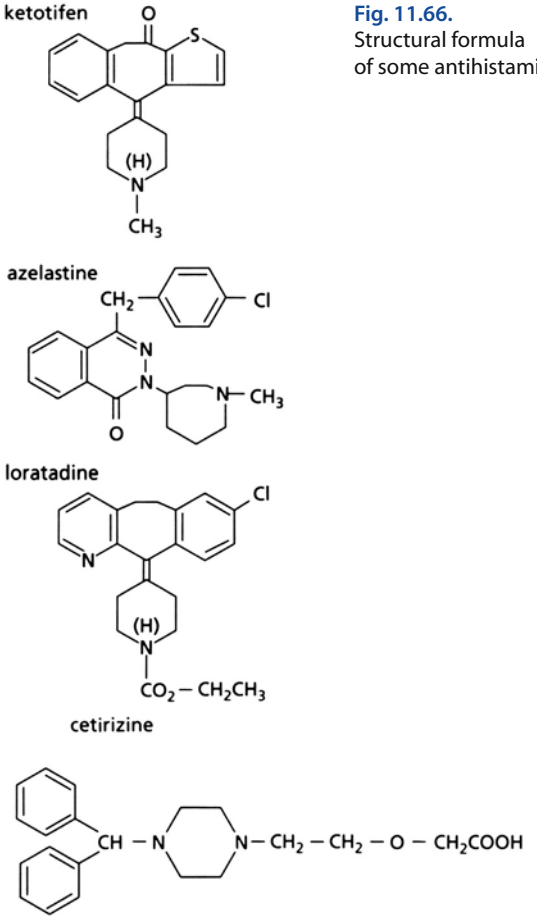


Fig. 11.66. Structural formula of some antihistamines

Table 11.59. Possible antiasthmatic effects of second-generation antihistamines

Antihistamine	Inhibition		
	Histamine release	Eosinophil chemotaxis	PGD and LTC release
Azelastine	+	+	+
Cetirizine	+	+	+
Ketotifen	+		
Loratadine	+	+	+
Oxatomide	+		+

Data from [105, 139, 303, 528].

drugs; therefore we will simply clarify a few points (see Chap. 12). These drugs fulfill a protective activity related to their half-life with a variable interval of time, ranging from 4 to 12 h [105, 139].

Azelastine

Azelastine has proved to be a powerful inhibitor of histamine-induced bronchoconstriction. Once optimal serum levels have been reached, however, it is not also able to antagonize LTC_4-induced bronchoconstriction, though it is able to significantly reduce SP levels in BALF [439]. A randomized DBPC study has concluded that azelastine is capable of significantly inhibiting IAR, without, however, having any noticeable impact on LAR [662].

Cetirizine

Various studies on humans have shown that cetirizine can reduce eosinophil, neutrophil and basophil recruitment by 75 % [303], as well as PAF-induced bronchoconstriction, without leading to a similar LAR reduction in the airways, as demonstrated by PFTs, [636]. An antiallergic effect is produced on epithelial cell CD54 expression [81]. Recent evidence suggests that it inhibits eosinophil adhesion to endothelial cells [322]. A randomized DBPC study among adults has shown that cetirizine is able to reduce the number of eosinophils in BALF and inhibit their enrollment [519]; cetirizine's ability to induce consistent reductions of FEV_1 and of histamine-induced bronchoconstriction is also known. In 348 children and adolescents with grade II and III asthma, cetirizine reduced the number of days with symptoms as well as the need for other drugs, on a par with cromolyn [303]. The formulation in drops has allowed us to treat very young children (aged 6 months and older), always with very good results.

Long-term *Levocetirizine* has proved to have a first-rate preventative activity in >200 asthmatic children aged 5–6 years and older treated by us (one tablet = 5 mg/day).

Fexofenadine is a new drug that can be given to children. It is more useful in the treatment of AR and has no effects either on the cardiovascular system or the CNS.

Loratadine

In vitro, loratadine significantly inhibits PAF-induced eosinophil activation, superoxide generation, and nasal secretion of histamine and PGD_2 after the challenge [165]. Another long-term antihistamine is *desloratadine* (a loretadine metabolite), with anti-inflammatory and anti-allergic activities in addition to its antihistaminic activity, it has a 27-h elimination half-life, which facilitates once-daily dosing. Desloratadine does not cause sedation or prolong the corrected QT interval, can be administered without regard to concurrent intake of food and grapefruit juice (Chap. 12), like the other antihistamines. We have prescribed doses of 2.5 mg (1.15 ml of syrup)/day to children aged 2–5 years and of 5 ml (2.5 ml of syrup)/day to children aged 6–11.

Oxatomide

In addition to having an antidegranulation effect on mast cells, inhibiting even the activity of serotonin at the receptor level and, partially, of LTs with spasmogenic activity, oxatomide reduces the bronchoconstriction induced by methacholine and by physical exercise, as has been demonstrated by a DBPC study in asthmatic children [301].

The formulation in drops has allowed us to treat young children, always with effective results.

Nonpharmacological Therapy

Asthmatic children are strongly encouraged to participate in a sport. As a preventive measure, the family doctor should be consulted on the choice of the most appropriate activity. Though not everyone agrees [359], *swimming in a swimming pool* is most certainly advisable because the effort is well balanced, warm and humid air is inhaled, it helps to develop respiratory functions and it tones up the body. It is useful to know in advance the percentage of Cl in the water, because high levels could be an irritant. We have seen how an airway cooling down linked to hyperpnea can provoke EIA. As previously suggested, a preventative measure for this form of asthma – *a filtering mask* – could protect bicycling or motorbiking asthmatics or those engaged in long-distance skiing in the winter, by covering the nose and mouth and thus avoiding the continued inhalation of asthmogenic cold and dry air [618]. It is possible that the increased prevalence of asthma in long-distance skiing [335] is dependent on the lack of attention given to preventive measures.

Other Interventional Measures

The disease impact on the asthmatic's everyday life: information on asthma and on its treatment provided to the family (in the manner deemed most appropriate) enables children to better face their disease and follow their medical therapies with greater diligence; moreover, performing their breathing exercises more frequently to help them relax, leads to a possible reduction in the sometimes excessive use of their medications [272].

Greater education also improves the quality of life: by reducing problems such as cough, wheezing, trouble

Table 11.60. Limitations on the quality of life in children and adolescents

Limitations (%)	[657]	[213]
Limitations in physical activity, playing and sports		
Running	85	
Running up hills		78
Playing with pets	36	
Swimming	33	45
Sleeping	30	
Playing with friends	30	
Basketball	27	
Bicycling	26	55
Playing sport	20	63
Soccer	29	
Surfing		33
Emotional function		
Feeling frustrated	62	
Feeling uncomfortable	58	
Feeling different from friends	54	
Frustrated not having a normal life	53	
Feeling concerned/troubled about asthma	52	
Feeling frightened by an asthma attack	49	
Mad or angry because of asthma	45	
Problems related to school		
Days missed at school due to asthma (1–99)		78
Asthma attacks at school		43

breathing, activity limitation, anxiety, loss of sleep or frequently interrupted sleep and worry over the physical symptoms of their disease and the possibility of future attacks, the array of stressors that a child has to face as a consequence of suffering chronic asthma [448], the patient can be ensured a life that is as normal as possible, even regarding school, sports and above all social activities, etc.

The overall situation can be made easier and improved *if pediatricians act promptly and effectively* not only during the critical attacks, but *also in the intercritical times,* via focused treatments aimed at preventing future attacks. We are referring to the serious problems faced by a child prone to EIA who will return to play and take part in sporting activities as a direct result of an adequate preventive therapy. A good pediatrician–patient relationship will undoubtedly be beneficial to the psychology of asthmatic children and adolescents and will assist them in facing the disease and daily activities.

In adolescents, psychological problems occur more frequently: the doctor should treat these special aspects of the disease, including the refusal to acknowledge symptom presence, widespread emotional problems including lack of cooperation, social aspects, excessive recourse to inhalers, smoking, etc.

Even some aspects *related to school* can require the doctor's assistance. In schoolboys, schoolgirls, young adolescents, problems related to modifications of behavioral attitudes or learning disabilities caused by medication are often present. It is for this reason that doctors must educate both children and parents about exceptional cases of disturbances possibly occurring, and that such problems often last not less than 2 weeks [711].

To better illustrate the seriousness of the problems relating to pediatric asthma, the results of two studies done on 100 asthmatic children aged 9–13 years [657], and in 4,161 adolescents (958 asthmatics and 3,203 nonasthmatics) and 1,104 of their teachers [213] (Table 11.60 [213, 657]) are reported. The trial showed that 42%–59% of the subjects believed that asthmatics can become addicted to their drugs, 70%–82% that there could be fewer problems if the drugs could be taken in class, 36%–45% that asthmatics are embarrassed about using their inhalers and 47%–56% that teachers are worried about taking asthmatics on school outings or to summer camps. Even if comprehension toward asthmatics was greater among students than among school teachers ($p<0.0001$), the teacher's positive participation in asthmatics' problems is significant [213]. The results of a subsequent trial on 381 students aged 8–18 years are more optimistic: the findings showed that there is only a 30% restriction on their participation in youth activities [41]. Nevertheless, it is difficult to quantify that many young children will not be able to fulfill their dreams, for example that of being *able to run and play football* like so many other children with no restrictions whatsoever.

Table 11.61 [284, 435] summarizes the advantages and disadvantages of the main therapeutic methods outlined so far.

Outcome

We have gathered the relevant data in Table 5.15, defining positive outcome in 41%–47% of cases. In the last few years, a high percentage of children (43%–76%) whose asthma persisted as they grew into adulthood has been noted. A large number of children or adolescents who remain asymptomatic can have relapses and/or anomalies in their PFT that do not return to normal, even after 3 years [219, 575]. It is important that these children are regularly examined by their pediatrician, enabling a timely identification of those subjects who are at risk of suffering from relapses on reaching adult-

Table 11.61. Advantages and disadvantages of medications for treating pediatric asthma

Medications	Advantages	Disadvantages
β-Agonists	The most potent and rapid bronchodilator drugs available at the moment	The regular use may mask the airway inflammation and disease progression
Corticosteroids	The most potent anti-inflammatory drugs for treating both asthma and airway hyperreactivity currently available, topical steroids offer the most secure therapeutic range	Oral: the side effects suggest their use as a maintenance therapy in the most severe forms Inhaled: unconfirmed growth hypothalamic-pituitary-adrenal axis suppression.
Theophylline	The best available drug to add an extended bronchodilation and anti-inflammatory effects	Narrow therapeutic range, variable clearance, requiring careful monitoring of theophylline levels, unconfirmed negative effects on temper, learning, etc.
Antihistamines	See Table 11.59	Continual anti-inflammatory therapy
Anticholinergic	Delivered by nebulizers may be added to β-agonist therapy in acute conditions	Modest bronchodilators less potent than β-agonists
Cromolyn	Reduces symptom scores, airway hyperreactivity and necessity of other medications	Prophylactic, is less potent than corticosteroids, not effective in all children
Nedocromil sodium	Reduces symptom scores, airway hyperreactivity and necessity of other medications	Prophylactic, is less potent than corticosteroids, not effective in all children
Allergen avoidance	Allergen-specific, may reduce and eliminate symptoms	None, major discomfort for children and families
Immunotherapy (Chap. 13)	Definitive cure of asthma, abatement of symptoms, airway hyperreactivity and need of medications	Poor compliance in small children, high cost, children should be observed 30 min after injection should be started early, well before that asthma aggravates

Data from [269, 419].

hood. On this matter, in the studies cited in Chap. 5, various parameters were predictive of asthma at an adult age. Up to the present time, no study has demonstrated that pharmacotherapy is able to modify the natural history of asthma, whereas SIT is able to do so. On the contrary, as has been noted, the discontinuation of a therapeutic cycle is followed by a reappearance of symptoms [671], which also confirms our experience. The inflammatory process at the basis of asthma persists even after years of CS treatment, even if it can be reduced [652]. Nonetheless, it can resurface displaying all its functional characteristics [671]. As we have seen, there are various antiasthmatic treatments available for use at an infant age and we could additionally recommend ensuring a timely diagnosis and early adoption of preventive measures.

Present and Future Prospects

In the long term, anti-inflammatory strategies could include nonactivation of T lymphocytes or of IL_4, inhibition of the isotypic conversion after the second signal emitted by the cytokines, and direct elimination of B_{IgE} cells through anti-IgE monoclonal antibodies [24, 301]. There are two treatments that *cure* asthma, in addition to SIT.

Anti-IgE

In a randomized DBPC study, a cohort of 334 entrants aged 6–12 years with moderate or severe allergic asthma received a recombinant humanized monoclonal antibody that binds to free IgE at the same site as the high-affinity (FcεRI) receptor (omalizumab). A 28-week treatment reduced the requirement for ICS while protecting against disease exacerbation, while serum free IgE was reduced in 95 %–99 % of cases [405]. Children in the omalizumab-treated group reported significant improvements in the activities and symptoms domain scores as well as in the overall asthma-related quality of life compared with placebo [342], but also formoterol [183]. In a pooled analysis of three multicenter, randomized DBPC studies, omalizumab reduced the rate of serious asthma exacerbations and the need for unscheduled outpatient visits, ED treatment, and hospitalization in children with moderate-to-severe allergic asthma [118].

Leukotriene Modifiers

Anti-LT could have a positive influence on ASA and/or block bronchoconstriction responses in BPT, leading to a decrease in CS use [329]. Figures 11.60 and 11.61 sug-

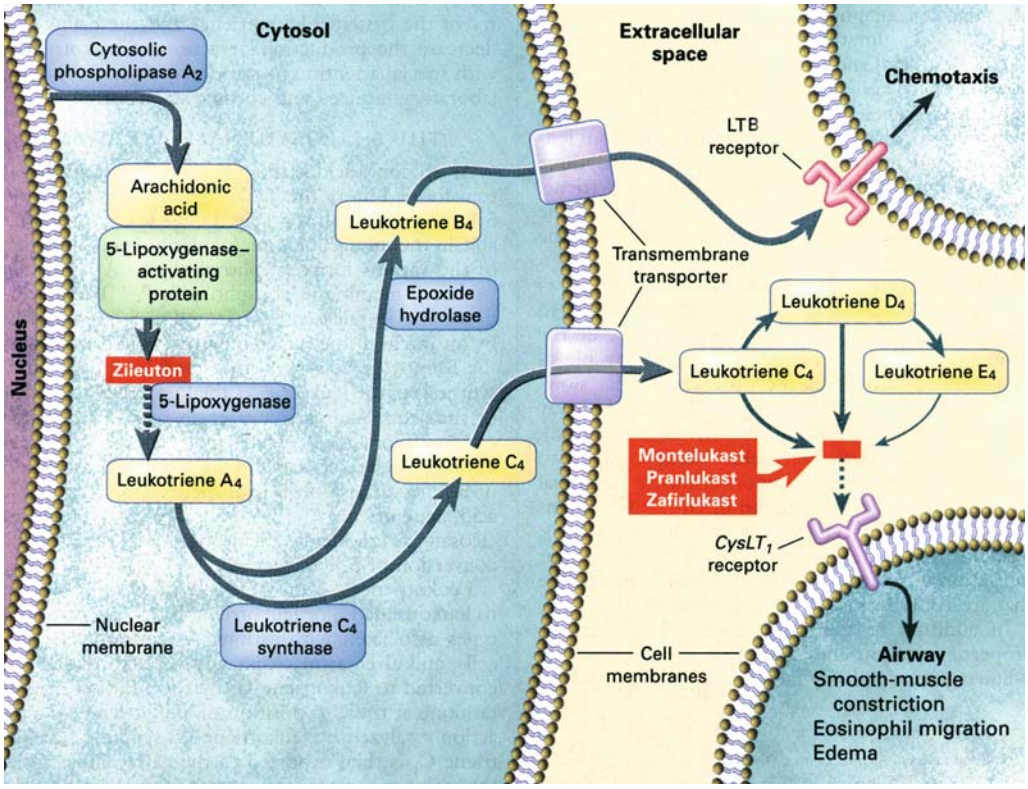


Fig. 11.67. Biochemical pathways of LT formation and action and sites of action of LT-modifying drugs. Enzymes are shown in *blue*, products in *yellow*, essential cofactor in *green*, and drugs in *red*. Although the synthesis of LTB4 and LTC4 probably takes place in close proximity to the nuclear membrane, for clarity they are shown throughout the cytosol. *LTB* denotes the LTB receptor. An individual cell may produce the cysteinyl leukotrienes, LTB4, or in rare cases both

gest the use of anti-LT drugs, though they have yet to be thoroughly tested: zileuton, an inhibitor of LT synthesis and montelukast, pranlukast and zafirlukast, competitive LT receptor antagonists, as well as pobilukast, tomelukast and verlukast, though the last three are more properly inhibitors of the activation of cysteine-derivatives receptor (LTC_4, LTD_4 and LTE_4). The spirometric data of children of 6–14 years with chronic asthma, who were treated with montelukast (oral monodose of 5 mg), demonstrated a net improvement [308], added to BUD in 279 children aged 10.4 ±2.2 years, it induced a clinically relevant decrease in the number of asthma exacerbation days [591]. A DB, multicenter, multinational study at 93 centers on 689 children (aged 2–5 years) with persistent asthma given 12 weeks of treatment with 4 mg of oral montelukast has shown significant improvements compared with placebo in multiple parameters of asthma control, including daytime and overnight asthma symptoms, percentage of days with and without asthma symptoms, need for β-agonists or oral CSs, physicians' global evaluations, and peripheral blood eosinophils [309]. We have treated 40 children aged 3.1–5.1 years (mean, 3.7 years) with montelukast. Compared to 41 controls, there was a significant reduction in the mean incidence of day (74%) and night (69%) wheezing in addition to a significant amelioration in EIA compared with controls, and in younger children a significant increase in weight. A mean difference between study children and controls was evidenced by spirometric data. We conclude that in pediatric asthma management, chewable tablets of LT modifiers can be used as substitutes of long-acting β-agonists and ICSs, especially in young children who may have difficulty in using in-

Table 11.62. Antileukotriene drugs: route and doses of administration in children

Anti-LT	Route	Doses/Ages
Montelukast (at bedtime)	Oral	4 mg once daily/2–6 years
		5 mg once daily/6–14 years
		5 mg once daily/6–14 years
Zafirlukast (1 h before or 2 h after a meal	Oral	10 mg bid/ 5–12 years
Zileuton	Oral	Children aged 12 years and older

Data from [159].

halers. Montelukast was significantly effective when cat-sensitive children aged 6–14 years with mild persistent asthma were exposed to high levels of cat allergen [492]. Table 11.62 reports the related doses, and Fig. 11.67 shows the biochemical pathways of LT formation and action. It should be noted that in 32 children 6 to <24 years old the 4 mg dose was equally effective and free of untoward adverse effects [402].

Montelukast administration also reduced the increased Th2-like T cell IL mRNA expression in lung tissue and protein in BALF found in OVA-sensitized or challenged mice, and markedly reduced the increased lung mRNA expression of Th2-like T cell ILs [246]. In other animal studies, CD54 modulation during treatment with CSs could represent a new and more selective treatment for the control of the chronic airway inflammation and BHR that characterize asthma. *The effect of CD54 could be associated with significant reductions of* eosinophilia and consequently, of *BHR* [230]. Apart from other immune effects, CSs (Figs. 11.43, 11.44) inhibit IL action on eosinophils, and cetirizine and picumast inhibit their activation in vitro, even though not originally introduced for this purpose [528]. The initiative to positively alter the levels of PAF by means of SIT [265] or ketotifen [528] and, finally, that of blocking BHR by inhibiting iNOS by means of CSs [443] is a stimulating prospective. Of special interest would be a method that could monitor NO production in infants and young children, aiming at focusing on the role of the inflammation in early asthma and fostering a strategy for timely intervention [759].

The list of new compounds for the pharmacological control of asthma is long and, in addition to new applications of antihistamines, also include PDE IV inhibitors, anti-TXA_2 and anti-TXA_2-synthetase, tachykinin antagonists, inhibitors of peptide releases from C-fibers, K^+, Ca and Cl channels deactivators, M_3-selective antagonists, inhibitors of IL_5, CD antagonists of adhesion molecules, and inhaled NSAIDs [418]. Also foreseeable are antagonists of PAF, tryptase, quinines and of some chemokines, inhibitors of FLAP and of PLA_2, antagonists of α-adrenergic receptors, modulators of transcription factors and new antimuscarinic drugs. Finally, the antagonists of tachykinin receptors could eliminate bronchial smooth muscle contractions [426] and cold-induced bronchoconstriction [744].

Most strikingly [243], an anti-IL_{17} mAb (monoclonal antibodies) treatment regimen has been shown to abate bronchial neutrophilia in parallel with reduction of bone marrow and blood neutrophilia. This treatment also raised eosinophil counts in the bone marrow and bronchial IL_5 production, without alteration of allergen-induced BHR.

A crucial role may be played by TARC (thymus and activation-regulated chemokine) CCR4 receptor expressed by Th2 cells in bronchial airway epithelium. Asthmatic patients exposed to a relevant allergen release large amounts of TARC in their BALF; costimulation with IFN-γ, but not with the duo IL_4–IL_{13}, stimulated human bronchial epithelial cells to further increase TARC mRNA and protein expression. TNF-α amplifies IFN-γ ability to induce TARC 30-fold [38]. TARC concentrations are elevated in childhood asthma [345], thus TARC up-regulated in bronchial epithelial cells may play a role in the pathogenesis of allergic asthma [38]. This marker is also linked to plasma total IgE levels and cat allergen sensitization [345]. As a consequence, CCR4 antagonists may have a substantial impact in treating allergic asthma.

In another area of increasing interest, administration of a stable analog of lipoxin A4 (LXA4) blocked both BHR and pulmonary inflammation, as shown by decreased leukocytes and mediators, including IL_5, IL_{13}, eotaxin, prostanoids and cysteinyl LTs [349]. Moreover, blocking IL_{13} [150], receptors, or the downstream signaling pathway activated by their ligation, could provide one strategy to improve the specificity of asthma treatment [321].

In the animal model, TrkAd5 is able to modify the airway late hyper-responsiveness to histamine, brg sequestering endogenous NGF. Notably, the TrkAd5 administration causes the contractile response to histamine to be lower than control after ovalbumin challenge, thus showing potent effects in allergic asthma [686a].

Other Pediatric Allergic Lung Disease

These are pulmonary interstitial disorders having an immunological pathogenesis, caused by the inhalation of various antigenical agents active in subjects who are particularly vulnerable; such disorders are rare at the pediatric age.

Extrinsic Allergic Alveolitis

Also known as hypersensitivity pneumonitis, farmer lung disease (described by Ramazzini in 1713, Chap. 4), and pigeon breeder's, EAA can occur in children aged 3–15 years [111, 319, 395]. It is rare in infants [168] and >80 pediatric cases are known [111, 137, 168, 223, 248, 319, 389, 411, 748]. The pathogenesis is of sensitization to inhaled allergens, generally thermophilic actinomycetes or avian antigens [111, 319]. In addition to the classic cases of actinomycetes that contaminate fodder [111] or of other mycetes that pollute houses [319], there is evidence of a growing number of cases related to contaminated heating and air-conditioning systems [132] and to birds in cages, pigeons, etc., in the barn, house, and the child's bedroom [248, 319]. The latter form is the most widely studied in children, with cases caused by free-roaming city pigeons [137], a problem in most countries. There can be type III reactions with formation of antigen–antibody complexes at the alveolar level [411], but by studying BALF a significant lymphocytosis

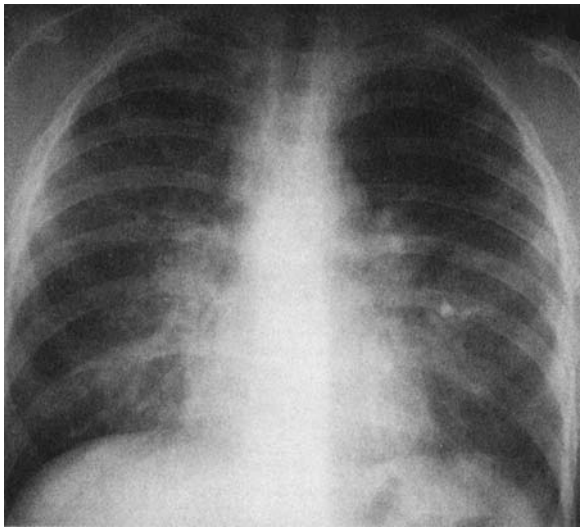


Fig. 11.68. Chest radiograph of an 11-year-old boy with EAA (for details see text). After a short course of oral corticosteroids a complete resolution is seen with fading pulmonary parenchymal shadows

Table 11.63. Differential diagnosis of EAA

Idiopathic forms
Alveolar proteinosis
Autoimmune disease
Ciliary dyskinesia
Congenital cardiopathy
Cystic fibrosis
Gastroesophageal reflux
Hamman-Rich disease
Pulmonary hemosiderosis
Sarcoidosis
Exogenous forms
Chemical substances
Drug-induced pulmonary conditions (Chap. 19)
Eosinophil pneumonia
Interstitial pneumonia (by *Mycoplasma*, *Chlamydiae*, etc.)
Pneumoconiosis
Psittacosis

Modified from [319].

during the acute period, with CD8 predominant intervention, has been demonstrated. These reactions could therefore assume a more significant pathogenetic role [319]. Pulmonary damage is the result of the immune response characterized both by antigen-specific antibodies and cellular hypersensitivity with IL releasing PBMCs. Complement activation in the airways and the irritating effect of thermophilic actinomycetes [95] should also not be overlooked.

The *insidious onset of acute forms,* secondary to exposure to high concentrations of avian antigens, includes increasing lethargy, weight loss, a febrile bronchopneumopathy with a prevalence of respiratory symptoms such as breathlessness and cough, which can also be spasmodic, and effort-induced dyspnea appearing 4–8 h after exposure, with auscultatory findings of rales at pulmonary bases [111, 319]. Complications may occur in the form of sudden respiratory failure [111]. If exposure is prolonged or repeated, the process becomes chronic with aspecific insidious signs such as worsening of the overall general condition, effort-induced dyspnea and even at rest [411], and marked weight loss [319]. More frequently, the picture is subacute with coexisting respiratory and systemic symptoms; among these a general feeling of ill health, anorexia, asthenia, etc. [111].

Diagnosis depends on history of exposure to birds, clinical findings, positive avian precipitins, restrictive defects on PFTs, and a suggestive chest X-ray appearance [223], and in children exposed to birds or playing in barns with a history of breathlessness, or strolling amidst city pigeons [137]. X-ray results reveal a fine miliary pattern, diffused to both lungs with interstitial infiltration in the lower fields and hilar enlargement in acute forms, and diffused interstitial fibrosis with accentuation of the network in chronic forms (Fig. 11.68), but may be unremarkable [248]. PFTs show a restrictive ventilatory defect with reduced lung volumes and compliance [111, 319]. SPTs positive to pet danders provide both immediate and delayed results [411]. Lymphocytosis is found in BALF (>50%), a net reduction of both CD4 and CD8 rates and of NK cells, with 87% sensitivity and 72% specificity [319], or 85% of CD8 expressing the activation marker HLA-DR and 32% of $CD4^+$ and 16% of $CD56^+$ [389]. Follicle-like aggregates of B cells in the lung interstice may indicate that local antibody synthesis may be involved in an antibody-dependent cellular cytotoxic mechanism [389]. Differential diagnosis is illustrated in Table 11.63 [319] and should be extended to children presenting with unusual respiratory symptoms and signs early [223].

Avoidance of triggering agents entails that clinical findings subside within a few days [425]. CSs are effective and resolutive, especially if accompanied by environmental clearance [75], unless insufficient child compliance or a relapse due to a new exposure to specific allergens occurs [319].

Allergic Bronchopulmonary Aspergillosis

Though rare in children, ABA is often found in adult studies reporting the onset of symptoms in childhood [75]. Often it is caused by airway colonization by the mold *Aspergillus fumigatus* (AF) (with 18 different allergens, Table 1.74), which proliferates in soil in great numbers where it can be isolated (Fig. 1.77). In addition to being found in wheat fields and more generally wherever there is vegetation, it is also found in humid houses, trash cans, vegetable substances, rotting wood, freshly cut grass, old hay, fallen leaves, in bedding and

Table 11.64. Differential diagnosis of allergic bronchopulmonary aspergillosis (*ABA*)

Diagnostic criteria	Comments	ABA	Allergic asthma	Cystic fibrosis
		(%)		
Chest X-ray infiltrates	Present in some studies	100	0	100
SPT+ to *Aspergillus fumigatus*	Diagnostic, not specific	100	13–38	30
Raised total serum IgE	Markers of ABA activity	80–100	50	20
Precipitant antibodies to Af	Not specific to ABA	60–90	25	35
Eosinophilia	Absent if treated with steroids	100	40	20
High specific IgE/IgG to Af	Essential and specific to diagnosis	100	<5	<5

Data from [95].

in common household floor dust [95]. Since it is very small, with an average diameter of nearly 3 µm, inhaled spores can reach the peripheral airways where they can proliferate, aided by internal temperature [95]. In ABA other species of AF, *Candida albicans*, *Mucor*, *Penicillium* spp., *Cladosporium herbarum, Helminthosporium* spp., *Stemphylium* spp., *Torulopsis* spp., *Curvularia lunata, Rhizopus* spp., *Drechslera* spp., *Pseudallescheria* spp. [416] are likewise implicated. Mold growth is accompanied by an intense type I, III and IV immune response: antigen release with production of IgE and IgG antibodies. Activation of Th2 T cells orchestrating pulmonary inflammation has also been shown in ABA together with the expression of genes for several ILs present on the *5q* chromosome [95]. They are restricted by HLA-DR2 and HLA-DR5 and produce high concentrations of IL_4 but few of IFN-ɣ [99].

ABA is characterized by five stages: I acute, II remission, III recurrent exacerbation, IV progressively ingravescent and steroid-dependent, which, if not treated, evolves into stage V, with diffuse pulmonary fibrosis [75, 95]. Some 28 pediatric cases have been reported [75, 292, 369, 416, 425, 631, 693], aged 0.11–18, including 3 girls aged 0.11–8 with cystic fibrosis [292, 369, 425], two girls aged 6–7, with GER of asthmatic origin and multiple sensitizations to mycetes [75] and a 6-year-old boy with *cladosporiosis* [416]. The symptoms are an asthma-like syndrome with recurring afebrile bronchospastic episodes, coughing, dyspnea, wheezing, and pulmonary infiltration [95].

ABA diagnosis is made on the presence of classic hyphae in the sputum (Fig. 11.69) and on X-rays pulmonary parenchymal wedge-shaped shadows, typical of ABA can be observed depending on the phase of activity (Fig. 11.70a). In the pediatric age, a CAT scan is more appropriate, as it permits a more rapid diagnosis [580]. The presence of marked eosinophilia serves as a guideline. The presence of precipitant antibodies, findings of fungal hyphae in the sputum and fungal isolation in cultures [95] may have diagnostic value. SPTs reveal immediate and/or delayed reactions to the molds. For the diagnosis of the condition, high levels of total IgE (>1,000 ng/ml) [369] and IgE antibodies are relevant

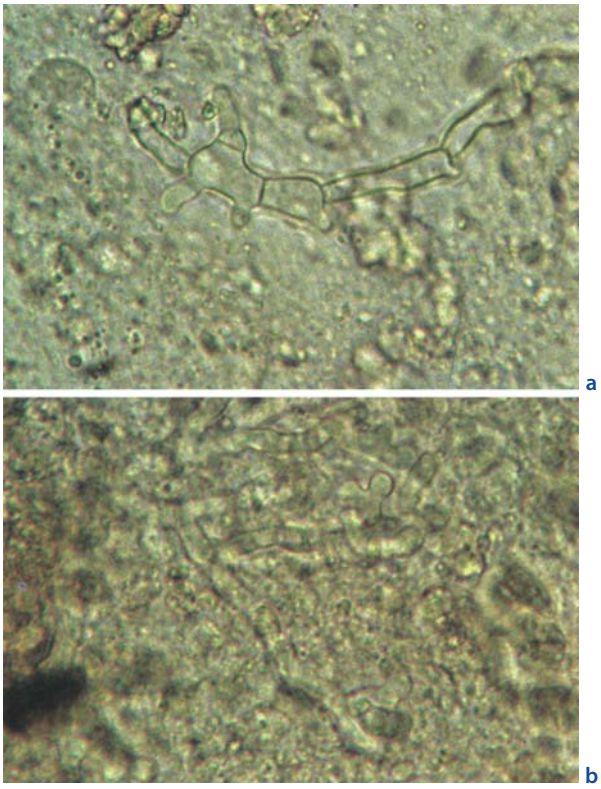


Fig. 11.69 a,b. Allergic pulmonary aspergillosis. Note the characteristic appearance of the hyphae (branching separate structures) in the sputum of a 10-year-old girl

such as the significant association with sIgE and IgGs to AF [75] (Table 11.64) [95]. Children with CF [292, 369] also have anti-AF sIgE [369]; therefore, to obtain a higher specificity, it is safer to take sIgE for CD46 measurements using ELISA [355], but a child with CF and acute symptomatic ABA with low serum IgE levels has been reported [369].

Steroid therapy for a few months causes remission (stage II), which continues after 10–15 months of follow-up [75, 416, 693]. Under steroid treatment, clinical findings improve, regression can be seen in the X-rays (Fig.

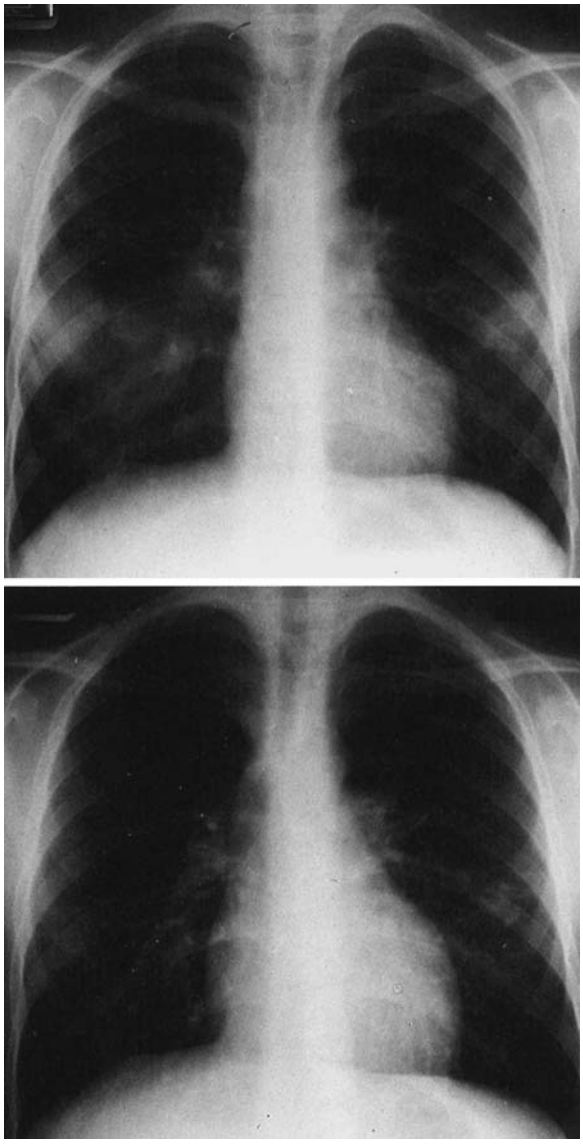


Fig. 11.70. **a** Chest radiograph of an 8-year-old girl with ABA. **b** Note the improvement of the girl after 2 weeks of treatment with high dose oral corticosteroids

11.70b), peripheral eosinophilia serum IgE titers are reduced, and fungal hyphae are cleared from the airways [75]. Total IgE decrease from 1,596–2,900 kU/l to ≈1,000 ng/ml after treatment [75, 416] and blood eosinophil count from 1,690 mm^3 to 980 mm^3 [416]. Monitoring total IgE and sIgE/IgGs titers is an excellent index of ABA activity, since their levels are newly increased in case of progression from stage II to III [95]. However very high titers of serum IgE may also be present in several other illnesses, including AD and parasitic disorders.

Pediatricians and Pediatric Asthma

Apart from pulmonary disease caused by mycetes, the secret of successful management in asthmatic children is to avoid prescribing only symptomatic therapies, but to *try to find the casual factors first,* specific to each child; therefore every form of treatment should *be tailored to the individual child.* The negative psychological factors to which we have already referred can trigger asthmatic symptoms, aggravate the attacks and negatively influence compliance with the therapeutic regimen. From our examination of doctor–patient relationships the importance of the pediatrician's role emerges. This doctor must not appear to be simply one who prescribes drugs or tests, but must also fulfill the role of being a friend full of enthusiasm, encouragement and understanding, both to young patients and their families. He must also instill a responsible attitude toward the disease and bring them to an understanding that asthma that is not treated effectively can, more often than not, result in the child or adolescent growing into an invalid adult. Especially in cases such as these, pediatricians should be particularly close to children and their family. Following an asthmatic child for a long time provides an opportunity for acquiring important information on the course of disease and the physical and mental repercussions asthma can have on those involved. Problems related to quality of life and limitations to leading a normal life, or playing and participating in sports are problems with which children must contend. Here we underline the different character of both asthmatic children and adolescents. The real difference is school absenteeism, chronic infirmity, the causes of delayed growth and the effects of HPA-axis depression, which should be considered carefully, especially if alternative treatments are available. The positive aspect, which we have always preferred, is that of giving value to the intercritical periods rather than to side effects, recommending the practice of sports, which can improve quality of life. On the other hand, self-treatment programs have yielded disappointing results, or are based on cycles of hospitalization and do not always achieve positive results. In other cases that we have regularly noted, treatment of children may involve their families in a better understanding of the clinical effects after giving an informative overall picture.

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Allergic Rhinitis

The Airway Entrance

Allergic rhinitis (AR) is a very common disease. This condition, which predominates during the childhood years [48], is found most often in boys up to 10 years of age, and at equal rates with girls from the ages of 10–20 years. Studies conducted in many countries in recent years, thanks chiefly to the ISAAC study (Table 5.21), have revealed a significant increase in its prevalence, especially among 13- to 14-year-olds, *reaching a rate of 80% in Paraguay*. There is an increase in prevalence from the age of 15–17 years, of 18% in girls vs 14% in boys [184]. As for the minimum age of onset, Table 5.5 shows that children *as young as 1–3 months are sensitized to pollens*. At this age children may be sensitized to HDM (house dust mite), pets and pollens [78]. While 50% of cases were diagnosed within the 1st year in the United States, this took place in an area with a dry climate and thus lacked the most well-known allergens [149]; however, the case of a little girl shown in Fig. 5.23 is eloquent. A recent study from Denmark [174] during a 6-year follow-up in a population of 7- to 17-year-old subjects reported a significant association between the SPT (skin prick tests) sensitization to grass (RR=2.6) and HDM (RR=2.7) or a positive IgE screening test (RR=2.4) and the development of AR. Children of primary schools with pollen sensitization have a significantly higher risk of developing AR symptoms (OR, odds ratio = 2.63) and of getting pollen asthma, but only *0.69% of doctors* have diagnosed their symptoms as such [139]. This is a sound demonstration that AR is a disease largely neglected in the literature as well as in clinical care. We have seen how pollutants are a contributing factor in the onset of pollen-induced rhinitis (Fig. 4.18) and we wonder to what point other similar interactions increase its prevalence. The complications due to unexpected cross-reactions (Table 1.73) also between distantly related plants are commonly attributable to a group of proteins, such as profilins, present in 13 different pollens (Table 1.72). AR can be a source of serious problems for both the child and the family, since it is quite *frustrating* at all ages, albeit for different reasons. For example, in the youngest children, a loss of the sense of smell, a complication that frequently goes undiagnosed, may result in a loss of appetite, increasing emotional tension in the other family members at mealtimes [171]. The continuous throat-clearing, noisy breathing, annoying sniffing, snorting, and coughing often lead to social isolation at school and conflicts at home [181], all of which have negative reflections on the already suffering mind of the child, who is inclined to *skip school,* just like his classmate affected with atopic dermatitis (AD). It must also be considered that children, in particular the youngest ones, do not complain about their problems in the same way as adults do. Furthermore, for various reasons, they are often out of their parents' sight and care and, as a result, their symptoms may go unnoticed, resulting in an obvious delay in providing an appropriate treatment [120].

Nose Physiology

The airways start in the nose, one of the most vascularized organs of the body, and an integral unit that functions together with other sections of the airways, continuously under attack from contaminants and various stimuli. Tables 12.1 and 12.2 [48, 76] show the make-up of its secretions and their purposes. Thanks to its peculiar anatomy, it functions as an organ of respiration, *as a first line in host defense,* as the organ of olfaction, and to provide resonance [116]:

- It cleans and humidifies inspired air.
- It purifies, by mucociliary clearance, the air of foreign particles, thus acting as a first line of defense against respiratory tract pathogens.
- It holds moisture and heat in the front part of nasal cavities, where it is relatively cool.
- It acts as a resonance chamber for the voice.

The heating of the inspired air, made possible by the great blood flow within the nose (42 ml/100 g of tissue/min) [188], brings it to a temperature (T) of 30–32 °C in the pharynx. Environmental air is also humidified through the addition of 0.5–1 l of H_20, since the relative humidity is 79% in the nose, and 95%–98% in the trachea [180]. Thus conditioned, the air can proceed to the delicate structures of lower air passages without being irritating for them. To be able to carry out these functions, the nose must have an adequate surface area: first of all, it is practically *doubled by the septum,* together with the *three turbinate bones,* so that the nasal mucosa cover a total area of from 100 to 200 cm^2, with a thickness varying from 10 to 15 µm [180].

Table 12.1. Constituents of human nasal secretions

Mucous cell products
Mucous glycoproteins (10%–15%)
Serous cell products
Aminopeptidase
Lactoferrin (2%–4%)
Lysozyme (15%–20%)
Neutral endopeptidase
Peroxidase
Secretory leukoprotease inhibitor
sIgA (15%) and secretory component
Uric acid
Plasma proteins
Albumin (15%)
Angiotensin-converting enzyme
Carboxypeptidase N
Immunoglobulins
IgA (not secretory) (≈1%)
IgG (2%–4%)
IgM (<1%)
Kallikrein
Indeterminate source
CGRP
Urea
Inflammatory mediators
Bradykinin
EDN
Histamine
LTC_4
MBP
PGD_2
TAME
Tryptase

See abbreviations list.
Date from [48, 76].

Table 12.2. Functions of human nasal secretions

Protective functions
Antioxidant
Humidification
Insulation
Lubrication
Provision of adequate medium for ciliary function
Waterproofing
Barrier functions
Macromolecular filter
Microorganism and particulate matter entrapment
Transport and elimination of entrapped materials
Defense functions
Extracellular site for multiple enzyme functions
Extracellular source of IgA/IgG antibody
Antimicrobial function
IgA/IgG antibody
Lactoferrin
Lysozyme
Rapid deployment of multiple plasma proteins

Data from [48, 76].

The purification of inspired air is provided first by the vibrissae of nasal vestibule, which block particulate material >15 µm, and then by the mucociliary action, which effectively eliminates material <10 µm. Particles and harmful gases contained in 20 m^3 of inhaled air are taken up by mucus and drained passively toward the pharynx by ciliary beat movement (400–800/min), at a speed of 6 mm/min [180]. The cilia of the front cavity *move forward,* while those in the rear cavity *move rostrally,* to remove secretions in both directions. In nasal mucosa, the epithelial surface is protected by a mucous bilayer, a superficial gel layer (85% of mucous glycoproteins) and underneath a thin liquid sol layer. The outer gel layer takes inhaled solid particles and/or debris up, while the cilia, which need a fluid medium to perform their function, grab the gel layer with their tips during the forward movement, thus ensuring the progression of solid particles; so the particles move easily within the sol layer, which, in turn, enables the beating of the cilia [188]. The mucous layer is secreted, removed, and replaced constantly: its rapid turnover contributes to the *barrier functions* [76]. Therefore, when harmful external stimuli pass across the mucosal barrier, not only do they damage the mucous membrane locally but, by interfering negatively on humectation, filtration, and heating of inspired air, they can cause disturbances in other parts of the *tracheobronchial tree:* for example, inactivation of the mucociliary system (Fig. 11.17) leads directly to sinusitis (Chap. 15).

As a consequence, it can be understood that particles >5 µm are filtered almost 100% by the nose, so few intact pollen grains would be expected to reach the lower airways provided that the nose functions properly. Amylaceous granules making up grains (<3 µm), as well as smaller fungal spores (5 µm), may reach the bronchi, causing asthma.

Pathophysiology

The human nasal mucosa is lined by ciliated pseudostratified columnar epithelium and nonciliated goblet cells. A lamina propria rich in seromucous acinar glands, blood vessels, and nerves is beneath the epithelium. An area rich in sinusoids is deeper in the tissue. The *rich vasculature* of the submucosa contributes to the onset of rhinorrhea and nasal congestion. In fact, the vascular architecture is characterized by arteriovenous anastomoses and cavernous sinusoids, which, when they become engorged, may lead to nasal obstruction [122, 187] (Appendix 12.1) [107]. The tissue may swell due to *increased vascular permeability* or sinusoidal engorgement; it may secrete mucous, serous, or goblet

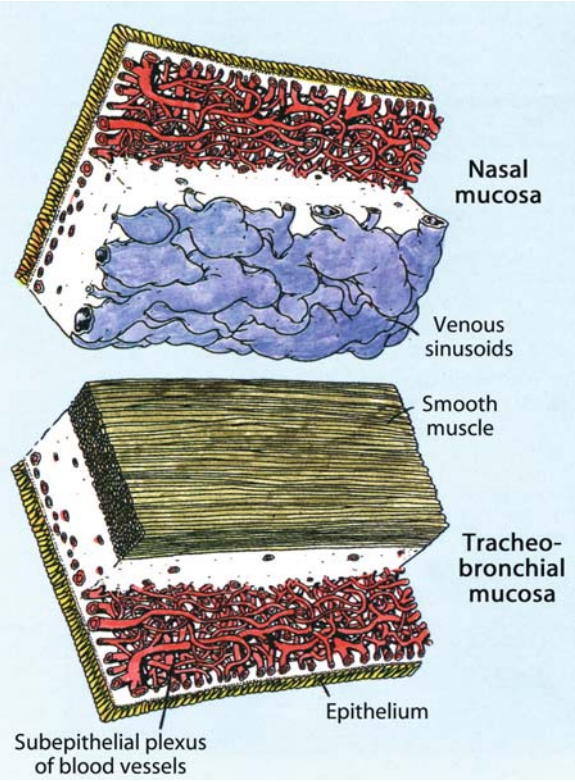


Fig. 12.1. Differences and similarities between nasal and bronchial airway tissues. Differences and similarities are emphasized: a central obstructive mechanism of nasal passage is filled with venous sinuses; tracheobronchial smooth muscle constriction in the lower airways is comparable . However, the epithelial lining and the abundant subepithelial network of microvessels are similar in upper and lower airways and so are exudative and absorptive mechanisms

cell products. The differences and similarities between nasal and bronchial tissues can be seen in Fig. 12.1 [122]. Furthermore, the openings of epithelial and periglandular capillaries permit the rapid blood circulation inside the vein walls. It is believed that these peculiarities aid in controlling the moisture and T of inspired air [48].

Histamine, an important mediator acting on nasal mucosa vasculature, may directly increase permeability, causing rhinorrhea [131, 165]; however, other authors report that histamine causes neither this increase nor that of nasal blood flow [166], whereas still others confirm the increase in permeability after histamine challenge [187]. Rhinorrhea and congestion may also be influenced by histamine indirectly [68], by stimulating glandular secretion, as deduced from the homolateral nasal provocation test (NPT), which provokes in atopics a contralateral increase in sIgA (secretory IgA) [187]. Also possibly implicated in causing rhinorrhea are histamine challenge released secondary mediators, such as bradykinin and TAME (tosylarginine methylester) [165]. TAME reproduces the clinical sequence of sneezes, and the production in nasal cavities of histamine, leukotrienes, and PGD_2. High concentrations of kinins may induce vasodilation and increase in vasal permeability and increase capillary flow [98], together with hyperalbuminemia in nasal discharge during a Rhinovirus infection [126].

The autonomous nervous system (ANS) plays an important role in rhinitis symptoms [68, 151]. Nasal mucosa is richly innervated by sympathetic neurons containing norepinephrine and NPY(neuropeptide Y) with vasoconstrictor action and by parasympathetic neurons containing acetylcholine, PHM (peptide histidine methionine), VIP (vasoactive intestinal peptide) inducing vasodilation, serous cell secretion, mucous cell secretion, and epithelial secretion [187]. Other transmitters such as CGRP (calcitonin gene-related peptide), SP (substance P), NKA (neurokinin A), and GRP (gastrin-releasing peptide), are released in the nose by C-type sensitive nerves [130] (Table 11.9). *Neuropeptides* are all mainly distributed to submucous glands, arterioles, and sinusoids. On the other hand, the binding sites are very different: the arterioles bind CGRP and NKA, the epithelium and submucous glands bind GRP, and the vessels, glands, and epithelium bind SP [130]. In other words, the sensitive fibers prime a neural reflex that stimulates nasal secretion. In AR the parasympathetic antagonists inhibit rhinorrhea, but not nasal congestion or sneezing [4]. Challenge with a parasympathetic agonist, methacholine, increases secretion, while pretreatment with atropine inhibits it; moreover, methacholine has no effect on nasal flow, which is, instead, reduced by oxymetazoline. Therefore *glandular secretion is under cholinergic* control, while *the vasculature is under adrenergic control* [131]. Cholinergic stimulation causes arteriolar dilation, thus enhancing passive diffusion of plasma protein into the glands by 50%–100% [76], while adrenergic stimulation of the mucosa has little or no effect [106].

Lastly, nasal response cannot be divided into distinct categories; in fact, even if histamine is classically identified as a mediator released by metachromatic cells, NPT with cold air causes an increase in both histamine and tryptase levels, and both itching and congestion [127, 173]. Moreover, NPT may produce a delayed response in the same way as allergens [68]. Antihistamines have no effect on cold air NPT, while they inhibit nasal response to NPT with allergens [172]. Interestingly, the allergic reaction intensity is aided by the so-called *priming effect:* repeating the NPT on consecutive days in subjects with ragweed pollinosis, but outside of pollen season, a progressively lower quantity was necessary to provoke the same clinical symptoms that appeared after the first NPT (Fig. 12.2) [38]. Repeated exposure to a pollen to which a subject is sensitive induces *nasal hyperreactivity* (NHR), not only to the pollen studied, but also to a second pollen not immunologically correlated with the first. The concept has been revisited in children with perennial or persistent AR (PAR) from Der p, in whom two subsequent NPTs with allergen doses, too low to

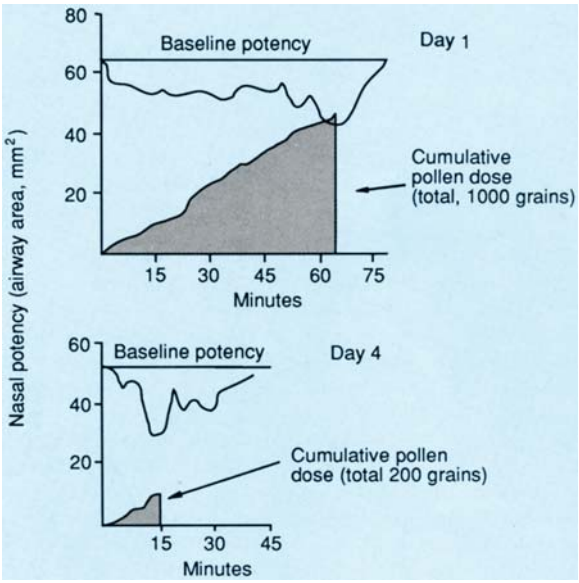


Fig. 12.2. Effect of nasal priming. On 4 successive days, an allergic rhinitis (AR) patient was given a decreasing dose of ragweed pollen (1,000, 800, 400 and 200 grains). On day 1 >60 min of exposure and 1,000 pollen grains produced a similar reduction of nasal potency as 15 min at 200 grains on day 4

cause significant responses, led to an increase in both immediate and late response [80]. Since the priming is not correlated with cumulative doses, but with repeated exposures during the natural pollen season, it may explain the symptom's chronic nature and persistence. Another important issue to be considered is the mucociliary system, which may suffer from the insults of allergens, viruses, cationic proteins, etc. In particular, in AR, allergen contact with the sensitized nasal mucosa causes the ciliary movement to progressively slow down and then stop [108].

Nasal Immunology

The mucosa covering the nasal cavity is in close contact with the *NALT* (nasal-associated lymphoid tissue), anatomically equivalent to Waldeyer's ring (Chap. 15), and is part of the mucosa-associated lymphoid tissue (*MALT*). B cell clones endowed with a rather immature memory, but with the potential to express the J chain, are initially stimulated in both tonsils and adenoids, and then in the NALT. They migrate into the glandular tissues, where they differentiate, turning into Ig/plasma cells (immunoglobulins). The sIgA antibodies localized to serous cells, as an integral part of the immune exclusion mechanism [14] (Chap. 9), are charged with the crucial duty of *defending the nasal epithelium* from infectious agents and outside irritants. In this function, sIgA is supported by aspecific defensive mechanisms such as the mucus forming a mechanical barrier against particulate material, together with the mucociliary system, lactoferrin, and lysozyme, secreted selectively by the serous glands with *bacteriostatic and bactericidal properties,* and several other substances and secretions [9] (Tables 12.1, 12.2). IgA antibodies are mainly gland-associated (76.7%) and less surface-associated (46%), whereas in inflammatory rhinitis the proportions change to 62% and 24%, respectively [14]. Other Ig isotypes are present in nasal mucosa: IgG antibodies are found in the lamina propria and are largely produced in nasal mucosa, even if no inflammation is present. On the contrary, there are few IgM antibodies that seemingly predominate in the GALT (gut-associated lymphoid tissue); moreover, many more IgD antibodies are found than in other sites of MALT and are probably tonsillar in origin [14] (Table 12.3) [9, 14, 191]. IgE/plasma cells are practically absent, even if they apparently increase in allergic subjects [58]. Whether these IgE are really associated with both mast cell phenotypes or with *Langerhans cells* (LCs), rather than deriving from an autochthonous production, is largely disputed [70]. It is not clear in what structure the isotype switching takes place and whether B cells produce IgE in nasal mucosa [4]; however, there are IgE antibodies [58], and their nasal localization is demonstrated by their cultivation [202].

Another immune function of nasal mucosa is the *defense from aeroallergens* through the production of antibodies. The immune exclusion may act by preventing microorganism adhesion, including *Haemophilus influenzae*, *Streptococcus pneumoniae*, etc. to nasal and nasopharyngeal mucosa [149]. Immune events occurring in nasal mucosa during infections or AR are connected with an IgG increased secretion in interstitial

Table 12.3. Percent distribution of nasal immunocompetent cells

Localization	CD4	CD8	B	IgA	IgG	IgM	IgD
Epithelium	65	31	4				
Subepithelium	57	22	21	50	50	1	1
Glandular area	39	50	11	80	20	2	2
Deep vascular area	57	30	12	80	20	2	2

Data from [9, 14, 191].

tissues, with consequent transudation of serum IgG into nasal secretions. These IgG responses have been designated as immune elimination [14], since the properties of these antibodies act as a *second line of defense* against invading antigens. Nevertheless, as is well known, IgG persistence may be deleterious to nasal mucosa, if immune complexes increase capillary and epithelial permeability, thus allowing bystander antigens such as food, bacterial, and viral antigens to penetrate into nasal mucosa. The vicious circle that ensues may lead to a persistence of inflammation in nasal mucosa [9].

All immunocompetent cells are represented, most lymphocytes are T cells: on the average there are 110 $CD3^+$/mm of basement membrane (BM), without differences between allergic and control subjects. Of these, 66/mm are $CD4^+$ and 23/mm $CD8^+$; therefore $CD4^+$ are 2.87-fold the $CD8^+$ [70]. Generally speaking, CD4 T cells predominate in the mucosal superficial layers, while CD8 are prevalent around the submucous glands (Table 12.3), where numerous Ig-producing cells are found, among which the IgA^+ antibodies predominate [130]. Others have demonstrated that these proportions correspond to the lamina propria, while in the epithelium of allergic subjects, CD8 T cells are 1.9-fold of CD4 [58], and NK cells account for only 5% [119]. Of the CD3 T cells, TcRγδ cells total 3% of the Vδ1 family, are more adherent to epithelial surfaces [167], where they are significantly more numerous, and are activated since they express $CD45RO^+$, but are absent in the serum [119]. We emphasize these findings, which reconfirm that *blood lymphocytes do not reflect their number in inflammation sites*. $CD25^+$ account for 0.6%, so in allergic subjects the main lymphocytes are $CD3^+$, $CD4^+$, and CD25 [70], while for $CD14^+$, $CD19^+$, and CD22 there are no significant differences [70, 110]; HLA-DR antigens were few or none in the epithelium but were present in striking numbers in the lamina propria [58]. In the serum, a reduction has been recorded of CD4, CD45RO, CD45RA, and CD19/CD23, with significant differences as compared to nonallergic subjects [58, 70]. B cells ($CD22^+$) and monocytes/macrophages ($CD14^+$) are found more in the lamina propria and perivasal sites [58, 70]. More specifically, there are 16 macrophages/mm of BM, 27 plasma cells [76], 16 mast cells, 90% of which are tryptase and chymase-containing mast cells (TCs) and 0.5/mm of lamina propria, 80% T cells, one-third of which are IgE^+ [130]. These cells are heterogeneous, yet they produce many interleukins (ILs): IL_3–IL_8, $IL_{10,}$ and TNF-α, while it is not clear which phenotype produces IL_{13} in the lamina propria [119].

Basophils and eosinophils are the main cells responsible for nasal allergic response [43, 110]. In atopic children, the close correlation has been demonstrated between the increased prevalence of these cells (highest at age 4 years in the presence of atopic disease, mainly AR), sensitization to inhalants and foods, and high IgE levels [200]. During the pollen season, activated eosinophils accumulate in both submucosa and epithelium [8], and mast cells migrate with basophils to nasal mucosa epithelium. Mast cells only penetrate [59], while basophils reach the submucosa, but neither lymphocytes nor basophils increase in number, unlike epithelial mast cells, which increase from 0–10% at rest to 40% during the season [69]. In normal subjects, there are 7,000/mm^3 in the tissues and only 50/mm^3 in the epithelium [188].

Immunopathology

In the nose, there is again a predominance of Th2 lymphocytes, and the mechanism that causes their supremacy has been clarified. Thymus and activation-regulated chemokine (TARC) has been shown to facilitate the recruitment, activation and development of Th2 polarized cells, as well as *Th2-like ILs in nasal mucosa.* Combined stimulation with IL_4 and TNF-α, as well as IL_{13} and TNF-α, synergistically induced TARC expression in epithelial cells and fibroblasts. Significantly, IL-induced TARC was higher in epithelial cells obtained by AR patients than in those from nonallergic patients [170].

Nasal mucosa is the first point of contact with inhaled allergens, and act in concert with the NALT. Since a considerable quantity of antigens penetrate into the nasal cavities, these cavities need structures that guarantee an effective protection against the harmful action of such invaders. The mucous layer is *the first innate defense mechanism,* which carries out its functions by taking up and eliminating the potential antigens via an important engagement of secretions; a crucial protection is constituted by antigen-specific sIgA. In the mucosal epithelium, the encounter between mast cells/IgE^+ and the antigen takes place, followed by histamine and other mediator release, as demonstrated by mast cell degranulation (Fig. 12.3) [69]. This has an effect not only on the mucosa, but also on the vasculature, the calyciform cells, and nasal neurons [124]. The interactions between antigens, nasal mucosa, and NALT depend on the nature, quantity, and frequency of antigen contact, and on an injured epithelial tissue. Airborne allergens penetrate into the nose and deposit on nasal mucosal epithelium, drained by superficial cervical lymph nodes. There they are taken up and processed by APC (antigen-presenting cells) LCs, which travel to the posterior lymph nodes and the adenoids, where antigen-specific T cells, homing in on the nose, are stimulated [108]. Therefore an excess of antigen material could make its way directly through the posterior cervical lymph nodes and reach the nose. In general, the inflammatory alterations of seasonal rhinitis (SAR) are milder and less persistent than those of PAR [13], as seen in the data collected on 1,002 patients before treatment (Table 12.4) [99]. The results of a study conducted on 50 children 2–7 years of age are even clearer (Table 12.5) [98]: the high levels of eosinophils and basophils in AR and those of neutrophils in nonallergic rhinitis are evident.

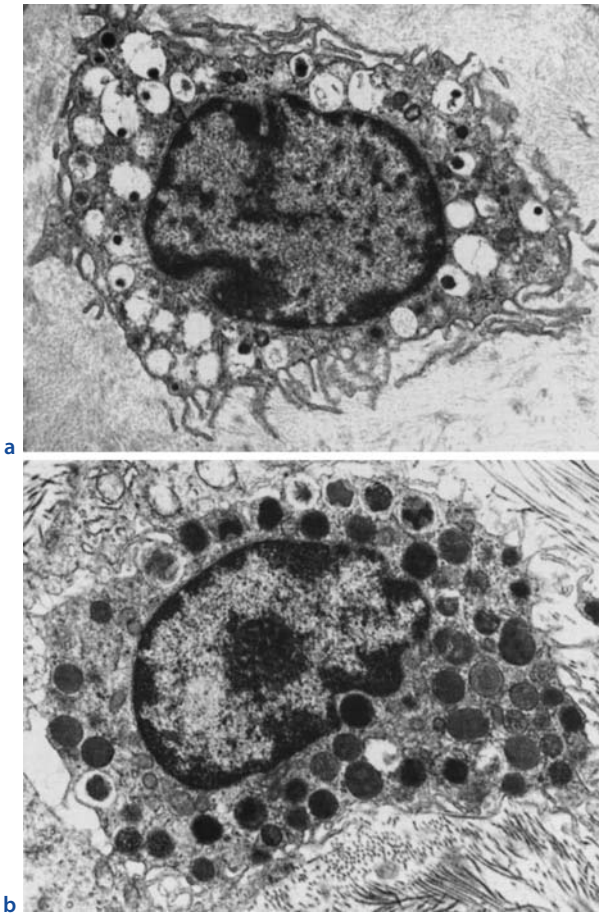


Fig. 12.3. Electron micrograph of nasal mast cells from patients with SAR: out of season (**a**, ×10,000), and in season (**b**, ×8,000) identifying evidence of degranulation in season

Figure 12.4 [14, 26, 50] summarizes a hypothesis of the pathogenesis of nasal allergic inflammation.

Immediate Phase

A secondary nasal antigen challenge triggers immediate allergic reaction (type I), which peaks within 15 min (with a corresponding peak of eosinophils), and then gradually subsides, with a consequent cascade of stored or newly synthesized mediators in nasal mucosa (Fig. 12.5). The duration of clinical manifestations is usually shorter than 1 h; however, cellular, vascular, glandular, and nervous reactions resulted in typical clinical manifestations in nasal mucosa. *Nasal obstruction* is highlighted by an increased nasal mucosal volume provoked by parasympathetic neurons inducing blood vessel dilatation. Blood levels of many mediators decrease during the initial phase, then in 20% of cases increase again 6–12 h later during the late phase [12], which explains the *symptom recurrence*. Complement *in loco* activation may also be followed by a further metachromatic cell mediator release by binding to anaphylotoxins, which have direct effects on the vascular epithelium [2]. *Histamine* plays a salient role in the immediate phase, where it is released by mast cells, while in the late phase it is released by basophils, the participation of which has been studied closely [124]; in the first hours the histamine passes from 4.3%±2.7% before challenge to 10.3%±3.8% at the 4th h [73]. There is a considerable difference with histamine in normal and nonallergic children, compared to children with AR: ≈0.2 vs ≈10 pg/µg protein [98] and, also with tryptase, in ragweed-allergic subjects and symptomatic subjects

Table 12.4. Mean percent of 1,002 patients with each cell type examined per field in the nasal cytograms of patients at the start and end of five studies (mean)

Allergic rhinitis	Eosinophils		Basophils		Neutrophils	
	Start	End	Start	End	Start	End
Seasonal (SAR)	80.1	40	47.4	22.6	71.3	63.1
Perennial (PAR)	100	44	55	12	92	82.5

The data refer to three SAR studies and two PAR studies.
$p<0.05$ to $p<0.01$ in patients receiving active treatment vs placebo.
Data from [99].

Table 12.5. Mean number of each cell type (standard error of the mean) per field in the nasal cytograms of 50 children

Disease	Eosinophils	Basophils	Neutrophils
Normal	0.11 (0.07)	0.15 (0.07)	4.87 (1.93)
Allergic rhinitis	6.96 (2.16)*	6.72 (2.40)*	11.93 (5.75)
Nonallergic rhinitis	1.08 (0.44)	0.14 (0.09)	20.41 (7.76)*

*$p<0.05$ in comparison to the other groups.
Data from [98].

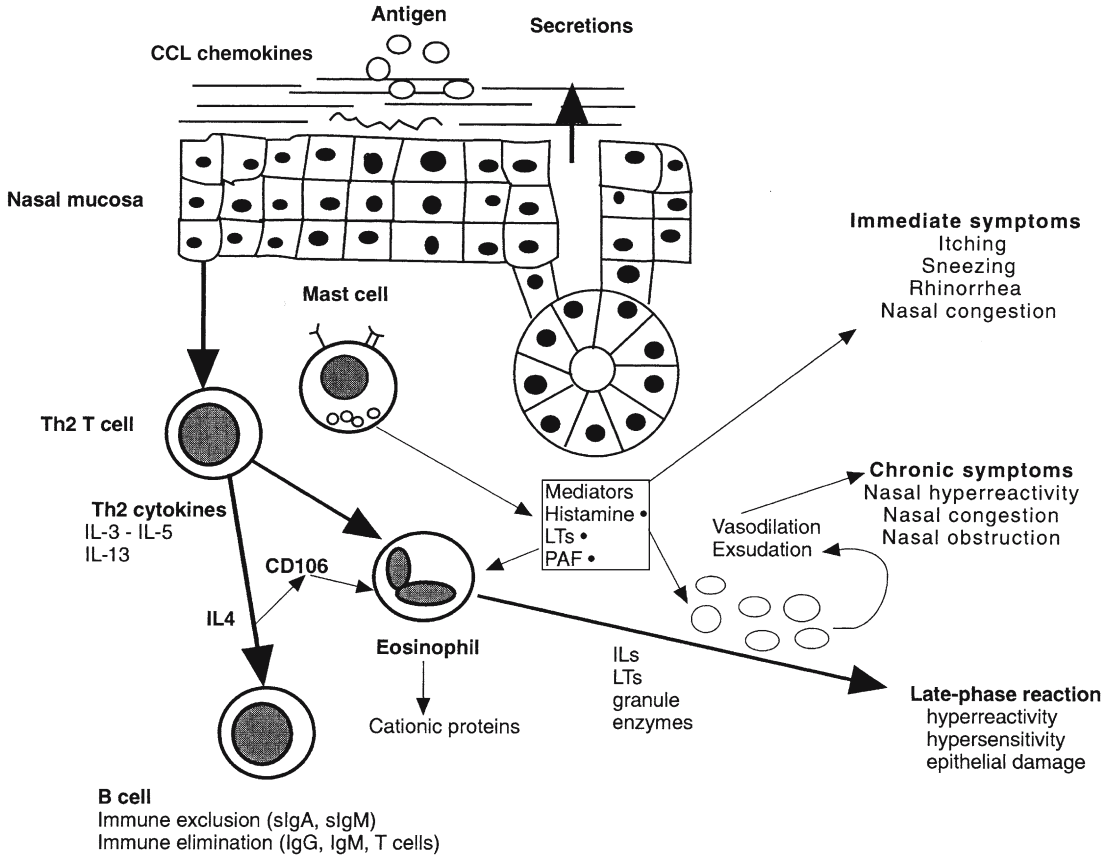
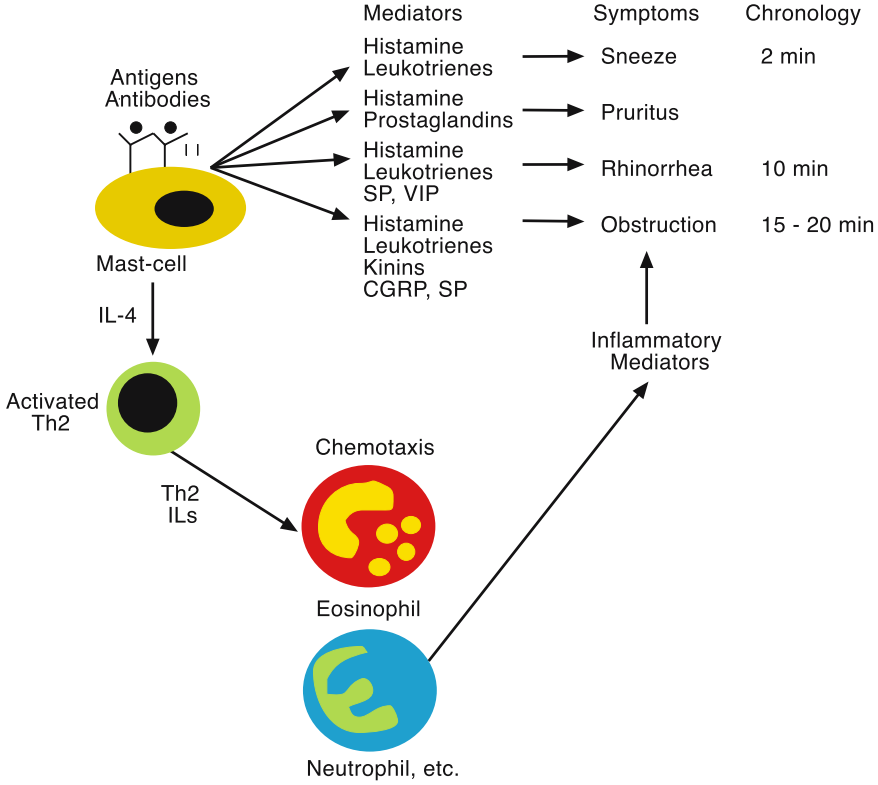


Fig. 12.4. Scheme proposed for AR pathogenesis (see text). *Potential targets for corticosteroids. (Data from [14, 26, 50])

Fig. 12.5. Direct role played by mediators in the acute symptoms of AR. *CGRP* calcitonin gene-related peptide, *VIP* vasoactive intestinal peptide, *SP* substance P. (Modified from [5])



compared to control subjects [84]. After the initial phase, allergens interacting with T lymphocytes increase locally activated metachromatic cells expressing CD40L, the amount of which decreases in parallel with the histamine, from 10.1±5.4 before challenge to 4.4±2.2 by the 2nd h [73]. The *mast cell role is significant:* thanks to fixing on FcεRI, they release IL_3 (activation of basophils), IL_4 (synthesis of IgE by isotype switching of B lymphocytes piloted by Th2 T cells), and IL_5 (which along with GM-CSF interacts with eosinophils via endothelial adhesion molecules). This suggests that a positive feedback mechanism is triggered, increasing the inflammatory response. Mast cells are the only cells of nasal mucosa that carry IgE [70, 130]. They are localized in strategic sites, represented by inflammatory *foci* and small vessels, therefore near HEV (high endothelial venule) receptors, which respond with increased vascular permeability, near glands that respond by secretion, and near sensory nerves with H_1 sensory receptors that respond by starting an itching sensation, thus eliciting the sneeze reflex [51]. Mucosal mast cells produce IL_4, IL_5, and IL_6, which proliferate in the allergic epithelium [4], in addition to histamine, release PGD_2, kinins, and tryptase, responsible for the immediate symptoms: the release of these mediators shows the priming effect [172]. Compared to the total number of mast cells, the percentage of T positive cells is 26.4 in allergic subjects and 7.4 ($p<0.01$) in control subjects [70]. Most effects associated with AR symptoms can be reproduced by histamine challenge: histamine and other vasoactive substances induce vasal permeability, thus causing *immediate symptoms* such as rhinorrhea, as well as stimulating sensitive fibers that provoke sneezing, by reflex stimulation of sensitive nerves of nasal mucosa. LTD_4 (leukotriene D_4) and PAF (platelet hyper activating factor) are substances with opposite effects: LTD_4 causes nasal obstruction, while PAF reduces nasal resistance. LTs generated by 5-lipoxygenase further increase vasodilation, vascular permeability of the microvasculature, and mucous secretion [69].

Studies using *in situ* hybridization have confirmed, in parallel with skin studies, the presence of *Th2-like cytokines* (IL_3, IL_4, IL_5, IL_9, IL_{10}), with a highly significant correlation between IL_5 and activated eosinophils [4, 50]. Their recruitment is thus associated with cells expressing the mRNA for Th2-like ILs, including GM-CSF [50]. *In childhood, nasal eosinophilia is significantly correlated with allergic symptoms* [99]. The Th2 cell-driven inflammation is amplified and maintains itself (immediate-type alterations, unlike in asthma, prevail over late-type alterations) by activating eosinophils and neutrophils with their store of harmful proteins, proteolytic enzymes, and toxic O_2 radicals, with limited effects compared to asthma [99]. Further *basophil* release of HRF (histamine release factor) -mediated histamine is observed, as well as eosinophil chemotactic factors released by epithelial and endothelial cells (altered by mediators in case of long-standing inflammation),

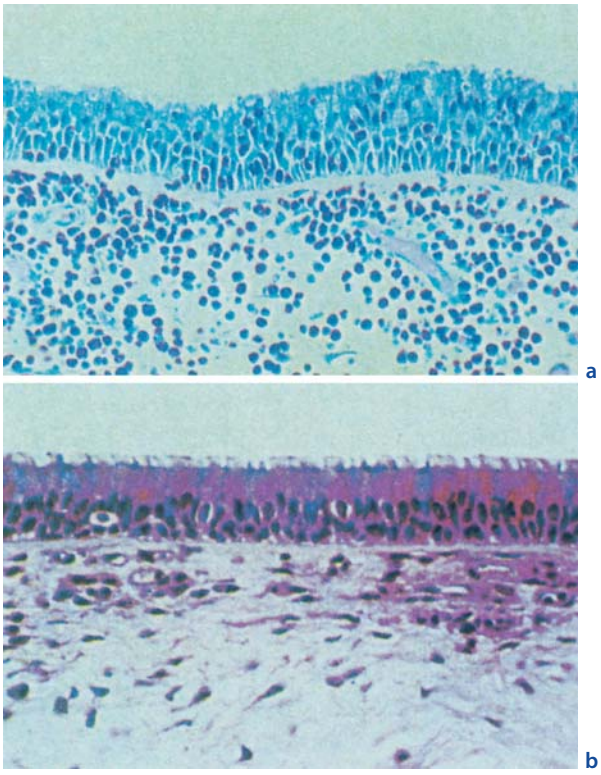


Fig. 12.6. **a** Photomicrograph of Giemsa-stained nasal tissues showing marked eosinophilia. ×400. **b** Anti-RANTES staining. ×400

tachykinin subsets including SP and CGRP, which increase capillary blood flow, fibroblasts and epithelial cells activated by TGF, IL_1, TNF, and CCL chemokines such as MIP-1α and -β [4], MCP-1 [84] and RANTES [169] (Tables 1.54–1.56). MCP-1 is significantly increased in allergic subjects during the season, while IL_8 levels are unchanged [84], and RANTES generated *in loco* plays a crucial role in *eosinophil* infiltration into nasal mucosa [169] (Fig. 12.6). The migration of inflammatory cells, chiefly eosinophils carrying VLA-4 (CD49d/CD29), is ensured by vascular endothelium only by VCAM-1 (CD106) (Table 1.2), the expression of which on the endothelium 24 h after challenge *is* significantly fostered by CD62E and CD54 [87]. Eosinophil recruitment is selective because CD49d/CD29 cannot be found on neutrophils (Table 1.45). At the pollen season peak, budesonide (BUD)-treated patients had significantly lower nasal lavage fluid IL_5, EDN and ICAM-1, and eosinophil-CD11b was suppressed, with no effects on IL_4 and IL_6 levels, ragweed-specific IgE seasonal increase, and nasal fluid IgA antibodies [79]. Unlike in asthma, there is no BM thickening [110]; the epithelial cells do not vary in number during the various phases of rhinitis. Commonly cationic proteins of migrating eosinophils promote their adhesion to leukocytes, stimulating interactions with CD11/CD18, counter-receptors of CD54 [26], induced by IL_5 in AR patients and not in

nonatopic controls [168]. The pro-inflammatory IL_{18} is up-regulated because of natural allergen exposure in nasal secretions in SAR, showing a similar pattern to IL_1, with a slow but persistent increase during the course of the pollen season. Persistence of IL_{18} high concentrations in PAR compared to SAR suggest its role in persistent allergic inflammation [177]. Instead, in children with atopic asthma or AR, polymorphisms in $IL_{12}\beta$ are not likely to be associated with the development of atopy-related phenotype [117].

Late Phase

Several hours after allergen exposure (on the average from 4 to 8) the *late reaction* occurs, with *a hyperinflux of inflammatory cells and mediators.* For example, LTs increase considerably up to the 12th h [168]. At the clinical level, sneezes and rhinitis are replaced by nasal obstruction [168]. There is also a quantitative demonstration of the different incidence of late reaction: 68% in asthmatics vs 23% in rhinitic subjects, with similar differences in FEV_1 drop (24% vs 11%), without conducive factors as in asthma such as sIgE (specific), severity of immediate reaction, and degree of methacholine response [106]. Histamine reaches a peak at ≈10 h (increasing from ≈10 ng/ml of baseline to ≈50, which corresponds to an eightfold increase of metachromatic cells, up to 79.7±39, mostly represented by basophils, as verified in vivo [73], since they do not produce PGD_2. This is further confirmed by the high correlation between increased histamine ($p<0.0001$) and CD18 coexpression, which identifies basophils in 90% of IgE^+ cells arising in the late phase [110]. The HRF can be seen bound to basophils (which increase 8- to 12-fold by 12–24 h), capable of perpetuating its flow [110]. Eosinophils are well represented [73], from $0.22\pm0.10\times10^3$ before challenge to $49.10\pm27.30\times10^3$ at the 8th h [168], more specifically from 1–2 cells/cm (baseline) to 18 at the 8th h [118]. They degranulate, leaving their entire array of cytolytic cationic proteins: ECP (eosinophil cationic protein), EDN (eosinophil-derived neurotoxin), EPO (eosinophil peroxidase), and MBP (major basic protein), which even at low concentrations reduce the frequency of ciliary beating [99]. ECP increases during both phases, more markedly in the late phase [168], where, however, it produces no harmful action [110]; nevertheless ECP levels correspond significantly to histamine levels in symptomatic SAR patients [84]. Eosinophilia can have a considerable significance in the nasal mucosa, like the change of cellular density, since the more cytolytic hypodense phenotype binds to sIgE more easily, playing a significant role in nasal inflammation [61]. Indeed, it is seen to a greater extent in the moderate to severe forms compared to those present in asymptomatic patients or those with mild symptoms, thus indicating that hypodense eosinophils may participate actively in AR physiopathology [61]. These data have not been subsequently elucidated. Furthermore, in asthmatic patients, LTC_4 synthesis stimulated by Th2-like ILs is a more sensitive parameter of the activation of density change. To date, the most endorsed hypothesis tends to reduce the finding, considering it an epiphenomenon secondary to eosinophilia (Chap. 11). It has been demonstrated that both phenotypes react to the same extent to PAF chemoattractant action, believing that normodense cells become hypodense under the action of inflammatory mediators, and that both respond to chemotactic signals in the same way [91]. Nevertheless, in childhood these eosinophils are mostly immature, which is the reason for the numerical increase observed (Fig. 1.35c). Neutrophils, with still far from understood functions, reach nasal mucosa, doubling their number [118], but they are few in both epithelium and lamina propria [55], while they persist for a long time even during periods of symptomatic remission [99].

The late phase may be regulated by *extensive interactions between APCs, nasal LCs, T lymphocytes, and ILs*: the APCs are well represented by dendritic cells forming a network below the BM, macrophages arranged in the subepithelial tissue and around adjacent glands, and PBMCs (peripheral blood mononuclear cells), which migrate from epithelial vessels after the allergic challenge [125]. Epithelial $CD1^+/IgE^+$ cells ($p=0.01$ vs controls) [58] increase quantitatively during the NPT only in allergic subjects and may bind allergenic peptides [138]. The role played by *T lymphocytes* has recently been re-evaluated, since 24 h after the reaction in nasal mucosa, there is an increase of $CD4^+$ and $CD25^+$. In both epithelium and lamina propria of nasal mucosa, T cells appear to be well expressed in all their components in atopics, but not in controls, and more in subjects suffering from PAR than in those with SAR [58]. After activation induced by allergen encounter, nasal T cells express $CD45RO^+$ and, after a new exposure, IL_4 influences mast cell proliferation (emphasizing their participation in this phase also) as well as IgE synthesis [66]. It is still being studied whether CD45RO surface antigens come from circulation or reside in the NALT or in nasal mucosa [66]. Lymphocyte infiltration has opened a new chapter in AR pathogenesis: T cells bind to HEV epithelium and then roll (Fig. 1.59) with adhesion molecule interactions; afterwards vasodilation increases [4, 87, 168]. An important contribution on the cells that are active in this phase demonstrates that *eosinophils* and *basophils* remain at high levels until the 24th h, with a *close correlation between cell count and increase in nasal resistance and obstruction* at the 8th and 24th h [118]. In the case of continuous allergen exposure or exposure to aspecific irritants, especially in patients with the most severe forms, *the late phase may take on an aspect of continuity*: in fact, in nearly 50% of patients, repeated daily exposure increases their responsiveness, associated with basophil influx and increased histamine release, significantly correlated with sIgE levels [17]. Continuing

	Resistance of nasal airway	
	Increased (↑)	Decreased (↓)
Nasal cycle (2–6 h)	↑	↓
Posture variation		
Erect to supine	↑ ipsilateral side	
Lateral recumbency	↑ dependent side	↓ upper side
Skin pressure		
Axilla or chest side	↑ ipsilateral side	
Temperature		
Skin	↑ on warming	↓ on cooling
Nasal inspiration of cold air	↑	
Respiration		
Breath-hold TLC		↓
Deep inspiration (mouth)		↓
Forced expiration (mouth)	↑	
Hyperventilation	↑	↓
Hypoxia/asphyxia		↓
Hypercapnea		↓
Exercise		↓
Additional reflexes		
Sexual stimulation	↑	
Stress	↑ (chronic)	↓ (acute)
Ocular stimulation	↑	

Fig. 12.7. Factors affecting the resistance of nasal airways in normal subjects via the autonomous nervous system. *TLC* Total lung capacity. (Modified from [48])

this exposure, the number of mast cells in nasal mucosa also increases [59]. It is significant that β1 integrins such as CD49d/CD29 and VLA-5 (CD49e/CD29) [119], also common to eosinophils [87] and vitronectin receptor, are involved in nasal mast cell activation, IL up-regulation, and mast cell survival [119]. Furthermore, eosinophils and basophils respond well to CD103 [87], and IL_4/IL_{13} can strengthen its expression in epithelial cells. Therefore lymphocytes and mast cells may indirectly contribute to eosinophil and basophil recruitment into nasal mucosa [124]. It is known that the development of late phase is connected with the severity of initial challenge and not with the greater or lesser responsiveness to allergen exposure; this may be related to the heterogeneity of late-phase inflammatory phenomena [161].

It was also seen in Chap. 11 that there are other, currently not fully elucidated mechanisms that could come into play in the pathogenesis, for example ANS and neuropeptides that provide beneficial effects in addition to negative ones. Figure 12.7 [48] sums up the recent acquisitions on factors influencing the resistance of nasal airways through the ANS. Stimulating parasympathetic nerves cause an *increased glandular secretion,* not only rich in sIgA, lysozyme, lactoferrin, and other enzymes, but also useful for humidifying, lubricating, waterproofing, etc. the mucosa, combating together with the enzymes and sIgA the pathogenic microorganisms coming from the inhaled air [76], an important function in nonspecific defense. On the other hand, nociceptive nerve responses (associated with pain), even if degraded by epithelial NEP (neutral endopeptidase), in turn inhibited by cigarette smoke, viral infections, etc., are able to send the CNS (central nervous system) negative messages, initiating axonic responses: *their activation induces itching as in AD,* congestion, glandular secretion, etc. (Fig. 12.8) [4]. Neuropeptide activation involves other effects, of greater or lesser functional sig-

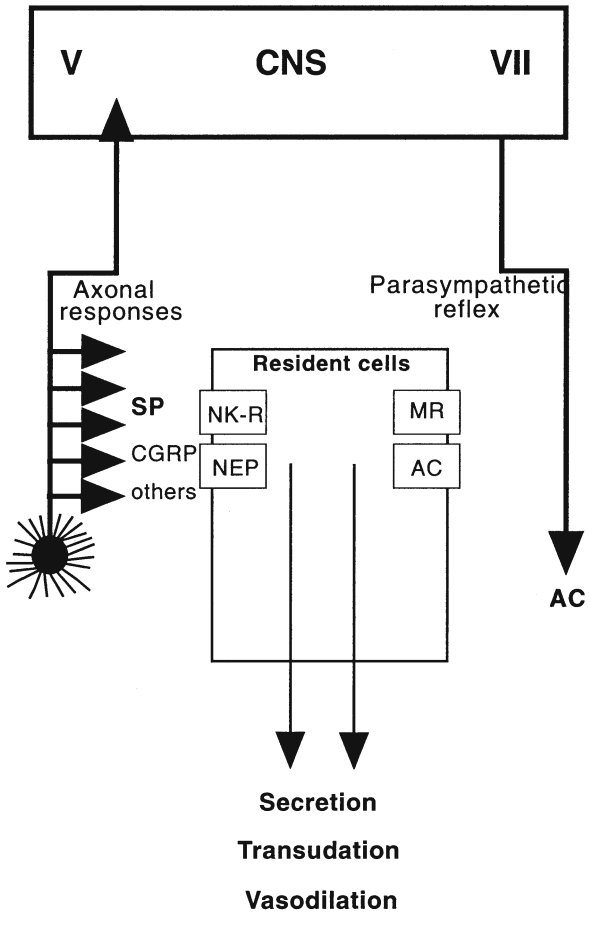


Fig. 12.8. Activation of nociceptor neurons in AR. Activation may induce axonal responses mediating neuropeptide release acting on pain centers and clinical symptoms. *AC* acetylcholine, *CGRP* calcitonin gene-related peptide, *CNS* central nervous system, *NEP* neutral endopeptidase, *NK-R* neurokinin receptor, *MR* muscarinic receptor, *SP* substance P. (Modified from [4])

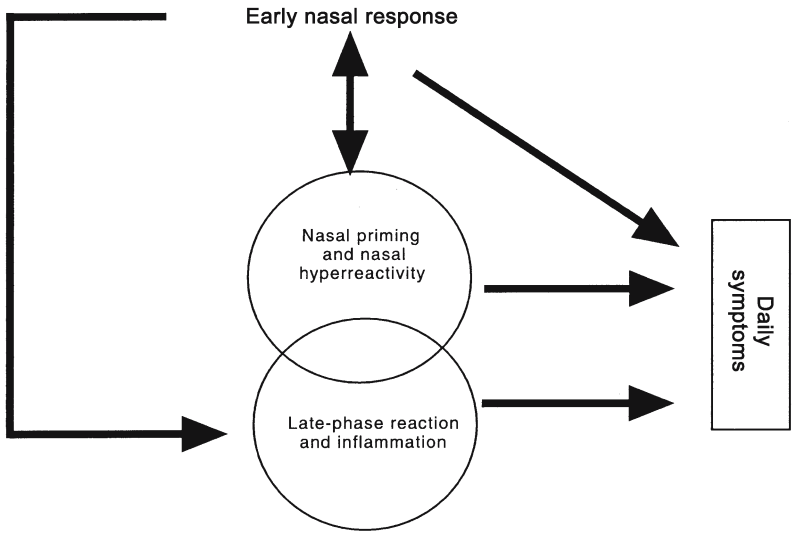


Fig. 12.9. Relationship between nasal hyperreactivity and nasal allergic reactions. (Modified from [62])

nificance such as vasodilation, edema, BM exposure, migration of leukocytes, thickening of the mucosa, and reduction of nasal patency [187]. *Neurogenic inflammation may be up-regulated by neurotrophic ILs,* such as NGF (nerve growth factor) and IL_{11} may participate in the pathogenesis of both AR and asthma [4]. Epithelial cells not only act as a barrier, but are also capable of secreting mediators both to aid and combat inflammation. Their interactions with CD54, aided by MBP, ECP, and IFN-α and -γ, which substantiate the intervention of eosinophils in nasal mucosa [124], explain the peculiar susceptibility of epithelial cells and nasal mucosa to Rhinovirus infections, which have in CD54 their receptor [1], whose counter-receptors on leukocytes are CD11/CD18, thus ranking CD54 first in the pathogenesis, as demonstrated by its absence in healthy volunteers [36].

The sIgE are significantly higher in allergic subjects than in controls in both epithelium and lamina propria (p=0.0001 and 0.005) [58], and also in the subepithelium (17.4 vs 3.9 cells/mm of tissue, p<0.001) [70]. On the other hand, it is possible that B lymphocytes isolated from the nose produce sIgE, distinguishing between allergic individuals and nonallergic subjects [202], as also demonstrated by the presence of anti-ragweed sIgE (and IgG_4) in nasal washing fluid [123]. Experimental data explain why, despite serum sIgE's rather short half-life, allergic symptoms can be induced in pollen sufferers when the pollen counts at the end of the season are lower, since even a few nanograms of IgE can trigger effector mechanisms [163].

NHR Role

Hyperreactivity indicates an increased mucosal and submucosal response to nonspecific irritants such as histamine, methacholine, bradykinin, hypertonic saline, and other provocational agents, also permitting the avoidance of those irritants. Therefore the onset of nasal symptoms following exposure to large quantities of dust or irritating substances, or to great T variations, is a common phenomenon. In patients with AR, these manifestations are more frequent and are triggered even by mild stimuli [62]. The priming effect, attributed to an increased nasal permeability that facilitates the allergenic invasion, is characteristically caused by exposure to immunological stimuli [62]. Recently, various studies have evaluated the role of NHR in relation to IgE-mediated allergy, specifying that when it is not possible to demonstrate its involvement the term "aspecific NHR" is used, supported for example by a change in the position of the head, changes in posture, and by nasal exposure to abrupt T changes, or irritants, cigarette smoke, perfumes, and paint smells [4]. It has also been observed that NHR to histamine correlates well with NHR to allergens, thus suggesting that there is a common pathogenesis for both. The underlying mechanisms have not yet been fully understood, so only explanatory hypotheses can be presented [62]:

- Increased epithelial permeability, possibly facilitating the direct stimuli toward the sensory nerve endings, the vessels, and nasal glands
- Hypersensitivity of sensory nerve endings (nociceptor nerves), possibly leading to an exaggerated response to normal stimuli
- A directional change in impulses afferent to SNC, possibly translated into a relative predominance of the parasympathetic system over the sympathetic system
- An increase in number and sensitivity of the receptors, especially muscarinic receptors, which could be the reason for the increased nasal secretion in patients with AR
- Mediator release after aspecific nasal stimulation, for example with cold, dry air [62]

Figure 12.9 [62] illustrates the relationships between NHR and nasal allergic reactions.

Etiological Factors

Genetic Factors

Atopic parents, mothers with asthma, and increased IgE levels at 9 months and 6 years of age were statistically very significant (p=0.01 to p=0.00002) [196]. Mean family history (FH) for atopy (FHA) was as follows: paternal 70.5%, maternal 62.2%, both 32.8% [78]. Moreover, there is an inverse relationship between rhinitis and number of children, with the only son most at risk [164]. A linkage between IgE responses, AR and chromosome *11q* as well as other markers on 12 chromosomes are reported in Table 4.2. Genotyping data of 400 microsatellite markers suggested linkage of SAR to chromosomes *1p36.2*, *4q13.3*, and *9q34.3*, as well as of serum total IgE levels, and of orchard grass RAST IgE levels [198]. It is suggested that IgE antibody responses to pollen may be controlled by genes associated with a particular HLA haplotype [10]. Epidemiological studies have suggested that AR and asthma sometimes occur together in the same family, indicating that some overlap exists in the genetic factors that contribute to the development of the two diseases [42, 65, 90]. However, PAR is associated with AR in the offspring with an OR=2.16 [105].

Predisposing Factors

Of particular importance are pollens in SAR and dust mites and animal danders in PAR.

The most common pollens [148] are grasses [34], weeds (*Compositae*) such as *Parietaria* (P) [41] and ragweed [33], trees including olive [24], birch and *Betulaceae* [40], and *Cupressaceae* [201]. SPT positive results on 129 [196] and 157 atopic children [199] are summarized in Table 12.6 and Table 12.7 [101] summarizes the prevalence of emergent pollens [101].

This demonstrates the prevalence of type I pollen allergy, a group of glycoproteins with a MW of 26–32 kD, consisting of numerous homologous and immunologically correlated isoallergens (Table 1.74). We stress that if the daily pollen count is 50/m^3 of air, 25 pollens/h are inhaled, =1 µg of Lol p (rye grass) during the whole season, but thanks to the filtering action of the nose and mucociliary system, only 0.1 µg reaches the airways [185], =1%–2% of the airborne particles of a diameter >10 mm; to cause clinical manifestations in a sensitized subject, just 1×10^{-4}–5×10^{-5} µg of pollens/h are sufficient [94]. The chief pollens have dimensions that range from 15–16 µm in *P. judaica* to 31–35 µm in *Dactylis glomerata*; in any case the microgranules have a diameter of 2 µm and rain can reduce them by 30%–60% [34]. Several indications on pollens [71, 112] and the pollen calendar (Fig. 12.10) follow:

- *Grasses* [34] (Figs. 1.68, 1.73), common and widespread practically everywhere, produce the dominating pollens, from the allergenic standpoint; flowering is in spring-summer, mostly starting in January in Australasia, April–May in northern and southern US and Europe, continuing until September in northern US and Europe, and until October in Southern US, and between October and December in Australasia [148]. Some pollens may be perennial allergens (Bermuda grass in Southern California and Florida [148]). In Europe, grasses are common in the center and south, starting in late April or early May in the north, and continuing until the month of July in the center and south and beyond in the north. There may also be a modest and inconstant increase of certain species in September with a second summer-autumn flowering).
- The *weeds* flower in Europe from July to September, in northern US from May to mid-September, in southern US from June to mid-November, and in Australasia from January to March and from October to December [148].

Parietaria [41] (Figs. 1.75, 1.76), the most widespread species of which are *P. officinalis* and *P. judaica*, is of particular importance in central and southern Europe (the Mediterranean area). Pollination goes from February–March to November, with an attenuation in July–August and a return in August–September (2nd peak), while in the north it starts in mid-May and continues without interruption until autumn.

Mugwort and ragweed [33] (Figs. 1.67, 1.74), with the first widespread and the second generally limited to northern Italy, flowers from the second half of July to late August–early September, continuing until mid-October [112]. Ragweed typically begins pollinating in mid-August and stops in October. All species contain the major allergen Amb a 1 (Table 1.74), so sensitized children may experience symptoms out of season when visiting regions where different species pollinate perennially [148]. It seems that ragweed has become considerably more widespread, in particular around airports, spread by the seeds clinging to the shoes and luggage of travelers coming from the United States.

Table 12.6. Prevalence of positive SPT in children

Pollens	[199]	[196]
Alternaria		48
Bermuda		69
Birch	46.5	
Compositae	32.5	
Grasses	99	
Mulberry		29
Olive		38
Parietaria	39.5	
Ragweed		39

Data from [101].

Fig. 12.10. Tree pollens appear in March or April, grass pollens from April to June

Table 12.7. SPT positive to emergent pollens positive in 2,344 Italian adults

Pollens	SPT positivity (%) Mean
Total	28.2
Alder	21.2
Birch	18.8
Cypress	10
Hazelnut	19.6
Hornbeam	21.2
Ragweed	14.4

Data from [101].

Among the *Euphorbiaceae, Mercurialis annua*, which pollinates between April and September, with a peak during the months of June and August is noteworthy.

- The pollen trees flourish in Australasia from January to February and from August to December, from mid-January in southern US to May, from mid-February to May in northern United States, and from April to June in Europe [148].

Of the *Plantaginaceae,* the best known is *Plantago lanceolata*, known as plantain, which flowers from April to September, giving earlier and longer-lasting symptoms than the grasses.

The *Betulaceae,* most important in the north-northeast (Table 12.7), start to pollinate in February-March, reach their peak in April, and decline until they disappear in early May; in central regions the flowering goes from February to April–May and in the south from January to April–May [40].

Olive pollen, very common in the south, especially in Italy around Bari (54% of the territory), and in Liguria, reaches its peak in May–June; in the latter month the counts reach 1,000–2,000 granules/m^3 of air, with a peak of 4,000 [24].

The *Cupressaceae* [201], common in Mediterranean Europe and central and southern Italy, with an early season from February to late April, should be included in the SPT panel, considering how widespread cypresses are, not only as ornamental plants according to the classic usage, but also as dividing hedges and windbreaks, and without latitudinal limits.

Table 12.8. Differential diagnosis of the types of rhinitis that are more frequent in childhood

Findings	Type of rhinitis				
	Allergic		Vasomotor	Eosinophilic	Infectious
	Seasonal	Perennial			
Age at onset	Childhood	Childhood	Adulthood	Childhood/adulthood	Childhood
History					
Familial	+	+	–	±	–
Personal	+	+	–	±	–
Frequency	Seasonal	Perennial	Perennial	Perennial	Sporadic
Etiology	Allergens	Allergens	Irritants	?	Virus/bacteria
Symptoms					
Fever	–	–	–	–	+
Congestion	±	+	±	+	±
Itching	+	+	–	±	–
Sneezing	+	+	±	±	±
Rhinorrhea	+	+	–	+	±
Anosmia	±	±	±	+	–
Medical examination					
Nasal mucosa	Pale	Pale/pink	Pale/obstructed	Pale/obstructed	Red/obstructed
Swollen turbinates	±/+	±/+	+	++	±
Nasal discharge	Watery profuse	Mucoid	Minimal/mucoid	Watery profuse	Yellow/grayish purulent
Associated symptoms					
Allergic salute	+	+	–	–	–
Ocular	+	±	–	–	–
Respiratory	+	+	?	±	±
Examinations					
Skin tests/RAST	+	+	–	–	–
Eosinophilia >10%	+	+	–	++	–
High IgE levels	60%	+	–	–	–
Response to therapy					
Allergen avoidance	+	+	–	–	–
Antihistamine	Good	Fair	Limited	Fair	Limited
Decongestants	Limited	Limited	Fair	Limited	Limited
Cromolyn	Excellent	Good	Limited	Limited	None
Corticosteroids	Excellent	Good	Limited	Excellent	None
Immunotherapy	Excellent	Excellent	None	None	None

– None, ± moderate, + good, ++ marked.
Data from [108, 149].

• Other moderately allergenic pollens are characteristic of other trees, basically the poplar, willow (*Salicaceae*), plane or sycamore (*Platanaceae*), the dust of which comes from the fruits and is not really pollen, and horse chestnut (*Sapindaceae*), with the last two often used for decoration along streets and roads; less allergenic are fir, pine (*Coniferae*), and elm (*Ulmaceae*); hazelnut (*Corylus avellana*), hornbeam, alder, and others are emergent (Table 12.7).

A comprehensive study in 2148 children has found that sensitization to birch (OR, 6.0), timothy (OR, 2.8), and horse (OR, 4.1) were significant risk factors for rhinitis [140].

It must be kept in mind that at an elevation of 1,000–2,000 m pollination is delayed by about 1 month; however it may be *accelerated everywhere by ozone depletion* (Chap. 4).

As a result of these studies, it is reasonable to conclude that exposure to a high level of birch pollen in early infancy increases the risk of sensitization to the same allergen, as well as the risk of allergic asthma [78].

The role played by food allergy is demonstrated by food-related (fresh fruit and vegetables) AR within the oral allergy syndrome (Chap. 9), for example 39 out of 157 children (24.8%) [199]. Due to the action of profilins, also with the mediation of latex, patients sensitive to *Betulaceae* often have symptoms associated with various fruits and vegetables and correlated trees such as hazelnut and alder [40] (Table 8.14).

Children sensitized to pollens and trees may require, in 9%–13% of cases, a visit to an emergency department (ED), and in 13% of cases have been diagnosed with asthma [25].

Among fungi, spores of basidiomycetes are associated with rhinitis, especially because the spores are present between April and October, providing a longer time to exert their influence, and are severe enough to prompt children to present to an ED [25].

Other aspecific factors that may influence AR onset are summarized in Tables 5.22, 5.24, and 5.25, especially HDMs [20, 37, 85]. *Household pets* were present in 30.0%–33.3% of cases at age 0–3 months and over 3 months in 34.4%–36.7%, cats in 13.8%–15.8% and 18.4%–18.7% of cases, respectively; thus 29 cats (5%) entered the house after childbirth [78].

Another important risk factor in PAR is *passive smoke:* mothers of allergic babies smoke during pregnancy (18.9%) and when the baby is 0–3 months old (18.3%), and over (26.8%), as well fathers, more after childbirth (22.5%) than later (17.7%) [78]. The first night spent by *neonates in a nursery* may be a risk factor for developing AR (OR, 1.46) [105].

Anatomical–Physiological Factors Differentiating Children from Adults

At the otolaryngological level, apart from the different dimensions of the respiratory tract already explained in Chap. 11, the differences may be quantified as follows [48, 71]:

- *Nasal resistance* 4-fold higher than in an adult: from a maximum of 12 cm H_2O, it decreases until it reaches adult values at 16 years of age.
- *Nasal cycle:* the circadian rhythm of congestion and decongestion, related to changes of the sympathetic tone, lasts an average of 3–4 h in the adult; in the child it is limitless because of the immaturity of the vasomotor control of nasal mucosa.
- Dependence on *nasal breathing:* in the child, alterations of nasal function may negatively influence general body growth and, locally, the development of splanchnocranium and lower airways.

Classification

With regard to the allergen responsible and the temporal evolution, AR symptoms may be distinguished as follows (Table 12.8) [108, 149]:

- Seasonal, taking place only in certain periods of the year, from pollen sensitization
- Perennial, or present all year round, as in the forms caused by Der p

We continue to use this classification, because dividing the symptoms into intermittent (the symptoms are present <4 days a week or for <4 weeks) and persistent (the symptoms are present >4 days a week and for >4 weeks) [12] seems to be impractical. Also, the difference between SAR and PAR may be unfeasible since symptoms do not necessarily occur strictly in conjunction with the allergen season because of the different prevalence of pollens and molds, many being perennial allergens, and since symptoms of PAR may not always be present all year round [12].

Seasonal Allergic Rhinitis

Definition

SAR is a condition caused by pollen allergy, occurring seasonally and characterized by itching and sneezing, emission of serous or seromucous secretion, and persistent nasal obstruction for over 3 weeks, in the absence of similar symptoms in other family members [108]. SAR prevalence, within pollinosis, increases in parallel with age, until it becomes one of the most frequent clinical manifestations (Table 5.21).

Etiopathogenesis

This is related to pollens, as explained in the pathogenesis. Depending on the period of onset, the following forms can be identified in Europe:

- Early or pre-spring, from sensitization to arboreal plants
- Spring or spring-summer, the most frequent from sensitization to grasses *Parietaria* and *Oleaceae*
- Summer-fall, rarer, mainly due to *Compositae,* also referred to as end-of-season pollinosis
- Combined forms from sensitization to different families of pollens

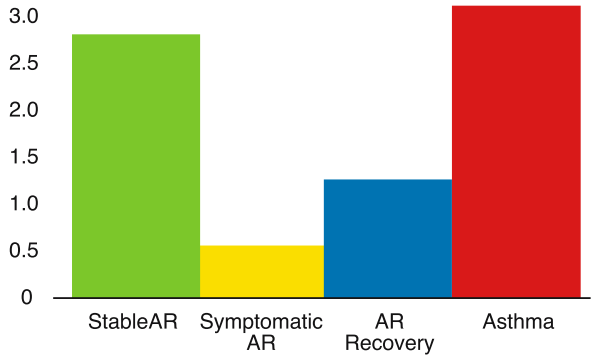


Fig. 12.11. Circulating basophil and eosinophil progenitors related to control. A large number of progenitors is evident in the off season, *stable AR*. With recovery, levels start to return to a high level, whereas in asthma sufferers, the levels of these progenitors are chronically high. *AR* allergic rhinitis. (Modified from [46])

Figure 12.11 [46] shows how the levels of hemopoietic progenitors are correlated to symptom variations.

Clinical Presentation

The characteristic symptoms, most frequent in the morning in 60%–70% of cases (p=0.008) and in the first 4 months, based on circadian rhythms and chronobiology [137], are made up of:

- Fits of sneezing (5–20 sneezes at a time)
- Rhinorrhea
- Nasal congestion
- Itching [27, 194]

With regard to chronology, itching and sneezing begin 1–2 min after an NPT, rhinorrhea after 5 min, and nasal obstruction after 15–20 min; the latter may continue even for 1–2 h [71].

Fits of sneezing, most frequent in the early morning and evening, are a frequent cause of social handicap [194].

Rhinorrhea is clear, watery, and abundant (the nose produces 20 ml of secretion/h) [194] and can become mucopurulent by secondary infection. The persistence of nasal drip (runny nose) can lead to an external irritation of nostrils, with a possible subsequent impetiginization. Postnasal drip, on the other hand, can irritate the retropharynx, which can cause a troublesome, even paroxysmal, cough. Nocturnal postnasal drip causes frequent awakenings and, as a consequence, can leave the child exhausted during the daytime.

Nasal congestion (stuffy nose) is usually less evident than in PAR: the obstruction may be accompanied by rhinolalia, a sensation of headache, and nosebleed. Because of frequent mouth breathing, the child snores while sleeping [111]. Nasal congestion may cause the infant or young child to become fretful, restless and easily tired.

Itching often precedes sneezing; when the soft palate itches, it means that the mucociliary system has transported the allergens into the nasopharynx [71]. Itching of the nose and especially the eyes is a cardinal manifestation of pollen sensitivity. In some children, the constant pruritus, excessive nasal blowing, sneezing, and picking or rubbing of the nose produces an increased frequency of epistaxis. Some complain of ear itching; this can be explained as due to the common innervation of the pharyngeal mucosa and the ear, by the glossopharyngeal (IX) nerves.

Characteristic Symptoms

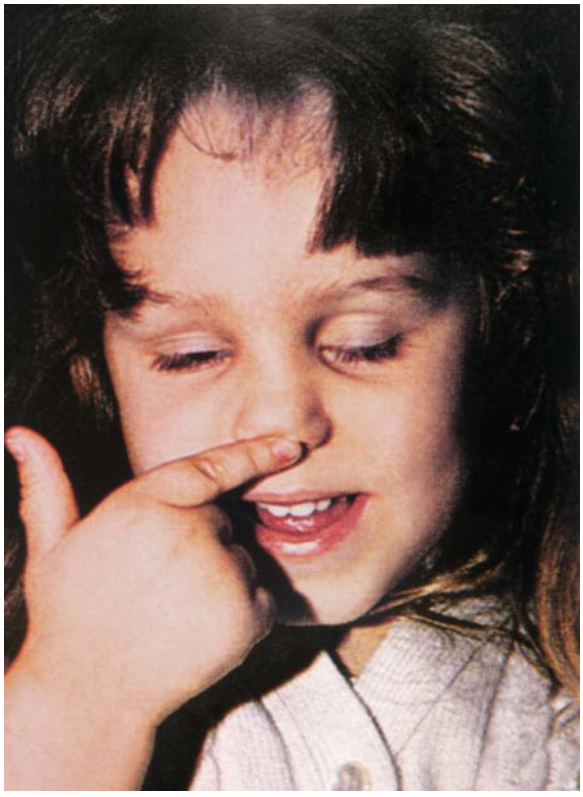
The allergic salute and other particular signs pertain exclusively to SAR, most often to PAR. Moreover, several facial characteristics connected with repeated nasal rubbing, mouth breathing, and the presence of allergy-related circles under the eyes (allergic shiners) may be seen [19].

The *allergic salute* is frequently exhibited by children, who to alleviate intense, intolerable nasal pruritus (as well as to allow air into the nostril) move their nasal lobe upward and downward using a finger or the palm or dorsum of their hands (Figs. 12.12, 12.13); the continuous repetition of this movement may eventually result, above the anterior nasal extremity, in a permanent *transverse nasal crease,* across the mobile and bony part of the nasal pyramid (Fig. 12.14), which may be reduced by folding the nasal tip downward. For the same reason other mannerisms are observed, such as nasal wrinkling and mouth stretching by contracting the buccinator and orbital muscles [19].

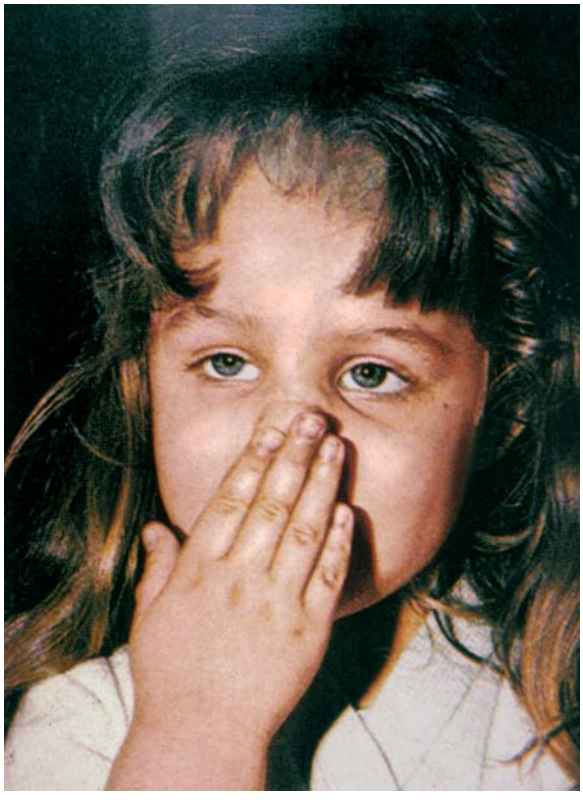
Often *orbital darkening* (*allergic shiners*) also appear. These bluish, brownish to grayish discoloration of the lower eyelids, and swollen infraorbital tissue, most likely result from venous engorgement and stasis due to impaired infraorbital vein flow (Fig. 12.15), which anastomose with the veins draining the nasal turbinate swollen mucosa [48].

Because of persistent mouth breathing, with protrusion of the tongue and the resulting secondary hypertrophy of the peribuccal muscles, a *dental malocclusion* and the appearance of dysodontiasis may appear. With the passing of time, if chronic nasal obstruction (CNO) persists, in some children *craniofacial modifications* can be seen (Chap. 15).

Eye symptoms (itching, watering, photophobia, sensation of foreign bodies) are almost always associated with nasal symptoms (rhinoconjunctivitis) and in some cases can take on a predominating role; they are always more frequent and/or serious in SAR than in PAR caused by Der p or molds [114]. Similar symptoms are also caused by using *contact lenses* (Chap. 14), which are a risk factor 5-fold higher for SAR patients than for healthy subjects.



a



b

Fig. 12.12 a,b. Allergic salute. **a** The girl pushes the tip of her itchy nose laterally with a finger, **b** or pushes the nose upwards with the palm of her hand to relieve the itching

Fig. 12.13. Allergic salute. The child rubs his itchy nose all the time

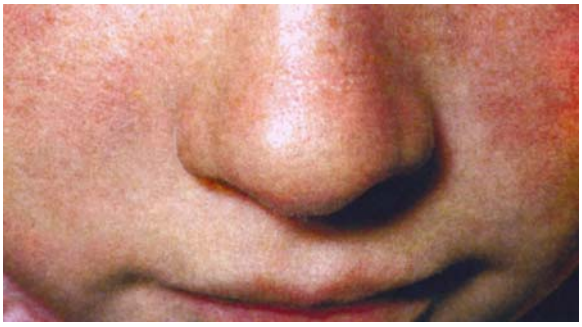


Fig. 12.14. Typical aspect of a girl with AR showing transverse nasal crease across the lower third of the nose

Oral allergy syndrome (Chap. 9) symptoms are possible owing to pollens and fruits with shared allergens and vegetables [199], as are cases of *contact urticaria* in young pollinosis sufferers who play outside in fields (Chap. 8).

The symptom presentation may be different:

- Symptoms appear during specific periods of the year.
- During the pollen season, symptoms are proportionate to the quality of environmental pollens, attenuated if it rains (pollens fall to ground), or if the child remains at home or spends time at the seashore (reduced pollen presence or absence), and accentuated by nice weather

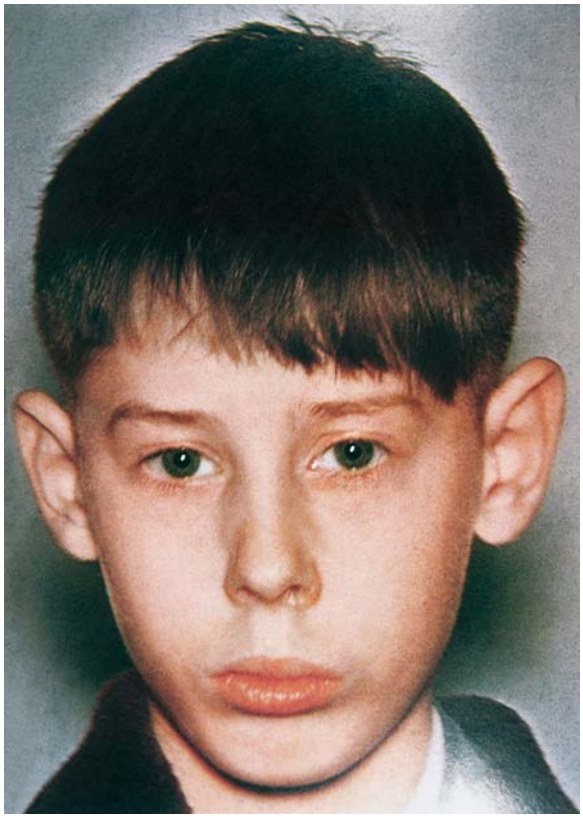


Fig. 12.15. Allergic rhinitis: note the pallor, discoloration of the lower palpebral area, mucous nasal discharge and lip edema

and wind (prolonged pollen suspension in the air), country walks, etc.

- The evolution is totally variable, with a possible remission of symptoms 1 year and worsening another year. In children with rhinoconjunctivitis of a certain severity, the appearance of asthmatic symptoms during the pollen season is frequent, but acute asthmatic episodes can occur even if no eye or nasal symptoms are present [71].

Table 12.9. Selected points of patient history in pediatric AR

Contributing factors
Atopy
Male gender
Month of birth
Early pollen/pet exposure
Symptom frequency
Daily
Seasonal
Episodic
Persistent
Symptom persistence
Weeks
Months
Years
Symptom severity
Mild
Annoying
Accentuated
Symptom type
Sneezing
Pruritus
Nasal blockage
Rhinorrhea
Cough
Postnasal drip
Aspect and color of secretions
Mucoid, opaque
Purulent, discolored
Watery, clear
Precipitating factors
Allergens
Environmental pollution
Irritants
Associated factors
Local or systemic disorders
Medications
Viral infections

Data from [97].

Diagnosis

Medical history may reveal a positive FH [78] (Table 4.7) and/or personal history for other allergopathies. SAR may be suspected on the basis of typical symptoms, arising on the occasion of pollen exposure (remember the month of birth; Fig. 4.11), and the child's characteristic appearance will help confirm the diagnosis (Table 12.9) [97]. Some children labeled as adenoidism sufferers actually have forms of nasal obstruction stemming from a chronic edema of the turbinates [194].

Medical examination is facilitated by data summarized in Table 12.10 [97]; conjunctival examination shows its hyperemia, with eyelid edges raised due to edema. Chest auscultation must not be overlooked, to discover possible wheezing.

The *anterior examination* with a hand-held rhinoscope equipped with a head light or head mirror or a headlamp with nasal speculum allows visualization of the anterior one-third of nasal airways. Children with AR have a pale, swollen and congested mucosa, and nasal turbinates that are often hypertrophic show a certain degree of obstruction [51]. Watery mucoid or opaque material may be noted in the nasal cavities and posterior pharyngeal wall. Findings are normal outside the pollination period. In small babies, it is usually not possible to examine the nasopharynx with mirrors [181].

Table 12.10. Evaluating children with suspected rhinitis

Nose
External aspect
Nostril inflammation
Secretion quality and quantity
Septal position
Transversal nasal crease (allergic salute)
Turbinate appearance
Eyes
Blepharitis
Blepharospasm
Conjunctival injection
Increased lacrimation
Mucus discharge
Palpebral edema
Periorbital darkening (allergic shiners)
Photophobia
Symmetrical creases below the lower eyelids (Dennie-Morgan infraorbital folds)
Ears
Eustachian tube dysfunction
Infections
Secretions
Mouth
Dental malocclusion and overbite
Lip and/or gingival inflammation
Orthodontic abnormalities
Secretion quality and quantity
Thorax
Auscultation suggesting asthma

Modified from [97].

The *cytological examination of nasal secretion* (Table 12.11) [97], obtained by having the child blow his or her nose directly onto two slides, or smear collected secretion using a cotton swab passed delicately over the mucosa and left *in situ* for about 2 min, may reveal the presence of a large number of eosinophils. Eosinophilia values >10% are considered positive [179] (see Appendix 12.2). The absence of eosinophils does not, however, exclude the diagnosis of AR, nor does their presence confirm it (Appendix 6.5); in fact, in nonallergic rhinitis with eosinophilia syndrome (NARES), there is also a pronounced eosinophilia (Table 12.11).

Therefore these studies have no absolute discriminatory value. For example, repeated respiratory infections may clearly modify the local picture indicated above and may also cancel the normal aspect observed during the intervals between SAR episodes [149].

As needed, further examinations and tests may be prescribed, including (Table 12.12) [116]:

- *Otoscopic examination,* which usually reveals retracted tympanic membrane and signs of fluid in the middle ears.
- *Rhinomanometry,* which records the air flow and endonasal pressure during respiration, allowing an objective measurement of the degree of nasal obstruction. Anterior rhinomanometry may be used in children, while posterior rhinomanometry is difficult in this age group [183].
- *Tympanometry,* which in children <7 months of age, indirectly measuring the compliance and impedance of the tympanic membrane and middle ear ossicles, reveals any existing alterations of the tympanic membrane system – middle ear – and the eustachian tube.

Table 12.11. Nasal cytologic diagnosis

	Cell increase			
	Eosinophils	**Basophils**	**Neutrophils**	**Goblet cells**
Allergic rhinitis	+	+	+	
NARES	+	+		
Aspirin intolerance	+	+		
Nonallergic rhinitis with basophilia		+		
With intracellular bacteria[a]			+	
With ciliocytophoria[b]			+	
Without bacteria[b]			+	
Nonallergic rhinitis				+
Infectious rhinitis				+
Vasomotor rhinitis (?)				+

Data from [97].
NARES nonallergic rhinitis, eosinophilic subgroup.
[a] Rhinopharingitis or sinusitis.
[b] Viral upper respiratory tract infections (URTI).

Table 12.12. Additional tests for pediatric diagnosis of rhinitis

Cytologic examination of nasal secretions and scrapings
Nasal provocation test
Otoscopy
Rhinolaryngoscopy by flexible rhinoscopy or fiberoptic endoscopy
Rhinomanometry
Skin prick tests
Total and specific IgE
Tryptase dosage in nasal fluids
Tympanometry

Modified from [116].

- *X-ray examination,* with projections for paranasal sinuses if sinus (mucosal thickening, sinus opacification, presence of hydro–air levels) or adenoidal hypertrophy are suspected.
- *Rhinolaryngoscopy* with fiberoptic endoscopy, particularly useful as a complement to preceding examinations if the pediatrician suspects sinusitis. The primary function is the visualization of the upper airways, detecting any nasal polyps, endonasal foreign bodies, adenoidal hypertrophy, etc. In small babies, fiberoptic rhinoscopy is impractical since heavy sedation with local anesthetics is required [181].

Allergy Diagnosis

Complementing data recorded by medical history and physical examination, it may be useful to refine a specific etiological diagnosis, make SPTs with standardized allergenic preparations, outside of the pollen season to avoid the risk of triggering unpredictable reactions. If, considering the aerobiological calendar and where the child lives, the symptoms:

- Are *exclusively seasonal,* the test will be on grass and/or *Compositae* and/or *P. officinalis and P. judaica* pollens and, as necessary, the most common trees in the region of residence [179].
- Are *perennial or nearly perennial,* the test will include Der p and *P. officinalis and P. judaica*: in fact, *Parietaria* does not always have a circumscribed season, since it is present almost all year round in southern regions, with high pollen counts from February to November. In some areas cockroaches should be also included [179].

It should be added that:

- In a study on 1,100 schoolchildren, the SPT and RAST sensitivity and specificity showed a significant SPT difference for specificity and very poor positive and very high negative predictive values for both. With the increased cut-off point of 1.5 kU/l, almost identical predictive values for SPTs and RAST were obtained. SPT and RAST perform better in the negative than positive prediction of AR cases in epidemiological studies [147].
- In polysensitized children, the pollination season is longer, so SAR symptoms may overlap PAR symptoms.
- A child with double sensitization to pollens and dust mites [149] may show more severe and prolonged symptoms because pollen exposure increases airway reactivity to dust mites.

IgE and PRIST tests are reserved for cases where the medical history has not produced any valid indications and SPT results are not clear and unequivocal, while a NPT (Chap. 6) may be advisable in doubtful cases, for which the other examinations and tests have produced controversial results.

Specific Examinations and Tests

- Study of mucociliary transport may reveal a more or less pronounced slowdown.
- Mediator counts in nasal washing fluid.
- Studying tryptase levels makes it possible to evaluate the degree of activation of the mast cells [127] and of differentiation between active and nonactive forms [132].
- Nasal ECP level [84, 145] is an aspecific indicator of inflammation [133], in significant correlation with histamine levels in SAR patients [84].
- The NPT is the only reliable test if food allergy is suspected.

Because children, especially young children, do not or cannot complain of symptoms in the same manner that adults do, and because children are often out of sight of parents for prolonged periods of time, symptoms and signs of rhinitis may go unnoticed. This *may delay care for these children* [120].

Complications

AR can cause numerous complications, including otitis media with effusion (OME) [23], an abnormal skeletal development of the face, with orthodontic problems, dysfunction of the eustachian tube, and sinusitis. Otitis and sinusitis are results of the *continuum* formed by nasal, middle ear and paranasal sinus mucosa. *Paranasal sinusitis* is considered a factor that contributes to the development of bronchial asthma (Chap. 15).

Perennial Allergic Rhinitis

Definition

PAR is caused by allergy to non-pollen inhalants, with a nonseasonal recurrence, almost always a source of uneasiness, with symptoms that are more pronounced and longer-lasting than SAR, the most common of

which is nasal obstruction (Table 12.4). In the absence of controlled studies, it is assumed that about 2%–4% of the population suffers from it. The onset occurs at around 2–3 years of age, reaching the maximum prevalence during school age.

Etiopathogenesis

The most significant differences from SAR are mostly environmental aeroallergens, such as Der p (the main etiological agent), fungi, animal dander, cockroaches, etc. Compared to SAR, with continuation of the disease, chronic and irreversible local alterations can be seen, such as mucosal thickening, epithelial hyperplasia, more pronounced cell infiltration, and connective tissue proliferation [120].

The clinical pattern can be maintained or complicated by the possible presence of a large number of nonimmunological inciting agents, considering nasal hypersensitivity induced by chronic inflammation [48]:

1. T changes (even minimal), especially cold air, and weather variables (humidity, rain, storms)
2. Cigarette smoke
3. Perfumes and pungent odors (detergents, disinfectants, paints, especially if oil-based)
4. Alcohol
5. Gas (automobile exhaust fumes)
6. Various chemical substances
7. Bright light
8. Newspaper ink
9. Air pollutants
10. Changes in position of the head and/or body
11. Stress or mental factors in general

These stimuli, like URTI, often contribute to symptom chronicity, although the causal agent may have been eliminated [48]. Among air pollutants, it was recognized as early as in 1871 that O_3 in a particular state of activity becomes highly irritating, especially on the nasal mucosa, and excites nose secretion [32].

Clinical Presentation

Clinical features overlap SAR symptoms (except for the year-round duration), with the two differences previously listed: less frequent itching, especially of the eyes, and more pronounced nasal obstruction. The duration of the single episodes may be highly variable: from a few minutes to several hours. Generally speaking, the symptoms are less intense, and the association with bronchial asthma, urticaria, etc. may be less frequent.

Nasal obstruction is worsened by the supine position. Rhinorrhea and bouts of sneezing are more frequent and pronounced in the hours after awakening in the morning [120]. The allergic salute, transverse crease, allergy-related under-eye circles, and dental malocclusion are, in this form, even more common. The symptoms are tolerated much more by children than by adults [120].

Other signs and symptoms, mostly connected with CNO, frequently underestimated by family and pediatrician, and difficult to identify by children [183] are [120] as follows:

- *Asthenia* also caused by restless sleep
- *Sore throats* related to poor humidification from open-mouth breathing
- *Anorexia,* nausea, and sometimes vomiting, caused by the exaggerated swallowing of nasal mucus
- *Hearing loss,* mainly of transmissive type, owing to recurrent episodes of OME or nasotubal infections with intermittent occlusions of the tube. It is especially important that it is detected and treated in infants to avoid the ensuing delayed onset of speech causing inevitable learning difficulties and behavioral problems [181].
- *Anosmia* (often associated with sinusitis) and hypogeusia, complications frequently remaining undiagnosed [39], possibly leading to further appetite loss, increasing the emotional tension of family members at meal time
- *Headache,* especially frontal, caused by the chronic edema occluding the opening of the eustachian tube and paranasal sinuses
- *Restless sleep* because of nasal congestion, snoring, headaches
- *Epistaxis* (in 40% of children >12 months of age), caused by the traumas to the delicate nasal mucosa from the continuous rubbing/scratching, and from forcefully blowing the nose
- *Adenoidal facies* deriving from nasal obstruction and consequent chronic mouth breathing
- *Development of a high, narrow palate,* to which both chronic edema and venous stasis may contribute
- *Nasal voice* and other language defects, also caused by nasal obstruction
- *Poor school performance* due to concentration problems because of the continuous respiratory symptoms
- *Problems with parents,* relatives, teachers, and peers, causing social isolation at school and domestic conflicts because of frequent halitosis and continuous sniffling

Diagnosis

The diagnosis (Table 12.8 and Appendix 12.1) of aperiodic forms is based on medical history information and a clinical history of typical symptoms. The characteristic appearance of the child is also very indicative, and knowledge of the month of birth may offer some help.

A *physical examination* using a rhinoscope, *unlike with SAR,* shows nasal mucosa that may be variable in appearance Generally it is rather edematous and pale, nasal turbinates are hypertrophic, and *obstruction is pronounced* (Fig. 12.16); secretions are mostly mucoid.

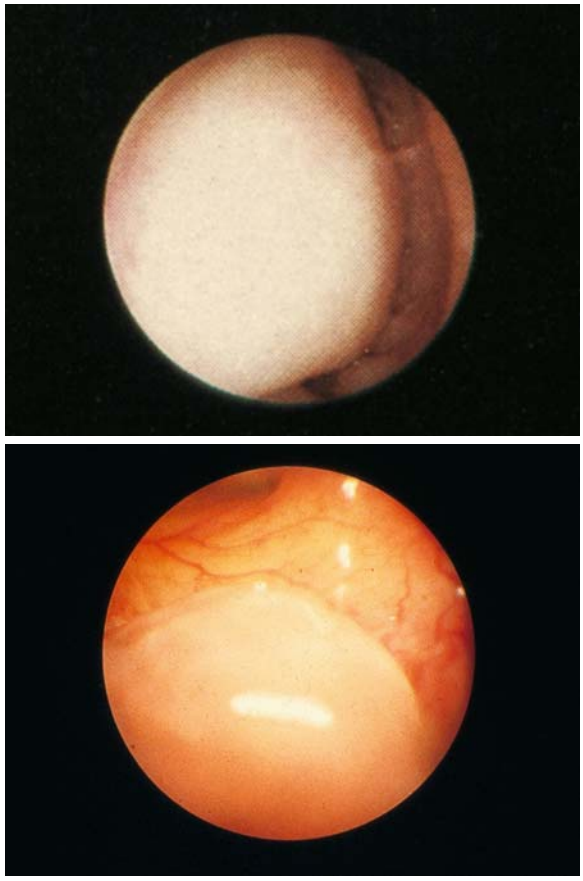


Fig. 12.16 a,b. Children with AR have a pale, swollen and congested mucosa; nasal turbinates that are often hypertrophic show a certain degree of obstruction

A similar problem of delayed care exposed in SAR babies has applicability also to PAR babies.

The diagnosis is confirmed, as in the preceding case, by otoscopy, tympanometry, and rhinomanometry; audiometric tests are discussed in Chap. 15.

Nasal cytology is useful for distinguishing between infectious and noninfectious forms (recurrent infections may be confused with PAR). Moreover, given the variable results, usually two or three tests are needed, preferably performed by the same examiner.

Allergy Diagnosis

SPT and RAST: to select the allergens to be tested, the standard probable causes are Der p, fungal spores, animal dandruff, or other allergens implicated based on medical history of individual children. The mycetes, as seen in Chap. 4 were positive in up to 19.5% of these children. Tables 5.18 and 5.19 show the high prevalence of related allergy. Pediatric studies report that Der p-positive IgE agree with SPTs, nasal IgE and NPT results agree in 82% of cases [11], and nasal sIgE appear to be a more sensitive index than PRIST for evaluating the exposure-avoidance of dust mites [144]. Therefore NPT may be a useful complement.

Specific Examinations and Tests (see "SAR")

Differential Diagnosis

Differential diagnosis (Table 12.8) may be made essentially based as follows [149, 181]:

- *URTI:* short clinical course, possible presence of fever, lymphadenopathy and neutrophils in nasal secretions
- *Chronic infectious rhinitis:* mucopurulent secretion, with fever, sore throat, cough, sickly feeling, etc. If relapsing and associated with other infections, may warn of underlying defects in host immune defense such as primary ciliary dyskinesia (two congenital syndromes) or secondarily, cystic fibrosis, and immune deficiencies.
- *Nonallergic rhinitis:* it occurs in children with intermittent and perennial symptoms, seasonal acute phases, and affects nasal mucosa, where altered vasomotor control makes it reactive to sudden changes in body T, neurogenic stimuli, and changes in environmental factors such as weather and T variations, high humidity, atmospheric pressure, odors, smoke, and pollution. It clinically mimics AR symptoms: scarce, often mucoid, rhinorrhea, nasal obstruction, pale and edematous mucosa, anosmia and hypogeusia. SPTs are usually negative.
- *NARES:* noninfectious pathology wholly similar to SAR, which is manifested with bouts of sneezing, rhinorrhea, nasal obstruction, nasal and eye itching, etc., triggered by aspecific stimuli.
- *Rhinitis medicamentosa:* congestion of nasal mucosa caused by a rebound effect following overuse of nasal decongestant drops or sprays; symptoms recede when treatment is discontinued [74].
- *Nasal obstruction* caused by deviation of nasal septum: the predominance of the symptoms affects only one nostril. A thorough inspection of the nose with a rhinoscope can help make a differential diagnosis.
- *Rhinitis from foreign bodies:* unilateral, purulent, foul-smelling nasal discharge with exceedingly unpleasant halitosis occurring after several days. It is mostly observed in young children who insert various objects into their nose; often weeks or months go by before a precise diagnosis is made.

Appendix 12.1 lists the main causes of CNO in childhood.

Complications

- *Bronchial asthma* arises in around 30% of all cases, after a variable period of time from the first AR episode. A 23-year follow-up has demonstrated that students who reported nasal symptoms but no evidence of asthma

when first enrolled developed asthma >3-fold more than students without rhinitis [146].

- In *paranasal sinusitis* particular care must be taken to detect a possible sinusitis, with an X-ray of the paranasal sinuses and, as needed, a CT (Chap. 15).
- *Other allergic diseases,* with SAR in nearly 30% of cases, determining a PAR pattern with periodic seasonal exacerbations.

Treatment

There are different treatment options (Tables 12.13–12.15) [116]. Figure 12.17 [27, 48] presents a simplified algorithm for therapy. With regard to the scientific bases of cromolyn and sodium nedocromil, see Chap. 11.

Identification and Elimination of Principal Allergens

While the elimination of allergens in the external environment is not possible (for example pollens abundantly present in the lower layers of the atmosphere, or the ubiquitous spores), the inhalants present in homes can be eliminated with precise environmental controls [19].

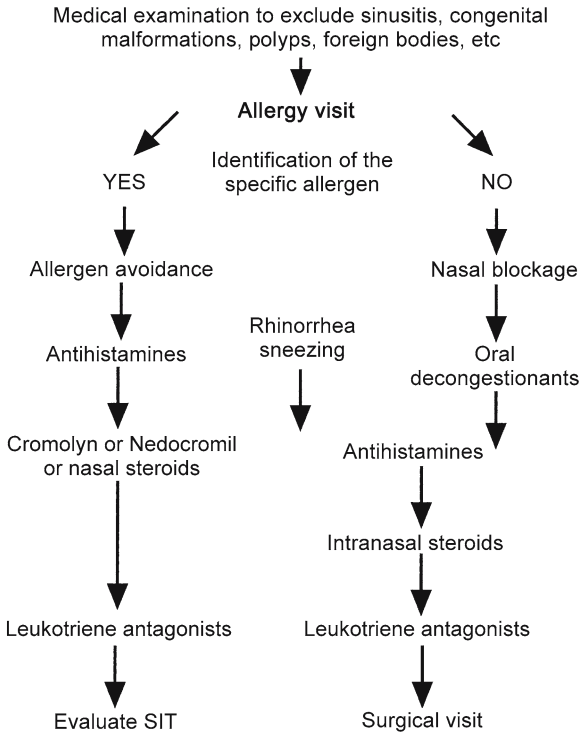


Fig. 12.17. Simplified algorithm for the management of allergic rhinitis. *SIT* specific immunotherapy. Antihistamines include azelastine, cetirizine, ebastine, levocetirizine, desloratadine, fexofenadine, etc. (Modified from [27, 48])

Table 12.13. Treatment strategy for pediatric allergic rhinitis

1. Allergen identification and avoidance
2. Antihistamines
3. Antileukotriene medications
4. Chromones
5. Immunotherapy
6. Topical corticosteroids (severe cases)

Modified from [116].

Table 12.14. Drug treatment of pediatric allergic rhinitis (and associated conjunctivitis)

Drug	Topical		Oral
	Nose	Eyes	
Chromones	+	+	–
Antihistamines	–	+	+
Corticosteroids	+	+	+
β-Adrenergic agents	+	+	–
Anticholinergic	–	–	–
Antileukotrienes			+

Data from [116].

Table 12.15. Effectiveness of drug treatment of pediatric allergic rhinitis

Drug	Sneezing/ pruritus	Hyper-secretion	Blockage
Antihistamines	+++	+++	+
β-Adrenergic agents (not for long-term treatment)	+	++	+++
Chromones	+	+	+
Topical steroids	+	++	+++

Data from [116].

Antihistamines

There is no doubt that antihistamines (see also Table 11.59) have been the starting point of therapy, and are still the most valid drugs, especially with regard to the new generation of H_1-specific receptor antagonists, lacking sedative action. They mainly reduce rhinorrhea, nasal and eye itching, watering, and sneezing, while their effect on nasal obstruction is much lower. Their easy use makes them useful in any circumstance [109,

153]. An advantage of the oral preparations compared to medications for topical use is their effectiveness on the eye symptoms that invariably accompany SAR. The last-generation antihistamines – acrivastine, azelastine, cetirizine, ebastine, fexofenadine, ketotifen, levocabastine, levocetirizine, loratadine, oxatomide – compared with those of the first generation, have practically no sedative effects, since they do not cross the hematoencephalic barrier; nor do they significantly jeopardize children's learning capacity [22]. More precisely, ORs (adjusted for age and sex) for the incidence of sedation were 0.63 for fexofenadine; 2.79 for acrivastine, and 3.53 for cetirizine compared with loratadine [92]. Their dosage is shown schematically in Table 12.16. Recently several cardiovascular effects induced by terfenadine and astemizole, that is the capacity to block the cardiac K channels that permit cardiac repolarization, determining an ECG pattern (prolongation of the QT interval) known as torsades de pointes, have been reported [49]. Terfenadine has been withdrawn from the market. However, a panel of experts expressed a positive opinion on the drug, because in certain conditions the risk of adverse events is rare, especially during the pediatric years [45]. In fact, among 25 patients who had had problems there was a 16-year-old who had intentionally taken an overdose [195]. The average age was 53.3 years. Concerning the variables contraindicating terfenadine and astemizole prescription (Table 12.17) [28, 49], children may be at risk due to a coprescription of macrolide antibacterials, antifungals, etc., especially if they are cardiopathic or hepatopathic, or drinking grapefruit juice. The risks for acrivastine, cetirizine, and loratadine are even rarer, limited to $0–13\times10^6$ daily doses [89]. However, 80 atopic children aged 5–12 years, all suffering from AR and with SPTs positive to Der p, were treated with astemizole, cetirizine, loratadine and terfenadine associated with erythromycin without any cardiotoxic effects, and the increase in QT interval, caused by terfenadine, was no longer statistically significant after correction by the Bazzett equation (for heart rate correction) [45]. It is to be hoped that these data will receive further confirmation. Astemizole, the other medication withdrawn from the market, was an effective drug, well tolerated even in the youngest children [20]. However, its onset of action occurs within 2 days and its peak of effectiveness in 4 days [49]. Its half-life means that a single daily dose is sufficient to ensure 24-h protection [19]. Untoward effects could be due to its long delay in reaching therapeutic concentrations, thus some doctors could be tempted to prescribe higher initial doses, with a further increase in serum levels. A new molecule, tecastemizole, lacks CNS effects and cardiotoxic potential [153].

Acrivastine is a triprolidine metabolite, available in nasal spray form. With a half-life of 1.4–2.1 h, it requires the intake of more frequent doses than other second-generation preparations. It has no effects on the CNS [154], nor is it implicated in torsades de pointes [89].

Azelastine appears to have a triple-action effect: it is an H_1-receptor antagonist (antihistamine effect), stabilizes mast cells (mast cell-stabilizing effect), and inhibits inflammation (anti-inflammatory effect). It was evaluated in an unpublished multicenter study on 4,018 patients, 490 of whom (12.2%) were children aged 2–12 years; 87% of patients showed no undesired reactions, and the drug was effective and well tolerated by 82% of patients and 85% of physicians, with comparative results in the child cohort, data confirmed by two studies in adults [100, 134]. It has no undesired cardiovascular effects, without differences between 12- to 18-year-olds and >18 years of age [16]. Data from 489 children <13 years, including children aged 2–6 years, show that it is well tolerated and effective [86]; it is also marketed in the form of eye drops. The efficacy and safety in the treatment of both PAR and SAR have been evaluated in 211 children <13 years [192]. Azelastine efficacy was assessed by the changes in severity of ten individual symptoms of rhinitis and symptoms were rated according to a four-point scale: 62% of children exhibited a decrease in the total ocular score. General tolerance was evaluated as very good or good by 97% of the treating physicians [192]. In a DBPC trial, 125 children suffering from PAR were treated for a period of 6 weeks and the results were higher than placebo in the relief of all symptoms assessed, namely sneezing, nasal blockage, nasal itch and rhinorrhea [67].

Cetirizine possesses pharmacological actions that enable it to reduce both the infiltration of inflammatory cells and, in particular, chemotaxis and activation of eosinophils. Unlike others, which are metabolized by the liver, it is excreted by the kidneys [154]. Several studies have confirmed its clinical effectiveness, in adults and children, in treating both pollinosis and urticaria, concluding that its effects are very rapid, appearing within 40 min of intake [183]. In an unpublished study we conducted to evaluate its effectiveness and tolerability in children, a clear, significant improvement of all nasal and conjunctival symptoms was noted, while the cytological examination of nasal secretion showed a reduction in the average percentage of eosinophil (43%) and neutrophils (25%) numbers; drug tolerability was excellent. A prospective, randomized, parallel-group, DBPC study has demonstrated the safety of the H_1-antihistamine cetirizine, particularly with regard to central nervous system and cardiac effects, in infants age 6 to 11 months [158] as were several babies we treated and reported in Chap. 11. In two multicenter studies on 293 children 6–12 years of age with SAR [96] and PAR [72], very significant differences were noted in overall improvement. In three multicenter US studies on children 6–11 years, the cetirizine dose was too low (5 mg/day) for its effects to be evaluated; however, it was safe and well tolerated [75]. It is also known that cetirizine can induce sizable reductions of FEV_1 and histamine-induced bronchoconstriction; therefore we conclude that it may be successfully used in children, since it has

Table 12.16. Antihistamine H_1 commonly used for children with allergic rhinitis

Antihistamine	Doses	t_{max} after single dose (h)	Sedating	Peak plasma level after standard dose	Duration of action (h)
Acrivastine (children >12 years) Capsule 8 mg and syrup 0.8 mg/ml	8 mg × 3	1.4–2.1	–	?	8
Azelastine Tablets = 2.2 mg and nasal spray	1 Nasal spray × 2	5.3	±	?	12
Cetirizine, two similar medications Tablets = 10 mg and syrup 1 mg/ml	0.2 mg/kg/day	1.0	–	0.3 µg/ml	24
Desloratadine	5 mg	1.3	–	5.09 ng/ml	24
Dimethindene Nasal spray = 0.14 mg solution	1 Nasal spray × 2				
Ebastine Syrup	5–10 mg/daily	3.6	–		24
Fexofenadine (children >12 years) Tablets = 120 mg	30–60 mg/b.i.d.	2.6	±	1.5–2 mg/ml	24
Levocabastine two similar medications, nasal spray	1–2 Nasal sprays × 2	1–2	–	?	12
Levocetirizine Tablet 5 mg	5 mg/daily	0.5–1.0	–	?	24
Loratadine Tablets = 10 mg and syrup 1 mg/ml	0.2 mg/kg/daily[a]	1	–	5–7.78 ng/ml	24
Oxatomide Tablets = 30 mg and suspension 2.5 mg/dl	1 mg/kg/daily	2.5	–		

Children have a metabolic capacity much greater than that of adults; nasal spray means in both nostrils.
? Unknown.
[a] Or children aged 2–12 years weighing ″ 30 kg, 5 ml/day; if >30 kg, 10 ml/day in single administration in the morning. Compiled from the cited references and from [153].

Table 12.17. Risk factors possibly associated with adverse cardiovascular effects of astemizole/terfenadine in children

Concomitant medications involving liver cytochrome P450 metabolism
Azole antifungal agents: ketoconazole, itraconazole Macrolide antibiotics: erythromycin Cimetidine, delavirdine Natural flavonoids: grapefruit juice
Overdose
Heart abnormalities
Prolonged QT interval Ischemic heart disease Congestive heart failure Anti-arrhythmic medications: quinidine
Metabolic abnormalities
Hypokalemia: use of diuretics Hypomagnesemia Anorexia, fluid protein diet Severe liver disease

Data from [28, 49].

proved to be a rapidly active medication without major sedative effects, similarly to studies done in adults [182]. In a DBPC, randomized study on 69 PAR children 6–12 years with mite allergy, cetirizine was more effective than oxatomide and ketotifen in relieving nasal congestion and rhinorrhea, and significantly decreased the eosinophil number of a post-treatment nasal smear [85]. In 544 SAR children 6–11 years old, cetirizine 10-mg syrup provided significant reductions in mean symptom severity, but above all significant improvements on health-related *quality of life* (HRQL) outcomes were noted [63]. In mite-allergic children, cetirizine administered daily for prolonged periods decreases symptoms of and drug prescriptions for AR and asthma compared with symptomatic treatment [37]. The efficacy and safety of cetirizine and loratadine were compared in a prospective, randomized, DBPC, longitudinal study of 80 children, 2–6 years, with PAR caused by house dust mites or pollens: cetirizine produced significantly greater inhibition of the wheal response compared with loratadine; eosinophil counts were improved to a comparable degree with both agents. Cetirizine and loratadine produced comparable improvements in symptoms; however, cetirizine was more effective than loratadine in relieving the symptoms of rhinorrhea, sneezing, nasal obstruction, and nasal pruritus [150]. In children aged 6–11 years, cetirizine 10 mg has a rapid onset of H_1-antihistaminic activity, a 24-h duration of action, and greater H_1-activity than fexofenadine 30 mg [157].

Levocetirizine has an onset of action within 1 h and provides significant peripheral antihistaminic activity for 28 hours after a single dose. Once-daily dosing may be optimal in children aged 6–11 years. Levocetirizine tablets 5 mg reproduce and extends the cetirizine effects in children aged >6 years based on our extensive experience.

Ebastine is a new piperidine-containing, relatively nonsedating second-generation H_1-receptor antagonist. In a DB, parallel-group study of a single 5-mg or 10-mg dose of ebastine syrup used to treat AR in 20 children 6–12 years of age, both doses significantly reduced the histamine-induced wheal-and-flare areas for up to 28 h compared with predose values [154]. In 30 PAR children, ebastine improved the clinical symptoms, SPT was inhibited and sensitivity of the shock organ was reduced; no secondary effects were seen on the CNS and the tolerance of the drug was very good [6].

Fexofenadine, registered for children >12 years, is a new drug that is 80% eliminated in the feces and 10% in the urine, free of cardiovascular and CNS effects, and has been used in 8- to 12-year-olds, in whom the 30- or 60-mg single dose reached the maximum concentration in 2.4±0.2 h and inhibited the histamine-induced erythema and wheal in 2–24 and 1–8 h, respectively [156]. Fexofenadine, 15, 30, and 60 mg twice daily, was safe and well tolerated in 865 children 6–11 years old for treatment of SAR; no statistically significant mean change from baseline in any ECG parameter after fexofenadine treatment was recorded [64].

We have discussed the properties of *ketotifen* in Chap. 7 and its dosage in Table 7.19.

Levocabastine, available in nasal spray and eye-drop form, is characterized by a fast start of action and a prolonged half-life. Moreover, it does not alter the functionality of nasal mucosa [44], while it protects from symptoms caused by nasal and conjunctival challenge [176]. Data concerning 154 children 6–15 years indicate a good to excellent effect in 54% of the cases (65% in 13- to 15-year-olds), and thanks to its tolerability it can also be prescribed for children >3 years of age, at the dosages stated in Table 12.16. In another cohort of 110 entrants 6–15 years old, it had a significant effect (compared to cromolyn) on eye watering and nasal obstruction [178].

Loratadine possesses all the qualities listed so far [52] and, what is more, it reduces symptoms considerably within the 1st h. The half-life is long, so in children (from 2 years up) a single dose in the morning is sufficient, thus increasing compliance [153]. The peak plasma concentrations were 7.78 ng/ml, at 1.17 and 2.33 h after administration of loratadine. The areas under the plasma concentration–time curve to the last quantifiable time point for loratadine was 16.7 ng/h/ml; ECG parameters were not altered [141]. A meta-analysis of three studies, comprising a total of 784 children aged 3–12 years, demonstrated that loratadine is superior to placebo (61.7% vs 38.3%) with significant differences between the basic symptom score and the 7th day of treatment [77]. In 60 children 3–12 years old with AR caused by dust mites, both total symptom score and daily card scores of the loratadine syrup group 5 mg or 10 mg once a day at day 7 and day 21 was lower compared to the placebo group (DBPC study); no adverse

reactions were recorded [197]. In 25 children with pollen-induced AR, weed and tree pollens treated for 4 weeks, tryptase, IL_5, and ECP levels decreased significantly while the decrease of RANTES and TNF-α levels was not significant. Loratadine was shown to be an effective anti-inflammatory drug that affects the early and late phase of immediate hypersensitivity [82]. In 63 children 8–10 years with a history of SAR and attending a laboratory school, treated with diphenhydramine or loratadine, no treatment-related differences emerged on the verbal instruction score, reading test score, reaction time, or somnolence scale [7].

Oxatomide is 75% eliminated in the feces and has a long half-life. Like ketotifen it interferes with the mast cell degranulation and has similar clinical applications; its action inhibits serotonin and, partially, LTs [153].

Desloratadine is the loratadine metabolite and has a potent unique profile of potent antiallergic and anti-inflammatory activity, in addition to a safe pharmacological profile [141]. Desloratadine 5 mg once daily relieves the symptoms of both SAR and PAR and provides the added benefit of efficacy against nasal obstruction in SAR [175]. In 29 patients 12 to <18 years, desloratadine rapidly and safely reduced the symptoms of PAR, and its efficacy did not diminish during 4 weeks of treatment [157]. Only azelastine, fexofenidine, desloratadine and cetirizine are approved for the PAR treatment in the US [156].

Dimetindene 0.1% spray in 100 children <14 years of age suffering from SAR was compared to levocabastine in a multicenter single-blind, PC trial: symptoms associated with pollen rhinitis, nasal rhinorrhea, itching, sneezing and congestion, and ocular symptoms, lacrimation, ocular itching and red eyes were statistically reduced for both treatments [5].

Anticholinergics

In nasal mucosa there is a cholinergic innervation, so the use of these drugs is justified [21]. *Ipratropium bromide* (IB) is considered useful in vasomotor rhinitis and is effective in reducing aqueous hypersecretion, proving to be a safe and effective alternative product for patients with rhinorrhea-dominated rhinitis. On the contrary, it has no action on itching and sneezing or on nasal obstruction. Since it does not cross the hematoencephalic barrier, it has no sedative effects on the CNS; it is absorbed very little by both oral and nasal mucosa [68]. IB was safe and effective in controlling rhinorrhea and diminishing the interference by rhinorrhea in school attendance, concentration on school work, and sleep, and pediatricians may prefer a medication less likely to produce side effects [102].

Cromones

Cromolyn has an important place in AR because it leaves the mucociliary function intact, an overdose is virtually impossible, and it has proved effective and safe in various studies [21] in nasal spray form. The effect on eosinophil recruitment and/or activation is probably secondary to the inhibition of mast cell infiltration and mediator release into the nose [145]. It is particularly suitable for children, both because it has no side effects and it avoids a frequent use of steroids. Its clinical effectiveness has been proven both in SAR and in PAR, reducing rhinorrhea, nasal itching, and sneezing, while it has no effect on CNO [109]. Cromolyn reduces symptoms of AR, and, when used prophylactically, can prevent symptoms from occurring: because it is poorly absorbed systemically, it is well tolerated and not associated with drug interactions [135]. In children with AR, we regularly prescribe cromolyn spray as a prophylactic measure, two to four times a day, as needed, and cromolyn eye drops if AR is associated with ocular manifestations, usually with excellent results.

Various studies have demonstrated that *nedocromil sodium* acts on both immediate and delayed phase, with highly significant differences vs placebo [121], inhibiting almost entirely the inflammatory cell overflow and reducing their number after NPT [162]. It is available in nasal spray form. It reduces symptoms, including nasal congestion, seems to have a more effective and longer action than cromolyn, and it is tolerated just as well [68].

Corticosteroids

Corticosteroids (CSs) are very effective, used topically, in the treatment of SAR and PAR. They have an anti-inflammatory effect and reduce capillary permeability, mucosal edema, and mucus production. The rational use of this class of drugs resides in their capacity to block A_2 phospholipase, and therefore the first step in the production of LTs and prostaglandins; with the same mechanism PAF formation is prevented [26] (Fig. 12.8). They are used as needed by inhalation and in small doses, making it possible to bring the drug directly to the target organ (Table 12.18) [48, 51]; 80%–90% of all children with SAR improve and are able to reduce or replace the use of other drugs [30]. The therapeutic action is most successful with CS regular use, with pollinosis sufferers starting about 1 week before the AR season starts. In the case of severe CNO, antihistamines and/or vasoconstrictors may be associated (only for a few days, to avoid a rebound effect and dependency), to help the drug effectively reach the site of action on nasal mucosa [109]. However, ICSs, beyond the concerns for suppression of growth, bone metabolism, and the hypothalamus-hypophysis-adrenal (HPA) axis, etc., it is indisputable that their use is purely symptomatic.

Table 12.18. Dosage of pediatric inhaled nasal corticosteroids

Drug	Formulation	Dose (μg)
Beclomethasone dipropionate	Nasal spray	42
	Aqueous	42–84
Budesonide	Nasal spray	32
	Aerosol	32
Dexamethasone	Nasal spray	
Flunisolide	Aqueous	25
Fluticasone dipropionate	Aqueous	50
Monometasone furoate	Nasal spray	50
Triamcinolone acetonide	Aqueous	55
	Aerosol	55

All these formulations are marketed for children aged ″6 years, all by nasal spray or aerosol 1–2 puffs bid, all aqueous 1–2 sp bid.
Data from [48, 51].

BDP nasal spray proved effective and well tolerated in a study by us conducted on 16 PAR children aged 5–14 years, showing a significant reduction in nasal, but not peripheral, eosinophils after 1 and 3 months [53]. BDP proved more valid than the decongestant association [113], attenuated asthma symptoms [136] and reduced histamine and eosinophil levels [109]. Compared to IB, child and physician assessment favored BDP in the control of sneezing [101]. Long-term clinical use of ICS BDP in children aged 24–117 months (mean, 70 months) and treated for an average of 36 months was not associated with decreased height growth. Careful height measurements are recommended every 6 months [93].

BUD nasal spray is a topical CS, which at doses of 64–256 μg once daily has been found to be effective in the treatment of SAR in children. In a recent pediatric study, BUD reduced all symptoms, with significant differences compared to controls [60]. In another, administered by Turbohaler (200–400 μg in a single dose), it proved effective without causing problems detectable by knemometry [193]. In 202 children 6–16 years old with PAR, BUD ICS spray, 128 μg once daily, was significantly more effective than placebo in improving peak nasal inspiratory flow (PNIF), combined and individual nasal symptom scores, and the overall evaluation of treatment efficacy. The onset of action was found to occur within the first 12-h time interval evaluated for combined nasal symptoms and within 48 h for PNIF [60].

Deflazacort, in the form of oral drops, helps in personalizing the prescription (1–2 mg = 1–2 drops/kg/day) and in the compliance of the youngest children, overcoming the problem of a lack of cooperation in the administration of other forms of medications.

Flunisolide ICS proved significantly effective in reducing the clinical symptoms, number of nasal and peripheral eosinophils, and concentration of total and nasal IgE in 22 children suffering from PAR and two from SAR, with no significant variation of the number of peripheral eosinophils, a result that confirms the absence of systemic symptoms with flunisolide [32]. Other studies produced positive results in 80% of 49 children 5–16 years old vs 8.3% of controls [21], with an improvement statistically higher than placebo and a reduction of ECP nasal levels in 38 other entrants, aged 4–14, while cromolyn remained at the limits of statistical significance [145].

FP studied in a wide range of SAR children 4–17 years old was used with excellent effects vs placebo, with statistically significant effectiveness and tolerability, in four randomized DB pediatric studies [18, 115]. This drug is credited with clinical reduction of both early and delayed reactions and of immunological reduction of T lymphocytes, $CD25^+$, and IL_4 [129], with a decreased peripheral eosinophilia as an added result [18]. An interesting meta-analysis study in 1,002 patients documented the statistically significant reduction of nasal eosinophils in SAR and PAR [99] and comparable results in 499 children 4–11 years of age, in whom the single 100-μg/day dose was equivalent to the 200-μg/day dose [3], and in 106 more children treated the same way with the 100-μg/die dose [115].

Monometasone furoate (MF) has been evaluated in 990 pediatric patients studied in phase I, II, and III clinical trials. The 100- and 200-μg daily doses of MF were found to be more effective than 168 μg BDP or 25 μg MF given daily. In clinical efficacy and safety trials, MF was given to 381 children aged 3–11 years for 4 weeks and was found to decrease symptom scores from baseline significantly better than placebo [47]. To examine MF effects on growth, a study on safety and tolerability of once-daily MF in children assessed short-term lower-leg growth by knemometry [15]. In a randomized, PCDB, multicenter study, 1 year of treatment with MF 100 μg daily was found to be well tolerated, with no evidence of retardation of growth or suppression of HPA-axis function in 98 PAR children as young as 3 years. The absence of systemic adverse events, combined with the established efficacy and safety profile of MF nasal spray in children, indicates that it may be an appropriate therapy for children of this age [143].

Neither *triamcinolone acetonide* (TAA) nasal spray 110 μg or 220 μg nor FP nasal spray 200 μg had a clinically significant effect on lower-leg growth velocity. In contrast to FP, TAA nasal spray did not significantly affect HPA-axis function when used over a 2-week period [160].

Table 12.19 [51] shows a comparison of all treatments discussed for AR. A review has compared ICSs with antihistamines: the former produce greater relief from most nasal symptoms than do antihistamines, are more cost-effective than oral antihistamines and both show

Table 12.19. Effects of medications on pediatric nasal and ocular symptoms

Drugs	Symptoms				
	Congestion	Sneezing	Discharge	Itching	Eye symptoms
Oral antihistamines	–	+	+	+	+
IN corticosteroids	+	+	+	+	+
IN cromolyn	–	+	+	+	–
IN antihistamines	+	+	+	+	–
Anticholinergic	–	–	+	–	–
Leukotriene antagonists	+	+	+	+	+

Data from [51].
IN intranasal.

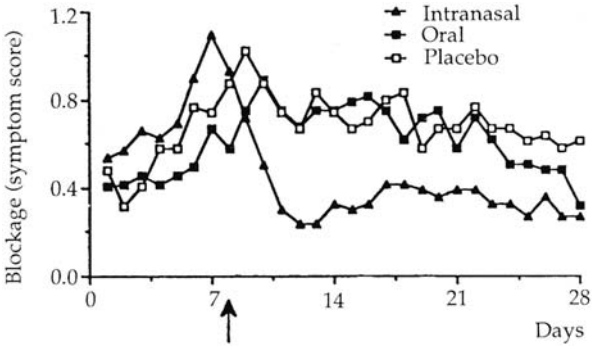


Fig. 12.18. Topical administration of glucocorticoids by nasal spray is effective in treating nasal obstruction in AR; an equivalent oral dose is no different from placebo

no difference in the relief of the eye symptoms of AR [186]. Figure 12.18 [88] shows the difference between CSs taken by young adults orally and by nasal spray.

Anti-LT

Anti-LT have been associated with nasal CSs in adult studies with variable results, favoring BDP over zafirlukast [128], and BUD over montelukast, but both treatments significantly reduced AR symptoms [189]. In the comparison with antihistamines, once daily fexofenadine as monotherapy was equally effective as the combination of once daily montelukast plus loratadine in improving nasal peak flow and controlling symptoms in SAR [190]. In 14 children with PAR, montelukast treatment reversed a typical Th2 cytokine pattern (IL_4 and IL_{13}) toward a Th1 (IFN-γ) predominance [35].

Specific Immunotherapy

Specific immunotherapy (SIT) in AR children is considered useless and expensive, and it is not substantiated by direct comparisons with pharmacotherapy [109]. We have achieved excellent results in children with pollen- and *Alternaria alternata*-induced AR, in whom control children were treated with all the usual medications, but without obtaining the excellent results of study children [29, 31]. Pollen SIT showed an OR of 2.52 (1.3–5.1) in favor of the hypothesis that it can prevent the development of asthma in SAR children [104]; thus SIT may be a means of *preventing asthma completely in AR children* [139]. In SAR, SIT is followed by an improvement in symptoms that entails cellular and humoral changes, and a switch from Th2 to Th1 lymphocytes [50]. Thus SIT has an important role in AR, especially in PAR and in mold-induced AR [142] and in cases not otherwise controllable.

Prevention

The absolute importance of environmental allergen avoidance will never be sufficiently emphasized [20]. A meta-analysis shows that exclusive breast feeding during the first 3 months after birth protects against AR in children, both with and without FHA. The summary OR for the protective effect of breast feeding was 0.74 (95% CI, 0.54–1.01). The effect estimate in studies of children with a FHA was 0.87 (95% CI, 0.48–1.58) [103].

Quality of Life

As in asthma, there is an aspect of the disease that affects the quality of life. In a study conducted on 83 youths 12–17 years of age with seasonal rhinoconjunctivitis, where the use of antihistamines alone or associated with other preparations was required in 73.1% of cases, serious limitations emerged (Table 12.20) [74]. In this study, a questionnaire was drafted, specifically focusing on everyday problems of children and adolescents, which was demonstrated to be specific to this age group, bypassing the divergences from similar questionnaires prepared for adults [74]. In the ISAAC study (Chap. 5), limitations of activities were present only in 3.7%–6.2%

Table 12.20. Limitations caused by pediatric problems related to quality of life

Limitation	%
Systemic symptoms	
Irritability	86.7
Poor concentration	86.7
Tiredness	86.7
Agitation	79.5
Exhoustion	78.3
Headache	74.7
Frustration	74.7
Impatience	69.9
Embarrassed by symptoms	68.7
Embarrassed by aspect	67.5
Embarrassment in general	62.7
Preoccupations	60.2
Nervousness more or less evident	53
Tension/depression	48.2–55.4
Specific symptoms	
Lack of good sleep	63.9–78.3
Nose symptoms	69.9–92.8
Eye symptoms	74.7–88
Practical problems	
Need to rub nose/eyes	94
Need to blow nose repeatedly	86.7
Need to have tissues/handkerchief always at hand	81.9
Limitations of social activities	20.5–44.5
Girls: problems with make-up	25.3
School and/or work	
Lesser efficiency	78.3
Poor concentration	74.7
Reduced capacity in general	69.9
Limitations related to leisure activities/sports	
Entertainment in general	81.9
Playing sports	65.1
Bicycling	61.4
Swimming	55.4

Adapted from [74].

of children aged 6–7 years and in 6.5%–14.2% of those aged 13–14 years. Symptomatic relief and a tolerability profile of cetirizine syrup daily converted into improvements in the HRQL of children with SAR [63]. In 57 teenagers with PAR, symptoms, practical problems, emotions, limited activities were all reduced 2 weeks after start of treatment with either loratadine or BDP, positively evaluating HRQL reproducibility [111].

Long-Term Avenues

Anti-IgE (*omalizumab*), a humanized monoclonal anti-IgE antibody, has successfully conferred a protective effect independent of the type of allergen, thus proving to be useful for the treatment of SAR, particularly for polysensitized children and adolescents [83]. In addition, anti-IgE therapy reduces LT release of peripheral leukocytes stimulated with allergen in children with SAR undergoing SIT, independent of the type of SIT allergen used: during both pollen seasons, the scores for daily medication use, daily nose symptoms, and daily eye symptoms were significantly lower in children receiving anti-IgE treatment compared with children of the placebo group [81]. Omalizumab treatment and concomitant SIT of 49 children with SAR resulted in *a reduction in the number of circulating myeloid DCs* (MDC) compared with control children during the grass pollen season. The MDC increase seen in the control children was paralleled by a dramatic increase in the severity score. Anti-IgE treatment might decrease both the amount of mast cell-derived IL_4 during the pollen season and the number of MDCs, known primers of Th2 cell differentiation, thus the observed effect of normal numbers of MDCs during the pollen season might provide an additional explanation for the clinical effects of anti-IgE treatment [55].

The studies by Terada et al [168] suggest that by using anti-CD49d/CD29 it would be possible to prevent the accumulation and activation of eosinophils in nasal mucosa, or monoclonal anti-IL_5 antibodies could be used to inhibit (in laboratory animals) eosinophilia and NHR. Otherwise it is possible to resort to anti-IL_4 to effectively control sIgE during the pollen season [163]. We wonder what actual, concrete applications can be drawn from this. Shorter-term prospects concern antihistamines, such as mizolastine and ebastine, epinastine, mequitamium, noberastine, rupatadine, and temelastine, which, however, are not yet marketed everywhere.

A novel approach to AR therapy is the application of antisense oligodeoxynucleotides (ODNs) to block proinflammatory IL production [54] by DCs [56] and increase the production of the Th1-related ILs [56]. PS (phosphorothioate-modified) ODNs had inhibitory effects on allergen-induced germline transcript RNA expression and mucosal eotaxin immunoreactivity, whereas PS-ODNs increased the amount of IFN-γ immunoreactivity, suggesting a combined mechanism of reduced synthesis of IgE and eotaxin. In conclusion, the IL_4 antisense PS-ODN effectively inhibits IL_4, IgE synthesis, and eotaxin 1, principal mediators of allergic inflammation, suggesting that mucosal PDCs may offer

a possible topical treatment for AR [56]. Plasmacytoid DCs (PDCs) stimulated allergen-dependent T-cell proliferation and Th2 IL production as efficiently as CD11c+ DCs. However, CpG-activated PDCs inhibited allergen-dependent proliferation of Th2 memory cells and markedly increased IFN-γ production in PDC/T-cell cocultures interestingly, PDCs efficiently drive allergen-dependent Th2 memory responses, thus suggesting that they play an active role in the allergic reaction. However, in the presence of CpG, PDCs produced high levels of Th1-related IFN-α/β and IFN-γ, indicating that mucosal PS-ODNs may be targets for CpG-based SIT strategies against airway allergy [54].

Pediatricians and Allergic Rhinitis

SAR is a highly prevalent chronic disease in childhood. In PAR children, the persistence of symptoms causes limitations in daily activities, not only because of the discomfort originated by the nasal symptoms and the associated symptoms such as migraine, disinclination, reduction in the capacity of to concentrate, etc. The limitations to social conviviality, sometimes erroneously interpreted as indifference, apathy, inattention, mainly at school age, rebound negatively on the emotional welfare of these children. A disease with a prolonged course deprives the individual of a great deal of sources of personal pleasure, as it interferes with self-estime, control of one's own body and in interpersonal relationships. The repercussions of a chronic disease in children and adolescents also affect their family as a whole, and the consequent long-term problems may further damage the image of the whole group. A certainly striking phenomenon is that AR is often *underdiagnosed*, with the prevalent concern of families being over the asthma symptoms, without taking into account the fact that for asthmatic children, AR is a triggering and aggravating factor, to the point that 75% of these children suffer from it; consequently, the impact as a cause of morbidity is also often underestimated [171]. In modern homes, various environmental factors, in addition to external ones, have contributed to increasing its prevalence. Pediatricians will certainly feel the evident need to suggest, where there is a greater concentration of pollutants and irritants, the preventive measures appropriate for concretely eliminating these factors [171]. The hygiene hypothesis [164] suggests that cross-infections might be protective against rhinitis. This is a provocative hypothesis as a wealth of studies have implicated viral infections in the pathogenesis of both rhinitis and asthma. Neonates spending their first night in a nursery are at risk of developing AR and more likely to experience low-dose and short-duration exposure to microorganisms [105]. In individuals >14 years of age with ≥2 siblings, the odds of suffering from SAR (but not from PAR) are less than the odds for people with no siblings [95]. However, AR prevalence is in a crescendo of striking data in children <14 years, and several studies referred to in Chap. 4 have linked AR to twelve different chromosomes and to several HLA molecules. Is it bad hygiene to inhale dust (Chap. 24) or pollens in early life? Should we refrain from inhaling pollens? On practical grounds, we can trust the positive influence of new studies on AR diagnosis and therapy, which will ensure a definite improvement in the quality of life, including *girls embarrassed with their make-up.*

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Specific Immunotherapy

Specific Therapy for Pediatric Asthma and Rhinitis

Although specific immunotherapy (SIT) or specific desensitization therapy was first introduced into allergy practice in 1911, only during the last 50 years have controlled trials been conducted [200]. Noon [166] and then Freeman [82] treated some patients affected with hay fever, which was the name of allergic rhinitis (AR), a term that still survives in the Anglo-American literature, by immunizing patients to a so-called toxin existing in certain pollens; whence the term "vaccine", which remained in current use. Before beginning any treatment, Noon [166] took notes about the dilutions of allergen extracts, which when instilled into his conjunctiva, produced an immediate local reaction, thus showing that the conjunctival reaction could be provoked only by instilling ever more concentrated allergen solutions. The treated patients manifested a marked reduction in symptoms and Noon consequently stated that they had acquired a growing immunity to such a toxin [166].

The term "immunotherapy" is justified since *SIT corrects the immunoregulatory dysfunction at the basis of atopy:* the major purpose is that of reducing the patient's hypersensitivity to aeroallergens, thus achieving the phenotypic modulation of immune responses to specific allergens. Currently it is the only available means to transform the immune response to allergic agents in a clinically helpful way. SIT is therefore the therapeutic option of the greatest benefit to children, whose use should be combined with a high level of consensus on its effectiveness based on the severity and duration of the underlying disease [44]. However, SIT has limitations in addition to benefits, since its undoubted validity based on yearly reports stressing the clinical improvement and fading of more or less severe symptoms in allergic children is matched by several questions on the exact mechanisms of action responsible for its effectiveness. Answers to these questions have remained elusive, even if the never-ending progress in the scientific field in the last few years has led to a better understanding of some of the principal immunological changes, both humoral and cell-mediated, induced by SIT [54]. Additional important aspects are the persistent necessity of disposing of standardized allergen extracts and of clarifying the duration of injection cycles. The search for a gold standard treatment for allergic disease continues to offer outstanding acquisitions. Outcomes in adult asthma may be determined primarily in early childhood [218]. Two risk factors for development of asthma in children and adolescents have been demonstrated, the 88.8% increased prevalence of pediatric asthma in 2003 compared to 2001 [47], and the wheezing that persisted from childhood to adulthood or that relapsed after remission in 26.9% of the study members. At 21 years 77.5% were sensitized to HDM (house dust mite) and 53.8% to cat allergen [218]. In addition, we first report the oral desensitization of food allergy (FA) [187]. *In children SIT is more effective* compared to adults [44] and *it positively influences the natural history of respiratory allergy* [40, 122]. SIT has also been suggested to inhibit progression from AR to asthma [118, 158], and even more reassuring, *adverse reactions are very uncommon and fatal reactions are statistically nonexistent.*

SIT methods of use and safety are often controversial. Some authors suggest that SIT should be routinely given to all patients with respiratory allergy, others that the associated risks do not justify SIT use at all, also considering the availability of newer medications [18, 30, 83]. This philosophy is reviewed by a BSACI (British Society of Allergy and Clinical Immunology) Position Paper [33], which practically restricted the use of SIT to cases of rhinoconjunctivitis and allergy to insect stings, specifying *on the basis of adult studies* that there is no justification to use SIT in infantile asthma [33]. Similar concepts are expressed by the WHO/IUIS Working Group [237], with the notable exception of the EAACI (European Academy of Allergy and Clinical Immunology) [115]. However, both the BSACI [33] and EAACI [115] advised against SIT utilization in children <5 years of age, and raised doubts in particular for the treatment of pediatric asthma. The WHO may focus on SIT and ask for an international consensus with *specialists on adult asthma* [267], which is clearly surprising since the *different results currently found in children being cared for by pediatricians are being methodically neglected* [8, 45, 46]. Abramson et al [2] conducted a meta-analysis of randomized controlled trials using SIT in allergic asthma, and they found only two pediatric studies [69, 264]. It is worth mentioning that at the 11th International Congress of Immunology (Stockholm, 22–27 July 2001), we were invited to deliver a lecture on pediatric SIT.

Table 13.1. Reasons for initiating specific immunotherapy in children

Presence of IgE-mediated disease proven to benefit from immunotherapy
a. Allergic rhinitis
b. Allergic asthma
c. Anaphylaxis following Hymenoptera stings
Documentation of sensitivity to allergens associated with symptoms

Symptoms of sufficient duration and severity
a. Seasonal symptoms despite avoidance measures and pharmacological therapy
b. Perennial symptoms failing trials of avoidance measures and chronic pharmacological therapy
c. Anaphylaxis following Hymenoptera sting except children with non-life-threatening anaphylaxis

Availability of allergenic extract of allergen responsible for sensitivity
Other considerations
a. Discussion of long-term nature of treatment and need for compliance
b. Discussion of risk vs benefit of treatment
c. Accessibility of facilities and personnel capable of administering treatment and evaluating and treating anaphylaxis
d. Emphasis on avoidance as treatment of choice

Adapted from [36].

In this chapter, we illustrate the SIT immunological bases, evaluating its effectiveness in the natural history of respiratory allergy and its safety in children, and analyzing indications, counter-indications, and limits (Table 13.1) [36]:

- Is SIT effective in children with respiratory allergic diseases?
- Does SIT prevent allergic inflammation?
- Does SIT decrease nonspecific bronchial hyperreactivity?
- Is SIT safe in allergic children?
- What is the best age to start SIT?

Definition

SIT, as it is known, is *the only pediatric allergen-specific treatment for respiratory allergy and hypersensitivity to Hymenoptera venom syndromes*, which positively interfere with their underlying immune mechanisms. IgE-mediated disease proven to benefit from SIT is prerequisite, including AR, allergic asthma, and anaphylaxis following Hymenoptera stings. The immunologist says that the shift from Th2 to Th1-type interleukin (IL) predominance redirects the lymphocyte response toward the Th1 T cells and can change the ILs produced by Th2 cells in the respiratory tract [71, 155, 193, 203, 253]. The clinician says that at variance with drugs, SIT is a preventive treatment that has been shown to have long-term efficacy even several years after withdrawal [70], and modifies the natural history of respiratory allergy by *preventing new allergic sensitization* [60, 158, 179], or even *asthma onset* and exacerbations of disease [44, 118]. SIT has also been suggested to inhibit the *progression from AR to asthma* [16, 158]. SIT consists in the administration by subcutaneous (SC) injection of gradually increasing graded doses of the allergen to which the child is sensitized, which will certainly cause clinical manifestations, in order to modify the immune response and reduce the sensitization to the given allergen [37]. Despite its use in clinical practice, more personalized and safer allergy preparations are required to desensitize infants, children, and adolescents [43, 44].

Indications

SIT finds an elective indication in children, as shown by 44 studies (Table 13.2) [1, 7, 23, 26, 38, 40, 43–48, 60, 69, 90, 93, 98, 99, 103, 118, 122, 127, 130–133, 140, 154, 156, 158, 161, 174, 175, 179, 198, 207, 212, 222, 236, 240, 246–248, 268, 264]:

1. SIT is indicated in *allergic respiratory disease* (asthma and/or allergic rhinoconjunctivitis) [115] when the diagnosis is well documented, by skin prick tests (SPTs) and/or specific IgE (sIgE) positivity and, in certain cases, by the response to specific provocation test (PT), and when *the correlation between offending allergens, history and clinical symptoms is well proven.* In our opinion, SIT use should be instituted in the early course of disease [115, 265], *before both morbidity and duration of symptoms are aggravated in young sensitized children*, due to the widespread atopic march (Tables 5.18, 5.19) and more so in all children in whom allergic manifestations are so severe as to provoke school absences and/or poor progress in school as well as problems related to the *quality of life.* Independently of these prerequisites, SIT should be used when both allergen avoidance and drug treatment are inadequate and/or insufficient, or when children are sensitive to allergens that cannot be effectively avoided and need daily treatment for longer periods [115]. SIT is instituted when standardized extracts of true effectiveness of the allergen in question and specialists to perform it are available. Clinicians must also take into account the willingness of parents or responsible persons to accept SIT, their compliance to treatment as well as their socioeconomic status. Arguments have been put forward that suggest that early SIT institution provides a better opportunity for preventing rather than reducing chronic inflammation, and the protection achieved within months or years should be weighed against the increased likelihood of adverse reactions [115]. For perennial asthma, the treatment is also widely accepted even though only one controlled trial [3] was unconvincing (see below).

Der p/f allergy is more frequent in children, and SIT is able to efficaciously control the related symptoms [1, 7,

Table 13.2. Forty-four clinical studies (40 controlled) including only children who have demonstrated SIT efficacy

Authors and reference	Year	Allergens	Duration (years)	No. of cases		Age (years)	Blind	Random	Results Eval	Efficacy	
				A	P					Clin	BR –/= CPT-
Sanders [212]	1966	Pollens	0.5	16	16	4–16	D	Yes	SS	Yes	NT
Johnstone et al [122]	1968	Various	14	67	63	<16	S	Yes	SD	Yes	NT
Aas [1]	1971	HD	3	52	28	3–14	D	Yes	SPT, BR	Yes	–
Taylor et al [236]	1974	Der f	0.2	21	21	6–15	D	Yes	PE, PFT	Yes	NT
Warner et al [264]	1978	Der p	1	27	24	5–14	D	Yes	SS, PFT	Yes	–
Berg et al [23]	1980	Phl p	4	48	0	6–16	No	No	SS, BR	Yes	–
Kjellman et al [127]	1980	Phl p	3	19	17	6–11	D	Yes	SS, DU	Yes	NT
Goldstein et al [90]	1981	Various	0.6	13	0	8–16	No	No	DU, PFT	No	–
Hill et al [103]	1982	Lol p	2	11	9	9–14	No	Yes	SS, DS	No	NT
Cantani et al [45]	1984	Lol p	3	67	57	2–14	No	No	SS, DS	Yes	NT
Price et al [198]	1984	Der p	2	27	24	5–15	D	Yes	SD, DS	Yes	NT
Valovirta et al [246]	1984	Can f	1	15	12	5–18	D	Yes	BR, CPT	NT	–
Murray et al [161]	1985	Der f	1	8	5	11*	D	Yes	SS, BR	No	=
Kuhn et al [133]	1985	pollens	3	26	26	6–14	No	Yes	SS, SPT	Yes	–
Möller et al [156]	1986	pollens	3	39	0	6–16	No	No	SS, SPT	Yes	–
Dreborg et al [69]	1986	Cla h	0.9	16	14	5–17	D	Yes	SS, DS	Yes	– SPT, BR
Sundin et al [207]	1986	Can f, Fel d	1	12	8	8–18	D	Yes	SPT, BR	NT	– (Fel d)
Cantani et al [46]	1988	*Alternaria*	3	39	40	5–14	No	No	SS, DU	Yes	NT
Wahn et al [268]	1988	Der p	2	24	0	8–15	No	No	SPT, BR	Yes	–
Lilja et al [140]	1989	Can f, Fel d	2	12	8	8–18	D	Yes	SPT, BR	Nt	–
Bertelsen et al [26]	1989	Can f, Fel d	0.8	14	13	7–15	No	Yes	SPT, BR	Yes	–
Van Bever et al [248]	1990	Der p	2	9	10	8–15	D	Yes	BR	NT	–
Hedlin et al [98]	1991	Can f, Fel d	3	12	8	8–18	D	Yes	SPT, BR	NT	–
Tsai et al [240]	1991	Der p	3	25	30	5–13	No	Yes	SS, DS	Yes	NT
Van Bever et al [247]	1992	Der p	5	48	42	5–11	D	Yes	PFT	Yes	–
Calvo et al [40]	1994	Der p, pollens	10	166	248	4–12	No	No	PE, SPT	Yes	NT
Koker et al [130]	1994	Der p	1	10	11	4–15	No	No	SS, SPT	Yes	NT
Businco et al [38]	1995	Der p, pollens	3	1,056	0	4–16	No	No	PE, SS	Yes	NT
Jacobsen et al [118]	1996	Phl p, Bet v	4	75	74	7–13	No	Yes	PE, BR	Yes	NT
Des Roches et al [60]	1997	Der p	3	22	22	5*	No	No	SS	Yes	NT
Cantani et al [44]	1997	Der p, pollens	3	151	149	3–7	No	No	SS, DS	Yes	NT
Ohashi et al [174]	1998	Der p	10	19	12	6–10	No	No	SS	Yes	NT
Hedlin et al [99]	1999	Der p, pollens Fel d	3	13	12	7–16	D	Yes	PE, BR	Yes	–
Gruber et al [93]	1999	Der p	2	14	12	7–16	No	Yes	FPT	Yes	–
Altintas et al [7]	1999	Der p	2	28	5	4–18	No	No	SS	Yes	–
Pajno et al [179]	2001	Der p	6	75	63	5–8	No	No	SS, SPT	Yes	NT

Table 13.2. (Continued)

Authors and reference	Year	Allergens	Duration (years)	No. of cases		Age (years)	Blind	Random	Results Eval	Efficacy	
				A	P					Clin	BR –/= CPT-
Milgrom et al [154]	2001	Der p, Bla g Can f, Fel d	0.6	225	109	6–12	Yes	D	SS, DS	Yes	–
Kuehr et al [132]	2002	Bet v, pollens	0.3	108	114	6–17	D	Yes	SS, DU	Yes	NT
Möller et al [158]	2002	Bet v, pollens	3	208	97	10.7*	No	Yes	PFT	Yes	NT
Kopp et al [131]	2002	Pollens	0.3	46	46	6–17	No	Yes	SS	Yes	–
Pifferi et al [175]	2003	Der p	3	14	14	6–14	No	Yes	SS	Yes	–
Cantani et al [47]	2005	Der p, pollens	3	592	590	2–7	No	No	SPT, IgE	Yes	–
Cantani et al [48]	2005	Pollens	3	28	28	3–6	No	No	SS, DU	Yes	NT
Shim et al [222]	2003	Der p	1.1	14		6–13	No	No	SPT, SS	Yes	–

Kjellman and Lanner matched the children treated with purified pollen extracts (*S*) with those treated with crude pollen extract (*C*). Kuhn et al [133] compared those treated with pollen extracts or allergoids and Businco et al [38] with those treated with Der p extract. Sandin et al, Lilja et al [141] and Hedlin et al [99] evaluated the results of the same cohort after 1, 2, and 3 years: the positive results for Fel d were paralleled by those for Can f, even if at lower statistical levels. Shim et al [222] compared the final results with the basal data. Calvo et al [40] evaluated quality of life that was significantly better in the SIT-treated children. Gruber et al [93] performed cold dry air challenges. The children reported by Van Bever et al [248] come from the study by Cools et al [53]. In the study by Kuehr et al [132], 114 children received SIT + anti-IgE (omalizumab) and experienced even more favorable results. Milgrom et al [154] found IgE levels reduced by 95%–99% at the end of the treatment.
A active, *P* placebo, *Rand*, *R* randomization, *S* single, *D* double, *Eval* evaluation, *Clin* clinical, *BR* bronchial reactivity, *CPT* conjunctival provocation test, *DS* drug scores, *DU* drug use, *PE* physical examination, *HD* house dust, *PFT* pulmonary function tests, *NT* not tested, *SD* symptom diary, *SS* symptom scores, *SPT* skin prick tests, * median age, – no reduction.

38, 40, 44, 60, 93, 99, 130, 154, 164, 174, 175, 179, 194, 198, 222, 236, 240, 247, 248, 268, 264], reducing reactivity to nasal or bronchial provocation, increasing specific IgG (sIgG) serum levels and reducing serum IgE levels. Therefore it is not documented that SIT with Der p is less effective than SIT with pollens [18, 83, 164].

Pollen allergy is a SIT classic and well-documented indication both for AR and asthma [23, 38, 40, 43, 44–48, 99, 103, 118, 127, 131–133, 154, 156, 158]. We suggest that SIT use is justified in seasonal rhinitis (SAR) caused by grass in Europe and ragweed in the USA, recognizing that repeated allergen contact during successive seasons may lead to ever-increasing sensitization and to onset of more severe symptoms. SIT effectiveness is demonstrated with other pollens such as olive and *Parietaria*, although results of controlled clinical trials with these allergens in typically pediatric case reports are lacking. We have successfully treated several such children.

Mold allergy responds well to SIT only in cases of IgE-mediated monosensitization to *Alternaria alternata* [46]: its principal allergen (Alt a 1) is well characterized (Table 1.74) and extracts of good quality are available [46]. SIT for *Cladosporium herbarum* reduced medication use in asthmatic children, but not symptom score [69].

In *animal dander* (Can d 1 and 2, Fel d 1) [26, 98, 140, 154, 228, 246], SIT is warranted not only for the spread of related allergies (Tables 5.11, 5.18), but also in specific cases, including ineffective allergen avoidance, psychological shock for the child in case of animal removal, offspring of veterinarians, laboratory technicians, animal dealers, etc. However, while SIT with cat dander extract reduces the reactivity to bronchial PT and increases IgG_4 serum levels after 9 months [26, 98, 204], SIT with dog dander extracts was positive in five studies [26, 98, 140, 154, 246] and induces cat allergen tolerance [98]. These results could depend on a lesser potency of dog compared to cat allergen extracts [96] and on the only recent isolation of Can f 1 and 2 allergens (Table 1.74).

2. *Hypersensitivity to Hymenoptera venom* syndromes represents an elective application of SIT, especially when the history is positive for severe and life-threatening systemic reactions (SRs) to insect stings, and venom sIgE has been demonstrated. SIT should be given following specific schedules and with great caution (rush SIT) and performed in the hospital under observation, in a unit staffed to undertake emergency measures and with personnel capable of administering treatment and evaluating and treating anaphylaxis, especially in the initial

stages. However, the high effectiveness (>90% of cases) in preventing anaphylactic reactions from insect stings justifies the risk [83, 160]. In children, reactions localized solely or extensively to skin do not require SIT, since the prognosis is very favorable [184].

The allergies (almost always pseudoallergies) to drugs and chemical substances usually are not an indication for SIT, especially when IgE-mediated mechanisms do not predominate (exercise-induced asthma, hypersensitivity pneumonia, pulmonary infiltrates with eosinophilia, etc.) [37]. Allergy to foods [187] and to latex [186] can be desensitized.

Child Selection

SIT prescription should be the result of:

- Careful clinical examination (type, severity and frequency of symptoms) (Table 11.34)
- Allergy diagnosis (SPT, sIgE, spirometry, BPTs)
- Respiratory status
- Availability of allergen extract of allergen responsible for sensitivity
- A favorable risk-benefit ratio

Child Age

Although there are no sound and definitive data in the literature indicating the minimal age to begin SIT in children (Table 13.3) [1, 38, 43–48], 10% of pediatricians believe that SIT should be started at 2–3 years, 32%–42% at 5 years and 38%–42% at 10 years of age [207]. However, the most widely held opinion is that children should be at least 2 or 3 years of age [115], the age of several children in a number of studies [1, 38, 43–48] and in sublingual SIT (SLIT) [62, 209]. Although there is no appropriate age, age-related limits should be established [265], to take advantage of SIT's preventive potential [118], especially in children with AR, to prevent the risk of getting pollen asthma [130], but these children are not quickly diagnosed as such [208]. Carefully following both counter-indications and security measures specified in "Adverse Reactions", SIT

Table 13.3. SIT age at start

Data in favor of early treatment with SIT:
The most severe cases with asthma have an early onset of the disease
The allergic component of asthma is most pronounced in children and adolescents
SIT of children with rhinitis reduces the risk of developing asthma
Even slight asthma is accompanied by desquamation of bronchial epithelium, probably due to the ongoing allergic inflammation in the bronchial mucosa
Elastic fibers in the bronchial wall are destroyed in cases with long-standing asthma

Data from [1, 38, 43–48].

can be administered to children of all ages [60, 61, 118, 130, 208], when considering the early onset of more severe cases of asthma (Table 5.5) and the substantial allergic background of pediatric asthma. Above all, SIT can begin at a very early age, in children aged 3–5 [61, 182]. SIT might be a means of preventing asthma completely in such a situation [208]. When an irreversible deterioration in lung function has occurred in the child, and less severe forms are accompanied by bronchial epithelium desquamation caused by ongoing inflammation, or when the bronchial wall elastic fibers are disrupted in long-term asthma, it is too late to start (Fig. 13.1) [265]. Eosinophilic inflammation and even some early signs of airway remodelling might be present in children with developing asthma *before a reliable diagnosis of asthma could be made* based on clinical features [196]. Adult asthma severity is associated with childhood disease severity and indices of atopy, suggesting that adult outcomes are decided *at a very young age*. By the time a child requires medical treatment, the course of disease may already be established *demonstrating that no early intervention may lead to optimal outcomes* [141]. During early childhood the asthma persistence and severity predict disease presistence into later childhood and adulthood *in a least 25% of children* [218]. These are valuable clues to desensitize children as soon as possible. When treating young children with

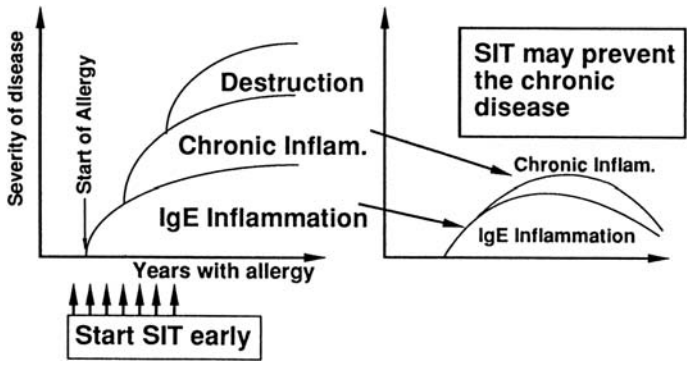

Fig. 13.1. Early start of specific immunotherapy (*SIT*) may change the natural history of respiratory allergy in children

SIT, physicians should know how to evaluate and treat SRs as well as have a well-equipped office [115]. The limiting recommendations made by several experts in adult asthma [78, 237], who report many SRs and the rare fatal cases, *are based on statistically nonexistent results in children.* The implications of SRs related to children stem from the *continuing extrapolation to children,* with these as well in other conditions, of results related to studies in adults.

Counter-Indications

Potential counter-indications may be absolute [38, 115]:

- Severe immunodeficiencies, primary or secondary
- Autoimmune and lymphoproliferative diseases
- Severe chronic and debilitating diseases
- Severe infectious diseases, ongoing or recent
- Other significant medical conditions
- Diseases in which epinephrine use would be contraindicated when necessary due to onset of anaphylactic shock
- Extremely severe sensitization such as that triggered by anaphylactic reactions to SPT
- History of severe reactions during past treatments with SIT (except cases of anaphylaxis to Hymenoptera venom)
- Absolute absence of compliance from children and their parents

Allergen Extracts

Allergen extracts used for SIT are of different types:

- *Aqueous extracts* with rapid absorption are heterogeneous mixtures of allergenic and nonallergenic materials; however, these vaccines can be standardized and used in special conditions such as rush or cluster SIT [267]. In traditional SIT, a greater number of shots is necessary before the maintenance dose is achieved [7].
- *Alum-precipitated* extracts, with stable, delayed and uniform absorption, therefore requiring fewer total injections before benefits are manifested [45]. Adsorbed extracts may be preferable in childhood because of efficacy, safety, tolerability and fewer shots. Lesser adverse reactions are produced and usually limited to the first injections, eliciting good clinical responses together with very good IgG antibody responses [7].
- *Allergen standardization* (Chap. 1) to obtain a complete suppression of IgE synthesis is permitted by a more precise characterization of the allergenic components of available extracts. The advantages of allergen standardization are well known; thus SIT performance with standardized extracts as well as alum-absorbed extracts ensures beneficial effects, and reproducibility and comparability, on condition that the extracts are expressed in International Units (IU), such as BU (biological units), BAU (Chap. 1), and subject to ongoing progress in the field of recombinant allergens (RA) (Tables 1.70, 1.71). It is certain that SIT with pollen extracts is effective when the extracts are lyophilized, of high potency, standardized, and correspond to international standards [115].

SIT Efficacy: Clinical Effects

To best appreciate the significant importance of clinical trial results, it is necessary to understand that allergen-specific IgG, or blocking antibody concentrations, are very low in both serum and secretions of atopic subjects. By administering the same sensitizing allergens, by gradually increasing doses and for a sufficient time span, SIT can increase a child's ability to produce blocking IgG antibodies that are necessary when children come into contact with offending allergens. Consequently, IgG levels rise progressively and unceasingly at the start of treatment and are correlated with administered allergen extract dose: actually *SIT efficacy is dose-dependent, compared to total delivered cumulative dose and not to treatment duration* [54, 96]. No statistically significant effect for ragweed below the maintenance dose of 2 µg Amb a 1/injection was demonstrated, although at a threshold dose for ragweed SIT protective effects begin to progress on both clinical and immunological components [85, 148]. In patients treated for 3 years with the highest immunizing dose, the eosinophil migration was distinctly dampened compared to control subjects, with a significant statistical difference; in addition, the clinical improvement is correlated with the maximal dosage received [85, 148]. In children <5 years, asthma attacks decreased significantly during the 1st year of treatment [61], and those treated for >1 year showed a decrease in the number of yearly acute exacerbations, in hospital admissions and in drug requirements for bronchial asthma [182].

Table 13.2 shows that 40/44 (90%) clinical trials based on a total of 2,909 out of 2,941 children and 592 children seen by us between 2000 and 2003 [47] were positive, with a total of 3,533 children and approximately the same number of control subjects (Fisher, p=0.0000), 1,933 from our studies (54.7%), have incontrovertibly established SIT efficacy in the treatment of respiratory allergy, *positively influencing its natural history in children*. There are four negative trials (9.1%): in one the sample was restricted in absolute and there was a second sensitization to pollens in 11 out of 13 children [161], another treated slightly older children with preseasonal SIT and highly variable doses [103], and the third [90] treated children for 0.6 months. In a further study with supposedly negative results [3], 61 children and 60 control subjects received a mixture of *up to seven aeroallergen extracts* (were they multisensitized?) or placebo for 2 years only, stressing that they suffered

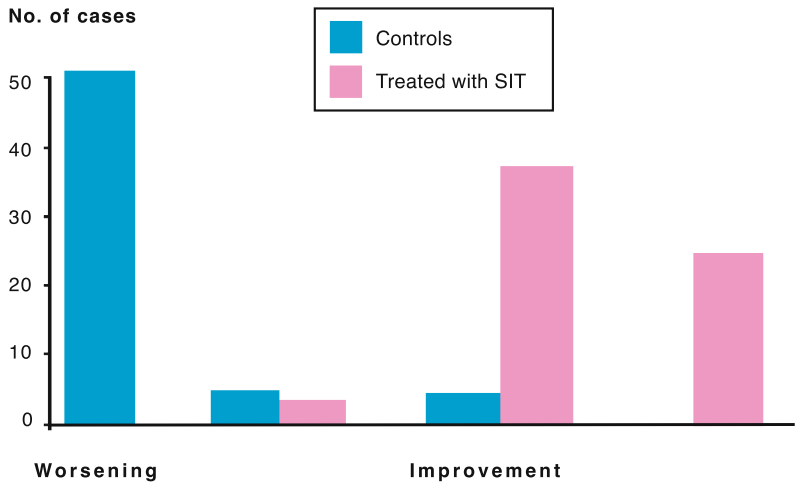

Fig. 13.2. Final results in 124 children with grass-induced asthma 67 treated with specific immunotherapy (SIT) and 57 control subjects treated with pharmacotherapy. Follow-up, 3 years, p=0.0001. (Data from [43])

from severe asthma requiring daily medications, but reporting a significant rise in IgG levels and a reduction in SPT diameter [3].

Six studies are especially noteworthy, two [40, 174] followed children over 10 years, one [157] confirming at most SIT efficacy, at re-evaluation after 5–10 years of SIT injections, the risk of frequent asthmatic symptoms was 3-fold higher in the controls than in the SIT-treated group; the rates of decrease in levels of sIgE and IL_4 were correlated with the rates of decrease in symptom scores after 5 and 10 years of treatment [174]. In another study, the frequent use of antiasthmatic medication was less statistically significant than in the control group even 12 years after SIT termination [40]. Also reduced were the number of days and nights free of asthma [45, 47] as well as the bronchial hyperreactivity (BHR) [99]. In asthmatic children monosensitized to HDM (house dust mite SIT has been shown to prevent onset of new sensitizations [163]. In a 3-year course of SIT there is evidence that SIT may hinder progression from allergic rhinoconjunctivitis to asthma [142]. Anti-IgE grass SIT (omalizumab) significantly reduced daily medication use, eye symptoms, and symptom load, whereas birch SIT significantly reduced nasal symptoms and symptom load [116]. Effectiveness was rated excellent or good for 31.5% and 44.7% of the omalizumab group vs 16.3% and 32.7% of the placebo group [139].

A study in 210 asthmatic children followed for 14 years has demonstrated that during adolescence 72% of SIT-treated patients outgrew their asthma for at least 1 year, while only 22% of control children were asymptomatic [122]. As early as 1968, this study demonstrated that SIT is able to modify the natural history of allergic disease, despite criticism raised because of its methodological limitations. These results were matched by a study reporting that SIT-treated children had fewer new sensitivities to inhalant allergens than those who did not receive SIT [60], by results on 688 children, 75% of whom outgrew asthma in the adult age [200], and confirmed by recent studies [44, 60, 118, 158]. Asthmatic symptoms faded after 5 years of therapy in 66% of SIT-treated pollen-allergic children, and increased by 16% in placebo-treated control subjects [121]. Several trials have been criticized because they were not double-blind (DB) studies using nonstandardized extracts, were not placebo-controlled (PC) and did not take into account that spontaneous improvement in asthma is commonly seen during childhood [18], although this is certainly not true in children with persistent asthma (Table 5.15). Table 13.2 shows that there are only 4 out of 44 (9.1%) pediatric studies that were not PC over 39 years. Several authors have demonstrated in long-term studies that a substantial proportion of non-SIT-treated children continued to present with asthma in their adolescence, thus establishing SIT efficacy in actively treated children with respiratory allergy [51].

In a study conducted in 87 children with pollen-induced asthma and/or rhinitis [43], treated for 3 years with SIT using a base of pyridine-adsorbed and alum-precipitated allergen extracts, we found very good results in 39% of cases and good results in 55% of cases. In 10% of control subjects, the improvements varied from good to poor (p=0.0033–0.0001) Figs. 13.2 and 13.3 show that the result is practically identical in children with pollen asthma or rhinitis [43]. Figure 13.4 [43] shows that *the clinical result is dose-dependent.* In a further study in 39 children with monoallergy to *Alternaria alternata* treated with SIT for 3 years and evaluated as above, we observed very good results in 80% and good results in 20% of cases (and in 0% and 2.5% of control subjects) (p=0.0001) (Fig. 13.5) [46]. In 300 Der p- or pollen-sensitized children, we again found significant differences between SIT-treated and control children and a reduction in the number of days with asthma (Fig. 13.6) [43]. In a trial on 28 children, we measured the mean daily grass-pollen counts (Fig. 13.7) [48] and among the clinical results we found a decrease in antihistamine use (Fig. 13.8) [48]. In the most recent trials

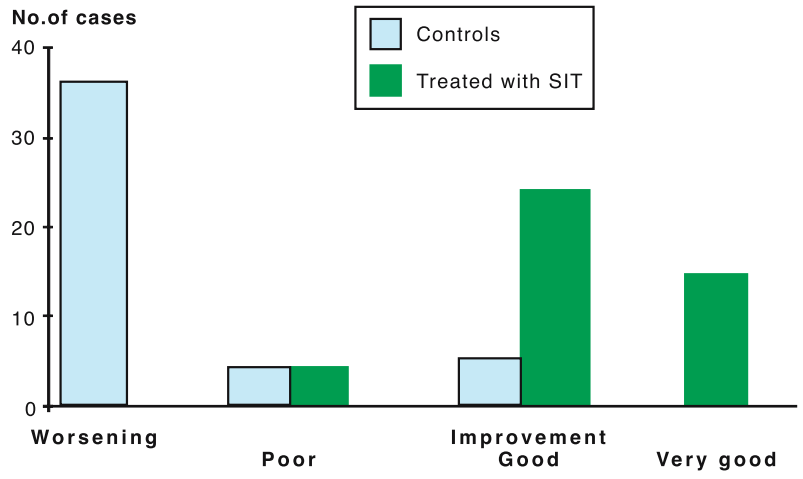

Fig. 13.3. Final results in 41 children with grass pollen-induced rhinitis, 20 children treated with SIT and 21 control children treated with pharmacotherapy. Follow-up, 3 years, p=0.0033. (Data from [43])

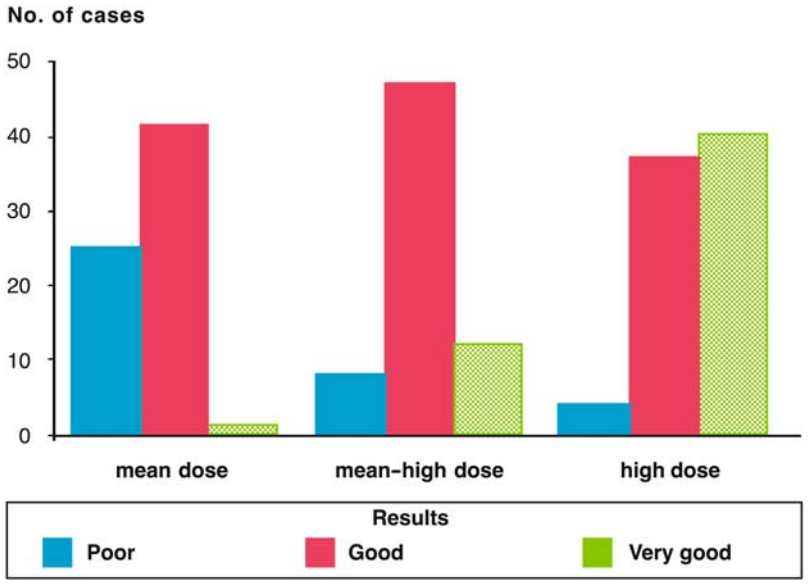

Fig. 13.4. Correlations between clinical results and allergen doses administered to 67 children with grass-induced asthma and rhinitis treated with SIT. Follow-up, 3 years, p=0.0001. (Data from [43])

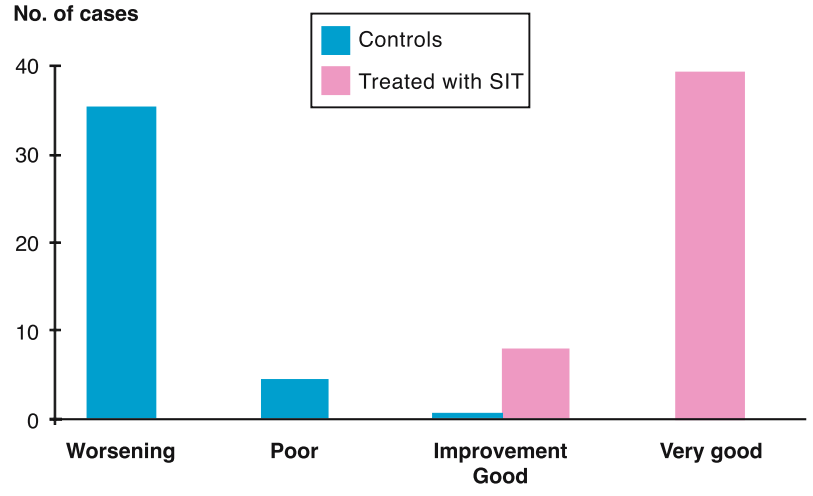

Fig. 13.5. Final results in 39 children allergic to *Alternaria alternata* treated with SIT and 40 control subjects cured with medications. Follow-up, 3 years, p=0.0001. (Data from [46])

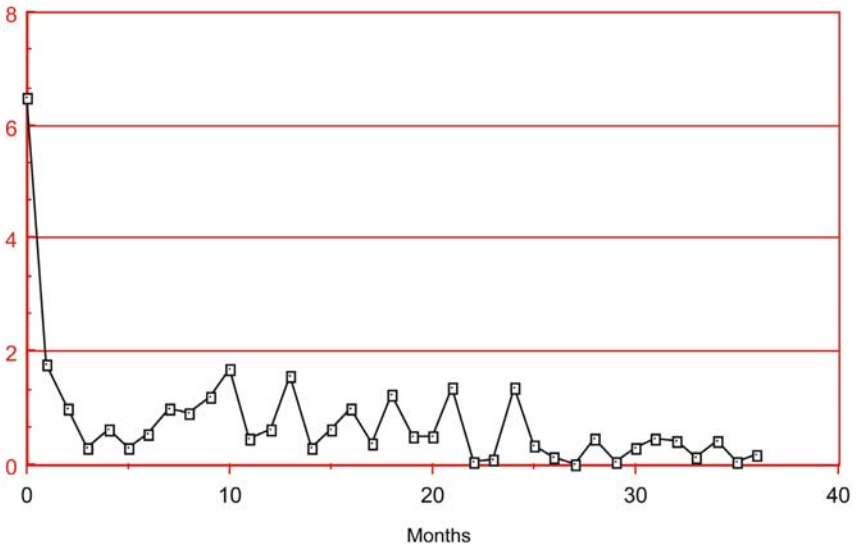

Fig. 13.6. Significant decrease in number of days with asthma after 40 months with asthma plotted in 151 children. (Data from [43])

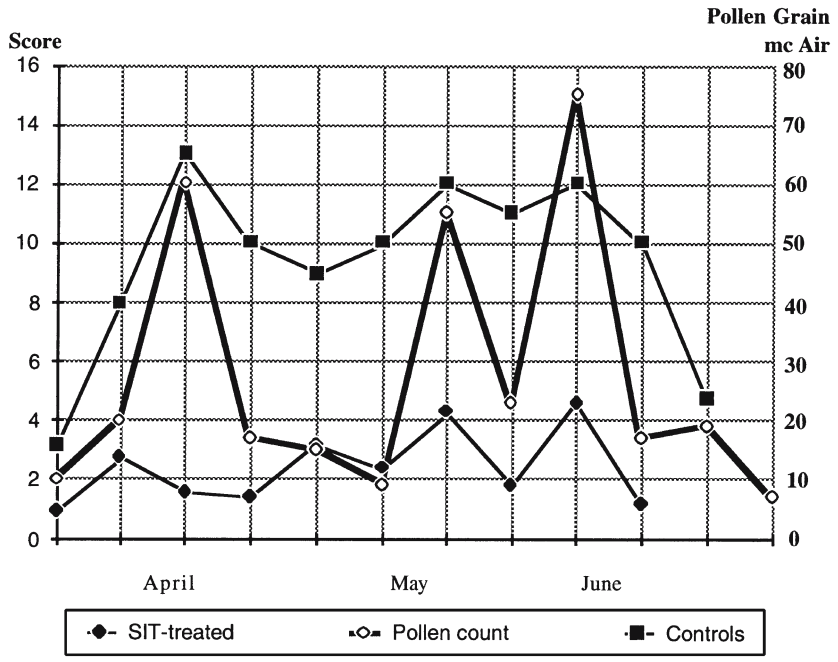

Fig. 13.7. Symptom rate (week mean) during grass pollen season in 28 children aged between 3 and 6 years with respiratory allergy treated with SIT and control subjects. Follow-up, 3 years. (Data from [48])

[43, 47] we have demonstrated the effect of asthma desensitization. As in the other trials, the control group formed by a sample comparable for all parameters to the study children was treated with all available medications. Results were evaluated based on a differentiated score of asthmatic and rhinitis symptoms. At the second yearly control the study children had a significantly greater reduction as regards the number of nights with asthma, school absences and the amount of rescue medication used as compared with drug-treated children [49]. In addition, AR draws a great benefit from SIT [43, 118], as demonstrated by statistically significant increases in symptoms and asthma development in drug-treated control subjects [118]. SIT for Hymenoptera stings was particularly effective both in adults [129] and in children: 9% of untreated subjects underwent SRs compared to 1% of those undergoing SIT [216]. DB, PC (DBPC) trials in children raise notable ethical problems [45] and very few parents would agree to give informed consent [45]. Recently a new problem has emerged, that pollen-treated children, with no asthma before commencement, developed asthma after *SIT*, in a dramatic number of cases [170] in contrast with a 6-year study [179]. In the PAT study the proportion of pollen-treated children developing asthma was 24.7% in the study group vs 39.7% in the controls [158]. In a *pre-seasonal* trial *23% of children* developed new sensitization to perennial allergens after a 13-week treatment, *and 61% 8 years* after start of SIT [72]. Notably, the percentage of children who had asthma after 3 years of

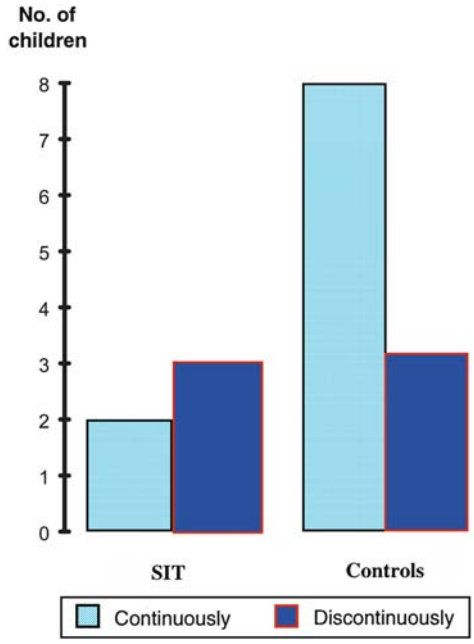

Fig. 13.8. Number of children aged between 3 and 6 years with respiratory allergy treated continuously or discontinuously with antihistamines in the study group and control group (Fisher = 0.0219). Follow-up, 3 years. (Data from [48])

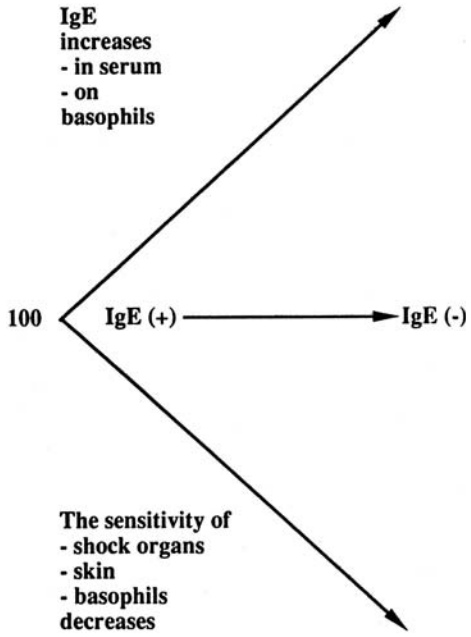

Fig. 13.9. In vivo results from clinical trials conducted with patients undergoing SIT with an effective improvement, controlled with in vitro investigations. These data correspond well to the hypothesis that the SIT mechanism works on a switch from IgE (+) to IgE (–)

SLIT *varied from 10% to 63%* [170]. We have treated hundreds of children with pollen-rhinitis, who never developed asthma, as seen in the frequent controls as detailed before [38, 44, 45, 47]. An investigation on SIT between Italian pediatricians showed that those who administered SIT evaluated SIT efficacy in 92% of cases and those who did not administer SIT in 78% of cases [207]. A recent study on the quality of life has found high ORs when assessing 27 children 6 months after SIT treatment, in pruritus (OR = 6.8), nasal obstruction (OR = 5.9), and also in runny eyes and nose (OR = 7), nose blowing (OR = 4.8), irrtability (OR = 6.2), and ocular pruritus (OR = 3.1) [159].

SIT Efficacy: Immunological Effects

SIT elicits immunological modifications, both humoral and cell-mediated [70, 94, 164]:

Humoral modulations

1. Regulation of IgE synthesis (Fig. 13.9) [68]
a. Long-term decline in antigen-specific IgE [54]
b. Decrease in seasonal IgE boost [54]
2. Increase in allergen-specific IgG [86]
3. Production of anti-idiotype antibodies [50]

Cell-mediated modulations

4. Reduced mediator release by sensitized cells
a. Decreases in human blood basophil reactivity to antigen-specific histamine [94]
b. Decreases in production of chemotactic factors for eosinophils and neutrophils [163, 202] and eosinophil adhesion [95]
c. Reduced mast cell numbers in the skin and respiratory tract
5. Reduction/inhibition of effector cell activity and tissue responsiveness
a. Decreased antigen-specific cutaneous response [164]
b. Decreased BHR [1, 93]
c. Decreased nasal responses to mediators
6. Decreased cutaneous late-phase response to specific allergens (LAR) [264]
7. Production and/or stimulation of lymphocyte populations and subpopulations [231]
8. Possible switching from Th2 to Th1 with ensuing down-regulation of Th2 and up-regulation of Th1 responses [253]
9. Induction of IL_{10}-producing regulatory T cells [79, 169]
10. Reduced expression of CD23 on peripheral B cells [123]
11. Increase in associated IFN-ɣ production [71, 149]
12. Reduction of adhesion molecules [113]
13. Increased antiendotoxin antibody [215]
14. Other IL effects

Taken together, these studies suggest that SIT may act by modulating T-lymphocyte responses to allergen exposure, compatible with the induction of T-lymphocyte tolerance in vitro. Future strategies directed at development of peptides that induce T-lymphocyte tolerance

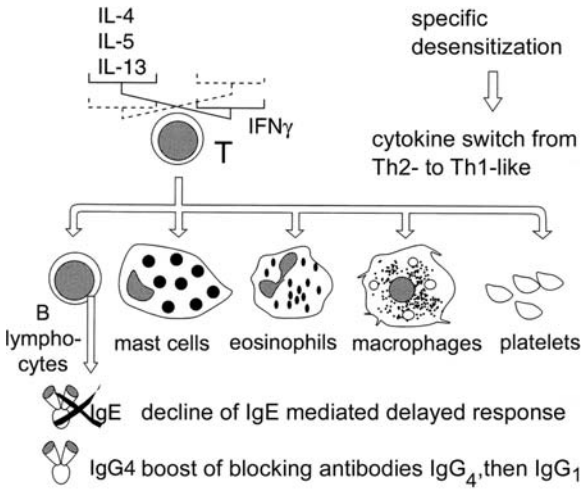

Fig. 13.10. Immunological effects of SIT

but do not interact with antibodies may improve the efficacy and the safety of immunotherapy, minimizing the risks of anaphylaxis [45]. Figure 13.10 outlines SIT immunological effects.

Humoral Modulations (Fig. 13.11)

Regulation of IgE Synthesis

Suppression of Allergen-Specific IgE Response

Initially in response to treatment, for some months a significant increase in IgE is observed, if SIT is continued for years, followed by a gradual lessening of seasonal increment and the further decrease in allergen-specific IgE synthesis after 5 years of SIT [174]. Alternatively, total serum IgE levels decrease after 4 months of SIT and increase 9 months after SIT starts. Since there is no correlation between IgE decline and clinical improvement, IgE monitoring during SIT is usually of no value, also because the response to SIT of a given patient may be highly variable [249]. SIT-induced sIgE reduction can acknowledge several mechanisms, among which the most fitting is the preferential induction of IgE- and allergen-specific CD4 or CD8 T lymphocytes [231], and production of ILs capable of modulating IgE synthesis, including IFN-α, IFN-γ and TGF-β (Fig. 1.58). The initial, rapid IgE increase is not accompanied by a matched increase in allergen-specific IgG, which occurs in a significant way only subsequently, as IgE response is evoked by low doses of allergen extracts, whereas for IgG antibody higher doses are necessary and hence a prolonged period of SIT injections. This discrepancy can explain the exacerbations of respiratory symptoms occasionally present in the first months of therapy, which therefore would always necessitate pharmacological prevention [45]. The bulk of evidence shows the need for receiving SIT for long periods and with adequate doses to obtain a firm and significant IgE reduction; however, IgE levels may increase a year after stopping SIT [70]. Surprisingly, in 225 children treated with anti-IgE, median free IgE ranged from 133 to 790 IU/ml at baseline and was in the range of 6–9 IU/ml at week 24 of the treatment period. This corresponded to a reduction in free serum IgE ranging from 95% to 99% related to subgroups. Omalizumab binds to free IgE at the same site as the high-affinity (FcεRI) receptor. Although it attaches to free IgE, it does not bind to cell-bound IgE, nor does it induce cross-linking of cell-bound IgE that would lead to the release of allergic mediators [154]. In conclusion, the reduction in IgE levels never corresponds to the levels found in control subjects: it is known that minimal ng levels of serum IgE are sufficient to induce mast cell degranulation. In a recent study [48] (Fig. 13.11) in children allergic to aeroallergens treated for 3 years, there was an inverse correlation between IgE and IgG antibodies, with highly significant differences [48], data confirmed by an adult trial [270].

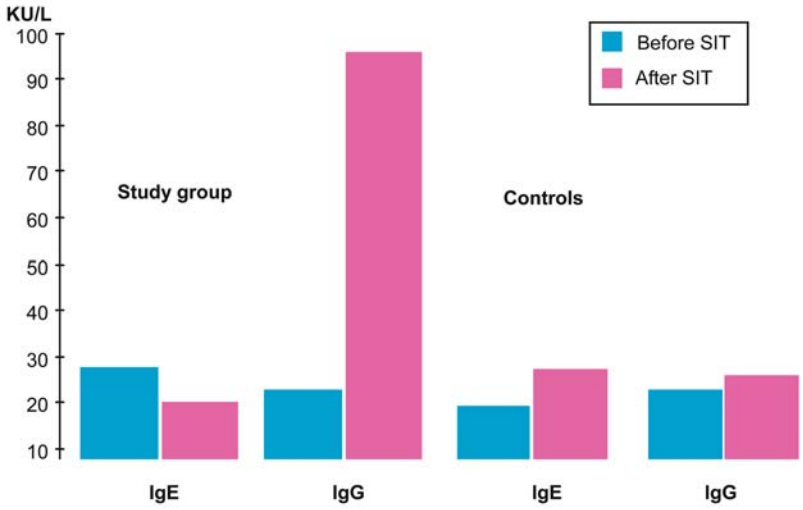

Fig. 13.11. Decrease in IgE and increase in IgG antibody levels before and after SIT in 28 children and 28 control subjects aged between 3 and 6 years with respiratory allergy. Follow-up, 3 years. IgE and IgG levels before vs after SIT; p=0.00001. (Data from [48])

Decrease in Seasonal IgE Increase

In pollen-allergic subjects, SIT significantly reduces the common seasonal increment of allergen-specific IgE with time. Only rarely will these levels disappear [48].

Production of Allergen-Specific IgG Antibodies

IgGs are identified with IgG_4, found in very low concentrations in serum and secretions of atopic subjects. IgG_4, equipped with receptors for metachromatic cells, are called *blocking antibody* (thus protective) because following the administration of allergen extract they do not trigger histamine and other mediator release but, on the contrary, compete with IgE for allergen binding. As a consequence, mast cell degranulation and mediator release is halted, with the ultimate result of intercepting specific allergens before reaching mucosal surfaces in the respiratory tract. Actually, the studies reported above suggest that these could be anti-IgE IgG antibodies complexed with sIgE (erroneously interpreted by RAST as IgG, see Chap. 6), provided with higher levels than supposedly free anti-IgE antibodies that are similar to sIgG, and that they might be at the basis of some negative results [120]. In addition, 25% of children could be deficient in one or more subclasses, or may be due to the use inadequate devices (Chap. 22). IgG, by competing with IgE, can therefore alter RAST results, by disguising and thus misrepresenting the true IgE concentration. IgG increase progressively and constantly at the start of treatment and are correlated with immunizing doses of administered allergen extract [174]. After 2 years of SIT, a mean 8.8-fold increase in the level of IgG allergen antibodies was noted [3]. Elevated serum levels are obtained only by high doses, whereas when discontinuing the treatment, IgG titers decrease rapidly. Among SIT-induced immunological changes this is understandably the most convincing and significant, strictly correlated with clinical efficacy (Fig. 13.11). The hypothesis that IgG may block an IgE-mediated condition is not decisively proven, there being cases with an evident clinical improvement preceding IgG rise [48] and inversely cases of negative clinical results associated with an IgG net increase [51]. It has been recently confirmed that SIT induces blocking IgG antibodies that inhibit IgE-facilitated allergen binding by B cells and presentation to T cells [257]. Moreover, mite-specific IgG_4 and IgA increased significantly in asthmatic children after SIT and is thus a marker of effective treatment [84]. Thus, IgG production (and production of IgA) may be a marker of effective treatment, even more so when allergens are diffused by blood, as is the case of SIT with Hymenoptera venom, but also seen in inhalant allergy. Increased titers of Der p-specific IgG_4 during SIT have reached a highly significant correlation with serum antibody levels and the clinical improvement in 96% of children after 2–3 years of injections [240]. Mite-specific IgG_4 and IgA increased significantly in asthmatic children after SIT and is thus a marker of effective treatment [84]. Similarly, after 1–2 years of oral SIT, IgE levels were decreased and IgG_4 levels were increased [187]. However, it is also true that follow-up studies of SIT-treated patients has shown that the SIT success persists, whereas allergen-specific IgG have returned to the starting point [120].

The role played by *IgG subclasses* has been studied over several years by many authors: in the first SIT stage, IgG_1 (mean half-life, 29 months) predominate and, after about 1 year there is an IgG_1 switch to IgG_4 [191], which does not influence clinical manifestations; thereafter IgG_4 (half-life, 9 months) prevail [191]. IgG_4 selective rise, in case of long-term SIT, for example with pollens, is significantly correlated with clinical improvement [11, 86, 233]. Other authors are not convinced that this rise depends on the production of antibodies directed against epitopes or antigens unrelated to IgE response [51]. Further data [225] show that the early appearance of IgG_1 (at 12 weeks) is inversely correlated with IgE immune response to certain antigens, whereas this response appears for additional antigens when the early IgG_1 rise is lacking. Moreover, in the reaction to certain antigens the IgE increase corresponds to a drop in IgG_4 values, and in other cases the contrary occurs. It is surprising that with unchanged IgE levels, the increase in IL_{13} titers is positively reflected on both IgG_4 titers and the improvement of Der p-allergic children [144]. There should be a common regulatory mechanism between IgE synthesis and either IgG_1 or IgG_4 [225], with a significant proof of a correlation between IgG and IgE after 2 years of SIT [191]. Among the immunological changes more recently described in association with grass pollen SIT are the allergen-specific IgG antibodies that can inhibit IgE-facilitated allergen binding by B cells and presentation to T cells and disrupt formation of allergen–IgE complexes that bind to APC (antigen-presenting cells) [257]. Bet v 1-specific IgG_4 levels were significantly increased after SIT. None of these changes were observed in the placebo group. When the sera were tested for their ability to induce serum IgE-facilitated allergen presentation, a complete abrogation of this effect was noted in the sera from patients receiving active treatment [250]. In particular, following SIT peripheral T cells cultured in the presence of grass pollen extract produced IL_{10} [169]. Post-SIT serum exhibited inhibitory activity, which co-eluted with IgG_4 and inhibited IgE-facilitated binding of allergen-IgE complexes to B cells [169] and presentation to T cells [257]. Both the increases in IgG and the IgG blocking activity correlated with the patients' overall assessment of improvement. Thus, grass pollen SIT may induce allergen-specific, IL_{10}-dependent protective IgG_4 responses [169]. In thinking about some antigens binding to IgE and expressing major allergens to which patients frequently react [46], IgG antibodies could be essential in preventing the start of side effects during SIT, thus decreasing patient reactivity to *progressively increasing doses of*

allergen extract. Recent data show that the capacity of IgG to counteract IgE is probably the central mechanism behind successful allergen desensitization. If the Fc fragment of IgG in the immune complex produced by allergen-specific IgGs in response to immunization bind to the inhibitory receptor FRcεIIb, this, in turn, inhibits the activation pathways activated by FcεRI. Classic desensitization may fail in a subgroup of patients because activating FcεRIIa prevails over inhibitory FcεRIIb [126, 275].

Production of Anti-Idiotype Antibodies

Idiotype–anti-idiotype interactions play a qualitative and quantitative role in regulating IgE production: concentrations of anti-idiotype antibodies were much higher in the serum of patients treated with SIT compared to control subjects [50]. However, an inverse correlation was found between titers of both IgE and anti-idiotype antibodies characterized by reduced IgE and elevated anti-idiotype antibodies in SPT-negative and RAST-negative nonatopic patients and by an inverse result in atopic subjects [50]. In complex, anti-idiotype antibodies increase in SIT-treated atopic subjects, whereas IgE decrease. Hence, SIT could stimulate an active production of anti-idiotype antibodies capable of inducing a complete and persisting suppression in sIgE [50, 244], functioning in atopics as antigens of the network [244], therefore in practice mimicking the structure of antigens that are apparently not correlated (Fig. 1.21). It seems, moreover, that further studies are necessary to correlate the development of anti-idiotype antibodies to SIT clinical efficacy, therefore clarifying the mechanisms that regulate this production.

Cell-Mediated Modulations

Reduced Mediator Release by Sensitized Cells

Reduced Histamine Antigen-Specific Release by Basophils

According to several investigators, SIT protracted for years and at high doses, modulates target cell function and number by reducing histamine release by basophils despite allergen–IgE interaction on their molecules, possibly depending on a synergistic action of other aspecific stimuli that contribute to a reduced histamine release. More precisely, basophils are the target of chemokines that, interacting with sIgE on basophil membrane, induce their releasability [30, 97] and hence histamine release [94]. Thus, basophils have the potential to play an outstanding role in decreased IgE-mediated histamine releasability during SIT [222]. Changes in cytokine production induced by venom SIT may also affect effector cells such as basophils and inhibit antigen-induced basophil histamine release [193]. HRF spontaneous production occurring in patients following histamine challenge, causing a ≥20 % drop in FEV_1 (PC_{20}), has demonstrated a significant correlation between HRF-induced histamine release by basophils (found only in atopic asthmatic subjects) and symptom severity during late reaction [114]. The clinical relevance of these results has been documented in pollen-allergic patients treated with SIT for 2 years (but not in control subjects) by an outstanding reduction in HRF spontaneous activity, correlated with an equivalent FEV_1 improvement [135]. Thus SIT can inhibit HRF seasonal increase by ragweed-stimulated mononuclear cells, as well as seasonal activation of nasal monochromatic cells. The elucidation of the mechanisms at the base of SIT and the clinical results require further suitable investigations, taking into account that a clear-cut likeness between histamine release and effects on IgE concentrations or on IgG production has not yet been recognized [35].

Reduction of Eosinophil Chemotactic Activity and Neutrophil Chemotactic Activity and Eosinophil Adhesion

ECA and NCA reduction can be correlated with eosinophil and neutrophil reduced attraction to the airways with evident clinical benefits. In patients with SAR treated for 3 years by the highest immunizing dose, eosinophil rates were clearly lower than in the control subjects, with a statistically very significant difference: by analyzing BALF (bronchoalveolar lavage fluid) it was demonstrated that SIT blocks eosinophil recruitment and ECP (eosinophil cationic protein), ECA and NCA accumulation in both nasal and respiratory mucosa, but *only in patients treated with elevated doses*, that is a maintenance of 10^5 BU [202, 203], likely for switching from Th2 to Th1. In 20 asthmatic children aged 5–17 years, half SIT treated and half not, only in SIT-treated patients was a significant reduction in eosinophils clearly shown [155]. The demonstration that SIT blocks eosinophil influx to the target organ, combined with inhibition of ECA generation [163, 202], a very sensitive marker of allergen exposure, could lead to the identification of etiopathogenetic mechanism of SIT [202]. This study has measured in the BALF cells and proteins the levels before and during two succeeding pollen seasons, demonstrating that *inhibition of eosinophil traffic is determined by the parallel inhibition of ILs released by peripheral blood mononuclear cells* (PBMCs) [202]. SIT prevents eosinophil adhesion to CD54 and CD106 molecules, but only in patients with pollen asthma, but neutrophil adhesion continued to progress, another event correlated with the switching from Th2 to Th1 [95].

Reduction/Inhibition of Effector Cell Activity and Tissue Responsiveness

Reduced Antigen-Specific Cutaneous Response

The reduced antigen-specific response is in part correlated with the reduction of late responses [165], due to raised IgG levels, as demonstrated in untreated atopics by low antibody levels and a 94% incidence of late responses, while in SIT-treated patients, IgG concentrations were elevated and the incidence of late responses dropped to <50% [94].

Reduction in BHR

Specific challenges given during a check-up done after a prolonged SIT cycle demonstrated a rise in the BHR threshold to the responsible allergen, an evident clinical improvement [1]. As a whole, these modifications speak for a general attenuation of effector cells actively involved in allergic reactions [30]. BHR reduction was recorded in adults with Der p-induced asthma without remarkable side effects [147]. In a placebo-controlled study, no significant change in BHR to histamine was found after 1 year of SIT with a standardized HDM extract [224]. Others observed an insignificant increase in BHR to histamine after HDM-SIT, while BHR decreased in subjects without SIT [161]. In a subsequent study [93], 2 years of SIT caused a significant reduction in BHR in HDM-allergic pediatric asthma patients. The difference in outcome [93] could suggest that a SIT course of 1 year [161, 248] might be too short to bring about a significant therapeutic effect on BHR. A 3-year course of SIT in children with allergic rhinoconjunctivitis significantly improved BHR [158].

Decreased Nasal Responses to Mediators

In the nasal lavage fluid of SIT-treated pollen-allergic adults, a significant reduction in histamine level and a reduction in immediate and late kinins and TAME-esterase reactions was observed after pollen challenge, always more evident as SIT progressed from the lowest to the highest tolerated immunizing dose [114]. Similarly to the cell-mediated field, eosinophil migration into the nasal cavity is inhibited with reference to dosage increase, by reaching reduced levels with increasing doses, as pointed out above [85]. This reaction can also be demonstrated in the skin [54]. SIT inhibits eosinophil infiltration in the nasal mucosa and activation, as well as a significantly increased expression of IFN-γ, unlike *control subjects who experienced highly significant increases in IL_4 and IL_5* [71]. Parallel results have been found during nasal SIT by an evident reduction in both eosinophils and patients' reactivity to the challenge [183]. Moreover, since PAF (platelet-activating factor) levels were remarkably increased during asthmatic attacks in children and as much decreased after SIT, this treatment may have interfered at the level of the metabolic pathway with the production or secretion of inflammatory mediators [111].

Decreased Cutaneous Late-Phase Response (LPR) to Specific Allergens

The decreased cutaneous LPR is responsible for chronic and aspecific hyperreactivity frequently seen in asthma and AR, hence the SIT potential for positively influencing the LAR is a very significant result, due to the known interconnection with symptom severity. Studies in cedar-allergic adults [77, 165, 252] have demonstrated a very significant reduction in cutaneous LPR, of $CD3^+$, $CD4^+$, activated eosinophils and switching from Th2 to Th1, which correlated with clinical improvement (Fig. 13.12) [252]. Three studies on exclusively pediatric entrants have evidenced a positive effect on the LAR after 1 year of injections: the classic study by the Soothill school [264], which was the first to show a SIT selective effect on the LAR, noted LAR suppression in 54.5% of children; the second in 33.3% vs none of the control children [247]. In a further study, the LAR was reduced in 88.8% of children, who fared far better in the 2nd year [248].

Production and/or Stimulation of Lymphocyte Populations and Subpopulations

SIT efficacy on regulatory T cells has been documented in several trials and not in others. According to the prevalent philosophy, SIT corrects CD8 depression (which does not respond to IL_2) in allergic subjects: thus SIT positive results can be associated with an *increasing T suppressor activity* [231]. T cells generated during SIT are activated by antigens, thus leading to the in vitro *suppression of sIgE synthesis, an event not seen in patients not undergoing SIT* [231]. In Der p-sensitized asthmatic children, SIT inhibited T-cell activation, and not only proliferative responses, but also T lymphocyte sensitivity to IL_2R (they are CD4 Th2) but not in the non-hyposensitized children [155]. Two studies in pollen-allergic subjects have instead reported a highly significant increase in $CD3^+$ and $CD4^+$ in BALF compared to control subjects, contrasting with nonsignificant variations in CD8 T cells [203]. On the contrary there was a significant decrease in $CD3^+$ and $CD4^+$ and an increase in $CD8^+$ in the skin compared to control subjects [253]. These data should be revised in light of T-cell switch.

Possible Switching from Th2 to Th1

In situ hybridization studies have shown at the cutaneous level an increased number of ILs, both Th2-like (IL_4 and IL_5) and Th1-like (IL_2 and IFN-γ), compared to control subjects, Th1-like ILs only in SIT-treated patients, without differences in IL_4 and IL_5 expression [253]. This DBPC trial, based on the new pathogenetic immune mechanisms of allergic disease, that is a Th2-to-Th1 shift that down-regulates IgE production, thus

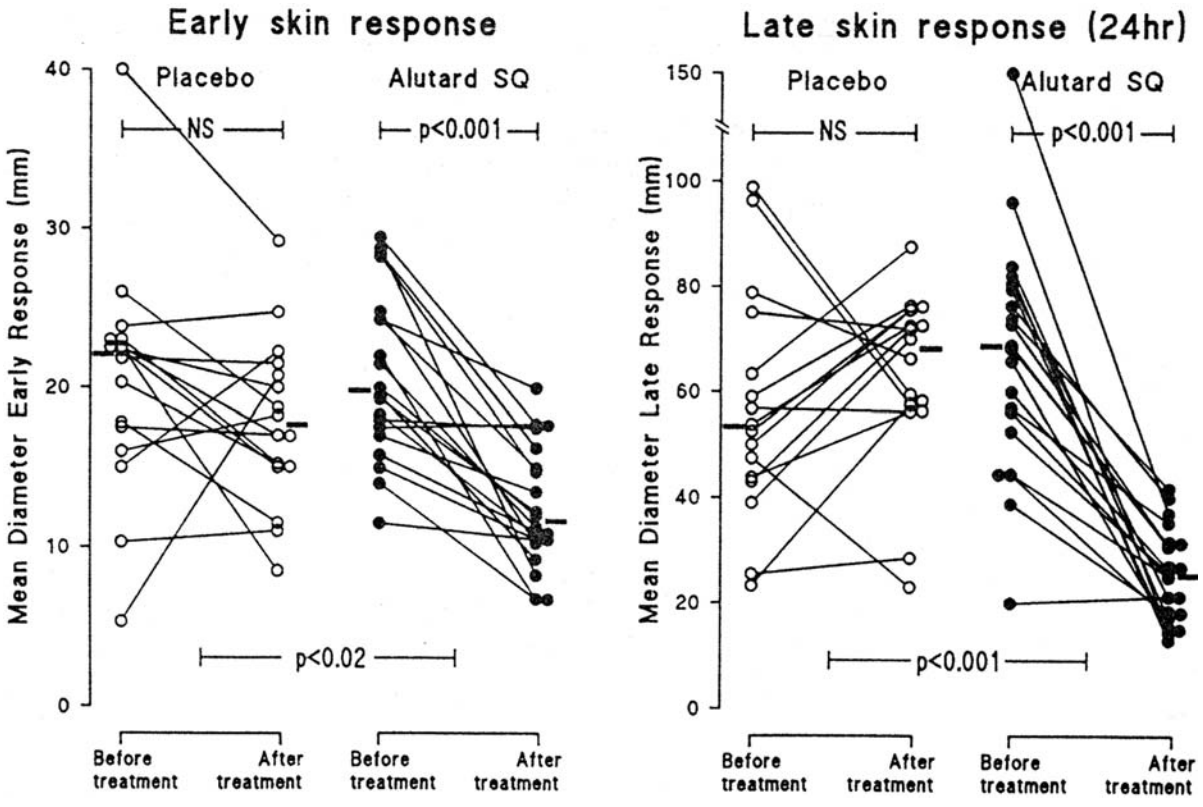

Fig. 13.12. Effectiveness of grass pollen SIT in reducing both early (*left*) and late (*right*) cutaneous responses to grass pollen SPT after 9 months of treatment

matching the results from Jujo et al (Chap. 7), has confirmed SIT efficacy in severe SAR [253], in severe pollen [95], and mite [84] asthma and in venom SIT [193]. This study has been confirmed, since both IL_4 expressing $CD4^+$ and $CD8^+$ T cells were significantly lower in asthmatic children after SIT compared with those before treatment and the normal control subjects. Thus significant decrease of mite-specific Th2 cells might be closely correlated with the SIT regulatory mechanisms [84]. Studies on skin biopsies using immunohistochemical methods have detected in SIT-treated patients a parallel decrease in cutaneous LPR mediators, $CD4^+$ and eosinophils and additionally a significant increase in $CD25^+$ cells, the α chain of IL_2R, HLA-DR and mRNA for IFN-γ [253]. Since IL_2 and IFN-γ are part of only the Th1 secretive model (Table 1.10) and IFN-γ is produced during SIT [71], already after the dose-increase phase and a further rise after 1 year of treatment [149], a possible *shifting of Th2* (that dominate LAR) *to Th1-IFN*-γ ensues with *inhibition of IgE production* (Fig. 13.13) [70]. Opposing evidence can be found in pollen-allergic subjects successfully treated with SIT: the down-regulated Th2-dominated late response is accompanied by increased IL_{12} levels compared to control subjects, by an IFN-γ correlated increase (Fig. 13.14) [70] and an IL_{10} increase in venom SIT [193]. Examining the pattern of T-cell-produced ILs by atopic adults, their clinical improvement was correlated with an allergen-specific IL_4 reduced generation up to the levels seen in nonatopic subjects, but there were no discernible effects on IFN-γ production being CD8-dependent [220] between untreated and SIT-treated groups [84]. Importantly, rates of decrease in IL_4 levels correlated well with rates of decrease in sIgE [174]. We may conclude that SIT modifies both allergen responsiveness and presentation, steering it to IL_{12}-producing cells, which inhibit IgE synthesis and promote the differentiation of Th1-type cells [70]. Thus SIT may arouse a state of immune tolerance by modulating the IL local outline [70] and sIgE production [48, 174].

Induction of IL10-Producing Regulatory T Cells

Among the immunological changes recently described in association with SIT, it has been shown that following SIT, peripheral T cells cultured in the presence of grass pollen extract produce IL_{10} [169], an anti-inflammatory IL produced by T cells and dendritic cells, which down-regulates both Thl- and Th2-type responses [169]. The presence of peripheral blood T cells that produce IL_{10} in response to allergen stimulation after SIT has, however, emerged as a consistent finding from studies of venom SIT [21, 193]. An increase in IL_{10} production after venom SIT was superimposed on a suppression of T-cell ILs and proliferative responses [5]. More specifically,

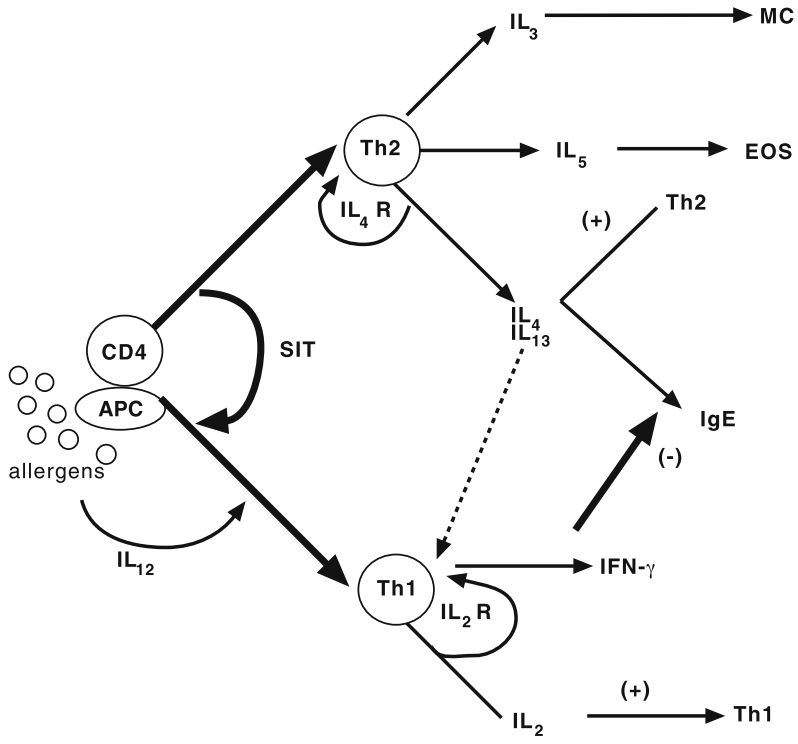

Fig. 13.13. Mechanisms of SIT: schematized encounter between allergen (*Ag*) and T lymphocytes, with a probable shift from Th2 to Th1 T cells. *MC* mast cells, *EOS* eosinophils. (Modified from [70])

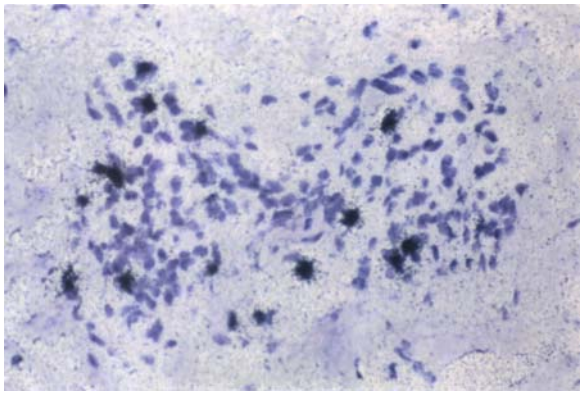

Fig. 13.14. Autoradiograph showing IL_{12} mRNA in a skin window 24 h after allergen provocation in a patient subjected to SIT

patients undergoing SIT produced significantly more IL_{10} than atopic control subjects, and exogenous recombinant IL_{10} did inhibit grass pollen-stimulated T-cell responses. Only T cells from patients undergoing SIT were positive for intracellular IL_{10}, and these were almost exclusively $CD4^+CD25^+$ cells, thus the number of $CD4^+CD25^+$ cells identified after allergen stimulation was greater in the SIT group than in the control subjects [80]. As a consequence, grass pollen SIT results in a population of circulating T cells that express the $IL_{10}(^+)$ $CD4^+$ $CD25^+$ phenotype in response to allergen re-stimulation [80]. The rapidness of allergen-specific IL_{10} mRNA expression in PBMCs is essential for the early and beneficial outcome of SIT [169] There is evidence that IL_{10}-induced tolerance could be a key phenomenon in SIT [5, 21].

Reduced Expression of CD23 on Peripheral B Cells

SIT leads to an inhibition of the CD23-mediated serum IgE-facilitated allergen presentation needed to obtain optimal T cell activation at the low allergen concentrations present in vivo [250].

Der p-sensitive asthmatic subjects have shown a significantly increased number of CD23-expressing B cells compared with healthy children, which were significantly reduced by SIT; however, no significant change was found in clinical improvement [164]. FcεRII/CD23 expression on peripheral B cells in grass pollen-sensitive patients was not found to be altered by successful SIT, but when grass pollen-induced *FcεRII/CD23 expression was correlated,* it was found to be *suppressed by SIT.* Severity of allergen-induced manifestations and drug requirement were reduced in the next pollen season, and correlated closely with a SIT-induced reduction in the percentage of FcεRII/CD23 B cells [123]. A subsequent study has found that CD23 levels were reduced when SIT duration was >36 months compared with a duration >3 to ≤36 months. In the first group, the CD23 mean value was 1.59 (1.0–1.9, 25th and 75th percentile), but in the group with shorter SIT duration, the corresponding values were 2.31 (0.5–1.7, 25th and 75th percentile) [6].

Increased IFN-γ Production

In other treated subjects, SIT increases the number of cells expressing messenger RNA for IFN-γ activity [71], which is also deduced, since it inhibits CD23 expression as compared to control subjects and thus IL_4 or IgE expression [123]. These results may be apparently discordant, but they attest to IL synthesis by CD4 memory cells that can be modulated by in vivo treatments [148, 220]. IFN-γ increased from 224.6±153.9 pg/ml before venom SIT vs 531.1±479.9 pg/ml after the initiation of venom SIT [193].

Reduction in Adhesion Molecules

Recruitment of leukocytes from the peripheral blood into tissue requires a series of cell-adhesion molecule-mediated interactions between the surface of the vascular endothelium and leukocytes. We demonstrated in Chap. 1 that several adhesion molecules play a critical role in the recruitment and migration of leukocytes to sites of inflammation in various diseases. Important adhesion molecules expressed on leukocytes or endothelial cells include CD54 and CD62L. A recent study has demonstrated that with long-term SIT, the percentages of $CD54^+$ T lymphocytes, $CD69^+$, an early marker of T-cell activation, and serum CD62L were significantly lower in children with a good response to SIT. Thus the decreased expression of adhesion molecules on T lymphocytes and the number of activated T lymphocytes in asthmatic children receiving treatment with SIT may decrease the recruitment of the activated T lymphocytes into the airways, resulting in a decreased airway inflammation [113].

Increased Anti-endotoxin Antibody

Endotoxin is a ubiquitous and potent proinflammatory agent (Chap. 11). Anti-LPS-antibodies of IgA, IgM, and IgG classes were investigated by ELISA in 22 bronchial asthma patients and 98 healthy control subjects. On the 70th day of SIT, higher levels of IgA antibodies to endotoxin of *Escherichia coli* (*E. coli*) K 30 were noted than before SIT, significantly greater than in the normal control subjects. A positive clinical effect of SIT in patients with bronchial asthma may be associated with an elevation in anti-endotoxin antibody, including an increase in IgA to endotoxin of *E. coli* K 30, suggesting an increase in Th1 responsivity [22].

Additional ILs and Chemokine Receptors

During specific pollen SIT, there is a mucosal shift from Th2- to Th1-type IL predominance, and IL_{12} plays a major role in this shift [169]. Moreover ovalbumin-IL_{12} can convert immune responses characterized by high IL_4 and high IgE synthesis into Th1-dominated responses in an antigen-specific manner [125]. In intermediate samples, taken in PBMCs of AR patients when the maintenance dose was reached, the peak expression of allergen-induced IL_{18} mRNA was associated with the most favorable outcome of SIT. Thus IL_{18} is an important IL in the SIT of pollen AR patients [214]. Chemokine receptors play an important role in the migration of leukocytes to sites of allergic inflammation in humans. An increased expression of the chemokine receptor CXCR1 was found on $CD4^+$ T lymphocytes obtained from patients with atopic disease. The expression of CXCR1 was dramatically decreased in patients undergoing successful treatment of AR by SIT [79].

In conclusion, the clinical improvement of symptoms cannot explicitly be correlated with any of these immunological modifications considered individually, but is likewise the result of a variable mixture of the aforementioned factors, and tentatively of other as yet unknown, but perhaps not less intriguing factors. Actually, in ragweed-allergic patients treated for 3 years with the highest immunizing dose, the rates of eosinophils were definitely lower than those found in subjects either not treated or treated with lower than threshold doses [85]. Likewise, IL_4 levels were correlated with SIT duration [220].

Considerations Before Initiating SIT

- Presence of IgE-mediated disease proven to benefit from immunotherapy
- Allergic rhinitis
- Allergic asthma
- Sound documentation of sensitivity to allergens associated with symptoms
- Symptoms of sufficient duration and severity
- Seasonal symptoms despite avoidance measures and pharmacological therapy
- Perennial symptoms failing trials of avoidance measures and chronic pharmacological therapy
- Anaphylaxis following Hymenoptera sting, except children with non-life-threatening anaphylaxis
- Availability of standardized allergen extracts of allergen responsible for sensitivity

General Criteria of SIT Execution

Before writing a prescription for SIT, pediatricians or allergists should inform the parents, in an adequate, complete and intelligible way, of the following points [37]:

- Duration of treatment

– Discussion of long-term nature of treatment and need for compliance

• Necessity of associating a pharmacological prevention with SIT to control symptoms completely
• Possible risks associated with SIT's overwhelming benefits
– Accessibility of facilities and personnel capable of administering treatment and evaluating and treating anaphylaxis
• Monitoring children to ensure they are waiting in the facility where they receive SIT for a time interval of 30 min, also during maintenance treatment
– Emphasis on avoidance as treatment of choice
• Inefficacy of SIT in a certain percentage of treated children

Tinkelmann et al have studied both chronology and type of reactions: *within 30 min of waiting* after each shot they observed 71.4% of overall reactions, 76.4% of respiratory reactions, 72% of cutaneous reactions and mostly important, 100% of cases of shock [239].

In conclusion, pediatricians or allergists must ask parents for their informed consent, adapting the proposal shown in Appendix 6.7 to SIT.

Technique of Administration

The elective way of SIT administration is the SC route. Within the practical principles of treatment, we describe the traditional technique as it is widely used, and then rush SIT.

Before administering SIT it is necessary to [37]:
• Ask whether the child had side effects after the previous injection.
• Attentively examine the clinical conditions of the child and carry out the physical examination, excluding the child from SIT if symptomatic and/or with intercurrent infections [8].
• Check whether name and surname of the child, batch number, allergen extract vial, concentration, and expiry date are correct: usually on the vial both batch number and expiration date are shown, because the child's name and the type of allergen extract may be specified on the box and not on the vial.

During the injection [115]:
• Before administering injections, make sure that epinephrine is readily available, and antihistamine and corticosteroids (CSs) are within reach to permit an immediate intervention if necessary.
• Any injection should be made with a single dose 1-ml graduated tuberculin syringe with incorporated needle, not an insulin syringe; however, if this type is available and for contingent reasons it is impossible to use the recommended syringe, in a 1-ml insulin syringe, scale 40, 0.10 ml corresponds to four marks.
• Lyophilized extracts should be accurately reconstituted with a suitable diluted dose, avoiding air bubbles, clots, etc., according to the manufacturer's instructions.
• Clean the area with an alcohol swab before injection.
• Accurately and gently roll and shake the vial before the injection.
• Carefully withdraw the proper amount from the appropriate vial with the syringe.
• Take care to avoid intracutaneous, intramuscular (IM), or IV injections.
• Inject subcutaneously in the middle third of the forearm, dorsal aspect; either arm may be used or the arms may be alternated, and when concurrently administering two allergen extracts use only one extract for each arm. The choice of forearm is dictated by the likelihood of placing a tourniquet upstream of the point of injection in case of severe local or systemic reactions.
• The needle should be introduced subcutaneously, with an angle inclination of about 40°; in this manner an intradermal inoculation is avoided; otherwise granuloma formation is caused along with a possible unsuitable absorption and reactions to the subsequent administrations.
• Before injecting the dose, gently draw the plunger, and repeat the aspiration once or twice depending on the volume to be sure that the needle has not inadvertently penetrated a vessel, then repeat carefully the aspiration during the gradual injection.
• If the needle accidentally penetrates a vessel, withdraw the needle and select a new injection site.
• Withdraw the needle at the end without stroking the inoculation site, to avoid a quicker absorption, which could increase the risk of SRs, apply pressure over the injection site for 15–20 s.
• After every injection, record the date of administration, hour, place (if different from the doctor's office), dose injected, the arm (right or left or both) and side effects (nature, timing, severity and treatment given).
• It is mandatory for children to wait in the doctor's office for at least 30 min after each shot, because the more severe reactions take place during this time [8, 101, 239];
• After 30 min, the injection site is inspected and the child may leave the office, after being instructed to avoid intense physical exercise such as running and climbing, and more importantly hot bathing, sauna, etc. on the day of injection: such activities may induce vasodilation with consequent accelerated absorption of allergen extracts.
• Allergen extracts in solution or lyophilized should be kept at a stable temperature between +2 °C and +4 °C at all times except when actually in use, absolutely avoiding both freezing and heat exposure.
• Exposure at ambient temperature (T) for short times does not disprove extract validity.
• When the extracts are not employed routinely, it is timely to change them after 4–5 months, even if the expiration date is still valid, since the proteins may denature, especially those in watery solution, and stick to vial walls (wall effect), with a consequent reduction in potency of the extracts.

- Beginning a new maintenance vial, we suggest reducing by 30%–50% the next administration dose, increasing the dose gradually, since the just opened vial may have a greater allergen potency than the vial in use [115].

Administration Schedules

As a general rule, doctors can follow the manufacturer's schedule enclosed in every box of allergen extracts prepared by specialized laboratories according to the results of specific allergy tests. Of course, a standard schedule cannot be used, since each subject differs, notably in the reaction to injections. Thus, to counter the impossibility of establishing a standard schedule valid for all patients, Appendix 13.1 shows a SIT average schedule, which should be used as a general guide. Pediatricians or allergists can modify the schedule according to age and the child's reactivity and allergen nature, to reach the highest tolerated dose, in other words the optimal dose, by personalized increments.

Generally each preparation of allergen extract contains three vials with increasing allergen concentration (build-up phase). The build-up phase involves the administration of incremental doses of allergen extract at 1- to 1.5-week intervals, up to the maintenance phase based on the *highest tolerated dose* that does not elicit side effects [45]. Initial doses should be reduced in children who are very sensitive to the allergen in question, and subsequent doses are regulated based on the first injection results; however, when no reaction is provoked, or no more than a mild local reaction, the dose may be increased by 50% or even doubled based on the doctor's judgment. Additional guidelines for dose modifications are as follows:

- No increment of allergen dose in case of local reactions of diameter >3 cm or in case of large local reactions >8 cm, delayed or lasting >24 h, or causing inconvenience to the child. In practice the dose of allergen extract should not be increased, repeating the last previously tolerated dose.
- The dose should be reduced in relation to the preceding dose, for example, in case of increased environmental exposure to allergens (grass pollination during seasonal SIT) or changing to a new vial of allergen extract.

Different guidelines may apply regarding immediate, local or large reactions, with a diameter >5 cm, which may warn of future immediate SR, therefore:

- If SR (wheezing, urticaria, shock, etc.) occurs SIT should be re-evaluated (see below).
- If the child does not receive the injection in due time for foreseeable or health reasons, that is if the child is within 2 weeks of being overdue, the prior dose should be repeated, but if the child is 1 month overdue the dose should be reduced by one step. If the child is 5–6 weeks overdue the dose should be reduced by two steps. Any lapse >6 weeks should result in a consultation with the physician [115].

The injection should be postponed contingent upon the following:

- Respiratory tract infections within the last week
- Intercurrent pathological situations within the last 3–4 days
- Exacerbations of atopic dermatitis (AD) (SIT administration may exacerbate cutaneous symptoms)
- Urticaria in the symptomatic phase (for the same reasons)
- Acute respiratory symptoms with evident functional impairment: namely, FEV_1 reduction with <70% of predicted value despite adequate treatment

The magnitude of dose reduction depends on severity of the reaction [115].

In SIT for Hymenoptera, the rush method is used (see "Rush SIT") [217]. We suggest that the doctor not exceed the maximal dose shown in the manufacturer's standard schedule. The units adopted by the single manufacturer are valid in relation to the extract used (Chap. 1) and do not allow interchangeability of extracts of different manufacturers in the same patient, even if titrated by the same method.

Particular precautions should be adopted in patients with *multiple sensitizations*: SIT should be undertaken in this case with two distinct hyposensitizing extracts, each to be injected on a different lower arm [161]. Also in multiple sensitizations only to pollens, no mixtures of allergens belonging to different families should be employed because of potential cross-reactivity, a possible cause of unsuccessful SIT [3]. In mold allergy, SIT should be proposed only to patients allergic to *Alternaria alternata* [44].

Treatment Chronology

SIT is done according to three procedures:

- Preseasonal
- Continuous or perennial
- Rush therapy

In pollen allergy, it is possible to provide either a preseasonal treatment with progressively increasing doses, to be completed before the start of the pollination period, or a continuous treatment, without interruptions during the injection schedule. SIT for perennial allergens can be started in any year period by following a continuous schedule. In both cases, the continuous treatment is the most effective and widespread method. SIT for Hymenoptera venom is begun in fall or winter to desensitize patients before spring, the insect season; in case SIT is started late or in high-risk patients a rush SIT may be instituted [68].

Preseasonal SIT

This unusual type of SIT may be suggested to patients with pollen sensitization, limited to the months of October to May or September to June. The goal is to reach, by scalar doses, the patient's maximal tolerated dose before plants begin to bloom and produce pollens to which the patient is sensitized. At this point, treatment is rescheduled to restart injections in the subsequent period, for several years straight. When SIT is started ahead of time, and its cycle is concluded 1–2 months before the expected onset of clinical symptoms, an advisable measure is to continue the maximal tolerated dose every 3 weeks, up to the pollination period. Eight years after commencement of preseasonal SIT, 61% of the initially pollen-monosensitized children had developed new sensitization to perennial allergens compared to 100% in the control group [72]; thus the long-term results are positive in only 39% of the treated children.

Continuous or Perennial SIT

Continuous or perennial SIT, a more common type of SIT, requires a larger consensus and is the SIT method that we recommend. This is prescribed to pollen-allergic children, particularly to those sensitized to *Parietaria* (with almost perennial blooming), and to children allergic to aeroallergens. The child is initially administered allergen extracts SC by scalar weekly doses up to the maximal tolerated dose (build-up phase), continuing subsequently by biweekly intervals for 1 year, then gradually extending to monthly intervals. This type of SIT is never stopped for the entire treatment. Clinical studies and scientific evidence indicate that a continuous treatment allows children to tolerate a higher cumulative dose, which ensures a greater protection than the preseasonal schedule. In SIT perennial for pollens, as discussed in the preceding section, it is advisable to begin 5–6 months before plants bloom. The dose may be reduced only in children in whom SRs develop presumably to seasonal and injected allergens to which children are sensitized, and then to return gradually, as the subsequent season approaches, to the previous maximal dose. Children sensitized to other aeroallergens, for example, Der p/b, can start SIT at any period deemed appropriate.

Each of these two types has advantages and disadvantages, apart from the possibility of personalizing SIT. The patient is more motivated with preseasonal treatment, since SIT begins before the pollination period, when disturbances peak, and ends after a relatively short time. With a perennial treatment, the total number of injections is in the end substantially lower, with the added advantage of spacing doses. *Neither in our studies nor in our daily practice have we suggested a preseasonal schedule.*

Rush SIT

Rush SIT is a method applied almost exclusively to children allergic to Hymenoptera venom, when a rapid immunization is necessary [70]. Rush SIT is done exclusively *in the hospital or under observation* in a unit staffed to undertake emergency measures and with personnel capable of administering treatment and evaluating and treating anaphylaxis.

Two methods are suggested:

- Normal rush (rush IT) with doses every week and the maximal dose at the 7th and last week in the hospital and under continuous control
- Modified rush (modified rapid means clustered SIT), which includes a first accelerated session (repeated injections at 30-min intervals), followed by another six injections (one every week) with conventional chronology (see Appendix 13.1) [160, 184 and Chap. 17]
- Accelerated in 3–5 days and semi-rush in 2–8 weeks [204]
- Ultra-rush for Hymenoptera-allergic adults and children over 3.5 h by administration of six increasing doses at 30-min intervals up to a total dose of 100 µg (Appendix 13.2) [27, 116, 160, 184 and JO Steiß, pers. comm., 2005]

Some authors distribute the first series of injections over 2–4 days [100, 101]. We deem that with the rush protocol the reactions are more frequent in absolute, resulting in a SR risk, especially in children [197, 204], which are prevented with adequate premedication [197, 236] and making admissions necessary or at least appropriate [101, 197]. Moderate adverse reactions (28.6%) [100], anaphylaxis (15.3%), serious reactions (37.3%) and strong reactions (34%) occurred in children [204]. Premedication reduces SR incidence up to 27% [197]. *Without premedication* SRs become significantly more frequent in children 3–5 years old [100] and in PC-treated subjects 6–18 years of age [197]. The SIT maintenance interval is usually 4 weeks. Lengthening the maintenance interval for children up to 6–8 or even to 8–12 weeks in case of Hymenoptera allergy [129] is likely to decrease SR prevalence.

SIT Duration

No ideal duration has been ascertained or established; however, Table 13.2 offers 44 studies related to pediatric cohorts: in our studies [38, 43–48] and in another 22 studies SIT duration was ≥3 years, so we estimate that SIT should be administered for at least 3–5 consecutive years of maintenance, or until a satisfactory remission of symptoms is noted over at least a 1- to 2-year period [43–46]. SIT for perennial allergens should be discontinued after 5 years [48], yet for seasonal allergens a shorter maintenance could be sufficient to achieve long-term results. We have decided to continue SIT for 5 years in either case, especially in children with initially severe

symptoms, obtaining definitive results in 97% of cases [47].

There is no consensus to define guidelines regarding the optimal duration of SIT for Hymenoptera hypersensitivity. In adult trials, 5 years would be adequate to ensure prolonged tolerance [91], provided that cases of severe anaphylaxis or other symptoms are not experienced [88]. The 5-year limit ensures the reduction of clinical sensitivity and, above all, that no SR will follow subsequent insect stings [91]. The likelihood of discontinuing SIT before the usual term remains to be evaluated [216] as well as the option of whether or not to extend the procedure to children, provided the intentional stings are done in a hospital environment to control the tolerance state and thereby determine the SIT duration (Chap. 17). Children who have experienced no previous life-threatening and/or exclusively cutaneous reactions are not necessarily subjected to SIT [216]. SIT-treated children at 9±3 years reported no SR at 26±5 years, while SRs occurred in 14% of untreated children, thus suggesting that over the long term children benefit from venom SIT [85].

When SIT-treated children do not respond with a clinical improvement within 2–3 years after receiving appropriate allergens, a diagnostic re-evaluation is indicated. If children switch to placebo after receiving 1 year of active treatment with Der p SIT the allergy relapses within months, whereas continued active treatment ensures ongoing improvement [264]. When clinical symptoms relapse, with heavy interference on school life and/or *quality of life,* SIT may be resumed subsequently, after a new diagnostic assessment.

Anti-IgE SIT has revolutionized the treatment duration: 3 [131, 131] to 6 [154] months has provided a 90%–95% reduction.

Possible Association of Medications with SIT

SIT association with levo-cetirizine, cromones, or long-acting theophylline may be necessary for complete symptom control. In particular, the association with oral cromones has been shown to be remarkably valid in preventing symptoms. Levo-cetirizine, antihistamine a new-generation is helpful because of its antihistamine activity. CSs and bronchodilators should instead be employed only in the treatment of clinical manifestations of a certain magnitude, and not as preventive drugs, since they could disguise the mild reactions that are useful signals for regulating the therapeutic schedule. Remarkably, a significantly greater proportion of children treated with omalizumab reduced the inhaled CS dose compared with children in the placebo group [154].

Monitoring SIT Efficacy

The tests proposed to monitor SIT-induced improvements, including total and specific IgE evaluation, SPT modifications and increases in both IgG and subclasses are not always correlated with clinical results. Specific challenges (reduction of nasal and bronchial reactivity to allergens) and variations in in vitro release of histamine or mediators released by basophils in response to specific allergens, are procedures that are not easily performed in children and are also not standardized. It is trivial to measure IgG periodically to monitor both dosing and frequency of administrations [115], as is the case in Hymenoptera allergy, although it was recurrently proposed [88], since there is no correlation with clinical symptoms [91]. Varney et al have shown the decrease in cutaneous immediate allergic reaction (PAR) and even more of LAR as possible markers of SIT efficacy [253]; the clinical results remain to be demonstrated. Therefore, sound clinical observation of children and the quantification of subjective and objective clinical parameters are the only reliable means to appraise SIT progress and the ensuing benefit. This assessment is based solely on the improvement of respiratory symptoms, which can be achieved, for example, by the daily recording of objective parameters such as PEFR [115] and mean daily drug usage, or of subjective parameters such as surveying daily scores of symptom severity [43–48]. The values of related subjective and objective parameters should be compared from time to time.

Causes of SIT Failure

As a general principle, the causes of SIT failure (Table 13.4) [37] are manifold, often a result of the child's or the family's lack of cooperation or unreasonable expectations, the premature conclusion that SIT has failed, which is often related to insufficient avoidance of allergen, resulting in an ongoing and overwhelming exposure to that allergen. More rarely, SIT failure may stem from the administration of inactive or expired extracts, or inadequate dosing (SIT efficacy is always dose-dependent), or on an inexact etiological diagnosis. Other explanations include poor concordance of in vitro results with clinical manifestations [1], the expression of new sensitizations or other triggering allergens, and SIT ineffectiveness in itself. Causes of SIT failure can be found in significant titers of microbial allergens that bind highly to IgE, instead of Der p, or in Eur m 1 homology to Der p 1 and Der f 1 (Table 1.73) in conventionally assembled extracts from cutaneous scaling. Pollen extracts could deteriorate if inappropriately stored at a T >4 °C [115]. Allergen mixtures may also be a cause of failure, because some allergens possess enzymatic activity that can alter the proteins of other pollens, thus reducing extract potency [267]: the employment of therapeutic extracts containing mixtures of up

Table 13.4. Potential causes of SIT failure

1.	No compliance from parents
2.	No compliance from children
3.	Incorrect SIT administration
4.	Administration of inaccurately prepared extracts
5.	Administration of inactive/expired extracts also because of long-term storage
6.	Administration of inappropriate doses
7.	Interventions for allergen avoidance inaccurately performed, with continuous and massive exposure to allergens
8.	Unreasonable expectations by children leading to a premature conclusion of SIT failure
9.	Mistaken etiological diagnosis or no clarification of all the allergens actually responsible forthe ongoing disorder
10.	Subsequent new sensitization of the child by additional allergens or intervention of additional triggering factors
11.	SIT inefficacy in a given child
12.	SIT inefficacy in itself
13.	Possible false-negative results of laboratory investigations

Modified from [37].

Table 13.5. Causes of a possible erroneous evaluation of SIT results

A.	Different pollen concentration in the atmosphere due to climatic influences, resulting in greater or lesser variations in clinical manifestations, in terms of both duration and severity
B.	Modification of patient reactivity, which may underlie a greater or lesser variability of disease severity, also independently of treatment
C.	Changes in the patient's habits, possibly inducing changes in the course of the disease
D.	Difficulty in the objectiveness and/or quantification of symptoms, also related to diverse therapeutic steps carried out in the time considered
E.	Complications related to the method of study and/or in balancing the patient cohorts

to seven unrelated allergens (pollen, molds, and HDM) may explain some negative results [3]. It is not surprising to find that mixtures containing variable doses of potent allergens elicit negative results [164], since both cross-reactions and/or administration of low concentrations of primary allergens are foreseeable [115, 267]. Dilution of multiple allergens may result in suboptimal doses of single allergens, and the potency of individual allergens may be reduced more rapidly when diluted or mixed with other extracts [258]. SIT is definitely *ineffective in multisensitized patients treated for a single allergy* [267] *or for seven allergies* in *the same extract* [3], or sensitized to *Plantago*, generally associated with grasses, but easily differentiated by the atypical calendar (Chap. 12). Therefore, apparent SIT failure may stem from imprecise scientific planning, without considering the variables that can influence an exact final evaluation (Table 13.5).

Adverse Reactions

The prevalence of adverse reactions has been analyzed at 99% in adults [232]. SRs occurred in 0.1%–1.7% of injections and in 1%–50% of patients treated with non-standardized extracts, with a greater frequency for these patients, the rush method and some types of polymerized extracts or allergen mixtures [252]. A comparison with the data in the literature shows that anaphylactic shock has an incidence of 0.01% of patients and 0.0013% of injections. Some experts not familiar with pediatric allergy [78, 237] ascribe a great number of severe or even fatal reactions to children without specifying they were reporting on adults [78]. As a consequence, the CSM "has eliminated SIT entirely as a therapeutic option," because it was believed that the risks would be higher than the potential positive effects. However, in most of these cases adequate cardiorespiratory equipment necessary for resuscitation was not available [78]. Several authors maintain that "SIT is dangerous in children and a cause of anaphylactic shock" [30, 178], since they quote the study by Hejjaoui et al [100], without pointing out that 30 of 105 children aged 3–15 years experienced SRs *in a rush study*, which should be done in hospital facilities [46] because it carries a risk for SRs [28], *especially with no premedication* (Table 13.6) [100]. As a rule, it is intriguing to extrapolate the results of rush studies and compare them with a normal SIT; however, in other children, when applying the due precautions, *no SRs were recorded* [47, 247, 248], and several trials *demonstrate the exact contrary of the CSM* in terms of SIT danger in children and in the time span considered here [38, 45–48]. To date we have treated thousands of children with SC SIT and have recorded only one case of anaphylactic shock in a multicenter trial [38] plus some cases of mild skin reactions.

- In a multicentric study, mild symptoms such as asthma and urticaria were observed in 0.08% of injections, and only one case of shock in 0.002% of injections; such reactions occurred in children with Der p allergy, giving a much more significant statistical difference than in children with pollinosis (Table 13.7) [38].
- In another prospective and controlled study lasting 3 years and including 300 asthmatic children, *we recorded no cases of shock*, and only mild reactions in 2.5% of cases and in 0.066% of treatments [44].

Table 13.6. Pediatric study by Hejjaoui et al

1. The children were subjected to a rush protocol, which is known to provoke more reactions
2. The children had neither premedication nor preventive measures; the authors were conscious of the risks, therefore the patients were hospitalized for the first night of treatment
3. The authors hypothesized that the hospitalization increased the rate of SR because of its psychological impact

Data from [100].

Table 13.7. Systemic reactions in 1,056 children

Extract	No. of cases	(%)	No. of injections	(%)
Mite	29/689	(4)	29/29,091	(0.09)
Pollens	9/291	(3)	9/12,286	(0.07)
Parietaria	3/109	(3)	3/4,602	(0.06)

Data from [38].
p (mite vs pollen) = 0.0001.

- In a cohort of 42,457 entrants undergoing SIT between 1957 and 1992, 3,187 SRs to SIT occurred with traditional allergens (7.5% of patients), 139 with modified allergens causing 8 SRs (8.4%), and 2,422 during rush procedures causing 393 SRs (16.2%). It is notable that in the USA $\approx 10^7$ injections are given annually and neither systemic nor fatal reactions were observed in children undergoing the conventional protocols [227].
- In another trial, nonfatal SRs were observed in 3 out of 374 children aged 2–9 (0.8%) and 37 out of 1,097 aged 10–19 (3.37%), a total of 139 cases with 513,368 injections (0.027%); pollen prevalence (50% grasses and 21% ragweed) in comparison to 1% attributed to dust (Der p is not named) [142].

The incidence of SRs in children is summarized in Tables 13.8 [38, 142] and 13.9 [4, 38, 177].

- In patients with a premedication regimen, over a 4-year period with 22,250 injections, an edema <5 cm in 1.9% of patients and >5 cm in 0.17% of cases and a mild SR in 0.023% of cases were reported [262].
- Of a total of 108,000 injections given over 4 years, only three children aged 7–15 years experienced reactions (rhinitis, conjunctivitis, urticaria), with a 0.0027% incidence, with only five injections responsible for symptoms in 0.0046% of cases [66].
- Tinkelman et al [239] concluded that SRs occurred in 2.14% of patients and in 0.06% of injections: taking into account only subjects between 1 and 20 years, the figures are reduced to 0.5% and 0.014%, respectively.
- In a SIT trial for sting allergy, only 1/62 children (1.6%) experienced a severe SR, explained by the fact that he had discontinued treatment 7.5 years earlier, after undergoing SIT for a total of 2.5 years [92].
- In a SIT trial for mold allergy after several months on maintenance therapy, following an earlier tolerable allergen injection, 3/106 children (2.9%) suddenly developed severe, nearly fatal anaphylactic reactions to Cla h, Alt a and Phl p for which ICU treatment with IV theophylline and epinephrine was required (all recovered without sequelae), and another three severe anaphylactic reactions also successfully treated with epinephrine [177]. In children treated with SIT for Cla h [69] and Alt a [46], *no such reactions were provoked.*
- In a retrospective trial for Der p and Der f allergy, SRs occurred in 12 children, successfully treated with epinephrine or clemastine, while one child also required intubation [4] (Table 13.9).
- Concerning the discussed issue of fatal cases, between 1945 and 1992 there were 45 cases throughout the USA [143, 227]. In 24 cases that were reported with sufficient data [143] and 17 from 1985 to 1989 [206], including 7 children aged 7–18, five cases can be related to specific errors, especially the delay in epinephrine administration, leaving the doctor's office too soon after the injection, and wheezing in progress and in the preceding 24–48 h (Tables 13.10, 13.11) [9, 143, 206]. In the remaining two children, there were no known errors: by introducing statistical corrections including SIT execution in a site different from the doctor's office (40% of cases), administration during the pollen season (33%) [227], insufficient experience on the part of the physician [263] and the number of injections per year [227], the FR incidence approximates 0% (Tables 13.9, 13.11). The shock onset was within 20 min in 29 cases, between 20 and 30 min in 2 cases and after 30 min in 4 cases, with no specific information regarding the other cases [143, 206]. The case record was updated in 2001 [9]. Recently, a 12-year survey of fatal reactions to SIT injections was conducted between 1990 and 2001, showing that the rate of fatalities per SIT injection has not changed much over the last 15 years. Fatal reactions were estimated to occur at a rate of 1×2.5^6 injections, with an average

Table 13.8. Systemic reactions (*SR*) in children during SIT

Authors and references	Year	No. of SR	Age (years)	No. of injections
Lin et al [142]	1993	3/374 (0.8%)	2–9	NS
		37/1,097 (3.37%)	10–19	139/513,368 (2.9%)
Businco et al [38]	1995	41/1,443 (3.7%)	2–14	41/45,979 (0.08%)

Table 13.9. Anaphylactic shock in children during SIT

Authors and references	Year	Age of children (years)	(%)	Injections (%)	Treatment
Akçakaya [4]	2000	8.5	1.1%	0.017%	Epinephrine, ICU, full recovery
Businco et al [38][a]	1995	8	0.09%	0.0016%	Epinephrine, hydrocortisone, no hospitalization
Ostergaard et al [177][b]	1986	7–13	2.8%	NS	Epinephrine, theophylline, ICU, full recovery

NS not specified.
[a] Additional data: cases of shock=0.089% of 1,119 treatments.
[b] See notes in the text.

Table 13.10. Fatal reactions (*FR*) in 7 children aged 7–18 years during SIT (1945–1989), updated to 2001 [9]

Cases	Cause
1.	Known error of SIT administration and an incorrect dose of epinephrine
2.	Probable dosage error and delay in the treatment
3.	Did not wait in the doctor's office
4.	Did not wait in the doctor's office
5.	Wheezing at time of injection and during the previous 24–48 h
6.	No known error
7.	No known error
8.	Epinephrine timing of administration not clear
9.	Left by father early before end of waiting period

Conclusion: 2/9 FRs not related to doctor during SIT from 1945 to 1989, plus 2 children aged 5 and 12, cases 8, 9 (1990–2001) (Bernstein, pers. comm., Apr 8, 2005).
Data from [9, 143, 206].

Table 13.11. Doctor-unrelated FRs in 9 children aged 5–18 years during SIT (1945–1989) updated to 2005

	Based on years considered	Based on injections
1 case	22.5 years	12×10^{18}

Data from [9, 143, 206, cases 8, 9 (1990–2001) (Bernstein, pers. comm., Apr 8, 2005)].

Table 13.12. Risk factors for systemic reactions to SIT

Related to children:
History of previous systemic reactions
Respiratory tract infections within the last week
Intercurrent disease, presence of symptomatic asthma within the last week
High degree of allergen sensitivity
Related to environment:
High allergen exposure
Related to extracts:
Passage to subsequent dose
Injections from a new vial
Rush SIT
Related to doctor:
Change of vials
Incorrect technique
Errors in dosage or administration
Mistaken clinical evaluation
Inadvertent intravenous injection of dose
Injections made during seasonal exacerbations

Moreover unchanged doses at the moment of switching to a vial with extracts of greater potency or with the new vial with maintenance doses; early months of injection course in very sensitized children; unchanged doses during pollen season; excessive increase in doses; excessive shortness of the intervals between administrations, excessive extension of these intervals, no concern for transient or permanent contraindications; disregarded prerequisite of not performing vigorous exercise before injection.
Data from [38, 115, 267].

of 3.4 deaths per year. Interestingly, 3 of these reactions occurred at times >30 min after the injections. There was either a substantial delay in starting epinephrine or that epinephrine was not administered at all in many of the fatalities [24]. Two children aged 5 and 12 were included (D Bernstein, pers. comm., Apr 8, 2005). Thus the pediatric rate is 0.16 deaths/year.

Conclusions that can be drawn from the data analyzed to date are as follows:

- Children do not have coronary heart disease and thus *tolerate epinephrine well*: CSM [78] and WHO-IUIS [237] guidelines for SIT are based on adults.
- We stress that in *analyzing the CSM's fatal cases* [78] *no children were found!*

- The rare SRs in children aged <18 years were *restricted to rush SIT and to Hymenoptera SIT* [227].
- Even fatal reactions may occur following administration of antibiotics, anesthetics, iodized contrast media, muscle relaxants, and foods (Chaps. 19 and 20).

The lesser prevalence of these reactions in children, apart from the hypothesis that children are less exposed compared with adults for age-related reasons, speaks in favor of the particular attention and supervision parents give to their children, more than to themselves. If properly instructed on the model of allergic diary charts, parents will clearly report any reaction to SIT such as potential intercurrent infections, massive allergen exposure, allergic symptoms, although temporary, and SIT contraindications. Moreover, children tolerate a possible antishock therapy better than adults, who, in addition to a greater statistical risk of coronary heart disease, can react unfavorably to epinephrine, because of increased numbers of mast cells lining the walls of their coronary arteries. Upon allergen challenge, these mast cells release mediators into the myocardium [68]. Severe adverse reactions with no apparent cause sometimes develop in children who have tolerated the long-term maintenance dose well; these SRs, although rare, can occur in any SIT stage, including administration of preparations with relatively low biological activity. Such reactions usually appear within 30 min [239]; commonly less severe delayed reactions complete the picture [51]. *Several risk factors,* well identified as causes of reactions to SIT and whose knowledge is vital to bring about an effective prevention, are summarized in Table 13.12 [38, 115, 267].

Clinical Reactions During SIT

Clinical reactions during SIT may be [37, 46, 115] mild local reactions or systemic and are thus schematized as follows:

- *Mild local reactions* include burning, warmth, bruising, redness, rashes, wheals of discrete entity, edema 5 cm in diameter, often accompanied by intense itching and tension in the injection site. These reactions occurred in 37.5% omalizumab- and 36.6% placebo-treated patients; the frequency and severity of local reaction tended to decrease over the course of the study [154]. These reactions usually resolve within a few hours (immediate reactions) or at the most within 24–48 h (delayed reactions). The immunological basis is unclear, but the reactions are considered within normal parameters and are not a reason to interrupt treatment; however, their monitoring for subsequent doses is useful.
- *Large local reactions* can be >5 cm in diameter within 30 min, a local swelling of part of the forearm, or a large swelling, but limited to the area of injection. These reactions commonly do not predict subsequent future SRs because they fall within the limits of normality. In this case no increment of allergen dose is suggested, unless the lesions cause a disturbance in the child. If the reactions recur it is necessary to check whether the injection was appropriate, since the extract can occasionally be injected in the hypoderm instead of SC tissue.
- *Moderate SRs,* usually sporadic and of short duration, localized to the target organ: urticaria, rhinitis and wheezing indicate that the tolerability threshold has been surpassed. A maintenance therapy should be reduced in relation to the preceding injection.
- *Severe SRs* (statistically less frequent) are acute, persistent, with the onset of manifestations localized to different organs and tissues, indicating that the tolerability threshold has been surpassed.
- *Immediate SRs* (very rare) are complicated by glottis edema and anaphylactic shock (statistically exceptional occurrence). They are almost always caused by errors in dosage. The early signs and symptoms of anaphylactic shock may include pruritus of the palms, soles and scalp, conjunctival injection, mild edema, cough, etc., which are the alarms invariably followed by systemic symptoms. Immediate reactions appearing within 30 min and with size >5–10 cm could be a premonition of impending SRs [37].

Also, in case of SRs (immediate or late), the dose should be reduced in relation to the preceding injection. Anaphylactic shock may be a SIT counter-indication and it is advisable to reduce the dose in case of uncertainty. Guidelines suggest decreasing the dose by 50%. If the reduced dose is tolerated, increase the dose by 0.05–0.1 ml weekly and resume the previous schedule. If reactions occur again the schedule should be re-evaluated [115].

Treatment of Local and Systemic Reactions

Even if SRs are severe and usually nonexistent in children, doctors should have all the recommended equipment necessary to protect children if such an occurrence should arise.

Equipment Recommended for SR Treatment

The medical office where SIT is administered should be supplied with appropriate equipment for treating SRs and medications required for resuscitative measures with proper expiration dates noted (Table 13.13) [8, 115].

It is of crucial importance that epinephrine, antihistamines and CSs be ready in syringes before beginning SIT procedures, in order to accelerate treatment in case of need. The periodic verification of the equipment and its maintenance or replacement as components or medications expire is recommended, making note in an appropriate registry.

Table 13.13. Equipment and drugs needed for office treatment of severe reactions

Stethoscope and sphygmomanometer
Equipment for administering O_2 with availability of a mask
Ambu bag with masks
Oral airways of the appropriate size
Tourniquets
Syringes and disposable needles
Tongue depressors
Endotracheal tube
Intravenous setup with large-bore catheter
Aqueous 1:1,000 epinephrine 1-ml vials for both injection and inhalation to be preserved at about 5 °C (expiration no later than 6 months) and/or Epi-pen
Equipment for administering IV fluids
Antishock solutions
Nebulizer for aerosoltherapy
Normal saline 10-ml vial for epinephrine dilution
Additional medications:
Antihistamines, anti-H_1 and anti-H_2, oral/injectable
β_2-mimetics, aerosol/injectable
Diphenhydramine injectable
Corticosteroids for IV and IM injection with rapid action

Data from [8, 115].

Treatment of Adverse Reactions

Ready recognition of SR and *immediate epinephrine administration* are the cardinal points of therapy [8]. The immediate therapeutic approach is as follows [115]:

1. *Mild local reactions:* most require no specific therapy. Oral antihistamine or topical CSs may be sufficient.
2. *Large local reactions:* in addition, a venous tourniquet applied above the injection site may decrease absorption of the injected allergen, and epinephrine administration. Observe for 60 min.
3. *Rhinitis:* oral antihistamines. Observe for 60 min.
4. *Mild urticaria:* oral or IM antihistamines, observe for minimum of 60 min;
5. *SRs* (asthma, ocular rhinitis, angioedema or generalized urticaria):

– Apply a venous tourniquet above the injection site and give 1:1,000 epinephrine immediately .
– Children should remain in observation under regular surveillance for clinical parameters until symptoms subside.

6. *Severe SR* – anaphylaxis (see Chap. 20).
7. When the clinical response to treatment is not immediate, *additional measures* should be taken before and after admission to a first aid station. The recent AAAAI (American Academy of Allergy, Asthma and Immunology) Position Statement suggests that specialists in these procedures should be immediately available [8]. If needed, the child should be hospitalized immediately in a pediatric emergency department or other suitable unit for at least 24 h to monitor anaphylaxis progression, because of the risk of a delayed shock [115], and to start an appropriate therapeutic approach.

Recommendations on Proper SIT Administration

Several guidelines and WHO [52, 115, 237, 267] have cautioned that risks and/or burdens to patients may evolve from SIT prescription and/or execution, as outlined below:

- *SIT prescription*
  – Superficial or mistaken indications
  – Specific contraindications
- *SIT execution*
  – Superficial, insufficient or absent evaluation (history and clinical examination) of the degree of child sensitivity to the allergens to be administered
  – Insufficient identification of a child at risk for severe reactions
  – Omitted, superficial or insufficient clinical monitoring of a child, in particular with high degree of skin reactivity to allergen inoculations, in which case adequate dosing of allergen extract is necessary
  – Inadequate equipment in the doctor's office regarding instruments, or the availability of medication necessary to deliver first aid, or the omitted or insufficient control of both validity and expiration of drug used, etc.
- *Risks associated with allergen extract*
  – Inadequate storing
  – Excessive potency
- *Risks relative to each individual child*
  – Temporary contraindications, apart from adverse unforeseeable reactions

In clinical practice, problems can arise when an injury, objectively demonstrable, results from negligence, inexperience, imprudence, and where a cause–effect rate between action, blamable omission and harmful accident exists. Jurisprudence with regard to a physician usually considers blamable error in the following issues:

- Examples of *negligence*
  – Inadvertent exchange of allergen extract vials
  – Insufficient alacrity in delivering treatments
  – Inadequate evaluation of the clinical status of each child before SIT administration
  – Incomplete and/or inaccurate clinical and personal history
- Examples of *inexperience*
  – Errors in administration of extract
  – Errors in dosage
  – Delay in carrying out emergency treatments

- Examples of *imprudence*

– Inadequate resuscitative instrumentation as detailed above

– Dose increase despite the child reporting large local reactions and/or SRs at the last SIT injection

– Improper SIT supervision by the administering physician

The attending physician should act with care, foresight, and experience in having all necessary equipment at hand, as well as complete, efficient, and precise knowledge as to the preventive measures which must be taken to avoid any risk factor and of course a complete understanding of all the therapeutic approaches. It is always preferable to take ten more precautions than one less to limit the number of unexpected situations or emergencies that any doctor should be able to handle. A study conducted on Italian pediatricians has shown that 79% of them were informed of the recommendations set forth on the proper SIT administrations that should be followed in their offices [207].

We underline the suggestion forwarded to minimize the risks and improve SIT efficacy [8]: 1. SIT prescription by specialists only; 2. SIT administration by physicians to cope with SR including anaphylactic episodes.

Comparison Between SIT and Pharmacotherapy

Disregarding the above-mentioned contraindications, we examine the SIT cost–benefit ratio, taking into account the main advantages and disadvantages, notably in children compared to adults (Table 13.14) [30, 267].

Cost–Benefit Ratio

The major problems that need to be addressed are symptom severity and frequency on the one hand, and costs and risks of SIT on the other. For proper evaluation one should compare SIT with the other available therapeutic options, taking into consideration several elements:

- Potential severity of the disease to be treated with SIT
- Relative effectiveness of available treatments
- Cost and duration of each type of treatment
- Risks of the disease and related treatment

SIT Advantages

- Specificity of effect
- Monthly frequency of administration (after the maintenance dose is started)
- Less medication usage
- Duration reduced to a few weeks with omalizumab [132, 154]
- Conventional SIT abates symptoms
- Anti-IgE treatment abates IgE levels
- Positive influence on the natural history of respiratory allergy

SIT Disadvantages

- High cost
- Administration only by physicians
- Costly instruments and continuing controls by physicians

Table 13.14. Advantages and disadvantages of SIT and pharmacotherapy in the treatment of respiratory allergy

Items	SIT	Pharmacotherapy
Specific cause	++	–
IgE synthesis	Decreased	–
Effect duration	Long-term	Short
Effect localization	Systemic	Local
Administration	Medical doctor	Patient
Frequency	Weekly/monthly	Daily (2–4 doses or more)
Duration	5 years	Lifelong
Mechanism of action	++	+ (?)
Side effects		
Short-term	±	±
Long-term	–	– (?)
Effectiveness	90%–95%	[a]
Materials used	Defined/undefined	Defined
Cost[b]	High	Moderate/high

[a] Effectiveness requires lifelong treatment.

[b] The SIT cost is divided between 5 years, the cost of medication may be reduced, but must be multiplied for each year of life.

Data from [30, 267].

- Time availability by parents/escorts (missed hours/days from work) and child (missed hours/days from school/study)
- Invasiveness
- Questionable availability of purified and standardized extracts
- Potential risks of SRs, in children statistically nonexistent severe reactions (Tables 13.10, 13.11)

Advantages of Pharmacotherapy

- Less invasiveness
- Substantially lesser cost in the European Union; in the USA SIT cost represents 32.1% of the cost of medications [25]
- Physician presence unnecessary
- Instruments and particular controls almost nonexistent

Disadvantages of Pharmacotherapy

- Aspecific effects mostly localized and of short duration
- Effect cessation when discontinuing treatment [256]
- Administration one or more times a day and often by different routes
- Risk of adverse reactions also in children
- Even early therapeutic intervention after the onset of symptoms may be too late to alter the natural progression of the changes in the airway wall [196].

In children with AR, SIT was substantially more effective in the long term compared to control subjects treated with cromolyn [174]. In our studies [38, 43–48] SIT-treated children fared much better than pharmacologically cured control children. The disappearance of symptoms after long-term SIT implies one more economic advantage [25]. Therefore, effectiveness can be evaluated, even in single cases, including the duration of pharmacotherapy that may last a lifetime [30], whereas SIT duration most often lasts 5 years [43–46]. The two treatments are not antithetic, each having particular characteristics that may act in a synergic and complementary way. However, no studies have demonstrated that pharmacological treatment alone with CSs, modifies the natural history of childhood asthma. Yet after 5 years, medication alone would be effective in controlling symptoms but not in modifying the underlying allergic disease, the major advantage of SIT [25, 44]. In contrast with pharmacotherapy, SIT offers the chance of addressing the fundamental pathophysiological process of allergy, mast cells and eosinophil mediator release upon contact with specific allergens up to a switch from Th2 to Th1 type immune response. In children who were not treated with SIT, CSs may reduce Th2 lymphocytes but not augment Th1 responses [70], nor abate IgE antibodies as in SIT [48, 256]. Several trials have instead concluded that 75% of pharmacologically cured children continue to suffer from asthma in their adult life [115, 121, 122, 200] and that discontinuing treatment makes the symptoms reappear [256]. Several authors failed to evaluate this issue, in particular those employing multiallergen extracts [3]. Compliance is improved in SIT , increasing with age (monthly injections that accustom to regularity), whereas the daily dependence on medications could have a negative impact on the quality of life, when noncompliance can lead to undertreatment in allergic children [115].

A recent study addressing the *sustained effect of SIT after 5, 10, and 16 years* (Table 13.2) re-evaluated 48 patients who had been treated with SIT in their childhood after a mean cessation 9.3±2.76 years. SIT-treated patients had fewer asthmatic features and needed fewer antiasthmatic medications than the non-SIT-treated control subjects (OR, 6.14–6.67); *the risk of frequent asthmatic symptoms was three times higher in the control group than in the SIT-treated group* [53]. This confirms that once established, SIT will give long-lasting relief of allergic symptoms, whereas the benefits of drugs only last as long as they are continued [83]. However, an analysis of the economic advantages of SIT would be incomplete without taking into account issues pertaining to *quality of life*, which may be important references for assessing the impact of disease morbidity on daily life activities [25]. The issue of quality of life was assessed in children treated with anti-IgE. In 54 children aged 7–17 years, a high OR was found when assessing children 6 months after SIT treatment, especially in nasal pruritus (OR, 6.8) and obstruction (OR, 5.9) and for carving eyes and nose (OR, 7.0), nose blowing (OR, 4.8), carrying disposable tissues (OR, 4.7), throat itch (OR, 4.0), irritability (OR, 6.2), and ocular pruritus (OR, 3.1). Patients without SIT were likely to use more drugs (OR, 6.4) than those receiving SIT [159].

Present and Future Perspectives

In the last few years, SIT has been the object of stimulating revisions, expressed in the formulation of several consensus documents, though only one for the pediatric age groups, as demonstrated by several recent studies and by the attention shown by scientific societies [37]. The points that deserve more consideration appear to be the following:

- SIT represents *the only allergen-specific treatment* of IgE-mediated respiratory allergies.
- The *hyposensitizing allergen extracts* available so far are much better purified and standardized and certainly in the near future there will be further innovations in this respect, especially concerning the titration in international units (Chap. 1).
- *Severe side effects* are wholly negligible in the pediatric field between 0% and one case of shock, equal to 0.0016% of injections and to 0.089% of treatments [38] and seem to stem not so much from a treatment that is dangerous in itself as from errors in execution, apart from unforeseeable reactions [46].

- *The cost-benefit ratio* is in favor of benefit, as documented.
- *The major progress* may originate from *current perspectives* such as new forms of therapy or from *future perspectives:* well-characterized and standardized allergens and new approaches to the modulation of IgE response.

Present Perspectives

The innovations include oral, sublingual, conjunctival and nasal or bronchial inhalatory and ID SIT. The idea of using the oral route for SIT is more or less as old as SIT itself.

Oral SIT (allergen immediately swallowed) was experimented in 1900 [56], does not elicit anaphylactic reactions, and avoids the inconvenience and unpleasantness of SC SIT while ensuring greater compliance. Oral administration of allergens leads to gastrointestinal lymphocytes and to GALT (gut-associated lymphoid tissue) [68], equipped with specific sites that fit to receptors on distant mucous membranes and glands by an intense cell exchange. It is known that orally absorbed medications reach systemic levels more rapidly than those parenterally administered. Lymphocytes stimulated in the oral or gut mucosa, or in regional lymph nodes, are then homing to the respiratory mucosa where they might induce favorable immune responses; however, the exact mechanism remains uncertain [108]. There is also an objective difficulty in overcoming the modifications that extracts undergo in the enteric environment, related to their breakdown, absorption procedures and contact with the immune system [178]. Positive results have been demonstrated mainly for birch [68], while for grasses the efficacy is unquestionably less than that of SC SIT [242], probably because of rapid enzymatic degradation of allergens in the gut [68]. Several DBPC studies have confirmed oral SIT efficacy and found it to be the most reliable route of administration (keratinized capsule or watery solution), including administration rate and doses to be reached. Adverse reactions reported up to now and typical of this method are principally gastrointestinal (diarrhea, dyspepsia, etc.), related to the overall necessity of achieving a clinically significant effect with doses as much as 100- to 400-fold higher than in SC SIT, typical of this method. For this reason the cost-benefit ratio is 4,000 times more costly than the benefit [115]. Positive results have been found in a Der p study in 18 children 3–13 years old, DBPC after 12 months of treatment revealed no local or systemic adverse effects [87]. A clinical improvement of rhinitis was seen only after 2 years of treatment, and of asthma symptoms only at the third year [87]. Allergen-specific IgG antibodies, especially IgG_1 and IgG_4 subclasses, increase, and the seasonal and postseasonal rises in sIgE antibodies are blunted [87], as in SC SIT (Fig. 13.11). The sIgA antibodies were studied in a cohort of ten children, but no significant differences were found between treated patients and control subjects [235]. In young adults, a comparison with local IT, though only in SC, SIT results were positive, with significant statistical differences as and clinical results [192]. The good compliance and the lack of severe adverse reactions of the oral route could favor, in the near future, this as the preferred route in the treatment of infantile respiratory allergies, especially if three conditions are satisfied:

1. It substantially reduces the cost.
2. It clearly defines the degree of risk. The results obtained to date are insufficient, because they are too inconsistent, even with enteric coated tablets, in terms of the rate of allergen release and because allergen denaturation makes the calculation of absorbed allergen difficult.
3. It makes safe home administration possible [115].

However, oral immunization of allergic children requires large quantities of protein, and for many allergens it would not be feasible to provide sufficient quantities of native allergen to immunize children. Moreover, the pharmakokinetics of allergens is in general still controversial [74].

Another possible oral route [187] is the *desensitization to natural foods diluted in H_2O,* starting with incrementing doses of the prepared solutions and continuing with purified foods: cow's milk (CM), egg, and fish (Appendix 13.4) [187]. Entrants were 34 patients, ten of whom were aged <12 years; the administrations were preceded by cromolyn medications. Twenty-seven of 34 patients (79.4%) completed the 4-month therapy (mean), which was positive in 25 of 27 (92.6%). Complications included abdominal pain, pruritus and urticaria treated in the doctor's office. In 32 children, the success rate was as high as 84.8%. A similar method was used for a *rush desensitization to CM* in a 13-year-old girl (Appendix 13.5) [20]. This desensitization route could meet with very good compliance in children with food allergy. The duration is similar to anti-IgE, but wholly noninvasive.

Sublingual IT (SLIT) is available in solutions of allergen extracts, and administrable in drops and soluble tablets [180]. The concept is promising, because of a consistent supply of lymphoid tissue in both the throat and neck, partly related to NALT: we can hypothesize that allergen extracts via the sublingual mucosa link to Langerhans cells (LCs) draining into regional lymph nodes. The rationale could be that LCs releasing IL_{12} modulate Th0 switching to the Th1 phenotype. However, we deem that the drainage of the allergen into regional lymph nodes is difficult to test in humans. In a DBPC study in 47 children aged 5–12 and 39 control subjects with monosensitivity to Der p, no variation in CD40 and ACTH, but a significant decrease in ECP, IL_{13} and prolactin were observed after 6 months of successful therapy [117]. In 24 children aged 4–16 monosensitized to Der p, SLIT was able to avoid the spontaneous increase in both nasal sIgE antibodies and in local aller-

Table 13.15. Twenty pediatric studies (13 controlled) including only children who demonstrated sublingual immunotherapy efficacy

Authors	Allergens	Duration	No. of cases		Age (years)	DB	PC	Disease	Results	Efficacy
			S	C						
Tari 1990 [233]	Der p	1.5	30	28	5–12	+	+	A/AR	C	+
Hirsch 1997 [105]	Der p/f	1	15	15	6–16	+	+	A/AR	C	–
Vourdas 1998 [255]	Ole e	2	34	32	7–17	+	+	A/AR	C	+
Di Rienzo 1999 [64]	Pollens	[a]	36	12	5–12	–	–	AR	C	+
La Rosa 1999 [136]	Par j	0.6	20	21	6–14	+	+	AR	C	+
Pajno 2000 [180]	Der p/f	2	12	12	8–15	+	+	A	C	+
Caffarelli 2000 [39]	Pollens	0.4	24	20	4–14	+	+	A/AR	C	+
Bahcecilier 2001 [16]	Der p/f	0.6	8	7	7–15	+	+	A/AR	C	+
Marcucci 2001 [152]	Pollens	0.6	30	20	7–12	–	–	S/AR	C	+
Della Volpe 2002 [59]	Pollens, Der p	3	288	–	5–8	–	–	A/AR	C	+
Silvestri 2002 [223]	Der p/f	2	10	–	5–12	–	–	RC	C	+
Di Rienzo 2003 [62]	Der p	4–5	35	25	3–17	–	–	A/AR	C	+[b]
Ippoliti 2003 [117]	Der p	0.4	47	39	5–12	+	+	A/AR	C	+
Marcucci 2003 [151]	Der p/f	1	13	11	4–16	+	+	A/AR	C	+
Wüthrich 2003 [269]	Pollens	2	10	12	6–13	+	+	A/AR	C	+
Pajno 2003 [181]	Par	1.1	15	15	8–14	+	+	A/RC	C	+
Arikan 2004 [12]	Der p	0.6	32	5	5–11	–	–	A/AR	C	+
Bufe 2004 [34]	Pollens	3	68	64	6–12	+	+	A/AR	C	+
Novembre 2004 [170]	Pollens	3	54	59	5–14	–	–	A/AR	C	+
Rolinck 2004 [209]	Pollens	2.7	39	39	3–14	+	+	SAR	C	+

S = Study children, *C* = control children, *A* = asthma, *AR* = allergic rhinitis.

[a] Two pre-coseasonal treatments.

[b] The children were followed-up up to 10 years after start of treatment. *RC* = Rhinoconjunctivitis, *SAR* = Seasonal allergic rhinoconjunctivitis.

gic inflammation in basal conditions [151]. An open study in ten children confirmed that SLIT was able to reduce CD54 expression on nasal epithelial cells and to decrease methacholine responsiveness [223]. No change in the mucosal level of tryptase and ECP was detected in pollen-allergic children [152], but tryptase levels were significantly increased in untreated children as well as nasal IgE [151]. Table 13.15 shows the results of 20 pediatric studies including 820 children [12, 13, 16, 34, 59, 62, 64, 105, 117, 136, 151, 152, 170, 180, 181, 209, 223, 233, 266, 269]. In two DBPC studies in 5–12 years olds, with monosensitivity to Der p, a significant reduction in asthmatic episodes was observed [223, 233] along with urticaria and asthma in some children [233]. Studies on children aged 8–12 with Der p asthma have demonstrated a statistically significant reduction of clinical symptoms and a very significant increase in IgG_4 antibodies [199]. Positive results were reported in 80% of 288 children aged 3–14, after 3 years of SLIT [59], and in children aged 4–16 either with allergic rhinoconjunctivitis to Par j [136] or to mites [12, 13, 16, 59, 62, 105, 117, 151, 180, 232]. SLIT performed poorly in two studies [104, 105] or the clinical efficacy of SLIT combined with fluticasone was equal to that of fluticasone alone [181]. In a DBPC study in 161 children after 1 year of SLIT, no significant difference between treatment and placebo was observed [34]. Additional DBPC trials were conducted in mite-sensitized [16, 151] and grass-sensitized [39, 209, 269] children, in whom SLIT was well tolerated and significantly reduced drug consumption during the 2nd [269] and 3rd [170] year of treatment. Importantly, SLIT is effective in children and maintains the clinical efficacy for 4–5 years after discontinuation: 8.6% of children were taking medications after 10 years vs 96% of control subjects [64]. SLIT was effective only in children with severe clinical symptoms and after 3 years of therapy [34]. Two studies involved treatment for 2 years [180, 266], 3 for 1 year [105, 151, 181] and 6 for <1 year [12, 13, 16, 39, 117, 136, 152].

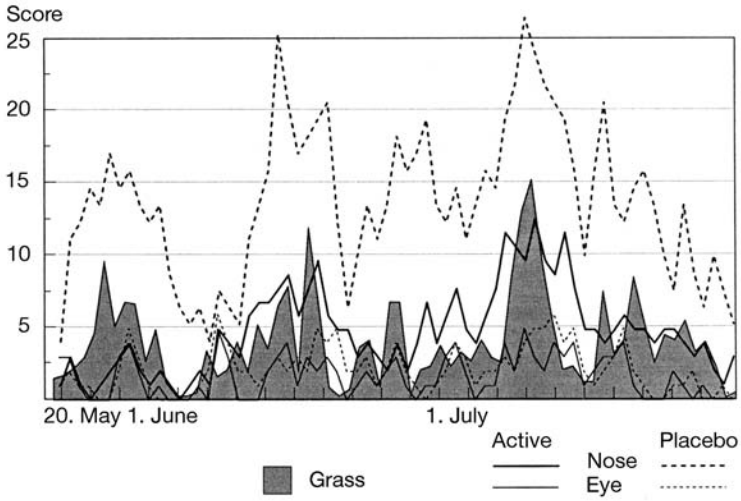

Fig. 13.15. Local nasal SIT: symptoms during the first season

Some studies are relatively small (8–15 children) [16, 105, 151, 180, 181, 223, 269] (as are 14/44 [31.8%] studies in Table 13.2) and about 18%–25% of the patients dropped out of the study or were lost to follow-up [34, 136]. Doses of allergens used in SLIT are usually fivefold to 20-fold greater than the doses required for traditional SIT [195]. Moreover, the cumulative dose of allergen used was fivefold [94] to 20-fold [41], and up to 375-fold [136], the doses given in conventional SIT, with a significant rate of gastrointestinal complaints [136].

The extract is usually kept under the tongue for 1–2 min [180]; then swallowed (sublingual swallow). The dose, especially in young children, is absorbed mainly by the gastrointestinal tract, thus making an objective evaluation of SLIT effects somewhat difficult. If the allergen is not absorbed in the mouth, the allergen contact with the oral mucosa is critical [15]; thus an almost complete degradation of the allergen should occur in the duodenum in humans [41]. The adverse reactions so far observed, in addition to more or less intense oral pruritus, are related to the digestive, respiratory and cutaneous systems [68]. However, 115 systemic reactions (5.2% of patients and 0.06% of injections) were observed [201] and 45 children (22.5%) had side effects: 20 oral itching or burning, 15 gastrointestinal symptoms and ten cases of rhinitis [81]. All side effects were successfully managed [81, 201]. A postmarketing study in 268 children aged between 2 and 15 years and receiving SLIT for up to 3 years showed that the overall incidence of systemic side effects was 3% of the children and 1 of 12,000 doses [63]. Thus more conclusive data are required [68]. In Appendix 13.6 we show a *4-day SLIT protocol to desensitize latex-allergic patients.* The desensitization was so successful that after 3 months all patients could wear latex gloves without any symptoms [186]. A Cochrane review has found that there was no significant reduction in symptoms and medication scores in four studies involving only children, but total numbers of participants were small, casting doubt on the validity of the conclusion [255].

Conjunctival IT, which refers to original experiments [166], has been experimented with good clinical results in adults with allergic conjunctivitis; however, a daily intraocular instillation of a given allergen in scalar doses for 8–30 months [137] does not meet childhood compliance, since this method has no effects on lacrimal IgE and SPT [137]. This kind of IT should be adapted for use in older children and adolescents.

Local IT includes nasal or bronchial inhalation of allergens: the rationale is to restrict the allergen immunological impact and the resulting immune responses to mucosal sites and to distant sites via MALT (mucosa-associated lymphoid tissue).

Nasal inhalatory IT utilizes treatment when symptoms are manifested. The allergens can be absorbed through the nasal mucosa and reach the bloodstream [72]. Treatment should start prior to the pollen season, to avoid allergen sensitization in the nose provoking symptom worsening that could be protracted beyond the season. The proposed hypothesis that the patient self-manages treatment appears unwise, because a nasal spray may trigger a psychological mechanism as seen in asthma therapy, that is an incentive to augment or increase the dose frequency: a new sensitization can produce symptom worsening secondary to a *priming effect.* To bypass the problem inherent in molecules that are too large to be absorbed through the mucosa, PEG (polyethylene glycol) modified allergens can be used to penetrate the nasal mucosal barrier better than unmodified allergens. They combine efficacy and reduced allergenicity; however, their results have been contrary to expectations [68]. A new technique is to incorporate allergens in granulometrically controlled powder form, which has shown encouraging results [108]. The predosed micronized allergen powder contained in capsules is sprayed in the mouth and applied to the nostrils by insufflators. The efficacy of this technique was demonstrated for grasses [108] (Fig. 13.15), *Parietaria* [10, 183], Der p and birch [267] and with DBPC studies. Only two studies were done in children with grass

Table 13.16. Conjunctival provocation test (CPT) threshold dose according to the treatment regimen

	No of cases Placebo		EPD	
Der p SQ-U ml	Baseline	Last follow-up	Baseline	Last follow-up
10	0	0	0	0
100	1	1	0	0
1,000	0	0	0	0
10,000	7	5	7	2
100,000	2	4	3	8
Negative with 0100,000		0	0	1

Statistics: Placebo baseline vs EPD baseline = N S, nonsignificant; placebo last follow-up vs EPD last follow-up Fisher extract test, p=0.0286; EPD baseline vs EPD last follow-up Fisher extract test p=0.0349.
Data from reference [49].

pollen [17] and Der p AR [150]. The efficacy is limited to nasal symptoms [10, 183], but no effect was seen on the eye or on lower airways, often associated symptoms [108, 192]. Incidentally, a discrepancy between nasal and eye symptoms should be noted: nasal lymphocytes could actually induce a tangible switch or modification also at the eye level (and cutaneous via the links between NALT and SIS). A significant increase in sIgA antibodies was observed in nasal secretions but not in serum and not correlated to the clinical picture [192]. An important result is the reduced response to nasal challenge, despite exposure to pollens and absence of treatment and CD54 presence within 30 min [183], most likely deriving from previous deposits. An original study has indirectly demonstrated the validity of this type of IT by inhibiting an immune response to Der p in mice [110].

Bronchial inhalatory IT: in a DBPC study in adults, the available positive results show a decrease in the clinical symptom score and a significant increase in bronchial tolerance to allergens and IgG levels. Before subjecting patients to treatment, it is necessary to perform respiratory function and bronchial provocation tests with the allergen, to ascertain the provocation threshold and the protective effect of associated cromolyn [115]. Further studies are required to adapt this method to children.

In both cases, the administration should be preceded by a premedication with cromolyn to prevent the onset of undesired effects produced by the inhalation of allergen vaccine.

Enzyme potentiated desensitization (EPD) consists in applying on scarified skin a very low dose of allergen premixed with a small amount of the enzyme β-glucuronidase, which, pretreated with other chemical substances, would gain a configuration able to ensure in a given subject a non-response or a tolerance to administered allergens; the hyaluronidase added to EPD would allow better transcutaneous diffusion of the formulation. Since a technical period of about 30–60 days is predicted to obtain the greatest level of immunological non-response, each pediatrician allergist should check, in case of need, the pollination period of the locality. The first controlled study in adults has given not very significant differences between actively treated subjects and controls [75]. Instead, significant results were noted in a pediatric DBPC study [49] in 20 children with Der p allergy and a median age of 8.5 years. The children were randomized to receive either two ID placebo injections or the active material with an 8-week interval. The ID injection of EPD (0.05 ml) contains 0.01 ml of β-glucuronidase (40 Fishman units) and 0.04 ml of a mixture of inhalant allergens (1 Noon unit). The EPD-treated children had significantly fewer days with asthma. In addition, the EPD-treated children used significantly less medication for the management of asthma attacks (p=0.0000 for both) [49], a greatly significant result of the conjunctival provocation test (CPT Table 13.16) [49] and of clinical evaluation by the investigators (Fisher = 0.0349) (Table 13.17) [49]. No significant local or systemic side effects were reported. The results of this study provide further data on the effectiveness and safety of EPD in patients with asthma.

Table 13.17. Global clinical evaluation by the investigators and parents related to the type of therapy

	Investigators		Parents	
No. of children	Placebo	EPD	Placebo	EPD
Improved	3/10	8/10	4/10	6/10
Not improved	7/10	2/10	6/10	4/10

Fisher exact test: p = 0.0349, not significant.
Data from [49].

Comparison of Traditional (SC) SIT and Other Routes

Even if these methods have been investigated in DBPC studies, at present there is no study that has compared the efficacy of these alternative SIT methods on a large scale and with the same methodological and statistical characteristics as traditional SIT by the SC route *in pediatric populations exclusively*. Various studies have examined the advantages and disadvantages of oral and local IT [58]. Oral IT yielded positive results only in the studies carried out over an average of 12 months, but in only one pediatric study [157], and negative results in the shorter trials, again in only one pediatric study [242]. In SLIT several studies found positive results, including 19 pediatric studies plus one covering a 10-year period [62]. For nasal SIT, there were several positive studies, including two pediatric studies [17, 150] with different types of extract; especially those employing dried-powder extracts appear devoid of side effects [58]. Unless the contrary is demonstrated, SIT is supported by better clinical and experimental evidence and still remains the most valid course of treatment for infantile allergies.

The crucial issue on the most effective route for SIT, speculative motivations appear to be subordinated to clinical results, which may favor the administration of SC or SLIT routes. SC SIT is asthma desensitizing [44, 118], whereas SLIT favors the asthma development in 15%–63% of nonasthmatic children [180]. Nonetheless, elucidation of factors influencing the shift between subsets of Th cells favoring Th1 migration to particular tissue sites and the influence of the microenvironment on Th1 polarization will be critical when making this decision. However, the induction of high levels of IFN-γ in the milieu of injected allergen, for example, offer interesting alternative approaches limited to SC SIT. The relative significance of generating IFN-γ-producing regulatory Th1 cells must also be evaluated, supported by the evidence that current SC SIT regimes associated with increased IFN-γ production may correct the IFN-γ defect in atopic children (Chap. 4). Thus, application of new SIT strategies should be proportional to careful consideration of actual immunological data.

Future Perspectives

The future is likely to bring increased focus on new developments: perhaps SIT, as it is practiced today, represents one of the first uncertain steps on the manipulation of immune responses. Advances in the knowledge of the cellular and molecular basis of immunity have greatly increased in the last few years and can prove very useful for therapeutic purposes in a more or less near future. The hallmarks are preparation of more standardized extracts and different approaches to the manipulation of T-lymphocyte functions and suppression of IgE response. In brief, future therapeutic options may feature novel vaccine delivery systems and modifications of allergens to reduce allergenicity (Table 13.18) [145, 154, 195].

Table 13.18. Future directions of specific immunotherapy (*SIT*)

SIT with native proteins
Crude extracts
Purified proteins
Recombinant allergens
Modified allergens
Allergoids
Urea denatured
Formaldehyde or glutaraldehyde
Polyethylene-glycol
Peptides
Short: up to 26 amino acids can be synthesized
Longer: produced as recombinant peptides
Recombinant proteins expressed in bacterial or yeast vectors
Natural sequence including isoforms
Altered proteins
Disulfide bonds causing misfolding of the molecule
Amino acid substitutions in epitopes producing subtle changes
DNA vaccines
Boost antibody and depending on DNA sequences can alter response
Controlled expression, site and continuity
Oligonucleotide immunomodulatory sequences
Anti-IgE
Decrease in serum IgE levels
Immunization early in life[a]
Unaltered antigen
Antigen linked to a Th1 adjuvant such as IL_{12} or bacterial proteins
Antigen incorporated into a plant (e.g., transgenic bananas) for oral IT

Data from [145, 154, 195].

[a] We suggested starting SIT as soon as possible (see text).

Necessity for More Standardized Extracts

As is logical, convincing advantages will evolve from allergen standardization and characterization and their titration in the more diffused BU, or IU and from application of techniques of molecular biology and genetic engineering (recombinant DNA [rDNA] technology). Extract manipulations facilitate the research on extracts with reduced allergenicity.

Allergoids

Modified extracts constituted by allergoids are allergens polymerized through a treatment that partially modifies their characteristics. The major benefit of allergoids

is to produce an immune response with less risk of anaphylaxis. However, these molecules in which formaldehyde has caused cross-linking both within proteins and between proteins induce IgG antibodies and have T-cell epitopes. Therefore they may not represent a major benefit for these patients [195]. Urea denaturation was therefore proposed as a method of preserving T-cell responses while reducing the risk of anaphylaxis. In mice, glutaraldehyde-polymerized allergens can down-regulate IgE antibody production through a mechanism that involves increased IFN-γ secretion. However, differences were observed in the availability of T-cell epitopes between allergoids and unmodified allergens, which are most likely due to structural changes within the allergen molecule [65]. The characteristic features of these chemically modified allergens are their strongly reduced IgE-binding activity compared with the native form and the retained immunogenicity [124]. These molecules have a greater MW (molecular weight) and are released more slowly from the injection site, circulate over a longer time, are not excreted by urine as are aqueous extracts, but are stored in the reticuloendothelial system (RES) of various organs. The well-known advantage is that of administering reduced doses, especially with delayed vaccines. Allergoid-based studies resulted in the reduction of allergic symptoms [185], especially when using high-MW allergoids [185], which, however, may be a shortcoming if these allergoids are administered in such a way as to induce their passing via the mucosal barrier. Several similar trials followed, with good clinical results. In an open, multicenter study of 90 children and adolescents 6–17 years old, short-term SIT using four injections of pollen allergoids adsorbed to L-tyrosine and adjuvanted by monophosphoryl lipid A adjuvant was shown to induce *significant reductions in symptom scores and medication use* compared with the previous pollen seasons. Pollen-specific IgG was significantly increased, whereas little change was apparent in pollen-specific IgE [67]. Two DBPC studies on the *Parietaria* pollen allergoid extract resulted in an IgE decrease and an IgG_4 increase [11] or was limited to IgG_4 antibodies [234]. Recently, it has been shown that using dendritic cells (DCs) and macrophages as APCs, allergoids exhibited a pronounced and reproducible T cell-stimulating capacity. Responses were superior to those with PBMCs, and isolated B cells failed to present allergoids. Considerable IL_{12} production was observed only when using the DCs as APCs of both allergens and allergoids. The amount of IL_{10} was dependent on the phenotype of the respective T cell clone. High IL_{10} production was associated with suppressed IL_{12} production from DCs in most cases. So the adoption of the allergoid principle in the development of genetically modified allergens with reduced IgE reactivity by retaining T-cell reactivity will provide a promising basis for the development of newer therapeutic vaccines for SIT [124]. Allergoid particularity is revealed also in the manipulation of B and T lymphocytes.

Liposomes

New approaches to SIT, designed to increase efficacy and safety, include encapsulation in liposomes and conjugation of allergens to immunostimulatory sequences (ISSs), short base-pair segments of DNA with a basic sequence of purine-purine-cytosine-guanosine-pyrimidine-pyrimidine. Stimulating perspectives are opened up by using allergen extracts incorporated into *liposomes,* which may be a vehicle for safe and effective SIT, as multiple injections of liposome-entrapped allergen have been shown to reduce sIgE response and induce specific IgG response in mice [219], even with allergen doses that were not too high. These are small, biodegradable phospholipid vesicles that shift the immune response toward Th1 and induce IgE-selective unresponsiveness [241]. Liposomes are formed by phospholipids disposed within compartments found between membrane-like lipid bilayers that fold concentrically; uni- and multi-lamellar liposomes alternate and water- or lipid-soluble molecules are within their interior. H_2O is able to entrap water-soluble substances, are biocompatible, biodegradable, stable and prolong the half-life of many drugs. The rationale behind the use of liposomes as allergen carriers is that since allergens are water-soluble, these will be sequestered inside the aqueous space and thus will not be capable of interacting with local IgE, whose levels will decrease by natural degradation processes. Moreover, liposomes are too voluminous to be absorbed by blood capillaries, so when they are SC injected they will follow the lymphatic route to lymph nodes, where the macrophages will phagocyte liposomes and process the antigens, thus minimizing the SR risk, since the protective bilayer inhibits interactions with antibodies [261]. This method seems to be promising, since it was demonstrated that in mice liposome treatment potentiates the specific IgG response rather than IgE and CD8 T cells [14], and IFN-γ more than IL_4, thus showing a Th1 dominance [219]. The problem is how to regulate and control the progressive release of allergens by liposomes to reach an effective therapeutic concentration [261]. Clinically, in a DBPC study asthmatic patients sensitized to mites were randomly assigned vaccination with Der p extract encapsulated in liposomes or empty liposomes for a period of 12 months. Asthma-free days increased from 10.5% to 64.5% in the active group and remained at 18% in the placebo group [19].

Recombinant Allergens

Using the rDNA technology combined with PCR (polymerase chain reaction), it has become possible to investigate the tertiary structure of allergens both by modeling based on sequences and because RAs can be produced in sufficient quantities for X-ray crystallography or nuclear magnetic resonance [215]. Therefore, the molecular cloning and sequencing of a striking number of well-defined RAs has made it possible to isolate the primary amino acid sequence of >60 different allergens (Table 1.70), although the identification of the complete

repertoire of allergenic epitopes may remain a critical task [164]. Concerning SIT, the use of RAs is promising, although the wealth of available allergens for SIT may never be transformed into practical results, considering the complexity of allergen extracts, and the incomplete characterization of the major allergen repertoire and the possible incomplete availability of RAs [215]. RAs can be produced on a large scale and with the assurance of purity compared to the traditional ones, which often contain additional allergens or histamine-releasing compounds, suggesting that children avoid receiving these proteins and allergens because they may increase their sensitivity [215]. Moreover, a widespread use of RAs will avoid untoward effects, new allergies and cross-reactivity, since RA extracts are prepared specifically for the exact number of potential allergens to which a given patient is effectively sensitized. In addition, it is now possible to produce commercial quantities of these RAs and in many cases to assemble molecules that are immunologically very similar to the native molecules [195], so that RA derivatives with greatly reduced allergen activity can be produced. Hypoallergenic RA derivatives can then be used for patient-tailored therapy, and, given their reduced anaphylactic activity, they hold the promise that patients can be treated with fewer injections of high-allergen doses and with a lower risk of side effects. RAs have undergone exhaustive clinical trials to become available for clinical use [215]. Recently, recombinant Fel d 1 (rFel d 1) chains have been successfully co-expressed as mature proteins with comparable immunoreactivities to natural Fel d 1. The rFel d 1 can be targeted to APCs through CD64. These constructs will facilitate structural studies of Fel d 1 and the development of improved allergy diagnostics and therapeutics. In particular, these allergens will also provide essential tools for basic and clinical studies of the immune response to cat allergens [243]. Similarly, the rBet v 1 derivatives exhibited a reduced capacity to activate basophils and mast cells by cross-linking of Bet v 1-specific IgE, and genetically engineered hypoallergenic rBet v 1 derivatives induced significantly lower activation of eosinophils by means of surface expression of CD69 and ECP release (and levels of GM-CSF and IL_4) compared with rBet v 1 wild type. The derivatives may therefore be candidate molecules for SIT of birch pollen allergy with reduced risk of inducing allergenic or inflammatory side effects [167]. Zhu et al [275] engineered a chimeric molecule in which the truncated human IgG Fcγ1 is fused to the rFel d1. This fusion molecule binds both FcεRIIb and the IgE specific for Fel d1 thus inducing dose-dependent inhibition of Fel d1-driven IgE-mediated histamine release from cat-allergic donors' basophils and sensitized human cord blood-derived mast cells. Such inhibition was associated with altered Syk and ERK (extracellular signal-regulated kinase) signaling. Similar fusion molecules could be designed to counter other types of allergy with an even stronger effect on countering allergy if they are used to ablate IgE-producing B cells, the source of immunoglobulins which also express FcεRIIb. This approach would induce true desensitization for example, by replacing Fel d1 with the major peanut allergen [126].

Plasmid or DNA Vaccines

Plasmids expressing allergens referred to as naked DNA could also be used for SIT. These plasmids are circular DNA molecules found in an assortment of bacteria, which rely on enzymes and other proteins in the host cell for replication and transcription. DNA can be inoculated as "naked" DNA or coating some sort of microparticle that facilitates cellular uptake. Plasmid vectors encoding specific proteins may be IM injected, where they are taken up by APCs and expressed, preferentially activating a Th1 response, probably because the antigen produced from the inoculated DNA is processed as an intercellular antigen presented on HLA class I molecules. When introduced into an animal, they can bring about a source for functional genes in addition to those on the chromosomes and can provide a novel form of immunization for allergic disease. Plasmids encoding Hev b 5, Ara h 2, and Der p 2 have already been tested in experimental animals [35, 112], and more food allergens are being identified and cloned. In DNA-primed mice with a plasmid DNA, encoding the protein β-galactosidase could not only switch off an ongoing IgE antibody response to the coded protein, but also produce a 66%–75% reduction in sIgE antibodies in 6 weeks [205]. However, the amount of protein produced is very small, and it appears that a major problem will be scaling up DNA vaccine from mice to humans. In addition, it appears that most plasmids will only continue to express proteins for a few weeks. Thus, it is still difficult to assess the potential role of gene vaccination for treating allergic disease; however, the primary goal is to switch T cells toward a Th1 profile. DNA plasmids can achieve the same effect, inducing a Th1-dominant response over the Th2 response [195]. Studies in mice have demonstrated that the immune response to plasmid-DNA SIT is strain-dependent [139]. Vaccination of mice with plasmid DNA encoding Der f 11 induced Th1 responses characterized by $IgG_{2\alpha}$ responses, and spleen-cell secretion of IFN-γ prevented the induction of IgE responses and could inhibit ongoing IgE responses [189]. Similarly, the lowest IgE and highest $IgG_{2\alpha}$ levels were found in mice vaccinated with the combination of plasmid IFN-γ and IL_{12} as an adjuvant [134]. If DNA vaccines could also change pre-existing immune responses, the inhibition of specific IgE antibody formation [188] is dependent on the specific ISSs in plasmid DNA, which suppress IgE synthesis and promote IgG and IFN-γ production [210]. Moreover, in a mouse, a substantially lower dosage (50 μg) of grass allergens, when administered along with a plasmid DNA IL adjuvant, can induce effective immune deviation and *a protective airway response* in comparison with allergens alone [134]. A combination of CpG (cytosine-phosphate guanine) + plasmid

DNA immunization may be effective in antagonizing Th2 responses. The effect of CpG motifs in vivo depends on a variety of parameters such as the nature of the antigen and the immunization modality [106].

Immunostimulatory Sequences

Studies have shown that ISS CpG motif DNA sequences can redirect an anti-allergen Th2 response toward a nonallergic Th1 response both in animal models of asthma and allergic diseases and in allergic patients [226]. ISS CpG oligodeoxynucleotides (ODNs) including unmethylated palindromic motifs have been shown to decrease airway hyperreactivity, lung eosinophilia (IL_5), and as effectively as 7 days of CSs and sIgE production if administered during allergen sensitization. This was associated with induction of a Th1 and inhibition of a Th2-like IL response [32]. CpG ODNs were capable of redirecting the Th2 to a Th1 response to allergen in mice inhibiting airway eosinophilia and BHR. CpG ODN led to induction of antigen-induced Th1-like ILs and of RANTES and suppression of eotaxin. An established atopic eosinophilic airway disease can be effectively overcome by a combination of CpG ODN and allergen [128]. Administration of CpG-ODN with antigen through the skin may provide the basis for a novel form of SIT of both allergic asthma and AD since treatment elicited an antigen-specific, Th1-predominant immune response and enhanced the production of IFN-γ and drastically suppressed Th2-like IL_4. IL_4-regulated IgE production was also suppressed [116]. However, while 61.5% of NC/Nga mice did not exhibit dermatitis after CpG ODN was administered intraperitoneally every 2 weeks for a total of five times, 38.5% of mice treated with CpG ODN exhibited an *exacerbation of dermatitis* accompanied by the hyperproduction of IFN-γ, although Th2-like ILs were suppressed in both spleen and lymph node cells and culminated in a decrease in the serum IgE level [230]. ISS conjugates to allergen (such as Amb a 1) are being developed as SIT for allergic asthma and AR [109, 213]. They have therefore been tested as immunoadjuvants for various vaccines including T-cell independent antigens. Using liposomes as a carrier for CpG ODN to improve the immune response to biotinylated liposomes and incorporating PEG-modified lipid in liposomes enhanced the immune response. In conclusion, liposomes are a useful delivery vehicle for CpG ODN as an immune adjuvant for T cell-independent antigens [138]. It is significant that, probably owing to steric interference by the attached ISS-ODN with the reaction of antigen with IgE, allergenicity is decreased, defined by decreased binding to IgE. Using ISSs as Th1-inducing adjuvants is conceptually derived from unmethylated CpG motifs in bacterial DNA. ISS conjugates to allergen (Amb a 1) are being developed as SIT for allergic asthma and AR [109, 213]. Recently, ISS–allergen conjugates were shown to induce stronger antibody and Th1-type immune responses, while reducing mast cell degranulation, anaphylactogenic potential, and delayed Arthus-type reactions than either allergen alone or allergen mixed with ISSs but not conjugated [109]. In this context, by exploring the relative immunogenicity and allergenicity of allergen/ISS–ODN conjugates of varying ratios in mice, an important result was that allergenicity and, in particular, anaphylactic potential were reduced significantly, although higher levels of ISS–ODN conjugation might reduce the immunogenicity of allergen conjugates slightly. Thus, *ISS–ODN is being explored as a safer and more effective approach to SIT* [109]. The chemical conjugation of ISS–ODN to Amb a 1 confers on the allergen a new property that makes it an excellent candidate for human SIT. Amb a 1–ISS conjugate induces strong Th1 and high IgG responses, even in the face of an ongoing Th2 response [213].

Manipulation of T and B Lymphocyte Functions

- *Manipulation of T Epitopes*

A suggestive hypothesis speculates that inhibiting interactions between TcR and HLA class II molecules by *synthetic peptides* could offer an alternative treatment to conventional SIT with allergen extracts. The rationale for using T cell-reactive peptides of this kind is that they would be loaded onto HLA class II molecules in vivo and presented to T cells in such a way that they would alter the T-cell responses in vivo. The assumption of a potential peptide vaccine is based on present acquisitions on CD4 recognition of antigens previously processed by macrophages or other cells with a specific task (APCs) and their transformation into peptide fragments. TcR triggering by peptide-HLA class II molecule complexes on APC surface establishes the activation of T lymphocytes along with B cell cognate help for the production of IgE antibodies, thus a point of attack could exemplify either the TcR or HLA molecules (Fig. 1.66). A possible use of synthetic peptides to block IgE binding to mast cells, and as a consequence the release of mediator and the development of anaphylactic shock is intriguing [251, 265]. These could be the starting base to make T cells tolerogenic in vivo in human beings [273]. The possible reasons why peptides reacting with T cells in vitro do not produce a clinical response in vivo include a) that chosen peptides covered too few of the relevant T-cell epitopes, b) that peptides are so rapidly decayed or removed in vivo that they cannot interact with the immune system, and c) that these peptides are presented to T cells in vivo in a form that fails to produce the solicited response [195].

- *T cell anergy or clonal deletion* [273]

Figure 1.22b illustrates T-cell anergy due to the absence of the second signal, which is obtained in the animal model by employing immunogenic peptides provided in particular of immunodominant T epitopes. Stimulating results have come out of this research, but so far they have not provided insight into the immunological

events that occur in human subjects. In rodents, tolerance was induced by delivering sequential doses of a peptide with T epitopes and subsequently the same peptide with an adjuvant. Conversely mice receiving saline continued to mount significant T-cell responses accompanied by significant antibody responses [221]. Very demonstrative studies have shown that sensitized animals in a first phase with Fel d 1 elicited similar results [31], and in the other phase intranasal inhalation of low levels of peptide containing a dominant T-cell epitope inhibited ongoing immune responses in mice sensitized to Der p 1 allergen [110]. If a T-cell peptide is able to induce peripheral T-cell tolerance in rodents to a subsequent challenge with allergenic proteins, peptides provided with T cell-dominant epitopes appear to be much more effective tolerogens than those with only minor epitopes, since in the first case a single exposure is all that is necessary instead of three exposures [221]. The potential use of a peptide-induced T-cell non-response is facilitated by the demonstration in animal experiments that peptides without B-cell epitopes are able to suppress immune responses [153]. However, using immunodominant peptides may have negative effects on the human immune system [172]. These effects include stimulation of peptide-specific Th1 T cells with the possible onset of fever, skin rashes, and/or delayed but not *systemic or anaphylactic reactions,* since Th1 cannot link IgE because it is sequential [57]. Similar peptides able to block a wide spectrum of different HLA molecules have not yet been identified, also because the studies on restriction have not been concluded exhaustively. To respond to practical applications, one should consider that in peptides provided with T-cell epitopes, one or two are immunodominant: therefore the functional regions of several allergens have been mapped, relative to such epitopes, thus identifying the peptides with an amino acid sequence identical to the allergenic one and able to stimulate T lymphocytes by making them unresponsive [171]. Perennial and seasonal allergens have been determined as well as others with T-cell epitopes (Table 1.76), also including Fel d 1, egg lysozyme, myeline basic protein, type II collagen, pollens, mites and Api m 1 [31, 215, 260]. The key point in preparing a peptide vaccine is that usually the single allergens possess several B- and T-cell epitopes. Thus peptides could be fragmented to utilize only the immunodominant regions, for example, small fragments with a few amino acids [260], thus avoiding the larger ones, which could activate both IgE and metachromatic cells; while inhibiting Th2 T cells IgE responses were eliminated or minimized [251]. Small peptides may equally bind IgE, activate basophils and platelets, and T cells can produce chemokines and related mediators, thus priming basophils and mast cells [57]. *Therefore T-cell immunodominant epitopes may act as potent inducers of Th2 unresponsiveness in humans* [260] *by activating Th1 and/or suppressor CD8 T cells* [215]. This is a very promising prospective, since it is easily understandable that peptides provided only with Th1 epitopes can effectively contribute to a therapeutic strategy that provides for the induction of a Th2 sound anergy without stimulating mast cells, which would result in nearly or entirely absent side effects [57]. However, clonal anergy is not the only mechanism involved in down-regulating T-cell responses, since anergy should presumably only affect T cells specific for the epitope used for treatment [221].

In keeping with that, it has been demonstrated that an analogical peptide was able to inhibit in vitro the proliferation of T-cell clones specific for Der p allergens, avoiding T lymphocytes both the interaction with HLA class II molecules and their recognition [171]: the concrete result is that DPB1*0401-restricted T-cell clones become unresponsive and are therefore no longer capable of secreting IL_4 and instead *produce IFN-*γ [172]: this is the switch from Th2 to Th1 that precludes IgE synthesis. The functional T-cell inactivation, made tolerant by use of such peptides, would be translated into a therapeutic potential in atopic disease, where Der p-reactive T cells are restricted by HLA-DP molecules [102]. By inactivating both HLA-DP- and HLA-DR-restricted T cells, SIT with allergen peptides may provide a therapeutic potential, also independent of whether it is associated with HLA class II molecules [102]. A practical question to be resolved is the more frequent immunogenicity of Der p 2, such that polypeptide fragments should be made ready in B-cell sites that have been inactivated along with peptide residues provided with a sIgE anti-Der p 2-linking epitope [173]. However, in applying this method we must recognize the impossibility of unmasking all relevant epitopes in allergen molecules [251]. Even if we could do so, we should point out that the antigen-specific T-cell epitopes are different from those recognized by IgE [274]. Thus, an IgE cross-linking to injected allergens should not occur in theory. Moreover, T-dependent IgE synthesis is reduced during SIT (Fig. 13.13). In addition, we must emphasize the necessity of mapping the T-cell epitopes of a given patient and typing HLA molecules [273]. A possible complication stems from the 85% homology between Eur m 1 and Der p (Table 1.74), while it is significant for SIT efficacy when using T-specific peptides derived from Fel d 1 in patients with this allergy [164]. In contrast, modifying the allergen and stimulating the priming of naive T cells results in a selective turn to the Th1 phenotype: for example by using glutaraldehyde, a high-MW compound is obtained, thereby increasing 20-fold the connection of IFN-γ with IL_4 and IL_{10} [271]. Reprogramming the Th2 response of allergic subjects to a Th1 response, thereby intervening in the signals that modulate naive T-cell differentiation is considered even more critical.

Further strategies include utilizing isolated, purified and mixed IgG antibodies with Der p allergen: thus autologous circulating immune complexes (CIC), possessing a potentially low allergenicity, would elicit a minimal SR, and in addition the greater part of the aller-

gen's biological activity would be neutralized by excess antibodies. This method would appear to be useful in eliminating the production of neutralizing antibodies, even evident after repeated administrations of recombinant cytokines or monoclonal antibodies [172].

A novel form of SIT that makes use of T-cell peptides derived from Fel d 1 has been developed for patients with asthma who are allergic to cats and are injected IT with short, overlapping, T-cell peptides derived from Fel d 1. The patients received either Fel d 1 peptides (90 µg in increasing divided doses) or placebo. Four of the 16 patients on Fel d 1 peptides had initial late asthmatic reactions, but could be desensitized to the higher dose of peptide [176]. Two studies [168, 188] first attempted to treat cat-allergic subjects by SC injection of two peptides (called IPC1 and IPC2), which spanned a large proportion of chain 1 of Fel d 1. However, IPC1 and IPC2 were 27 residues long and associated with immediate and late allergic symptoms that occurred between 10 min and 6 h after SC injections, which may have been the result of cross-linking of allergen-specific IgE. Therefore the Fel d 1 peptides in a subsequent study were of relatively small size (16/17 residues) and had a linear configuration to enable them to be presented to T cells [176]. To investigate the possibility of a peptide epitope-induced switch from a Th2 to a Th1 cytokine profile, the primary proliferative responses and cytokine production of PBMCs to both the individual peptides and whole cat dander was assessed. A single 5-µg ID injection of Fel d 1 peptides was associated with a reduction in both proliferation and production of *IFN-γ* and of IL_4 and IL_{13}, and the amount of proliferation *significantly decreased* between baseline and second follow-up, and the concentration of IL_{10} was significantly higher in patients on peptides [175, 176]. A hybrid peptide comprising seven T-cell peptides has been shown to possess the capacity of inducing T-cell proliferative responses that is superior to the potential of a mixture of the T-cell peptides and comparable with that of Cry j 1 and Cry j 2. Cry j (*Cryptomeria japonica*) the hybrid peptide will be of use in SIT as a potential therapeutic agent for pollinosis [104]. Instead of covering the entire length of several major allergens in a combination treatment, a DBPC phase I clinical trial was developed for patients hypersensitive to bee venom, a novel strategy of venom SIT based on three long synthetic peptides (LSP) mapping the entire 140 amino acids of PLA_2 (phospholipase A_2), a major Api m allergen. SIT with LSPs derived from PLA_2 was able to induce T-cell anergy and immune deviation toward a Th1-type T-cell IL response and enhanced IL_{10} secretion and specific IgG_4 production [76]. These results may thus represent a novel and safe approach to SIT [76]. LSPs offer the advantage of covering all possible T-cell epitopes for any HLA genotype, and can be considered candidates for a novel and safe approach to SIT [76].

- *Manipulation of B epitopes*

Extending the technique to B epitopes, the whole amino acid identification of the allergens via the technique of rDNA has surprisingly disclosed only limited information on the partial sequences necessary for IgE binding [195]. Although IgE antibody binding is severely hampered by the lack of monoclonal IgE antibodies, some B epitopes on allergens have reacted to monoclonal antibodies [215]. This result could be explained at least in part by the probability that special conformational peptides are necessary for IgE binding; thus certain allergens may even constitute an entire epitope. This was demonstrated for Der p 2 and seemingly also for Bet v 1. Subjecting a complementary DNA (cDNA) clone coding the latter allergen, via techniques of cross-hybridization and cloning, the resulting fragments had no IgE-binding peptide; likewise the allergen binding site was abolished by trypsin or thrombin treatment [245]. Weighing these results globally, we should point out that data raising uncertainty on the short term employed by such techniques should be taken into consideration.

Manipulation of IgE Responses

Studies related to IgE response show that if glutaraldehyde-modified allergens push the balance to the Th1 phenotype, they regulate IgE antibodies negatively. Various restrictions are predictable: since they are epitopes recognized by B cells they are conformational so that peptides representing small linear epitopes may not be able to trigger IgE-mediated responses. If peptides are recognized by allergen-specific IgE sequentially distinct from T-cell peptides, this enables the dissection of an allergen that respects the protein sequences, but abrogating the structural integrity of the molecule necessary for IgE-binding [57]. The discussion centers on the class-specific and antigen-specific suppression of IgE responses. In the first case, evidence is emerging that implies IL_4 inhibition by monoclonal antibodies, mutant proteins, etc., while IFN treatment results (Chap. 7) are controversial.

Antigen-specific suppression, as Fig. 1.66 indicates, may include:

- *Functional inactivation of T-cell clones* or subpopulations with different specificity, but with commonly characterized TcR
- *Replacement of epitopes* related to the disease by "inert"peptides via peptide-HLA molecules combinatory SITS;
- *Switching from Th2 to Th1* that down-regulates IgE synthesis
- *Idiotype manipulation of IgE*-specific responses
- *Engagement of antigen-antibody CICs* to functionally inactivated B cells, and to stimulate production of anti-idiotype antibodies to suppress IgE-specific responses

In the first case, a human $CD8^+$ regulatory subpopulation induced by CDR2 peptide modulates $CD4^+$ activity, as demonstrated that in depressing $CD8^+$, not only this modulatory capacity is terminated, but it additionally increases peptide responses [119]. Interestingly, the anergy of selected components of the T lymphocyte-specific repertoire, realized by a TcR peptide, based on the sequence of the *CDR2 region of TcR-Vβ3* gene segment inhibits human T-cell polyclonal responses to Der p [119]. In another trial, TcR expression (as measured by CD3) is reduced on anergic cell surface similarly to observations on activated cells. However, the TcR modulation is not capable of inducing a non-response since CD3 reappears on the surface within 60 h, while T-cell anergy persists. The effect of peptide-mediated anergy on T phenotype was studied on membrane proteins. In this context, CD25 expression was raised in tolerant cells, that of CD28 appeared to be down-regulated and not equally widespread [172]. CD25 increment is in line with the Th2-to-Th1 switch [253]. Thus, peptide-mediated tolerance results potentially from the signaling alteration via TcR/CD3, independently of whether this complex is expressed on the cell surface [251].

Especially stimulating are the experimental studies done on idiotype regulation of IgE response, which have demonstrated that anti-idiotype antibodies have the capacity of controlling IgE-mediated immune responses. In humans, important observations have been reported. Effects stimulating immune responses, such as the result in allergic subjects of markedly reduced titers of anti-idiotype antibodies and increased during SIT, and in other cases a suppression of this response, have been reported. Preliminary data appear to indicate that anti-idiotype antibodies recognizing idiotypes within the paratope are able to replace an antigen in certain experimental conditions, thus potentiating the immune response. Instead, when idiotypes were not associated with paratope, anti-idiotype antibodies appear to induce preferentially suppressive signals. However, an idiotype manipulation of IgE response contrasts with practical obstacles, while the question remains unsolved whether and which side effects follow a treatment based on these antibodies, even if no SRs were noted in the animals studied [195].

A randomized DBPC study was conducted in pollen-allergic adults, who, beginning a month before the season, were treated for 13 weeks with autologous allergen-IgG complexes. The allergen incrementing doses (containing the 12 most frequent European pollens) were on average 100- to 350-fold lower than the doses normally employed in SC SIT. What appears intriguing is that after a few injections the effects were notable: abolition of sIgE increase that was either transitory (present at the start of conventional SIT) or took place during the pollen season. Clinically, drug use as well as eye and bronchial, and to a lesser extent nose symptoms were significantly reduced [146]. In Der p-allergic children, good tolerance to the injections has been shown, thus suggesting that allergen-antibody complexes might be worth considering in mite-sensitized children, with the advantage of long-term improvement after SIT administration and a significant influence on nonspecific inflammatory changes both in the airways and skin [211]. In conclusion, it is suggested that:

- In addition to the rapid and sharp reduction in the levels of specific anti-Der p antibodies, there is a clear correlation between a decrease in IgG levels and clinical improvement.
- Treatment with allergen–antibody complexes boosts the production of corresponding anti-idiotype antibodies, with a loose correlation, however, between levels of anti-idiotype antibodies and clinical improvement, indicating a potential positive role for this therapeutic mechanism; an unsatisfactory explanation of the mechanism of action persist's.
- Suppression of anti-allergen antibody production may be the result of an epitope-specific mechanism; therefore antibodies experiencing a down-regulated production are of the same specificity as those administered in the form of complexes.
- To bypass this obstacle, anti-allergen antibodies of the polyclonal type should be produced, or antibodies to B-cell epitopes of Der p allergen may be envisaged: however, B epitopes are conformational, which renders their proper identification difficult [211].

Anti-IgE

A new concept of SIT to treat allergic disease has been developed to inhibit IgE function. The monoclonal *anti-IgE antibody, omalizumab,* lowers levels of serum-free IgE in human beings and reduces IgE-mediated symptoms, regardless of the allergen specificity and biological role of the IgE involved [154]. It binds to free IgE at the same site as the high-affinity receptor. Although it attaches to free IgE, it does not bind to IgA, IgG, or cell-bound IgE. It therefore does not induce cross-linking of cell-bound IgE, which would lead to the release of allergic mediators. It has been reported to decrease serum IgE levels in a dose-dependent manner, inhibit PAR and LAR, and cause a down-regulation of FcεRI receptors on basophils, as well as blocking the mechanisms responsible for the release of the cascade of mediators involved in early-phase and late-phase asthmatic responses to allergen aggression. Efficacy of anti-IgE vs placebo in reducing the severity of symptoms of SAR and in improving rhinoconjunctivitis-specific *quality of life* has been reported. Efficacy in patients with allergic asthma has also been reported, anti-IgE being used as an IV-administered formulation [30]. In 225 children aged 6–12 [154] and in 221 aged 6–17 [132] with inhalant allergy, treatment with monoclonal omalizumab and SIT was more effective than SIT alone and was well tolerated [132]. The proportion of days with allergy symptoms was also significantly lower in the SIT + anti-IgE group vs placebo (median percent of days of the entire pollen season, 58.5 vs 79.3 [132]). Treatment with omalizumab

allowed children with moderate to severe asthma who required CS therapy to reduce the need for inhaled and oral CSs. Median free IgE ranged from 133 to 790 IU/ml at baseline and was in the range of 6–9 IU/ml during the treatment period [154]. An increase noted in total IgE represents the persistence in circulation of biologically inactive omalizumab–IgE complexes. These low MW complexes lack the biological activity of IgE because the Fc portion of the molecule is blocked [154]. The anti-IgE therapy might be a novel strategy for further reducing symptoms and additional rhinitis medication requirements in patients for whom SIT is indicated. The lack of dependence on allergen specificity makes anti-IgE especially suitable for use in multiplesensitized allergic patients as well [132, 154].

Long-Term Perspectives

Further studies could explore*IL complete or partial inhibition* via anti-cytokines or tolerogen allergens capable of priming IgE response, by intervening on IgE synthesis [226]. IL_4 and IL_{13} inhibition by an IL_4 mutant protein (Chap. 1), as well as similar experimental results may eventually make it possible to augment the IgG_4 antibody levels by IL_{13} [144]. In effect, IgG_4 inhibits IgE antibodies, and therefore the ultimate benefit to patients would be the selective induction of IgG_4 antibodies [254]. To monitor SIT efficacy, it is very helpful to establish a correlation between modifications of T-cell function or IL generation and clinical symptoms. The induction of a rapid and strong allergen-specific IFN-γ release, as well as the IgG antibody formation to injections of low doses of allergen, are both desired properties that would enhance the beneficial response to an immunotherapeutic agent [238]. Other adjuvants that induce deviation of antigen-specific Th2-to-Th1 responses and include antigen-linked ILs, such as IL_{12} and IL_{18} [214], the latter expressing IFN-γ, or heat-killed *Listeria monocytogenes* [272].

Holt has proposed an immunization for young infants [107], in line with data from Varney et al [253], to include a protein extract provided with Th1-like ILs in a vaccine, to stimulate a Th2-to-Th1 switch, or allergens in bacterial or viral carriers, which induce Th1 responses [251]. It is a surprise that an edible, inexpensive transgenic plant could be used as an antigen vehicle for SIT. This technique would be best utilized by using a fruit or vegetable that is eaten raw, such as *transgenic bananas* expressing the major Der p allergens, which could be a very simple approach to inducing immunological tolerance in children [145]. Recent results demonstrate that oral immunization of transgenic rice to express and orally deliver specific peptide epitopes of tree pollen allergens expressing A1aB1b-Crp-1 and -2 protein resulted in the generation of systemic unresponsiveness with the inhibition of IgE-associated Th2 ILs, including IL_4, IL_5 and IL_{13} and of allergen-specific Th2-mediated IgE responses and histamine release. Inhibition of Th2 responses can occur without anaphylaxis [229]. Oral immunotherapy with transgenic lupin seeds expressing sunflower seed albumin dramatically *suppressed the developement of experimental asthma* by suppressing the production of Th2-type Abs, a response critically dependent on the development of the CD4+ $CD45R^{Blow}$ suppressor T cell population and the production of IFNγ [224]. In conclusion, since the future is this very moment, we can refer to the therapeutic perspectives presented in Chap. 1, which demonstrate stimulating long-term progress for SIT.

From a clinical point of view, we summarize in Table 13.19 the results of an epidemiological study of our Division centered on the SIT crisis, an alarming prospective since we have demonstrated that only 5% of children with allergic respiratory disease will undergo

Table 13.19. Epidemiology of allergic disease in Italian children. SIT in 323 asthmatic children

SIT	Children (%)
Traditional SIT	5
Oral SIT	1

Personal data.

Table 13.20. Conclusions on SIT efficacy

Efficacy in 93% of trials (Table 13.2) in the treatment of respiratory allergy as demonstrated by:
Improvement of clinical symptoms
Significant reduction of medication use
Improvement of spirometry results
Threshold raising of provocation tests
Opportunity to prescribe SIT to children with allergic rhinitis despite correct medication
Early reduction of asthmatic symptoms during SIT of children with pollinosis, *but not in chronic asthmatics*
Symptoms reduced in dose-dependent manner
Necessity of prescribing SIT early in the course of asthma, before permanent damage to the bronchi has developed
Reduction of allergic components of allergic rhinitis and asthma provided doses that are high enough are used
Several positive immunological changes
May induce allergic reactions, why proper monitoring is most important
Comparison SIT/pharmacotherapy: SIT effects persist after stopping injections, but medication effect is abrogated when medications are discontinued

Modified from [68].

SIT, stressing the significance of this result, since as analyzed in Table 5.16, 41.3 %–47.5 % of asthmatic children but 95 % of those with severe asthma continue to suffer from asthma when they reach adulthood. Not rarely have physicians stopped using SIT because they do not believe in the treatment [1], or fear (severe) side effects [1], in 5 % of cases [207], or because they consider the allergen avoidance and pharmacotherapy more effective [207]. This is a contradictory result because for 67 % of interested physicians, SIT positively modifies the long-term natural history of pediatric asthma [207]. However, we must point out that the very high number of reactions credited to SIT leads one to question the results presented in the 44 pediatric studies (Table 13.2). Table 13.20 [68] summarizes data on SIT as a conclusion of this chapter.

Pediatricians and SIT

A new page in the relationship between pediatrician and patient points to children in treatment with SIT and the pediatrician who cares for them. Given the long duration of SIT, over a period of years, it offers the pediatrician an exclusive occasion to set up good terms with young patients and their parents and/or relatives and/or escorts, which goes beyond a simple medical visit. The pediatrician is therefore a support figure for the child's entire family who lives the disease with comprehensible insecurity and fear. In other words, the pediatrician must explain the nature of the condition comprehensively, the positive SIT target, recovery from the disease, and the necessity of setting up an effective allergen avoidance regimen. This would be very helpful to the child thus able to accept the SIT course, in addition to parents who often transfer their anxiety into an overly protective attitude toward their child undergoing SIT. What worries us is that several authors have concluded that SIT results are controversial, whereas a thorough evaluation based on experience leads us to believe that SIT's critics are often supported by statements that are often clichés, including the controversial results of well-controlled studies, the limits of its efficacy, the improvement of drug therapy, and the potential danger. These clichés often resemble a repetition of the statements of various experts. Above all, pediatricians should be convinced and they should assure parents of *SIT efficacy, and safety,* which we have demonstrated, data in hand, in 99.9 % of cases (Tables 13.10, 13.11). Rhinitis symptoms are abated after a 5-year course, and are reduced in a dose dependent manner. Asthmatic symptoms are reduced early in the course of SIT in children with pollinosis, but not in chronic asthmatics when permanent damage to the bronchi has developed. Thus, SIT has the potential to provide a positive step forward in the treatment of respiratory allergic disease.

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Eye Allergy and Immunology

Eye Disorders

The eye and the ocular membranes are frequently prone to allergic reactions in sensitized patients, because of the common ectodermal origins of the skin and several ocular structures, easy access for aeroallergens, and because of potential conjunctival immune reactivity. In this chapter we examine the various forms that involve the eye from an allergic-immunological viewpoint.

Pathophysiology

The eye surface having an area cavering several hundred square millimeters [139] is naturally exposed not only to microorganisms but also, and above all, to allergens for long periods, thus representing a relatively large collection window [139]. The eyelids defend the eyes from external attacks by closing, both in response to stimuli (blinking reflex) and automatically at short intervals (a few seconds) to avoid external substances, also allowing allergen removal and causes of inflammation due to the tear fluid layer. The tear film, which is continuously renewed, is most important for protecting the eyes and preserving tissue integrity [18]. Since the eye has a particular structure, the tissue most exposed to type 1 reactions is the conjunctiva. The eye is an immune *privileged site* since inflammatory reactions cannot normally occur in the cornea, the anterior chamber, the crystalline lens, or vitreous body, protected by their anatomical position, which makes the eye's interior inaccessible to external agents. The cornea in turn is protected by the conjunctiva from the immunological viewpoint [181]. This occurs because the optical axis needs only to cross transparent elements to ensure normal vision: therefore the privileged structures, to ensure protection from immune reactions that are potentially dangerous, have no blood vessels, no normal lymphatic drainage or inflammatory cells, which are instead numerous in the conjunctiva and the eyelids [67, 80]. The privileged immune sites are protected from invading pathogens and from infiltrating lymphatic cells by an active process that relies on the intraocular set-up of Fas/CD95 counter-receptor, Fas-L, capable of promoting apoptosis of activated ocular T cells [80]. The resident ocular dendritic cells (DCs) and macrophages cope with the immune privilege by producing interleukins (ILs) such as TGF-β [195].

Early DC activation by allergen within the conjunctiva is a very early step in disease pathogenesis [139]. The *conjunctiva* maintains a drainage system into the nose through the nasolacrimal duct, *thus representing the upper extremity of the respiratory system* [139]. The conjunctiva is a thin, transparent, vascular mucous membrane, which is divided into three portions: the bulbar conjunctiva, the palpebral conjunctiva, and the fornix, exposed to the external environment and protected by the tear film. It is endowed with a relatively limited capacity for inflammatory reactions. The eye normally appears reddened due to dilatation of conjunctival vessels, hence chemosis occurs, or conjunctival edema, due to fluid exudate across blood vessel walls; exudated cells, mucosal secretion and tears provide consistence to ocular discharge [167]. As the inflammation progresses, the palpebral conjunctiva develops follicles or papillae; subepithelial accumulations of lymphoid tissue, which cause papillary hypertrophy usually described as Roman cobblestones, typical of vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC). Elongated microvilli with microplicae and medium-sized lymphocytes with no plasma cells are associated with papillary hypertrophy [16].

When inflamed, the *cornea,* which is normally clear and transparent, presents infiltrations in the stroma and granular opacities: these are initially marginal, forming close to the newly formed vessels and assuming a more central position as the inflammation progresses [36].

Another eye peculiarity is the accessory lymph node, represented by the *uveal tract:* the eye *in itself* has no lymphatic drainage and it is assumed that the external portion of the globe, including the conjunctiva, is richly supplied with lymphatics capable of responding to foreign antigens. The uveal tract (a continuous layer of iris, ciliary body, and choroid) constitutes the eye's vascular tunic and is the main deposit for lymphocytes; the limbus also accepts sensitized cells: repeated exposure to antigens, both at the ocular level and in other more distant sites, can set off local antibody reactions [67]. Lymphatics from the lateral conjunctiva drain into the preauricular nodes (parotid node) just anterior to the ear tragus. The nasal conjunctival lymphatics drain to the submandibular nodes and sensitized lymphocytes return to their original locations [14].

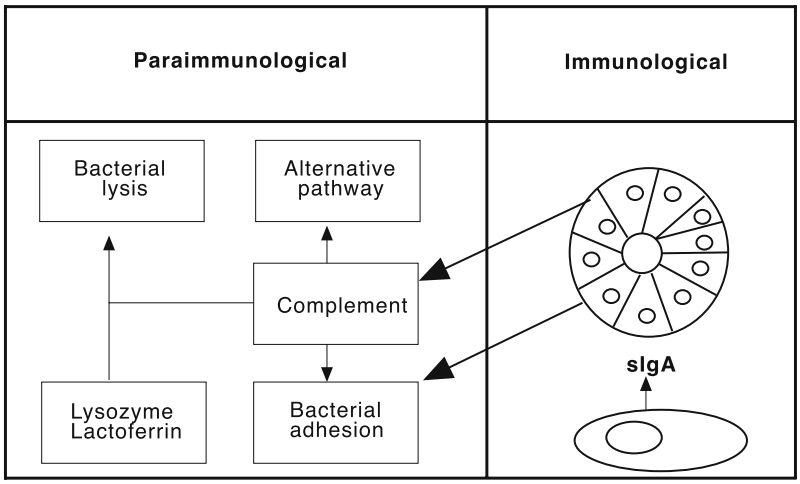


Fig. 14.1. Interactions between immunological and paraimmunological defense systems. (Modified from [36, 166])

Table 14.1. Natural structures involved in mucosal defense of the eye

1. The orbital skeletal structure minimizes potential trauma.
2. The eyelid architecture is relatively impermeable to macromolecules.
3. The eyelid blink reflex and ciliary movements rapidly clear foreign objects from the ocular surface.
4. The resident conjunctival populations of nonpathogenic bacteria, including aerobes and facultative and obligate microbes, may curtail the attachment and colonization of invasive bacteria.
5. The tear flow and reflex tearing remove microorganisms and cellular debris by hydrokinetics and drainage into the nasolacrimal duct.

Data from [166].

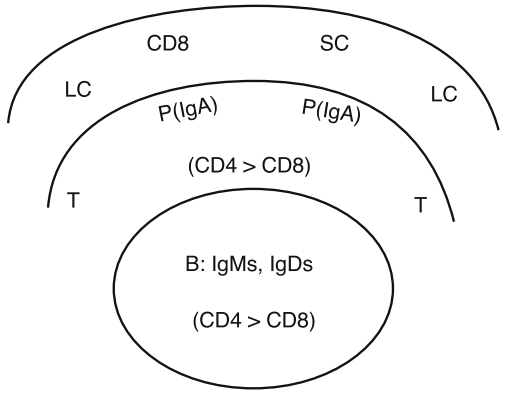


Fig. 14.2. Schematic representation of a conjunctival follicle. *IgMs*, *IgDs* surface IgM and IgD, *CD8* T suppressor/cytotoxic, *SC* secretory component, *P* plasma cells, *LC* Langerhans cells. (Modified from [170])

Table 14.2. Additional specific and aspecific agents in human tears that support ocular mucosal immunity

1. Lysozyme, among major tear components, possesses antibacterial activity.
2. Lactoferrin, another major tear component, may also prevent activation of the classic complement pathway by inhibiting C3 convertase.
3. A heat-stable antichlamydial factor prevents *Chlamydia* attachment.
4. Peroxidase may exert bactericidal, viricidal and fungicidal activity.
5. Plasminogen activator is chemotactic for leukocytes.
6. Specific tear prealbumin and transferrin may exert antibacterial activity.
7. Ceruloplasmin may reduce viral infectivity and act as a superoxide-dismutase.
8. A system producing superoxide radicals may also exert antibacterial activity.

Data from [166].

As with other organs and systems, there are natural defense factors for defending ocular immunity, schematized in Table 14.1 [166]. Furthermore, tears contain various specific and aspecific factors provided with antibacterial activity, which work together with the specific defense systems: these are illustrated in Fig. 14.1 [36, 166] and in Table 14.2 [166].

Ocular Immunology

Conjunctiva-Associated Lymphoid Tissue

The ocular immune system relies on the CALT (conjunctiva-associated lymphoid tissue), a structure which includes the conjunctiva and the lacrimal glands. The epithelial layer and the follicle-associated epithelium (FAE) overlying lymphoid follicles [77] demonstrate an intraepithelial pocket with the presence of $CD4^+$ cells

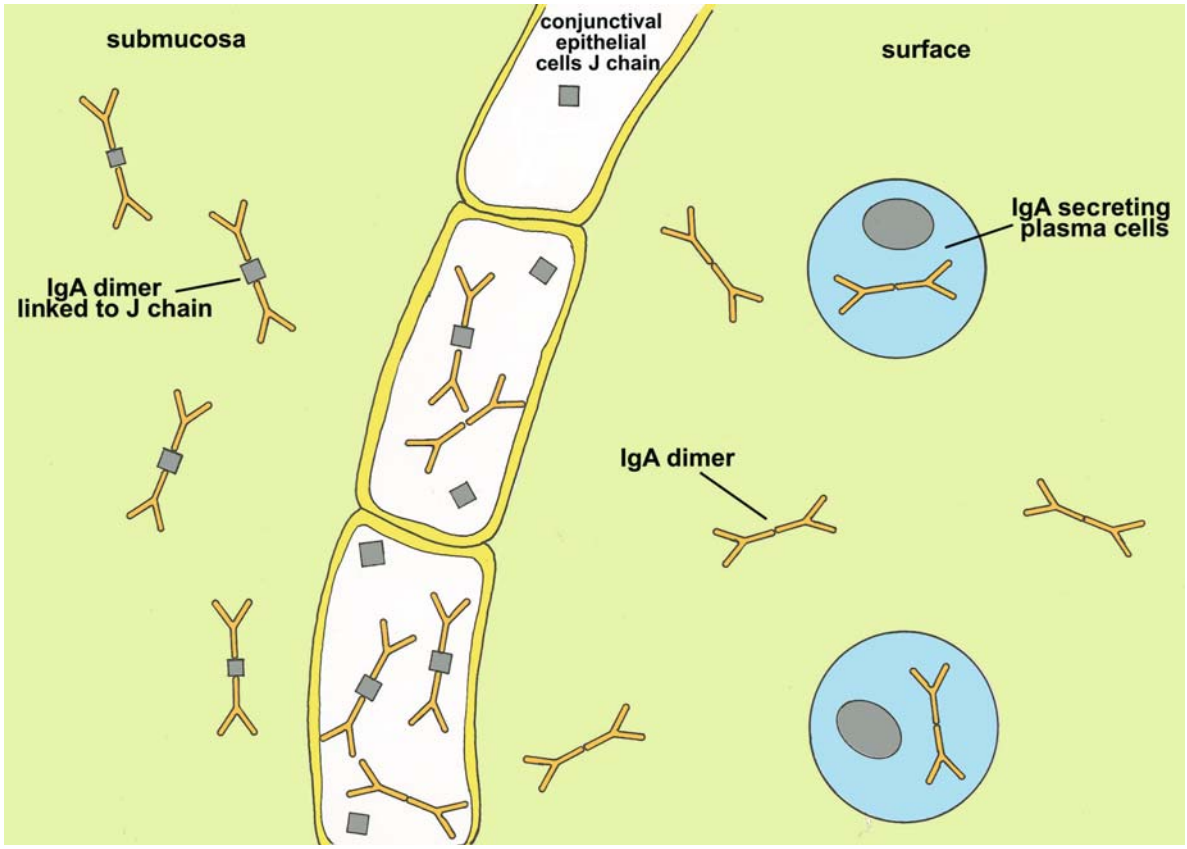


Fig. 14.3. Transportation of IgA antibodies across the conjunctival epithelium

[155]. In addition, the distribution of lymphoid follicles containing CD4, CD8 [155], B lymphocytes and a considerable number of IgA antibodies (Fig. 14.2) [170] shows morphological and functional features analogous to mucosal-associated lymphoid tissue (MALT), functionally linked to other structures such as BALT, NALT and GALT. In animals, CALT has a histological structure similar to Peyer's patches (PP), including the dome-shaped surface covering PPs, as well as for the significant proportion of B cells switching into B_{IgA} similarly to PP B cells [65]. The FAE overlying CALT demonstrates the morphological characteristic of M cells, including attenuated apical cell surface with blunted microvilli and microfolds [77]. DCs in the CALT follicular area were identified to be FDCs (follicular dendritic cells) [160]. The lymph nodes predominating in the CALT share several characteristics common to other MALT structures: 1. They are usually numerous and provided with small and medium-sized lymphocytes. 2. Frequently larger lymphocytes undergo mitosis. 3. The lymphocytes are also placed in adjacent lymphatic channels [36].

Lymphoid tissue with similar characteristics, including secondary follicles, was also observed inside the lacrimal drainage system [87]. Human plasma cells, most of which were IgA-positive, formed a thin layer in the lamina propria. The overlying epithelium produced *secretory component* (SC) [102]. Lymphoid tissue with similar characteristics, including secondary follicles, was also observed inside the lacrimal drainage system [77]. The sIgA (*secretory IgA*) antibodies, locally synthesized, prevent bacterial colonization, interfere with parasitic infestation, and defend the eye by reducing antigen changes to the membrane, while the remaining immunoglobulins (Igs) inhibit viral infection and internalization [57]. High endothelial venules (HEV) were present in all types of lymphoid tissue [102]. Thus the secretory immune system protects the eye from allergic, infective and inflammatory affections, ensuring ocular integrity and preserving visual acuity [118, 181].

Immunopathology

Tears contain high concentrations of proteins that are immunologically active and mediators such as lactoferrin, lysozyme, histamine, prostaglandins, complement and ILs, and also B and T lymphocytes, DCs and macrophages [76, 146]. Fifty-four percent of mononuclear cells in lacrimal glands are plasma cells [192], which mostly secrete IgA antibodies, with a subclass distribution generally corresponding to that of MALT [68]. Dimeric IgA, positioned in the glandular interstitium, after synthesizing the J chain and polymeric IgA,

Table 14.3. Mean lacrimal immunoglobulin levels from a meta-analysis of 38 studies in normal individuals

Immunoglobulins (μg/ml)				
IgA	sIgA[a]	IgG	IgM	IgE[a]
70–630	374–2,020	Traces –690	0–120	0.25–0.06 0.06–<5 IU/ml

Data from [166].

[a] The studies on sIgA and IgE antibodies are a meta-analysis of seven studies. In the six studies analyzing IgD levels the result was 0.

are transported across the epithelial cells where they bind to SC [27, 101], which facilitates IgA transportation into the tears and protects it from degradation by enzymes localized in the tears [36] (Fig. 14.3). Concentrations of IgG, IgM and IgD are rarely present [192]. Other cells are B lymphocytes (6%), organized in lymphoid aggregates at the center of primary follicles for 40% by T lymphocytes positioned in the follicle and parafollicular area, with a CD4/CD8 ratio of 0.56. CD8 T cells are more numerous than the CD4 T cells also at epithelial and interstitium levels [141, 192]. DC LCs and macrophages are present; the glands also express HLA-DR on B cells, DCs, and ductal epithelium [128]. In patients with allergic conjunctivitis (AC), IgE were positive in 56% of eyes compared with 21% and 25% of eyes with nonspecific conjunctivitis and dry eyes, respectively. IgE levels were significantly higher in the allergic group than in the other two groups [12] and in 64.7% of children with AC aged 10 months to 15 years. HLA-DR positivity in epithelial cells was negatively correlated with tear IgE [12]. Table 14.3 [166] indicates average Ig levels in the tears of normal patients. To a great extent, these levels depend on collection and processing methods and the measure of stimuli transmitted to tears [175]. Table 14.4 [166, 194] summarizes the effects of conjunctivitis on tear Ig levels.

The *conjunctiva,* the CALT's second component, which contains all components necessary for a complete immune response [102], plays an equally important role in the inductive and effector stages of the ocular immune system [170]. Unlike previous results [153], the presence of IgA plasma cells with the SC in conjunctival follicles (Fig. 14.2) confirms that the conjunctiva is part of MALT [170], hence of CALT (Figs. 14.4, 14.5) [36, 166]. Under normal conditions here too T and B lymphocytes are disposed in specialized lymphoid follicles, with IgA plasma cells outnumbering IgE plasma cells in a 6:1 ratio [117] and other Ig classes [5]. The connective tissue surrounding the eye provides an additional barrier with its large MALT supply [117]. The CD4/CD8 ratio is =0.75 and the CD3 T cells are 189±27/mm^3 [163]. There are higher numbers of leukocytes in the bulbar than in the tarsal area, highest for CD3$^+$ T cells and CD57$^+$ NK cells. In both bulbar and tarsal conjunctiva, B cells and neutrophils were seen in the epithelium and substantia propria, together with plasma cells, NK cells and mast cells.

Table 14.4. Effect of conjunctivitis on lacrimal immunoglobulin levels

Conjunctivitis	Lacrimal immune response
Allergic conjunctivitis (seasonal)	↑IgE
Atopic keratoconjunctivitis	↑IgE
Vernal keratoconjunctivitis	↑IgE, IgG, IgM, IgA[a]
Giant papillary conjunctivitis	↑IgE, IgG, IgM
Hard contact lens wear	↑IgA
Soft contact lens wear	≈IgA, IgG, IgM
In comparison:	
Malnutrition (severe)	↓IgA, SC
Stevens-Johnson syndrome	↑IgG ≈ IgA, IgM

Data from [166, 194].

≈ Unmodified levels, *SC* secretory component.

[a] IgA levels were increased in one study out of five.

CD8$^+$ cells are more abundant than CD4$^+$ cells in the epithelium, but this was reversed in the substantia propria. Conjunctival intraepithelial lymphocytes (IELs), like gut IELs, are predominantly CD8$^+$, are found in the basal epithelium, are human mucosal lymphocytes-1 (HML-1$^+$) and have very high expression of CD45RO [83]. Significantly increased numbers of CD4$^+$ coexpress, CD45RO$^+$, CD45RA$^+$, and HLA-DR$^+$ T cells are found in the conjunctiva of patients with AKC, VKC and giant papillary conjunctivitis (GPC), whereas CD4$^+$ T cells in AKC are predominantly CD45RO$^+$ CD45RA$^-$ with a corresponding up-regulation of markers present on APCs (antigen-presenting cells). These findings suggest that like allergic conditions in the skin and lungs, CD4$^+$ memory T cells are involved in the regulation of the immunopathology of chronic allergic eye responses [126].

There is a large number of *T mast cells,* on average 9,342±3,472/mm^3 in the substantia propria (90% of the total) in normal patients aged between 1 and 25 years [88]; in mice these are more numerous in the palpebral rim [123], which produce LTC_4, similar to the BALT and GALT sister cells [167]. In the event that inflammatory processes should become chronic, TC mast cells will

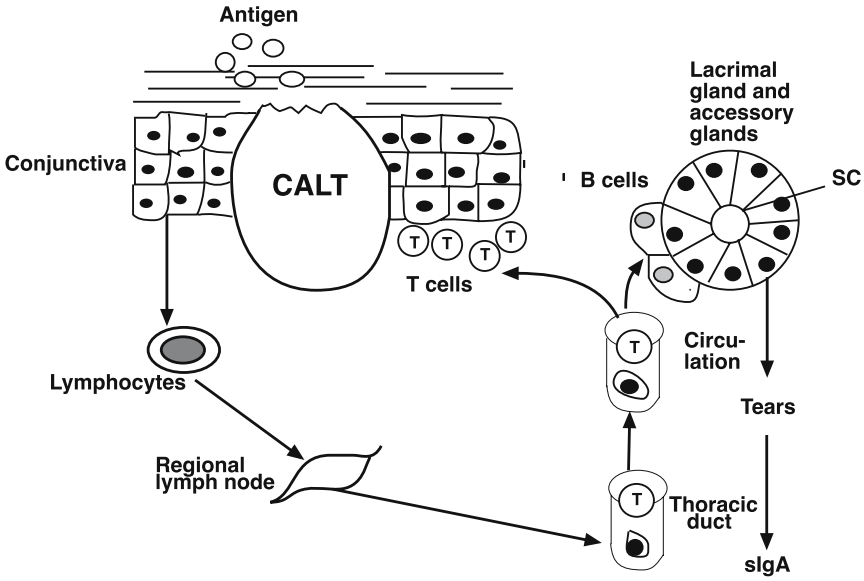


Fig. 14.4. Ocular defense based on CALT (conjunctival-associated lymphoid tissue). *SC* secretory component. (Modified from [36, 166])

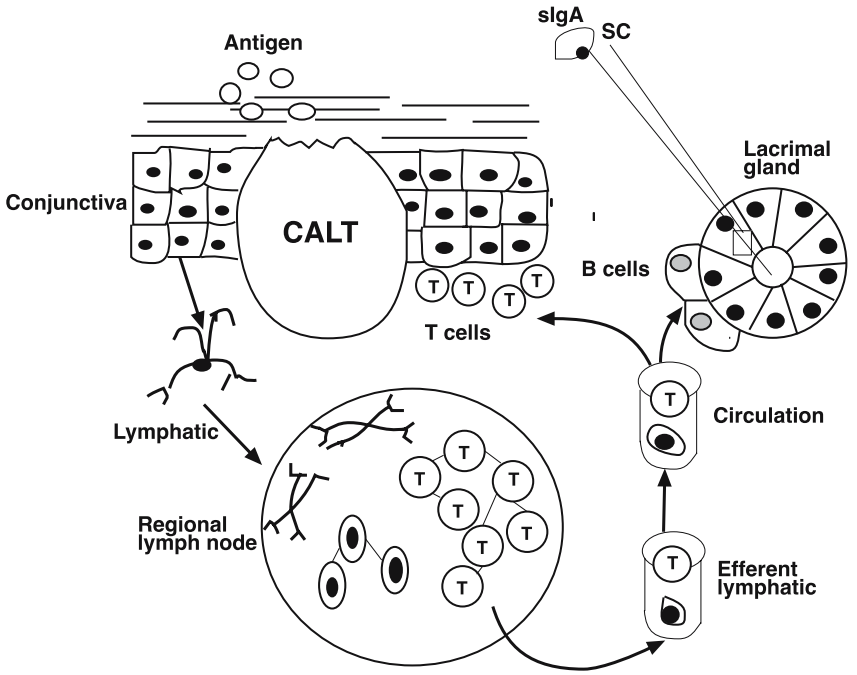


Fig. 14.5. Ocular defense based on CALT, Langerhans' cells and sIgA with the *SC* of the lacrimal glands. (Modified from [36])

increase [126]. Both the CALT and CLs are involved in the antigen presentation at the ocular level. The ocular CLs, situated in the epithelium [170] (85±16 CD1) [163], totally identical to the epidermic ones, seem to be more efficient than the macrophages: since they are antigen-activated, they are efficient APCs, taking the antigens up and migrating to draining submandibular and cervical lymph nodes [195]. If cell-mediated reactions also involve even simple haptens, this can result in important occasions for contact reactions [36]. Both T and B lymphocytes stimulated within regional lymph nodes migrate into the circulatory flow (Fig. 14.5), in which case T cells return to colonize submembranous conjunctival sites, while B cells localize in sites adjacent to the lacrimal epithelium and in accessory glands [65]. The presence of lymphatic glands in the two defensive structures allow local lymphocyte trafficking, thus avoiding their recirculation, although circulating lymphocytes may maintain contact both with other MALT structures such as the PPs and regional lymph nodes [192]. Some of the blood vessels associated with conjunctival follicles and lamina propria have HEV features and may play a significant role in lymphocyte homing to the conjunctiva, aided by the vascular adhesion protein-1 (VAP-1) [82]. In conclusion, the eye has unique immunological characteristics, reflected by *corneal avascularization,*

capable of receiving allogenic transplants with very little immunomodulation, producing antigens belonging to the HLA group, with a subsequent heterograft rejection [14].

In laboratory animals, the ocular secretory immune system is regulated by ILs. For example $IL_{1\alpha}$ and $IL_{1\beta}$ and TNF-α, but not IL_6 or IFN-γ, stimulate both SC synthesis and discharge [101]. IgA antibodies increase the production of TNF-α monocytes [52], while both IL_5 and IL_6 stimulate IgA synthesis in explants of lacrimal tissues, also increasing secondary responses of lacrimal IgA and pneumococcus antigens [143, 144].

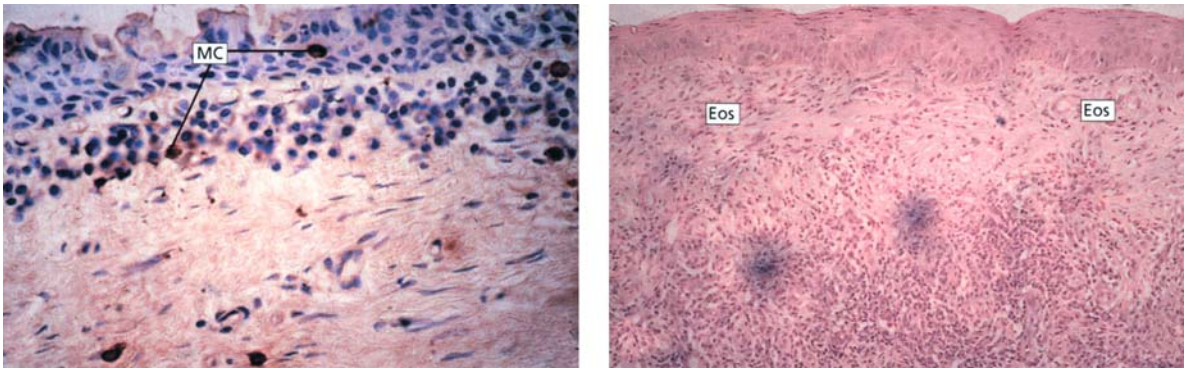
Immune Functions

Immediate Reactions

In the substantia propria of a normal conjunctiva there are once again inflammatory cells, such as mast cells, set out together with plasma cells, neutrophils and lymphocytes, these last ones also found in the epithelium, while basophils and eosinophils are characteristically absent. When a small dose of allergen comes into direct contact with an allergic child's conjunctiva, there is an encounter with the sIgE (specific IgE), which capture it and by cross-linking to mast cells, set off an immediate type I reaction with eosinophils and neutrophils in first position [14]. The quantity of allergen necessary for inducing a reaction is usually *10- to 100-fold higher than the amount needed for the skin*. Depending on the concentration of allergens the reaction can end within 1 h or persist for several hours (late reaction), with increasingly amplified symptoms when the dosage is 100- to 1,000-fold higher (320,000 BU/ml) [19]. Histopathology has been studied using the conjunctival provocation test (CPT) [34], both in conjunctival scrapings and in tears. After 20 min, there is a marked increase of neutrophils (from 4 to 21.5 on average), a more modest presence of eosinophils and lymphocytes (0.5 and 1.5, respectively), while monocytes are absent [18]; eosinophils predominate at all stages [21]. By instilling pollen in the conjunctival sac in atopic dogs, within 5 min conjunctival injection and edema appeared [68].

T Cells and Mediators

Th2 lymphocytes, with contribution from a few ILs, enhance IgE hyperproduction ($IL_{4)}$ and both mast cell (IL_3) and eosinophil (IL_5) differentiation and activation. In chronic cases, the numbers are higher, they are activated (CD25), and have a higher level of HLA-DR, CD1a (DC) and CD68 (macrophages) [126]. Unlike the skin, where Th1-induced delayed reactions are often prevalent, there is an *immune deviation priming Th2 T cells* [195]. *In situ* hybridization studies of ocular tissues have also shown Th2-like T-cell IL cluster (IL_3–IL_5) in subjects with VKC and GPC but a shift in IL profile toward a Th1-like pattern (IL_2) in subjects with AKC [127]. Tears from allergic donors contained significantly less IL_{10} and had significant increases in the ratios of TNF-α/IFN-γ, IL_5/IFN-γ and IL_5/IL_{10} [47]. Specifically, the tear IL_4 level in patients with AKC was significantly higher than those in VKC, SAC and controls [179], and a significant elevation compared to normal controls was shown in SAC, VKC and GPC [71]. Such findings suggest that all chronic ocular allergic disorders have a slight shift in mRNA expression to the Th2-like T-cell IL profile [127]: local lymphocytes express the Th2 phenotype and, to a lesser degree, the Th0 phenotype [113]. A subsequent study has found a greater localization to eosinophils for VKC and AKC than for GPC. RANTES, TGF-α, and TNF-α were localized to eosinophils in all disorders [84]. Tear TNF-α levels of VKC patients as well as TNF-α positive cells in VKC tissues were significantly increased compared to controls and significantly correlated with the severity of the disease. No differences were found between SAC and control tear samples [114]. Different patterns of eosinophil-IL localization were found to be prominent in VKC IL_3, IL_5, IL_6, and GM-CSF; in GPC IL_5; and in AKC IL_4, IL_8, and GM-CSF [84]. There are higher levels of *inflammatory mediators* including histamine, kinin, albumin, LTC_4, PGD_2 and TAME-esterase in tears immediately after CPT [146]. Histamine and PGD_2, at an average tear concentration of 5×10^{-7} and 5×10^{-8} mol/l, respectively, are directly responsible for the two cardinal symptoms: swelling and pruritus [146], also contributing to damaging the ocular structure [195]. There are also higher albumin and kinin levels, indicating the surprising rise in the ocular allergic reactions involving vasodilation and vascular permeability [143]. Histamine, when applied to the ocular surface, causes the typical clinical picture with a dose-dependent mechanism [18], and can be measured in high concentrations in the tear fluid of patients with VKC, but not in those affected by GPC [5]. The mediators released by metachromatic cells are involved either in immediate reactions or in delayed stages, recruiting inflammatory cells such as eosinophils, basophils, lymphocytes and platelets [3]. This action, up-regulated at the tissue level by *endothelial adhesive molecules* (ICAM-1 = intracellular adhesion molecule 1 = CD54, VCAM = vascular cell adhesion molecule = CD106, Fig. 1.59), results in the release of other mediators and the subsequent epithelial damage is worsened, together with other inflammatory products, causing fibroblast proliferation and collagen deposition [43]. IL_4 and IL_{13} increased production of collagen and modified the equilibrium between matrix metalloproteinase-1 and its inhibitor. These effects were partially opposed by IFN-γ and TNF-α [118]. In particular, CD54 expression on conjunctival epithelium was significantly more increased in 17 allergic children than in control children [138] after specific CPT [43] and during the pollen season [44]. CD54 seems to play a leading role in ocular inflammatory reactions, produc-



a b

Fig. 14.6. **a** Mast cells (*MC*) demonstrated immunohistochemically in the epithelium and submucosa in PAC. **b** Large numbers of eosinophils (*Eos*) present in the edematous submucosa of a patient with SAC

ing interactions between inflammatory cells and target organs [43]. A significant correlation between CD54 expression on conjunctival epithelium and ECP tear levels suggests that CD54 is locally up-regulated in inflammation, mediating eosinophil activation and migration to conjunctival epithelium [138].

Mast cells and *eosinophils* play a considerable role in ocular allergy (Fig. 14.6). In children's tears, both histamine and major basic protein (MBP) are present; ultramicroscopic studies have also documented mast cell degranulation [123]. Eosinophils are usually absent in conjunctival scrapings in healthy patients [5]; therefore *the presence of even only one cell is considered a diagnostic marker* in VKC and GPC, and in AC, where the absence of eosinophils on conjunctival scraping cannot rule out the diagnosis of AC because eosinophil infiltration may be deeper in conjunctival tissues [3], also the case if their numbers are lower because of intrinsic factors or environmental pollution [97].

In AKC the prominent IL_4, IL_8, and GM-CSF [84] are activated cells as proven by the presence of eosinophil cationic protein (ECP) also *in the tears* of patients with AC and VKC [66], significantly in the 17 children with AC but not in serum [138]. It is not the extent of eosinophil infiltration *per se* but the extent of eosinophil activation and the pattern of IL expression that is indicative of disease severity, as well as clinical variations such as involvement of the cornea [84]. Subjects with AKC and VKC had significantly higher tear ECP values than subjects with GPC and SAC, with a significant correlation between ECP values and disease severity in all disorders [131]. The release of IgE-induced eosinophil peroxidase (EPO) can be primed by incubating peripheral eosinophils of allergic patients along with specific allergens [66]. This process of activation/degranulation illustrates the concept of eosinophil releasability; furthermore tear MBP levels appear to be strictly associated with disease severity, especially in VKC and GPC [174]. Mast cells are not present in unaffected conjunctival epithelium [134], while in the bulbar and tarsal substantia propria either phenotype is found [134], 5% of T (tryptase) and 95% of TC (tryptase + chymase) [88]. TC mast cells are numerous in different types of conjunctivitis: in VKC epithelium they are as high as 96% and in GPC 100%, in the substantia propria they amount to 82% in VKC and to 98%–100% in others [88]. *Tear histamine* level (normal, 5 ng/ml) [9] varies on average between 3 and 4×16 ng/ml. The lowest levels are found in AC and in rigid-lens wearers; the highest levels are found in VKC. *Tryptase* concentrations (normal, ≤40 ng/ml) are high in tears, a marker of conjunctival mast-cell activation [66]. Mast cell activity is also well documented by histamine and tryptase high levels found in conjunctival epithelium, in AC and even more in VKC. This test is useful for diagnosis [73, 120]. Interesting contributions focus on a potential mast cell activation by *non-IgE-induced mechanisms* [17]. Approximately 50% of patients with VKC do not have a medical or family history of atopic diseases (FHA), and do not show IgE sensitization, suggesting that this disease is not solely IgE-mediated [25]. Moreover, it is possible an intervention of chemokine released by CPT stimulated lymphocytes and macrophages, not only by allergens but also by viruses, bacteria and unidentified antigens. These factors are equally capable of priming metachromatic cells to release mediators [17].

Late-Phase Reactions

Bonini et al [19] have provided the first evidence of a *conjunctival late-phase reaction* (LPR) in the eyes, although less accentuated than the immediate reaction, documented by *conjunctival eosinophilia 6 h after CPT.* Pertinent data on this LPR is summarized in Table 14.5 [18, 19, 109]. After allergen challenge, the conjunctival histological evaluation revealed a marked neutrophil influx, as was that of eosinophils and lymphocytes during the LPR (over 6–24 h), with a marked mediator release in tears collected after 20 min in patients affected by

Table 14.5. Features of the ocular LPRs

1. Persisting symptoms over 6 h (up to 24 h) after the immediate reaction
2. Continuous persistence and no biphasic aspect, or biphasic in 33%, multiphasic in 25% and monophasic in 41% of cases
3. Continuing recruitment of inflammatory cells, early neutrophil and lymphocyte accumulation and later increase of eosinophils, concomitant with symptoms
4. Mediators present in lacrimal fluid
5. Dose-dependence of clinical and histological changes (reduced with low allergen dose, higher with high allergen dose)

Data from [18, 19, 109].

conjunctivitis [18]. This pathogenic hypothesis correlates with experimental evidence from animal studies [109] and, indirectly, with an increase in vascular permeability after histamine challenge [41] and loratadine protective effects on CPT-induced LPR symptoms [42], extended by cetirizine to the immediate reaction [45]. Studies on IL involvement in the early and LPR of ocular allergy have given surprising results in knockout (KO) mice. IL_{12} KO mice and anti-IL_{12} antibody-treated wild-type animals failed to have a cellular infiltration into the conjunctiva. On the contrary, IFN-ɣ KO mice had a significantly stronger immediate hypersensitivity reaction and prolonged infiltration into the conjunctiva after ragweed challenge than control animals. The data suggest that the presence of IL_{12}, although predominantly implicated as a Th1-inducing IL, is important for the development and regulation of LPR pathological features in ocular allergy, whereas IFN-ɣ may have a protective effect on the LPR cellular infiltration of ocular allergy [121]. It is too early to reach definite conclusions from these studies, although the association of these two different ocular immune mechanisms (immediate and delayed hypersensitivity) is the most plausible hypothesis.

Conjunctival Hyperreactivity

An aspecific hyperreactivity to histamine recently identified in patients suffering from VKC may be helpful in characterizing some forms of VKC, and also in GPC, in which no elements of clinical sensitization were evidenced. An elegant study carried out on patients affected by VKC and asymptomatic AKC and in controls has reported this hyperreaction [20]. Injecting increasing histamine doses in one eye and normal saline in the other, widespread dose-dependent swelling appeared after a few minutes, but only in the eye treated with the mediator and more evident in patients than in controls; the quantity of histamine needed to elicit the reaction was considerably lower in VKC sufferers [20]. We suggest that this new pathogenic picture can explain symptom variability, which is not necessarily correlated with the allergen-induced changes. Equally, aspecific natural agents, such as the wind, dust and sunlight can contribute to an aspecific hypersensitivity.

Allergic Conjunctivitis

Definition

Although AC is the most common cause of ocular inflammations, ocular features deriving from immunological mechanisms have different characteristics in terms of severity and frequency of clinical manifestations. The causes can include IgE-induced as well as type III and IV reactions. The tissues involved in AC are summarized in Table 14.6 [30].

AC generally affects children and young adults and is the most widespread condition in ocular allergic pathologies, with a preponderance varying between 20% and 40%, with no distinction between males and females [16]. In a large sample, 32% of children presented ocular allergy as the only manifestation of atopy [189]. In our practice, children with AR (allergic rhinitis) also have AC in 80% of cases, dependent on sensitization to grasses. Inversely 12% of an unselected pediatric cohort were affected by AC. AC prevalence was 9.7%–10.1% in children aged 6–14, with no difference between highly or less polluted areas [182].

Table 14.6. Tissues involved in allergic conjunctivitis

	Tissues involved			
Conjunctivitis	Tarsal conjunctiva	Limbus	Cornea	Lid margin
Atopic keratoconjunctivitis	++	++	++	++
Giant papillary conjunctivitis	++	±	–	–
Perennial allergic conjunctivitis	+	–	–	–
Seasonal allergic conjunctivitis	+	–	–	–
Vernal keratoconjunctivitis	++	++	++	±

Modified from [30].

Classification

AC is caused by aeroallergens and is often seasonal (SAC) (pollens), but can also be a perennial condition (PAC) (dust mites, fungi, animal danders, rarely food), or present occasional peaks throughout the year depending on contact with the allergen (especially animal danders). In PAC exposure to Der p is far more frequent than SAC (42% vs 0%). The symptoms (conjunctival injection and itching) are more evident in SAC than in PAC [15]. Because of individual differences, some pollen-allergic patients report a prevalence of ocular symptoms, while the majority have a prevalence of nasal symptoms [35]. The association with AR is more frequent in PAC (72% vs 12%) [15]. In young adults, PAC is less common and does not depend on seasonal variations [15]. An association with ragweed is highly significant, but sensitization to fungal spores is diffused in 4.9%–9.9% of children with conjunctivitis: an aggravation that prompts these children to visit an emergency department (ED), where 8% were asthmatic. The risk could be sevenfold greater for fungal spores than for pollens [32]. Statistically significant risk factors for the development of pediatric AC may be FHA, negative antecedent of breast feeding, asthma or AR [33]. Secondhand tobacco smoke was present in 12.6%–36.2% of children's houses [182].

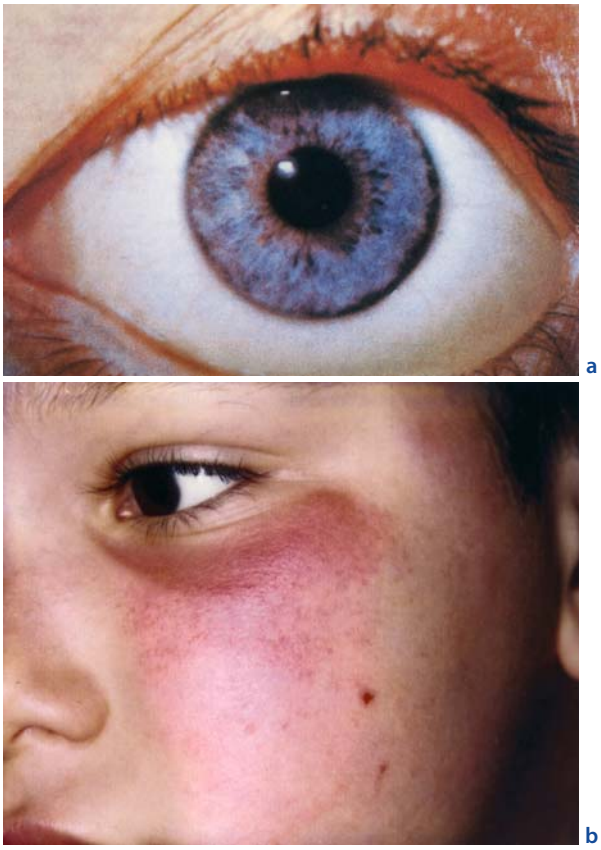


Fig. 14.7. **a** SAC in a child, with slight injection of the bulbar conjunctiva and **b** marked conjunctival edema. Despite the objectively mild manifestations, SAC may cause quite severe symptoms, with itching and profuse and persistent lacrimation, often affecting children's quality of life, especially girls

Pathogenesis

Since the eye is in direct contact with the outside environment, aeroallergens quickly melt in the tear film, provoking a type I immune reaction involving conjunctival mast cells [9] (Fig. 14.7a). In the tarsal conjunctiva mast cells can be found in increased numbers (15,380±2,830/mm^3 in the substantia propria = 100% of TC phenotype mast cells) [88] and widely degranulated, along with neutrophils and pollen granules [67]. Mast cell degranulation releases histamine, which can reach tear levels of >100 ng/ml [9], and other vasoactive mediators, preformed and newly formed, directly responsible for vasodilation and increased vascular permeability, with the addition of leukotrienes, kinin and PGD_2 and intensified symptoms [18]. The vasodilation causes conjunctival swelling, edema and pruritus (Fig. 14.6b), which are indistinguishable from the characteristic of the disease itself. Nasal irritation also causes conjunctival vasodilation that is transitory and without pruritus; lacrimation in turn can be stimulated in a reflected manner both by the conjunctival and the nasal mucosa. The *role played by the eosinophils* is remarkable: MBP levels are linked to disease severity [63]. These are found in the scrapings of 43% of patients with SAC and between 25% and 84% of those with PAC [15], as well as in tears together with ECP [138]. Recently the pathogenic role of the adhesive molecule CD106 [138] as well as the role of *chemokine eotaxin* has been emphasized [164]. IL_8 and the eosinophil chemoattractant RANTES (Tables 1.55, 1.56), are stimulated by TNF-α and IFN-γ in vitro, and upstream by NF-κB (nuclear factor of the κ chains of B cells) [74], which selectively recruit eosinophils via its chemotactic activity in IgE-mediated AC, also related to patient age and sex [90]. Moreover, IL_4-induced *eotaxin production in keratocytes* may play an important role in eosinophil recruitment to corneal ulcers in allergic ocular disease [75]. Following an experimental intraocular instillation of histamine, the allergic pathogenesis finds confirmation in the possible passive transportation of the disease and in the association with other allergic affections such as AR and asthma. AC is perhaps the only ocular pathology that can be considered an IgE-induced reaction [64].

Clinical Presentation

The clinical features are subject to even daily variations in relation to exposure to sensitizing substances. The objectivity is characteristic: red, swollen, itchy eyes and conjunctival edema (Fig. 14.7b). Itching is a distinctive

characteristic, also because there are very few ocular diseases linked to itching, which interferes with daily life and causes a great deal of inconvenience, as well as increased swelling and edema: without itching, a condition cannot be classified as ocular allergy [189]. This is caused not so much by the direct action of histamine as by a mechanical action of rubbing the eyes continuously, which frequently leads to increased susceptibility to infections and superinfections. Thus a *vicious circle* is created because the itching returns, acquiring greater drive [20]. Both eyes are involved, often asymmetrical in pollen allergies; unilateral in rare cases, for example when patients rub their eyes with hands to which animal hairs are stuck [18]. If the cause is pollens, the pruritus is mainly localized in the medial corner of the eye where the granules accumulate because of blinking [18]. Photophobia is absent and the mucus discharge scarce, and also microclimate variations causing symptom improvement when the weather is wet and cold and worsening in hot and dry climates [35].

Diagnosis

Diagnosis is based on positive results of a family and personal history. In children aged 6–14 years, FHA and personal history were positive in 58.9%–79.7% of cases [182], since the particular clinical symptoms are often negligible and *may go unnoticed.* Skin prick tests (SPTs) are positive in 67.7% of children: Der p in 52.9% and Der f in 47% of children [138], and if pollens are involved the botanical species are easily identifiable [57]. CPT is very useful: agreement between the two tests and the RAST (radio allergosorbent test) is positive in 71% of cases [110]. Eosinophils and sIgE are present in the serum in 59% of cases [138], and 89% of patients with PAC have sIgE for the Der p vs 43% of SAC sufferers [15]. In 50 children with SAR, SPTs were positive to Der p, Der f, Lol p, Atriplex bacteosa of the HDM group and pollens [33]. Thus PAC is more likely than SAC to be associated with AR, exposure to Der p is far more frequent compared to SAC (42% vs 0%) [19]. Additional triggers implicated in PAC that might elicit this reaction are animal dander and feathers [14]. Tear fluid presents high levels of total IgE, as well as sIgE to different aeroallergens [3], in 85% of patients with AC and in 10% of controls [86]. Tear IgE are mostly of local origin [85] and do not correlate with PRIST [17, 85, 86], for which in some cases there is no difference between allergic entrants and controls [86], while in others the correspondence was with tear IgE levels [175]. *Tear IgE levels* are reduced in normal cases (Table 14.3) [166], vs 0.25–25 IU/ml in allergic and therefore diagnostic [85], also stressing the difference between PAC and SAC regarding Der p: 78% vs 0% [15]. The *correlation of tear IgE and symptoms* is highly significant [86]. Tear eosinophil and ECP levels are diagnostic in children [138].

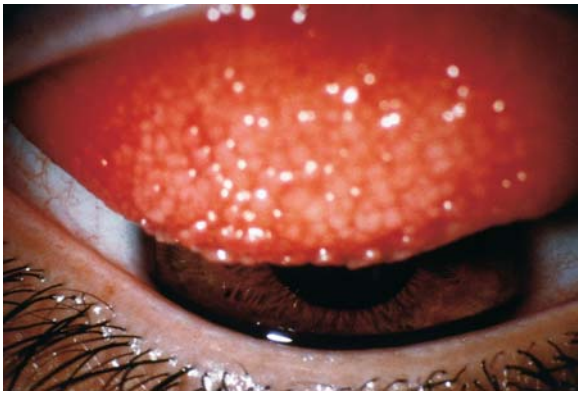


Fig. 14.8. Upper tarsal conjunctiva in a patient with SAC

The *ocular examination* takes place using a strong flashlight or a slit-lamp microscope that allows fine viewing of the smallest details that may be helpful to the doctor in making a diagnosis and following the AC course. The allergic conjunctiva appears inflamed and edematous: normally in tarsal conjunctiva moderate swelling and chemosis are observed (conjunctival edema), accompanied by small papillae (Fig. 14.8), symptoms that are less obvious in PAC [19]. Rather than intense redness and blood vessel prominence, there is a pinkish or milky coloring, caused by blood vessel vasodilation and chemosis; in acute and severe allergic reactions palpebral edema may also be observed [19]. Venous stasis produces bruising of the skin of the lower lids, called allergic shiners (Fig. 12.18). Unlike other forms of conjunctivitis, the cornea and the limbus are never affected [16].

In sum, the condition can be correctly diagnosed on the basis of five fundamental points:

- FHA and/or personal history
- Cause–effect relation with the pollen season in VKC
- Modest to intense pruritus
- Widespread conjunctival hyperemia from slight to modest
- Almost constant presence of sIgE

Differential Diagnosis

Differential diagnosis occurs with bacterial, viral or fungal infections (Gram stain for bacteria, and Giemsa stain for various organisms, bacterial and fungal culture). If a superinfection is present the bacterial culture may be positive (see Appendix 14.1 for examination of conjunctival discharges and their interpretation) [91].

Typical *bacterial conjunctivitis* is easily differentiated by the objectivity dominated by modest conjunctival swelling, chemosis and palpebral edema, the absence of pruritus, purulent mucus discharge and positive cultures (negative results do not exclude it). In bacterial

infections and in *Chlamydia* infections, leukocytes predominate, while lymphocytes are predominant in viral conjunctivitis (*Herpes simplex, Adenovirus*): the reaction is follicular in both cases. There is no evidence of IgE, nor of eosinophils [66]. In teenagers, sexually transmitted conjunctivitis should be ruled out [79].

Other causes must also be taken into consideration: irritation caused by toxic substances or *contact dermatitis* caused by drugs, cosmetics, and environmental pollutants, subpalpebral foreign body (left eyelid), *and the chemical conjunctivitis of neonates*.

Treatment

General Principles

No treatment is necessary if the disease is self-limiting and does not cause any particular discomfort. If necessary a conservative treatment can be applied for the first 3 or 4 days [13]. Treatment first of all must take the *hygienic aspects* into consideration. Primary treatment includes avoidance, cold compresses and lubrication. If the eyelids are encrusted with exudate or scaling, a bland liquid soap or also suitably diluted non-irritant pediatric fluid, applied using a tampon or a gauze can facilitate removal. Ocular washes are useful for mechanical allergen elimination, also using normal saline. Cold compresses reduce superficial vasodilation and provide notable relief from ocular pruritus [189].

Common eyewashes can be prescribed: medical eyewashes and decongestant/vasoconstrictors are useful aids if used in moderation. It is necessary to make the child understand the usefulness of eyewashes (young patients always have a negative reaction to the use of this form of treatment) instead of constantly rubbing their eyes: the eyewash must be used before rubbing, otherwise the drug will cause intense burning sensations caused by the preserving agents it contains [63].

Decongestants (vasoconstrictors) may be applied topically bid–qid as needed. We suggest oxymetazoline, which has a faster onset of action, longer duration of action, and a better decongestant effect than naphazoline and tetrahydrozoline.

Lubrication with artificial tears can be applied topically two to four times a day as needed (the solution should be refrigerated before application to improve symptomatic relief) [15].

Oral Medications

Cetirizine is used successfully for children with rhinoconjunctivitis [122], also for its capacity to modulate in vitro the eosinophil activity [158]. In 20 children with mite allergy, cetirizine was able to reduce nonspecific conjunctival hyperreactivity related to allergic inflammation compared to the placebo group [173]. We also prescribe levocetirizine to these children, with or without associated nedocromil eye drops, usually with optimal results.

Table 14.7. Topical treatment of pediatric allergic conjunctivitis

Topical medication	Daily dosing
Antihistamines	
Azelastine hydrochloride 0.05%	1 drop bid
Emedastine difumarate 0.05%	1 drop qid
Levocabastine hydrochloride 0.05%	1 drop bid qid
Antihistamine/mast cell stabilizers	
Ketotifen fumarate 0.025%	1 drop q8–q12 h
Olopatadine hydrochloride 0.1%	1 drop bid
Mast cell stabilizers	
Cromolyn sodium 4%	1–2 drops q4–q6 h
Lodoxamide tromethamine 0.1%	1–2 drops qid
Nedocromil sodium 2%	1–2 drops bid

Data as quoted in the text [191].

Eye Drops

Antihistamines in eye drops (Table 14.7 [191]) reduce pruritus locally and are usually associated with a vasoconstrictor to reduce hyperemia; by mouth they work after 20 min and the therapeutic effect persists, depending on patients, for 4–12 h. Antihistamines in eye drops are azelastine, emedastine and levocabastine, which reconfirm the properties illustrated in Chap. 12.

Azelastine eye drops in 20 children with mite allergy showed a significant reduction in nonspecific conjunctival hyperreactivity compared to the placebo group [46]. In 113 children aged 4–12 suffering from SAC or rhinoconjunctivitis, the mean response rate was significantly higher than in placebo children and comparable with levocabastine-treated children. Mainly local irritant effects were reported in 23% of placebo-treated, 35% of azelastine-treated and 38% of levocabastine-treated children [152].

Emedastine 0.05% and levocabastine eye drops are extremely selective H_1-receptor antagonists that are excellent for ocular pruritus. Emedastine administered twice daily is more effective than levocabastine 0.05% eye drops in the prevention and treatment of the signs and symptoms of AC in children ≥4 years. Both medications were well tolerated [185]. In other pediatric entrants, results confirm the potent and long-acting efficacy of emedastine, which was significantly superior to levocabastine for the relief of redness, itching, eyelid swelling, and physician's impression score [157]. Emedastine effectively decreased the extent of itching of

the eye and conjunctival hyperemia in 20 children; its effects were felt within minutes [62].

Levocabastine (0.5 mg/ml) can be used twice a day [186]; not associated with vasoconstrictors [4] it is effective for reducing hyperemia and pruritus in children aged 3–16, as confirmed on a total of 279 children [104, 130, 137, 150, 186, 193], proving to be effective in reducing pruritus, eye lacrimation and hyperemia, with good to excellent results in 83%–85% of cases. Its effectiveness was comparable [130, 137, 193], or better than that of cromolyn and placebo in CPTs [150] after 2 weeks, but matching after 4 weeks [186]. Levocabastine has a rapid onset of action in children with SAR and the topical application of medication does not irritate the conjunctival mucosa and nasal cavities [104]. In adults it proved to be significantly better than placebo [4] and cromolyn [7], especially for ocular pruritus, eye lacrimation and more severe eye symptoms [162].

Topical mast cell stabilizers include cromolyn and nedocromil, lodoxamide, and pemirolast. Ketotifen and olopatadine have H_1-receptor blocking activity as well as mast cell-stabilizing activity. These eye drops are suited for children with moderate to severe allergic eye conditions that in the past only steroids would help [79].

Cromolyn 4% ophthalmic solution in eyewash is particularly effective because of the high number of T mast cells. Well known as a membrane stabilizer and significantly more effective than placebo, the drops should be used three or four times a day. Cromolyn is helpful as a preventive measure for long periods, as there are no side effects [93]; some patients fare better immediately, others after 2 [107] or 6 weeks [93]. We regularly prescribe cromolyn eye drops for preventive purposes in children with eye allergy, as in those with AR (Chap. 12).

Nedocromil sodium is equally valid, in 2% eye drops [106, 107, 124]. In the first pediatric trial [130], it was statistically better than placebo for pruritus, eye tearing and the total score of symptoms (p=0.0097), with the additional advantage of limiting its use to twice a day. In experiments its effectiveness appeared to be closely linked to its capacity to reduce IgE levels, eosinophil infiltration, and mast cell numbers, also in degranulation and the subsequent IL release [125]. In adults with ocular allergies, headaches are reported and ocular burning, while in 7.4% of children [130], the effects were only light and passing.

Ketotifen (KF) fumarate has a dual mechanism, being a histamine H_1-receptor antagonist and mast cell stabilizer, as well as inhibiting eosinophil chemotaxis and activation and adhesion molecule [2]. In a double-masked, multicenter, placebo-controlled, CPT trial 133 children aged 8–16 with AC received KF 0.025% ophthalmic solution bid for 4 weeks. KF significantly inhibited ocular itching compared with placebo (p<0.001) and also significantly reduced hyperemia, chemosis, and lid swelling (p=0.031) in 79% of children. No KF-related systemic adverse events were reported, and ocular adverse events were comparable to placebo.

Lodoxamide at 0.1% concentration is a more potent mast cell stabilizer than cromolyn sodium in the prevention of histamine release, to be used qid, also for children aged <2 [165]. It is ≈2,500-fold more potent than cromolyn in the prevention of histamine release [15], and is effective in reducing tryptase levels and inflammatory cell recruitment (clusters of neutrophils, eosinophils and epithelial cells) in tear fluids after CPT [23].

Olopatadine is a recently introduced ophthalmic NSAID with mast cell stabilizing and antipruritic effects and has mast-cell stabilizing and antihistamine properties [79]. The 0.19% ophthalmic solution bid had a significantly greater effect on the ocular signs and symptoms compared with cromolyn 2% ophthalmic solution qid, but olopatadine had better local tolerability in children aged <11 [100]. Olopatadine relieves ocular itching and has a half-life of about 3 h. The dosage is one or two drops in each eye bid in children as young as 3 years [191].

Pemirolast potassium 0.1% is a more potent mast cell stabilizer than cromolyn sodium in the prevention of histamine release, inhibits eosinophil activation and chemotaxis [79]. It is effective and safe in preventing ocular itching in patients with AC during the allergy season [1].

Topical corticosteroids (CSs) are highly effective in the symptomatic treatment of acute and chronic forms of AC and are required for control of some of the more severe variants of conjunctivitis, including AKC, VKC and GPC [15]. CSs must be used carefully [37], because they can worsen pre-existing affections [123], foster glaucoma and cataract development if chronically used [13], and mask ocular infections [189]. However short cycles as needed have proved to be without undesirable side effects [4, 15].

Specific immunotherapy (SIT) proved to be effective in forms caused by grass pollens (Fig. 13.3) and *Cladosporium*, in CPT-tested children [56], but SIT is not effective in PAC [92].

Vernal Keratoconjunctivitis

Definition

VKC is characterized by a bilateral, chronic and recurrent conjunctival inflammation, but can also persist over both summer and fall, especially in warm and dry climates worldwide [117]. VKC is a Th2 lymphocyte-driven chronic inflammatory and potentially blinding disease [25], and a challenge for ophthalmologists since the pathogenesis is unclear and antiallergic therapy often unsuccessful [108]. This is because VKC is a chronic disease that requires prolonged treatment to control the inflammatory process [19]. Palpebral and limbal VKC are considered to be expressions of the same disease and only occasional histopathological differences are described between the two forms [148].

Epidemiological and Genetic Aspects

VKC mainly affects children and adolescents (82% <10 years old) with an average age at onset of about 8 years, rarely after 20–25 years [29, 117]. VKC burns out spontaneously in most cases after 5–10 years and vanishes after the age of 30 [10]. Otherwise 60% of patients are between the ages of 11 and 20 and of 0.1%–1% of cases experience other ocular pathologies [15]. The mean age at onset was 7.83 years and 10.94 years at presentation [28]. In 110 children, the mean age was 8.3 years, range 3.2–18 [148]. In 10 children aged 9.12±2.45, the mean duration of disease before the enrollment was 3.96±1.96 years [187]. VKC is common in some Mediterranean countries, the Middle East, the Far East, and South America, where VKC is perennial and the association with atopy is less prevalent [117]. Children are males more often than females (85% vs 15%); 75% have a personal history of atopic disease, and 67% a FHA [28].

Pathogenesis

VKC is a Th2 lymphocyte-mediated disease [108]. $CD4^+$ T cells are found abundantly in conjunctival scrapings and biopsy specimens [108]. These $CD4^+$ cells had been cloned and were demonstrated to exhibit Th2 phenotypes [119]. Plasma cell, neutrophil, T-lymphocyte proliferation, and especially *eosinophil and mast cell* proliferation is evident in the conjunctival stroma (16,000/mm^3 of epithelium) (Fig. 14.9). CD4 clones from conjunctival infiltrations express Th2-like ILs, with a subsequent massive migration of lymphocytes to the conjunctival epithelium (Fig. 14.10). $CD4^+$ T cells are found abundantly in conjunctival scrapings and biopsy specimens [119]. These $CD4^+$ cells had been cloned and demonstrated to exhibit Th2 phenotypes that produce IL_4 [119], IL_3, IL_5 and IL_{13} when compared to normal tissue [17, 108]. Expression of $IL_1\beta$, IL_1 Rα, IL_4–IL_6, IL_8, TGF-β1, TGF-β2, TNF-α and INF-γ were positive in 3–6 cases out of 6. IL_2 and TNF-β were negative in all patients, thus proving that the Th2 ILs play more important roles than the Th1 ILs in VKC inflammation [133], T lymphocytes in VKC are activated and express several activation markers that might contribute to the pathogenesis of VKC, including *potent T-lymphocyte chemoattractants* such as PARC (pulmonary and activation regulated chemokine), with numbers higher than macrophage-derived chemokine (MDC) and I-309 [60]. The numbers of $CD25^+$ T lymphocytes were higher than the numbers of $CD26^+$, $CD62L^+$, $CD71^+$, and $CD30^+$ T lymphocytes [60]. The chemokine receptor CXCR3 has a predominant role in the regulation of lymphocyte recruitment within the conjunctiva of VKC patients [59]. Eosinophil chemoattractants such as RANTES [73] and eotaxins [116] are constitutively expressed. Tear eotaxin-1 and eotaxin-2,

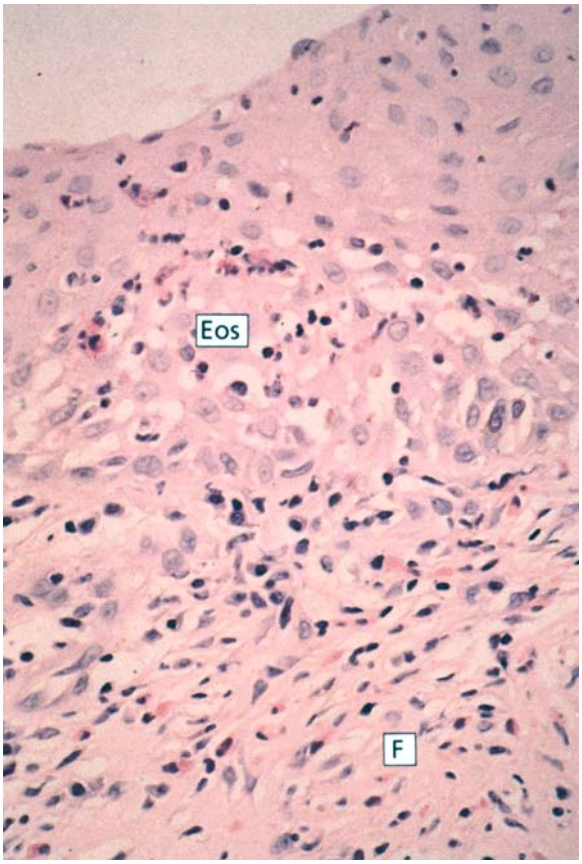


Fig. 14.9. Eosinophil (*Eos*) and mast cell infiltration in epithelium and increased fibroblast (*F*) activity in submucosa of a patient with VKC

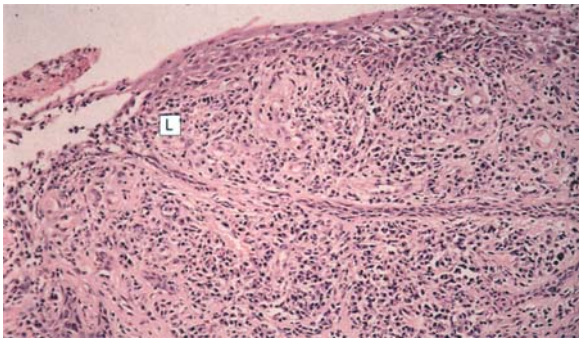


Fig. 14.10. Massive lymphoid infiltration (*L*) in a patient with VKC

potent eosinophil chemotactic and activating peptides, *correlated significantly with the eosinophil recruitment* in VKC and AKC patients compared with tears of normal patients [116]. A significant tear up-regulation of substance P (SP) a well-known neurotransmitter, was shown in patients with SAC and VKC compared to healthy controls and AD patients [72]. In concert with other changes, lacrimal CD54 levels appear to be higher in the severe limbal forms than in the palpebral forms

[48]. Eosinophils, serum IgE, ECP, and EDN are significantly higher in VKC than in control subjects [113]. Serum *ECP is a useful marker* of disease activity in tarsal and mixed forms [148]. Mast cells are mainly found in the substantia propria (24,689±7,748/mm^3, 82% of TCs), but also in the epithelium (7,994±2,120/mm^3, 96% of TCs) [88], 80% in degranulation [29]. There are high tear levels of IL_4 [71], total IgE and sIgE for aeroallergens, histamine and other vasoactive mediators, as well as MBP, Charcot-Leyden crystals, basophils and eosinophils (90% of cases) [180]. Therefore Th2 cells with IL_4 foster IgE production, mast cell and B lymphocyte accumulation and eosinophil activation [17]. The widespread polyclonal IgE activation even in the absence of IgE antibodies to common allergens have led to hypothesizing an IgE down-regulation, associated with up-regulation of the ILs of chromosome *5q*, which through its products (IL_3, IL_4, IL_5 and GMF (granulocyte/macrophage-colony-stimulating factor) regulates Th2 prevalence, IgE production and mast cell and eosinophil growth [22], thus suggesting that VKC may be *a Th2 rather than an IgE-induced form* [22]. Thus, IgE- and non-IgE-mediated immune mechanisms may coexist in the conjunctiva of VKC patients. Although high IgE antibody levels are found in tears, these IgE may stem from the local production by degranulated mast cells, and by low levels of plasma cells [14]. However, 57.8% of patients had a positive SPT and 52.2% had a positive RAST for common allergens [24]. On the one hand, interactions between specific (IgE- and Th2-mediated) and nonspecific triggers and mechanisms [108], and the percentage of Th2 lymphocytes are significantly correlated with the severity of the disease [113]. On the other hand, an overall eosinophilic response is present in VKC independently of IgE-sensitization. Tarsal and mixed forms, unlike bulbar forms, were associated with significantly high levels of total IgE: 10/16 VKC subjects showed evidence of IgE-sensitization [148]. Moreover, the amount of messenger RNA encoding ILs and inflammatory cell markers in VKC did not correlate with IgE-sensitization. [132].

Eosinophil levels, significantly high compared to controls, are present both in IgE- and non-IgE-mediated forms [17], regardless of positive or negative SPTs and RAST [22], and patients have tear and serum ECP levels [111] and tear MBP [111, 180] in the conjunctiva as well [174]. Tear histamine levels are fourfold higher than the levels found in GPC patients [3]. Recently high tryptase levels [120] have emphasized even more the pathogenic role played by mast cells. C3, C3a and high eosinophil levels (90% of cases) and plasma cells secreting IgA and IgE antibodies (with a 2:1 ratio) are characteristic of active stages; moreover, basophils, microvascular alterations of epithelial cells and deposits of fibrin are also found that prove the increased permeability [64, 111, 117]. Vast deposits of collagen drive the thickening of both conjunctival epithelium and stroma. Association with the hyper-IgE syndrome, characterized among other elements by a severe deficit of CD8 lymphocytes indicates a poor involvement of these lymphocytes in VKC etiopathogenesis [29]. In addition, histocytological examination demonstrated SP-positive cells in the conjunctiva of patients with VKC, but not in normal controls, thus suggesting that SP-increased tear levels may contribute to VKC pathogenesis and severity [72]. Structural cells such as epithelial cells and fibroblasts are involved both in the inflammatory process and in the tissue remodeling phase, eventually resulting in the formation of giant papillae [108]. Recently, the expression of toll-like receptors (TLRs) was investigated in healthy and active VKC conjunctiva in 9 patients with VKC and 10 healthy subjects. In VKC conjunctival epithelium and stroma, TLR-4 was up-regulated, TLR-9 was down-regulated, and TLR-2 was slightly decreased relative to normal tissues. TLR-4 was localized on CD4$^+$ lymphocytes, eosinophils, and mast cells, whereas lymphocytes and eosinophils, but not mast cells, expressed TLR-9. Thus the TLR up-regulated or down-regulated presence on inflammatory cells suggest their role in VKC [26].

Clinical Presentation

Children complain of conjunctival reddening, intense pruritus, tearing, abundant discharge of yellow-white dense and filamentous mucus, photophobia, burning and the annoying sensation of a foreign body [57] (Fig. 14.11). The intense and at times exasperating pruritus exacerbated by exposure to wind, dust, bright light, and hot weather [5] is the fundamental symptom, to the extent that its absence makes diagnosis difficult, the continuous eyelid rubbing while attempting to find relief amplifies hyperemia and burning [16]. Pruritus gets

Fig. 14.11. Photophobia is a common discomfort in a girl with VKC, especially on waking in the morning. She needs to wear a *hat with a brim* and spectacles with photochromic lenses

worse in the evening and also if exposed physical activities associated to hypertranspiration in addition to atmospheric disturbances [5]. In the mornings, patients are frequently totally unable to open their eyes [117].

Diagnosis

Allergic involvement in child VKC can only be assessed through a detailed evaluation, leading to specialized ophthalmic and allergic management [48]. Of the 10 children, 9 had concomitant AR and 2 asthma [187]. At the examination, conjunctival injection, epiphora, little mucus discharge, palpebral edema are seen, and in more severe cases also pseudogerontoxon and pseudoblepharoptosis (Fig. 14.12). Biomicroscopic examination emphasizes a very hyperemic conjunctiva, with a milky fibrinous discharge and many papillae at the lower tarsal level [16].

Two forms can be identified that depend on which conjunctival portion is mainly affected [16]:

- *Palpebral form* affecting the eyelid. One observes the giant papillae, reaching 7–8 mm in diameter, occurring at the upper tarsal plate with a polygonal shape and flat surface that are typically described as Roman cobblestones (Fig. 14.13), consisting of dense fibrous tissue with epithelium thickening and eosinophil infiltration, that by their weight can cause a mechanical type of ptosis; these cobblestones become extremely swollen during the active phase, commonly in the spring, and persisting during the quiescent phase of the disease [15].
- *Limbal form* at the upper sclerocorneal junction (the point of transition from corneal to conjunctival epithelium). *Trantas dots* can sometimes be observed (Fig. 14.14): gelatinous whitish or pale pink/grayish dots, collections of neutrophils and eosinophils, persisting for 2–7 days, more often seen in VKC and in black patients [3].

In the initial stages, the typical symptoms are not accompanied by giant papillae (acute forms). A biopsy, however, reveals these typical lesions [5].

In more severe cases, especially when the upper eyelid is everted and exposed to heat, pseudo-membranes (Maxwell-Lyon sign) punctate epithelial keratitis, and corneal lesions such as superficial ulcers, often close to the limbal papillae, may be seen. Keratitis is frequent, perhaps linked to MBP release, involves the upper half of the cornea and induces a reduction in visual acuity: the cornea becomes covered with a solid layer of mucus, fibrin and epithelial cells. Should the ulcer persist, with time the lesion will form scar tissue, neovascularization and a subsequent reduction in visual acuity up to 20/200 [5].

About two-thirds of patients display SPTs positive for aeroallergens, normally for pollens [63], and 50% have positive sIgE to more allergens [22]. SPTs were positive in 9/10 children (Der p 7, acacia 2, cat 1, cockroach

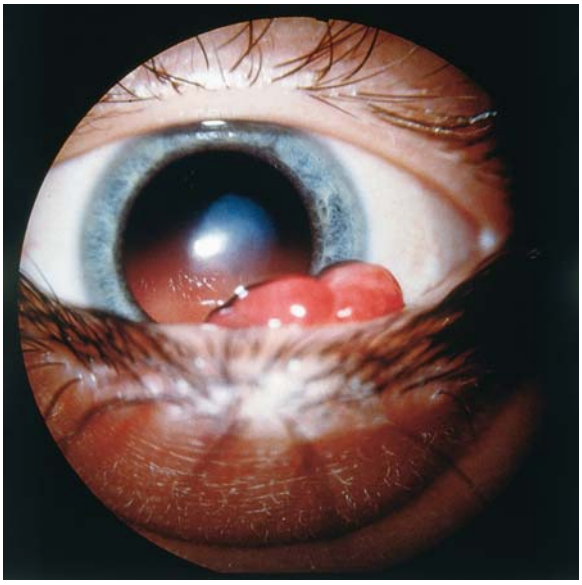


Fig. 14.12. VKC in active phase

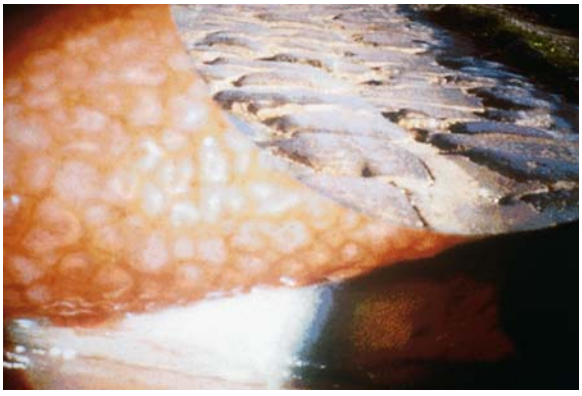


Fig. 14.13. Upper tarsal conjunctiva in VKC showing giant papillae

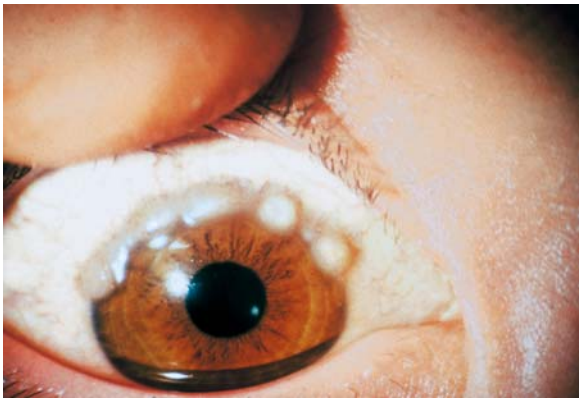


Fig. 14.14. Trantas dots at the limbus in VKC

1, and grass pollen 1) [187]. Eosinophils are diagnostic as mentioned above, since they are absent in normal eyes [5]. Serum and tear IgE levels are high. In acute stages tear tryptase levels are also high (14.5±13 µg/l), but not in late reactions [120]. For the examination of conjunctival discharges see Appendix 14.1.

The study of conjunctival hyperreactivity to histamine is useful for assessing either the pathogenesis of a number of clinical aspects or VKC outcome, and the hypersensitivity to natural agents observed in these patients [20].

Differential Diagnosis

For a differential diagnosis with atopic keratoconjunctivitis (AKC) and GPC, see Tables 14.8 [5] and 14.9 [3, 4, 31], respectively. Note that phenotype T mast cells are found only in VKC, in the epithelium and in the substantia propria (4% and 18%, respectively) [88]. Serum CD106 can be used as a marker to differentiate VKC from nonproliferative ocular allergic diseases [178].

Table 14.8. Differential diagnosis of allergic conjunctivitis (*AC*), atopic keratoconjunctivitis (*AKC*) and vernal keratoconjunctivitis (*VKC*)

	AC	AKC	VKC
Seasonal variation	+	±	+
Age involved	All	10–20	3–25
Long course	±	+	+
Itching	+	++	+++
Discharge	–	+	++
Predominant involvement			
Upper eyelid	–	+	
Lower eyelid	–	+	–
Corneal involvement	–	+	±
Eosinophils	+	+	++
Eyelid atopic dermatitis	–	+	–
Papillary hypertrophy	±	+	++

Modified from [5].

Table 14.9. Differential diagnosis of vernal keratoconjunctivitis (*VKC*) and giant papillary conjunctivitis (*GPC*)

	GPC	VKC
Seasonal variation	±	±
Discharge	++	+
Pruritus	++	++
Cobblestoning	++	++
Eosinophils/basophils/mast cells		
Conjunctival epithelium	±	+
Substantia propria	±	+
Tear MBP levels	±	+
Mean lacrimal histamine levels (ng/ml)	2–7	16
Lacrimal tryptase levels	+++	–

Data from [3, 5, 31].
MBP major basic protein.

Treatment

A specific treatment should be established in children, possibly based on allergen eviction [48]. Treatment includes cold compresses, natural tears, avoidance of specific/nonspecific triggers, and application of topical ophthalmic preparations ranging from antihistamines to mast cell stabilizers [14]. Some children may fail to respond satisfactorily to sufficient trials of conventional VKC treatment [197]. Considering VKC's good prognosis and the spontaneous recovery within 5–10 years in most patients, treatment must be conservative and aimed at alleviating subjective symptoms, without the development of a reduction of visual acuity because of the disease or the treatment. Children should live, study, work or sleep in fresh or air-conditioned environments: a cold, humid climate is more suitable than a hot, dry one. Ideally, treatment of VKC should be targeted toward reduction of Th2 activity [25].

Montelukast treatment significantly decreased physician-rated hyperemia, secretion, and chemosis as well as patient-rated burning, tearing, photophobia, secretion, and redness. Effects persisted 15 days after discontinuation of treatment. Clinical changes were associated with a significant increase in LTB_4 in tears and a significant decrease in LTE_4 in urine after 15 days of treatment [105].

Cromolyn led to full control in 19% and to partial control in 65% of patients [112] and is as effective as nedocromil sodium; another mast-cell stabilizer is ketotifen [61].

Antihistamines are also indicated [19] but are used only with children with a positive allergic evaluation [48]. *Lodoxamide* 0.1% significantly reduces ECP tear levels and thus eosinophil activation, and is more effective than cromolyn in reducing clinical signs and symptoms [6, 112]. In 15 children, both symptom scores and clinical signs were significantly lower than in controls. *Loratadine* had a greater effect on the $CD4^+$ cells, thus directed against the $CD4^+$ cells, which play a pivotal role in the pathogenesis of VKC [6]. Lodoxamide and *levocabastine* 0.05% eye drops, instilled four times daily for 3 months, were effective, safe and well tolerated by patients with VKC, but lodoxamide was significantly superior to levocabastine [184]. The drug can also be used in children <2 years, and is more useful in VKC, AKC, and GPC [165].

We have prescribed *cetirizine* drops and *levocetirizine* tables to children with rhinoconjunctivitis with a high clinical effect. Among the NSAIDs, the preservative-free *diclofenac sodium* in 0.1% eye drops has demonstrated its efficacy and safety in controlling the signs and symptoms of VKC in prolonged treatment [49].

Topical CSs such as the above-mentioned fluorometholone are to be used in resistant cases, but for *short periods* the potential undesirable effects can be reduced by associating the drug with chromones, with a parallel dose reduction [68]. CS therapy may cause ocular hypertension or open-angle glaucoma [28, 51], as in a young girl who presented with raised intraocular pressure and headaches due to the prolonged administration of nasal and inhaled CSs [51]. Therefore, CSs should be doctor-prescribed, to verify localized effects.

Cyclosporine 2% eye drops were effective and safe for treating 25 children with severe VKC, without causing major side effects After 2 weeks, most of the therapeutic effect was achieved and was maintained for the next 3 months [147]. Ten children with VKC were treated with cyclosporine, lodoxamide, olopatadine and emedastine [187].

SIT can be used successfully in a certain percentage of patients [48, 68]. Excision of the giant papillae is not advised [29].

Atopic Keratoconjunctivitis

Definition

More frequent in males, appearing <3 years and in the late teens, AKC prevalence among ocular pathologies varies between 2.5% and 10% [16]. It is the ocular counterpart of AD. AKC is associated with a 95% prevalence of concomitant AD and an 87% prevalence of asthma, and occurs in 20%–40% of individuals with AD. This condition is more prevalent in men than in women [37]. Like VKC, AKC is characterized by pruritus, tearing and a more chronic course than PAC, which intensifies during the winter. Complications may appear with structural eyelid changes. It is a potentially severe form in which secondary infections and corneal vascularization are a threat for eyesight integrity [3].

Pathogenesis

AKC histopathologic findings are diagnostically specific. They include degranulating conjunctival mast cells with the release of vasoactive mediators, and a marked papillary infiltration of monocytes, eosinophils and lymphocytes in conjunctival scrapings. T cells (CD3, CD5) and activated Th1 cells (CD25), CD4, macrophages with CD11b/CD18 and CD14, and $CD1^+$ cells are found in the epithelium; in the substantia propria there is a greater presence of inflammatory cells, as well as CD8 T cells and B lymphocytes (CD22) [64]. Histologically, epithelial hyperplasia can be observed with an increase in calyciform cells, and inflammatory cell infiltration in the limbus, especially eosinophils, with subsequent vascularization. It has been recently reported that eosinophil-released ILs in delayed stages, and also in VKC, attack the ocular cells, later causing corneal involvement [195]. Consequently, along with IgE-induced mechanisms (elevated serum and tear IgE concentrations), changes to cell-mediated immunity (CMI) are observed [5]. The immune pathogenesis is confirmed by frequently positive FHA and the presence of other allergic disease in addition to AD (asthma, urticaria, and rhinitis).

Clinical Presentation

Only a few patients with AD present bilateral eye symptoms, including evident hyperemia, intense itching, burning, tearing, watery or purulent mucus discharge, the feeling of the presence of a foreign body, photophobia and eczematous lesions on the eyelids, sometimes exuding such as the basic disease [57]. Ocular symptoms present with little or no seasonal variation (as opposed to VKC, which is seen mostly in warm weather). In addition to edema, lids may show thickening and tylosis, crusting, fissures and ptosis [37]. The conjunctiva may show small or medium-sized papillae, hyperemia, edema, and excessive mucin [5]. Children complain more about the *intolerable external aspect* that makes the eyes so red due to palpebral edge involvement than about itching. Scratch marks are noted around the eyes, the forehead and the cheeks. There is often canthi maceration because of continuous hypertearing: inflammation can distance the eyelids from the optical globe to the point that the lacrimal point no longer corresponds to the lacrimal lake; consequently the tears do not drain through the nose and fall outside [5]. Both eyelashes and palpebral edges often exhibit chronic blepharitis (Fig. 14.15), often induced by *Staphylococcus aureus* [63].

Diagnosis

Ocular examination shows conjunctival inflammation, often associated with upper tarsal conjunctiva papillary hypertrophy (Fig. 14.16). Sometimes giant papillae may form, caused by the infiltration of inflammatory cells and the accompanying edema. The eyelids appear reddened and bruised with palpebral margin hardening and lichenification. Both conjunctiva and cornea may develop scarring in the later AKC stages. With more active symptoms, Trantas dots can be observed around the upper limbus, all the more common in VKC [176]. Conjunctival biopsy specimens reveal excessive eosinophils, mast cells and goblet cells [37].

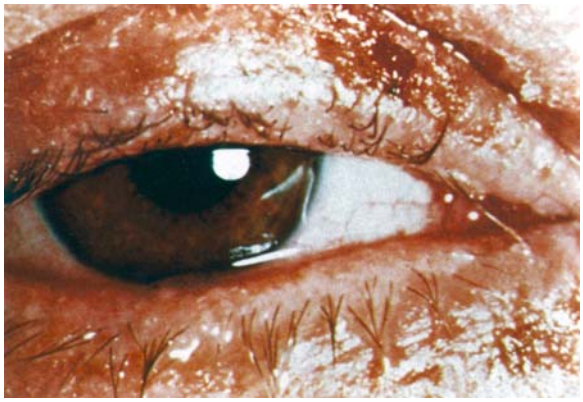


Fig. 14.15. Blepharitis complicating AKC in a child; the risk of corneal involvement is great. Therefore the infection should be treated urgently

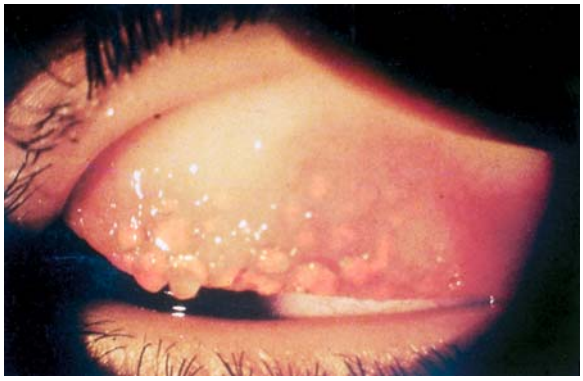


Fig. 14.16. Appearance of upper tarsal conjunctiva in AKC

SPTs are mainly positive to food, pollens, etc., but the meaning of these findings is as yet unclear. Total IgE levels are usually increased and eosinophils are frequently recognized [14].

Differential Diagnosis

Differentiation from AC is quite simple: in these patients the eyes are normal during intercritical periods, while in those with AKC the eyelids are thicker and harder, with scaling skin and scratch marks. Furthermore, there is the potential corneal involvement. Table 14.7 summarizes differential diagnosis with VKC.

Complications

Unlike patients with AC, progressive keratitis punctata with proliferation of the limbic vessels around the cornea may develop. If keratitis and neovascularization progress up to involving the visual axis, *eyesight is reduced and patients may go blind* [37]. About 10% of patients present opacity of the crystalline lens [5]: because of AKC persistence and excessive use of topical CSs, *a cataract* may appear extending to most of the anterior crystalline lens; the opacification can become complete within 6 months. Should it progress to prejudicing the eyesight, its extraction becomes necessary. Should a keratoconus also form, the cornea is highly astigmatic [181]. Correction of eyesight defects includes contact lenses: conservative treatment is insufficient over the long term in avoiding a cornea transplant. It is thought that the keratoconus is caused by the continuous eye rubbing because of itching and also perhaps by a congenital defect in collagen synthesis [176].

Treatment

Rinsing the palpebral rims twice a day with a saline solution and if necessary the application of antibiotic cream make up the usual treatment. Antibiotics will sometimes have to be prescribed systemically. Massaging the eyelids is useful, especially the lower one, which can be pulled externally to re-establish the connection between the lacrimal point and lake [5]. Often the severe clinical features require the use of topical CSs. Complications are seen more rarely with fluorometholone and rimexolone than with other CSs [15]. Some patients find cromolyn helpful, and it is certainly advisable for continuing treatment [3]. In the event of a relapse, the above-mentioned treatment should be continued for a long period of time [3]. Topical 2% cyclosporine has proven to be effective for exacerbations. Low-dose maintenance therapy (5 mg/kg q4d) may be required in refractory cases [37].

Giant Papillary Conjunctivitis

Definition

Originally described in contact lens wearers, both rigid and soft, more often with the soft lenses and in young females, GPC was found in patients with ocular prostheses, exposed corneoscleral sutures following surgery to the upper limbus, band keratopathy, etc. Probably these stimuli and/or the lens edges have a chronic microtraumatic effect on the upper tarsal conjunctiva. Between 10% and 15% of patients using soft lenses, and between 1% and 5% of those using hard ones were found to suffer from GPC [67, 145]. GPC is less serious than VKC or AKC, as corneal surface disease does not occur [127].

Pathogenesis

Although a great deal of data points to the coparticipation of an irritative mechanism activated by foreign bodies present on the outer ocular surface, associated

with the use of contact lenses, the current hypothesis most supported by data proposes a unifying immunological pathogenic mechanism underlying two different subtypes of a single category of conjunctival changes: VKC and GPC [29]. GPC has a risk factor that is fivefold higher than in healthy patients. FHA is frequently positive (67% of cases) as well as the presence of other atopic disease (AD, asthma, AR) in 75% of patients [29].

The immune pathogenesis is associated with markedly increased cells expressing HLA-DR antigen and LC infiltration [39] and with the presence of conjunctival infiltration, a mixture of plasma cells, mast cells and eosinophils, with participating basophils and lymphocytes [99]. The infiltration of eosinophils, neutrophils and granules is most prominent, with a lesser infiltration of mast cells [39]. Histologically, mast cells, basophils, eosinophils, neutrophils and a few lymphocytes are seen invading the conjunctival epithelium rich in papillae; the stroma shows only basophil and eosinophil infiltration, lower, however, than in VKC [53]. Mast cells are 17,313±3,801/mm^3 in the substantia propria and 3,814±2,052/mm^3 in the epithelium (100% of TCs): in asymptomatic patients, mast cells are only found in the first tissue, while in symptomatic patients or those with VKC, mast cells are found in both tissues [88]. Tear tryptase levels have provided further credit to GPC immune pathogenesis [31], proving that these might be an early diagnostic marker [73, 120]. Eosinophil infiltration in the conjunctiva, MBP and other cationic proteins are linked to the severity of clinical features and the presence of typical symptoms in young atopic people with SAR and who wear contact lenses [98]. Locally produced IgG, IgM and IgE antibodies are found in tears, but are absent in asymptomatic patients [53]. Excessive collagen formation leads to the flattening and movement of the micropapillae, resulting in conjunctival ulceration. Furthermore, contact lenses, like any foreign body, work as an abrasive surface that causes repeated trauma to the upper tarsal conjunctiva: the immune sensitivity of a traumatized conjunctiva is raised, allowing and promoting environmental antigens entering the conjunctiva, especially bacteria, which are normally avoided [53]. Reduced levels of lactoferrin in patient tears inhibited the formation of converted C3 (which splits the C3 into C3a and C3b) and therefore complement activation in the classic pathway [10]. In other studies these levels were increased [11, 53].

The condition is often set off or intensified by one of more of the following variables [5, 38, 54]:

- The use of thimerosal (Chap. 8) in fluids used for cleaning lenses (Fig. 14.17)
- The material accumulating on the lenses, especially if protein-like
- Edge design
- Surface properties
- Fitting characteristics
- Excessive lens use
- Individual sensitivity to a certain kind of lens

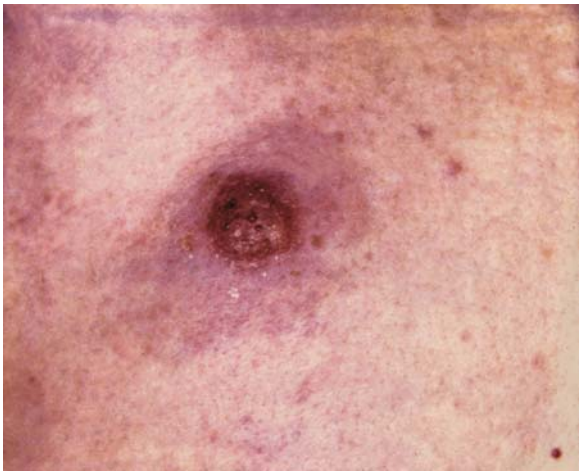


Fig. 14.17. Thimerosal hypersensitivity with vascularization of upper cornea

- Larger lenses providing a larger adhesive area for antigenic matter
- Genetic predisposition to an antigen linked to the lenses used

Other incriminated substances in addition to thimerosal in the solutions include sorbic acid, papain, benzethonium chloride, Hg phenylnitrated, etc.

Clinical Presentation

The symptoms are classic: pruritus especially after removing lenses, pain, burning, tearing, mucus discharge on awakening and the sensation of a foreign body.

Two more common forms of GPC are a localized form in which the papillae are confined to one or two areas of the tarsal conjunctiva, near the lid margin, and a generalized form similar to that seen with conventional soft contact lenses [54].

GPC course is divided into four stages, but individual variation is very common [5]:

- *Stages I and II* (preclinical, mild): symptoms are modest and can go unnoticed. If the lenses continue to be used, one enters the next stage.
- *Stage III* (moderate) consisting in abundant mucus formation and increased itching, to the extent that lenses cause intense annoyance because patients find it difficult to keep the lenses clean and they feel the sensation of a foreign body. Continuous lens movement on each blink results in fluctuating and blurred vision.
- *Stage IV* (advanced): the lenses are no longer tolerated, the mucus discharge increases to the point of covering the eyes, so that the patients' eyelids can stick together in the morning, the lenses are often decentered, the sensation of a foreign body becomes unbearable (Figs. 14.18, 14.19).

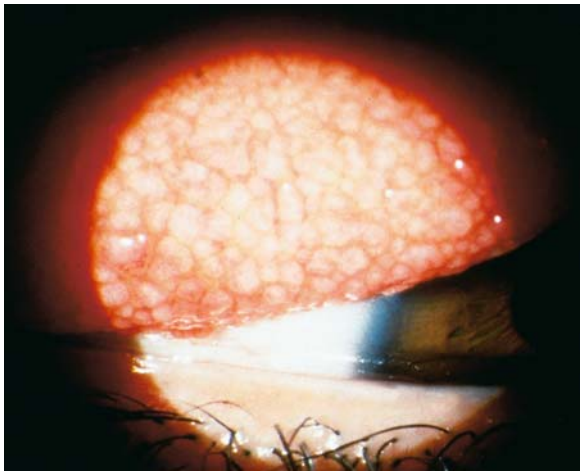


Fig. 14.18. Appearance of upper tarsal conjunctiva in CGP

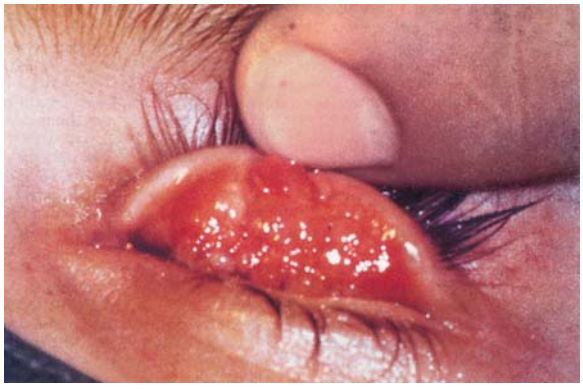


Fig. 14.19. Limbal inflammation in CGP

Diagnosis

Biomicroscopic ocular examination makes it possible to follow how the alterations evolve [5]:

- In *stage I* the lesions are mild.
- In *stage II* micropapillae can be observed.
- In *stage III* giant papillae are seen (>1 mm), which increase in number, size and height, forming the Roman cobblestone effect (present in 5%–10% of those who wear soft lenses and in 3%–4% of those who use hard ones).
- In *stage IV* the apices of giant papillae look flattened and epithelial damage is visible. Punctate keratitis or erosive lesions may affect the cornea.

The micropapillae, with a diameter of 0.3–1 mm, are seen in 80% of GPC patients [5].

A patch test with thimerosal and the other indicated substances can be carried out [145].

Differential Diagnosis

Differential diagnosis of GPC and VK is schematized in Table 14.8. Although the histopathological picture recalls that of VKC, the cornea is not affected, and rear histamine and MBP levels are normal or only reduced [3] and those of tryptase are high [31].

Treatment

Treatment will only be effective if the diagnosis is made early. Changing the lenses, or if necessary, removing the prosthesis or eliminating the sutures, improves the situation surprisingly. If the patient cannot or will not give up wearing contact lenses, a type that does not prove troublesome should be chosen [34] and solutions *without thimerosal* should be used for cleaning them once a week.

Cromolyn eye drops has proved to be very effective. The drops should be used in the morning before putting in the lenses and in the evening after removing them. If necessary the lenses should be removed during the day for a few moments.

Loteprednol etabonate 0.5% ophthalmic suspension may be an appropriate treatment for GPC, by ensuring a rapid therapeutic response combined with the low incidence and transient nature of any intraocular pressure increase [69]. In case of documented hypersensitivity, children are advised not to use levocabastine, lodoxamide or olopatadine while wearing contact lenses [191].

Prevention

The Committee on Health Risks of Contact Lenses from the Health Council of the Netherlands has recommended that permanent-wear lenses should not be used, warning that a very serious complication of contact lens wear is infectious keratitis, caused by bacteria or fungi. This is most common in users of permanent-wear soft lenses (20×10^4 persons/year) and can lead to a severe loss of vision [156]. Young people can wear contact lenses safely only if monitoring takes place at least twice a year [100]. In patients who are at high risk for GPC, replacing lenses at intervals of 1 day to 2 weeks appears to offer a better strategy in avoiding GPC than incorporating enzymatic cleaning into their care system [55].

Ocular Contact Allergy

Definition

This is a typical form of conjunctivitis caused by xenobiotics, due to continuous and direct exposure to a variety of environmental stimuli, in particular to sub-

stances present in the surrounding atmosphere, with eye irritation despite the eyelid and tear protective action.

Pathogenesis

This late-type hypersensitivity is set off by whatever allergen or hapten comes into contact with the eye and periorbital area: eye drops and ocular drugs for topical use can be especially responsible [68]: in 62.5% of cases the culprit is phenylephrine [133]. Antibiotics are also on the top of the list, including neomycin, as are anesthetics, antiviral drugs, antimycotics, dilating agents such as atropine and its derivatives, etc., and also soap, various detergents, shampoo, cosmetics, hair spray, chemical substances, preservatives contained in solutions for contact lenses and eye cosmetics, eyelashes, etc. [68]. Some typical examples are rubbing the eyes with dirty hands, or after touching soap, detergents, cosmetics, etc., and the use of mascara, eyeliners, suntan lotion for the face, nail polish, etc. [68, 190] (Table 8.9).

Clinical Presentation

Symptoms can appear from within a few minutes to 24–72 h after allergen contact. Swelling of eyelids, which are inflamed and pruritic, is characteristic. Ocular symptoms vary from mild irritation to severe symptoms with marked hyperemia [3] and photophobia, sometimes so unbearable that the patient keeps the eyes obstinately closed, making the ophthalmologic examination impossible. In severe cases, caused for example by the continuous use of drops containing the sensitizing substance, corneal erosions also form. Some patients can develop psychological disorders stemming from the disease's effect on appearance.

Diagnosis

Diagnosis is made difficult because of photophobia. Irritation and variable hyperemia, tearing, papillary reaction, and chemosis are present, and in the most severe cases a thin punctate keratitis. The identification of sensitizing agents can be complicated, especially if it is a cosmetic or eyewash whose composition is not always specified. Benzalkonium chloride in nose drops is not inoffensive (see Chaps. 8, 20). Patch testing is useful in some cases [16].

Treatment

Treatment consists in the immediate elimination of contact agents. Both hot and cold compresses are often helpful and if necessary, topical CSs can be used in moderation, warning the patient that side effects of an increased use can be worse than the disease itself.

Acute Edematous Conjunctivitis

Acute edematous conjunctivitis is a form of angioedema localized in the conjunctival membrane, showing acute unilateral and bilateral chemosis, normally caused by direct contact of the conjunctiva with dust or vegetables and/or animal danders [57, 190].

Blepharitis

Most chronic blepharitis in children is of allergic origin. Initial symptoms consist in palpebral rim hyperemia, with thin scaling, becoming furfural near the eyelashes. It can be associated with Zeis gland hypersecretion with formation of yellow scales. Depending on the predominance of one or the other aspect, it is described as scaly or eczema-form blepharitis.

Pathogenesis

There are three main factors at the origin of eczema-like blepharitis: refraction defect and binocular vision, atopy and aeroallergens, especially animal danders.

Clinical Presentation

The features are classic for blepharitis: burning, tearing, photophobia, and sometimes blepharospasm. It evolves to tylosis (thickening of the palpebral rim), madarosis (loss of eyelashes), and sometimes trichiasis (inward growing of eyelashes).

Treatment

Eliminating the causes is fundamental, but not always easy. In the scaly forms, freeing the palpebral rims from the microcrusts with dry gauze is suggested. Local treatment involves the use of zinc oxide or antibiotic creams, limiting the use of topical CSs to the more resistant cases. Self-medication should be discouraged [57].

Keratitis

Allergic keratitis is more difficult to treat than AC because of differences in the corneal tissues, their fragility, the abundant innervation and transparency that may appear changed [66]. Because the cornea is avascularized, hypersensitive reactions may take place only in the limbal barrier, which is the sclerocorneal junction [66].

Lymphatic drainage is also absent, unless there is central cornea neovascularization. After penetrating the limbus, the allergens can spread to the cornea; this shows how the passage of macromolecules such as IgM is prevented, and all other Igs are present, mostly concentrated in the stroma [183]. The antigen–antibody reaction of the Arthus type, which damages the collagen fibrils, also occurs in the avascular cornea. In rabbits, by injecting the allergen into the cornea, within 3–5 days there is a reaction possibly of a delayed hypersensitivity type; after 7–14 days CIC (circulating immune complex) deposits follow, accompanied by a Wessely phenomenon [181]. Predominantly mononuclear cellular reactions are observed, although basophils, eosinophils and neutrophils are also seen, leading to the hypothesis that a reaction parallel with cutaneous reactions is also induced by basophils [67]. Lymphocytes and differentiated CLs are basically absent [159].

Allergic keratitis appears in inflammatory forms divided into superficial or subepithelial, deep or interstitial punctate.

In the first case, they etiology is led by aeroallergens, as in AKC, and mycetes come in second. Symptoms are bilateral, with photophobia, tearing and the sensation of a foreign body in the eye. It lasts for 1–2 weeks, characteristically receding to recur after 4–6 months [181]. The best-known forms of keratitis are AKC and VKC and those associated with GPC and contact conjunctivitis. Treatment is based on topical CSs, with careful use of contact lenses [57].

Interstitial keratitis appears in children after the age of 5 years, and is usually monolateral. Symptoms are milder than in the punctate forms and consist in photophobia and a more or less pronounced loss of visual acuity. With a biomicroscopic examination, one observes deep deposits that make the cornea look like frosted glass. The epithelium is intact and the stromal lesions can assume different appearances depending on the extension, causing nodular sectorial or total keratitis. In these forms, when they are persistent, there is a stromal vascularization starting from the limbus [57].

Uveitis

Definition

Uveitis is defined as an inflammation of the uveal tract, which encompasses the iris, ciliary body, and choroid. Uveitis is an entity most pediatricians and ophthalmologists rarely encounter and has been reported to cause increased rates of visual loss compared with adult patients [140]. An improved knowledge of disease patterns and associated morbidity will help in the care of children with uveitis [177]. Although its diagnosis and management present a distinct clinical challenge for the pediatrician, its early diagnosis and management are vital in the preservation of vision [177].

Epidemiology

The incidence of uveitis in district hospitals in children <16 years of age was 4.9×10^5 [58]. In a referral practice in south India, a total of 31 (6.29%) pediatric uveitis cases were seen among the 493 uveitic cases in the year 2000 [135]. Of 219 patients, 112 were girls, with a mean age of 7.4±4.2 years, and 107 were boys, with a mean age of 8.3±3.4 years. The mean follow-up time was 37±6.2 months [95]. In referral cohorts, the most frequent diagnosis was juvenile idiopathic arthritis-associated uveitis (67%) [58]. Uveitis in children represents only ≈5%–10% of all cases: uveitis associated with juvenile rheumatoid arthritis (JRA) was the largest group (41.5%), followed by idiopathic uveitis (21.5%) and pars planitis (15.3%) [177]. The most frequent type of uveitis in 0- to 7-year-olds was chronic anterior uveitis, posterior uveitis in 8- to 15-year-olds, and acute anterior uveitis in 16- to 19-year-olds [58].

Classification

Uveitis affects the iris, ciliary body, and choroid and represents an immune ocular disorder characterized by type III (CIC) and IV reactions [70]. The uveal tract commonly contains B and T lymphocytes, mast cells and fibroblasts [154]. In patients with uveitis, one can observe infiltrating T cells also in silent stages, unlike in controls, with a CD4:CD8 ratio >1:0, proving that there are CD4 linked to the inflammation, while when it is receding there are higher CD8 levels [154]. CDs of the posterior uvea look like veiled cells (Fig. 1.39). During a chronic choroidal inflammation, plasma cells, phagocytes and lymphocytes link up to form pseudo-lymphoid follicles or granulomas; inflammatory cells enter the anterior chamber and the aqueous humor [70]. No HLA antigens are in the uvea; however, IFN-α can induce both class I molecules and IFN-γ [81]. From an immunogenetic viewpoint, anterior uveitis is associated with HLA antigens like other immune pathologies with ocular symptoms; acute iritis is a classic symptom in this disease and is linked to the *HLA-B27* genotype. However, 45% of patients with acute anterior uveitis have this allele without the clinical or biological stigmata of other disorders [189] (Table 14.10) [78, 168, 171]. *HLA-B27* uveitis is associated with approximately 16% of pediatric cases of anterior uveitis [140]. *HLA-DR1*, absent in posterior uveitis, can be considered a protective factor, while 100% of patients with panuveitis (involvement of all three portions of the uveal tract) express *HLA-DR4* [169]. The anatomical classification of uveitis into four types [151] was confirmed by two recent papers. In one paper, anterior or iritis or *iridocyclitis* was seen in 56.9% intermediate or *cyclitis* or peripheral uveitis (20.8%), posterior or *chorioretinitis* (6.3%) and diffused or *panuveitis* was observed in 16% of cases depending on the area affected by inflammation.

Table 14.10. HLA alleles in disorders with ocular manifestations

Disorder	HLA allele	Relative risk [168, 171]	Relative risk [78]
Anterior uveitis	B27	10.4	8
Ankylosing spondylitis	B27	87.4	80
Reiter's syndrome	B27	37	40
Behçet's syndrome	B5/B51	6.1	
Multiple sclerosis	DR2	4.1	
Sjögren's syndrome	DR3	9.7	6
Pemphigus	DR4	14.4	

The most recent HLA nomenclature can be found in Appendix 1.1.

Nongranulomatous (77.6%) and noninfectious (85.7%) were the most frequent types of inflammation. The process was bilateral in 74.4% of patients [103]. In the other an equal number of children had anterior, intermediate and posterior uveitis and 4 children had panuveitis [135]. It is agreed that any uveitis lasting for >3 months should be considered as chronic.

Clinical Presentation

Clinically, uveitis is characterized by the more or less extended reduction in visual acuity, sometimes by conjunctival injection and ocular pain, by possible complications that are more frequent in children (cataracts, glaucoma, corneal calcification and globus atrophy), and the chronic and recurring course of uveitis [57, 188]. Moreover [140, 142]:

1. *Acute anterior uveitis* will frequently present with sudden onset of conjunctival injection, tearing, photophobia and a peculiar injection seen adjacent to the limbus, perilimbal flush (which can be confused with AC) [189]. Pain is temporofrontal and exacerbated by light; the pupil is myotic. In *chronic* forms, the symptoms are less evident. The loss of eyesight, to variable degrees, is reversible in the acute forms but can be aggressive in chronic forms, including ankylosing spondylitis, JRA, and Crohn's disease.
2. *Intermediate forms* are generally bilateral, presenting a chronic and insidious course. There are no or few symptoms until complications occur, consisting in black floaters (myodesopsia), occasional cloudy eyesight and transient myopia. Vitreal cells and cellular aggregates can be seen with direct ophthalmoscopy, while inflammatory cells in the anterior chamber, protein exudation in the aqueous humor (flare), keratic precipitate and exudates with membranes covering the ciliary body can be seen with indirect ophthalmoscopy and the split lamp.
3. *Posterior uveitis* is heralded by floaters. The humor darkens because of inflammation, causing loss of visual acuity and cloudy vision. There are inflammatory cells in the vitreous, retinal perivasculitis and papillary edema, with the option of evolving into optical atrophy. Chronic forms are associated with JRA.

In most cases, the allergic origin of uveitis is hard to prove; the cause is *idiopathic* in 29%–50% of cases [142]. A relapsing tendency following contact with a given allergen is clearly very attractive, but rarely demonstrated. Current research is orientated toward ocular immunopathology. However, uveitis onset in childhood can be insidious, without clear symptoms. Therefore knowledge of this potential complication and subsequent ophthalmologic tests are important for the prevention of severe consequences. Carrying out tests on children until the age of 15 is recommended [57].

Diagnosis

Table 14.11 [140, 142] gives a panorama of the different forms of uveitis in childhood. Although its diagnosis and management present a distinct clinical challenge for the pediatrician, its early diagnosis and management are vital in the preservation of vision [177]. Accurate diagnosis requires history from both children or adolescents and parents or caregivers, a complete ophthalmic examination that may require general anesthesia, and carefully selected investigations [161]. However, in 24.2% of cases, uveitis was idiopathic, since no etiological factor could be ascertained [95] or this was the most frequent diagnosis (78%) [58].

Complications

Uveitis in childhood is a potentially blinding disease, in the majority of patients characterized by a chronic course and a high complication rate: 26% of the eyes had <20/200 visual acuity at the time of first referral at ≤16 years of age [177]. Visual loss (any eye <6/12) occurred in 17% and was not associated with age [58]. Three patients (2%) with the onset of ocular disease <16 years became legally blind and an additional 20 out of 121 (17%) had one legally blind eye caused

Table 14.11. Different forms of uveitis in childhood

Anterior uveitis
Ankylosing spondylitis
Drugs
Herpes-associated uveitis
Heterochromic iridocyclitis (Fuchs)
Idiopathic
Juvenile rheumatoid arthritis (pauciarticular)
Kawasaki disease
Reiter syndrome
Sarcoidosis-associated uveitis
Spirochetal (syphilis, leptospiral)
Stevens-Johnson syndrome
Trauma-related uveitis
Tuberculosis
Ulcerative colitis
Intermediate uveitis
Behçet's syndrome
Lyme disease
Sympathetic ophthalmia (trauma to other eye)
Vogt-Koyanagi-Harada syndrome (uveo-otocutaneous syndrome: poliosis, vitiligo, deafness, tinnitus, uveitis, aseptic meningitis, retinitis)
Posterior uveitis
Idiopathic
Ocular toxocariasis
Sarcoidosis
Subacute sclerosing panencephalitis
Syphilis-associated uveitis
Toxoplasmosis-associated uveitis
Tuberculosis
Viral (rubella, herpes simplex, human immunodeficiency virus, cytomegalovirus)

Data from [140, 142].

by uveitis. The most frequent causes of blindness were chorioretinal scars in the macular area and glaucoma [50].

Treatment

Noninfectious anterior uveitis generally responds to topical CS and mydriatic therapy. Although used frequently in adults with posterior uveitis, systemic CSs may cause serious adverse effects, including growth retardation in some pediatric patients. Methotrexate is the most commonly used systemic immunosuppressive agent for pediatric uveitis. It is effective, generally well tolerated, easy to administer, and inexpensive. Cyclosporine has also been used successfully in children with uveitis, being associated with a low risk of renal toxicity when used at standard doses. Alkylating agents are generally contraindicated in children because of risks including secondary malignancy, sterility and bone marrow suppression [161].

New Therapeutic Perspectives

The antagonism of specific chemokines, their receptors, or both might be of value in the treatment of ocular allergy [132]. Therapeutic strategies that block CXCR3 may inhibit T lymphocyte recruitment and suppress adverse inflammatory reactions [59]. In parallel, antagonizing the MCP-1/CCR1 interaction or signaling from chemokine receptors holds promise for the treatment of both acute- and late-phase reactions [172]. In the murine model of ocular allergy, it has been shown that the antagonism of the receptor for eotaxin-1 (CCR3 = common to eotaxin-2) might inhibit both early- and late-phase inflammation [138]. A different way of neutralizing eotaxin-1 is by using the humanized anti-eotaxin-1 antibody (CAT-213) that can inhibit the activation of human conjunctival mast cells in an *in vitro* passive sensitization system [134]. Topical application of blocking antibodies to $IL_1R\alpha$ have a profound inhibitory effect on the recruitment of eosinophils and other inflammatory cells essential for the immunopathogenesis of ocular atopy in animal models [100].

Nonsteroidal ocular preparations have without doubt attracted great interest, among them cromolyn, nedocromil sodium, antihistamines such as levocabastine, effective and safe alternatives compared to the risks linked to CSs.

Tacrolimus (Table 7.21), a macrolide antibiotic that has potent immunomodulatory properties, was effective in several ocular immune-mediated disorders, including keratitis and uveitis [15]. Application of *tacrolimus 0.1 % ointment* on eyelid skin may be effective for treatment of severe AD of the eyelids, and may have secondary benefits for AKC and VKC [149]. The ointment was applied to the lower conjunctival sac of both eyes once a week then twice a week and also daily in one child with VKC without any significant side effects [187].

Recently, a novel anti-IgE antibody (omalizumab) was developed that is directed against the receptor-binding domain of IgE. This binding is specific toward free IgE, thereby preventing it from attaching to the mast cell and its subsequent activation. In adolescents and children with SAR, there was a significant reduction in the nasal and ocular symptoms, with a side effect profile no different from the placebo [8]. Recently the systemic or conjunctival administration of immunostimulatory sequence oligodeoxynucleotides (ISS-ODN) has

been shown to significantly inhibit both eosinophilia and neutrophilia in the late-phase reaction in a mouse model of ragweed-specific conjunctivitis. This indicates that ISS-ODN may be an effective novel approach that may be useful in the treatment of SAC and VKC [129].

Effective treatment of contact lens-associated GPC might be possible in the future by employing inhibitors of LT synthesis and action [89].

Pediatricians and Eye Allergy

The eye is often considered as an incompletely defined accessory during childhood, but when inflamed – and generally when it is reddened – it automatically becomes the recipient for creams, eyewashes, etc. (hardly ever prescribed by a doctor), commonly for esthetic reasons, with less concern for eyesight [189]. AC, VKC, and AKC usually affect the child's quality of life, also because the illness is clearly visible, and can become the cause of an inferiority complex in older children, but this aspect, as all the others emphasized here, is normally ignored. Limitations in normal, relational and emotional spheres of life emerged from investigations concerning the quality of life for children suffering from SAC due to symptoms [94]: a pediatrician knows how these problems are also important for adolescents and to what extent they affect their quality of life. The clinical manifestations are important; however, a correct consideration of the emotional aspects will reflect effectively on the symptoms. The treatment of ocular disorders has focused to date on symptomatic relief, but just as we have a better understanding of the mechanisms, we now have better therapeutic interventions. With further discovery in the basic understanding of these conditions, we may eventually develop even better preventative therapeutic strategies [15].

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Allergy to the Venom of Hymenoptera and Other Insects

Historical Data

Hymenoptera, an order of bees and wasps, were known and written about by the early Latin and Greek scholars: Aristotle (384/383–322 B.C.) accurately reported on the bees, Aristophanes (445–385 B.C.) titled one of his comedies "The Wasps" and Virgil (70–19 B.C.) described them in the "Georgics," although they first appeared in a hieroglyphic of 2641 B.C., attributing the death of Pharaoh Menes to a wasp sting, and a note in the "Babylonian Talmud" reports an analogous fatality [15]. In France in 1765, the first reported case of mortal anaphylaxis in a 30-year-old who had twice suffered near fatal episodes was published [19]. In 1914, the anaphylactic shock induced by insects was identified with the anaphylaxis described by Portier and Richet [134]. From the scientific point of view in 1978, during a prospective study of the disease, the venom was recognized to be the causative agent responsible for Hymenoptera sting hypersensitivity [53]. As a result of a deeper understanding of the mechanisms of this illness, a radical change in both the identification of venom and allergen components and in the diagnosis and treatment has been reflected in a positive sense towards the practice of SIT (specific immunotherapy). Recently, interest in other insects by researchers looking at this study has broadened.

Definition

Allergy is an abnormal response to the bee sting, especially that of the order Hymenoptera. This abnormal response is not so much a consequence of the toxic effect of venom constituents in honeybee, yellow jacket, hornet, and wasp venoms, but rather is secondary to an IgE-mediated reaction to those constituents.

Epidemiology

The pediatric prevalence of insect allergy is reported in Chap. 5. Insect allergy is relatively more common during the pediatric age group up to about 20 years of age, with a male to female ratio of 2:1 and in those who are atopic, it is on the order of 33% [109]. The incidence in children of about 0.4%–0.8% [96]. Classical textbooks defined systemic reactions as uncommon in children: three studies by Settipane et al on 10,057 boy scouts and girl scouts demonstrated a prevalence of 0.4% (medical diagnosis) *compared to 1.9% of those questioned* [1, 116, 118], with a slight increase in the atopic population [118], while those in the general population revealed an increase of 3% [32]. In one study on 3,236 subjects who had an adverse reaction to insect bites, the prevalence during the pediatric age was 31.8%, with a net percentage of moderate or severe reactions of 56% [62]. In the Swiss Canton of Bern, the annual incidence of anaphylaxis ranged between 7.9 and 9.6×10^5 inhabitants per year. Hymenoptera stings (58.8%) were the most commonly identified culprits for anaphylaxis [46]. Among 229,422 children and adolescents with a median age of 12 years studied between March 1, 1991, and December 31, 1997 there were 30.8% of probable and 15.2% of possible cases of anaphylaxis to Hymenoptera stings and 2.8% of probable and 1.9% of possible cases of anaphylaxis to toxic effect of venom [7]. Ninety five episodes of anaphylaxis occurred in 76 Italian children and 36.4% were for bee, 36.4% for wasp and 27.2% for Polistes [77]. The lethal episodes at minimal levels are more frequent in adults [97] ($\times 10^6$): Switzerland 0.45, France 0.43, Denmark 0.25, Germany 0.18, USA 0.14–0.16, Great Britain 0.09 [70]; in total the rate does not exceed 50–100 cases per year, which translates into 40 in the USA, 20 in Europe [70], 37 in Japan [2], etc. In Australia the mean annual incidence rate was of 0.02 deaths $\times 10^6$ per year [67]. As regards the death incidence related to single insects, in Sweden, this is 0.95/year for wasps and 0.05/year for bees [56], in Denmark is 1.3/year for wasps and bees [69], in the US wasp and bee sting fatalities occur with approximately equal frequency [4]. As to the pediatric prevalence, in an average decade the number of fatal cases regarding children <10 years of age in the US was 1%, those 10–19 years was 0.5% [38], while in 163 autopsies performed, 1% referred to children who were 4–10 years old and 5% to those who were 11–25 years old with a male-to-female ratio of 5:1 [74]. Postmortem blood tests of a 10-year-old girl who collapsed on the street and died after a bee-sting revealed increased total IgE level as well as increased levels of sIgE (specific IgE) against bee and wasp venoms [75]. A retrospective study of a series of 94 suddenly deceased subjects in the absence of other determinant illness revealed that, in 23% of these (two of whom were 15 and

Table 17.1. Taxonomy of stinging Hymenoptera important in clinical allergy

Order	Hymenoptera						
Suborder	Apoides		Vespoidea				Formicidea
Family	Apidae		Vespidae				Myrmicidae
Subfamily	Apinae		Vespinae	Vespula	Polistinae		Myrmicinae
Genus	*Apis*	*Bombus*	*Vespa*	*Vespula*	*Dolichovespula*	*Polistes*	*Solenopsis*
Species	*A. mellifera* *A. dorsata* *A. scutellata*	*B. agrorum* *B. lapidarius* *B. terrestris* Megabombus Pyrobombus	*V. crabro* *V. orientalis*	*V. vulgaris* *V. flavopilosa* *V. germanica* *V. maculifrons* *V. pennsylvanica* *V. rufa* *V. acadica* *V. atropilosa* *V. austriaca* *V. consobrina* *V. intermedia* *V. vidua* *V. squamosa* *V. sulphurea*	*V. albida* *V. artica* *V. arenaria* *V. maculata* *V. norvegicoides*	*P. annularis* *P. exclamans* *P. fuscatus* *P. gallicus*	*S. geminata* *S. invicta* *S. richteri*
Scientific and common names							
	Apis mellifera Honeybee	*Bombus pennsylvanicus* Bumblebee	*Vespa crabo* European hornet	*Vespula* spp. Yellow jacket	*Dolicho-vespula* Yellow hornet[a], white-faced wasp[b]	*Polistes* spp. Paper wasp	*Solenopsis* spp. Red fire ant[c], black fire ant[d]

There is a marked cross-allergenicity between the genera *Vespula* and *Vespa*, broad cross-reaction between *Vespula* and *Polistes*, and negligible cross-reaction between Vespidae and Apidae families.
[a,b]These are more properly the names of *V. arenaria* and *V. maculata*, respectively.
[c,d]These are more properly the names of for *S. invicta* and *S. richteri*.
Data from [82, 138].

20 years old) sIgE was present for at least one type of Hymenoptera venom [114], suggesting that *a number of cases remain misdiagnosed.* This is indirectly confirmed by significant reports of parents who were unaware whether their young children, aged 15–39 months, had had a previous allergic reaction to imported fire ant sting [3] and in 15% of 269 adults who presented with an unexpected positive skin prick tests (SPTs) [32]; therefore a few subjects who were bitten did not consult with a physician [32]. For these reasons, we can not offer complete epidemiological studies.

Characteristics of Hymenoptera

Taxonomy and Entomology

The order of *Hymenoptera* contains about 16,000 species in only North America [41], of various forms and dimensions endowed with a unifying characteristic, two pairs of membranous wings [41].

The diffuse species found in Europe are essentially part of the families of *bees and wasps* (Table 17.1) [82, 138] (Figs. 17.1–17.4), while those found in the US also include some formicids (ants) [124]. Only the females of the pointed Hymenoptera are equipped with stingers and venom sacs, which are formed following atrophy of the reproductive apparatus [82]. However, it has been disputed that the various venom components demonstrate a significant homology with proteins derived from the male reproductive system, suggesting that this derives from a separate venom apparatus (Fig. 17.5) and not from a modified ovipositor, as traditionally believed [54].

Bees (Figs. 17.3 and 17.4) in general are rarely aggressive towards man, because since ancient times the bee has been domesticated for honey and wax, thereby resulting in a close relationship with man [80]. The domestic bee is distinguished morphologically by its stout body, which is covered with fuzzy hair and a black-brown coat with brown-yellow transverse stripes [41]. The aggressive instinct changes according to the colony

a b

Fig. 17.1. **a** Wasp. **b** Wasp detail

a b

Fig. 17.2. **a** Honeycomb. **b** Honeycomb: detail

Fig. 17.3. Bees

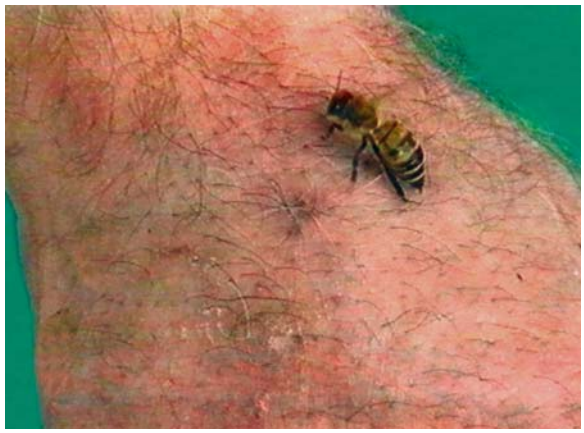

Fig. 17.4. Bee detail

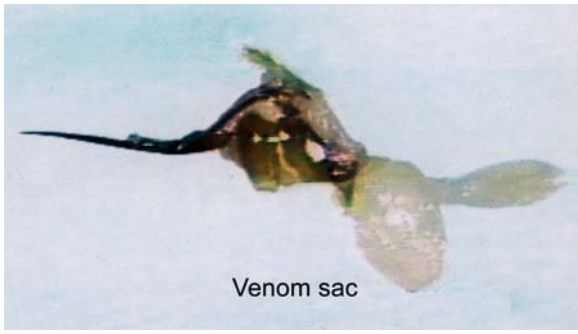

Fig. 17.5. Venom sac (for details see text)

and is habitually influenced by age (older bees are more aggressive), by adverse atmospheric conditions and also by periods of insufficient gathering of pollen [5]. The bees in fact have a particular activity that they perform known as pollination, in which they diffuse or spread the pollen of the flower chosen because of its nectar and in so doing they guarantee the reproduction of those particular flowers [80]. Bees are provoked or stimulated by brilliant colors, perfumes, vibrations, sharp movements, distinctive noises, etc. [10], but above all by traversing the corridors of flight or flight paths used by foragers to perform waggling dances containing information about the distance and direction to food sources [22]. The food-location information in the dance is presumably important when food sources are hard to find, variable in richness and ephemeral, whereas colonies with disoriented dances (lacking directional information) recruit less effectively than do colonies with oriented dances [106]. The bee is the only insect to leave the stinger (Fig. 17.6) embedded in the skin of the victim: in fact it does so because of the stinger's anatomical form of a serrated edge (Fig. 17.7). The stiner detaches and remains in the flesh together with the entire distal segment of the bee's abdomen, various muscles, and the venom sac, thereby causing evisceration of the insect and as a consequence its death [68, 80, 132]. The sting consisting of two lancets with curved barbs continues to inject venom after separation from the bee because the detached stinger is attached to a nerve ganglion that continues to coordinate its movement and in so doing, the barbs provide one-way traction and the sting continues to pump venom deeper into the flesh [132].

The *bumblebee* is distinguished by its larger dimensions, its black coat or alternating yellow or orange stripes and its layer of fuzzy hair. Allergy to bumblebee venom is a rare form of Hymenoptera venom allergy. Since this insect is increasingly utilized for the pollination of greenhouse plants, allergic reactions are increasing subsequent to its stings [41], especially when it attacks in order to defend its nest. The bumblebee moves

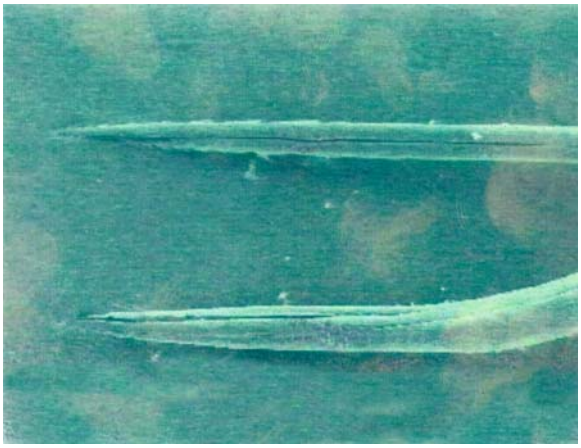

Fig. 17.6. Stinger seen at EM: *top* bee, *bottom* wasp

Fig. 17.7. Stinger seen at EM (for details see text)

Fig. 17.8. Polistes gallicus

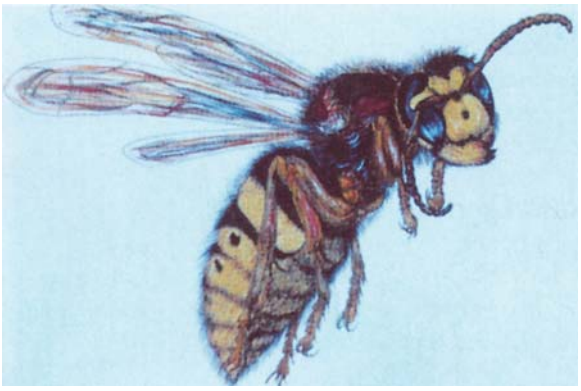

Fig. 17.9. Dolichovespula media

Fig. 17.10. Hornet

very slowly and in so doing man has time to make an escape long before the defender has left its nest [41]. Among the vespids (Figs. 17.1, 17.2) (yellow jacket, hornet and wasp), the genera *Vespula* and *Polistes* have a fusiform body and smooth coat, with black and yellow stripes (Figs. 17.8, 17.9) [68]; in addition, the *Vespula* has a distinct abdominal form, which is joined to the thorax by a thin stalk. These Hymenoptera nest underground, for example in the interior of caves that are not being used by other animals (mice, moles, etc.), or externally in hollow logs among the roots and debris of trees. They have a pugnacious attitude, especially in late summer or autumn, stinging even though not provoked; therefore they are responsible for numerous allergic reactions [10, 41, 68] The *Polistes* possess long legs characteristically distended during flight. In addition, the position of the nest is typically located above or around inhabited buildings, high wall cavities, roofs, windows or the branches of trees, in general of small dimensions and constructed of paper-like materials leading to the English denomination of paper-wasp [10, 68, 80]. In the vespids, the stinger lacks barbs (Fig. 17.6), which allows the extraction from the skin without further damage, providing that no traumatic events such as crushing are involved [80]. The formicids, especially the red imported fire ant (*Solenopsis invicta*), provoked a systemic allergic reaction in 94% of children (aged 15–39 months) and *Solenopsis richteri* in 100% of cases) [3] represent a serious allergic problem in the USA [124], less in Europe [115]. The potential for anaphylactic events caused by *S. invicta* is greater than for native ants because of its unusual venom [123].

The *hornet* (principal allergen Vesp c 1) (Fig. 17.10) is recognizable by its larger dimensions (>3 cm), builds its nests in the trunks of hollow trees, decaying logs, stumps or in wall cavities and may sting children of nursling age. It frequently flies at night and is attracted to light [41].

Hymenoptera Venom

The injected venom contains immunogenic proteins and nonallergenic substances, including biological amines and irritants (Table 17.2 [73, 82, 138] and Table 1.74 for the allergens). The most important, including those most studied, are Api m 1 and Api m 2, two enzymes that constitute 7%–15% and 0.5%–1.5% of the dry weight of the venom, respectively [51], and Api m 3 (melittin) corresponding to 50% [59]. Api m 1 is a very powerful allergen, even through inhalation, and 95% of patients with allergic reactions are sensitized to the bee sting [59]. We point out that all the allergens with the # 1 correspond to phospholipase A2/B, with the # 2 (Table 17.2) to hyaluronidase and with # 5 to progressive antigen 5. The numerous nonallergenic substances help to explain the prevalent vasoactive reactions and are responsible for the normal local reactions caused by the bite [70]. For example, bradykinin reinforces the inflammatory activity of the venom and contributes to the pain that it causes and hyaluronidase, constantly active (as Api m 2, Dol m 2), favors the intracutaneous penetration of the venom [84]. Many venoms contain histamine and some kinins; melittin has a potent histamine-releasing activity [54]. The presence of leukotrienes, above all LTC_4, accounts for the possible persistence of edematous reactions [17]. The cross-reactivity between the bee and wasp venoms seems to result from the presence of IgE anti-hyaluronidase [51]. Finally, the quantity of venom injected, from 2 to 100 µg (Table 17.3), with a single puncture varies in relationship to the species and age of the insect [10], although some wasps have been known to secrete as much as 330 µg [113].

Table 17.2. Venom nonallergenic substances

Apis mellifera	Bombus	Vespinae		Polistes	Solenopsis
Api m 1	Bom m 1	Dol m 1	Ves m 1	Pol a 1	Sol i 1
Api m 2	Bom m 4	Dol m 2	Ves m 2	Pol a 2	Sol i 2
Api m 4		Dol m 3	Ves m 5	Pol e 1	Sol i 3
Api m 6		Dol m 5	Ves g 5	Pol e 5	Sol i 4
Apamin		Dol a 5	Ves p 5	Pol f 5	
Peptide 401		Kinin	Ves s 5	Pol m 5	
Noradrenalin		Histamine	Ves v 1	Serotonin	
Dopamine		Serotonin	Ves v 2	Histamine	
Histamine		Adrenaline Cholinesterase Dopamine Histidine decarboxylase Noradrenalin Protease Tyramine	Ves v 4	Kinin	

In addition to the allergens (Table 1.74) some more or less allergenic substances are shown.
Data from [73, 82, 138].

Table 17.3. Venom amount injected by each puncture

Insect	Venom amount
Honeybee	50–100 µg
European hornet	100 µg
Paper wasp	2–10 µg

Modified from [9].

Genetic and Environmental Factors

The sensitization and the natural history of the illness are influenced by a composite interaction of genetic and environmental factors.

Genetic Factors

It has been seen that in 13.1% of families in which allergy to Hymenoptera is present there are subjects with the same type of sensitivity, vs 3.1% of the controls (p=0.0195) [30]. With the study of HLA alleles, analyzing the skin infiltrates of 23 Hymenoptera venom-allergic patients, the subcutaneous (SC) application of Hymenoptera allergens induced a perivascular and periadnexial cutaneous mononuclear cell infiltrate consisting mainly of $CD4^+$, $CD45RO^+$ and $HLA\text{-}DR^+$ cells [57]. Moreover, basophilic granulocytes in allergic subjects, compared with those in healthy persons, showed elevated expression of CD32, CD122, CD124, CD130, CD154 (Table 1.2), and HLA-DR [120].

The prevalence of atopy is considerable at all ages. In 688 boys with a family history of atopy (FHA) and positive SPTs, atopy was 32% [62], in 19 toddlers it was 63% and a history of atopic disease was elicited in 47% [3]. In one Italian study, a personal history of atopy was present in 22.7% of children [82]. *Serum sIgE to Hymenoptera* venom was *significantly associated with young age* (children vs adults, odd ratio (OR), 2.80 [104]). In one survey of 525 adults, atopy was present in 25% and serum IgE levels were ≤100 kU/l in 48% [94]. The analytical goal of four studies of non-beekeeping adults revealed the percentage of atopy: on the basis of personal case histories it found between 10.8% and 65.9%, while in three cases of beekeepers and their families the rate was between 29.6% and 47.1% [94]. An increased prevalence in families of non-beekeepers is anomalous, because a clearly increased risk is expected in a family of beekeepers, or may in general be expected with life in the country [82] or suburbs. FHA was negative in ten children with negative SPTs to Hymenoptera venom [76].

Environmental Factors

Not to be ignored are the risks associated with environmental factors, listed in Table 17.4 [63]. Besides the correlation between exposure and risk of sensitization, a peculiar aspect is represented by the chronology of the stings. The study of 120 patients who had demonstrated a systemic reaction for the first time to a Hymenoptera sting and of 100 healthy controls showed that the shortness of the interval of time (<2 months) between the last two punctures represented a significant risk factor for the development of allergic reactions [95].

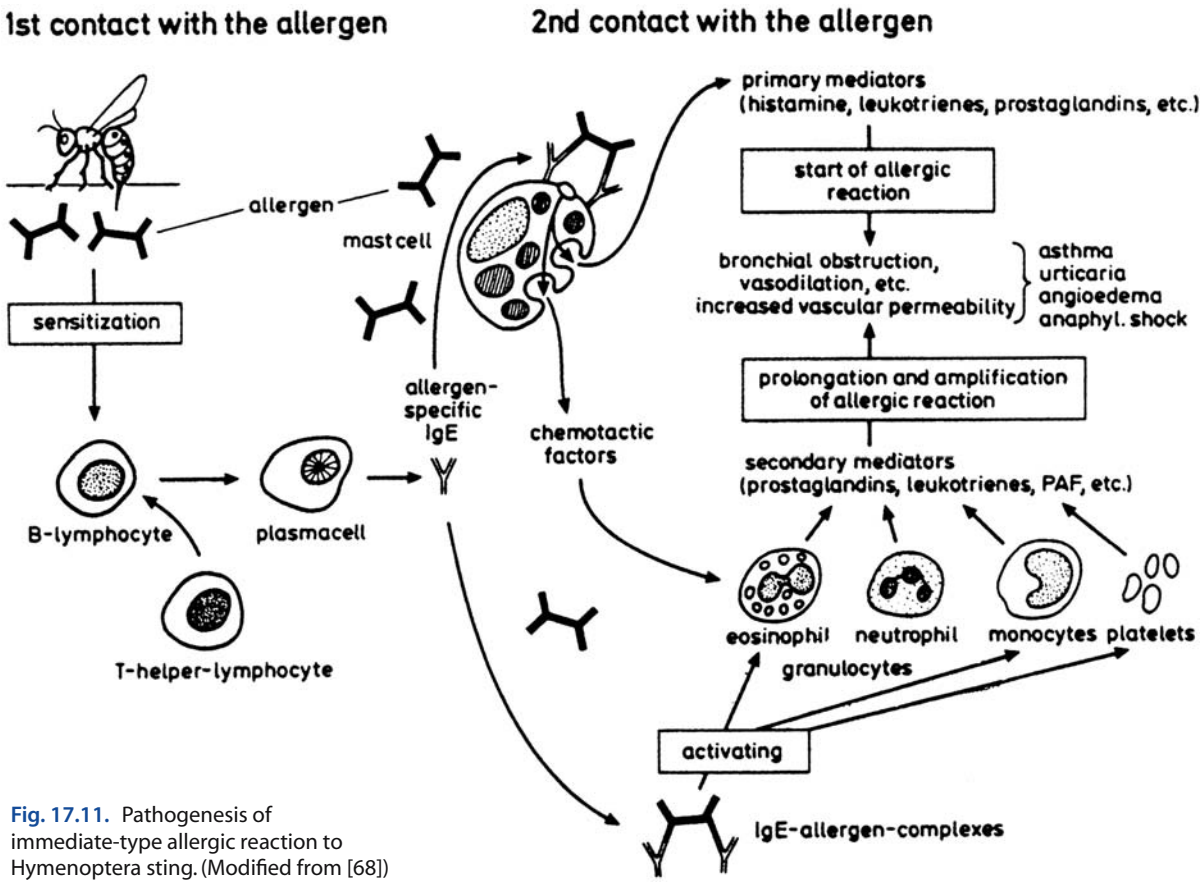

Fig. 17.11. Pathogenesis of immediate-type allergic reaction to Hymenoptera sting. (Modified from [68])

Table 17.4. Great risk of anaphylaxis to Hymenoptera sting in familiar surroundings

Activity at risk	%
Outside a building	92
Inside a building	8
Alone	85
Not alone	15
Gardening or mowing grass	36
Playing sport	13
Walking through the grass	7
Outside but not in the grass	6
Riding in a car	5.5
At poolside	3
Picking fruit	3
Picnicking	3
Riding bike	3
Fishing	2

Data from [63].

Natural History

The natural history demonstrates that babies and adolescents have *a risk = 10%–20% of reporting systemic reactions* after successive bites, therefore less than that of adults, who show a risk of 27%–57% [40, 110, 116–118, 121, 127, 129]. A much debated problem stems from the variability of the response: it is possible that some patients with progressive serious allergic reactions to the first puncture present no reactions to successive bites and are not in the desensitized state; in other subjects it is possible that a second puncture causes an increase in sIgE levels, thereby provoking more serious reactions [97, 99]. It has been observed that the risk of presenting with a serious reaction after a localized puncture is 5% [70, 99]; in addition, after a serious systemic reaction it is possible, at least in 40%–65% of cases, that a successive puncture is better tolerated and less consequential [53, 70]. This raises the question of whether in a baby serious reactions, after a cutaneous lesion, can be verified, even with a very favorable prognosis. Three pediatric studies (2–17 years) demonstrated that after localized reactions, the risk of systemic reactions, that is without acquiring characteristics of particular severity [129], as a consequence of successive stings is minimal [110], 2% [40] or 9.2% [110] of the cases. In another case report [98], the percentage of

reactions, independent from the period of time from successive punctures, was 46% in 112 children ≤16 years of age and 74% in adults. Usually, clinical features range from urticaria to anaphylaxis [96].

Etiopathogenesis

Bee and wasp venom extracts contain potent allergens able to induce severe clinical reactions [136]. The sequence of events in the allergic reaction of the immediate type is summarized in Fig. 17.11 [68]: the first contact with the allergen (preparatory) is not always followed by the second contact (provoked) [68]. The IgE-mediated pathogenesis is demonstrated by the presence, in subjects with Hymenoptera venom hypersensitivity, of a highly differentiated percentage of IgE^+, which is significantly greater than in nonatopics ($p<0.001$) [21]. After an extensive local reaction, such patients have SPT+ and, after a generalized reaction, venom-sIgE antibodies by interaction on mast cell surfaces [59]. However, there is the possibility, although rare, of non-IgE-mediated reactions caused by exogenous vasoactive amines delivered by the insect venom [97]. The contact with sIgE induces the liberation of histamine, leukotrienes, prostaglandins and other mediators from the metachromatic cells: in adults subjected to intentional challenge with insect venom [130], a notable increase has been demonstrated in the levels of histamine and tryptase (1.275±2.294 and 406±1.062 nmol, respectively), but not prostaglandin the level (specifically of PGD_2), an effect of Api m 1, which is also capable of interacting with specific lymphocyte clones with production of elevated titers of IL_4 and IFN-γ [20]. *Elevated levels of tryptase* have also been noted in the serum of recently deceased patients secondary to insect bites [138]. CD203c up-regulation on basophils activated by molecularly defined allergens was induced by defined Hymenoptera venom components in 35 of 39 patients with a diagnosed allergy to wasp and/or bee venom. Twenty-seven patients with wasp allergy showed *CD203c up-regulation in response to Ves v 5,* and 26 also reacted to Ves v 2 and 17 to Ves v 1 [7]. A recent trial observed that the lower the titers of angiotensin I and II the more severe the clinical symptoms in patients with a history of hymenoptera venom anaphylaxis [49].

From the events so far summarized, three phases can be discerned [70]:

- *Increased vascular permeability,* peripheral vasodilation, sequestration of fluids, therefore hypovolemic shock
- *Disseminated intravascular coagulation* with potential generalized hemorrhage and consequent aggravation of the hypovolemia
- *Hypoxia* induced by bronchoconstriction and cardiotoxicity secondary to the mediators from which the cardiac insufficiency and arrhythmias are derived, all contributing to shock

The IgE-mediated mechanisms are clearly evident when the symptoms are immediate. The allergic pathogenesis is more difficult when the reactions are localized, in so much as one cannot verify a progressive diffusion of the edema, which may not depend upon vasoactive amines whose half-life is too short to sustain such a manifestation but may imply instead an allergic mechanism. In addition, when the intensity of the reaction provoked by the sting increases dramatically, it is a sign that there is an IgE-mediated mechanism [70], which should set off alarm bells. Localized lesions at a distance from the initial puncture would have the ability to cause allergic processes [84]. Lacking allergenic stimulation, IgE levels diminish slowly, in variable measure from subject to subject, although they are capable of remaining high for years [99]. The release of secondary mediators from the inflammatory cells (eosinophils, neutrophils, macrophages, etc.) is probably responsible for the less common form of *protracted or biphasic delayed anaphylaxis* [80], whose incidence is about 3%–4%. The delayed reactions bring to light the intervention of other immune mechanisms, similar to type III reactions, which may appear within a few hours, or type IV reactions if the interval is 1 day [84], although they can also manifest themselves days or weeks after the puncture. A subject bitten many times within a short period of time may undergo a toxic reaction in which the symptoms are similar to IgE-mediated forms, but if the SPTs become positive the subject may be at risk of allergic reaction to a subsequent insect sting [97]. These venom-induced variants usually occur in nonatopic children [76].

Clinical Presentation

In normal subjects, the lesions consist of a localized reaction characterized by erythema, edema and pruritus, with a pain component of variable intensity. They regress spontaneously or with local therapy within a few hours, usually without leading to particular concerns in small children or relatives. In some cases the response is exaggerated with extension of the local reaction. The reactions are generally prevalent in the head and neck regions, although they may present to any other region of the body [70, 97].

The reactions are distinguished as *either immediate* (within 2–4 h of the sting) *or delayed* (>4 h); the immediate reactions are summarized in Table 17.5 [70, 72, 81, 97, 107] (Fig. 17.12). In 70%–80% of cases, the systemic reactions are not life-threatening [107].

The symptoms, for the most part, begin after 10–20 min *without any warning signs* [133]. The symptoms present either in isolation or grouped together. In the immediate type reactions, the rapidity of the insurgence is, in general, an indication of the severity of the clinical picture [80]. The most serious reaction caused by the puncture of the Hymenoptera is *anaphylactic shock* and

Table 17.5. Features of immediate reactions (local and systemic) to Hymenoptera stings

Immediate reactions [72, 81, 97, 107]	
Mild local reactions, with pain, localized to the sting site, transient erythema and swelling, usually last a few hours and involve 90% of children	
Large local reactions, with erythema and edema, extended up to half of the stung limb, type urticaria-angioedema, which peak within 24 h and last for up to 1 week (10% of children)	
True systemic reactions involve different organs and systems:	
Gastrointestinal: nausea, vomiting, abdominal pain, diarrhea	
Respiratory: laryngeal edema, rhinitis, dyspnea, bronchospasm	
Cardiovascular: hypotension, cardiovascular collapse and anaphylactic shock	
Toxic, nonallergic reactions, with onset after several stings, and include fever, nausea, vomiting, and other systemic symptoms	
Systemic reactions [70]	
Grade I	Generalized urticaria, itching, malaise, anxiety
Grade II	Any of the previous symptoms plus two or more of the following: nausea, abdominal pain, vomiting, diarrhea, generalized angioedema, bronchospasm, wheezing, dizziness or fainting
Grade III	Any of the previous symptoms plus two or more of the following: dysphagia, dyspnea, hoarseness, weakness, confusion, deep anxiety and sense of impending death
Grade IV	Any of the previous symptoms plus two or more of the following: cyanosis, hypotension, cardiovascular collapse, urine or stool incontinence, loss of consciousness

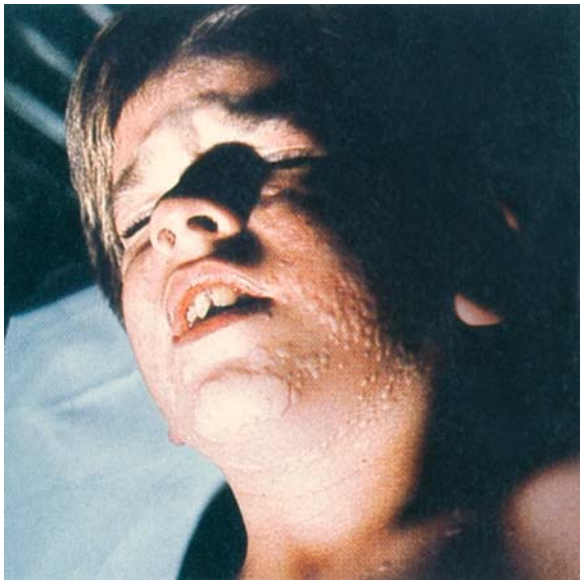

Fig. 17.12. Acute urticaria as part of a systemic anaphylactic reaction. In similar cases SIT for Hymenoptera is obligatory

it is not common to any particular age group [60, 61]; the symptoms have analogous characteristics to those observed in the reactions caused by other allergens: hypotension, bronchospasm, swelling of the epiglottis, gastroenteric symptoms, sphincteric incontinence and loss of consciousness [133]. This was the case of a 12-year-old boy who was stung by a swarm of yellow jackets. Immediately he developed severe pain at the site of sting and swelling all over the body. He complained of *constricting sensation in the chest within minutes.* His condition was so severe that he was *discharged after 30 days in the hospital* [126]. The delayed reactions may cause atypical reactions, including serum sickness accompanied by sIgE and IgG antibodies, renal complications, vasculitis and neuroencephalitis, transverse myelitis, myocarditis, vascular lesions which lead to vasculitis, nephrosis, etc., all of which are rarely fatal [100]. During the pediatric age, the following reactions present a notable risk, even though they are rarely observed after the sting of a wasp: collagenosis associated with arthritis, purpura, melena, terminal myocarditis, nodular pericarditis, nephritic syndrome or glomerulonephritis [84].

Imported fire ants defend their territory aggressively and make extensive use of pheromones or chemical signals to recruit other workers, synchronize attacks and initiate stinging. For this reason, multiple stings are the rule rather than the exception [123]. Imported black and red fire ants (Table 17.1) stings typically cause a pustule 24 h after the sting. *Red imported fire ants* (Fig. 17.13) are inconspicuous, reddish-brown ants with no distinguishing features visible to the naked eye. Superficially, they resemble many common native and exotic ant species present elsewhere. They range in size from 2 to 6 mm; their optimum habitats include grassed areas, gardens, sites near flowing and still water and recently disturbed soil [123]. The natural history of fire ant hypersensitivity in children who have only cutaneous manifestations has not been well elucidated. Because there is increased risk of fire ant stings in children who

Table 17.6. Clinical presentation in childhood insect bites

Related to severity					
No. of cases and reference		62 [39]	101 [82]	235 [110]	112 [98]	Mean
Type of reaction (%)					
Local extended		10	18.8	22.5	30	20.3
Mild systemic		56	44.6	52.3	58	52.7
Life-threatening systemic		34	35.6	25.1	47	35.4
Related to symptoms [35]	**(%)**					
Urticaria-angioedema	95					
Skin-limited symptoms	60					
Dyspnea/wheezing	40					
Hoarseness/dysphagy	40					
Hypotension	10					
Anaphylaxis	5					

Fig. 17.13. Solenopsis invicta. Photograph by courtesy of the Department of Primary Industries and Fisheries, Queensland, Australia

live in areas where fire ants are prevalent, SIT may be recommended for such children [93].

Müller has differentiated the systemic reactions into *four degrees of increasing severity* (Table 17.5), which helps to distinguish the reactions that are less life-threatening, reactions limited to the skin and gastroenteric apparatus (grades I and II), and reactions involving the respiratory airways, cardiovascular apparatus and finally sensorium (grades III and IV), which are reactions leading to a more severe risk, especially those caused by Solenopsis [12]. Table 17.6 shows the prevalence of these reactions, whose average is on the order of 20.3%, 52.7% and 35.4% in four pediatric cohorts [39, 82, 98, 110], and which is less frequent with respect to adults 43% vs 56% (p=0.0019) [39]; 59.6% of the cases are not life-threatening, a number less than usual [107] and similar to the numbers derived from other selected cases. The *increased prevalence of severe life-threatening systemic reactions* in the 112 babies [98] suggests that there is a relationship with progressive episodes of anaphylaxis and with nonadmission to SIT. In 10 of 76 children aged 3–13 (12%), Hymenoptera venom caused 11 episodes of anaphylaxis; sIgE against Polistes were detected in 3 cases, against wasps in 4 cases, and against bees in 3 [76]. In 107 children, Hymenoptera venom was the second most frequent cause of anaphylaxis (29%). Most of the patients with Hymenoptera venom anaphylaxis were male (80%) and more frequently they had no history of atopy [13]. Among 1,175 children, 228 (19.4%) had a history of Hymenoptera sting reactions [34] or 19.06% local reactions and four 0.34% local and systemic reactions) [77]. Quite differently, in 91 children the clinical symptoms were anaphylaxis (15.3%), serious reaction (37.3%), strong reaction (34%), and mild reaction (7.6%) [96].

The prevalence of the clinical characteristics of the reactions seen in infancy is summarized in the second part of Table 17.6 [35]. Usually, the reactions in children are generally *limited to grades I and II*, with a more favorable clinical outcome and a mortality incidence clearly lower than that of adults.

Müller has, in addition, stated that patients developing side effects during SIT are exposed to a significant risk of relapse [71], and factors such as age and type of the reaction influence the natural history of anaphylaxis to Hymenoptera stings. Recent observations indicate that for the majority of patients the event is self-limiting [98].

Diagnosis

A detailed case history is of capital importance; the investigation assumes particular prominence when there is positivity within the personal and/or family histories,

or when there are family activities or environmental characteristics where the patient lives that can increase risk. Besides the clinical aspects of the reaction and its progression subsequent to another sting, it is opportune to be able to identify the Hymenoptera that is the cause of the reaction, even though there may be great difficulty in doing so, because of the child's or relatives' inability to describe the particular morphology of the insect in question or their inability to remove the stinger [127]. It would be useful if the health provider working in the area, even though it may be risky, could provide photographs or specimens preserved in formalin to show the patient and/or relatives, with the aim of having valid criteria or evidence beyond that of the diagnosis before subjecting prospective patients to SIT [127]. In 47.7% of cases, there is a correct identification of the insect responsible, primarily by families of beekeepers [82]. However, *young children* present special problems in diagnosis because they *are usually not able to identify the insect.* The presence of a stinger, which is left by bees, or the presence of a pustule as a result of a fire ant sting (up to 24 h later) may help in insect identification [93].

SPTs are preferably done with caution and in a hospital environment because of the possible risks. Because the insect that caused the sting often cannot be identified, testing is usually done with all of the commercially available venom extracts [93]. It is suggested to employ the purified venoms corresponding to the national entomological origin when considering the taxonomy (Table 17.1). Lyophilized venoms exist for mellifera bees, *Vespula* species and *Polistes* species [138]. There are reactions in the US, besides those to the *Solepnosis* spp., to the *Halictidae*, of the Apid family, which are difficult to diagnose [85]. In the case of suspected fire ant hypersensitivity, SPTs should be done with whole-body extract [93]. SPTs are the first tests to be done in children, but several studies were prudently based on concentrations of 1 µg/ml, which in case of negativity were continued with intradermal reactions (IDR) performed at 30-min intervals [84]. The maximum concentration of 1 µg/ml for the IDR was used [18]. However, this procedure has led to a decreased rate of positive SPTs. Using SPTs with standardized venom extracts at a concentration of 100 µg/ml, 3.66% of 1,175 children had positive SPTs to any given Hymenoptera extract, 2.98% to honeybee venom, 1.45% to wasp, and 1.02% to *Polistes* venom [77]. Proper SPT execution ensures a positivity in the range of 95% in the absence of any complications [84]. However, although SPTs may be able to identify the different sensitivities, there is not a good correlation between the degree of response, the severity of the progressive reactions and the probability of a reaction to another sting [107]. Müller [70] recommends performing SPTs between the 1st and 3rd month after the acute allergic manifestations in order to guarantee a sufficient amount of mast cell mediators.

The RAST was negative in 15.4% of 52 children with a positive SPT [82], and is therefore less credible as a screening method [61]. RAST testing may be considered for those patients who have a convincing history of anaphylaxis after an insect sting and who have negative SPT responses [93]. RAST is very useful in the immunological monitoring of subjects who were both treated and not treated with SIT [81] because of RAST sensitivity to *Vespula* and *Polistes* species, both seen in 101 children (17.8% SPT and 10.9% RAST positivity) [82]. In another pediatric study, the SPT results were 80.7% positive and 71.7% positive to RAST, with a high concordance for both cases [81]. It should be noted in the interpretation of both SPT and RAST results that the reactivity to Hymenoptera venom compared to common inhalants increased in atopic patients [81]. A partial analysis by Müller determined that *both tests distinguish between positive and negative patients* (p=0.0001 and 0.0036, respectively), while the predictive value with respect to a successive puncture is less significant [71]. Other results appear to contradict this, disregarding the value of these tests [131], or recognizing a good concordance between SPT and RAST results for bee venom and a very good SPT sensitivity to wasp venom [26]. CAP has both a rapid and a good sensitivity, correlates well with the history, the SPTs and the RAST with which it has an increased sensitivity [55]. A method based on RAST inhibition distinguishes between the various RASTs positive for the venom and is attributable to IgE cross-reactivity [42]. ELISA is well correlated with RAST and is less costly [92].

We face SPT-negative results in about 10% of people with elevated RAST and RAST-negative results in about 20% of subjects with positive SPT results [36] *when we need to treat a child at risk.* Several adult trials have concluded that it is a self-limited process for difficult cases, where history, SPTs and RAST do not allow a clear decision regarding the relevant insect species for VIT [37]. In cases with contradictory test results, additional tests are recommended such as a test based on basophil CD63 expression as marker of activation in comparison with the basophil histamine release test and the cellular antigen stimulation [23] and cellular tests for IgE-mediated reactivity [24], which may not be available everywhere [37]. It is also true that in a trial on 149 people, only five (3.35%) with severe insect sting anaphylaxis had negative SPT reactions up to a venom concentration of 0.1 µg/ml [60]. In parallel, only 0.9% of 208 insect-allergic patients had both venom SPTs and RAST negative and still reacted to a challenge sting [34]. In adults, intentional provocation tests for diagnostic purposes [130, 131] were given in a hospital environment with live insect punctures to the skin in order to test the state of tolerance to successive stings by the same Hymenoptera (and to determine the indications for SIT [99]). In fact up to 72% of the adults with a history of prior reactions, even severe, did not present with any symptoms to a successive sting [99], while a 10%–15% positivity was found in a stimulatory test on skin-negative subjects [131]. According to these results,

the test was not in fact true and proper proof of whether protection was reached or not [99]. It was also demonstrated that if venom emission during the procedure was in the form of a spray, it could reach the patient in a negligible measure, explaining why at times a single challenge is not sufficient [29]. In other studies, 21% of adults who were negative to the first test proved positive to the second test with systemic symptoms in 50% of cases; nor was it proven that patients negative to this test were completely free of reactions to successive stings [131]. Therefore, fixed rules need to be instituted regarding the clear ethical problems [97]. In contrast, it could be useful diagnostically in subjects who are SPT+ to avoid subsequent bites and live a normal life [31]. In 21 children (aged 4–15 years) with bee-sting allergies, sequential challenges done to establish the need for VIT provoked on the first challenge a normal local reaction in 33% of the children, a large local reaction in 29%, and a systemic reaction in 38%. On the second challenge, the reactions were 67%, 22%, and 11%, respectively, in 18 out of 21 children [45]. However, *in children sequential bee-sting challenges have a high negative predictive value of 94.6% for the risk of further systemic reactions* [112] and may be a powerful tool to determine the necessity for VIT in bee-venom-allergic children [111].

One study on 113 children between 2–17 years answered these questions positively [44]. In particular:

- While 84% of the subjects had a history of severe systemic reactions and 16% of extended local reactions after the two challenges had comparable results, in 76%–78% the reactions were normal local, in 1%–5% they were extended local and in 13%–17% systemic.
- VIT was prescribed to all subjects with a history of severe systemic reactions or who developed them after the challenges.
- In the subsequent period after the procedure (2 weeks to 35 months) 40% were once again stung. Of the six treated with VIT, one presented with urticaria, while of the 39 not treated only one developed moderate respiratory symptoms.
- In comparison with other diagnostic evaluations such as SPTs, determinations of specific IgE and IgG antibodies and single-sting exposure, the dual sting challenge scheme appears to be the best predictor of reactions to subsequent stings [44].

Following this study, 78 of 92 children fulfilled the current criteria for VIT [26], but only 13 received VIT based on challenge studies.

- Therefore 65 of 78 children (83.3%) would have undergone unnecessary therapeutic interventions using the above guidelines.
- Two of the 13 children (15.4%) developed systemic reactions 1 year after VIT of 5 years, of which one was mild and one was severe.
- Among the 48 re-stung children who were not treated with VIT, three (6.3%) experienced mild systemic reactions, whereas 45 reported no more than a local reaction.
- The long-term predictive value of sequential bee-sting challenge tests for systemic reactions in children who are not SIT-treated remained at a level of 93.8% over a period of >5 years [111].
- Sequential bee-sting challenges are thus critical to establish whether to start VIT in bee-venom-allergic children [111].

Adult data justify the recommendation that patients with a history of a systemic reaction but negative venom SPTs should be evaluated by RAST, and if still negative, by repeat SPT after 3–6 months [34].

Treatment

The treatment for the reactions that result from insect bites and VIT, including first aid, is as follows [72].

If the sting remains within the puncture site it must be extracted delicately without producing any kind of pressure that may risk emptying the contents of its venom sac into the lesion [113]. *It does not matter what method is used*, but time to remove the venom sting is important [132]. The venom sac breaks in 90% of cases emptying in <20 s, and on average after 2–7 s 53 µg are released, after 8–16 s 133 µg are released, and after >16 s 148 µg; therefore *the quicker the reaction the better the prognosis* [113]. If a limb is involved it is convenient to place a tourniquet proximal to the puncture site in order to retard venom diffusion, releasing the pressure every 3 min until the elective treatment is started [84]. If the local reactions are not extensive they can be treated with cold packs or with antihistamine ointment. Cold compresses may help to reduce local pain and swelling. Local anesthetic cream and oral antihistamines are useful to reduce the pain or itching associated with cutaneous reactions [93]. In cases where the reactions are extensive or with manifestations of urticaria-angioedema, a tourniquet should be applied as described above and epinephrine injected subcutaneously (Chap. 20). Therefore, the cornerstone of emergency treatment to avoid death, which may ensue within 1 h in the majority of cases, is epinephrine administration, whose action is *immediate if injected IV* or within a few seconds if injected SC or IM [124]. For the treatment of anaphylaxis, see Chap. 20. Tables 17.7 and 17.8 report the treatment schedules for cutaneous and respiratory reactions indicated in the Position Paper of the specific EAACI (European Academy of Allergology and Clinical Immunology) subcommittee. If the symptoms progress towards anaphylactic shock it is imperative that children be hospitalized [26].

With reference to the use of VIT, it was demonstrated in 1978 that employing the entire body the treatment results did not differ from those found in placebo patients, while utilizing the venom resulted in positive results in more than 95% of cases [53]. However, *since 1956 Dr. Loveless has proven the superiority of VIT* [64, 65], using extracts of venom sacs removed from vespids that she

Table 17.7. Pediatric emergency treatment of Hymenoptera sting. I: Cutaneous reactions

Medical treatment	Additional treatment
Large local reaction	
Antihistamines H_1 oral (cetirizine, clemastine, loratadine, etc., dosage see Table 12.10)	Apply ice or cold compresses
Corticosteroids, oral (prednisolone 0.2 mg/kg bw)	Immobilization and elevation of the affected limb
Systemic reaction, urticaria-angioedema	
Antihistamines H_1 IV (clemastine) or subcutaneous adrenaline (Chap. 20)	Continued observation until the child improves for at least 1 h
Corticosteroids, IV (prednisolone 0.2 mg/kg bw)	

With protracted symptoms, readminister adrenaline every 10–20 min (Chap. 20).
Data from [26, 72].

Table 17.8. Pediatric emergency treatment of Hymenoptera sting. II: Respiratory reactions

Medical treatment	Additional treatment
Bronchial obstruction	
Epinephrine (Table 11.17)	Inhaled epinephrine
Albuterol or terbutaline to be repeated more times	O_2 supply by face mask, until full improvement
If severe, albuterol, IV (Chap. 20)	In severe cases hospital
Corticosteroids, IV (prednisolone 2 mg/kg bw)	Admission or intubation
Theophylline	
Laryngeal edema	
Epinephrine, SC or prednisolone/methyl-prednisone IV	

With protracted symptoms, readminister inhaled epinephrine every 10–20 min (Chap. 20).
Data from [26, 72].

had captured personally [65]. However, her studies were not taken into consideration because they were not conducted using double-blind controlled procedures [16], even though periodic deliberate stings were performed by Loveless [64], without convincing the medical community of the benefits of this approach [38]. Recently, her contributions have finally been recognized [14, 128] and nearly a quarter century after Dr. Loveless' pioneering publication, venom sac extracts were approved by the US FDA for the treatment of anaphylactically sensitive patients [128].

Normally during the pediatric age, the criteria for admission to a SIT treatment plan would include children with *life-threatening systemic, severe and strong reactions* (88.8% of 91 children) [96] and with positive SPTs, while excluding those cases with solely cutaneous reactions [83] (Table 17.9) [28, 80], with the aim of reducing the costs and avoiding the *possible toxic effects of long-term administration* [83]. In Chap. 13 we discuss the modality and the treatment schedules and examine the chronology of interrupting VIT [43, 58, 108]. It can be confirmed at this time that it is a completely decisive treatment, toward which children and their families can be directed without hesitation and/or concern and without necessity of modifying their normal routine [99]. As previously shown (Fig. 13.8), specific IgE levels initially increase and later decrease, as shown for wasp venom [135] and birch pollen VIT [48]. The follow-up

Table 17.9. Selection of children for VIT

Reaction to sting	Results of SPT/RAST	SIT?
Systemic, life-threatening (Müller stage IV)	Positive	Yes
	Negative	No[a]
Systemic, non-life-threatening (Müller stage III)	Positive or negative	No
Large local (Müller stage II)	Positive or negative	No
Normal (Müller stage I)	Positive or negative	No

SIT specific immunotherapy, *SPT* skin prick tests.
[a] Pastorello et al [80] suggest admitting to SIT children who are SPT+ but RAST-negative.
Data from [38, 80].

after 15–19 years of 1033 children, of whom 356 received VIT has shown that systemic reactions occurred in 3% of VIT-treated patients compared to 17% of untreated patients (p=0.007). Non-VIT-treated patients with a history of moderate to severe reactions had a 32% rate of reaction compared to 5% in the VIT-treated patients (p=0.007). VIT in children leads to a significantly lower risk of systemic reaction to stings even 10–20 years after treatment is stopped, and this prolonged benefit is greater than the benefit seen in adults [33]. Recently it has been shown that *VIT is able to protect insect venom-allergic children against life-threatening sting reactions* after a sting of the respective insect [111]. This effect is attributed to a shift from Th2 to Th1 (Chap. 13). Basophils in persons with wasp venom allergy are permanently activated, as illustrated by the expression of different activation markers, such as IL receptors and HLA-DR molecules. VIT immediately increases this activation, as shown by the rise in CD63 during VIT, which indicates a partial basophil degranulation with release of stored protein mediators. ILs released by T cells, which as a result of VIT change from a Th2 type to a more Th1 type, down-regulate the activation of the basophil granulocytes, which subsequently decreases to the normal levels seen in nonallergic persons. An initial rise in IgE may be caused by IL_4, which is released by the basophils during VIT. A significant decrease in the basophilic surface antigens CD11c, CD32, CD35, CD63, CD116, CD122, CD124, CD130, and CD132 was detected 1 week after the end of rush VIT [120]. Decreased large local reactions after long-term VIT was correlated with a significantly reduced recruitment of $CD4^+$ cells and $CD23^+$ cells as compared to large local reactions at the application site, whereas the number of $CD8^+$ cells, $CD11c^+$ cells, $NP57^+$ cells, and $CD25^+$ cells remained high [57]. The "rush" method is performed using diverse protocols and within a few hours [8]; because of the potential for systemic reactions, we recommend using a university or hospital center [11]. VIT is well reported, but protocols differ according to authors: ultra-rush in 3 h, accelerated in 2–5 days and semi-rush in 2–8 weeks [96, 125]. Venom-allergic patients underwent a modified ultra-rush desensitization protocol consisting in a *2.5-h ultra-rush desensitization by the SC route,* reaching the cumulative dose of 101.1 µg according to Table 17.10 [105]. The treatment caused a rapid variation of IgE and IgG_4 beginning the 15th day. SPT results became negative in 15 patients (27%) and decreased in 14 patients (25%). This *effective and safe ultra-rush desensitization can be adopted even for children and teenagers* [105]. A second protocol [125] reached a cumulative dose of 150.11 µg in 3 h plus a final dose of 100 µg on the 2nd day and was also effective in 14 children aged 6–18 (mean 9.7) (Table 7.10). A novel strategy of VIT based on long synthetic overlapping peptides (LSPs) mapping the whole sequence of PLA_2 (phospholipase A_2), a major bee venom allergen was safe and able to induce Th1-type immune deviation [27].

Table 17.10. Modified ultra-rush desensitization protocol

Dose administered in µg	Time (min)
0.1	0
1	30
10	60
20	90
30	120
40	150

The Maintenance dose (100 µg) was administered after 15 days and thereafter once a month.
Modified from [105].

Dose administered in µg	Time (min)
0.01	0
0.1	30
1	60
10	90
20	120
40	150
80	180
2nd day	
100	

The Maintenance dose (100 µg) was administered after 7–14 days, after 3 weeks, then every 4–6 weeks for 4–5 years.
Modified from [125].

Biting Insect Allergy

Beyond the Hymenoptera there are numerous other stinging insects that bite, including common or domestic flies, fleas, fire ants, mosquitoes and bedbugs, whose most common species is the *Cimex Lectularius* (Table 17.11) [138]. Mosquitoes are overrepresented worldwide, encompassing >40 mosquito genera and more than 3,000 mosquito species [122].

Pathogenesis

Reactions to mosquito bites are immunological in nature, with the involvement of IgE-, IgG- and T lymphocyte-mediated hypersensitivities [88] and are caused by the proteins in the mosquito saliva [87]. These reactions consist of inflammation caused by the trauma inflicted on the skin and the act of sucking performed by the insect while simultaneously introducing irritating and antigenic substances in the host [50]. Various studies have demonstrated the necessity of a *phase of sensitization* before the development of a bite reaction: the pro-

Table 17.11. Biting insects reported to cause allergic reactions

Order	Family	Genus	Common name	% Of anaphylaxis cases[a]
Diptera	Cimicidae	*Cimex*	Bed bugs	
	Muscidae		House fly	15.5
	Culicidae	*Aedes* *Anopheles* *Culex*	Mosquito	10.3
	Simulidae	*Simulium*	Black fly	18.9
	Tabanidae	*Crysops*	Deerfly	13.8
		Tabanus	Horsefly	15.5
Hemiptera	Reduviidae	*Triatoma*	Kissing or conenose bug	29.3
Siphonaptera	Pulicidae	*Pulex*	Cat or dog flea	3.4

[a] % Of reactions: see the AAAAI study [50] in the text.
Data from [138].

gression is schematized into *five stages* illustrated in Table 17.12 [50, 103]. A sensitizing bite is needed before a reaction can occur to a specific insect bite; a nonreactive period follows the first exposure. This type of reaction depends upon the number of bites, and time between initial sensitization and challenge leading to the acquisition of tolerance, which suggests the development of a protective mechanism [50, 103]. The role of the immune system in the pathogenesis is not clearly defined, despite the *evidence of IgE antibody involvement* in some reactions employing extracts from the insect salivary antigens [78, 89] or from insect saliva [102]. In the reactions to mosquito bites, IgE is involved *in the development of skin immediate reactions* in mosquito-allergic subjects [86]. Other studies on mosquito saliva have documented that 18% of North American healthy blood donors without any specific history of mosquito allergy had mosquito saliva-specific IgE levels >1 SD above the mean for mosquito bite test-negative subjects [91]. Although IgE does not cross the placenta, the IgE reactivity to the mosquito *Aedes aegypti* (Aed a) antigen is so strong that it was found in the cord blood probably because of a mixture of maternal and fetal blood during delivery [87]. In the saliva or salivary gland extracts from ten mosquito species, seven of which have worldwide distribution, 3–16 salivary allergens with molecular masses ranging from 16 to 95 kD were found in each species. *Species-shared and species-specific allergens that cause IgE responses* in subjects allergic to mosquitos are immunological in nature. Species-shared allergens are the most important for potential use in diagnosis and immunotherapy [87]. Recently, the gene encoding a 37-kD salivary gland protein of *Aedes aegypti* has been cloned and expressed, and using the antibody to the recombinant protein, the 37-kD *Aedes aegypti* saliva protein was found in *Aedes vexans* (Aed v) and *Culex quinquefaciatus* (Cul q) salivary gland extracts, confirming the existence of species-shared antigens in the three species, which may explain the cross-reactivity of skin reactions and IgE responses among different mosquito species [86]. In late reactions after 2 h, neutrophils and eosinophils are found, in addition to CD4 and CD8, which appear after 24 h and parallel other types of such reactions [103].

Table 17.12. Classification of cutaneous reactions to insects

Immunological stage	Reaction	
	Immediate: 15 min	Delayed: 24 h
Stage I (no sensitization)	–	–
Stage II	–	+
Stage III	+	+
Stage IV	+	–
Stage Y (tolerance)	–	–

Data from [50, 103].

Clinical Presentation

In addition to causing local and systemic reactions, biting insects serve as vectors for serious diseases [10]. Mosquitoes (order Diptera), including black flies, house flies, fruit flies, gnats, midges [50, 122, 138] (Table 17.11), and the tiger mosquito (*Aedes albopticus*) bite, which causes pruritus of variable intensity as well as swelling and blisters. Pediatric cases of yellow fever caused by the tiger mosquito have not been reported in Europe, but they have been reported in Southeast Asia where they originate [122].

The genus *Triatoma*, a night species found in illuminated homes, exclusively attacks vertebrates, although

Table 17.13. Clinical expression of mosquito bite reactions

Reaction types	Frequency
Immediate (up to 4 h after sting)	
Wheal and flare	Very common
Large edema	Not common
Anaphylaxis	Very rare
Delayed (>4 h after sting)	
Pruritic papules	Very frequent
Pustules or erythema surrounding hemorrhagic lesions	Quite common
Blister or tense bulla	Rare
Papular urticaria	Frequent in children
Erythema multiforme-like rash	Rare

Data from [103].

the bites are not painful and the victims are awakened by itching and respiratory distress, in contrast to the Tabanids whose bite is deep with notable bleeding [138]. The various types of clinical expression of mosquito bite reactions are summarized in Table 17.13 [103]. Anaphylaxis is, as a rule, a rare event [50, 66]: over 5 years of observation, the pertinent AAAAI committee has tested the serum of 132 patients who presented with 58 anaphylactic reactions, of which 36 out of 132 (27.3%) were RAST+ (Table 17.11), 47.2% were IgE-related and 29.3% were reactions to the bite of Triatoma [50]. In addition, there were 23 equivocal reactions in 14 patients, 6 of which to Triatome (26.1%) and 5 to deerfly (21.7%) [50].

Diagnosis

Diagnosis is made with nonstandardized whole-body extracts when salivary extracts are unavailable. SPT interpretation is compared to that of control subjects: the sensitization, specificity and positive or negative predictive values are not reported [66].

Both RAST and histamine release tests are useful. The frequent findings of *cross-reactions between insect and Hymenoptera stings* should be noted [50]. Up to 50% of patients with stinging-insect allergy have double-positive RAST results to honeybee and yellow jacket venom, and carbohydrate-specific IgE is a major cause for the double positivity seen in patients with Hymenoptera allergy. The *double sensitization and cross-reactivity* through venom hyaluronidases may be a logical explanation for this multiple reactivity [47]. Because of the lack of salivary preparations, allergic reactions to mosquito bites are *underdiagnosed and undertreated.* The diagnosis is also made difficult by the strong cross-reactive skin and IgE responses and species-shared antigens existing among the three mosquito species Aed a, Aed v, and Cul q [86]. However, the use of recombinant salivary mosquito allergens will greatly facilitate the diagnosis and, possibly, the SIT of mosquito allergy [90]. The tiger mosquito can be recognized by its black color, with white bands on its body and legs that give it its name.

Treatment

For local reactions, therapy is provided as detailed above with anti-inflammatory or antihistamine ointments; for anaphylactic reactions, refer to Chap. 20. Cetirizine has had a positive effect on the cutaneous reactions as well as on itching [101]. VIT is used with the *ultra-rush method* in cases of necessity with generally good results [66]. In a meta-analysis of ten studies, four of which studied 56 children aged 1–9 years, the results were positive in 41/56 (73.2%) cases [66]. VIT was also positive in children with severe skin inflammatory reactions to Aed a and Cul q mosquito bites [6]. The use of *repellents on clothes and skin* is suggested, especially for *infants and young children* [10]. However, one should select among currently available repellents those providing prolonged protection and those that can be relied on to provide effective protection in environments where mosquito-borne diseases are a substantial threat [28].

Prevention

Patients with hypersensitivity to Hymenoptera stings must, with precision, avoid all high-risk situations which include the summer season and during periods spent outdoors and in proximity to stimuli such as colors, perfumes or foods which attract the offender [10]. Bees are normally only aggressive and sting people only when alarm pheromone is released, while the Vespulae are particularly aggressive in the autumn when food is scarce [72]. Children at risk should wear a *MedicAlert bracelet or necklace,* and carry *Epi-Pen junior* for emergency self-treatment in case of future reactions caused by insect stings [93, 137]. These notes are expanded in Chap. 24 along with the proposed measures regarding other insects [10].

Pediatricians and Insect Allergy

The prevalence of Hymenoptera allergy has increased during the past decades. However, in the pediatric age group, insect allergy has a low prevalence, a propensity to elicit only mild clinical manifestations with a high assurance of definitive treatment. The vast majority of bites and stings cause little more than local pain and never require medical attention. We recommend that pediatricians who work in the emergency department

be prepared to treat the few children who present with anaphylactic reactions to Hymenoptera stings and recognize and treat those rare children who are severely bitten by poisonous snakes, spiders, or scorpions [52]. We also recommend that at-risk children always carry an Epi-Pen junior injector. Moreover, the prognosis of insect venom hypersensitivity in children is extremely favorable. Continuing our ideal conference, pediatricians should play down the importance of the condition by explaining to parents that both clinical characteristics and prognosis are different and even more favorable from those in adults. Although most insect stings produce only local discomfort, we stress that VIT may represent a definitive treatment. VIT is able to protect insect venom-allergic children against life-threatening sting reactions. Pediatricians can better provide information on the often self-limiting natural course in most of these children.

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Allergic and Pseudoallergic Reactions to Drugs

Pediatric Drug-Induced Disorders

The rapid development of new drugs to cure or control human illness corresponds to an expanding spectrum of adverse reactions to such agents. Before the introduction of sulfonamides in the late 1930s, the incidence of adverse drug reaction (ADR) approximated 0.5%–1.5% of all patients. Currently, 15%–30% of hospitalized patients experience an ADR [120]. The zenith is found in nurslings and toddlers. Drugs cause allergic and pseudoallergic reactions (PAR), even though other researchers, especially the Anglo-Americans, seem to consider it more suitable to call them ADRs, obviously shaped on the definition of adverse reactions to food, thus foregoing a pathogenic classification [2].

Definition

Adverse drug reactions may be defined as undesired and unintended noxious consequences of an appropriate drug that is administered in standard doses by the proper route for the purpose of prevention, treatment or diagnosis, defining as a drug any substance used for this purpose [67].

Prevalence

Prevalence in children ranges from 1% to 7.3% [41, 61, 90, 110], whereas in the general population it is estimated at about 15%, with figures ranging from 14% to 17%. Allergic reactions correspond to about 6%–10% of the total [51]. The age group most affected has been investigated. Data from a case study of 163 patients including 8-month old babies show that while ADR prevalence appeared to be higher during the first four decades of life, the figures in absolute terms for the first 10 years reached the highest peak [98].

Incidence is 5% in newborn babies, 11% in children aged 1–2, and 9% for children aged 3–10 [61], or 76% for those aged <7 [66] and 59.5% if the age is 7–12 years [211]. Therefore, *neonates are by no means unaffected,* with an incidence that sometimes matches 24.5%, mostly involving erythematous maculopapular rashes (67.1%) [90]. The majority of reactions is to antibiotics [23, 104, 224, 228], with a clear predominance of β-lactams [49], which in children account for 67.7% and 53.3%–55.2% of ADRs, respectively [81, 167], followed by aminoglycosides, tetracyclines, vancomycin, nitrofurantoin (9.8%) and macrolides (9.5%) [81], to which 35.1% of children in another case study reacted positively [167]. In 46 out of 214 children (21.5%), antibiotics-associated ADR (50%) predominated, followed by corticosteroids (CSs) (16%), tuberculostatic (4%) and immunosuppressive agents (4%). In five cases, an ADR was responsible for prolongation of hospital stay, and in four children ADRs were responsible for hospitalization [224]. However, incidence decreased in very large groups: 152 ADRs affecting 150 children were documented in a cohort of 12,628 children (1.2%), 60% of these occurring within 3 days after treatment initiation: 47% of reports were related to antibiotics, 31% to immunizations, 11% to respiratory, and 4% to gastrointestinal agents [104]. Table 19.1 [33, 44, 144, 148] reports the prevalence of allergic and PAR to several drugs

Table 19.1. Prevalence of allergic and pseudoallergic reactions to some drugs (range of values of several statistics)

Drug	Prevalence (%)
Acetaminophen	2–7
Antibiotics in general	34
Anticonvulsants	7
ASA	15–21
B vitamins	4–5
Disinfectants (oral, cutaneous)	2
Erythromycin	1
Insulin	2
Iodinated compounds	3
Neuroleptics	6
NSAIDs	39
Penicillin	6–29
Propionic acid derivatives	2–8
Pyrazolone derivatives	1–40
Sulfonamides	3–14

Data from [33, 44, 144, 148].

Table 19.2. Prevalence of allergic and pseudoallergic reactions to some drugs that required hospitalization of infants/children

Drug	% Of reactions	
	Total (*n*=76)	Severe/moderate (*n*=27)
Analgesics	9.2	11.1
Antibiotics	19.8	3.7
Anticonvulsants	3.9	11.1
Bronchodilators	10.6	7.4
Cardiovascular	10.6	14.8
Muscle relaxants	7.9	–
Sedatives	15.8	37
Steroids	7.8	3.7
Others	11.8	3.7

Data from [61].
ASA acetylsalicylic acid, *NSAID* nonsteroidal anti-inflammatory drugs.

and Table 19.2 shows the prevalence in children aged 4 days to 16 years [61]. Confusing figures include a high rate of errors in prescriptions and dosage [204], in the use of drugs beyond their indications [61] and the inclusion in pediatric statistics of cases of poisoning and/or oncology patients [61]. Overall, the frequency of drug-related allergies in infancy is underestimated [152] or assimilated to allergic reactions in adults [215]. The true impact is therefore much greater, since 99 substances listed among drugs, vaccines and diagnostics can cause anaphylaxis, considering that some are included within their class, or by examining the 1,000 most prescribed drugs, it is possible to calculate that *at least 70 % provoke ADR* [152].

Classification

Adverse drug reactions can be divided into in four main types [47, 97, 214]:

- *Type A:* an *increase* in *predictable* but undesirable clinical and/or pharmacological effects depending on the intrinsic properties of drugs, occurring in otherwise normal children. This type is dose-dependent, common (80 % of adverse drug reactions), generally not severe: collateral and side effects of drugs, toxic reactions, and drug interaction.
- *Type B: an increase in bizarre and unpredictable* effects, independent of drug action and dose, relatively infrequent (20 %), often severe (in 1902 a case of shock was due to acetylsalicylic acid administration) [75], and individual response by predisposed subjects: allergic, pseudoallergic, intolerance-induced, and idiosyncrasies [47, 97].
- *Type C: chemical* reactions that could be predicted from the structure of the drug or its metabolites. Examples include acetaminophen, which is bioactivated in the liver to a hepatotoxic quinone imine and azathioprine, metabolized to myelotoxic 6-mercaptopurine and must be further converted by the enzyme thiopurine methyl transferase [60].
- *Type D: delayed reactions* from drugs including teratogenicity and carcinogenicity [60], rarely found in children.

This classification has been somewhat overlooked by immunopharmacology, a recent science that has revealed how a certain number of drugs can modulate the immune response [124]. In most cases, an ADR can fit into one of the four classic types of hypersensitivity (Table 19.3) [46, 120], to which autoimmune reactions must be added (Chap. 18). It is believed that 25 % of cases of drug reaction are of *immunological pathogenesis* and in 75 % of cases are an *extraimmunological pathogenesis*, further divided into pseudoallergic, idiosyncratic and toxic reactions [214]. A more useful classification is nosological [205], both for the frequent mixing of these four types [23] and for symptom identification caused by allergic and pseudoallergic reactions [221].

Etiopathogenesis

Genetic Factors

Genetic factors play a vital role in hypersensitivity to drugs, conditioning the specificity of sensitization to a given drug or chemical substance. Various experimental studies have aroused understandable interest, by showing that ADRs are conditioned by specific genetic factors linked to HLA. At present, there are relatively few examples of restrictions or associations of HLA with respect to various allergies to drugs: *HLA DQw2* in patients with asthma caused by ASA, *HLA B7*, *DR2*, *DR3* in allergy to insulin, *HLA DR2/DR3* in allergy to D-penicillamine, *HLA DR4* in hydralazine-induced SLE [205], *HLA A2*, and *DRw52*, in patients with delayed hypersensitivity to aminopenicillins with a relative risk (RR) of 6.76–9.28 [169] and HLA-DR in eosinophilic pneumonia [13]. The rapidly evolving field of immunopharmacology has already clarified that many individual variations in drug metabolism are *genetically determined* [124]. Genetic factors can therefore act at various levels in the biochemical route to sensitization such as formation of reactive metabolites or selectively inherited capacity to develop specific antibodies to a given chemical substance [45]. A strong association of hypersensitivity to the HIV-1 reverse-transcriptase inhibitor, abacavir, was found with the 57.1 ancestral haplotypes *HLA-B*5701*, *HLA DR7*, and *HLA-DQ3*, such that there was a positive predictive value of 100 % and negative predictive value of 97 % in the presence of this haplotype [112]. A later replication study showed much poorer predictive value

Table 19.3. Classification of drugs most frequently implicated in allergic drug reactions

Organ System	Manifestations	Gell and Coombs type	Examples
Generalized	Anaphylaxis	I	Allergy extracts, chymopapain, penicillin, polypeptides
	Serum sickness-like reactions	III, I?	Antilymphocyte globulin, hydralazine, penicillin, polypeptides, sulfonamides
	Drug fever	II?, IV?	Barbiturates, CAF, cephalosporins, penicillin, phenytoin, quinidine, sulfonamides
	Vasculitis	III, IV?	Allopurinol, hypoglycemic agents, iodide compounds, NSAIDs, penicillin, propylthiouracil, sulfonamides
	Systemic lupus erythematosus	III?	Hydralazine, isoniazid, phenytoin, procainamide
Dermatological	Urticaria-angioedema	I, III?	Allergen extracts, foreign antisera, insulin, neomycin, organ extracts, penicillin, sulfonamides, thiouracil
	Morbilliform/ maculopapular	?	Allopurinol, ampicillin, barbiturates, CAF, erythromycin, naproxen, phenytoin, quinidine, sulfonamides
	Fixed drug eruptions	IV?	Barbiturates, penicillin, phenolphthalein, tetracycline
	Allergic contact dermatitis	IV	Antihistamines, neomycin, parabens, penicillin, thimerosal
	Erythema multiforme	?	NSAIDs, penicillin, phenylbutazone, phenytoin, sulfonamides
	Toxic epidermal necrolysis (Lyell syndrome)	IV?	Barbiturates, hydantoin, phenylbutazone, isoniazid, penicillin, pyrazolones, sulfonamides
	Purpura	?	Antihistamines, barbiturates, phenylbutazone, sulfonamides
	Exfoliative dermatitis	?	Allopurinol, carbamazepine, penicillins, sulfonamides
	Photosensitive reactions	IV	Furocoumarins, griseofulvin, psoralens, sulfonamides
Cardiac	Myocarditis	?	Methyldopa, penicillin, sulfonamides
Hematological	Eosinophilia	?	Aminoglycosides, carbamazepine, gold, phenothiazine
	Coombs + hemolytic anemia	II	Cephalosporins, chlorpromazine, insulin, methyldopa, penicillin, phenacetin, quinidine, sulfonamides
	Thrombocytopenia	II	ASA, carbamazepine, heparin, hydantoin, imipramine, quinidine, quinine, ranitidine, stibophen, thiazides
	Neutropenia	II?	Analgesics, antithyroids, penicillin, ticlopidine
	Lymphadenopathy	?	Phenytoin
Respiratory	Asthma	NI	ASA, NSAIDs, indomethacin, timolol, propanolol, naproxen
	Pulmonary infiltrates	?	Penicillin, sulfonamides, carbamazepine, cromolyn sodium
	Pulmonary edema	?	Hydrochlorothiazide, nitrofurantoin, opiates
	Hypersensitivity pneumonia	IV?	Gold salts, hydrochlorothiazide, nitrofurantoin, procarbazine
Hepatic	Acute hepatitis	IV?	Halothane, isoniazid, methyldopa
	Cholestatic jaundice	?	Phenothiazines
	Chronic hepatitis	?	Methyldopa, nitrofurantoin
Renal	Acute interstitial nephritis	II?, IV?	Cephalosporins, methicillin, NSAIDs, penicillin, sulfonamides
	Chronic interstitial nephritis	?	ASA, phenacetin
	Nephrotic syndrome	?	Captopril, paramethadione, penicillamine, trimethadione
Neurologic	Myasthenic syndrome	II	Penicillamine
	Encephalomyelitis	IV?	Live virus vaccines

Modified from [46, 120].
ASA acetylsalicylic acid, *CAF* chloramphenicol, *NI* nonimmunological mechanism, *NSAIDs* nonsteroidal anti-inflammatory drugs.

in another population, highlighting the limitations of such studies [74]. However, administering a single pharmacologically active molecule can sometimes lead to the formation of antibodies directed against different epitopes; consequently the association with HLA antigens would continue, in a certain sense, to be masked, nor are there sufficiently sensitive immunochemical instruments to evaluate the specificity of synthesized antibodies and the correlation with HLA antigens in drug-allergic subjects [124].

Allergic Reactions

Allergic ADRs occur when the immune system recognizes and reacts to a drug-derived antigen, which can also be represented by neo-epitopes formed by virtue of drug-induced clinical changes, or the epitopes may not be recognized as self by adjacent structures eluding immunosuppression [205], as we have seen in another context in Chap. 18.

We all know that only a very small quantity of antigen or drug is needed to trigger an allergic ADR [23]. Therefore, since low-molecular-weight (MW) (<1,000 Da) substances and most drugs fall into this category, given that they are not immunogenic in their native state and have low MW (<1,000 Da), to become effective immunogens, they must be covalently bound to high-MW carrier proteins and they should bind to a carrier protein, a hapten in order to acquire this property [38]. Two different kinds of drugs can be differentiated (Fig. 19.1):

- *Low-MW* molecules act as haptens, and should be conjugated with a protein carrier to become antigenic and elicit immune responses.
- *High-MW* molecules (>5,000 Da) act as complete antigens (Table 19.4) [2, 46].

B cells recognize soluble antigens by IgM and IgD receptors, processing and presenting antigens associated with class II molecules. T lymphocytes cooperate with activated B cells with the help of IL_{4-6} (Chap. 1): this mechanism accounts for the *hapten–carrier* bond, also explaining why TcR recognizes the antigens in association with HLA molecules [124]. Hence, the hapten drug, to acquire antigenicity, must bind to a carrier protein *to form an epitope* that is recognized by B and T cells, one group independently of the other [38] (Fig. 19.2). Another characteristic is found in the particular kind of bond. Drugs that are capable, and most are, form covalent bonds with tissue proteins, which are far more immunogenic than those that are relatively less reactive. This kind of bond, however, is not indispensable: even if not covalent, there may be sufficient affinity for the drug–carrier unit to remain intact during both processing and presenting stages. Nor is it necessary for the drug to be highly reactive, since its hydrolysis or biotransformation in the organism can serve as haptens [221]. The fact that some haptens can be derived from these changes makes epitope identification difficult.

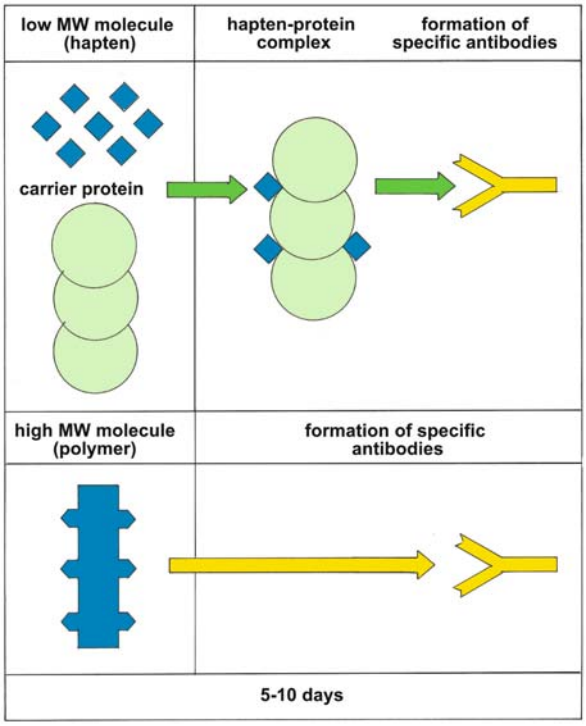


Fig. 19.1. Drug hypersensitivity: molecular weight (*MW*) and antigenicity

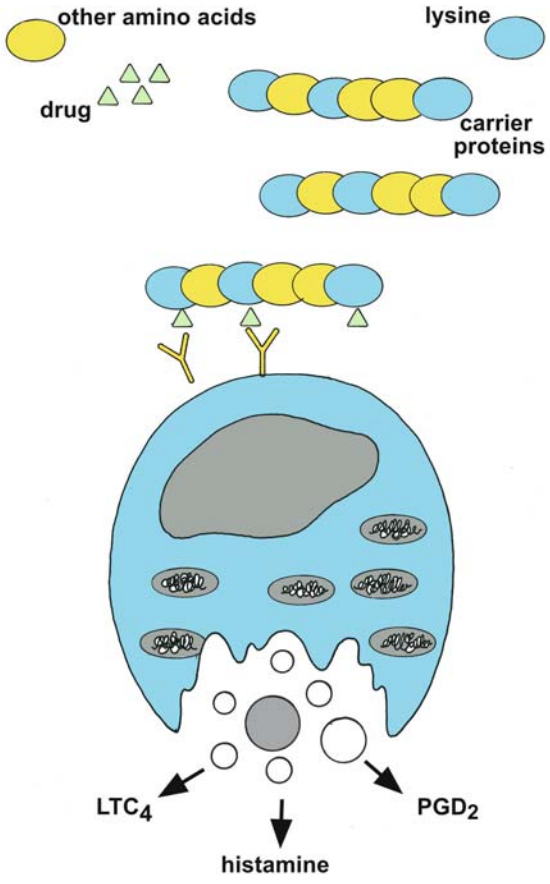


Fig. 19.2. Type I drug hypersensitivity

Table 19.4. Antigenicity of some drugs (complete antigens and haptenic drugs)

Complete antigens
Enzymes Asparaginase, bromelin Chymopapain, chymotrypsin Lysozyme, penicillinase Streptokinase, streptodornase
Dextran
Heparin
Heterologous sera
Insulin
Organ extracts (ACTH, hydrocortisone)
Vaccines
Haptenic drugs
Anticonvulsants
Antituberculotic drugs
β-Lactam antibiotics
Cephalosporins
Ethylene oxide
Local anesthetics
Muscle-relaxants
Non-β-lactam antibiotics
Penicillins
Protamine sulfate
Quinidine
Sulfonamides

Data from [2, 46].
ACTH adrenocorticotropic hormone.

Alternatively, *neo-epitopes* may form, as for example in kinin-induced thrombocytopenia, with synthesis of specific IgGs for quinine bound to platelets, therefore an antigenic neo-epitope not cross-reacting with other hematic cells or other tissues [221]. Hapten–carrier interactions, with formation of stable bonds with acyclic, amide or sulfhydryl bridges (*haptenization*) [38, 48, 197] create immunogenic bonds, which can form depending on the different ways outlined in Fig. 19.3 [221]. A classic example of haptenization is that of sulfonamides [197]. In other cases, a single unbound molecule should link to an IgE molecule, and the consequent mediator release. In theory, this takes place with molecules having two or more identical or similar epitopes or involving different epitopes belonging to the same molecule. Local anesthetics are an example of the first type, while thiopental, trimethoprim-sulfamethoxazole (TMP-SMX) and β-lactam antibiotics all have more than one epitope binding to IgE [2]. Such small T epitopes, consisting of a few amino acids, being unable to bind to specific IgE (sIgE), or of causing histamine release, cannot elicit anaphylactic reactions, like monovalent haptens (those containing only one epitope) [44, 45], and unlike the bivalent or polyvalent forms (Fig. 19.4). Drugs that have a high MW and are complete antigens (Table 19.4) are directly immunogenic [2]. As seen in Table 19.3, drugs also cause *type II reactions* with complement participation (Fig. 19.5), *type III reactions* with CIC (circulating immune complex) formation and *type IV* or cell-mediated reactions. It appears that like haptenic contact allergens, other drug haptens are not recognizable by T lymphocytes unless they are conjugated with a protein carrier [38]. Thus APCs (antigen presenting cells) process the hapten-modified carrier protein, degrading it to peptides that are complexed with HLA molecules and presented to antigen-specific T cells [60]. If presentation occurs in the context of appropriate activation signals, an immune-initiated inflammatory cascade ensues that leads to the development of clinically relevant drug-induced disease [158].

Consequently, the offending drug *may both possess and acquire antigenic properties*, binding to organic proteins and denaturing them: when it is possible to identify drug-specific antibodies, these would have diagnostic value. Broader knowledge of roles played by allergic reactions is in line with recent findings on drug recognition by specific T lymphocytes, meaning that APC before protein-hapten uptake and processing is unnecessary, because T-cell activated clones can recognize drugs with the HLA–peptide complex in a noncovalent manner. This type of presentation presents several interesting similarities with that of superantigens (SAs), but the drugs are incapable of stimulating PBMCs (peripheral blood mononuclear cells) in unsensitized subjects [218]. In addition, drugs have a MW that is much lower than that of SAs and consequently a T-cell stimulation is not as strong as that of SA. So T cells maintain an intermediate position between SA and peptide stimulation [218].

The role of T cells in ADRs is controversial because T cells recognize peptide antigens and not the low-MW compounds of which drugs are an example [184]. However, T-cell phenotype and cytokine release may correlate with the type of immune response involved in cutaneous drug reactions. Increasing in vivo and in vitro data indicate that cell-mediated immunity (CMI) is particularly involved in some cutaneous reactions such as drug-induced maculopapular exanthems and sulfonamide-induced reactions [67]. The involvement of T cells in the pathogenesis was demonstrated by a child with X-linked agammaglobulinemia who experienced a maculopapular rash after therapy with ceftriaxone, and an additional dose provoked a recurrence of the rash with severe itching, flushing, and hyperthermia. The child had no B cells or detectable IgE and negative SPTs to penicillin reagents and ceftriaxone, but further studies revealed a positive patch test to ceftriaxone and infil-

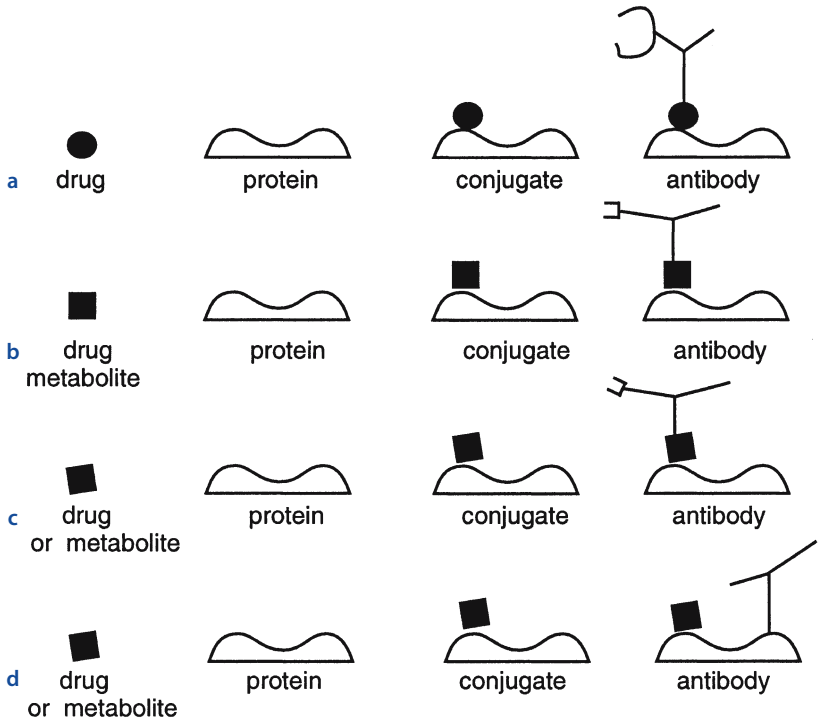


Fig. 19.3 a–d. The interaction drug-antigen may result in antibody formation (as shown) or T cells directed against epitopes (see text). The specific antibodies are directed against (**a**) the native molecule, (**b**) the drug biometabolites, (**c**) a new epitope formed by the interaction between the drug or its metabolites and the protein. **d** As a result, conjugation of the drug or its metabolites leads to a conformational change in the protein, which is then recognized as non-self by the immune system. (Modified from [221])

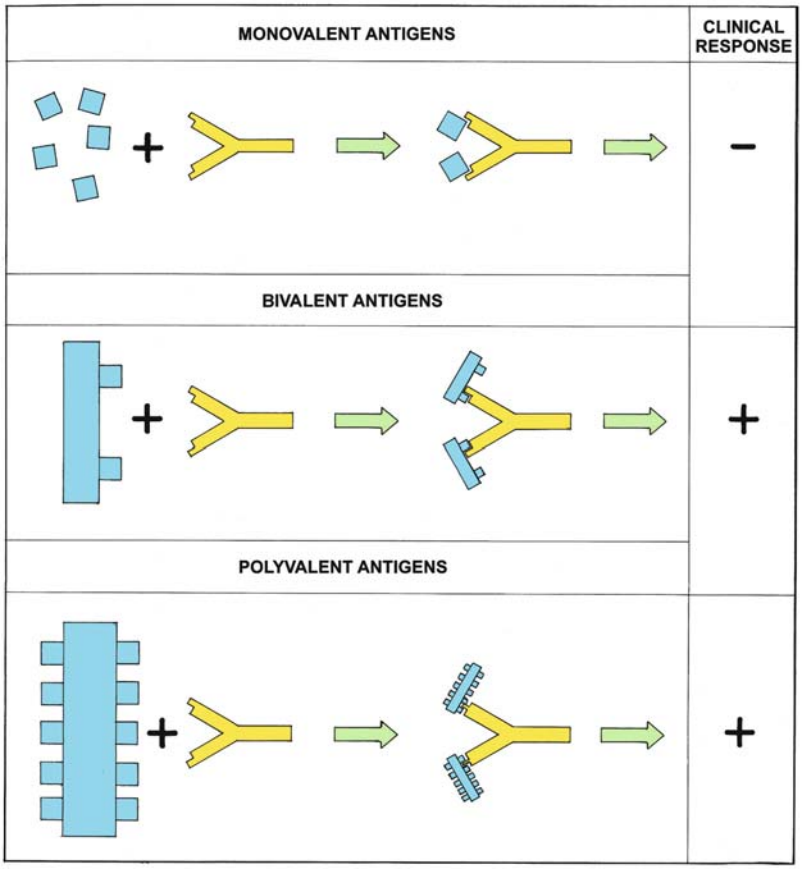


Fig. 19.4. Drug hypersensitivity: valency and antigenicity. Monovalent antigens including most drugs are incapable of eliciting an allergic reaction, which requires cross-linking of the antigen binding site on the Fab part of antibody molecules. To quickly cross-link, they must become bivalent or multivalent antigens, such as haptens attached to a carrier macromolecule, to becoming able to prime a reaction

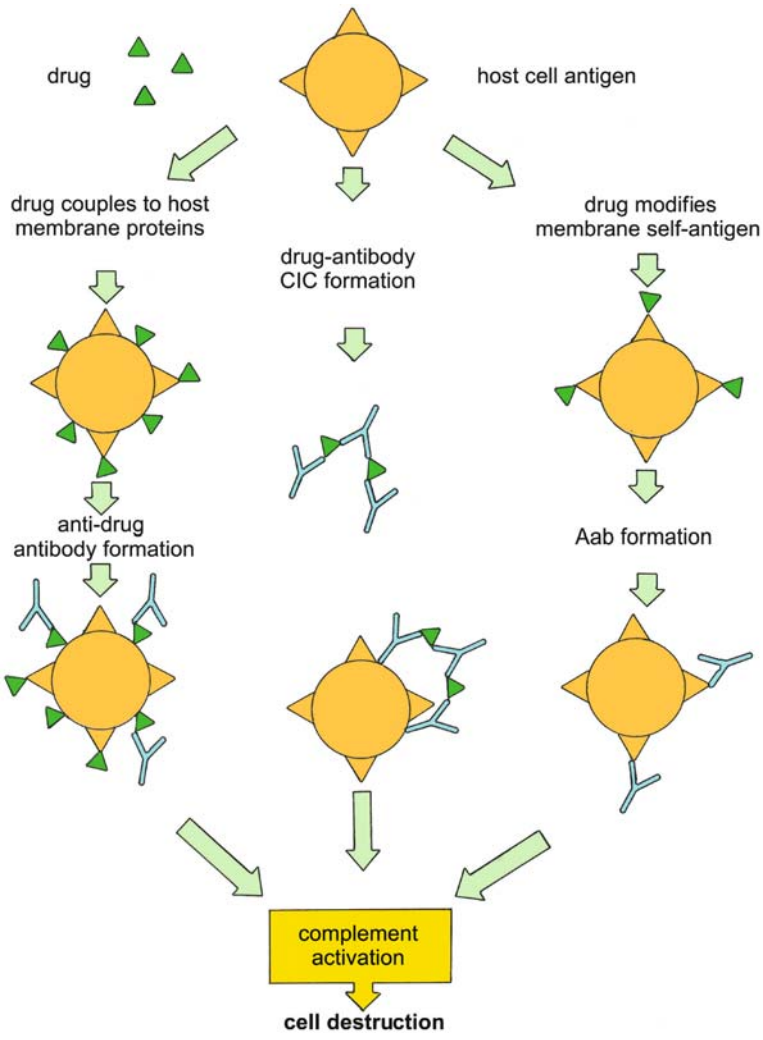


Fig. 19.5. Type II drug hypersensitivity. Complement-mediated cell destruction takes place in different ways. *Left*: Drugs covalently bind to host membrane proteins; anti-drug IgG antibodies bind to the drug and activate complement (such as, penicillin). *Middle*: Circulating drugs and IgG antibodies form CICs, which attach to cell membranes, thus activating complement cascade (such as cephalosporins). *Right*: Drugs bind to cell membranes, but immune responses are directed against the formerly altered host cells: Aabs are formed, also bind to the cell in drug absence, thus activating complement (such as α-methyl-dopa)

tration of the test site with $CD4^-$, $CD7^-$, and CD45RO-positive mononuclear cells, indicating that such rashes are T-cell mediated and occur in the absence of IgE [88]. The skin of patients with an exanthem may be infiltrated by T cells and show a marked enhancement of perforin and granzyme B immune staining, both expressed in T-cell $CD4^+$ and $CD8^+$ cells, partly located at the dermoepidermal junction and in the perivascular dermis [231]. In acute lesions, there was a large increase in TNF-α (9-fold), perforin (6-fold), and granzyme B (7-fold) with positive correlations with disease severity in patients with reactions to drugs in comparison with control subjects. FasL was expressed in PBMCs only in Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [158]. In the blister fluid of a patient suffering from cotrimoxazole-induced TEN, blister fluid cells were evaluated. Blister fluid lymphocytes were predominantly $CD8^+$, DR^+, CLA^+, $CD56^+$ T lymphocytes, perforin-positive and expressing preferentially two Vβ chains of the TcR repertoire. These lymphocytes were cytotoxic to autologous cells only in the presence of cotrimoxazole and some related metabolites [126]. Most phenobarbital-specific T-cell clones and lines from patients with cutaneous and extracutaneous involvement exhibited a Th2 phenotype, but those with Th1 or Th0 phenotype also constituted minor populations [70]. Further, strong immune reactivity for IL_{12} and IFN-γ was observed in the PBMC infiltrate, indicating that these ILs may be crucial in activation of cytotoxic T cells (CTLs) [231]. *Drug-induced maculopapular exanthems* express significantly increased numbers of eosinophils and amounts of IL_5 and eotaxin and markedly lower amounts of IL_8 in comparison with controls [232]. An elegant study has reported on the IL profiles of PBMCs collected from patients with an ADR. The PBMCs of patients with immediate reactions (urticaria or anaphylaxis) displayed predominantly IL_4. Those from patients with nonimmediate reactions (exanthema or SJS) displayed predominantly IFN-γ, TNF-α, and IL_2, thus showing *the classic Th1/Th2 paradigm* in vivo [157]. A subsequent trial in patients with nonimmediate immunological reactions (severe or mild) to β-lactams (40%) and anticonvulsants (30%) has confirmed that there was a high increase of IL_2, IFN-γ, and TNF mRNA

Table 19.5. Characteristics of drug-related allergic reactions

Previous treatments without drug-related reactions
The reaction occurs after several days of treatment even with no history of a previous drug-related reaction
Reactions are not reduced by doses well below the therapeutic range
Symptoms induced may be dissimilar to other classic allergic reactions
Reactions are encountered only in a small number of patients receiving the drug
Reactions occur in a restricted number of syndromes defined as allergic
Antibodies or T lymphocytes react with the drug or related metabolites in a few cases
Reaction may be reproduced by other agents possessing similar and cross-reacting chemical structures
Reactions may be reproduced even by a minute dose of the suspected drug or other chemically related molecule
Reactions should resolve after drug discontinuation

Modified from [207].

expression in both groups at the acute stage, with a significant relation between IFN-γ and TNF-α only in severe reactions. Thus, ILs appear to play a closely related role in mild reactions, whereas cytotoxic markers appear more relevant in severe reactions. The complexity of the Th1 phenotype after drug intake is fairly evident [159].

In conclusion, the *essential characteristics of drug-related allergies* can be outlined as follows, differentiating them from reactions that recognize dissimilar mechanisms [47] (Table 19.5) [207]:

- The phenomenon is *species-characteristic* and can usually be reproduced experimentally in all members of the same species. ADRs are objectively rare, involving few exposed subjects.
- To elicit an ADR, *previous sensitization to a given drug* is generally necessary (even if hidden) [47]. When the drug is administered for the first time, the *allergic reaction appears 6 and 10 days after start* of treatment. In the event of prior sensitization, the reaction occurs within a few minutes or several hours.
- Although the drug's chemical structure and therapeutic or toxic nature are widely different, *the clinical symptoms they cause,* having the same pathogenesis, *are uniform* and generally reproduce the classic symptoms of atopic diseases: shock, urticaria, bronchospasm, etc.
- *ADRs abate or fade several days after treatment discontinuance* (3–5 days on average), but reappear each time the same treatment is resumed [66]. ADRs *are reproducible* with similar chemical substances and are dose- or pharmacologically independent: they can be provoked by minute doses or can occur by taking other drugs with a similar chemical structure expressing cross-reactivity [47] (Table 19.5). Even if an allergy-related syndrome is recognized, it is often difficult, if not impossible, to prove the existence of prior sensitization, and it is equally difficult to prove that a drug can acquire immunogenic properties.

Nonallergic Reactions

Nonallergic reactions (NARs) are classified as unpredictable reactions: they are divided into PARs; those caused by intolerance and idiosyncrasies by definition do not recognize immune-mediated mechanisms.

In PARs there are three basic mechanisms [45]:

1. *Activation of mast cell mediators* by drug metabolites, often patient-specific, with a mechanism of direct basophil histamine release, as for example in radiocontrast media reactions [28, 165]. ASA-induced asthma involves mediator release from mast cells, as shown by the increase in neutrophil chemotactic activity following ASA challenge [137].
2. *Complement classic and/or alternative pathway* activation, with formation of C3a and C5a anaphylotoxins.
3. *Cyclooxygenase (COX) inhibition,* as in the case of NSAIDs that cross-react with ASA. This inhibition boosts the peptide-leukotriene release that stimulates bronchial hyperreactivity, a highly critical mechanism in ASA-induced asthma (Fig. 19.6) [163]. Recent data show that COX-2 inhibitors are unlikely to cause reactions in NSAID-sensitive patients [184].

Intolerance reactions are due to individual threshold lowering of pharmacological action, with a quantitatively increased effect, for example tinnitus caused by small doses of salicylate or an undesired drug effect produced by therapeutic or subtherapeutic dosages of the drug.

Idiosyncratic reactions occur in patients with genetically determined metabolic or enzymatic defects, after taking specific drugs: a deficit of G6PD is paradigmatic or an uncharacteristic reaction not explicable by known pharmacological actions of the drug [1, 67].

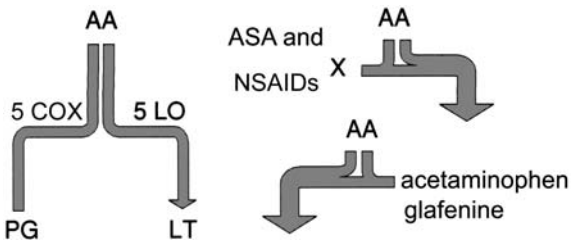


Fig. 19.6. Drug allergy/pseudoallergy: asthma induced by ASA and NSAIDs. *AA* arachidonic acid, *5 COX* 5-cycloxygenase, *LT* leukotrienes, *5 LO* 5-lipoxygenase, *PG* prostaglandins. Physiopathological hypothesis to explain ASA and NSAID-induced (pseudo)allergic reactions, as well as the reactions of intolerance to 5-LO inhibitors

ADRs that are considered predictable may depend on the following:

- *Overdoses* or toxicity. Examples include acetaminophen and toxic metabolite, aminoglycosides and nephropathies.
- *Drug interaction* from administration of two ≥2 drugs together with reciprocal modifications of their pharmacodynamic and pharmacological effects.
- *Undesired side effects* caused by pharmacological action directly linked to the therapeutic action such as first-generation antihistamines and sleepiness (Chap. 12).
- *Secondary effects* such as an indirect consequence of drug pharmacological action that often mimic a new pathology, for example oral candidosis by inhaled CSs (Chap. 11), alteration of oral and/or enteric flora by antibiotics [6, 46].

Predisposing Factors

Predisposing factors consist of atopy, age, sex, factors related to the drug itself and those related to concomitant infections or therapies [197] (Table 19.6) [20, 34, 77, 92, 97, 115, 145, 152, 164].

Atopy. It is not exact to state that atopic subjects are genetically predisposed to developing immediate ADRs [47]. The atopic phenotype relevance is inversely proportional to the sensitizing drug's immunogenic nature, while it is less decisive in strong immunogens [152]. Compared to nonatopic patients, there is no greater frequency in atopic patients of type I allergic reactions to injected allergens such as penicillin, Hymenoptera venom, or insulin compared to inhaled or ingested allergens [225]. The atopy prevalence in children is 16% [144], with much lower levels of 6% in adults [44]. Children of parents allergic to antibiotics have a 15-fold relative risk for histories of reaction to these drugs than controls with no positive history [9]. Confirmation comes from two studies on 1- to 15-year-old allergic to β-lactams with atopic heredity in 46%–60.3% of cases [66, 211] and 44.9%–45.2% with positive personal history [155, 211]. In addition, a questionnaire focused on family histories (FH) revealed cases of aspirin intolerance among parents, siblings, and children of ASA-intolerant probands [119]. In atopic children, systemic symptoms tend to be more severe compared to controls [155, 211].

Age. We have already noted that prevalence in newborns and in children is generally below the general population prevalence, indicating that they are less at risk than adults. This probably occurs because of the immaturity of immune responses or a lower exposure to drugs or smaller doses, in summary the necessary drug exposure for sensitization to occur [188]. Because drug pharmacokinetics and pharmacodynamics in children differ from those in adults, children are highly susceptible to ADRs. In a study in 278 children, the first episode occurred in *51.1% of children aged 0–4 years,* in 24.5% of those aged 5–8, in 16.9% of those aged 9–12, and in 7.6% of those aged 13–16, with no substantial differences between males and females (G. Patriarca, personal comunication). We found ADRs in 29.2% of 411 chil-

Table 19.6. Potential risk factors for drug allergy

1. Patient-related
Genetic factors
Atopy
Familial susceptibility
Previous history of anaphylaxis
Insufficient dehaptenization
Individual metabolic pathway
HLA phenotype
LMP polymorphism
TAP polymorphism
Age
Prenatal exposure
Maternal drug intake during pregnancy
Secondary exposure
Neonatal exposure
Child and adolescent exposure
2. Drug patterns
Chemical structure
Molecular weight and reactivity
Proteins and polypeptides
Small molecules
Reactive groups
Metabolites
Variability in drug metabolism
3. Drug-related
Cross-sensitizations
Multiple sensitizations
4. Pharmacological activity (nonimmunological)
Mediator release or activation
Autonomic activity
Enzyme activity
5. Treatment-related
Dose and route
Duration of drug treatment
Frequency of drug treatment
Patterns of drug exposure
Predictable ADR
Unpredictable ADR
6. Concurrent disease
AIDS
Asthma
Cystic fibrosis
Epstein-Barr virus infection
Immune deficiency
Infection-related factors
Endotoxins
Superantigens
Proteins with cytokine-like activity
Complement inhibition
Factors emphasizing the effector system

Data from [20, 34, 77, 92, 97, 115, 145, 152, 164].

Table 19.7. Cross-sensitivity related to the para group

Local anesthetics	**Preservatives**
Benzoic acid esters Benzocaine Panthesine Pantocaine Procaine	Parabens
Chemotherapeutics	**Dyes**
Para-aminobenzensulfonamide Para-aminosalicylic acid Sulfanilamide	Aniline derivatives Paraphenylenediamine (PPDA) Para-aminoazobenzene Para-aminoazotoluene Para-aminodiphenylamine Para-aminophenol Para-toluylendiamine Pyrazolone derivatives
Antibiotics of the neomycin group	**Antioxidants and vulcanization accelerators**
Gentamycin Kanamycin Neomycin Paromomycin	Dimethoxyphenylamine Dinaphtholpara-phenylendiamine Diphenylguanidine Hydrochinone benzyl ester Mercaptobenzothiazole Phenyl-β-naphthylamine Phenyl-α-naphthylamine Phenylcyclohexyl-paraphenylenediamine
Antimicrobial – Antifungal agents	**Substances possibly used for photographic development**
Substances of the quinoline group Quinine 8-Hydroxyquinoline Vioform	2,4-Diaminophenol Hydrochinone Metol Para-aminodiethylamine Para-aminophenol
Antiseptic agents	**Substances possibly used for heliographing**
Salicyl-anilide group Bithionol Hexachlorophene Tetrachlor-salicylanilide Tribromo-salicylanilide	Diazodiethylaniline
Additional drugs	**Substances possibly used as laboratory reagents**
Carbonic anhydrase inhibitors Sulfonylurea Thiazide diuretics	Benzidine Phenylhydrazine
Sunscreens	
P-aminobenzoic acid (PABA)	

Data from [182].

dren, 220 males and 191 females, aged 7 to 13. ADRs affected children more than food allergy (21.7%). Since pharmacokinetics has not been studied on a large scale in newborns and nurslings, many ADRs caused by prenatal, perinatal and postnatal exposure, especially in maternity wards, remain undiagnosed [68]. Moreover, since drug doses are not regulated on the basis of body weight (bw) and age (Chaps. 5 and 11), it is likely that in younger children there are higher drug blood levels, and in premature babies even higher levels compared to full-term babies and adolescents [68]. Age at time of the first ADR was *26.1±26.3 months* [143]. In addition, children often receive higher mg/kg doses than do adults [4].

Sex. According to some authors, females are more often affected than males [144], while others feel the opposite [211], the male/female ratio being 1.2:1 [90]. Reactions to anesthetics, ampicillin and ASA are more common in females [152].

The *individual drug characteristics* are outlined in the following sections.

Drug Nature

It is difficult to predict a drug's sensitizing capacity prior to widespread clinical use (experimental data in animals or healthy volunteers does not always coincide with practice). Considering the large number of drugs in use, a certain period of study is needed to ascertain which are implicated in ADRs and if these ADRs are scientifically based [204]. Reference is often made to chemical structures and the example of rigid-structure muscle relaxants, which induce allergic reactions less readily than those with a flexible structure [215].

Table 19.1 shows that many classes of drugs are implicated, the number of patients claiming to have allergy to

Table 19.8. Cross-reacting drugs

Drugs	Cross-reacting agents (examples)
Aminoglycosides	Gentamycin, kanamycin, neomycin, streptomycin
Aminopenicillanic acid	Cephalosporins, penicillins, synthetic penicillins
Aspirin	Other NSAIDs
Benzoic acid esters	Benzocaine, pantethine, pontocaine, procaine
Cyclooxygenase inhibitors	Aspirin, fenoprofen, indomethacin, NSAIDs
Ethylenediamine	Antazoline, aminophylline, promethazine,
Glafenine	Antrafenine, floctafenine, NSAIDs
Heterologous antisera	Antisera from other species
Insulin	Bovine, swine, DNA-recombinant
Penicillin, methicillin	Amoxicillin and other semisynthetic penicillins, ampicillin, cephalosporins, penicillamine
Procaine	Benzocaine, pontocaine, procainamide, tetracaine
Quaternary ammonium ion	Antibiotics, antihypertensive drugs, antiseptic agents, cosmetics, muscle relaxant anesthetics
Radiocontrast media Sulfonamides	Hypoglycemic agents, diuretics related to sulfa group, carbonic anhydrase inhibitor (acetazolamide) depending on the component to which allergic: egg, chicken, duck, rabbit, antibiotics, gelatin
Viral vaccines	

Data from [207].

one drug is equally surprising [78]. It should be noted that group B vitamins, often prescribed for nurslings, and acetaminophen, which is commonly recommended as antipyretic, even to children allergic or intolerant to ASA and NSAIDs, cause reactions in 4%–5% and 2%–7% of patients, respectively. In addition to severe reactions caused by overdoses [203], Acetaminophen has caused anaphylaxis in a 9-year-old girl [106] and in adults [102]. Moreover, repeated episodes of urticaria and angioedema have been reported in a 7-year-old child [43] and in 25% of children with NSAID hypersensitivity (OFC-ascertained) (oral challenge test) [86]. Other drugs, especially antibiotics, prescribed for children with fever, are often accused of causing skin rashes, whereas viruses are more frequently responsible for these rashes [204].

Cross-Sensitization

Once sensitization to a drug has occurred, the chance exists of reactivity either to drugs with a close structural chemical relationship or to immunochemically similar metabolites. This occurs since an epitope may be common to different substances with the same chemical group, for example it may share a free amino group in the para position of a benzoic ring, the β-lactam and 6-amino-penicillanic acid ring, or the benzene ring with the SO_2NH group of sulfonamides and correlated compounds, or it may share similar pathogenetic mechanisms (NSAIDs and cyclooxygenase) [193, 237] (Tables 19.7 [182], 19.8 [207]). Within this framework, a patient sensitive to a drug having these groups must prudently avoid other substances with the same group. Multiple sensitization enters into play in penicillin-allergic patients with a risk that is tenfold greater for ADRs to antibacterials not correlated with any penicillin drug [197]. A similar event occurred in a population of 120 children who, during antibiotic treatment, totaled 337 ADRs: 18.3% were sensitive to three different antibiotics and 1.6% to >3 (19.9%) [81], corresponding to 19.7% of another pediatric survey [61], opening the route to multiple sensitization in this case as well.

Route of Administration

- *Topical application* of some drugs is associated with a high incidence of sensitization and should be avoided, especially in inflamed or band-aid irritated skin, as disrupted skin often provides a compromised barrier to absorption in babies [68]. Cutaneous alterations increase the penetration into the organism of creams, ointments, lotions, eyewashes and their fixation on macromolecules such as seralbumin and cutaneous proteins [152]; in particular penicillin and sulfonamides are implicated. Furthermore, in nurslings and toddlers, the involvement of a larger surface area relative to volume increases absorption of topically applied drugs per unit mass [68].

- *Oral administration* can instead contribute to reducing the reactions associated with other routes since it is linked to antigen endoluminal degradation in low-MW constituents and to sIgA's protective role [152].
- *Inhaled drugs* are normally less immunogenic, despite challenging conclusions [152].
- *Intravenous (IV)* use should be evaluated on a case-by-case basis, because the risk of sensitization is either objectively reduced or it can increase the risk of severe immediate allergic reactions, becoming responsible for severe episodes of anaphylaxis [23].

Degree and Duration of Exposure

Sensitization is more likely when using a high dosage over a long period of time; two frequent events must be borne in mind:

- *Alternate or intermittent cycles* with reduced dosage are in perspective more sensitizing than a single, prolonged, and uninterrupted cycle.
- *During a single treatment* cycle, there is greater risk in the first 2–3 weeks of therapy [197].

ADRs are often accompanied by other symptoms of the immune type, including fever, skin rashes (50% of cases), eosinophilia, and especially shock and/or generalized urticaria in 0.1%–0.2% of cases [47].

Other mechanisms (Table 19.6) are related to concomitant diseases: asthma, cystic fibrosis (CF) and especially viral infections, active above all during infancy, including HIV (human immunodeficiency virus) and EBV (Epstein-Barr virus). Among the factors linked to infection a pivotal role is played by:

- *General factors* such as endotoxins [34], SAs [115], proteins with cytokine-like immunoregulating activities [77], complement inhibitors [92], and molecules activating metachromatic cells [145].
- Specific factors such as the elevated ADR frequency in patients with EBV or HIV infections due to the immunoregulating impact of respective viruses and to suppression or exaggeration of effector functions vs ongoing therapies [77].

Clinical Presentation

Based on the aforementioned classification it is possible to distinguish four types of symptom causes [1, 204]:

Symptoms Caused by Allergic Reactions

Type I reactions: IgE-mediated

- General: anaphylaxis, if IgE-mediated (Table 19.4) is caused by enzymes, insulin and other hormones, polypeptides and antibiotics.
- Respiratory: bronchospasm, rhinitis, etc.
- Cutaneous: urticaria/angioedema, etc.

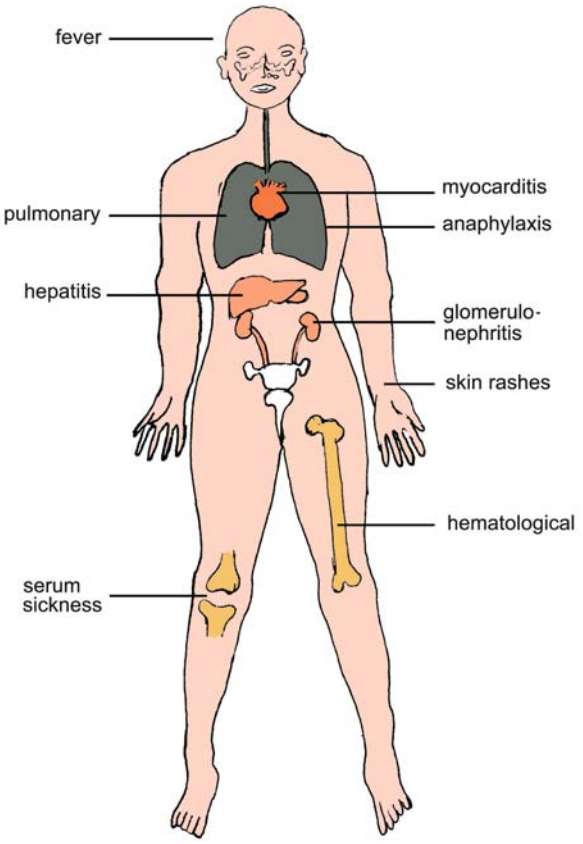


Fig. 19.7. Manifestations of drug allergy/pseudoallergy. Several organs are often involved, but in many instances the reactions are not confined to a single organ, the severity of the reaction can also vary

Type II reactions: cytotoxic IgG- and IgM-mediated

- Hematological: hemolytic anemia with a positive Coombs test, platelet disorder, agranulocytosis.
- Interstitial nephrites.

Type III reactions: caused by CIC

- Serum sickness (caused by any drug).
- Glomerulonephritis.
- Vasculitis.
- Drug induced SLE.

Type IV reactions: cell-mediated.

- Allergic contact dermatitis (ACD).
- Photodermatitis.
- Respiratory symptoms.

Table 19.3 and Fig. 19.7 summarize the clinical symptoms of allergic ADR divided according to organs and/or apparatus. The same drug may involve different mechanisms such as in the paradigmatic example of insulin: the 1st, 3rd and 4th type of immediate and systemic reactions are IgE-mediated and delayed reactions are Arthus-like dependent on IgG anti-insulin (Chap. 1). Antibiotics can cause immediate reactions (<1 h) or accelerated reactions (1–72 h), both belonging to type I and also delayed reactions (>72 h) of types II and IV [228]. Reactions can involve practically any organ or apparatus.

Symptoms Caused by Autoimmune Reactions

In some cases drugs induce auto-antibodies (Aab), often persisting at length even after treatment has been stopped (autoimmune hemolytic anemia, AIHA) (caused by α-methyldopa, pharmacological SLE, etc.).

Symptoms Caused by Reactions Probably Involving Immune Mechanisms

- *Cutaneous eruptions* (erythema, erythema multiform (EM), maculopapular rash, etc.)
- *Febrile mucocutaneous syndrome:* EM, SJS, TEN
- *Drug fever*
- *Eosinophil pneumonia*
- *Hepatitis and cholestasis*
- *Interstitial nephritis*
- *Lymphadenopathy*

Symptoms Caused by Pseudoallergic Reactions

- *Anaphylactoid reactions* (example: radiocontrast media)
- *Intolerance to salicylates* (in some cases caused by interference on production or mediator release)
- *Intolerance for various additives*
- *Rash caused by ampicillin*

Drugs causing anaphylactic or anaphylactoid reactions are summarized in Table 19.9 [207].

The *onset* of clinical symptoms after drug administration can be:

- *Immediate*, from a few minutes to 1 h, as in the case of anaphylaxis
- *Delayed* by a few days such as exanthematic rashes, or by a few weeks, as in the case of serum sickness

The *modalities of drug appearance* or related syndromes are:

- After the first administration: sensitization at first exposure
- After a later administration: successive, gradual sensitization [221]

Clinical Features

The data in Tables 19.3 and 19.10 [197] distinguish systemic and localized allergic reactions of individual organs or tracts, and Table 19.11 [33, 44, 107, 144, 167, 181] shows the frequency of different symptoms not yet identified and that most symptoms are cutaneous. In the 278 children, 75% of the reactions were cutaneous, 10% each were respiratory or gastrointestinal, and about 5% were unclassified.

Table 19.9. Drugs possibly causing anaphylactic or anaphylactoid reaction

A. IgE mediated
Proteins
a. Allergen extract Bermuda grass Buckwheat Cottonseed Ragweed
b. Antiserum Antilymphocyte globulin Antithymocyte globulin Diphtheria antitoxin Tetanus antitoxin
c. Hormones, enzymes Adrenocorticotropic hormone Chymotrypsin Insulin L-asparaginase Papain Penicillinase Relaxin Thyroid-stimulating hormone Trypsin
d. Vaccines Gelatin Influenza Measles Other egg-containing vaccines Tetanus toxoid
e. Venom Hymenoptera
Polysaccharides
a. Dextran
b. Iron dextran
Haptens
a. Antibiotics Chlortetracycline Demethylchlortetracycline Nitrofurantoin Penicillin Polymyxin B[a] Streptomycin Tetracycline
b. Vitamins Folic acid Thiamine
Miscellaneous
a. Cisplatin
b. Cyclophosphamide
c. Cytosine arabinoside
B. Arachidonate mediated
Aspirin[a] Indomethacin[a] Other nonsteroidal anti-inflammatory agents

Table 19.9. (Continued)

C. Complement mediated
Methotrexate
Transfusion reaction with IgA deficiency
D. Direct mast cell-releasing agent
Daunorubicin
Deferoxamine
Doxorubicin
Hydralazine
Opiates
Pentamidine
Radiocontrast media[a]
Rubidazone
Stilbamidine
Teniposide
Tubocurarine[a]
E. Enzymes
Asparaginase
Chymopapain
Chymotrypsin
Trypsin
F. Idiopathic
Bisantrene
Busulfan
Chlorambucil
Etoposide
Fluorouracil
Hydroxyurea
Mechlorethamine
Melphalan
Mitomycin
Procarbazine
Zinostatin

Data from [207].
[a] Drugs often causing anaphylactoid reactions.

Table 19.10. Classification of drug reactions usually considered allergic (see Table 19.6)

Systemic reactions
Anaphylaxis
Drug-induced fever
Lupus erythematosus systemic and other autoimmune reactions
Serum sickness-like reactions
Urticaria-angioedema
Organ-based effects
Dermatologic Allergic contact dermatitis Exfoliative dermatitis Fixed drug eruptions Morbilliform/maculopapular rashes Photodermatitis Stevens-Johnson syndrome Toxic epidermal necrolysis Urticaria-angioedema, not systemic
Hematological Eosinophilia Hemolytic anemia Neutropenia Thrombocytopenia
Pulmonary Pulmonary infiltrates Fibrotic reactions
Hepatic Hepatocellular Cholestatic
Renal Glomerulonephritis Nephrotic syndrome Interstitial nephritis
Not always drug-induced reactions Erythema multiforme minor and major Exfoliative dermatitis Vasculitis

Modified from [197].

Systemic or Mast Cell-Mediated Reactions (Table 19.10)

Generalized cutaneous reactions

The most frequent are *exanthematic rashes* and urticaria-angioedema [162]. Figures 19.8 and 19.9 show cases of allergic exanthema after taking phenolphthalein and delayed-release penicillin.

Iatrogenic urticaria (23% of cases), almost indistinguishable from forms of different etiologies, subsides in a few days when the drug is discontinued [57]. The purported drugs are summarized in Table 19.12 [213, 214]. Neomycin, which belongs to the aminoglycoside family, mostly causes urticaria, in >2% of treatments [213] and for this reason is involved in reactions to anti-measles vaccinations [31]. Of great concern are NSAIDs and ASA. The onset of penicillin-induced urticaria is the signal for oncoming immediate and generalized reactions, which rapidly turn into anaphylaxis [103]. Urticaria-angioedema reactions caused by β-lactams [66, 67, 121, 188] and by chemo-antibiotics in general are more frequent in children (72.1% of cases) [167]. In only two children could it be stated for sure that a medicament was the cause of urticaria (1.5%) [11].

Angioedema is caused by 1×10^4 treatments with penicillin, lethal in $1–5 \times 10^5$ cases. Incidence increased with the growth of prescriptions for ACE (angiotensin-converting enzyme) inhibitors, especially with delayed effect and in new patients during the first 3 weeks of therapy, equal to 2–10 cases $\times 10^4$, higher than penicillin incidence [172]. See also the subsection on sulfonamide.

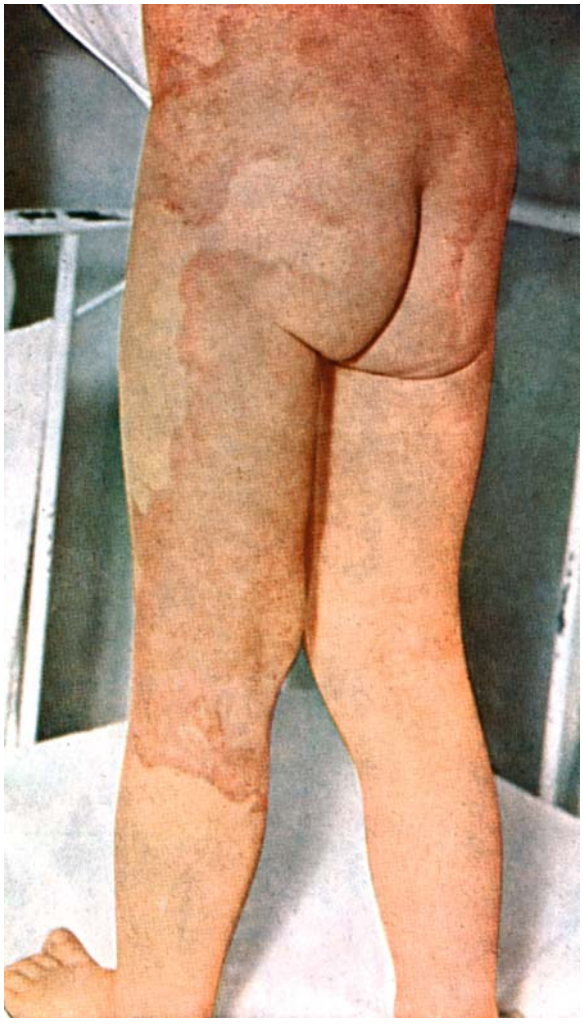


Fig. 19.8. Widespread allergic exanthem, partly scalloped, in a 2-year-old child, following administration of a phenolphthalein-based laxative, abated after drug discontinuation

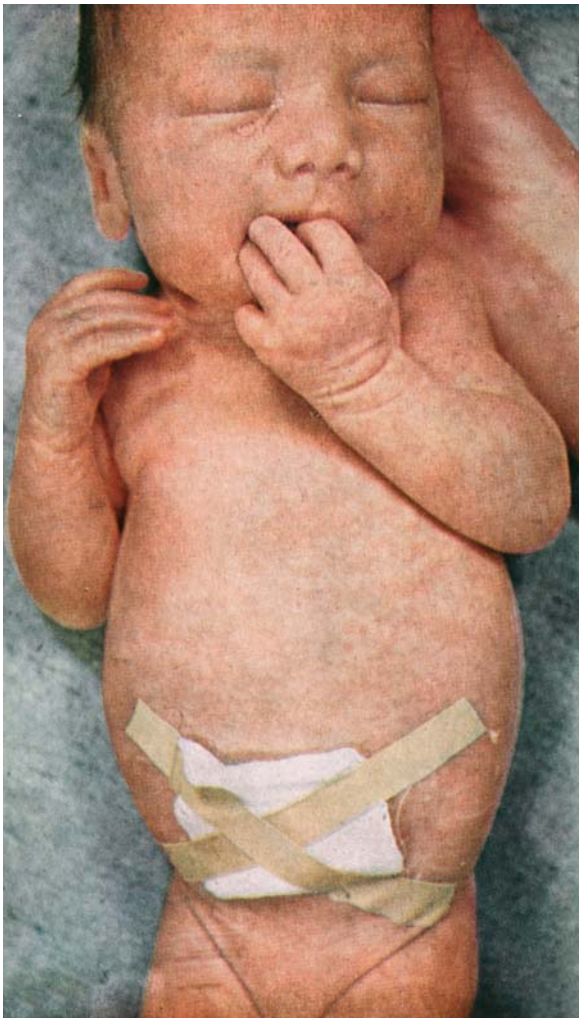


Fig. 19.9. Erythematous, roseoliform exanthem in an infant, 10 days after administration of slow-release penicillin

Serum sickness is a classic prototype of CIC-mediated ADR, with CICs diffusing into involved small vessels, activating complement and initiating inflammatory responses [172]. Currently the complete clinical expression has become rare since specific immunoglobulins (Igs) have replaced heterologous serum [47]. The cases reported are mostly caused by antithymocyte globulin (ATG) and antilymphocyte globulin [197]. Children may be as young as 0.8–0.9 years [84]. The primary form occurs 7–14 days (but also after 21) after the causative agents are administered; the latent period reflects the period of time necessary to synthesize a sufficient amount of antibodies and to elicit symptoms. Heralded by development of pruritus, fever and malaise are the cardinal manifestations (100% of cases), then urticaria-angioedema, morbilliform rashes and polymorphous exudative erythema in 90% of cases, arthralgias (50%–67%), glomerulonephritis (20%) and lymphadenopathy (13%), and at times edema [103]. A diagnosis is suggested, in addition to the typical clinical picture, by a marked fall in C3 and C4 levels. In 75% of patients treated with ATG for controlling transplant rejection, a serpiginous band of erythema was observed on their hands and feet at the margin or palmar or plantar skin, 12–48 h before a measles-like rash [46]. The drugs involved most often are summarized in Table 19.13 [116]. In children, the role played by antibiotics was ascertained, but the risk was modest, between 0.007 and 0.14% [71]. For cefaclor a prevalence of 0.02%–0.2% has been found, especially in children <5 [216], which may attain much higher levels [116]: up to 25% of all drug reactions [84]. A type III immune mechanism has been suggested, but there is no CIC evidence [73]. However, the symptoms and signs attributed to drugs may actually be caused by the disease itself, especially if the disease in question is viral [204]. Differential diagnosis as well as retrospective confirmation may be based on cefaclor cytotoxicity, via an in vitro lymphocyte-based cytotoxicity assay [84].

Table 19.11. Prevalence of clinical manifestations of adverse drug reactions

Manifestations	Prevalence (%)					
References	[33][a]	[44][b]	[107]	[144]	[167]	[181]
Anaphylactic shock		3–19		9.1		
Angioedema		9–26		3.7		
Asthma		0–5				2
CNS manifestations	15					
Contact dermatitis		1–5				
Drug-induced fever						11
Erythema multiforme			0.5			4
Fixed drug eruptions			26.5		2.8	
Gastroenteric manifestations	8.8					
Hematological disease	7.2	2–4				
Maculopapular eruptions					14.5	30
Respiratory distress	7.2					
Serum sickness		1–14				
Systemic exanthema		4–28	19		7.2	
Urticaria		33–39		75.5		
Urticaria-angioedema			38.5	7.4	72.1	18

Data from [33, 44, 107, 144, 167, 181].
[a] 42.3 were unspecified cutaneous reactions.
[b] Limits of three studies.

Table 19.12. Main causes of drug-induced urticaria

ACE inhibitors	Immunoglobulins (?)
Anesthetics, local	Levamisole
Antibiotics Aminoglycosides Ampicillin β-*Lactam* Cephalosporins Penicillins, etc. Polymyxin B Tetracyclines, etc.	NSAIDs
	Oral antidiabetics
	PAS
	Proteolytic enzymes
	Pyrazolone derivatives
ASA	Quinine
Desensitizing vaccines	Radiocontrast media
Drugs active on CNS	Substances with high MW
Heterogeneous proteins	Sulfonamides
Hormones	Vitamins
	Whole blood

Data from [213, 214].
ASA acetylsalicylic acid, *NSAIDs* nonsteroidal anti-inflammatory drugs, *PAS* *p*-aminosalicylic (acid).

Drug Fever. Body temperature (T) is regulated in the anterior hypothalamus preoptical region. In normal circumstances, the thermoregulatory center keeps body T within restricted limits by controlling local heat production and preservation via autonomous and skeletal neuromotor pathways [205]. The T rises when macrophages are stimulated to produce IL_1, which in turn stimulates PGE generation from arachidonic acid (Fig. 1.57): the PGE increase influences the thermoregulatory center, leading to a rise in T. Numerous factors are

Table 19.13. Drugs most often the cause of serum sickness

Cefaclor
Griseofulvin
Hydralazine
Penicillins
Phenylbutazone
Pyrazolone derivatives
Streptokinase
Sulfonamides
Thiazide diuretics
Thiouracils

Data from [116].

Table 19.14. Drugs provoking fever also in the absence of other allergic manifestations

Antimicrobials	Other drugs
Cephalosporins	Allopurinol
Chloramphenicol	Antipneumococcal vaccine
Erythromycin	Antithymocyte globulins
Griseofulvin	Barbiturates
Isoniazid	Blood products
Kanamycin	Heparin
Nitrofurantoin	Hydralazine
PAS	Hydroxyurea
Penicillins	Iodide
Pyrazinamide	Mercury diuretics
Streptomycin	Methyldopa
Sulfonamides	Penicillamine
Tetracyclines	Phenobarbital
Trimethoprim	Phenytoin
	Procainamide
	Propylthiouracil
	Quinidine

Modified from [207].

capable of stimulating the production of IL_1 [187]. The main inducers of nonimmunological fevers are the following:

1. Endogenous release of bacterial pyrogen
2. Administration of exogenous pyrogen
3. Secondary effects caused by an ADR producing toxic tissue injury
4. Increased tissue metabolism
5. Peripheral vasoconstriction and reduced heat loss
6. Central effect
7. Hormonal effect

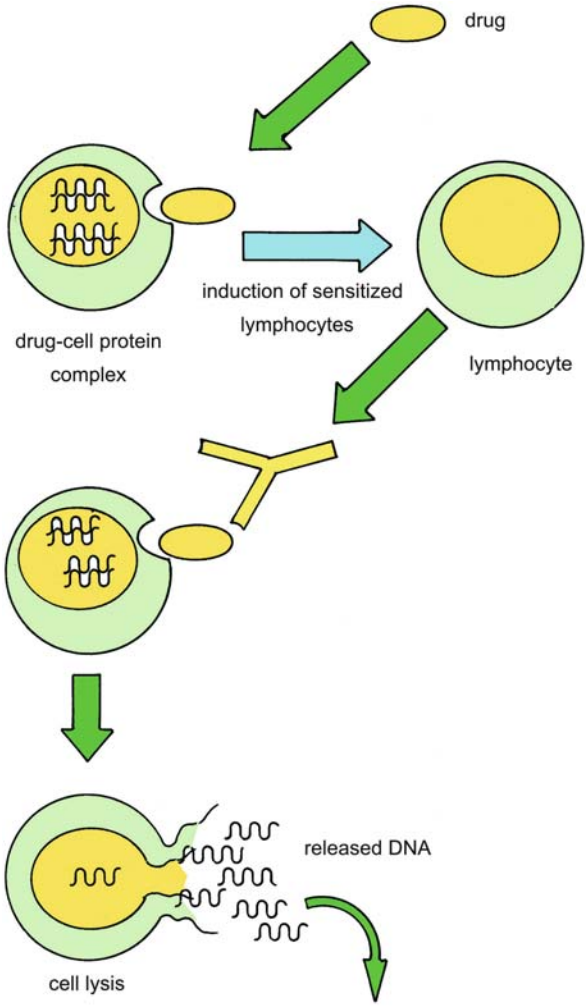


Fig. 19.10. Pathogenetic mechanism of drug-induced SLE

In children, epinephrine and norepinephrine are also involved [207]. A drug-induced fever and bone marrow suppression developed in a 12-year-old girl with CF [65]. Therapeutic progress comes with a price tag: the use of prostaglandins in treatment has led to feverish reactions: an example is alprostadil (PGE_1) to maintain the ductus arteriosus open in newborn babies with congenital heart disease, and with IFN-α to induce PGE_2 generation into the hypothalamus [205].

Fever may occur as the only manifestation of an ADR. In various cases the contrast with the patient's condition is peculiar. Table 19.14 [207] summarizes the drugs that most often cause a fever; the most involved among those are β-blockers, β-lactams and streptokinase [172].

SLE (systemic lupus erythematosus) (Chap. 18). In some children SLE is caused by an ADR (Fig. 19.10). The most incriminated among these are summarized in Table 19.15 [87]. In many cases SLE is self-limiting, clearing up after the offending drug is discontinued. The main symptoms, with a sudden onset a considerable

Table 19.15. Drugs incriminated in SLE

Antibacterial agents
Minocycline
Nitrofurantoin
Sulfadiazine
Tetracycline
Anticonvulsants
Barbiturates
Benzodiazepines
Carbamazepine
Ethosuximide
Hydantoins
Mephenytoin
Phenytoin[a]
Trimethadione
Valproic acid
Antihypertensive
Acebutolol
Atenolol
Captopril
Hydralazine
Labetalol
Methyldopa[a]
NSAIDs
Diclofenac
Ibuprofen
Phenylbutazone
Sulindac
Tolmetin
Miscellaneous
Chlorpromazine[a]
Hydrazine
Isoniazid[a]
Levodopa
Methylthiouracil
Penicillamine
Practolol
Procainamide[a]
Propylthiouracil
Quinidine
Sulfasalazine
Tolazamide

Modified from [87].
[a] Drugs with the most evident association.

number of months after therapy has been started, include fever, cutaneous symptoms, arthritis and pleuropericarditis. Antibodies against double-stranded (ds) DNA and anti-Smith antibodies and especially positive ANA (antinuclear antibodies) with antihistone specificity are diagnostic (90% of patients, while complement levels are normal [87] (Tables 18.25, 18.26).

Other Drug-Induced Autoimmune Diseases. Aab incidence during therapies involving certain drugs is clearly higher in the presence of a real autoimmune disease: D-penicillamine is not only associated with SLE but also with cases of hemorrhagic alveolitis simulating Goodpasture syndrome, severe myasthenia, polymyositis and IgA deficiency; practolol with Sjögren syndrome; ethyldopa and penicillin with AIHA, and, together with oxyphenisatin and perhaps also halothane, with hepatitis; and methicillin with tubular nephropathy [47, 62].

Reactions Localized in Individual Organs or Apparatus

Cutaneous Manifestations

ACD is caused by topical or repeated application of low-MW haptenic drugs, outlined in Table 19.16 [195], in addition to sunscreens that almost without exception contain PABA, tars, balms, additives and bases for topical drugs and adhesive plasters [26] (Tables 8.8 and 8.9). Deflazacort for topical use is also involved, associated with betamethasone, budesonide and hydrocortisone in a case of type IV hypersensitivity [129], and emulsifiers, hexamidine, clotrimazole and prednisolone contained in a cream applied by a boy suffering from atopic dermatitis (AD) [26]. The prevalence of positive patch tests for CSs is between 0.2% and 5% [15]. Table 19.17 synthesizes the prevalence of cutaneous reactions caused by drugs [6].

Fixed drug eruptions (10% of cases) are virtually pathognomonic of ADR. The onset appears in the form of solitary, pruritic, bright red or dusky red patches, round or oval, which can evolve into a livid discoloration, with clear margins, and central hemorrhagic pigmentation. Later the lesions spread to the oral and genital mucosa, the extremities, face and genitalia, more rarely to the chest, and are characterized by frequent duplicity. They are termed fixed because they tend to affect identical sites each time the specific drug is taken [12] (Fig. 19.11). In 15 children, the genital lesions were associated with several drugs [133]. Once the drug is stopped, the lesions subside, leaving desquamation and erythema, which darkens to become deeply colored, reddish-purple or brownish-pink. An IL- [187] or CIC-mediated mechanism has been identified (the latter implying that it is a EM variant) (see "Reaction Potentially But Not Definitely Drug-Induced") or by T lymphocytes [12]. Sulfonamides account for 43.3% and NSAIDs for 30.7% of cases [178]. More drugs and additives could be responsible, and those commonly involved are listed in Table 19.18 [71], in addition to nimesulide, which provoked extensive lesions showing cross-sensitivity to sulfonamides in a 10-year-old boy [177].

Macular and/or exanthematic lesions and/or generalized eruptions occur frequently: erythematous, maculopapular, morbilliform, and scarlatiniform lesions, associated with itching, generally appear at a later stage in the course of therapy and appear to be practically in-

Table 19.16. Drugs causing allergic contact dermatitis

1. Local anesthetics

A. Potentially cross-reacting with benzocaine

- Butacaine
- Butethamine
- Chloroprocaine
- Isobucaine
- Meprylcaine
- Metabutethamine
- Orthocaine
- Procaine
- Propoxycaine
- Risocaine
- Tetracaine

B. Not cross-reacting with benzocaine

- Bupivacaine
- Etidocaine
- Lidocaine
- Mepivacaine
- Prilocaine

C. Miscellaneous

- Cyclomethycaine
- Dibucaine
- Dyclonine
- Hexylcaine
- Phenacaine
- Pramoxine
- Proparacaine hydrochloride

2. Preservatives and components

- Chloroxylenol
- Diazolidinyl urea
- Formaldehyde
- Hydantoin
- Imidazolidinyl urea
- Merthiolate (thimerosal)
- Propylene glycol
- Propylparaben
- Quaternium

3. Topical antibiotics

A. More sensitizing

- Bacitracin
- Chloramphenicol
- Hydroxyquinolines
- Neomycin[a]
- Penicillin
- Sulfonamides

B. Less sensitizing

- Clindamycin
- Erythromycin
- Tetracycline

4. Topical antifungals

A. Imidazoles

- Bifonazole
- Clotrimazole
- Croconazole
- Econazole
- Isoconazole
- Ketoconazole
- Miconazole
- Oxiconazole
- Sulconazole
- Tioconazole

B. Nonimidazoles

- Ciclopirox
- Haloprogin
- Naftiline
- Nystatin
- Tolnaftate
- Undecylenic acid

5. Miscellaneous

- Ammoniated mercury
- Analgesics
- Antihistamines H_1
  - Benzocaine
  - Clonidine
- Corticosteroids
- Dyes in medications
- Estradiol
- Ethylenediamine
- Fluorouracil
- Idoxuridine
- Lanolin
- *P*-aminobenzoic acid (PABA)
- Parabens
- Scopolamine

6. Miscellaneous antibiotics potentially cross-reacting with neomycin

- Amikacin
- Gentamycin
- Kanamycin
- Netilmicin
- Paromomycin
- Streptomycin
- Tobramycin

Data from [195].

distinguishable from common exanthema. Anticonvulsants such as phenytoin, carbamazepine and phenobarbitone were implicated in 41.6% of patients with maculopapular rashes [178]. Some pediatric morbilliform and maculopapular rashes could present a CMI pathogenesis [166]. Recently, drug-specific $CD3^+$, $CD4^+$ and $CD8^+$ T cells in the skin and the peripheral blood of a child with maculopapular drug eruptions have been

Table 19.17. Prevalence of skin reactions caused by drugs prescribed to at least 100 patients

Drug	Prevalence (%)
Acetylcysteine	8.8
Allopurinol	7.7
Amoxicillin	51.4
Ampicillin	33.2
Atropine sulfate	1.6
Barbiturates	4.0
Bromhexine hydrochloride	6.4
Calcium hypodate	27.8
Carbocysteine	6.8
Cephalosporins	21.1
Cimetidine	12.8
Co-trimoxazole	33.8
Cyanocobalamin	17.9
Cyclophosphamide	4.8
Dihydralazine hydrochloride	19.1
Dipyrone	4.0
Doxycycline	4.7
Erythromycin	20.4
Gentamicin sulfate	4.5
Hydralazine hydrochloride	8.3
Hyoscine butylbromide	13.2
Indomethacin	2.1
Isoniazid	5.6
Mafenide	1.6
Metoclopramide hydrochloride	3.2
Nitrazepam	1.5
Penicillin G	18.5
Pentazocine hydrochloride	4.5
Phenazopyridine hydrochloride	8.8
Phenylbutazone	11.6
Quinidine	13.4
Vincristine sulfate	6.3

Data from [6].

identified through use of immunohistochemistry analysis [88]. In addition, all penicillin G-, ampicillin-, and amoxicillin-specific short-term T-cell lines showed high intracellular expression of IL_4, IL_5, and IL_{13}, but poor or no expression of IFN-γ, thus exhibiting *a clear-cut Th2 profile* [27]. Other common damaging agents are ampicillin, incriminated in 3%–7% of pediatric cases, aminoglycoside antibiotics, benzodiazepine, gold salts, sulfonamides [205], penicillin (Fig. 19.12) and barbiturates (Fig. 19.13).

Table 19.18. Drugs implicated in fixed drug eruption

Analgesics
ASA
Barbiturates
Gold salts
Iodide compounds
Metronidazole
NSAIDs
Phenolphthalein
Phenylbutazone
Pyrazolone
Sulfonamides
Tetracyclins

Data from [71].

TEN or Lyell syndrome, the most severe of the SJS-TEN spectrum, has an incidence of $0.2–1.2 \times 10^6$ and is in more than 80% of cases ADR-caused (Table 19.19) [12, 103, 172]. TEN can be linked to a constitutional or inherited defect in the detoxification of culprit drug-reactive metabolites [230] and in rare cases caused by GvHD (graft vs host disease). Culprit metabolites develop a specific mechanism activating immunocompetent cutaneous lymphocyte-associated antigen-positive (*CLA*) *T cells*, which migrate toward the skin and contribute to TEN. ADR-induced TEN and SJS are weakly associated with HLA-B12. Among patients reacting to a given drug, the stronger links were especially to *HLA-A29, B12, DR7* and sulfonamides [172]. The ensuing mechanism for anticonvulsant-induced TEN is associated with an immunological reaction originating in skin enabling CLA^+ T cells to migrate by a multistep adhesion mechanism involving CLA-CD62E, very late activation antigen-4 (VLA-4)/VCAM-1 (CD106) and lymphocyte function-associated antigen-1 (LFA-1) CD54 interactions in parallel with the severity of clinical symptoms [105]. After activation by Langerhans cells in the skin, T cells can release TNF-α and IFN-γ, leading to the necrosis of epidermal cells [105]. The widespread keratinocyte apoptosis from TEN patients results from interaction between the death receptor Fas (CD95) and the lytically active FasL [217]. Subsequent results indicate that soluble FasL (sFasL) secreted by PBMCs, not keratinocytes, plays a crucial role in the apoptosis and pathomechanism of TEN [1]. Notably, there is no correlation between sFasL levels and the degree of skin detachment in both TEN and SJS [1]. TEN affects schoolchildren or also older ones, unlike etiologic streptococcal forms that appear during the first stages of life; it can also be set off

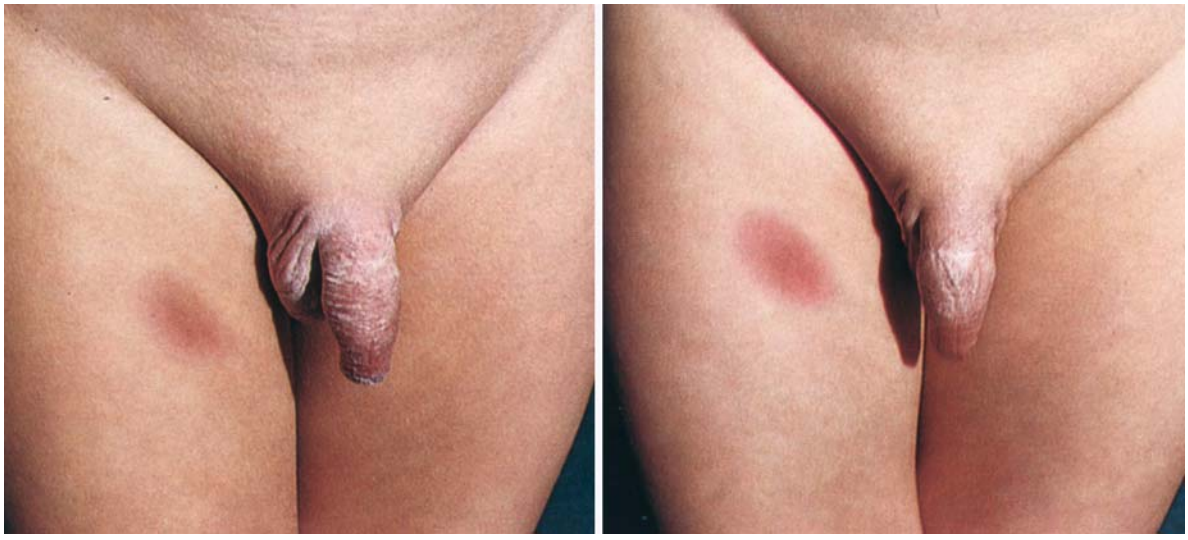


Fig. 19.11. Fixed erythema of the left thigh before (*left*) and during (*right*) exposure to sulfamethoxypyridazine: note the flushing of the previous reaction

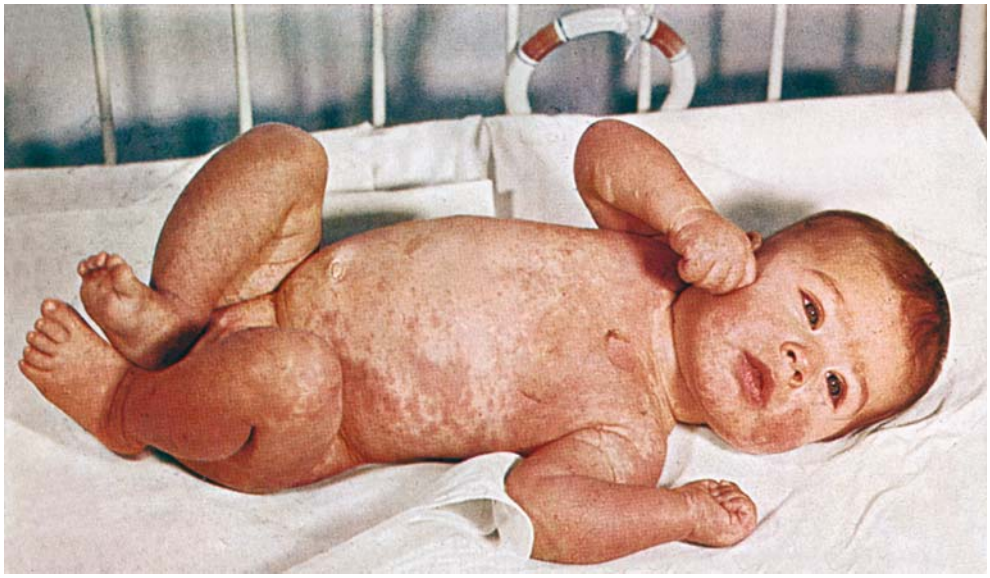


Fig. 19.12. Widespread allergic exanthem, first morbilliform, then rapidly confluent, assuming a bullous aspect in a 7-month-old infant, treated for 14 days with penicillin because of meningitis

in newborn babies by viral or bacterial infections. Cutaneous lesions involve 20%–100% of surface and mucous membranes in 70% of cases, oral mucosa in 95% and conjunctival mucosa in 86% of cases. Over a 10-year period, mucous membrane involvement was documented in 61% of children. Ocular involvement was seen in 39%. Complications occurred in 21% cases, all of whom had SJS or TEN. Only one patient died as a result of their skin condition [59]. Sometimes preceded by burning sensation, skin tenderness, fever and malaise, within a few hours and up to 2 h a sudden widespread erythematous eruption appears, evolving into bullae formation characterized by raised flaccid blisters which, full blown within 12–24 h, sloughs off into large *sheet-like denudation,* revealing a raw oozing surface as in a second degree burn, similar to streptococcal scalded skin syndrome) (SSSS) [12, 103] (Fig. 19.14). The consequent dehydration, severe electrolyte imbalance, septic complications and eventually disseminated intravascular coagulation (DIC), concur in determining a fatal outcome in 20%–30% of those affected [103, 172]. Long-term CS therapy delays TEN onset without blocking its progression [67]. In children, TEN was managed successfully in a burn facility, using early debridement and wound coverage with allograft skin as a biological dressing. The most common long-term sequelae involve the skin (100%), the eyes (40%), and include diffuse itching early after wound healing (53%) [191]. Mucosal involvement and ocular involvement occurred in 10/11 children (91%).The most common long-term morbi-

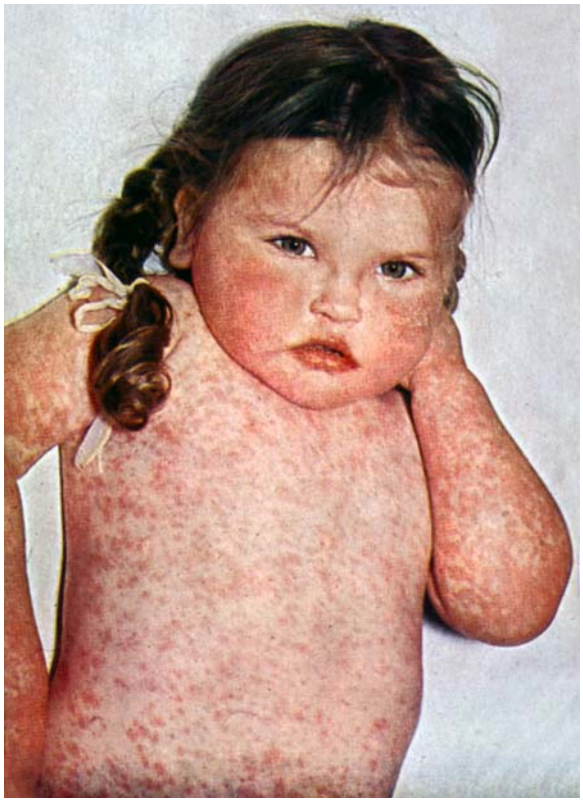


Fig. 19.13. Widespread allergic exanthem, first morbilliform, then with aspect of polymorphous erythema in a 3-year-old girl with whooping cough 10 days after sedative treatment

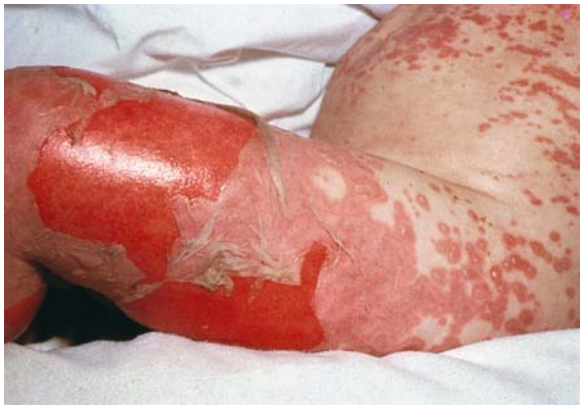


Fig. 19.14. TEN appeared after treatment with phenytoin

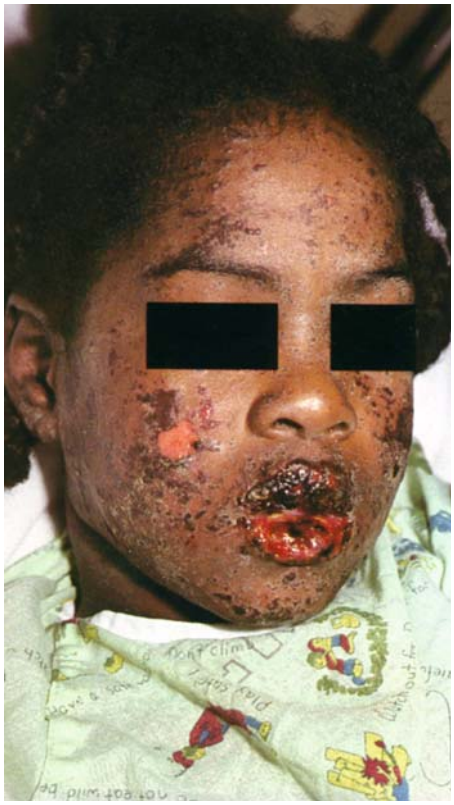
dity involved eyes (3 children), nails (4 children), and variegated skin depigmentation (11 children) [183]. Since antibodies present in *human intravenous immunoglobulins* (IVIg) blocked Fas-mediated keratinocyte death in vitro, ten individuals with clinically and histologically confirmed TEN were treated with IVIg; disease progression was rapidly reversed and the outcome was favorable in all cases [217]. No new blisters developed in children with SJS and TEN within 24–48 h after IVIg administration and rapid recovery ensued [122]. The average time to arrest the progression of lesions was 2.1 days and to complete re-epithelialization 8.1 days after IVIg treatment in children [202].

Table 19.19. Drugs incriminated in SJS and TEN

Allopurinol (6%–20% of cases)
Aminopenicillins
Amithiozone
Carbamazepine (5%)
Cephalosporins
Chlormezanone
Dapsone
Dilantin
Ethambutol
Isoniazid
Nitrofurantoin
Penicillin
Phenobarbital
Phenylbutazone (8%)
Phenytoin
Piroxicam (8%)
Quinolones
Sulfonamides (20%–66%)
Tenoxicam
Tetracyclines
Thiabendazole
Trimethoprim-sulfamethoxazole (69%)
Valproic acid

Data from [12, 103, 172].

SJS [192] is another fulminant form in which PBMCs secrete sFasL on stimulation with the causal drug. The induced apoptosis in cultured keratinocytes indicates that sFasL produced by PMBCs may contribute to the pathogenesis of SJS, as well of TEN [1]. SJS has an incidence of 0.4%–1.2%, equal to $1.2–6 \times 10^6$ patients per year, according to others 1–6 cases $\times 10^6$ [173] and $2.6–7.1 \times 10^6$ patients per year [196]. Among 42 children evaluated, 22 had EM (11 EM minor and 11 EM major), and 17 SJS, and there was a male predominance (28 boys and 14 girls), especially in the group of EM patients (16 boys and 6 girls, sex ratio 2.6:1). Mean age was 8.7 years (range, 8 months to 15 years). EM children were a mean 9.7 years old and SJS children 7.8 years [99]. In 25 years, 17 children had a diagnosis of SJS, in 64.7% of cases due to infectious agents [99]. Cutaneous lesions in SJS are preceded by fever, headache, prostra-



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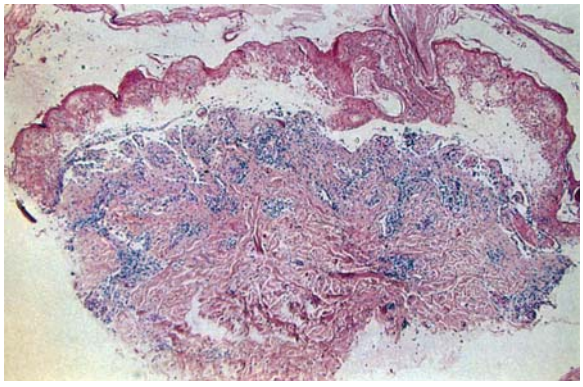

b

Fig. 19.15. **a** SJS with lip and oral mucosa ulcerations. The child also had widespread blistering lesions on the limbs and other mucosal sites. **b** Histological appearance of skin subepidermal separation and necrotic epidermis

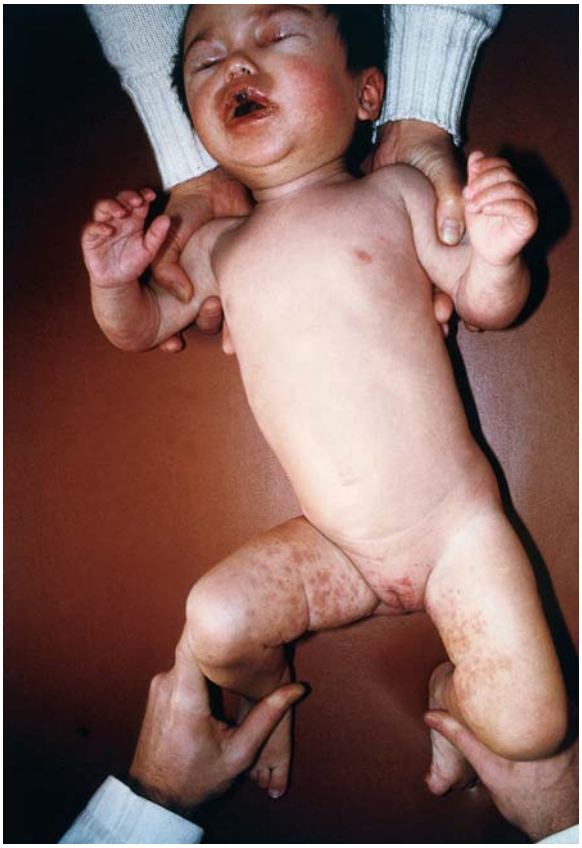

Fig. 19.16. SJS with widespread lesions

tion and characterized by the abrupt onset of a polymorphous eruption of erythematous or purpuric macules on the face and trunk, which rapidly and variably develop a coalescing darker center to form vesicles, bullae, and skin detachment of less than 10% of the body's surface area. The *widespread small blisters* are accompanied by involvement of ≥2 mucosal surfaces, for example, the eyes, oral cavity, upper airway or esophagus, gastrointestinal tract, or anogenital mucosa. Children report a burning sensation, edema, and erythema of the lips and buccal mucosa, which precedes the development of bullae, ulceration, and hemorrhagic crusting [8, 172, 173] (Figs. 19.15, 19.16). The oral, rectal, vaginal or conjunctival mucous membranes are always involved, as well as the lungs, gut, kidneys, and joints. This condition is *overdiagnosed* because of a mistaken interpretation of clinical data, to the point that 31.2% of children admitted to hospital for a suspected diagnosis of EM or SJS were misdiagnosed [99]. The detachment of <10% of the epidermis is suggestive of SJS, detachment of 30% is suggestive of TEN [173], and detachment of 10%–30% of body surface area suggests overlap of the two syndromes [67]. Approximately 50% of cases are associated with drugs, especially barbiturates, long-acting sulfonamides, NSAIDs, and anticonvulsants responsible for 43.8% of life-threatening reactions in TEN and SJS [178]. Generally it is difficult to confirm or exclude, also because the cause may be different, such as in HSV infections (the most frequent), *Mycoplasma* or other pathogenic agents, connective tissue diseases, tumors and radiological therapy [207]. In some cases, an immunological pathogenesis has been proven [172]. Because in SJS the same TEN-linked drugs appear frequently, it is believed that both may be elements of a common pathological spectrum [172]. In both these diseases, the highest risk of reactions per year is linked to the use of sulfonamides (0.0234%) and of co-trimoxazole (0.0224%) [173] or sulfonamides and penicillins (26% each) [59].

Table 19.20. Drugs most often incriminated in eosinophilia

Allopurinol
Ampicillin
Capreomycin sulfate
Carbamazepine
Chlorpromazine
Digitalis
Gold salts
Iodide salts
Nitrofurantoin
Pas
Phenytoin
Streptomycin
Sulfonamides
Tricyclic antidepressants
Vancomycin

Data from [213].

Diagnostic features include widespread blisters predominantly on the chest, presenting with erythematous or purpuric macules and one or more mucous membrane erosions. The mean duration was 18 days for SJS [99]. The serum sFasL level may be a good indicator for the early diagnosis of TEN and SJS [1]. Nursing should include meticulous skin and mucous membrane care, daily ophthalmological examination, and long-term follow-up when necessary [99].

Eosinophilia more frequently accompanies drug-induced diseases, but occasionally it is the sole manifestation, warning in this case of more severe complications to come. The drugs most commonly associated are outlined in Table 19.20 [213]: many of these can trigger mast cell degranulation (Table 10.13) or asthma and other conditions, albeit associated with eosinophilia, not, however, causing hypersensitive reactions such as digitalis, which if this case should not be discontinued. Differential diagnosis often includes many other diseases (Table 6.15).

Hematological Manifestations

Drug-induced AIHA (autoimmune hemolytic anemia) represents 16%–18% of all acquired forms. There are three different types depending on whether it is caused by haptenic reactions or is CIC-induced, or is caused by drug absorption or by autoimmunity. A lower number concerns nonimmunological mechanisms [62]. The most commonly cited causes of drug-induced AIHA are second- and third-generation cephalosporin antibiotics. The first case of cefuroxime-induced AIHA has recently been reported in a 4-year-old girl who 3 years later attends school and functions at her age-appropriate cognitive level [113].

Hapten-Cell Mechanism (Type II Immune Reaction). The most direct mechanism is via the drug or its reactive intermediate, binding to a cell membrane protein, thus typifying the hapten model. The prototypes are penicillin molecules incorporating into red blood cell (RBC) membrane by binding protein, thus forming a neoantigen. Anti-drug antibodies are formed of the IgG type, which react, more than with RBCs, with the membrane-fixed penicillin, which, adhering to the membrane, effectively has become a neoantigen. Additional drugs most commonly associated with hapten mechanism are ampicillin, carbenicillin, cephalosporin, tetracycline, and tolbutamide [109].

CIC-Induced Mechanism (Type III Immune Reaction)
Various stages develop:

- A tripartite antibody–drug–blood group antigen complex is formed (Kidd, Kell, Rh, I/i).
- To form a neoantigen, an antibody recognizes a drug only when it is associated with *a blood group antigen.*
- Antibody will not bind to RBCs deprived of the blood group antigen even if the putative drug is present.
- Then complement activation occurs.

It was previously believed that drug–antibody CICs linked to RBC membrane, either aspecifically, or via the C3b receptor, indicating that RBC acted as an innocent bystander during phagocytosis or cell lysis.

A further, severe variant involves second- and third-generation cephalosporin: the patient develops severe hemolysis with hypotension and DIC, but if re-exposed to cephalosporin the clinical manifestations worsen, with a possible fatal outcome [109].

Drug Absorption Mechanism. The drug absorption mechanism is proposed to explain the pathogenesis of penicillin-induced AIHA, and later extended to other compounds. The drug strongly binds via a covalent bond with the erythrocyte membrane, inducing later anti-drug IgGs that do not activate complement (Fig. 19.17). This reaction either induces a simple serological anomaly or hemolysis caused by extravascular removal of cells sensitized by IgG antibodies, by phagocytosis or, to a lesser extent, ADCC (antibody dependent cell-mediated cytotoxicity) by macrophages, especially if splenic, provided with the relative FcγR [109]. Hemolysis caused by penicillin is dose-dependent [62].

Autoimmune Mechanism. The paradigms for the autoimmune mechanism are α-methyldopa and mefenamic acid: the associated IgG-Aab are directed against RBC antigens with Rh specificity and inducing changes in the RBC surface membrane via a direct mechanism, with neoantigen formation, considered non-self by the immune system, and therefore capable of evoking an antibody response (Fig. 19.18). Simultaneously the drug,

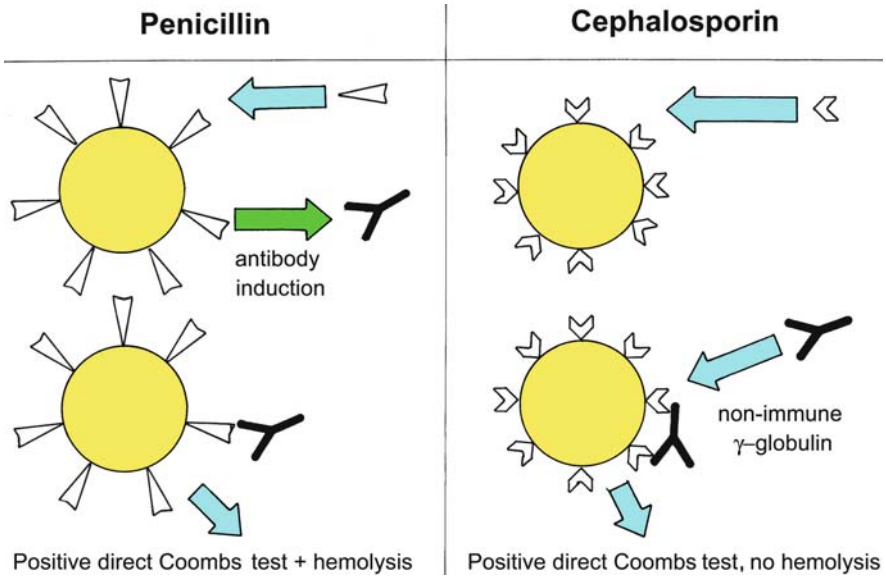

Fig. 19.17. Drug effects on circulating erythrocytes. *Left*: penicillin attaching to erythrocyte membrane causes specific antibodies to yield a positive direct Coombs test. *Right*: cephalosporin also yields a positive direct test but hemolysis does not occur, since the antibiotic induces erythrocyte membrane changes that expedite nonimmunological serum protein absorption

by increasing lymphocyte cAMP, inhibits the function of CD8 T cells, including those cells preventing uncontrolled Aab production, in this case directed against RBCs and not against the drug. Unlike the above mechanisms, these drug-induced Aab will react with normal RBCs even in the absence of the drug [109].

One further mechanism, present in <5% of patients is the *nonimmunological absorption of blood proteins* by nonspecific plasma protein absorption into the RBC membranes; cephalothin, the basis of this mechanism, does not cause hemolysis, even if it can cause it via the first mechanism [109].

Intravascular hemolysis occurs after complete complement cascade is activated: the often sudden onset is accompanied by hemoglobinemia and hemoglobinuria. Kidney failure can put the patient's life in danger. C3b-coated red cells are taken up by splenic and/or liver sequestration via C3b/C3bi receptors on macrophages. The drugs most involved in AIHA are recapitulated in Table 19.21 [62]. Two children aged 2 and 5 died from massive hemolysis 20–55 min after an IV injection of ceftriaxone, a third-generation cephalosporin [14, 96]. In one case, RBC cytolysis by a CIC mechanism was mediated by an anti-ceftriaxone-directed IgM antibody [14].

Thrombocytopenia type II was seen in 41.6% of children with ADR, and only in 0.46% of children without ADR. It is caused either by antigen–antibody interaction, especially of IgG [156]. Quinine antibodies are bound to platelet membrane glycoproteins (gp) GPIb/Ix and GPIIb/IIIa or V in association with the drug [62], thus generating a new epitope that does not cross-react with other cells or tissues [229]. The pathogenesis is best explained by the development of an antibody recognizing the *heparin* epitope bound to platelet factor 4 [109]. Heparin–IgG complexes bind to platelets and activate them via FcR recruitment; moreover this antibody causes platelet aggregation [103], and thromboxane (TX) biosynthesis and release [62]. Heparin is mostly responsible if IV injected (prevalence 5%). There are two different forms: early (before the 5th day) and delayed (between 6 and 25 days), associated in 30% of cases with skin necrosis and in 10% with thromboembolic complications. Within a few hours or days after the offending drug is administered, sensitized patients present fever, chills, widespread petechiae and ecchymoses, on occasion hemophtysis, hematuria, gastrointestinal and vaginal bleeding, and mucosal hemorrhages, while platelet counts fall but not to their nadir as in other drug-induced thrombocytopenia. Treatment must be stopped as in other ADR cases; diagnosis is often presumptive because platelet counts return to normal within 2 weeks [103]. Some cases of thrombocytopenia are induced by a type III immune mechanism. Table 19.22 lists the drugs that more commonly induce immunologically based forms: in pediatrics phenytoin, sulfonamides, TMP-SMX, valproic acid are most commonly used, heparin most rarely [62].

The rare *neonatal alloimmune thrombocytopenia purpura* is caused by development of maternal antibodies against allergens present in fetal platelets, shared with the mother and recognized as foreign by the maternal immune system. IgG alloantibodies are found against human platelet antigen (HPA)-1a in 75%–90%, HPA-1b in 3%–5%, HPA-3a in 4%–5%, HPA-5b in 6%–19% and against private platelet antigens in 3% of cases. Extensively testing children serologically is required to confirm the diagnosis, and their parents have to be typed for the important platelet-specific antigens, monoclonal antibody immobilization of platelet antigens and/or ELISA techniques [156].

Agranulocytosis appears 1–2 weeks after starting a new drug and is not dose-dependent. In patients previously sensitized the onset of neutropenia may occur within 24–48 h. Clinical characteristics include high

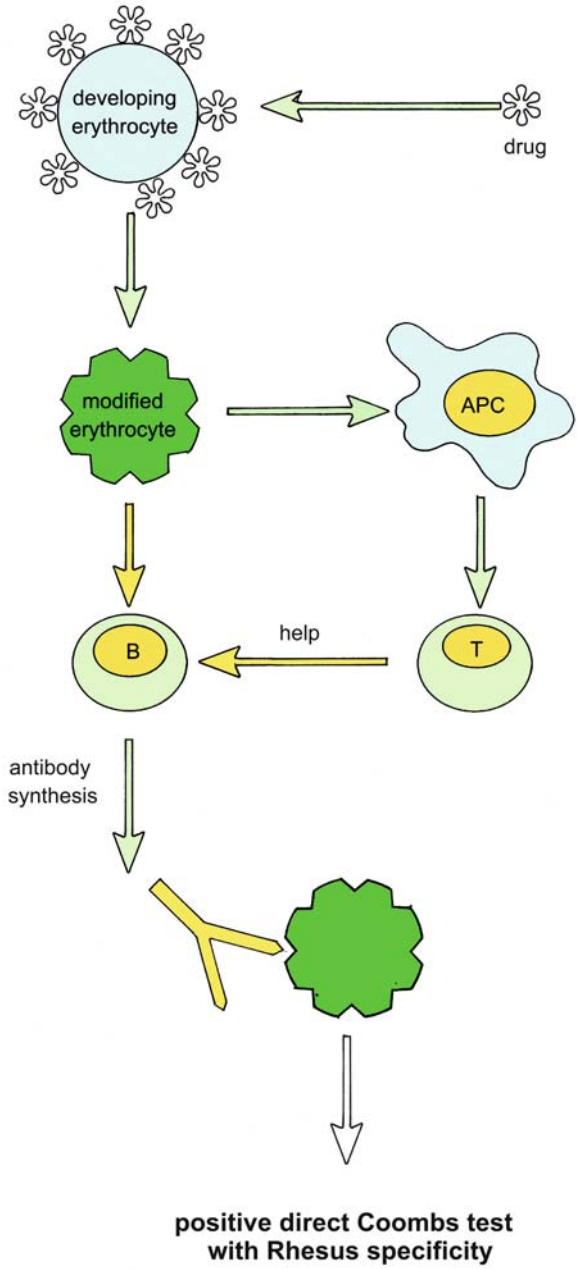

Fig. 19.18. Autoimmune hemolytic anemia

fever, acute chills, arthralgia, severe prostration, and neutropenia [235]. On the contrary, weeks to months after initial therapy with toxic effects, and readministration of even small doses may result in a fall in granulocytes [80]. Toxic or idiosyncratic forms caused by medullary depression may develop insidiously and are revealed by white blood cell count, requested for example to monitor a persistent infective form or in a child resistant to therapy. In an immune ADR in a 16-month-old male infant, there was no production of any type of granulocytes, and leukocytes began to increase from the 8th day after discontinuation of ethosuximide administration [80].

Table 19.21. Drugs implicated in AIHA

Hapten mechanism
Ampicillin
Carbromal
Cephalosporins
Methicillin
Penicillin
Tetracycline
Tolbutamide
Immune complex mechanism
Amphotericin B
ASA
Cephalosporins
Chlorpromazine
Chlorpropamide
Doxepin
Fenoprofen
Isoniazid
Nitrofurantoin
Phenacetin
Probenecid
Quinidine
Quinine
Rifampicin
Stibophen
Streptomycin
Thiazides
Thiopental
Tolmetin
Autoimmune mechanism
Diclofenac
Fludarabine
Levodopa
Mefenamic acid
Methyldopa
Procainamide
Teniposide
Tolmetin
Nonimmunological adsorption
Cephalosporins
Unknown mechanism
Acetaminophen
Chlorpromazine
Erythromycin
Ibuprofen
Isoniazid
Mesantoin
Nalidixic acid
Omeprazole
PAS
Phenacetin
Streptomycin
Sulindac
Thiazides

Modified from [62].

Table 19.22. Drugs most commonly incriminated in autoimmune thrombocytopenia

Analgesics and anti-inflammatory agents
Acetaminophen
ASA
Fenoprofen
Indomethacin
Noraminopyrimidine
Oxyphenbutazone
Phenylbutazone
Sulindac
Anticonvulsants
Carbamazepine
Diphenyl-hydantoin
Valproic acid
Antimicrobials
Aminosalicylate
Ampicillin
Erythromycin
Methicillin
Penicillin
Rifampicin
Sulfonamides
Tetracyclines
Trimethoprim-sulfamethoxazole
Cinchona alkaloids
Quinidine
Quinine
Diuretics
Chlorothiazide
Furosemide
Hydrochlorothiazide
Miscellaneous
Actinomycin
Amrinone
Bleomycin
Chloroquine
Chlorpropamide
Cimetidine
Cocaine
Danazol
Digitoxin
Gold salts
Heparin
Heroin
Interferon
Methyldopa
Procainamide
Ranitidine

Data from [62].

The immune mechanisms are as follows [42]:

- *Type II:* the drug or one of its metabolites can bind to the granulocyte membrane acting as a hapten, to which the resulting antibody binds, thus eliciting neutrophil splenic sequestration.
- *Type III:* drug IC and anti-drug antibody bind to Fc or complement receptors on granulocytes.
- The drug antigenically modifies the neutrophil membrane, *to create neoantigens* that are not recognized as self by the immune system, evolving into an Aab-induced reaction.
- The drug *induces Aab against neutrophils,* without reacting with neutrophils.
- *Drug-induced immune mechanisms* may interfere with T-cell regulation of granulopoiesis [62].

Via immune mechanisms, a number of drugs potentially cause *immune neutropenia,* associated with potential granulopoiesis inhibition in mature circulating granulocytes (aminopyrine, ibuprofen, hydantoin, quinidine, clozapine and ticlopidine); others evoke anti-neutrophil drug-dependent antibodies, reacting directly with mature circulating neutrophils (β-lactam antibiotics, cephalosporin, phenytoin, flecainide, procainamide, propylthiouracil). Other drugs acting as drug-dependent antibodies against mature circulating granulocytes are summed up in Table 19.23 [42]. CAF (chloramphenicol) has been responsible for seven lethal cases of aplastic anemia [90].

Pulmonary Manifestations

Prevalence of hospital admissions amounts to 1%–5% [171], several cases are not diagnosed or reported [134]. *Hypersensitivity lung disease:* following sensitization, ADRs develop within 4–6 h of exposure including an acute or subacute onset, rapid recovery after drug withdrawal and symptom worsening when reintroduced. Laboratory tests and radiographic findings are nonspecific and compared to other illnesses in different organs, it is characterized by rapid recovery after drug discontinuation. Laboratory findings include peripheral eosinophilia, elevated IgE concentrations, and increased lymphocyte blastogenesis resulting from positive lymphocyte transformation test (LTT) [134].

Various problems encompass drug-induced asthma and pulmonary infiltrates [46]:

Asthma may often occur as part of a hypersensitive ADR and rarely is the only manifestation of a systemic allergic reaction. ASA and NSAIDs are among the most common drugs underlying asthma: prevalence in asthmatics is 10% (Table 19.24) [79, 100, 141, 207]. Moreover, 24 children reacted to ibuprofen (63%), acetaminophen (46%), diclofenac (25%) and other NSAIDs (9%) [84]. Drugs can structurally damage the respiratory system by four mechanisms:

Alveolocapillary endothelium direct cytotoxic lesions

Oxidative lesions

Intracellular deposition of phospholipids produced by amphophil drugs

Immune-mediated lesions [171]

Table 19.23. Drugs most commonly causing immune agranulocytosis

Analgesics and anti-inflammatory drugs
Acetaminophen[a]
Aminopyrine
Gold salts
Indomethacin[a]
Penicillamine
Phenacetin[a]
Phenylbutazone
Antibiotics
Cephalosporin
Chloramphenicol[a]
Clindamycin
Gentamicin
Isoniazid
Noramidopyrine
Semi-synthetic penicillins[a]
Streptomycin
Sulfonamides[a]
Tetracyclins
Trimethoprim-sulfamethoxazole
Vancomycin
Anticonvulsants
Carbamazepine
Mephenytoin
Phenytoin
Antimalarials
Chloroquine
Dapsone
Quinine
Antithyroids[a]
Carbimazole
Methimazole
Propylthiouracil
Mercury diuretics
β-Blockers
Cimetidine
Ranitidine
Cardiovascular drugs
Captopril
Desipramine
Hydralazine
Methyldopa
Procainamide
Propanolol
Quinidine
Hypoglycemic agent
Chlorpropamide
Tolbutamide
Hypnotics and sedatives
Benzodiazepines
Chlorpromazine[a]
Desipramine
Doxepin
Imipramine
Meprobamate
Phenothiazine[a]
Miscellaneous
Allopurinol
Clozapine
Levamisole
Ticlopidine

Data from [42].
[a] Drugs most often quoted in epidemiological studies.

Asthma can be caused by various mechanisms:
Pharmacodynamic (parasympathomimetic, α and β-adrenergic)
Cycloxygenase inhibition (ASA and NSAIDs)
Hypersensitivity (ACTH, hydrocortisone, penicillin and other β-lactams, cimetidine, rarely aminophylline)
Local irritations (acetylcysteine, sulfites and sulfates) [79]

Pulmonary infiltrates characterize eosinophil pneumonia and infiltrative reactions (Table 19.25) [108, 141, 207].

Eosinophil pneumonia, rare in children, occurs with few clinical symptoms (mostly fever, dyspnea, wheezing), accompanied by a chest x-ray showing peripheral nodular infiltrates, marked eosinophilia, high IgE levels [108] and expression of HLA-DR molecules [13]. Two patterns have been systematized: in the acute pattern, in ≈90% of cases the reactions occur within 1 month; in the remaining cases with a chronic pattern the reactions occur within 2–5 months. In the acute form, resolution is usually the rule upon drug withdrawal; chronic cases, if complicated by interstitial fibrosis, which nosologically excludes them from eosinophil pneumopathology [152], are more difficult to deal with [108], especially if caused by antirheumatic [239] and chemotherapeutic drugs [234].

Infiltrative reactions normally develop 2–10 days after treatment is started, with acute symptoms characterized by cough, dyspnea, chills, fever and malaise, a maculopapular eruption is often also present; a few or basilar crepitant rales and, on occasion, a few focal rales and a harsh sound on lung bases may be appreciated. x-ray findings include widespread alveolar, reticulonodular, and focal migratory infiltrates. Sometimes patients present patterns similar to pulmonary edema, especially following treatment with nitrofurantoin [46].

Table 19.24. Drugs provoking or exacerbating asthma

Types of drugs causing asthma
1. Antibiotics
Cephalosporins Nitrofurantoin Penicillins Tetracycline
2. β_2-Adrenergic blocking agents
Topical or systemic
3. Cholinergic agonists
Bethanechol Carbachol Cholinomimetic alkaloids Muscarine Pilocarpine Echothiophate iodide Methacholine
4. Corticosteroids
IV hydrocortisone *
5. Diuretics
Hydrochlorothiazide/triamterene
6. Chemotherapeutic agents
Bleomycin Methotrexate, Podophyllotoxins Vinca alkaloids[b] Zinostatin
7. Nonsteroidal anti-inflammatory drugs (NSAIDs)
ASA Acetaminophen Indomethacin, etc. Phenylbutazone
Uncommon causes of drug-induced asthma
1. Agents experienced by airborne exposure in pharmaceutical manufacturing or in the hospital or laboratory place of work
2. Additives or propellants to inhalant medications
3. Agents inducing or aggravating bronchospasm via local irritant effect and via non-IgE mast cell activation
Colistin IV anesthetic agents Muscle relaxants Opiates Polymyxin Radiographic contrast media
4. Drugs causing or exacerbating gastroesophageal reflux
5. Sulfites and metabisulfites
Agents that mimic asthma
1. ACE inhibitors

Modified from [79, 100, 141, 207].
[a] In ASA-sensitive asthmatic children.
[b] In combination with mitomycin-C.

Table 19.25. Drugs causing eosinophil pneumonia and pulmonary infiltrates

1. Eosinophilic pneumonia
Ampicillin ASA Azathioprine Beclomethasone Bleomycin Captopril Carbamazepine Chlorpropamide Chlorpromazine Dilantin Glaphenine Gold salts Imipramine Mephenesin Methotrexate Naproxen Nitrofurantoin PAS Penicillin Phenothiazine Phenylbutazone Procarbazine Propylthiouracil Streptomycin Sulfonamides Tetracycline
2. Pulmonary infiltrates
Gold salts Hydrochlorothiazide Isoniazid Melphalan Methotrexate Nitrofurantoin Procarbazine hydrochloride Rifampicin Streptomycin Tetracycline

Data from [108, 141, 207].

The atopic individual may develop IgE to these antigens, and upon exposure manifest bronchial asthma caused by the response outlined above. A delayed response may be mediated by CMI [46]. CMI mechanisms and lymphocyte subset imbalances have been proposed without reaching definite conclusions [207]. As stated in Table 19.25, there is a multitude of drugs involved, but before making a diagnosis, we should exclude other potential causes, especially infective ones [134].

Pulmonary infiltrates with eosinophilia are a group of heterogeneous disorders with the common findings of lung disease and eosinophilia in the peripheral blood, bronchoalveolar lavage fluid, or pulmonary interstitium. Among 11 retrospectively identified cases, there were 2 drug-induced cases, 3 cases of acute eosinophilic pneumonia, 2 of infant pulmonary eosinophilia, and 5 of other diseases. The disorder is associated with signif-

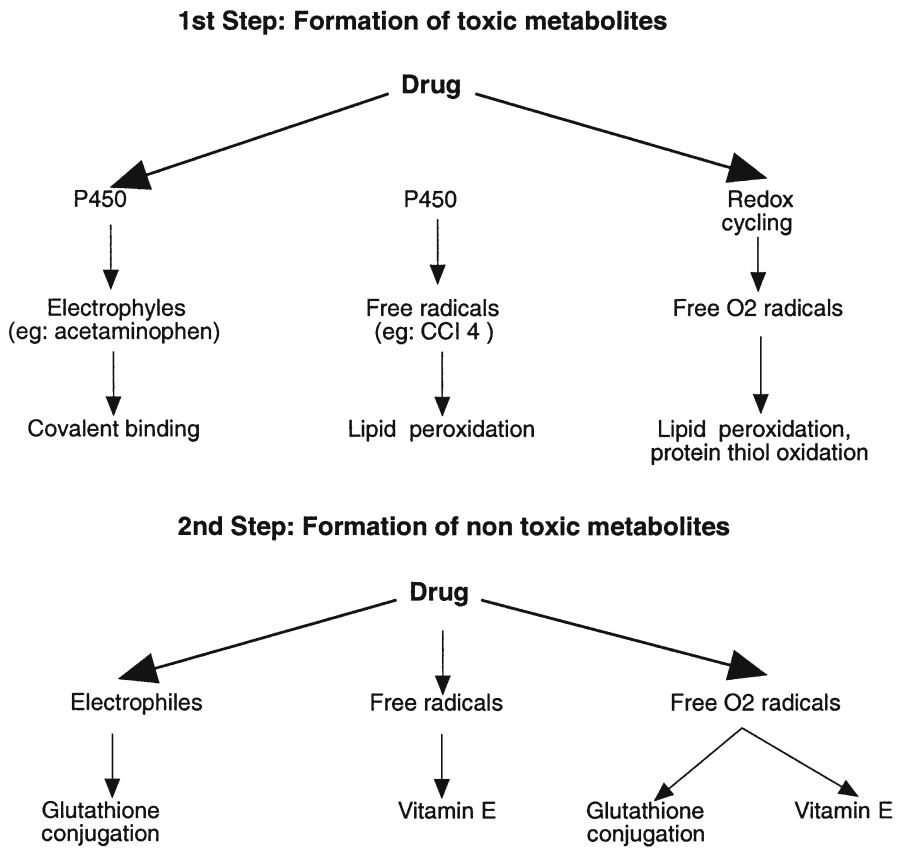

Fig. 19.19. Drug biotransformation up to detoxification (see text). Drug biotransformation is the conversion from a lipophilic to a hydrophilic compound to be filtrated by the glomerule or excreted by the bile. CCl4 C tetrachloride. (Modified from [229])

icant morbidity and mortality; therefore aggressive diagnostic evaluations are warranted, especially in children with respiratory failure of unknown etiology [135].

Hepatic Manifestations

The liver is the main organ concerned for drug metabolism and is specifically susceptible to drug injury as severe as liver failure [100]. Acetaminophen overdose was the most common apparent cause of acute liver failure, accounting for 39% of cases. Idiosyncratic drug reactions (isoniazid) were the presumptive cause in 13% of cases, viral hepatitis A and B combined were implicated in 12% of cases, and 17% of cases were of indeterminate cause [138]. The reactions are characterized by fever, cutaneous eruptions, eosinophilia and inflammatory infiltrates rich in eosinophils or granulomas in the hepatic parenchyma, with a sensitization period of 1–4 weeks and immediate exacerbation of hepatic function following readministration of even small doses of involved drugs [100]. Regarding liver injury, there was neither the ability to prove dose-related hepatic damage in laboratory animals, nor the presence of hepatitis-associated antigens, nor a correlation between drug dosage and cellular damage. We therefore wonder how a drug can be able to cause immune reactions, since most drugs are of low MW and nonimmunogenic [229]. To correctly classify this paradox, we must first confront a dilemma: most drugs provoking immune reactions are not reactive to the point of being capable of acting as *haptens*, forming drug–carrier conjugates that in turn interact with the host immune system [221]. Consequently it has been speculated that, via liver biotransformation products, various drugs may serve as *haptenic metabolites*, forming compound substances (step 1, Fig. 19.19) [229]. Having formed this conjugate, it becomes capable of acting as an immunogenic agent and activates a specific humoral or CMI response, or both. This acquisition, based on research on halothane-caused hepatitis, has stimulated the identification and characterization of such haptens involved in hypersensitive reactions. Currently these haptens have been purified and can be used as neoantigens for identifying at-risk individuals [229]. In the second step of Fig. 19.19, it is possible to observe how detoxification occurs (step 2): electrophiles are conjugated with glutathione, free radicals interact with vitamin E, while O_2 free radicals are eliminated by both mechanisms. These in turn lead to varying biochemical consequences, including covalent binding and lipid peroxidation. Different variables, endogenous and exogenous, modulate the delicate balance of hepatic drug metabolism in either direction, the main factors being genetic polymorphism, enzyme induction, nutrition, hepatic functional heterogeneity and enzyme polymorphism [223].

Table 19.26. Drugs and other substances incriminated in acute and chronic liver damage

Direct reaction
Acetaminophen
Carbon tetrachloride
Mushrooms
Phosphorus
Idiosyncratic reaction
Disulfiram
Isoniazid
Propylthiouracil
Toxic-allergic reaction
Halothane
Isoflurane
Ticrynafen
Allergic reaction
Amoxicillin
Clavulanic acid
Phenytoin
Sulfonamides
Primitive cholestatic reaction
Aprindine
Captopril
Chlorpromazine
Erythromycin
Estolate
Estradiol
Ethylchlorvynol
Haloperidol
Imipramine
Nalidixic acid
Nitrofurantoin
Papaverine
Phenothiazine
Sulfonamides
Sulindac
Troleandomycin
Primitive hepatocellular reaction
Amphotericin B
Ethacrynic acid
Furosemide
Gold salts
Griseofulvin
Halothane
Hydantoin
Ketoconazole
Methyldopa
Monoamine oxidase inhibitor
Nitrofurantoin
Oxyphenisatin
Para-aminosalicylic acid
Phenylbutazone
Propylthiouracil
Pyrazinamide
Quinidine
Rifampicin
Sulfonamides
Sulindac
Valproic acid
Microvesicular steatosis
Aspirin
Didanosine
Fialuridine
Tetracyclines
Zidovudine
Veno-occlusive disease
Chemotherapeutic agents
Cyclophosphamide
Herbal teas
Miscellaneous
Clozapine
Diclofenac sodium
Doxepin
Etoposide
Etretinate
Flutamide
Glyburide/glybenclamide
Ketoconazole
Labetalol
Methotrexate
Norfloxacin
Ofloxacin
Pentamidine
Phenytoin
Procainamide
Terbutaline
Ticlopidine hydrochloride
Trazodone

Data from [100, 141, 207].

A number of preparations are implicated in direct, allergic, toxic-allergic and idiosyncratic hepatic injury [100]. Histopathological studies show that hepatic lesions are of two kinds: either intrahepatic cholestasis or hepatocellular damage (Table 19.26) [100, 141, 207]. In the first case, a number of hepatic tissue components may become sensitized during treatment with phenothiazine and sulfonamides; the mechanism at the basis of hepatocellular reactions is even less clear. Histopathological studies document the microscopic lesions (Fig. 19.20). The prognosis is good and recovery is ensured by stopping treatment. Data on erythromycin shows that, with the exception of cases caused by estolate, rare cases of hepatic lesions based either on toxic metabolites or hypersensitivity reaction were reported [64].

Hepatocellular damage induced by halothane, isoniazid, methyldopa and oxyphenisatin can mimic chronic active hepatitis, but is different because after treatment discontinuation the hepatic picture returns to normal. Some hepatotoxic drugs instead cause a picture virtually indistinguishable from that of viral hepatitis, charac-

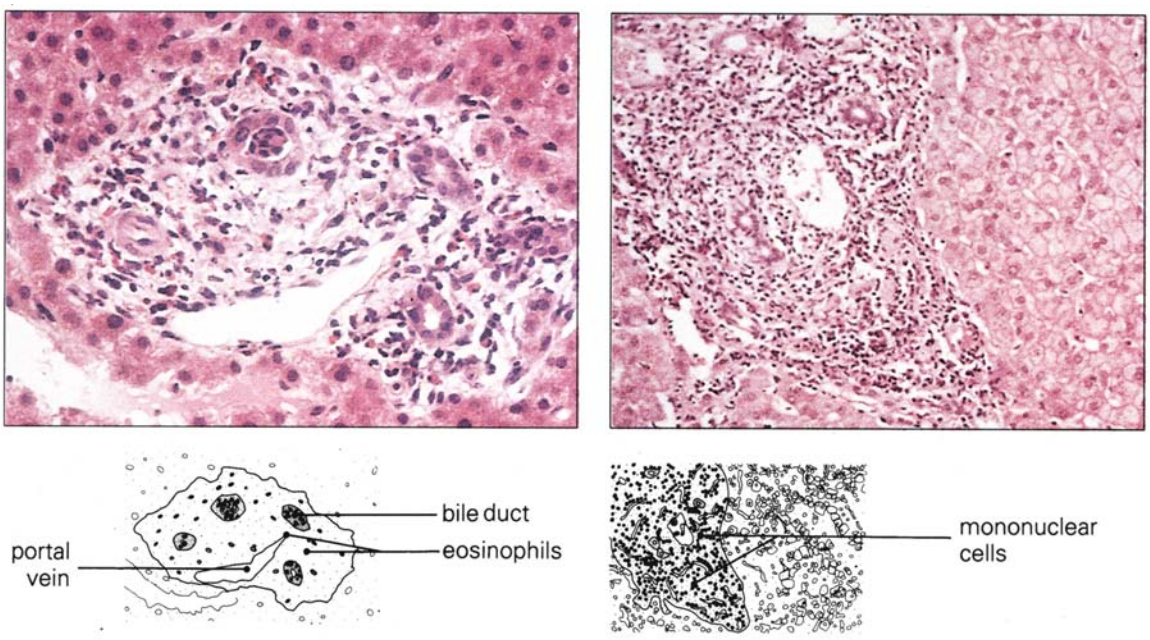

Fig. 19.20. Drug-induced hepatic disease with hepatic or cholestatic jaundice. *Left*: Chlorpromazine-induced cholestatic jaundice showing eosinophilic infiltrate in the portal tract. *Right*: Hepatic jaundice showing periportal mononuclear infiltrate

terized by a rapid onset of malaise and jaundice in association with elevated aminotransferase levels, fever, hepatomegaly, skin rashes and/or eosinophilia [100]: this is the reaction most often present in children treated with valproic acid. Recovery is good if liver injury is not severe [229]. Last, we mention Reye's syndrome, acute liver failure that may follow ASA intake during certain viral infections, particularly in children [63].

Renal Manifestations

The kidney is vulnerable to drugs because of its high blood flow and large capillary surface area, its role as the excretory route for many drugs and its detoxifying action, because any substance or drug present in the blood can easily come into contact with the glomerular endothelium and tubular epithelium [54]. Little is known about epidemiology of drug-induced disorders in the pediatric kidney [54]. Damage can be functional, as in the case of drugs acting on the pre- and postglomerular small arteries such as ACE inhibitors and NSAIDs, or organic with lesions that are more frequently interstitial [87] (Fig. 19.21).

Tubulointerstitial nephritis is most likely ADR-induced among renal manifestations; in 8% of cases this is the cause of renal failure [47]. The clinical picture, which appears 5–37 days (average 2 weeks) after starting penicillin, is marked by fever, maculopapular morbilliform rashes, arthralgia, eosinophilia and eosinophiluria, macro- or microhematuria, proteinuria, leukocyturia, pyuria and elevated IgE levels. Direct renal tubular toxicity has also been described with a number of new medications with unique effects on the epithelial cells of the kidney [153]. The drugs are listed in Table 19.27 [98] including the antiviral agents cidofovir, adefovir, and tenofovir as well as the biphosphate pamidronate [153]. Compared to adults, pediatric ADRs are more modest and do not last as long; regardless of the degree of severity, 90% of patients recover within 1 year. In acute immunological forms caused by penicillin and other drugs listed in the table, other symptoms have been identified such as eosinophilia, eosinophiluria, high total IgE levels and humoral and CMI mechanisms [46, 98]. In particular, the following have been identified: penicilloyl haptenic group of methicillin, antibodies to tubular basement membrane, complement (C3 bound to tubular basement membrane) and CICs; a positive LTT to methicillin, late reaction to a SPT for the drug and the interval necessary for the appearance of symptoms do indicate CMI [205]. Eosinophiluria is almost diagnostic [128].

ADR-induced *nephritic syndrome* (Fig. 19.22) can appear during treatment using anticonvulsants, heavy metals, penicillamine and puromycin. In some cases immunochemical data seem to indicate type III immune mechanisms [204].

In *glomerulonephritis* with the possible onset of serum sickness, cimetidine, D-penicillamine, dihydralazine, and NSAIDs, etc. are usually involved [87].

Hemodynamic renal failure may result from drugs that reduce renal PGs and thus renal blood flow and glomerular filtration rate [153]. An increase in the last 10 years in the involvement of drugs in acute renal failure has been observed in newborns [54].

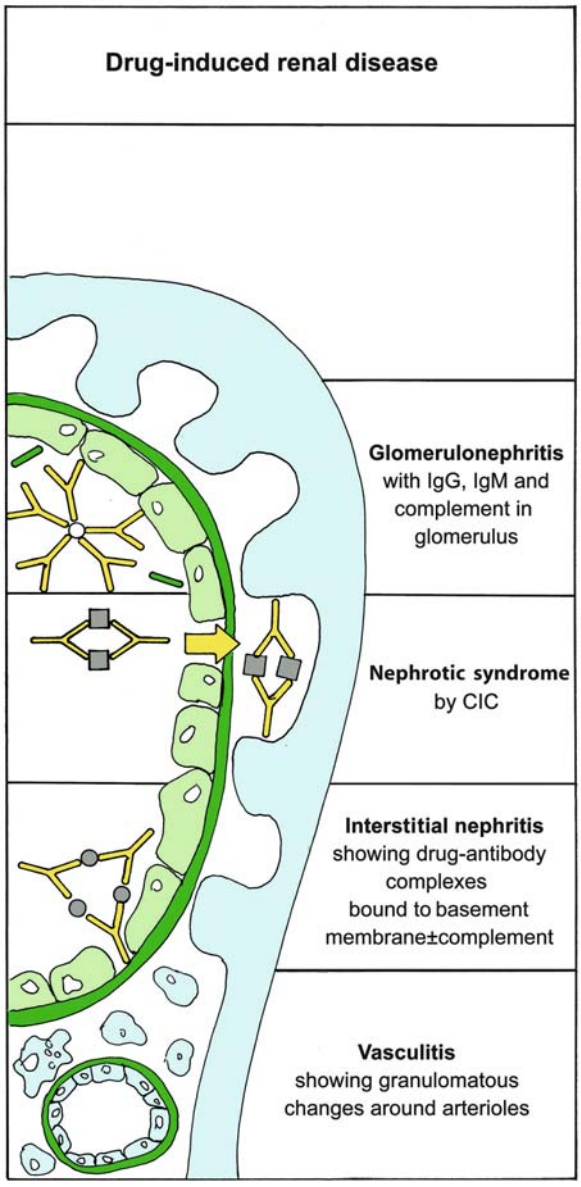

Fig. 19.21. Drug-induced renal disease

Table 19.27. Drugs implicated in interstitial tubular nephritis

1. Antibiotics
A. Penicillins and derivatives
Amoxicillin
Ampicillin
Carbenecillin
Methicillin
Oxacillin
Penicillin G
B. Cephalosporins
Cephalexin
Cephalothin
Cefoxitin
Cephradine
2. Additional antibiotics
Rifampicin
Vancomycin
Sulfonamides
Trimethoprim
Nonthiazide diuretics
Furosemide
3. Analgesics and anti-inflammatory drugs
Antrafenine
Diflunisal
Fenoprofen
Floctafenine
Glafenine
Indomethacin
Mefenamic acid
Naproxen
4. NSAIDs
Acetaminophen
Noramidopyrine
Phenazone
Phenylbutazone
Pyrazolone derivatives
Sulfinpyrazone
Tolmetin sodium
5. Additional drugs
Ajmaline
Allopurinol
Azathioprine
Captopril
Cimetidine
Clofibrate
Para-aminosalicylic acid
Phenindione

Data from [98].

Enteric Symptoms

Pseudomembranous colitis is linked to broad-spectrum antibiotics (ampicillin-amoxicillin, CAF, clindamycin, lincomycin, penicillin, tetracycline), which by altering the enteric flora favor hyperproliferation of *Clostridium difficile*. This elaborates B toxins, causing mucosal cytopathy in low doses, and is potentially lethal in high doses [161].

Anticonvulsant hypersensitivity syndrome (AHS) is a rare syndrome that occurs with exposure of variable lengths to anticonvulsant medications, which have a high risk of triggering hypersensitivity syndrome in susceptible individuals and their first-degree relatives. AHS may be associated with severe morbidity and sometimes even mortality [179]. It is characterized by fever, rash, edema, conjunctivitis, oral ulcers [179] and generalized lymphadenopathy along with visceral organ involvement [212]. Liver damage, in particular, seems to be associated with fatal outcomes. The pathophysiology of AHS is still uncertain, but it may be linked to a genetically determined inability to detoxify reactive drug metabolites. AHS has also been described with carba-

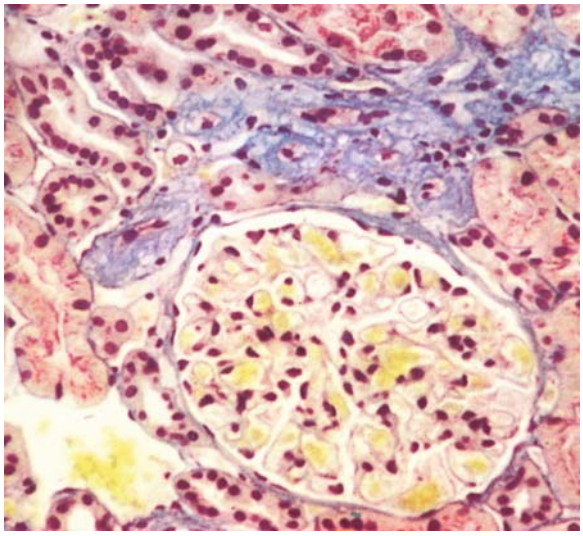

Fig. 19.22. Minimal change glomerular nephropathy. Histology usually shows normal glomeruli, but EM evidences fusion of epithelial foot processes in each glomerulus

mazepine, dapsone, phenobarbital, phenytoin, minocycline, and sulfonamides [212]. In addition to early withdrawal of the offending drug, treatment is mostly symptomatic. Recently, a 6-year-old boy showed a dramatic response to IVIg within 24–48 h, with an improvement in overall well-being and alleviation of AHS symptoms [179].

Reactions Potentially But Not Definitely Drug-Induced

Erythema multiforme is considered a variant of the pathogenetic process underlying urticaria. A minor acute form is known as well as a major form, which some have identified with TEN or SJS [172]. *EM minor* is perhaps the most common aspect of cutaneous allergic ADR (46%–94% of cases), with a usually benign course, rarely seen by doctors [227]. It is characterized by the abrupt onset of self-limiting cutaneous eruptions and by a wide range of symmetrical lesions with typical targets or raised edematous papules acrally distributed [12]. *EM major* exanthema is widespread, initially maculopapular, and later urticarious lesions involving one or more mucous membranes with blisters and bullae surrounded by a central elevated edematous ring in expanding erythematous macules or papules, spreading on dorsal surfaces of distal extremities [99]. EM lesions are fixed, and red papules, some of which evolve into plaques with central, dusky coloration and blister formation, called target or iris lesions, remaining in the same skin site for at least 7 days, are the hallmark of EM [227] (Fig. 19.23). Differential diagnosis with infections is indicated by finding rashes on both feet and hands in the first instance, while the lesions have an almost cyanotic aspect in the second instance. Furthermore, withdrawal of causative agents is followed by a quick recovery. Among the diverse etiological causes that have been purported, in 63.6% of 22 children were the infectious agents [99], the most reliable being HSV, mononucleosis, histoplasmosis and some drugs (Table 19.28) [12, 103], almost exclusively antibiotics and co-trimoxazole, often widely prescribed. Major EM, frequently used as a *synonym for SJS,* generally appears after HSV and *Mycoplasma* infections, and may be differentiated by one or more mucous membrane involvements [174]. The mean duration of EM disease was 12 days, 40% of children had recurrent disease. The mean disease duration was 16 days in ten children treated with steroids vs 15 days in ten non-steroid-treated children. A child with recurrent EM major was well controlled by thalidomide [99].

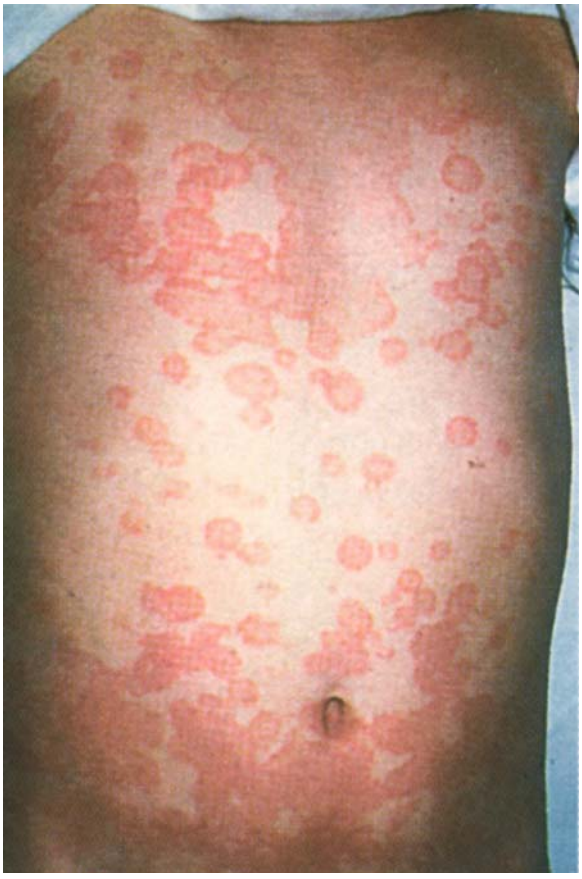

Fig. 19.23. Typical erythema multiforme lesions with target appearance

Exfoliative dermatitis or erythroderma is a potentially fatal cutaneous disease, characterized by macular (erythroderma) or maculopapular eruption over a period of 1–2 days and followed by an *extensive exfoliation* that progressively spreads to the whole body, accompanied by intense itching. If the affected areas are not quickly treated it may lead to edema and exudation, with severe dehydration. The lesions also provide a

Table 19.28. Drugs implicated in erythema multiforme

Cefaclor
Cimetidine
Codeine
Corticosteroids
Diphenylidantoin
Furosemide
Isoniazid
Ketoconazole
NSAIDs
Phenolphthalein
Quinine
Rifampicin
Sulfasalazine
Sulfonamides
Tetracyclines
Trimethoprim

Data from reference [12, 103].

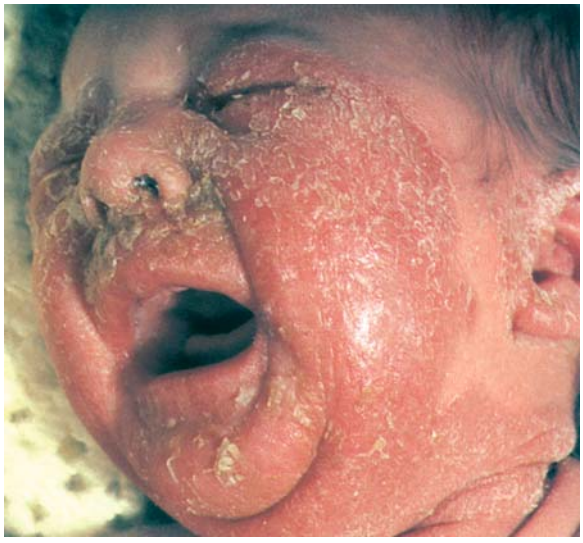

Fig. 19.24. Exfoliative dermatitis and extensive secondary infections in a baby following administration of phenobarbitone

fertile area for secondary infections. This could be a first stage of TEN or SSSS [103]. In infancy, a toxic reaction may be consequent to vitamin A poisoning following high-dose intake for some months [2]. About 10% of cases are attributed to drug-induced reactions (Table 19.29) [12, 207] (Fig. 19.24) or to cefoxitin therapy. From an immunological point of view, given the disease's eczematous nature, a mechanism mediated by T lymphocytes has been suggested [207].

Table 19.29. Drugs implicated in erythroderma

Allopurinol
Barbiturates
Carbamazepine
Idantoin
Penicillins
Phenothiazine
Phenylbutazone
Sulfonamides

Data from [12, 207].

Table 19.30. Drugs most commonly the cause of vasculitis

Allopurinol
Aminopenicillins
Cimetidine
Dilantin
Furosemide
Hydralazine
Idantoin
Ketoconazole
Levamisole
NSAIDs
Phenylbutazone
Propylthiouracil
Pyrazolone derivatives
Tetracyclines
Sulfonamides

Data from [12, 207].

Vasculitis (Table 8.19) – drug-induced forms mainly affect the small vessels and are subtypes of hypersensitive forms [12]. Some might be attributable to an immune pathogenesis most probably induced by CIC deposition. Other symptoms are systemic: fever, malaise, headache, arthralgia, myalgia, and abdominal pain [46]. Table 19.30 outlines the offending drugs more frequently purported in vasculitis [12, 172]. The overall picture that is most well-known in infancy is Schönlein-Henoch anaphylactoid purpura (Tables 8.20, 8.21 and Fig. 8.19). Drugs (antibiotics and thiazides), food, infections, vaccines, insect stings, etc. are usually incriminated. At the basis there is an immune Arthus-like mechanism (type III) with infiltration of polynucleates and the typical lesions of leukocytoclastic vasculitis. A high concentration of CIC-IgAs and serum IgA levels are reported while ANA is negative (Table 8.26).

Table 19.31. Drugs and related substances inducing PARs

Histamine-releasing substances
Polybasic compounds
Compound 48/80
Meperidine
Neuropeptide Y
Polymyxin B
Protamine
Somatostatin
Substance P
Vancomycin
Vasoactive intestinal peptide
Other drugs
Atracurium and tubocurarine
Codeine, fentanyl and morphine
Deferoxamine
Pentamidine
Radiocontrast agents
Thiopental
Vitamin K
Complement activators
Endotoxins
Foreign proteins
Gammaglobulins
Iodine-based radiocontrast agents
Plasma substitutes
Dextrans
Human albumin
Plasma expanders
Protein fractions
Enzyme inhibitors
ACE inhibitors
Anti-cholinesterase
ASA
NSAIDs
Drugs acting on CNS
Local anesthetics
Muscle relaxants
Systemic anesthetics
Sympathomimetics
Adrenergic β-blockers drugs
Cholinergic and anticholinesterase
Other antihypertensive drugs
Additional drugs
Additives
Antihistamines
Cimetidine
Chlorpheniramine
Ranitidine
Immunomodulators and immunosuppressors
Cyclosporine A
Sirolimus
Preservatives
Vaccines

Data from [206].

Pseudoallergic ADR

PARs involve several drugs (Table 19.31) [206], which cause symptoms suggesting allergy, but are rarely immune-mediated. As previously mentioned, the clinical similarity of these reactions to allergic responses is caused by the same effector cell involvement and the same mediators of allergic inflammation [221]. Some possible mechanisms are the following:

- Direct stimulation of metachromatic cell mediator released through nonspecific mechanisms (Table 10.13)
- Generation of C3a/C5a by alternate complement pathway
- Direct stimulation of platelets, involving the indirect mediator release through effector cells
- Plasma generation of bradykinin/quinine
- Nonimmunological stimulation of other effector cells (eosinophils, neutrophils, monocytes, lymphocytes)
- Release of inflammatory neuropeptides by neurons [45]

Differentiation from allergic reactions is shown in Table 19.5: PARs almost always appear after a first exposure, but only if the dose is sufficiently high, and often look similar to toxic reactions rather than an immune-mediated mechanism. It must be emphasized that, in addition to light reactions such as ampicillin-induced rashes, increased during viral upper respiratory tract infections (URTI) [184], there can also be severe reactions, sometimes fatal, because several drugs are capable of causing anaphylactic or anaphylactoid reactions (Table 19.9), also depending on prevalent widespread use. In particular, *anaphylactoid reactions*, caused by direct and nonspecific mast cell mediator release, are caused by iodized radiocontrast media, IV anesthetics, muscle relaxants, codeine, polymyxin, thiamine, tubocurarine, ASA and NSAIDs. The reactions are more severe if the offending substance is given rapidly and IV and/or high doses of drugs are promptly released into the circulation [206]. Prevalence is estimated at around 1 per 5000, with a rate of fatalities of 6 % among patients treated (Chap. 20).

Additional Drug-Induced Reactive Syndromes (Table 19.31)

Histamine-Releasing Substances [206]

Polybasic compounds

In humans, polybasic compounds release histamine only from skin mast cells and include:

- *Compound 48/80* is used by the pharmaceutical industry as a standard reagent to study the nonimmunological histamine release and mast cell heterogeneity.
- *Polymyxine B* is rarely used due to its nephrotoxicity.
- *Vancomycin* is an antibiotic that is highly effective against methicillin-resistant streptococcal infections and those caused by Gram-positive germs in penicillin-

allergic patients. It causes eosinophilia and the so-called *red man syndrome* similar to erythroderma. If too quickly IV-injected it may cause angioedema, pruritus and sometimes chest pain, dyspnea and hypotension.

- *Protamine* is IV-injected to counteract heparin anticoagulating effects and to treat overdoses of this hormone, with severe systemic reactions sometimes occurring [16]. There is also evidence of an allergic sensitization: in diabetics previously treated with insulin containing protamine, anti-protamine IgE and IgG antibodies increased risk to 95% and 38%, respectively. Very severe reactions were experienced by rechallenged patients, while 5/9 controls had only IgG. Protamine can potentiate in vitro basophil release of IgE-mediated histamine. In nondiabetic subjects, the presence of protamine IgG is significantly associated with an increased risk of acute protamine reactions, although many nondiabetic subjects had reactions but no IgG antibodies [226].
- *Somatostatin* is a compound that is no longer in use, and has been replaced by an analogous octa-peptide, provided with minimum effects on histamine.
- *Substance P* is provided with histamine-like activity.
- Neuropeptide Y is stored in noradrenergic neurons along with norepinephrine. The characteristics of histamine release are shared with those of substance P.

Other drugs [213]

- *Atracurium* and *d*-tubocurarine. The latter is the only muscle-relaxing agent triggering a clinically significant increased histamine release.
- *Codeine,* meperidine and morphine cause mediator direct release and cutaneous reactions to the same extent, the first two are also systemic.
- *Deferoxamine,* an iron-chelating agent elicits hypotension and anaphylactic shock if not slowly IV injected.
- *Phytonadione* (colloidal vitamin K), administered by IV, probably because of the effect of fatty acids used as emulsifiers, by releasing mediators and/or inducing vasodilation, may provoke shock, which should be a warning sign.
- *Pentamidine* causes 75% of IV-treated patients to manifest facial flush, pruritus, nausea, tachycardia, hypotension and syncope.
- *Thiopental* or thiopentone produces a noncytotoxic dose-related mast cell histamine release; the prevalence of reactions is estimated at between $1 \times 23{,}000$ and $1 \times 29{,}000$ administrations [213].

Complement Activation

Many substances can activate complement via nonimmunological mechanisms, including [213]:

- *Bacterial endotoxins* activate both pathways and zymosan only the alternative pathway if used for testing properdin.
- *Ig* activate the classic pathway generating anaphylotoxins. Previous use was limited due to a frequent onset of anaphylactoid reactions because of high-MW complexes. Reactions are more frequent in agammaglobulinemic patients, in particular with a selective IgA deficit (Chap. 22), or after fortuitous IV injections or repeated administrations.
- *Foreign proteins* such as peptone are used on laboratory animals.
- *Hemoderivates* and plasma substitutes are substances used as plasma substitutes. They are capable of direct mast cell activation.
- *Albumin* causes reactions due to aggregates formed during the polymerization process indispensable for preservation or stabilizing.
- *Dextrans* are available in three types with different MWs, which induce clinical symptoms in five stages, going from urticaria to a systemic reaction, and also cardiorespiratory arrest. The prevalence of severe effects is 0.008%–1% of administrations. They activate the complement alternative pathway, generating C2, C3 and C5. Compounds with lower MW are less immunogenic, but may react with anti-dextran antibodies belonging to IgG or IgM classes, formed in response to previous exposure to polysaccharides of bacterial or viral origin [213].
- *Protein fractions,* where rapid infusion determines a feeling of heat and a full head, constrictive chest pains, abdominal and back pains and hypotension are attributed to prekallikrein activators that stimulate the prekallikrein-kallikrein system during infusion [214].
- *Plasma expanders* such as fluid gelatins can be used as fillers in emergency situations and in measles vaccine [31]. Prevalence of reactions varies from 0.07% to 0.25% of infusions, while *severe reactions* range from 0.016% to 0.066%. From a pathogenetic point of view, histamine is released by metachromatic cells, mostly nonimmunological [85]. Clinical manifestations also are divided into five stages, with progressive worsening leading to death [213].

Enzyme Inhibitors

ACE inhibitors are practically used in managing hypertension. The first drug, captopril, produces maculopapular rashes in 50% of patients, attributed to kininase inhibition. New preparations have a lower effect, related to sensitivity of these molecules to sulfydryl groups. It has been ascertained that at least 10% of patients present at night a persistent, nonproductive cough, at times extremely irritating, and asthma worsening [206]. Anticholinesterase will be discussed in "Adrenergic and Cholinergic Substances."

Drugs Active on the Central Nervous System

Anaphylactic reactions caused by *general anesthetics* are better known [16, 20], while *local anesthetics* such as benzocaine can cause ACD immune type systemic reactions are very rare. Halothane, still used in the *induction stages in pediatrics,* causes reactions in 1 × 35,000 cases [141]. A 4-year-old child developed systemic anaphylactic reaction to lidocaine hydrochloride within 15 min after a dental procedure [35]. A 14-month-old boy developed anaphylaxis to propofol. The child was allergic to egg and peanut oil, and propofol formulation contains both foods [76]. Toxic reactions are more frequent, resulting from rapid drug absorption, inadvertent IV injection, or overdosage. Hence preservatives such as parabens and Na sulfite are blamed [214], more frequently during dental anesthesia or small surgical procedures [6]. Negative SPTs do not necessarily predict that a drug will be tolerated. Latex allergy (Chap. 8) should always be considered in the differential diagnosis of a perioperative reaction.

Additional muscle relaxants include reactions to *chymopapain* more frequent in atopic patients previously exposed to papain, for those who are candidates for chemonucleolysis, SPTs are extremely predictive [123]. *Suxamethonium* more often than other drugs causes IgE-mediated reaction, fourfold more frequently than with vecuronium and 2.5-fold more frequently than with atracurium, respectively inducing bronchospasm and hypotension [220]; others are discussed in Chap. 20.

Adrenergic and Cholinergic Substances [206]

- *Adrenergic β-blockers,* of which propanol causes or worsens asthma, 50% of asthmatics may experience bronchospasm, which does not improve with inhaled bronchodilators. There were reports in American asthmatics of 13 cases of deaths and 200 severe reactions to timolol maleate eye drops; β_1-blockers only partially avoid undesired effects [213]. Cases involving shock have been pathogenetically attributed to cAMP inhibition, by reducing its endocellular concentrations, and to the consequent lower threshold of mediator release by metachromatic cells. Moreover, these drugs decrease endogenous epinephrine secretion, both by blocking β_2-receptors at synapsis and inhibiting β_1 effects of endogenous and exogenous epinephrine on the heart [213]. All β-blockers increase in vitro the synthesis and release of anaphylactic mediators, as well as IgE levels [206]. They may be implicated in undesirable reactions that, albeit rarely (0.015% of cases), occur during SPT and SIT administration (specific immunotherapy) [72]. Definite data derive from a double-blind study on a statistically representative sample of patients. In the meantime, in collecting patient history in adult subjects, it is worth investigating any contingent use of β-blockers [69].
- *Other antihypertensive drugs,* for example, reserpine causes dose-related nasal obstruction and rhinorrhea and bronchospasm worsening at higher doses. Additional side effects are dry mouth and nasal mucosa from clonidine, asthma and nasal congestion from guanethidine, dyspnea from hydralazine, nasal obstruction and dry mouth from methyldopa [207].
- *Cholinergic agents* and *anticholinesterase.* The effects of methacholine are so predictable that it is used for bronchial provocation testing (Chap. 6). Another group, the *reversible inhibitors,* are used for managing paralytic ileus, urinary bladder atony, etc. These should be used by asthmatics with due care [206].

Characterization of Various Drugs and Categories of Drugs

Tables 19.32–19.34 [132, 186] summarize the best-known drugs containing sulfites and also rates of dye agents and aromas; those stemming from Na benzoate are shown in Fig. 10.3; sensitivity to tartrazine has been confirmed in Chap. 10, where we reported that of the 2%–35% the illustrative leaflets of several drugs did not specify the type of additive included. Not all oral preparations contain doses of sulfite capable of causing reactions: asthmatic reactions occur using injected products in very intolerant patients [186]. In Chap. 11, we analyzed side effects caused by CSs. Table 19.2 shows the rate of severe and moderate reactions. A meta-analysis reported approximately 100 published reports of immediate hypersensitivity reactions occurring after oral and parenteral administration of CSs [82]. Deflazacort, structurally similar to methylprednisolone, given to two 12-year-olds with severe rheumatic forms, with a dose of 1.5–2 mg/kg/day, caused a vertebral collapse [53]. Vaccines, in addition to the immunizing agent, can also contain small amounts of allergens, derived from production processes, such as egg albumin and other growth media for viruses, silk used for filters, antibiotics, preserving agents including phenol, mercurials, nystatin and gelatin, for example in the MMR (measles-mumps-rubella) vaccine [40] but no case of anaphylaxis [24]. A 28-month-old child experienced a severe anaphylactic shock following a testing dose of aprotinin during major orthopedic surgery [174a].

NSAIDs and ASA

NSAIDs and ASA inhibit cyclooxygenase, consequently blocking prostaglandin, prostacyclin and thromboxane synthesis from ASA, and the diversion down the 5-lypoxygenase pathway, finally producing leukotrienes [34]. NSAIDs, although possessing a chemical structure not remotely correlated to that of ASA, and also in the ab-

Table 19.32. Medications containing sulfites

Agent types	Denomination
Analgesics	Meperidine
Antibiotics	Amikacin, gentamycin, rifamycin, tobramycin
Antiemetic agents	Metoclopramide
Antipyretic drugs	Acetaminophen
Bronchodilators	Isoetharine, isoproterenol
Cardiotherapeutics	Dopamine, norepinephrine
Corticosteroids	Betamethasone, dexamethasone, hydrocortisone
Epinephrine	All forms
Eye drops	Dexamethasone, prednisolone, sulfacetamide
Local anesthetics	Lidocaine, procaine
Mucolytics	*S*-carboxymethylcisteine
Solutions for dialysis	
Solutions for parenteral alimentation	
Thorazine	

Data from [132, 186].

Table 19.33. Dyes in 650 medications

Dye	%
Sunset yellow (E 110)	5.5
Erythrosine (E 127)	2.3
Lady-bug red (E 124)	2.3
Quinoline (E 104)	0.3
Amaranth (E 132)	0.3

Data from [132].

Table 19.34. Artificial flavorings in 650 medications

Flavoring	%
Orange	16.4
Raspberry	12.4
Lemon	7.3
Strawberry	7.2
Banana	6.4
Vanilla	6.4
Cherry	6.3
Mint	5.6
All fruits	5.3
Sour cherry	5.2
Other flavorings	43.3

Data from [132].

sence of cross-reactions, can cause the same symptoms in ASA-sensitive subjects [3]. Thus, the similarity between NSAIDs and ASA should be pharmacological, with shared COX-2 inhibition, although the mechanisms proposed are not exhaustive; otherwise all subjects treated with NSAIDs would have adverse reactions [193]. In a study of 60 aspirin-sensitive asthmatic patients [194] and other studies in patients with cutaneous (243 patients) or respiratory (40 patients) reactions to NSAIDS showed that only a single patient developed mild urticaria [117, 127, 139]. Generally speaking, these are adult subjects suffering from asthma and/or nonallergic rhinosinusitis, often with nasal polyps and eosinophilia, less frequently in adolescents or children. Most at risk are children suffering from asthma or allergic rhinitis complicated by chronic sinusitis and nasal obstruction.

ASA can cause:

- Urticaria, angioedema, other skin affections.
- Rhinoconjunctivitis and bronchospasm; the nasal provocation with ASA induced itching and sneezing in intolerant subjects.
- Rhinitis associated with reserpine treatment, maculopapular and pruriginous rashes and an irritating cough confused with asthma, related to treatment with captopril; worsening of asthma when using β-blockers [193].
- Symptoms may be limited to the respiratory tract, while methylated ASA appears to be responsible for cutaneous and gastroenteric forms [238].

In a cohort of asthmatic children aged 9–13.6 years, 14.8% were allergic to ASA [193], with ≈25% of patients experiencing asthma worsening. In a group of *atopic children* suffering from rhinoconjunctivitis (81%), AD (31%), food PA (19%), sinusitis (81%), with SPT and/or RAST positive to inhalants (88%), 15/16 tested positive to a provocation test (PT), with ASA presenting asthma and three also urticaria (20%); 11/15 (73%) were previously reactive to NSAIDs [50]. Thus, this infantile population appears to have a number of particular features that distinguish it from adults:

1. Absence of nasal polyps
2. Higher prevalence of urticaria
3. High atopic background
4. High incidence of sinusitis [50]

In a group of children aged 0–21, ASA was the most commonly reported NSAID, and less common were pyrazolone and ibuprofen, and 69% of them reported a facial angioedema NSAID reaction occurring once or more due to inadvertent exposure. The reactions most frequently occurred in the group aged 16–21, although NSAIDs are more often used in younger children [32]. In conclusion, cutaneous reactions [32, 50] and sinusitis [50] appear to be prevalent in children. Two percent of NSAID-induced facial angioedema developed in 0- to 5-year-old children, 3.8% in 6- to 10-year-olds, 8.2% in 11-to 15-year-olds, and 21.8% in 16- to 21-year-old *atopic children* [32]. The 24 children presented com-

Table 19.35. Drugs cross-reacting with NSAIDs and ASA

Acetaminophen
Diclofenac
Diflunisal
Fenoprofen
Flurbiprofen
Ibuprofen
Indomethacin
Ketoprofen
Ketorolac
Meclofenamate
Mefenamic acid
Naproxen
Piroxicam
Salsalate
Sulindac
Tolmetin

Data from [184, 193, 213].

plaints to NDAIDs such as periorbital angioedeme (88%), urticaria (50%), wheezing (33%), perioral angioedema (21%), rhinorrhea (13%), facial swelling (8%), and a maculopapular rash (4%), developing 30 min to 4 h after ingestion of the offending NSAID. An oral challenge test (OCT) resulted in periorbital angioedema (100%), frank wheezing (38%), urticaria (38%), rhinorrhea (23%) and respiratory symptoms of tachypnea, wheezing, and cough (35.5%) [86]. A positive FHA was present in 86% of the children [86].

What a number of authors have recommended is a prudent use of *hydrocortisone* in subjects suffering from ASA-related asthma, only in emergencies such as status asthmaticus or anaphylaxis, since it might start reactions that in prospective may be very severe and hard to control. This is because, by inhibiting arachidonic acid release by phospholipids, the drug deprives the airways of PGE_2 and prostacyclin bronchodilator effects [160]. Subsequently a preferential sensitization to hydrocortisone was excluded (only 1.9% of adults presented rhino-ocular symptoms), suggesting that succinate was the case [55], possibly causing a fatal case (Chap. 20). Therefore there is no valid reason to avoid the use of this drug in those emergencies in which it is indicated. Caution imposes the inclusion of sensitization to hydrocortisone in the history. In those who are ASA-sensitive, it is possible to administer CSs either by mouth or by spray during anesthesia or during surgery [130].

NSAIDs and ASA cross-react with numerous drugs (Table 19.35) [184, 193, 213]: the strong COX inhibitors of cyclooxygenase have a rate of cross-reactivity of 90%. A challenge with COX-2-specific inhibitors resulted in mild periorbital edema and mild generalized urticaria in 4 of 25 children [86]. Acetaminophen is not tolerated by 6% of ASA-sensitive patients [213]. Many people who are ASA-intolerant do, however, tolerate salicylates, structurally similar to ASA. Furthermore, in ASA-intolerant patients platelets interact with ASA, creating cytotoxic products [214]. Additional data is lacking [3]. In 3/17 adults, mast cell involvement has been shown, since tryptase and histamine levels appeared to be higher [25]: negative results in the other 14 required considering an underlying heterogeneity as a pathogenetic model of ASA intolerance.

Ionic Radiocontrast Media

Ionic radiocontrast media are almost exclusively of the PAR type, independent of specific sensitivity to iodine compounds as such or present in the diet. They consist of erythema, bronchospasm, angioedema, syncope and hypotension; effects are multiplied by occasional hyperosmolality and/or medium viscosity: for this reason preparations with a reduced osmolality and viscosity have been introduced [222]. Age is a major risk factor, with children aged 5–15 having the highest sIgE levels, which are lower at age 25, but always the highest level for that age, as compared with the lowest levels at age 65–75 [97]. Reactions during contrast tests in children <10 years were as frequent as 0.063%, using both ionic and nonionic compounds, suggesting that, unlike adults, reactions do not increase test safety in children [83]. In the urine of patients suffering from previous reactions, histamine levels were higher than in controls [28]: hypersensitivity to radiocontrast media may therefore depend on their concomitant influence upon metachromatic cells, effectively activated by tryptase [165]. Yet such agents appear unable to react with tissue proteins to form immunogens [28]. Complement activation, observed in severe reactions, is potentially caused by elevated concentrations, but it has not yet been clarified whether this mechanism is due to direct actions or more likely to protease activation, in turn resulting in bradykinin generation, an effect modulated by kininase, particularly by ACE [28]. Contrast media seem to have the capacity to interfere with bradykinin catabolism [97], of activating the prekallikrein–kallikrein complex, of inhibiting platelet aggregation, and damaging the vascular epithelium [165].

Penicillin and β-Lactam Antibiotics

Immune mechanisms involved in ADRs to aminopenicillins in vivo are related to genetic markers of immune response and confirm that the presentation of penicillin-hapten determinants to lymphocyte is HLA-restricted [169]. Data suggest that *Th2-like T-cell ILs play an important role* in all β-lactam-induced ADR, even when late clinical manifestations occur and an IgE-mediated mechanism cannot be demonstrated [27]. It has been shown that at least three different epitopes

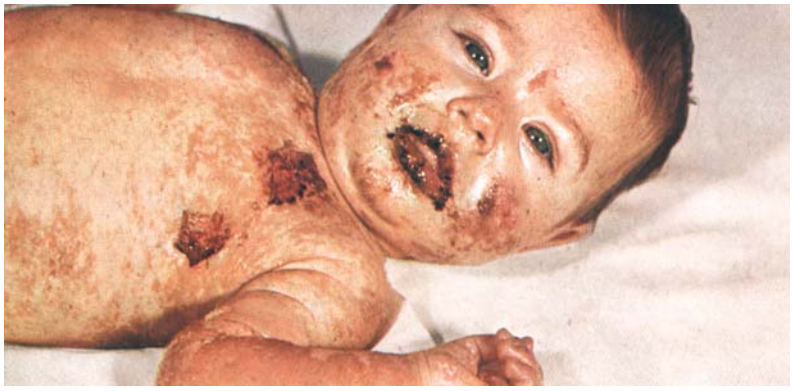

Fig. 19.25. Same infant as Fig. 19.12. After 5 days the exanthem assumes this hemorrhagic aspect

exist in the penicilloyl groups. They consist in the new antigenic determinants resulting from the opening of the β-lactam ring and conjugation to endogenous proteins. Penicillins form part of the epitopes contacting the antigen receptors of β-lactam-specific T cells in allergic individuals, as demonstrated by a peptide sequence derived from a natural *DRB1*1101*-binding peptide modified in vitro with penicillin G, which acquired antigenic properties [140, 236]. sIgE for penicillin have been identified in cord blood (Table 3.2) and also during the neonatal period [52], following pertinent therapies for pregnant and breast-feeding women [213]. sIgE levels to cefuroxime were not detected at the moment of the reaction but became positive 1 day after, increasing to peak at day 51, and still positive after 115 days [175]. Most IgE-dependent sensitizations were diagnosed by means of SPTs (86.4%) [155]. A high proportion of IgE antibodies to both benzylpenicillin and cephalothin suggested the presence of a *cross-reacting epitope* on each molecule [237]. Moreover, IgE-type reactions after SPTs or OCTs in 34% of children in whom 32%–60% suspected penicillin, amoxicillin, and cephalosporin reactions were shown to be IgE-mediated [154]. *Antibiotics are a paradigm of immunological reactions,* including IgE-mediated anaphylaxis, urticaria-angioedema, bronchospasm (type I), immune-mediated cytopenia (type II), serum sickness, urticaria, rashes (type III), ACD and other eruptions (type IV) [211, 214, 228], with a prevalence of cutaneous symptoms of 87.3% [211] to 96.6% [66]. The likelihood of β-lactam allergy was significantly higher for anaphylaxis (42.9% vs 8.3% in late reactions) and *immediate reactions* (25% vs 10% in other reactions) [155]. The risk was significantly higher in children with anaphylaxis (26.7%) and in those reporting immediate reactions (33.3%) vs 8.5% and 7.5% of the children with late reactions) [155]. Previous data suggest that Th2-like T cell ILs play an important role in all β-lactam-induced ADRs, even when late clinical manifestations occur and an IgE-mediated mechanism is apparently indemonstrable [27]. Specific IgE levels to cefuroxime were not detected at the moment of the reaction but became positive 1 day after, increasing to peak at day 51, and still positive after 115 days [175]. Most IgE-dependent sensitizations were diagnosed by means of SPTs (86.4%) [155]. A high proportion of IgE antibodies to both benzylpenicillin and cephalothin suggested the presence of a cross-reacting epitope on each molecule [237]. Of 72 children tested, 32% described their past cutaneous eruption as hives and 68% had other rashes; 96% of rashes were generalized [93]. ADRs to five classes of antibiotic were noted in 3.1%, to four classes in 10.3%, to three classes in 47.4% and to two classes in 39.2%. Most *children (85.6%) experienced an ADR to a penicillin,* while *71.1% reacted to a cephalosporin, 80.4% to a sulfonamide* and *35.1% to a macrolide.* Clinical presentations of the ADRs included urticaria or pruritus, rash, serum sickness-like reaction, angioedema or anaphylaxis, EM or SJS [143]. Indiscriminate antibiotic use *in animal fodder* to prevent infections may start a primary immune response, so that when the child receives one of the myriad of antibiotic cures, the *secondary antibody response occurs upon re-exposure to the same antigen* [39]. Cutaneous reactions form a large body of evidence, the study of patients reactive to ampicillin (7.6% with a positive history) [199], with delayed reactions to SPTs, led to the hypothesis of a type IV hypersensitivity [168, 199, 208, 219]. Opening the tetra-atomic ring and the linkage to a protein carrier occasions the classic form of haptenization (as in all low-MW chemicals), facilitated by ultrafiltrable serum factors [48]. Penicilloyl represents 90% of hapten–protein complexes and plays a nodal role in 75% of type I reactions to penicillin: the rest are divided between type II, III and IV reactions [197, 213] (Figs. 19.9, 19.12). The molecular nucleus, 6-aminopenicillanic acid, is found in penicillin and 7-aminocephalosporanic acid in cephalosporins and in all semisynthetic penicillins (first-, second- and third-generation cephalosporins) [1]. Four different groups of antibiotics, all sharing a tetra-atomic β-lactam ring in their basic chemical structure are commonly referred to as β-lactams (Fig. 19.26). Penicillins share a five-membered thiazolidine ring, and a dihydrothiazolinic ring is common only among cephalosporins [1]. The β-lactam group encompasses penicillins, cephalosporins, carbapenems, monobactams, and β-lactamase inhibitors [151]. New synthetic cephalosporins such as imipenem have the same characteristics [228]. An Italian 7-year old girl died because of an anaphylactic shock after taking a tablespoon of cefaclor (G. Tripodi, pers. comm., 2004), and a 9-year-old

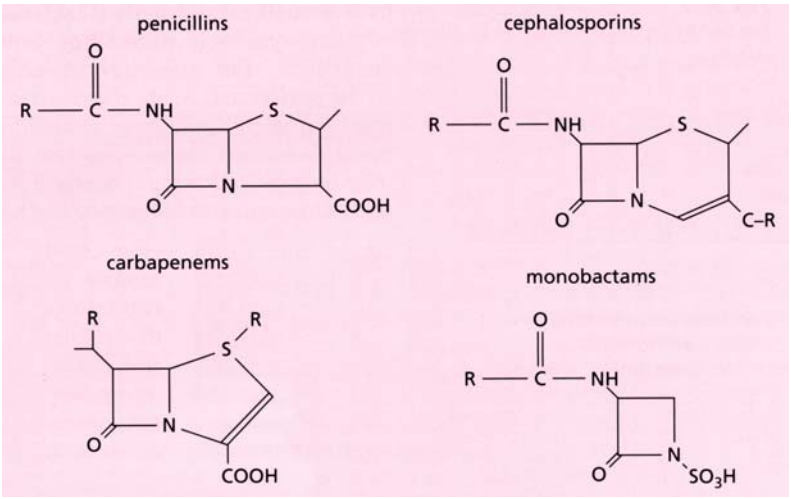

Fig. 19.26. Structure of 4 β-lactam antibiotics

boy after taking oral cefaclor presented with respiratory difficulty, throat tightness, and skin erythema, which required hospitalization. A dose of cefaclor taken 15 days earlier was uneventful (R. D'Ambra, pers. comm., 2005). A reaction was reported even in two 2-month-old babies [86, 111]. Aztreonam is a prototype of monocyclic β-lactams [20] with a production of IgE and IgG in 0%–3% of patients [23], but it is not tolerated by all β-lactam-allergic patients [209]. It follows that a patient who states to be allergic to penicillin can be allergic to all of them [49], as patients sensitive to NSAIDs are allergic to all drugs [149], while a patient reacting to one β-lactam can tolerate the others [114], also at a later administration [181]. Haptenization explains why some patients are allergic and others are not [48].

Several comments are necessary:

- Reactions that may be severe are caused by exposure to *hidden sources of penicillin,* nonmedically used penicillin (Table 19.36) [21, 136], or as an additive in cow's milk or in meat [21].
- Occasionally an allergic reaction may occur after a *first dose of penicillin:* this can also be explained by a prior exposure to antibiotic traces in food or vaccines that has remained unnoticed.
- Reactions can occur in subjects who have taken penicillin *up to a given moment,* an anomaly requiring investigation.
- It is absolutely imprudent *to prescribe penicillins and similar drugs,* as confirmed by various percentages, because the risk of severe and sometimes fatal anaphylaxis may be impending (Chap. 20) [189]:

– *Patients stating that they are allergic to penicillin* are 5%–20%; however, a meta-analysis on 295 articles, of which 30 fulfilled the inclusion criteria (15.6% of children) concluded that 33% of clinical history with SPT-positive patients had a vague penicillin allergy history; therefore such patients, like those with convincing histories, should undergo *penicillin skin testing prior to penicillin administration* [190].

Table 19.36. Hidden sources of penicillin or uncommon causes of exposure

Animal foods
Red meat Poultry Entrails, etc.
Dairy products: contamination during production
Cheese Yogurt
Dairy products: contamination during preparation
Cow's milk as such and powdered Cream Butter Additional dairy products
Penicillin added to foods as a preservative
Residue of animal foods
Surgical operation
Handling penicillin-containing capsules and syrups
Sexual contact
Use of contaminated glasses

We have not reviewed sensitizations by topical formulations. Data from [21, 136].

– *Subsequent treatment* causes a relapse in 6%–40% of subjects, but only in 6.8% of 298 children [66].

– *Reactions also occur* in 1%–10% of patients with a negative history, with the exception of adolescents [121].

– In particular, the prevalence of *SPT positivity in children* is approximately 4.7% (range 0%–8.8%) [66, 93, 121, 211], which with PT rises to 10% [66], confirming the risk run by a considerable proportion of pediatric populations; however, many children may be incorrectly labeled as penicillin-allergic, resulting in ongoing avoidance of penicillins [93], especially when there was

no positive response to SPTs, and only 4% of children had a positive response to intradermal testing [93].

- *Cross-reactivity* is impressive in vitro, but variable in vivo: although allergic reactions to first-generation cephalosporins in patients allergic to penicillin make up 5%–16% of the pediatric population, cephalosporins cannot be considered a safe and routine alternative, because antibodies against the immunogenic epitopes have been found in the lateral chains, so that many patients react to specific epitopes belonging to these chains [185]. The meta-analysis of all published studies that evaluated the risk of administering a cephalosporin to a penicillin-allergic patient indicates that patients who have known penicillin-specific IgE antibodies may be at increased risk for a reaction to cephalosporins [190]. However, the risk of suffering from an allergic reaction on administering cephalosporins to penicillin-allergic patients seems to be very low, provided that patients use cephalosporins with a different side chain to the penicillin responsible for the allergic reaction [131]. Moreover, hypersensitivity to β-lactams was suspected from clinical history in 30 (9.2%) children: SPTs and OCTs led to the diagnosis of β-lactam allergy *in 24 (7.4%) and 15 (4.6%) of the children*, respectively [155]. In a third pediatric trial, OCTs confirmed that 4% of reactions were caused by drug allergy [118]. These results conflict with another study reporting positive SPT or OCT results *in 23.5% of children* [154].
- *Lateral chains* have acquired importance related to the prospect of cross-reactions with other β-lactams and with similar ones, although the nucleus which defines the class is different [20].
- Studies with *amoxicillin* have proved that many patients can react to one antibiotic and yet tolerate others: this suggests a specific response, which for many patients seems to be correlated to amoxicillin lateral chains, as confirmed by SPTs and RAST [19, 20]. However, only 50% of patients are positive to PTs [210], indicating that the other half responds to another determinant correlated to amoxicillin.

The frequency of reactions to ampicillin and amoxicillin varies between 9.8% and 20% for the first and between 33% and 58% for the second [10, 185]: the higher figures can be related to the higher number of prescriptions in a specific country [1, 18]. Amoxillin alone or associated with clavulanic acid was most frequently reported to cause adverse reactions in children [155], similar to the 61.6% seen by a recent study, considering an additional 87 individuals who had allergy symptoms with penicillin exposure *despite negative penicillin SPTs* [201]. In a study involving 34 subjects allergic to β-lactams, eight (24%) suffered anaphylaxis, seven of these (88%) to amoxicillin: 2/8 (25%) were aged between 12 and 19; the only other boy in the controls presented a generalized maculopapular rash to amoxicillin [10]. In another study on amoxicillin 4/7 patients aged between 15 and 21 who underwent provocation testing presented generalized pruritus and facial or generalized edema [210]. Reactions often assume unusual forms: the amoxicillin-clavulanic acid association has caused generalized urticaria in two adolescents [56], angioedema by cloxacillin taken orally, with no reaction to amoxicillin simultaneously administered IV [200].

- In addition to the lethal cases reported above, three children aged 5–9 experienced an anaphylactic shock to ceftriaxone, two of them within 3–4 min [170].

Sulfonamides

Sulfonamides have been increasingly used for treating *Pneumocystis carinii* infections. According to some statistics, they cause 25.5% of reactions, and divided per apparatus, 25% of skin eruptions, 10% of allergic skin, hematologic, hepatic, respiratory and renal complications [81]; the incidence of skin lesions is 2%, rising to 6% for TMP-SMX [29]. *TMP-SMX* reactions are usually caused by sulfamethoxazole, with a case of anaphylaxis caused by TMP-SMX [29]. In the 25 children those reacting to antibodies were aged 4.8 years [86]. In children with AIDS, TMP-SMX reaches reaction

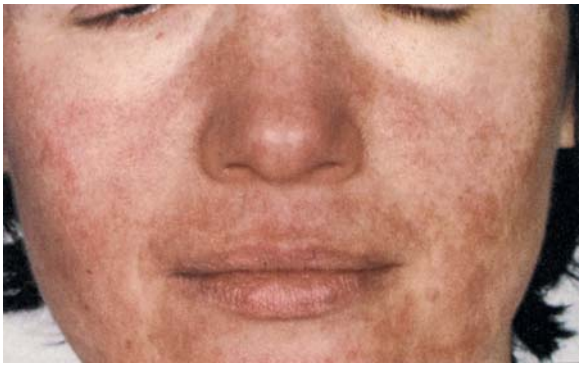

Fig. 19.27. Urticaria by TMP-SMX allergy

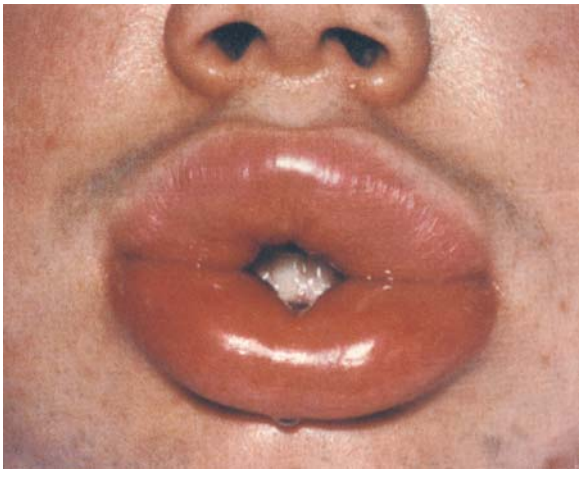

Fig. 19.28. Angioedema by TMP-SMX allergy

levels between 29% and 70% [5] and TMP-SMX causes 12% of skin reactions [125]. In view of clinical characteristics and the laboratory data available, it has been concluded that they cause various kinds of reactions [221], with a considerable role played by CICs in vasculitis and by cytotoxic mechanisms in cytopenia [213]; cases of serum sickness, urticaria-angioedema (Figs. 19.27, 19.28), ACD, etc., mostly reactions of immunological type.

Uncommon Reactions to Drugs

Two children have been reported who had reactions during etanercept treatment for systemic-onset JRA. A 12-year-old boy developed SLE with high ANA (antinucleus antibodies) and anti-ds DNA titers [101] and a 7-year-old girl developed diabetes mellitus (DM) 5 months after the initiation of etanercept therapy [22]. Therefore evaluation of ANA and anti-ds DNA titers in every etanercept-treated child is recommended [101]. A 9-year-old girl diagnosed with asthma developed SLE after treatment with zafirlukast [57]. A 9-year-old girl with SLE nephritis developed cutaneous bullae and mucositis while being treated with IV methylprednisolone. Skin biopsy specimen findings supported a diagnosis of SJS [176].

Diagnosis

The DAR diagnosis causes a considerable degree of difficulty, both because the pathogenetic mechanisms are little known and because of the patient's attitude. Often an adolescent, or frequently the parents, tend to apply the *post hoc, ergo propter hoc* concept (after this, so in consequence of this) [17]. One component that is often neglected is that of self-prescribing or of parent or family "prescriptions," in addition to over the counter products, in which the composition is not always mentioned [215].

History

This is the foundation of etiological diagnosis (Table 19.37) [218], although a multitude of diagnostic tests are proposed [189], starting with a detailed family history following specific points:

Family History

Have the parents and/or relatives ever presented reactions to drugs? If the answer is positive taking a properly meticulous collection of data is the best method to minimize allergic and nonallergic ADRs [207].

Table 19.37. Clinical prerequisites to diagnose drug-related reactions

The reactions do not represent a drug-dependent adverse effect
The reactions are usually similar to those attributed to other allergens
The initial exposure is followed by a latent period of about 7–10 days
The reaction may be reproduced by cross-reacting chemical agents
The reaction may be elicited by minimal doses of the drug
The finding of blood or tissue eosinophils may be associated
The clinical reaction should resolve after the drug has been discontinued
The allergic reaction should involve only a small percentage of subjects

Data from [218].

Personal History

Ascertain whether the child has ever suffered from atopic diseases and investigate any likely prior exposure to drugs. In this specific case, acquire detailed information on which drug or group of drugs has been taken, including dose, route of administration, duration of treatment, clarifying whether the child may have ever taken a medication, either regularly or intermittently, and which reactions occurred during the period in which any medication was taken. Taking a detailed history should include all associated clinical aspects and symptoms provoked by prior or present exposure to the same or structurally related drugs, the temporal relationship of drug administration and symptom onset, how long symptoms lasted and *whether treatment discontinuation* will lead or not to a reduction or abatement of symptoms. To identify all potential causes, pediatricians must be informed of all medication taken, prescribed or not, also over the counter, nonregistered, herbal, homeopathic, etc. Having completed this initial chart, pediatricians will try to differentiate predictable from unpredictable reactions, and analyze temporal relations, so manifold risk factors will be clarified [225]:

- *Verification of the child* and classification of reactions
- *Identification of all potential causes*
- *Analysis of the drug's innate capacity to cause the reaction* in question
- Proof of a *clear temporal cause–effect* relation
- *Verification of the effects* obtained by treatment withdrawal
- Evaluation of whether to prescribe an *antihistamine*
- *Possible re-exposure* and comparison with prior reaction(s)
- *Focused* clinical examination [164]

Table 19.38. Differences between allergic and pseudoallergic drug reactions

Features	Pseudoallergic	Allergic
Dose necessary to induce reactions	Elevated	Very little
Cumulative effect	Often necessary	Usually absent
Correlation between effects and pharmacological action	Often present	Absent
Reproduction of the effects by pharmacologically different drugs	Rare	Common
Clinical pattern	Uniform	Different

Data from [218].

Laboratory Investigations

In vivo and in vitro tests which have proved to be useful for differentiating allergic and nonallergic reactions (Table 19.38) [218] are outlined in the following sections [47, 164].

SPT

- *Immediate reactions:* reliable for high-MW drugs (complete antigens) (Table 19.4), unreliable for low-MW drugs (haptens), with the exception of penicillin.
- *SPTs with high-MW drugs* and with penicillin reagents may predict anaphylaxis and urticarial reactions.
- Children with a *suspicious history* and negative RAST.
- *Delayed reactions:* little correlation with medical history and clinical picture with a few exceptions.

Patch Test

Patch tests are rarely positive in drug-induced cutaneous rashes.

In Vitro Tests [46]

- PRIST: *total IgE:* to identify atopic children
- *IgE-mediated RAST reactions:* IgE to penicillin in nonatopic patients; children who cannot tolerate SPTs
- *IgG- and IgM-mediated reactions:* direct and indirect Coombs tests are usually useful, hemagglutination rarely in agreement with clinical picture

Other Tests

- *LTT, MIF* (monocyte migration inhibiting factor), production of cytokine, of little importance because these can indifferently give positive or negative results.
- *Lymphocyte stimulation test* (LST) is useful in the diagnosis of drug-induced hepatitis in 54.3% of cases [233].
- *Radioimmune testing* of histamine is efficient, also because of the rarity of false-positive and -negative testing [95].
- Various drugs have shown *specific antibodies*; Table 19.39 [37] shows the relevant tests.
- In adults, the *nasal provocation test* (NPT) has been evaluated in the diagnosis of hypersensitivity to ASA, with positive results in 38% of study patients compared to 8% in controls [147].

Table 19.39. Drug-specific antibodies and related tests

Drugs	Antibodies	Tests
Amodiaquine	IgG	ELISA
Anesthetics and muscle relaxants (alcuronium, gallamine, chlorprocaine, thiopental, tubocurarine)	IgE	SPB
Captopril	IgG	ELISA
Cephalosporins	IgG, IgM	HA
Cyclophosphamide	IgG, IgE	RBA
Chlorhexidine	IgG, IgE	ELISA
Dapsone	IgG	ELISA
Elliptio	IgM	RBA
Ethanol	IgG	IF
Ethynylestradiol	NK	RBA
Halothane	IgG	ELISA
Methyldopa	IgG	IF
Mianserin	IgG	IF
Morphine	IgG	RBA
Nomifensine	IgG, IgM	HA
Penicillamines	IgG	SPB
Penicillins	IgE, IgG, IgM	ELISA
Phenytoin	IgG	HA
Practolol	NK	RBA
Salicylates	IgG	HA

Data from [37].
HA hemagglutination, *ELISA* enzyme-linked immunosorbent assay, *IF* immunofluorescence, *NK* not known, *RBA* radiobinding assay, *SPB* solid phase binding.

- In selected cases a *challenge test* can be used.
- A number of *in vitro assays* have been used for ADR assessment, but many such assays, since they are specifically research tools, show little usefulness in clinical practice [164].

In conclusion, SPTs are the foundation for a diagnosis, tests in vitro support, but do not replace in vivo tests [60]: SPTs were positive in 68% of cases, a rate rising to 77% when combined with RAST+ cases, therefore the remaining 23% of patients need PTs [199]. The future in diagnosis is now.

The convergence of pharmacogenetics and rapid advances in human genomics has resulted in *pharmacogenomics,* that is the influence of DNA-sequence variation on the effect of a drug. The time is rapidly approaching when the sequences of virtually all genes encoding enzymes that catalyze phase I and II drug metabolism will be known. The same will be true for genes that encode drug transporters, drug receptors, and other drug targets. Thus the convergence of advances in pharmacogenetics and human genomics means that physicians can now individualize therapy in the case of ADRs [223].

Differential Diagnosis

Usually ADR clinical pictures are so dissimilar that, to permit an exhaustive differential diagnosis [172], a number of specific diagnostic charts have been proposed [215].

The first is represented by the cutaneous manifestations such as EM. Various algorithms are available for establishing whether the eruption in question represents a hypersensitive reaction supported by an immune-mediated process, and if so, whether it should be considered ADR-induced, differentiating it from other similar manifestations. The following data are useful for achieving this objective [103]:

- Drug-induced allergic reaction occurs in *only a small percentage of children.*
- The *morphology,* as well as associated symptoms and signs (fever, pruritus, eosinophilia, etc.), must be characteristic of that specific drug.
- With *reactions occurring after the first exposure,* there should be a latent period of 1–2 weeks between the start of treatment and the rash appearance, necessary for developing immune reactivity.
- *The rash must not be dose-dependent,* nor should it repeat a well-known toxic or undesirable effect, characteristic of the drug's pharmacological activity.
- *Symptoms should abate* when treatment is withdrawn, and relapse as soon as the child is rechallenged.

Drug-induced fever is sometimes accompanied by a maculopapular or urticarial eruption, often with eosinophilia, thus providing a clue. In other cases, the rise of fever is exaggerated compared to clinical features, or the concomitant use of a well-known potentially harmful agent should be suspected [46]. The differential diagnosis with other conditions is not always easy, while at times a prompt diagnosis is imperative, since fever often precedes the appearance of hepatitis, vasculitis, exfoliative dermatitis, and/or drug-induced hematological reactions. This repercussion often parallels a diagnosis of "a fever of a nature to be determined."

Table 19.40. Clinical and laboratory findings that should alert pediatricians that drug-induced cutaneous flare-ups may be dangerous

1. Clinical findings
Cutaneous
Confluent erythema
Facial or only central facial swelling
Skin pain or irritation
Palpable purpura
Skin necrosis
Blisters
Epidermal detachment
Positive Nikolsky's sign[a]
Mucous-membrane erosions
Tongue swelling
General
High temperature (>40°)
Enlarged lymph nodes
Dyspnea or wheezing
Hypotension
2. Laboratory data
Eosinophil count >1,000/mm^3
Lymphocytosis with atypical lymphocytes
Altered tests of liver function

Modified from [172].

[a] The apparently normal epidermis may be separated at the basal layer and rubbed off with lateral pressure.

Table 19.40 [172] indicates diagnostic criteria and laboratory data useful for an earlier and prompt identification of more severe cases. To differentiate TEN from EM and SJS, the following criteria [103], expanded in Tables 19.41 and 19.42 [172], are valid:

- *Bullae or erosions* should involve >20% of the body or cover at least three different areas, including mucous membranes.
- Lesions develop *on an erythematous base,* respecting sun-exposed areas, and within 48 h of the rash onset the skin is tender when touched.
- *Unlike exfoliative dermatitis,* in TEN the skin peels off in sheets of ≥3 cm in size, as in second-degree burns;
- *Fever is present and SSSS* should be excluded: *Staphylococcus aureus* is commonly recovered from the skin and nasopharynx, blood culture results are frequently positive, skin biopsy shows intraepidermal cleavage in SSSS, whereas in TEN cleavage reaches the basal cell layer and total denudation occurs. *Nikolsky sign* (skin that apparently looks healthy peels off following the lightest friction) is not differential [172].
- *Biopsy* carried out within 48 h from rash onset already shows basal layer local necrosis [103].

Table 19.41. Pediatric differential diagnosis of severe drug-induced cutaneous reaction

Disease	Interval (weeks)	Mucous lesions	Typical skin lesions	Other frequent findings	Differential diagnosis	Useful laboratory tests
Stevens-Johnson syndrome (SJS)	1–3	Erosions usually at ≥2 sites	Small blisters or dusky purpuric macules or atypical targets, detachment of <10% of body surface area	10%–30% of cases involve fever, lesions of respiratory[a] and gastrointestinal tracts	Postinfectious erythema multiforme major, by HSV or mycoplasma infection	Testing skin biopsy with immunofluorescence
TEN	1–3	Erosions usually at ≤2 sites	Lesions as in SJS, confluent erythema, Nikolsky's sign, diffuse epidermal necrosis, detachment of <30% of body surface area	Nearly always fever, acute skin failure[a], leukopenia, lesions of respiratory[a] and gastrointestinal tracts		Testing skin biopsy with immunofluorescence
Small-vessel vasculitis	1–3	Infrequent	Palpable purpura, most often on the legs, nodules, ulcerations, urticaria	In 30%–50% of cases gastrointestinal symptoms[a], fever, neuritis, glomerulonephritis[a]	Infections, rheumatic diseases	Skin biopsy, blood count, eosinophil count, liver function tests
Serum-sickness or similar reactions	1–2	Absent	Morbilliform rash, often with fever, arthralgias urticaria	Fever, arthralgias	Infections	ANA, rheumatoid factor
Angioedema	[b]	Often frequent	Urticaria or swelling of the central part of the face	Respiratory distress[a], circulatory collapse[a]	Insect stings, foods	Platelet count, specific IgE, antibodies to penicillin

Modified from [172].

[a] Potential causes of death.

[b] Heparin 5–10 days, NSAIDs 1–7 days, penicillin, contrast media, anesthetics a few minutes to a few hours.

Table 19.42. Differential diagnosis of SSSS and TEN

Lesions	SSSS	TEN
Pathogenesis	Group II staphylococcus	Usually drug-induced
Morbidity/mortality	Low	High
Mucosal involvement	Rare	Frequent
Nikolsky's sign	Positive	Positive
Target lesions	Absent	Often present
Blister localization	Subcorneal	Subepidermal

Data from [172].

Skin Test Indications and Application

Indications

When planning SPTs, they should always be done first. If there is a history of a previous serious reaction, the SPTs – if done – should be diluted to start with. If truly indicated and based on the above, they must be done in the following specified cases:

- Positive history for adverse reactions to a drug that is a *complete antigen* (Table 19.4)
- Positive history for adverse reactions to a drug that has *no alternative for the ongoing illness*
- Positive history for adverse reactions to a drug when administration of a *cross-reacting molecule* is necessary
- *Doubtful history* for adverse reactions to a drug when the same drug administration or when a cross-reacting molecule is necessary
- *Negative history* for adverse reactions to a drug in patients at risk for allergies related to the class of drugs to be taken
- *Positive history* for multiple sensitization to drugs

Based on the divisions in Table 19.4, one can use, depending on the case, SPTs or intracutaneous reactions. SPT is initially applied using graded dilutions. With all negative results, it is possible to carry out intracutaneous tests, these too with graded dilutions (never without diluting the drug!), following standard methods [189]. These tests must be applied with extreme caution because they are *potentially hazardous in sensitized subjects,* due to severe generalized and sometimes fatal reactions [69, 121]. However, SPTs using soluble β-lactams diagnose a significant number of immediate sensitizations to penicillins and β-lactams [237].

Particular Features of SPTs for Penicillin: Chemical–Immunological Requirements

The β-lactam ring opening forms a covalent bond with serum proteins and creates a neoantigen, the penicilloyl determinant that, reacting to penicillin in 75% of allergic patients, constitutes the major allergen (Fig. 19.29), which is commercially available, BPO (benzylpenicilloyl); 5% of the penicillin will conjugate with cutaneous proteins producing minor determinants (Fig. 19.30), those with a reduced rate of metabolites (penicilloate, penilloate, penicilloyl-α-propylamine, etc.), from which another commercial product is prepared using BPO and Na benzyl-penicilloate [51]. Because the precise haptenic determinants of cephalosporins are broadly obscure, the free individual drugs are used as SPTs to detect IgE antibodies reactive to cephalosporins [170].

Testing Method

- As usual the positive and negative controls are first carried out.
- In patients at risk, the SPTs are applied using BPO starting with a dilution of 1:100.
- If negative after 15 min *the SPT is repeated* with a dilution of 1:10 or, if necessary of 1:1.
- If negative after 15 min *the intradermal (ID) test is employed* with the same, also with an initial dilution of 1:100.
- If negative after 15 min *the mixture of minor allergens* is employed, starting with a dilution of 1:1,000.
- If negative after 15 min *the ID test* is employed using the same methods [48, 213].

The concentration of BPO is of 6×10^{-5} M, that of penicillin and of mixture of minor allergens is of 10,000 U/ml [49, 228]. For ohter β-lactams, SPTs can be done using 0.25 mg/ml, 2.5 mg/ml to 25 mg/ml, and 2.5 mg/ml, 25 mg/ml for ID test if SPTs are negative [155, 213]. The amoxicillin used in Europe is soluble at 20 and 200 mg/ml [17], whereas 2 mg/ml for both ID and SPTs were used in a US study [111] and it seems that this amoxillin cannot be diluted at the maximum concentration recommended by European authors [17, 19, 155].

To obtain highly reliable results, this significantly predictive test, whether positive or negative, must include both allergens. One must also test the child's reactivity to the specific lateral chains of the diagnostic reagents (semi-synthetic products) [185]. The meta-analysis of four studies shows that the rate of SPT predicting reactivity to challenge tests is of 0%–50% when positive and of 0.2%–4% when negative [49]. More recently, a challenge test only using allergens may result in 7% [19] or 20% [18] of patients missing the diagnosis (perhaps non-IgE-mediated reactions), instead identified with PTs with allergens [19]. Blanca et al [20] were the first to demonstrate the importance of side-chain diagnostic reagents, and consequently the need to include semisynthetic penicillins and cephalosporins, which are responsible for SPT reactions. Since these substances prime reactions caused by sensitization to common allergens with penicillins, by using semisynthetic penicillins and

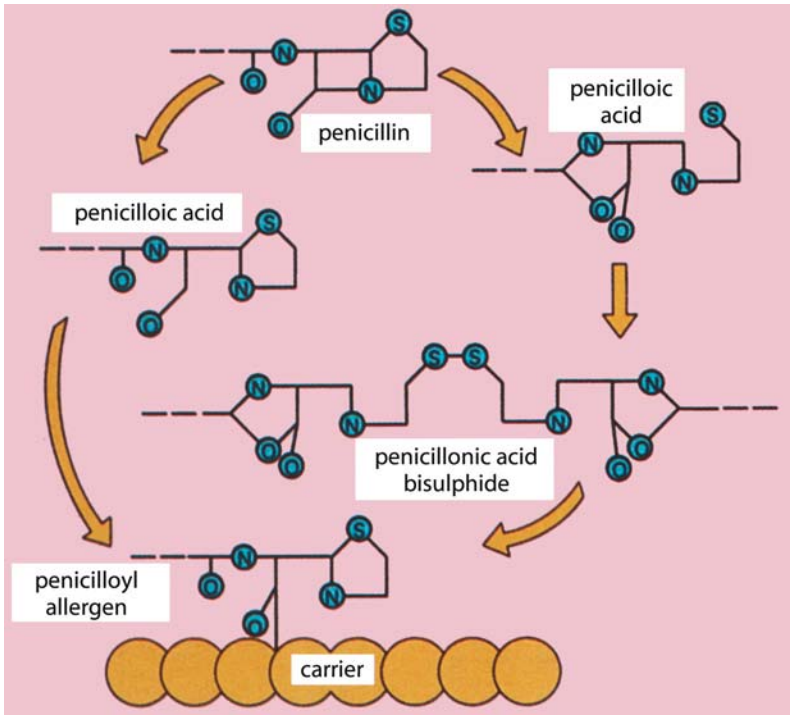

Fig. 19.29. Formation of major determinants

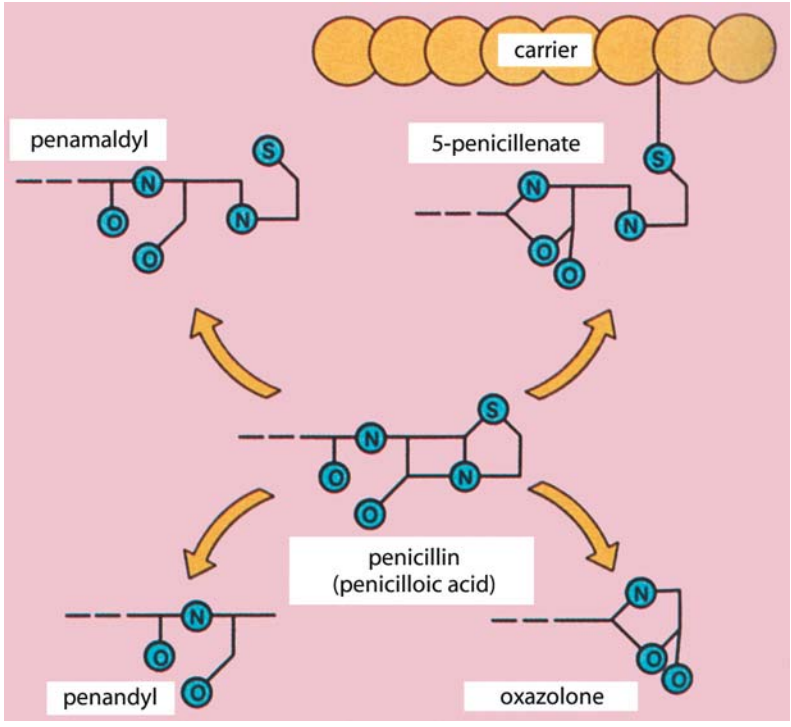

Fig. 19.30. Formation of minor determinants

cephalosporins for SPTs, cases of hypersensitivity to β-lactams are also disclosed, cases that otherwise were missed by using only penicillin major and minor allergens [18, 168]. Due to the well-known likelihood of causing clinical reactions simply by applying SPTs, it is possible to test the semisynthetic penicillin to be prescribed by eliminating the major and minor allergens involving penicillin to a certain extent, perhaps penicillin acid and a few more polyvalent compounds [18]. Allergopen, a kit for diagnosing penicillin-related allergies, is currently available.

Challenge Testing

Indications

Challenge tests are without doubt the only diagnostic tests for ASA and NSAID allergies: given the risks, it is only indicated if it is effectively necessary and urgent to administer the drug in question.

Requisites

- ADR must be *in remission.*
- The patient must abstain from taking any kind of drug *in the 24 h preceding the test* [155].

Testing Methods

A maximum degree of prudence advises to start with a dose of 1:1,000 of a single therapeutic dose (capsule or pill), increasing to 1:100, 1:10 with an interval of time between doses equal to that foreseen between drug administration and onset of reactions, which in turn will be suggested by history, therefore equal to or greater than the interval present in the specific case. If children present severe reactions it is advisable to begin with a dose equal to $1:10^5$ of the usual dose [47].

Tests are classified as follows:

- *Rapid challenge* test, with doses increased at least every 30 min, for example in ASA-induced asthma or urticaria
- *Slow challenge* test, with doses increased every 24–48 h as in fixed eruptions caused by sulfonamides [45]

In patients with late reactions to penicillin, test results may appear after 24 h in 45% of cases, and in 37% even after 48–72 h [199]. With SJS, TEN and agranulocytosis, such tests are excluded [47]; with PAs only alternative or less reactive drugs will be tested [36].

Treatment

There are three main periods of treatment:

- *Discontinuing* drug treatment, following specified instructions for a possible substitution. With antibiotics this measure must be adopted the moment a rash appears [5].
- *Skin eruptions* can be treated with oral antihistamines, and only in some selected cases could CS medications be applied locally or taken by mouth or parenterally, depending on the case [12].
- Treatment for *systemic manifestations* (Chap. 20).
- *ASA-sensitive children,* in addition to alternative drugs, can also follow an ASA-free diet following the indications in Table 10.11, but not all authors agree on this [147].

Table 19.43. Example of amoxicillin desensitization based on the weight of ±25 kg

Start with a 10 mg/ml solution		
Time (min)	**Dose in ml**	**Dose in mg**
0	1/10	1
20	5/10	5
40	1	10
60	2	20
90	5	50
In the absence of side effects go to the normal 100 mg/ml solution		
Time (h)	**Dose in ml**	**Dose in mg**
2	1	100
2 h 30′	1.5	150
3	2	200

The total dose reached over a period of 3 h corresponds to one-third of the daily dose for a child of 25 kg bw; for children above 25 kg bw, the last dose correlated to their weight, and calculated on the base of 50 mg/kg, shall be administered after 3 h.
Modified from [152].

- Treatment of *multiple sensitizations* is particularly difficult [81]: these children are the ideal candidates for desensitization when possible [149].
- Various *charts for desensitization* have been proposed:
  - For *children reacting to antibiotics,* ASA and NSAIDs there are several flow-charts, algorithms, and desensitization protocols that are effective also for adults [147, 198]. In ASA- and NSAID-sensitive children, the procedure is best carried out in settings where anaphylaxis can be recognized at its onset and treated immediately [146], we report an example structured on a bw of ±25 kg for amoxicillin desensitization (Table 19.43) [152]; in other cases the procedure is not based on bw or age of children.
  - Recently *five children and infants* with IgE-mediated reaction to TMP-SMX were evaluated; and 3/5 successfully desensitized [142].
  - For *drugs used in various diseases* in pediatrics such as sulfasalazine for juvenile rheumatoid arthritis [89] and enteric inflammatory diseases, ciprofloxacin [94] and tobramycin [180] for CF, and desferrioxamine for thalassemia [91, 150], charts are also available for other drugs [146].
  - For intervention *in cases of AIDS* prone to developing PAR.

Drug-Related Deaths

Penicillin causes 500 fatal cases each year only in the US: Fiori and Marigo [58] have published a series of 18 fatal cases, stressing that *unpredictability* is emphasized by the common administration in medical offices of ceftriaxone (two fatal and three anaphylactic cases) [96, 170]. DeSwarte [47] has clearly stated that 40% of fatal cases were prescribed penicillin treatment without any clinical indications. It is also believed that some 69 patients who died would not have died if all medical precautions suggested by individual cases were applied. The press announced that the MMR vaccine had been immediately taken off the market all over the world after the company producing it had ascertained that it caused meningitis-like complications in 1 case out of 11,000 instead of the predicted 1:300,000–1:500,000 [7].

Prevention

Prevention should always precede treatment: the best way is to prescribe drugs only if absolutely necessary [30]. Only 12 of 30 patients who died from anaphylactic shock caused by penicillin had a clear indication for this therapy [47]. Medications are often given without strict indication, thus sensitizing children to another drug, likely useful in the future. Prevention must follow a preestablished and approved order [48]. Research has also contributed, developing medications that are increasingly less sensitizing. Therefore *before administering a drug* known or suspected as hazardous, but that is considered indispensable for a certain patient an *accurate history* should be collected [46]. If this precaution had been followed rigorously, one-third of dead patients (or their relatives) could have warned doctors that they previously suffered from allergic reactions to penicillin [47]. One very severe observation is that 33% of drugs are used without respecting specific indications, dose and *child age* [61]. The *hidden sources* of penicillin (Table 19.36) and of drugs containing sulfites, dyes, and aromas have been mentioned above (Tables 19.32–19.34). *Small traces* of sulfonamides and penicillin can contaminate food and sensitize a predisposed child or cause reactions in sensitized children, although this event is rare [197]. *Unusual causes* are inhalation, cutaneous or mucous contact, sexual intercourse and intrasurgical contact [21]. To deal with any possibly severe reaction we suggest having at hand all equipment necessary to treat emergencies (Chap. 13); other extremely important preventive measures involve carrying out diagnostic challenge tests, desensitization measures, and premedication for at-risk patients if at all possible [149]. *Never trust a wheal test,* a practice that has proved to be potentially dangerous and also useless and not predictive [155]. Finally, one particular form of prevention is to control the *drugs taken by the mother* while breastfeeding a child who is at risk. It would also be extremely useful if legislators were to limit veterinary use of the more sensitizing drugs, from 100% penicillin *and to antibiotics that are in general totally useless, since they do not have the preventive effect for which they are used*, to avoid the risk of reactions and/or *drug-induced sensitization* [152]. To prevent reactions to x-ray examinations with contrast media, especially IV, follow the related analysis proposed in Chap. 20.

The future? The search for a safe, inexpensive, nonsensitizing, nonirritating, effective medication continues. Several therapeutic modalities both avoid side effects and keep costs low have been introduced in recent years. All these newer approaches to an effective treatment justify neither frustration, nor pessimism, nor multipragmatism in treating children, when profiting from the variety of available agents and using skilled techniques to avoid undesired side effects of drug treatment. We remember, however, that both time and patience are needed to develop the optimal treatment approach, and with regard to the tempting plethora of current therapeutic possibilities, that the ethical imperative *primum non nocere* remains binding [30].

Pediatricians and Drug-Induced Allergies and Pseudoallergies

The pediatrician's fundamental duty is to evaluate, taking each case individually, the possibility of avoiding the prescription for children of a drug suspected of having caused reactive symptoms in the past, even modest, or drugs with similar chemical structures that might cause cross-reactivity, or should it for some reason be necessary to prescribe it, this should be done with the necessary caution. There are no specific risk factors; neither age nor sex are significant. Reactions are less frequent in children although the clinical expression is not influenced and severe reactions are also always possible in nurslings and toddlers. However, expectations of pharmacological prophylaxis have a limited place since premedication can only be used for reactions to iodic contrast media.

During treatment a number of precautions concerning methods for administering drugs are useful [60]:

Inform parents or relatives of any possible eventual risks.

Write the prescription clearly and completely.

Prefer, if possible, oral administration.

To prevent some reactions or reduce their severity, administer antiallergic drugs simultaneously, including antihistamines, sympathomimetic, CSs, etc.

Unduly prolonged or intermittent exposure increases the potential for sensitization.

Children at risk, especially if treatment is injected, should remain under observation for 30 min.

After Administration. Children, parents, relatives, or caregivers should warn the doctor about the onset of

adverse reactions so that a suitable antiallergic therapy can be prescribed immediately. Pediatricians should provide the patient with a certificate including the treatment applied, drugs used, dose and administration as well as a description of episodes indicating an ascertained or suspected hypersensitivity to the drug. In more severe cases, children should carry the name of offending drug(s) reported on a card, or a bracelet or a tag that children should wear at all times (as often advised), and that will be useful to apply specific treatment in an emergency, even if children are in a state of shock or not accompanied by parents or informed persons. Also verify the composition of all drugs, including topical and/or over the counter medications [155].

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Allergy and Central Nervous System and Other Allergies

Introduction

In this chapter we provide noncomprehensive review of miscellaneous conditions associated with pediatric allergy. There are other phenomena that can rightly enter within the symptom patterns attributed to both gastroenteric and genitourinary systems.

For a while the existence of neurological manifestations is reported in children following food-induced anaphylaxis [167] and of easy fatigue found in children with food allergy (FA) and from inhalants [41]. A concept of a tension-fatigue syndrome (TFS) has been introduced [178]; varied symptoms have been connected to allergy such as migraine, hyperactivity, cognitive dysfunctions, emotional and behavioral troubles, besides insomnia and anxiety alternating with periods of restlessness and fatigue [41]. Above all hyperactivity has been associated with FA, widening it to so-called sugar allergy [39]. Proof has not been provided, however, for instance with a double-blind, placebo-controlled food challenge (DBPCFC) [92]. Although the central nervous system (CNS) may be a center of migraine, behavioral troubles and psychiatric alterations, there are no definitive proofs that these affections are immune-mediated. However, it has been postulated that dietary manipulations may affect some CNS symptoms; even if viewed objectively the psychological symptoms are not easily quantified and some patients are prone to show a placebo effect [27], exclusively revealing the scarce frequency of DBPCFC studies [126].

Also, in other disorders there is a reappraisal of FA that is spread to all the organs and systems [27]. We have previously mentioned some disorders or syndromes imputable to a true FA or pseudoallergy (PA); here we will appraise the positive changes in the contested field of the clinical ecology, long in the limelight. On the opposite side of food intolerance there is food aversion [197], while at the clinical antipodes is found the Münchausen syndrome by proxy, possibly linked with disorders related to child abuse. To understand how the enormous enhancement of allergic affections has met a notable impact on citizens of all countries and categories, it is sufficient to remember that the pathological manifestations constitute an ever more remarkable problem in the populations of industrialized countries [4]. Accordingly, it is excessive that new syndromes are always labeled as allergic, while it may be opportune to remember conditions that in the past have not been correctly identified and defined [27]. It is generally believed that FA is underdiagnosed, while the colleagues in this field reply in opposite terms. It continues with a distrust toward traditional medicine [126], which is shown to be incapable of full dealing with maternal anxieties of wholly physiological events [197]. Nevertheless, misinformation has led to an exaggeration of the danger of foods [27]: as a result, not since mesmerism and phrenology were in vogue has the public appeared so gullible and so vulnerable to fashionable nostrums [109].

A typical example is the Feingold diet [63], popularized at the beginning of the 1970s: although it lacked the confirmation of DBPCFC studies, this diet has benefited from ample publicity that has propitiated citizens' support to spontaneously publicize the shaky correlation between foods (and food additives) and behavioral changes.

In this chapter we will examine what else can rightly enter within the symptoms pattern attributed to both gastroenteric and genitourinary systems.

Migraine and Allergy

Definition

The Ebers papyrus (3,500 years ago) told of a sickness of "half the head," surely migraine. Headache is considered one of the most common painful syndromes and migraine is the most frequent form of illness, and both have been diagnosed using clinical impression. To address the limitations of this method, the International Headache Society (IHS) developed and updated defined criteria for headache diagnosis [165]. These criteria, however, have been criticized for incompletely diagnosing childhood migraine disorders, since rather than having two coexistent headache types, children and adolescents with chronic daily headache have a single syndrome that, in many cases, will paroxysmally worsen and gather migrainous features [96]. Migraine in children and adolescents is a common problem. *Migraine* is defined as a recurrent headache with symptom-free intervals and at least three of the following symptoms: abdominal pain, nausea or vomiting, throbbing headache,

Table 16.1. Epidemiology of migraine in children aged 4–19 years

Country	Reference	No. of children	Age (years)	Subtype	Prevalence/incidence
Greece	[125]	4,000	4–15		6.8% (P)
				Without aura	3.4%
				With aura	2.8%
Norway	[221]	8,255	13–18		7% (P)
				Recurrent	18%
Poland	[99]	1,759	6–19		8.4% (I)
				Tension-type	28.7%
Poland	[180]	659	15–19		28%
				Without aura	19%
				With aura	9%
Sweden	[23]	9,000	7		1.4% (P)
			15 year		5.3%
Sweden	[52]	402	7–15		23%
Sweden	[105]	131	7–15		11% (P)
				Tension type	9.8%
UK	[1]	67	5–15		10.6% (P)
				Without aura	7.8%
				With aura	2.8%
UK	[60]	811	7, 8.2%		
		1,511	11, 15.4%		
Israel	[139]	312	8.4		54%
				Tension type	22%
UAE	[21]	1,159	7.4–13	Without aura	12.6%
				With aura	8.2%
US	[153]	6,072	Adolescents	Recurrent	30%
Hong Kong	[98]	2,120	6–13		2.8%
India	[171]	233	11–15		11%
		1,308	11–15	Recurrent	19.5%
		233	11–15	Tension type	3.6%
India	[189]	46	7–15		8% (I)

Migraine occurs in 35.6% of American Indian adolescents and 32.1% of white American adolescents [210]. Among Norwegian children, 69.4% of boys and 84.2% of girls reported headaches during the last 12 months and 21.0% of boys and 36.5% of girls reported recurrent headaches [221], in the USA, 37.6% of girls and 21.3% of boys [153]. The prevalence was 9% in boys and 14% in girls [171] and increased with age, similarly in girls and boys up to 11 years, and thereafter only in girls [105]. By age 11, significantly more females were reported to have frequent headache [60]. In 189 children from Baltimore aged 13±3.1 years, mean headache was present 27.3±4.1 days per month, with a mean pain intensity of 5.9±2.1 on a 10-point scale [98].
From [1, 21, 23, 52, 60, 98, 99, 105, 125, 139, 153, 171, 180, 189, 210, 221].

unilateral location, associated aura (visual, sensory, motor) and a positive family history (FH) [53]. The factors that instigate *headache* are physical activity, relaxing subsequently to stress, skipping a meal, sleeping inadequately or excessively, consuming alcohol, or atmospheric changes, trips, traumatic events, bright light, hormonal factors and hypoglycemic states, etc. [53].

Epidemiology

Prevalence estimates are shown in Table 16.1 [1, 21, 23, 52, 60, 98, 99, 105, 125, 139, 153, 171, 180, 189, 210, 221], which demonstrates that migraine without aura is present in 42.8% of children vs 22.8% of migraine with aura.

Table 16.2. Foods involved in the pathogenesis of migraine and causing symptoms in 40 babies

Foods	% Of children reacting
Apple	10
Bacon	10
Banana	8
Beef	40
Carrots	8
Cheese	53
Chicken	8
Chocolate	55
Coffee	24
Cow's milk	68
Egg	60
Fish	23
Goat's milk	15
Grapes	10
Lentils	8
Maize	20
Malt	8
Melon	8
Nuts (mixed)	10
Orange	30
Peaches	10
Peanuts	13
Peas	8
Pineapple	8
Pork	23
Potato	10
Rye	30
Strawberries	8
Sugar	8
Tea	18
Tomato	33
Wheat	53
Wheat flour	8
Yeast	10
Benzoic acid	35
Tartrazine	30

2.5% of children reacted to avocado, cauliflower, cucumber, dates, leeks, lettuce, mushrooms,or rabbit.
Modified from [54].

Pathophysiology

From the first observations of Hippocrates, it is suggested that several foods, including ripe cheese, chocolate, red wine, citrus juice, salted fish, fat foods, bananas, peanuts, green tomatoes, avocados, and pineapple provoked migraine [150]. A mechanism of hypersensitivity to foods including cow's milk (CM), egg, wheat, vegetables, cereals may provoke some cases of migraine; in fact clinical remission follows appropriate elimination diets, with symptom relapse after the reintroduction in DBPCFC of the offending foods [200]. However, only the classic study of the Soothill school in 88 children, affected by severe and frequent crises of migraine (>1 week) and submitted to an oligoantigenic diet has shown with the DBPCFC the allergic pathogenesis of child migraine. In 88.6% of children, there was a complete remission and in 4.5% a gradual improvement, regarding particularly the migraines precipitated prior to the institution of the diet, excluding physical exercise, emotions, irritating odors, and cigarette smoke, with a significant improvement also of other associated symptoms, including abdominal pains, behavioral changes, asthma attacks and atopic dermatitis (AD) [54]. The children were subsequently submitted to a crossover DBPCFC in 45% of cases, 82.3% of whom were positive to foods vs 52% of placebo ($p<0.001$). The interval between food challenge tests (FCT) and a precipitated migraine lasted from a few minutes to >1 week, with a mean of 2–3 days. The principal foods to which there were positive results are shown in Table 16.2 [54], and other products never suspected such as in 33%–37% of reactions to benzoic acid and tartrazine and in 15% to goat's milk. In this study there was no relationship between SPTs (skin prick tests) and RAST, but FCTs were positive in 93% of cases.

Other pediatric cohorts endorse these results [67] of relationships between migraine and FA that has been confirmed in 49 out of 92 children with migraine (53%) who presented SPTs and FCTs positive to one or more foods, total serum IgE levels >2 SD for the age (82%). In 31 children, elimination diets induced a remission and in nine the improvement of symptoms, also after start the reintroduction of the causative foods 6–12 months after starting the diet, and at the follow-up after 2 years [117]. In another 12 children, the positive FPTs were not associated with SPT and specific IgE; for example 2/7 children positive to cocoa had total serum IgE levels under 2 kU/l and 7/7 had negative IgE levels [75]. However, FPTs were negative in 35 children with a suggestive history of migraine associated with food intolerance [24], and no DBPCFC was positive to suspected foods and food additives; the elimination diet followed by five children who complained about >2 episodes per week were effective only in two children [5].

Pathogenetic Mechanisms

It is believed that many drugs, foods and food additives [203] can provoke migraine, through an IgE-mediated mechanism or PA, by a direct or specific mast cell and basophil activation (Chap. 10), resulting in the release of vasoactive amines: a migraine attack often coincides with an increased amine metabolism [203]. Among the medications and other agents incriminated in migraine pathogenesis we find the following:

- Pharmacological action: 5-HT (5-hydroxytryptamine or serotonin) caffeine, ethanol, nitrites, phenylethylamine, phenolic flavonoids, tryptamine, tyramine
- Immunological mechanism: food proteins
- Uncertain mechanisms: aspartame, glutamate [203]

However, the diffused flow reduction during a migraine associated with aura is rather the expression of a decreased metabolic requirement [92]. During a migraine attack vasodilation is present: the dilated intracerebral vessels become impermeable and it is assumed that consequent exudation of polypeptide fosters a sterile perivascular inflammatory reaction, that would activate in turn the nociceptive trigeminal vascular terminations [150]. Further studies have underlined a subsequent release of histamine, $PGD_{2\alpha}$, $PGF_{2\alpha}$ [120, 140, 181], substance P and additional tachykinins, which play a salient role in the transmission of the nervous impulses implicated in migraine pathogenesis [4, 40]. The histamine pathogenic weight can be supported by the diamine-oxidase deficit that prevents its breakdown [195]. It is possible that CICs (circulating immune complexes) increase the platelet aggregation and 5-HT (5-hydroxytryptamine or serotonin) release.

In a group of migraine sufferers intolerant to foods, it was shown that CIC levels increased following FCT. Moreover, the early T cell activation after the FCT indicates an involvement of IL_2-related receptor in food-induced migraine. These results have reinforced the idea of immune mechanism involvement in food-induced migraine, but it seems to be localized at a different step from what has now been hypothesized, with the involvement of the complex cytokine network [123]. In a parallel work in 21 patients with migraine of food origin compared to 10 controls, the activated T-cell assay has shown their increase 4 h after the FCT and their reduction 72 h later [120]. Following FCTs, it is shown that 5-HT decreases and 5-HLAA (5-hydroxyindoleacetic acid) increases in patients with rheumatoid arthritis (RA) intolerant to foods: this data points out that 5-HT are released by platelets during FCT in patients with food hypersensitivity [123], and it is hypothesized that a similar mechanism can also be working in migraine sufferers [71]. The question remains: is it a true allergy [4]? The alternative between this and PA favors allergy, based on the following points [54]:

1. The symptoms are potentially provoked by an ample spectrum of foods that, as far as is known, fail to have common characteristics. Nevertheless, the significantly elevated number of subjects that have reacted to the food rather than to the placebo is the proof correlating the afflictions to foods, each responsible in any association.
2. The SPTs often negative to foods or in contradiction with the clinical manifestations of the 88 children suggest a non-IgE-mediated mechanism.
3. Atopy was present in 66% of children and 26.3% of 300 children prospectively seen by us had positive FH [29].
4. A positive FH of migraine was found in 35% [171], 46.5% [20] and 79% [96] of entrants.
5. In the pediatric age, remission can be of IgE-mediated allergy, not of PA.
6. Foods precipitating headache also induce cutaneous and/or respiratory symptoms.
7. The positive DBPCFC (as in other disorders subsequently examined) validate the allergic background of symptoms [134].

Compared to the last two points, a study on 158 children aged 0.5–13 years with food intolerance confirmed by elimination diets and FCTs has shown that headache was much more frequently associated with asthma and urticaria than with AD [12]. In 40% of 30 children and adolescents aged 11–17 years with the diagnosis of migraine with or without aura, the migraine was associated with allergy cases [204]. The prevalence of both headache and migraine was significantly and proportionally higher in children with atopic disorders compared to those without. A history of atopy (especially rhinitis) supports the diagnosis of migraine in young children with paroxysmal headaches [135]. So far, based on available data we can conclude that migraine has a frequent relationship with foods, which could be at the base in an undefined percentage of cases with mainly an IgE-mediated mechanism. Moreover, the positive effect of the diet in children with negative SPTs recalls the intervention of type III or IV mechanisms [67].

Diagnosis

Diagnosis consists in an accurate history, with an objective examination that includes pressure measurement as well as a neurologic, hematologic and ophthalmologic examination. For the diagnosis of migraine ask for recurrent headache with symptom-free intervals: there is an IHS three-question headache screen based on the following [165]:

1. Headaches interfering with work, family, or social functions
2. Headaches lasting at least 4 h
3. Presence of new or different headaches in the past 6 months

A diagnosis of migraine was suggested by a yes answer to points 1 and 2 and a no answer to point 3. The three-question headache screen identified migraine in 77% of the study population [28]. However, 46.6% of

children had some of their migraine attacks lasting for at least 1 h and 26.6% had some of their migraine attacks lasting at least 2 h [2], which does not fit this scale. A ten-point scale (10 being the most severe pain) indicates the average severity of the migraine [148]. Clinically, children with migraine report neck and shoulder pain, abdominal pain, back pain, and otalgia significantly more often than those with nonmigraine headache [7]. In children with FA, neither SPTs nor RAST have been useful, lacking a correlation with FPTs [54], and if the tests are performed in more days, DBPCFCs may also increase the number of false-positive tests [54] for the recurrent migraine symptoms and of false-negative tests for the delayed onset of symptoms. Testing children only with the offending foods or food additives (according to their parents) [9] introduces a potentially restrictive bias on the positive results. In the differential diagnosis should also enter celiac disease with a 1.1% pediatric incidence of the association [25].

Treatment

Preventive agents include amitriptyline, cyproheptadine, propranolol, valproic acid, naproxen, nimodipine, imipramine, and topiramate. Amitriptyline was the most commonly prescribed agent (58%) [112]. The most efficacious acute treatments of pediatric migraine include the nonsteroidal anti-inflammatory agent ibuprofen at 7.5–10 mg/kg per dose or nasal sumatriptan [111]. Migraine relief 2 h after the dose was significantly greater for adolescents using 5 mg of sumatriptan NS (66%) compared with placebo (53%). Complete relief 2 h after dose was significantly greater for patients using 20 mg of sumatriptan NS compared with placebo [209]. The treatment of the forms linked to FA includes elimination diets following the FCT results; cromolyn (Table 7.20) may be prescribed to allow the child to enjoy dietary holidays, especially on special occasions such as birthdays, or ketotifen, which reduces the frequency, severity and duration of the attacks by 72% [134]. In 12 children given an antiallergic treatment for 6 months (pharmacological or diet restriction), the migraine index decreased significantly from 2.45 to 0.33. Headache intensity decreased as well [204]. Similarly, the histamine-free diet can be recommended, which shows good results [196] (Table 10.12). Treatment in children consists mostly of trigger avoidance, rest, and simple analgesics. Behavior therapy, including relaxation-response training, may be an effective adjunct in managing both the frequency and intensity of migraine attacks. Use of medicines for abortive and prophylactic therapy has not been extensively supported by well-designed, well-controlled research. In general, use of these agents should be restricted to the small group of children with frequent, severe attacks [201].

Prognosis

Although migraine is rare before the age of 4 years, its prevalence increases throughout childhood, reaching a peak at about 13 years of age in both sexes, affecting approximately 5% of all children [201] and up to 54% in children aged 8.4 [139] (Table 16.1). Recurrent migraine has an impact on a child's life in a number of ways, including school absences and reduction in performance, decreased home and family interactions, and decreased socialization with peers [148]. Migraine interfered with play in 80 children (55%), aggravated by routine physical activities in 84 (58%), associated with nausea and vomiting in 48 (33%), photophobia/phonophobia in 77 (53%), and visual aura in two (1.37%), which are aggravated in children with severe symptoms by routine physical activity (80%) [171]. Children with migraine reported more often being bullied in school, stress in school, and problems in getting along with other children than children without headache. The association of stress in school with headache was strongest in girls with migraine [131]. The most common trigger was playing on a computer (45.9%), followed by loud noise (41.5%), and a hot climate (37.1%) [20]. Children with headache are at an increased risk of recurring headache in adulthood and may complain of other physical and psychiatric symptoms [60]. However, the quality of life of children with headache is significantly affected by their health condition [148]. Strategies for coping with psychosocial adversity in childhood may improve the prognosis in adulthood [60].

Psychological and Neurological Factors and Allergic Disease

Several troubles have been attributed to allergy, essentially to foods, in addition to headache: discomfort, pain in different parts of the body, palpitations, dizziness, paresthesia, depression, insomnia, irritability, anxiety or panic attacks, etc., but a clear-cut association with allergy was established only in a restricted percentage of cases. The behavioral changes for which a food pathogenesis is suggested are the following:

Tension-fatigue syndrome occurs mainly in young children and has already been described in the twenties [167]. Speer has specified its clinical pattern [178]: it consists in the alternation of complex alterations in cenesthesia, hyperactivity that can become maniacal, sleeplessness and anxiety, indifference, inability to concentrate, apathy, drowsiness, dizziness and easy fatigue. Other symptoms are arthralgia, myalgia, mild and intermittent or serious headache. The onset is often insidious; the diagnosis is difficult, both for symptom subjectivity and the absence of a reliable laboratory examinations. The gastroenteric troubles (more often generalized abdominal or epigastric pains, are sometimes so

intense as to interrupt the child's sleep). They are attributed to foods including CM, egg, wheat, corn, etc. [178]. In three patients with allergic TFS, RAST scores for CM and for buckwheat flour were negative or slightly positive, but PBMCs (peripheral blood mononuclear cells) responded well to CM allergens and to buckwheat flour, but not to ovalbumin. Only in patients with immediate allergic symptoms were RAST scores for offending foods positive, contrary to PBMCs. Thus, proliferative responses of PBMCs to food antigens are very useful in detecting offending foods in allergic TFS [97]. Thus TFS is correlated with an allergy pathogenesis, also for the frequent association with respiratory allergy [178], particularly asthma [12]. We deem that other DBPCFC studies are necessary to corroborate this hypothesis [126].

Attention deficit hyperactivity disorder (ADHD) is the most common neurobehavioral disorder of childhood. Up to 50% of children recover and are well in adulthood. Thus >50% of children may maintain symptoms of ADHD as adolescents and as adults [73, 152]. It is characterized by motor hyperactivity, restlessness, excitability, impulsivity, distractibility, short attention span, learning difficulty and perception of motor functions, learning disability, incommunicability with their peers that is likely to result in adaptive dysfunctioning [157, 218]. In adolescents, it may interfere with school performance, self-esteem, family relationships, and driving, and may predispose to high-risk behavior [154]. ADHD is a poorly defined disorder that generally arises before the conclusion of the 7th year and has a prevalence of 3%–10% [157] or 1%–5% [202] in subjects <18 years. However, ADHD may persist into adolescence for 70% of children, and some teenagers are diagnosed with ADHD for the first time during their 2nd decade of life [154]. The most significant measure of the public and family conscience [186] has been implicitly deduced by the notable 240% increase in the visits and 286% increase in the use of methylphenidate [185], a medicine safely administered for years to children for ADHD treatment [186]. The hypothesis of a mental defect is fading away, the biological origin has been privileged, but several added and/or overlapping factors (heredity, atopy, FA, chromosomal anomalies, cerebral disturbances, epilepsy, psychosocial troubles, anticonvulsant drugs, interventions in neonatal periods, hypersensitivity to food additives and/or salicylate, the mother smoking or drug- or alcohol-addicted during pregnancy, metabolic disturbances, malnutrition) may play a part, each of them, however, as a source of great handicap for the involved subjects [217], especially on the relational level [145]. Recently growing evidence has been found for an association between antenatal maternal anxiety and the neurobehavioral development of the offspring up into adolescence [192]. ADHD diagnosis is shown in Table 16.3 [55] when foods and food additives are involved, and Table 16.4 [26] outlines the necessary assessment according to a diagnostic score. The AAP practice guidelines suggest some screening questions [144]

1. How is your child doing in school?
2. Are there any problems with learning that you or the teacher has seen?
3. Is your child happy in school?
4. Are you concerned with any behavioral problems in school, at home, or when your child is playing with friends?
5. Is your child having problems completing classwork or homework?

The approach most likely to be efficacious encompasses both *psychosocial interventions and pharmacotherapy.* Treatment planning should include the identification of social and adaptive deficiencies when therapeutic targets are established [157]. Evidence suggests that individualized dietary management may be effective in some children [15]. There are four good trials [26, 31, 55, 162] that report substantial improvement and significant reduction of symptom scores in ADHD children on a hypoallergenic diet; >70% of children responded in each study. The results were confirmed with DBPC challenges [26, 31, 55, 162]; significantly higher scores were recorded during periods on challenge food rather than on placebo. Of course, an elimination diet works for some children with ADHD; there is good evidence for the effectiveness of the few-food and no-additive diet, but the extent of its effect may be unrewarding compared with that of medication [15]. We stress that dietary management cannot be neglected as a possible access to treating ADHD children, although it is effective in only a minority of such children [162].

Medical treatments include four options:

1. Stimulant medication management alone: 10.8% of the children received psychotherapy and/or mental health counseling alone, which increased with each age group (6.7%, 5–8 years; from 11.3% to 13.6%, 13–18 years)
2. Combination of medication (28.2%–37.3%)
3. A standard community care group
4. 15.1% received no treatment beyond the office-based visit [155], which was the case of 85% of the children who received a stimulant medication [86]

More girls (18.7%) were receiving no treatment option compared to boys (13.9%) [155]. A recent MTA (Multimodal Treatment Study of ADHD) study has compared medication management (MedMgt) alone, medication and behavior management (comb), behavior management (Beh) alone, and a standard community care group (CC): the MTA medication strategy showed persisting significant superiority over Beh and CC for ADHD. No significant additional benefits of CC over Med Mgt and of Beh therapy over usual CC were found [137]. In conclusion 15% of children receive no treatment beyond the enrolling visit [85, 155]. Methylphenidate based on a child's weight is prescribed in the range of 0.3 to 0.6 mg/kg/4-h dose, with a maximum daily dose of 60 mg [2]. The daily prescribing schedule

Table 16.3. Foods involved in the syndrome of the hyperactive child

Additive	79
Apple	13
Banana	8
Beans	15
Beef	16
Carrot	7
Cheese	40
Chicken	11
Chocolate	59
Citrus	45
Coffee	10
Corn	23
Cow's milk	73
Egg	39
Fish	23
Grape	50
Ham	20
Malt	15
Melon	21
Oat	23
Peanuts	32
Pear	12
Peas	15
Pineapple	19
Pork	13
Potato	11
Sugar	16
Tea	10
Tomato	20
Walnut	10
Watermelon	9
Wheat	49

Modified from [55].

Table 16.4. Diagnostic score for ADHD

1.	Fidgets and squirms, restless when standing, in adolescents it is sufficient to note that child is agitated or hyperactive
2.	Difficulty remaining seated as expected
3.	Easily distracted by extraneous stimuli
4.	Difficulty awaiting turn in games or group situations
5.	Frequently blurts out answers before questions have been completed
6.	Difficulty following through on instructions
7.	Poor attention span in schoolwork, play, chores
8.	Avoids activities or passes from one activity to another
9.	Difficulty playing quietly
10.	Talks excessively
11.	Interrupts or intrudes on others
12.	Often does not listen to what is being said
13.	Often loses things necessary for tasks or activities
14.	Often engages in physically dangerous activities, not conscious of possible consequences

To meet the DSM-IV diagnostic criteria of ADHD, a child must have exhibited 8 of 14 symptoms that had onset before 7 years of age and have persisted for at least 6 months to a degree that is maladaptive and inconsistent with developmental level.
Data from [26].

for short-acting methylphenidate (3–5 h) is 5–20 mg bid or tid, for intermediate-acting (3–8 h) is 20–40 mg or 40 mg in the morning and 20 mg early in the afternoon, for extended release (8–12 h), the dose is 12–72 mg. For short-acting dextroamphetamine (4–6 h) the daily dose is 5–15 mg bid or 5–10 mg tid, for intermediate-acting (6–8 h) is 5–30 mg or 5–15 mg bid, for extended release (daily) 10–30 mg [2, 155]. Despite the efficacy of stimulant medications in improving behaviors, only 38% of medically managed children in the MTA study received scores in the normal range at 1-year follow-up) [86]. Sustained treatment may lead to some growth failure [2] in addition to adverse affects including anorexia [73]. The association between reduced glucose metabolism in cerebral areas and ADHD and motor activity in adults that had suffered from ADHD since childhood and with children also affected requires clarification of whether the results are applicable to the whole subset of ADHD children; thus elimination of ADHD causes is preferable to ADHD pharmacological treatment [26].

- The *negative effect of food additives* on childish behavior was demonstrated 20 years ago with no scientific base. The Soothill school has shown (based on their parents as reporters) that with a markedly restrictive oligoantigenic diet for 4 weeks >81% of 76 studied children with ADHD had improved and 34% of them had regained a normal behavioral pattern: 28 children had been submitted to a crossover DBPCFC, resulting a positivity for different foods and even more for food additives and preservatives (79% of cases); also in this sample there was an unexpected positivity for some foods (Table 16.3). However, introducing parent reporting as a parameter to appraise the objectivity of improvements

[55] is an approach bias, also because an important cause of the hyperkinetic personality is really the relationship with their parents [82]. This again comes down to a classic work [147], the scientific demonstration of the impact of hyperkinesis in children on free diets, and the return to normal by an exclusion diet; currently such a position seems to be largely documented [26]. A significant behavioral improvement on an elimination diet and subsequent behavioral deterioration on DBPCFC was shown in 73% of hyperactive children. Atopic and nonatopic probands reacted to several preservatives and artificial colorings but the atopic children had a significantly greater response rate than the nonatopic group [26]. In a crossover study in which all food was provided to the families for 10 weeks, 58% of children (14/24) had a behavioral improvement with positive effects on their sleep, with a reduction in time falling asleep and the number of night awakenings [90]. In an intrasubject, 10-week crossover study in 24 preschool children [37], dyes and artificial aromas, preservatives, Na glutamate and any additional substances believed by parents to produce symptoms were excluded, sugar supplementation was reduced and the prevention of environmental inhalant and perfumes was associated. It is notable that up to 80% of parents of children with behavioral deterioration (37% of the sample) blamed food additives and preservatives [4]. The correlation between the syndrome and foods and food additives has subsequently been appraised by more studies in 3-to 12-year-old children. Of children subjected to a food- and additive-free diet, 76% improved; submitting 32% of them to a crossover DBFC demonstrated relation a cause–effect. In 74% of children correctly diagnosed and treated with an elimination diet, an improvement was also recorded, a result confirmed by DBPCFC (in 73% of cases). In this cohort, 40% of nonrespondents to the diet were atopic as well as 79% of the respondent: the foods incriminated were above all CM, artificial dyes permitted by the law, corn and wheat. In another sample a diet free of CM, egg and related by-products, and several foods of additive and/or histamine-releasers, produced a notable improvement of both attention and hyperkinesia in 79% of cases [196]. A double-blind study has shown a functional correlation between ingestion of tartrazine and behavioral changes (irritability, restlessness, and sleep disturbance) in 24 2- to 14-year-old atopic children; however the symptoms taken into account did not follow the strict diagnostic criteria of the syndrome (Table 16.4). Since 2 of 20 atopic controls on a dye-free diet also reacted to food additives in a DBPC dye challenge, the existence of *tartrazine hidden sources* is hypothesized. There is an evident relation between activity score and assumed doses; moreover doses of tartrazine >10 mg lead to prolonged behavioral troubles >12 h [160]. Entirely different is the randomized, DBPC study by Egger et al, who treated with EPD (enzyme-potentiated desensitization therapy) enriched with 45 foods, 10 dyes and preservatives, 40 children with poorly defined symptoms, 17 of whom (42.5%) were labeled as atopic. Also in this study, the results, statistically positive, were based on parents as reporters. In addition the poor description of symptoms and the exact composition of the medication make it impossible to reproduce EPD. The authors conclude that the treatment was able to stop the mechanism at the base, allowing children to consume the foods that were at first forbidden, even if eight children tolerated sugar after treatment, which nevertheless did not include it, underlining an aspecific effect of a therapy labeled as specific [58]. Certainly, the analysis of cost–benefit ratio is clearly for the natural method, which assures positive results in 58%–79% of children [26, 31, 90]. To us it seems that the problems related to dietary therapy reside rather in poor compliance, since it is difficult to carry out and too demanding for families [31]. In a recent, DBPC crossover trial, 52% of children following an additive-free diet worsened their behavior when challenged to calcium propionate, compared to the control children reacting to placebo (a preservative in bread consumed daily) (19%). Irritability, restlessness, inattention and sleep disturbance in some children may be caused by preservatives contained in daily foods [45].

- *Restless legs syndrome* (RLS) may affect >5% of the population. It is characterized by an unpleasant creeping sensation, generally felt between the knee and ankle, to which the subject finds relief continuously moving the legs about, particularly when falling asleep and/or while sleeping (periodic limb movements of sleep, PLMS); it is not associated with psychopathy [27]. Coffee, foods and drinks containing caffeine might instigate the syndrome, due to an increased sensitivity to caffeine [27], or to an effective allergy to coffee or more probably to substances contained in it [27]. Recently, it seems to be a current trouble in children 18 months to 16 years old with an autosomal dominant transmission [194] and in women affected with fibromyalgia or RA [215]. In this autosomal dominant mode of inheritance found in several large families, significant linkage on chromosome *12q* responsible for RLS has been identified [47]. The autosomal allele acts dominantly in RLS families with an early age at onset of symptoms and suggests that RLS is a causative heterogeneous disease [208]. Although little is known about the pathophysiology of RLS, it is generally segregated into primary and secondary forms. In at least 60% of primary RLS cases, a FH is reported which may increase from 64% to 92% when all first-degree family members are contacted and interviewed. This suggests that RLS is highly penetrant, although not always recognized [141]. Linkage studies have been hampered by the absence of any definitive diagnostic test, the subjective nature of the disease, the age-dependent penetrance, the possibility that several different pathologies result in a single phenotype, and the high frequency of RLS, which can often result in bilinear transmission. Furthermore, RLS symptoms may be caused by reduced iron stores, neuropathy, renal

failure, pregnancy, and possibly rheumatologic diseases, and cannot be reliably differentiated from primary RLS. Monozygotic (MZ) twin studies provide invaluable information regarding both genetic and environmental factors that can affect disease phenotype. In a study of 12 identical twin pairs, one or both members had RLS [141]. A study in 866 children (54.2% of boys) aged 2.0–13.9 years has recently ascertained that RLS may be associated with ADHD and PLMS and suggested that if either condition contributes to hyperactivity, the magnitude of the association suggests an important child health problem [32]. The prevalence of PLMS in 591 children with a preexisting diagnosis of ADHD was 7.1% [95]. Another pediatric study postulated that the amelioration of RLS and its associated sleep disturbance may result from the improvement in ADHD with monotherapy with levodopa or the dopamine agonist pergolide [195].

- The Soothill school has observed that *epileptic convulsions associated with migraine* and/or hyperactivity may also depend on the ingestion of small quantities of food allergens and that, even though resistant to pharmacotherapy, diet therapy responds to the diagnosis that has been set with crossover DBPCFC [56]. The finding that only children with the association of convulsions and migraine and/or hyperactivity have improved or have recovered with an oligoantigenic diet supports the existence of a syndrome as such, which is not that rare [56]. Even if only a minority of patients with epilepsy associated with migraine respond to dietary treatments, such data have a character of indubitable utility [117], considering that some patients could improve (or recover) simply avoiding specific foods. A component attributable to the hypersensitivity to given foods has been shown to be a pathogenetic factor of some psychic conditions, including neurosis, depression and certain forms of psychosis [143]. Certainly the cases of hyperactivity related to foods are a minority of mental disorders; however, this stimulating hypothesis can contribute to shedding light on the complex and partly unexplored genesis of psychopathies, or at least to a part of them.

Chronic Fatigue Syndrome

Definition

Chronic fatigue syndrome (CFS) was reported more than a century ago within neurasthenic forms. Beard [16] referred to fatigue as the "Central Africa of medicine – an unexplored territory which few men enter" and Seppilli [166] added that "the patient is fatigued from the very moment he gets up in the morning and fears being severely diseased." CFS is a chronic illness with unknown etiology characterized by persistent fatigue, exacerbated by minimal physical activity.

Epidemiology

The prevalence rate of CFS in children is 37.1×10^5 (5.5–9 years and 47.9×10^5 from 10 to 19 years) [115], or 33.4×10^5 in children aged 2–17 [120]; otherwise the median age was 14.3; 60% [30] 71% [101], or 73% [61] were female and 87% of mid or upper socioeconomic status [30]. In two open studies, the data were almost similar [100, 101], 50% of children aged 7–14 and 50% aged 15–21 [101]. In another case–control study, no differences between males and females were found [18]. This data provides a basis to understand how *the pediatric syndrome is different from adult CSF* [18, 30] (Table 16.5 [18, 30, 101, 175]).

Pathogenesis

It should be realized that the correlation with allergic disease was not thought to be sufficient to support a specific CSF pathogenesis, also because between 1985 and 1988 two unusual associations, one with EBV (Epstein-Barr virus) infection and the other with a clinical picture referred to as a chronic infectious mononucleosis were identified. Due to the CSF spread, many believed that the evidence provided was sufficient for the diagnosis of chronic EBV syndrome, patients were satisfied since the illness had a name and physicians because the search for a defined diagnosis might come to an end [91]. Subsequently, no consistent data have emerged, no specific infectious agents have been consistently linked to the illness [30, 175, 190, 205, 213], also because of the lack of diagnostic markers [205]. While the CFS etiology in children and adolescents is unclear, it was assumed to be multifactorial, so that symptoms such as depression, apathy, phobia, anxiety, cognitive dysfunction, difficulties with concentration, mood disturbances, confusion, headaches, and sleep disturbances can be found in several patients variously labeled, often convalescent from a systemic infectious disease [91]. Loneliness, social isolation and the stress of examinations, both independently and together, have been associated with *decreased natural killer (NK) cell* function in schoolchildren and/or medical students [88, 91]. Depressed individuals and/or those subjected to severe stress or psychological distress may undergo *immune deficits* [116]. However, apart from every possible stress, children and adolescents are often faced with significant psychological, emotional, social and existential dysfunctions, with considerable implications for normal psychoemotional development [30, 175]. In these children, a sign of their involvement is marked by depression, long histories of absence from school, accompanied by impairment in social and leisure activities, loss of peer relationships, heralded by an increased frequency of loss of peer group contact [205], different symptoms and sleep disturbances, the commonest symptoms of chronic fatigue (Table 16.5) [18, 30, 101, 175].

Table 16.5. Prevalence of symptoms in pediatric populations

References	[101]	[30]	[18]	[175]
No. of patients	58	20	21	15
Symptoms		(%)		
Fatigue	100	100	100	100
Headache	74	70	100	73
Sore throat	59	56	95	67
Abdominal pain	48	40	100	43
Fever	35	40	100	43
Concentration	33	56	67	67
Myalgia	31	51	100	27
Adenopathy	29	40	95	67
Diarrhea	29			23
Anorexia	29	40		30
Nausea/vomiting	24			67
Congestion	22			
Vertigo	19	51	62	57
Arthralgia	17		90	27
Sleep disturbance	86	30		40
Insomnia	50			30
Sleep ≥10 h	69			
School and relation with peers				
Excellent or good	50			
Attendance	17			
Relationships with friends				
Excellent or good	42			
Average	38			
Few or none	20			
Activities with friends				
Most/some	30			
Occasional	22			
Rare/none	48			

Mean age was similar: 11 [18], 14.3 [30] 14.6 [101], 14.5±1.7 [175].

Possible associations with FA are confirmed by the Straus et al data: 52.5% of patients show SPT and RAST positivity to food and/or inhalant allergens, 73%–83% a history greatly indicative of atopy [183, 184], 34% elevated levels of CICs (circulating immune complexes) [184] and serum total IgE [183]. Also in children, FH positivity (76%), history of allergies or asthma (71%) and recent ingestion of raw CM (81%) produced risk ratios of 23.3–44.3 [18]. Thus summing up the results, both genetic factors and increased IgE-mediated reactivity may be responsible for a rising expression of disease.

Several conflicting immune dysfunctions have been postulated, but no correlation was found with CFS pathogenesis. These include normal to decreased numbers of NK cells, reduction of IgG and subclasses, IgA and IgM, autoantibodies and CICs, reduced or increased CD4 and CD8 cell populations, with a decrease in circulating naive T cells and an increase in memory T cells, persistent CD8 activity and increased activation markers: CD38, HLA-DR on CD8 cells [104]. Evidence was found of a significant bias toward *Th2-type immune* responses in CFS compared to controls. Nonstimulated cultures revealed significantly higher levels of T cells producing IFN-γ or IL_4 in CFS patients [174]. Moreover, there was a reduction or increase in B cells also with significant evidence of HLA class II antigens. The class I antigen HLA-B61 and the class II antigen HLA-DR9 were positively associated with CSF (OR, 2.77 and 2.60, respectively) [85]. In a trial on 21 pairs of MZ twins discordant for CFS and 21 matched healthy control subjects, both sets of twins performed worse than the control group on all speed-dependent tests. Self-rated fatigue and dysphoric mood were only weakly correlated with cognitive performance [124]. Interestingly enough, significantly elevated ECP (eosinophil cationic protein) levels were found that link CFS to activity of eosinophil cells [36], suggesting that IgE antibodies and immune dysfunctions may be involved. In addition, such patients may display immune deficiencies as well as hypoactivity of the hypothalamic-pituitary-adrenal axis (HPA) following a CRH (corticotropin-releasing hormone) deficiency, which returns to normal levels following treatment [33].

Clinical Features

Prolonged fatigue after physical activity, headache, loss of ability to concentrate, inversion of the sleep–wake rhythm, excessive fatigue, myalgia following minor activity, nausea, abdominal pain, sore throat without coryza, *excessive dependency on parents,* and a feeling of *disturbed balance* were present in 87% of children, with symptoms rated as severe or moderately severe in >50% [30, 61]. Severe illness-related impairment (work, expectations, attendance), particularly through school nonattendance [69], or 94% attending school half-time or less [113], and high levels of illness-related school concerns was also recorded [69]. In children of a mean age of 11 years, there were statistically significant associations with allergic disease, *ingestion of raw milk* and other family members with CFS [18]. In the children referred to in Table 16.5, 60% *reported a history of allergies.*

There are no pathognomonic features or diagnostic tests to be assessed, but a complex interaction of physical and psychological components. Table 16.6 summa-

Table 16.6. Assessment of CFS

A. Criteria by Smith et al [175]
1. History of onset with an acute illness
2. Presence of profound persistent or intermittently prolonged fatigue in children or adolescents previously in good health
3. Symptoms for longer than 6 months and less than 3 years
4. Presence of at least three additional symptoms according to CDC criteria
B. Study by Carter et al [30]
1. The diagnosis of children was CFS for more than 2 months (instead of 6 months)
2. The syndrome should not be attributed to known causes
3. Symptom prevalence useful for assessment (in decreasing order) a. 55%–70%: headache, arthralgia, excessive sleep, concentration, sore throat b. 40%–50%: myalgia, dizziness, muscle weakness, depressed mood, decreased appetite, abdominal pain, fever
C. Oxford research criteria for diagnosis of CSF [151]
1. Syndrome characterized by fatigue of definite onset as the main symptom
2. The fatigue should have been severe and disabling, affecting physical and mental functioning
3. CFS symptoms should have lasted for a minimum of 6 months during which it was present for more than 50% of the time
4. Other symptoms may be present, particularly myalgia and mood and sleep disturbances
5. Patients with established medical conditions known to cause chronic fatigue should be excluded from the definition. All patients should have a history and physical examination
6. Patients with current diagnosis of schizophrenia, manic depressive illness, substance abuse, eating disorder or proven organic brain disease should also be excluded from the diagnosis. Other psychiatric diagnoses such as depressive illness, anxiety disorders and hyperventilation syndrome are not necessarily reasons for exclusion
D. Guidelines of CDC [80] **and of an international working group** [66] (see Fig. 16.1)
E. Protocol proposed by a joint pediatric-psychiatric team [193]
1. A definable onset of symptoms, potentially associated with a previous viral infection
2. Generalized fatigue
3. Easy fatigability
4. One of the following associated symptoms a. Headache b. Dizziness c. Moodiness d. Peripheral sensory change
5. Symptoms of sufficient severity to interfere significantly with normal social and/or school functioning
6. No detectable organic pathology to account for the above symptoms
E. Subsequent series of pediatric patients with CFS established the following [11, 100, 214]
1. Persistent fatigue was a common reason for referral to subspecialty clinics
2. The average patient was a young female teenager
3. More children reported antecedent illness
4. Results of physical examinations and routine laboratory results were normal
5. Children exhibited significant functional impairment and school absence
6. The majority of patients recovered

rizes North American and English classifications [11, 61, 80, 100, 175, 193, 214] compared with pediatric studies [18, 30, 121, 122, 151]. It should be noted that:

- The *Smith et al* suggestions [175] are more suitable to children aged 1–19, while CDC criteria [66, 80] are centered mainly on adults.
- The *Carter et al* study [30] introduces even more discriminatory pediatric characteristics: CFS patients score higher than healthy controls and depressed subjects on somatization; however, they experience less anxiety and depression, but less impairment in think-

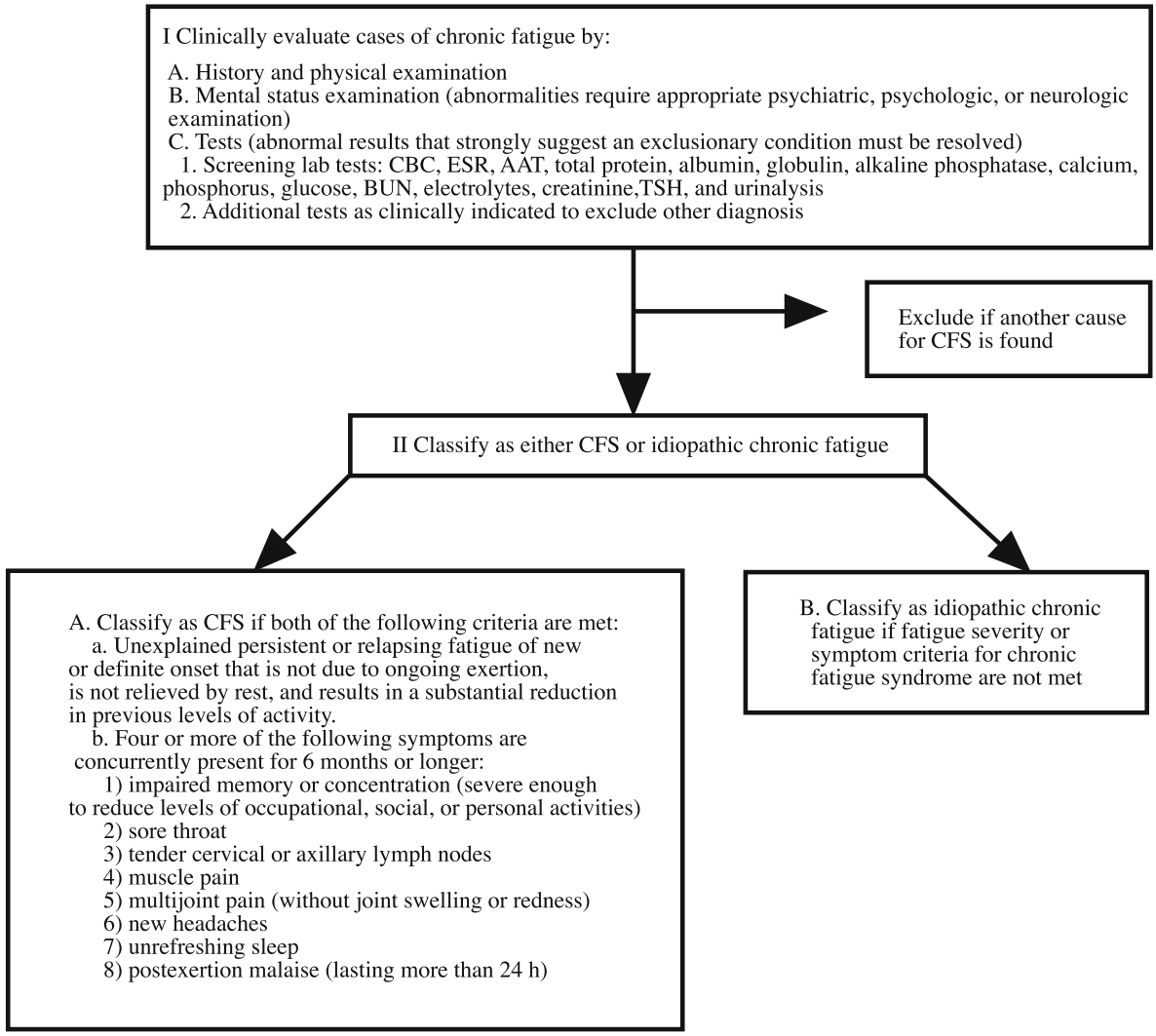

Fig. 16.1. Clinical evaluation and classification of CFS. *CBC* complete blood count, *ESR* erythrocyte sedimentation rate, *AAT* alanine aminotransferase, *BUN* blood urea nitrogen, electrolytes, creatinine, *TSH* thyroid stimulating hormone. (From [66, 80])

ing, and fewer social relationship difficulties than the conclusions of their parents.

- The *Oxford criteria* applied to 32 adolescents exclude psychiatric diagnoses [151].
- The *previous CDC criteria* [80] have been updated and simplified by an international working group [66] (Fig. 16.1).
- The *criteria proposed by a joint pediatric psychiatric team* [193] focus on psychiatric disorders, which should be excluded from the syndrome, even if they are usually present in some adult patients. Actually four of ten patients ranging in age from 10 to 16 years were affected with different forms of restrictions in physical mobility, which certainly cannot by related to the CFS constellation of symptoms.

Above all, pediatric studies establish more stringent criteria [11, 30, 61, 100, 122, 151, 175, 214]. Age was 4–21 years, follow-up 12–72 months, symptoms improved in 53%–100% of cases. Some researchers have rightly pointed out that the illness behaviors of children may be significantly influenced by parental expectations and responses [30]. The CDC criteria, which require 6 months of fatigue for the diagnosis to be made in adults, may be too long for children [121, 214]. Some pediatric studies have selected cases based on ≥6 months of fatigue [175], whereas others have used shorter durations such as 2 months [30] or 6 weeks [193].

Diagnosis

Children and adolescents are in a dynamic developmental state, and issues such as self-concept, autonomy, body image, socialization, sexuality and academic goals are of central importance. Early intervention in those individuals with persistent fatigue is therefore especially important [116]. There is an astonishing absence of specific physical signs and physical investigations fail to identify any organic pathology or current infection to account for the symptoms [175, 214]. The acute or nonspecific onset of the illness in children may cause problems for the doctor; moreover, symptoms often reported to be abrupt are marked by unexplained fatigue [175]. Furthermore, there is no available laboratory test [30]. Therefore, in children and adolescents, a diagnosis of CFS should be considered when unexplained fatigue persists for 3 months, rather than the 6 months stipulated in the adult case definition [116]. In a study in adult patients it emerged that some subjects with apparent postviral fatigue had complained of tiredness before their presentation with a viral illness [37]. EBV serological studies fail to ensure concrete results, also because several healthy individuals display high rises in antibodies to the early antigens of EBV, even years after a primary infection, thus a causal relationship with EBV cannot be found [30]. In a study using *in situ* hybridization, nine of 41 patients had a serological test positive for EBV; however, no statistical difference could differentiate males from females and control subjects [213]. The *differential diagnosis* should take into account several conditions that may mimic CFS: hepatitis B, fibromyalgia, multiple sclerosis, Lyme disease, AIDS and Münchausen syndrome by proxy.

Treatment

Acyclovir [183] has been tried, but the results could not reliably differentiate patients from placebo-treated subjects [183]. Interventions that reported adverse effects severe enough to cause withdrawal from the study encompassed several medications including acyclovir. Interventions that have shown promising results include cognitive behavioral therapy and graded exercise therapy [207]. Therefore, treatment tends to focus on targeting the symptoms of myalgia, sleep disturbances, affective symptoms, and fatigue. Evaluations of the effectiveness of different approaches suggest a variety of different outcomes. While there is some lack of agreement on management strategies, there is considerable agreement on elements of these strategies, even if terminology may suggest otherwise [214]. However, the management of CFS in children is not controversial [205]. *An active rehabilitation program should be offered to all cases* [181]. Actually, programs of rehabilitation rather similar to those now shown to be effective in adults, including some form of support, encouragement, behavioral management, and activation are now the mainstay of treatment in virtually all centers helping children with the syndrome and their families [205]. Receiving treatment was associated with increased school attendance, but one-third of subjects obtained no qualifications. Return to normal health or significant overall improvement was reported by 29/36 children [142]. Immediately following a multidisciplinary inpatient program (MIP) for CFS and up to 5 years after the program, 78% of 42 adolescents who were significantly incapacitated prior to entering the MIP had returned to school full-time or with occasional absences only and were functioning better in terms of physical activity and social interactions as compared with before the program [113]. The relapsing nature of CFS suggests that follow-up should continue for at least an additional 6–12 months after the intervention period has ended, to confirm that any improvement observed resulted from the resolutions of symptoms following the intervention and not to a naturally occurring oscillation in the course of CFS [17].

The *natural history is favorable:* the first follow-up study reported that about half of the children improved 2 years later [175], 24/31 (77%) returned to normal or improved with occasional relapse over a 2-year period [30], and 43% of families considered their child cured and 52% considered their child improved in a group of children and adolescents followed up for 4.5 years [101]. An overall good functional outcome was reported in 80% of participants an average of 13 years after illness onset [17]; in another cohort the rate was 74% 3 years after onset [151]. Follow-up of 23 children diagnosed as having CFS in a clinic found that most children had recovered after a median follow-up of 2 years [122]. Among these children, 76% reported a definite improvement at 2 years, with 38% continuing to have some occasional residual symptoms [122]. However, the participants who remain ill may have significant symptoms and activity limitation [31, 151], but after recovery several children may be left with mild to moderate persisting symptoms [31], or residual symptoms and handicap [151]. At the more severe end of the disability spectrum, 90% of 25 youngsters with CSF aged 15 years and 72% of their parents believed the illness was precipitated primarily by biological factors with an unrealistic view of normative fatigue levels. The tendency by children and parents to underestimate the children's actual levels of activity stressed distorted health perceptions or expectations with the children enhancing general disease conviction, in spite of medical evidence and reassurance to the contrary [68]. The family practitioner or pediatrician should seek the cooperation of the parents and other care givers in devising a supportive rehabilitation plan, including maintenance of peer contact and relationships with friends; academic and recreational activity; and physical activity, rest periods and sleep.

Clinical Presentation of Other Systems

Gastrointestinal Tract

Irritable bowel syndrome (IBS) is the most frequent nonorganic cause of chronic nonspecific diarrhea in children between 8 months and 3 years of age, due to a probable functional alteration in the intestinal motility, intensified or caused by diverse factors [76]. IBS is properly a heterogeneous term masquerading under a variety of affections highlighted by various gastrointestinal disorders in which there is alternating constipation and nonspecific diarrhea, or abdominal bloating, disordered defecation, and in many cases pain [191, 220]. IBS may be prevalent in girls following anatomical and positional abnormalities: ptosis of the transverse colon to the small pelvis [149]. In an IBS variant, constipation predominates (*spastic colon*) and gastrointestinal transit times are highly increased [93]. Often there is no radiological and/or histological evidence of organic disease. Heredity has been suggested to explain the finding that IBS tends to run in families: in 6,060 twin pairs, concordance for IBS was significantly greater in MZ (17.2%) than in dizygotic (8.4%) twins, supporting a genetic contribution to IBS [110]. IBS symptoms were noted by 17% of high school students and 8% of middle school students, representing 14% and 6% of all high school and middle school students, respectively [83]. Several patients with food intolerance have been reported [119]; however, the fraction of IBS sufferers with FA may be larger than believed [173]. Pediatric studies have confirmed this association [11, 220], to the point that a restricted diet improves symptoms in ≈77% of children, while the others found an improvement when cromolyn was added [11]. The incriminating foods in the improved children have been identified: CM, egg, wheat, spinach, tomato, etc. are most frequently involved [11]. An otherwise healthy 15-year-old girl presented with lower abdominal cramping, a variable bowel pattern, and autonomic signs (pallor and diaphoresis) [220]. Thus, IBS supportive symptoms include abdominal pain associated with stool changes, chronic history, normal physical examination and laboratory studies, while nonsupportive symptoms include weight loss and nighttime awakening [220]. The mechanisms at the basis of IBS could be those of a PA; however, the pleomorphic nature, variable in expression, as well as in day-to-day incidence [119], makes it difficult to demonstrate whether food intolerance has any role in IBS, because tests performed and interpreted improperly are liable to be misleading [126]. In several children, a correlation between symptoms and greater bloating, secondary to lactose intolerance and carbohydrate malabsorption have been recorded [136]. Similar conditions that may need to be considered in IBS differential diagnosis include gastrointestinal FA, carbohydrate deficiency, viral infections, inflammatory disease (Crohn's disease (CD), ulcerative colitis (UC), celiac disease) and cases where an excess of fibers or of unabsorbed food residues cause fermentation in the small bowel [220]. IBS diagnosis has been established by the Rome II diagnostic criteria of the condition, available at www.helpforibs.com/footer/rome_guidelines. asp (May 25, 2004). A differential diagnosis of IBS with organic disease was significantly associated with straining on defecation, diarrhea and abdominal bloating but not with pain in the upper abdomen, reflux and appetite loss. In comparison with extraintestinal allergy pain relieved by bowel movement, pain in the lower abdomen, pain in both the upper and lower abdomen, frequent pain and abdominal bloating were significantly associated. In comparison with FA, pain in the lower abdomen, relieved by bowel movements, frequent pain and abdominal bloating were also significantly associated [138]. Children are unlikely to comply with elimination diets, which would have to be carried out over a long enough period of time to exclude the likelihood of an improvement wholly related to chance [119]. Other useful adjuncts in individual children may be sequential dietary exclusion, use of hypoallergenic diets, cromolyn, and colonoscopic allergen provocation test [219]. In particular, oral cromolyn may be useful in chronic unexplained diarrhea and appears as effective as and safer than elimination diets [177]. May has discussed the difficulty of DBPCFCs with sufficient food antigens to provoke unequivocal symptoms [126]. Health care providers who work with these children need to be able to recognize the symptom complex associated with IBS, as well as the possible relationship to anxiety and depression [83]. Some children have their symptoms based on their malabsorption of carbohydrates and improve when placed on a diet without food high in fat, and caffeine, and those with predominant constipation should limit sorbitol, high-fructose corn syrup, stachyose, and raffinose [177]. Moreover, fat, fibers, fluids, and fruit juices should be normalized in alimentation [16, 136]. Thus the roles of *lactose and fructose intolerance* remain poorly understood [177]. If symptoms continue despite dietary changes, a variety of medications can be tried; however, the efficacy is often unclear [220]. Level II evidence is emerging for the use of probiotics and prebiotics in IBS, and in both UC and CD. The use of these therapeutic agents for the above disorders is rapidly moving into the clinical armamentarium [62]. The use of peppermint oil seemed to reduce the pain that children experienced during the acute phases of IBS [206].

Urinary Tract

The urinary tract as a shock organ for pediatric FA has been suggested by a few authors. Several children with respiratory allergy have coexisting *enuresis,* which disappears after elimination of certain foods and recurs after oral challenge. Enuretic children have been shown

to have associated allergic disorders more often than nonenuretic controls [214]. These studies have suggested a role of FA in the treatment of *nocturnal enuresis:* when a group of enuretic children was put on an avoidance diet as a complement of pharmacological treatment, the nocturnal enuresis ceased, the frequency of micturition decreased and the bladder capacity increased [65]. A number of articles report cases of cystitis with dysuria, nycturia and vesical tenesmus, secondary to FA, incriminating foods and food additives included CM, wheat, chocolate, cola, citrus fruit and colorants [35]. The impact of FA was stressed by the Soothill group who reported that enuresis in food-induced migraine and/or hyperactivity may respond in some children to withdrawal of provoking foods [57]; however, unusual therapies require further evaluation [65]. That an allergic reaction can occur in the bladder and result in *allergic cystitis* should not be surprising: histologically, the bladder resembles the respiratory and GI tracts because it is provided with a mucosal membrane, a submucosa rich in blood vessels and a smooth muscle layer. It is theorized that the *urinary bladder smooth muscle layer* may act as an allergic target organ, much as the airways' smooth muscle layer does in asthma, thus provoking a spasm causing decreased bladder capacity and thus enuresis [35]. However, due to the lack of well-controlled and statistically analyzed studies, the role played by FA has not been well documented.

The nephropathies purported to be induced or aggravated in some children by CM ingestion are as follows: orthostatic albuminuria, idiopathic nephrotic syndrome, certain cases of mesangial glomerulonephritis and urinary tract infections. The *lipoid nephrosis* or minimal change nephrotic syndrome is most common in young children [34]. The immunological abnormalities are very peculiar: IgG antibody decrease, IgE and IgM antibody increase, reduced responses to PHA and Con-A (concanavalin A), increase of β-microglobulin levels, a metabolite of HLA class I antigens, reduced IL_2 production and excess production of vascular permeability and immunosuppressor factors, respectively, by CD4 (Th2 T cells) and CD8 T cells [103, 163]. During the relapse phase, significant alterations were found of the NF-κB/IKBα regulatory pathway, whereas very low levels of $IL_{12}R\beta 2$ mRNA were detected, suggesting that T cell activation evolves toward a Th2 phenotype [161]. The Th2 predominance on Th1 T cells is fulfilled in this disorder, possibly with reference to HLA-DR7 allele preponderance absent in adults [103]. Several cases of lipoid nephrosis correlated with sensitivity to different foods, including shrimps, have been reported, with stable remission of symptoms following avoidance of the incriminated foods [50, 106]. Table 16.7 [106] outlines the responsible foods in 34 patients. In 6/17 children aged 1–15 with steroid-resistant nephrosis and minimal change or mesangial proliferation and CM intolerance (SPTs negative in three children tested), an improvement of renal lesions after 1 year on a CM-free diet was noted [172]; similar results have been reported in five subjects aged 7–26 [50] and in a 5-month old girl suffering from a clearly CM-induced nephrotic syndrome that remitted after the institution of a CM-free diet [43]. CIC glomerulonephritis was reported in a child with eosinophilic gastroenteritis and FA. CIC levels were >6,400 mg/dl) and contained κ-casein and bovine serum albumin (BSA). With strict dietary limitation of causative antigens and prednisone therapy, CIC levels decreased to 16,000 µg/dl and serum BSA antibody titer fell 32-fold over a period of 15 months. There was prompt symptomatic relief and amelioration of signs of nephritis [128]. Cromolyn associated with the elimination diet has a remarkable positive effect on the proteinuria, without the need for steroids and immunosuppressors [108]. In addition to food allergens, inhalant allergens are also present, Der p in 18% of pediatric cases [107]. Cross-reactions with different foods are possible when ragweed or grass pollens are implicated [103].

Table 16.7. Responsible foods in 34 cases of lipoid nephrosis

Food	No. of cases
Cow's milk	11
Gluten	6
Egg	4
Pork	4
Beef	2
Wheat	1
Chicken	1

Data from [106].

In cases of *mesangial glomerulonephritis,* IgA antigliadin antibodies are a possible marker of the condition; this specificity does not extend to antiendomysium or antireticulin IgA. IgA antibody anti-foods, lysozyme, ovalbumin, BSA, α-lactalbumin and anti-inhalants (Der p, pollens and feathers) have been observed [158]. History points out frequent cases of urticaria-angioedema and/or pollinosis; serum IgE levels are not higher than those found in the general population [107]. As regards treatment, food and gluten avoidance can lead to steroid withdrawal: in 30% of cases dietetic treatment associated with cromolyn is efficacious [38]. There is the risk that the nephropathy aggravates following a specific immunotherapy (SIT) for grasses due to possible cross-reactions between pollens and gliadin [107]. The case of *vaginal itching* as a manifestation of seasonal IgE-mediated rhinitis due to ragweed is interesting [48].

Scleroderma-Like Syndrome

Also known as toxic-oil syndrome, scleroderma-like syndrome began in an epidemic form in Spain in early May 1981, with clinical manifestations involving several organs in two clinical phases:

- *Acute phase* from May to October 1981: fever, pruritic skin rashes, nausea, vomiting, dyspnea, cough, malaise, acute pleural and lung disease (mostly pulmonary infiltrates), headache, myalgia, serum eosinophilia, lymphopenia, increase in lactate-dehydrogenase levels and elevated serum IgG [35]. Roughly 60% of children had flushed cheeks, enanthem and decreased salivary secretions were noted in some cases.
- *Chronic phase* after October 1981 (almost exclusively in the female sex; especially at risk were female patients with HLA-DR_3 and HLA-DR_4 antigens) [35]: neuromuscular and joint involvement, scleroderma-like skin eruptions, pulmonary hypertension, hepatic and pancreatic changes, disorders of the lacrimal and salivary glands, lymphadenopathy, weight loss, asthenia and hair-loss. Neuromuscular manifestations, the most severe, included myalgias, muscular weakness, yospasms, joint contractures, rapidly progressive muscular atrophies. Respiratory insufficiency was responsible for most of the deaths [187].

The physiopathology of eosinophilia-myalgia syndrome shares obvious contact points, mainly in the eosinophil activation [19]. A 6-year-old female presented with a rapidly progressive scleroderma-like syndrome (SLS) involving almost the entire integument. Initially clinical patterns and histopathological data of both eosinophilic fasciitis and SLS were present: thus both conditions might be subtypes of one disease entity [81]. A differential diagnosis is possible for the elevated IgE concentrations in 37%–50% of patients with SLS associated with tryptophan use [187] and not in the others [19]. The probable cause of this syndrome was the ingestion of rapeseed oil that had been diverted from industrial use and denatured by the addition of aniline and diluted with other seed oils and liquefied pork fat, fraudulently sold as pure olive oil by itinerant salesmen [187]. The oil contained anilines not removed by the treatment, and it is assumed that free radicals from aniline derivatives reacted with fatty acids, resulting in epithelial and vascular changes able to elicit the plurifocal lesions (Chap. 18). Over 20,000 people were ultimately affected, and >1,200 deaths from all causes have been recorded in the affected cohorts [187]. Finding this syndrome in an infant revealed the association with the toxic oil. The role played by high IgG, ICC and autoantibody levels is rarely elucidated; however, there could be a connection with autoimmunity [188].

Hypersensitivity to *Candida albicans*

The origin of this syndrome was the finding of IgE anti-*Candida* in atopic subjects, and above all from the popularity of the book *The Yeast Connection* [42], in which *Candida* is alleged to cause nonspecific and unproved findings, in other words, leading to everything except true allergy [22]. A causative factor should be the advent of antibiotics, essentially broad-spectrum antibiotics, which – to combat harmful microorganisms – also eliminate the useful bacteria, thus favoring yeast overgrowth. The yeast acquires the capacity of depressing the immune system by an increased toxin production; diets rich in carbohydrates and medications such as steroids could work similarly [22]. As a consequence, this exposure in susceptible individuals is alleged to produce multiple subjective symptoms that may involve any organ or multiple organ systems, as well as a sensation of chronic fatigue. Patients are therefore recommended not to take either the above medications or the yeasts, and to reduce their unrestrained craving for sweets and starchy foods [40]. It should be noted that oral nystatin, frequently prescribed for such a disorder, reduced symptoms significantly less than placebo [49]. The *AAAAI* finds that the very existence of this syndrome is unsubstantiated and that certain therapeutic approaches show no objective evidence to support the claim [5]. Moreover, no scientific proof from well-controlled studies has been provided [126], whereas the differentiation from the chronic mucocutaneous candidosis is important.

Allergy to Salt, Sugar, and Water

As pointed out several times, allergy has been very often cited inappropriately, and we are uncertain whether the zenith has been reached when reference was made to *sugar allergy,* a misnomer, as a phenomenon that adversely affects behavior and intelligence in children or when a nameless spokesman even incriminated *salt allergy* as a possible cause of hypertension. An additional chapter was opened up when some investigators mentioned carbohydrates and sweeteners as causative factors of this syndrome: a total of five studies investigating 108 hyperactive children aged 3–17 [74, 133, 211, 210] provided no sound basis for a cause and effect relationship. A DBPCFC study has demonstrated by significant statistical differences that no association exists between sugar consumption and children's behavior changes and a decrease in their cognitive performance [211]. Aspartame has also been questioned [168, 211] but it does not precipitate seizures or ADHD [10] as well as carbohydrates and sweeteners in general [94]. *Dietary salt* has been shown to increase asthma morbidity in children aged 9–15 years [146], whereas improvement in AD by reduced salt intake has been reported in a 4-year-old girl [13], a measure first suggested as long ago as 1912. Final-

ly, *water hardness* rich in Ca salts has been shown to be a risk factor for AD in children of primary-school age [129].

Münchausen Syndrome by Proxy

Münchausen syndrome by proxy is an extreme form of child abuse in which an adult repeatedly produces symptoms of illness in a minor under his or her care [14]. The presentations appear to be similar across the world with the exception of induced apnea, which emerged as notably uncommon [64]. Children can be victims even in a closely monitored pediatric intensive care unit (PICU) [89]. A child hospitalized for evaluation of confusing symptoms, was later shown to be a victim while on mechanical ventilation. The mechanism was intentional tracheal extubation by the child's mother [89]. Two cases were produced by poisoning with different substances (clozapine and clonidine) [14]. The differential diagnosis often includes this worrying syndrome of healthy children falsely presented by parents, especially by mothers [46, 160, 164], perpetrators in 76.5% [170] or 86% [64] of cases, according to two reviews, each including >400 cases [64, 170], who fabricate medical histories or cause symptoms by exposing the child to toxins, medications, infectious agents or physical injuries [46, 164]. The child is then presented to doctors as affected with hyperactivity, migraine, abdominal pains, enuresis, rhinitis, cough [198], *even totally simulated* [70, 130], although the true disease was FA or asthma [198]. In 15 children reported with false allegations of sexual abuse, cases of severe allergy, diarrhea, dermatitis, drug overdose and hyperactivity were found to be fabricated [130]. A more detailed investigation indicates that the children's families are usually involved, but mothers have an obsessive background, rave about the disease, showing an exaggerated desire to see the alleged diagnoses and related dietetic treatments confirmed, trying one doctor or specialist after another, finally coming to alternative medicine. The parents who urge the doctor (aggravating the symptoms) to prescribe a total RAST or additional, not invasive analysis, to find support for the supposed allergy, are also included [198]. The child is often taught to become an invalid [70], and ends up by colluding with the family delusion, and *from victim turns into a party to the falsification* [164]. Even if a child recognizes that it is wrong, victims may be afraid to speak up for fear of anger and more abuse at the hand of the abuser [77]. The annual incidence of these conditions in children followed up in a specialized department may be as high as 5%–8%, in children aged <16 is 2.0×10^5 [46] or 0.5×10^5 [127] and in children aged <1 is at least 2.8×10^5, the highest incidence [127]. Over 4 years a diagnosis was made in 23/41 (56.1%) children closely monitored by video surveillance [78]. As knowledge of the condition grows, a wider range of perpetrators is identified [170]. Typical victims may be either males or females [170] or males in 54% of cases [64], usually 4 years of age or even 4 months [160, 170] or between 3 and 13 years in 52% of cases [64]. Victims averaged 21.8 months from onset of symptoms to diagnosis [170].

This is one of the circles of innocents often subjected to incongruous and prolonged diets and/or inappropriate therapies, even with a great risk for their health and growth: 11 children with growth arrest had been forced to follow a severely restricted diet for an alleged food multiple sensitivity [156]. Most children (72%) present with multiple symptoms and over half (55%) have an underlying chronic illness [46]. The asthma prevalence is only 1%, but the pattern is different because, in addition to fictitious worsening due to the above-mentioned causes, even refusal of medical care and discontinuation of treatment in children with severe attacks have been recorded, until they comply with the battered-child syndrome, taking the shape of an induced worsening of symptoms [70]. However, the morbidity for the child in the majority of cases can be less severe, and following diagnosis there may be improvement or resolution of symptoms in 50% of cases [46]. On the other hand, medical conditions fabricated by children may go undetected for a variety of reasons or be diagnosed as somatization. Therefore clinicians should always be in contact with pediatricians, psychologists and social services teams [198].

Total Allergy

The media coined this label several years ago for *patients claiming reactions to several foods and different environmental factors*. Two surprising cases best exemplify the concept of total allergy: a 13-month-old girl with reactions to 12 different foods with an increase in circulating eosinophils and leukocytes [84] and a child with a double multiple sensitivity to food and inhalant allergens and anaphylactic reactions to foods [10]. A 6-year-old child (reported in the Italian lay press) had double allergy to inhalants (pollens and mites) and to numerous foods, so that he could eat only rice, beet, carrot, fennel, pumpkin, horse and lamb meat. He also could not tolerate odors and synthetic fibers. Many allergists and clinical immunologists manifest their widest skepticism for this syndrome that is taking root and flourishing [109]. However, given a large amount of attention received in the lay press, more and more patients are seeking a cure [109]. Typical is the aspect of a child with total allergy (Fig. 16.2). Over the years the spectrum broadened to include food additives and preservatives, and eventually petrochemicals, petrol fumes, environmental pollutants, perfumes, soaps and other make-up items [40]. The confusion ruling the syndrome has extended to a large number of hospitalized patients suffering from a myriad of complaints, but above all from malnutrition (Chap. 21). The symptoms

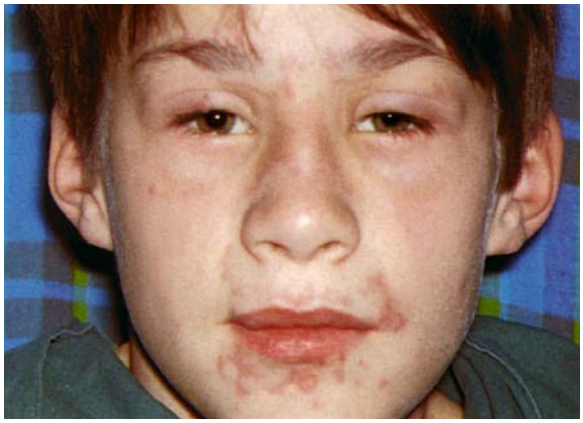

Fig. 16.2. A child with allergic rhinitis (allergic shiners, broad nasal bridge, nasal obstruction) conjunctivitis (puffy eyelids, injection in both eyes), eczema around the lips and elsewhere, asthma and multiple food allergy. A typical child with total allergy

include dyspnea or wheezing, arthralgias, transient skin eruptions, etc., with a lack of rigorous identification, but with a common characteristic: they cannot be attributed to any known allergic disorder. The current understanding refers to other diagnoses such as anorexia nervosa [197], thus implying a relationship with multiple food sensitization, or hyperventilation syndrome (in subjects with alleged FA) or current or past mood, anxiety, somatoform disorders, or finally a new chapter of Münchausen syndrome.

Clinical Ecology

The syndrome is also named the twentieth century disease, multiple chemical sensitivities (MCS) or clinical ecological illness, environmental hypersensitivity illness, cerebral allergy, chemical AIDS, sick-building disease, etc. [71], but sometimes it is also called the total *allergy syndrome* [93]. Clinical ecologists propose that chronic and recurrent complaints that superficially resemble those that apply to clinical allergy are instead imputable to low levels of chemical substances in the environment that are inoffensive to normal people [93]. Such theories may derive in equal parts from immunotoxicology (Tables 4.15–4.17) and from adverse reactions to food additives. The term "allergy" is understood in the broader sense, as opposed to the usual medical term, to include nonimmunological, pseudoallergic and other intolerance reactions. Clinical ecologists report that unrecognized immune system dysregulation develops after exposure to environmental pollutants (Chap. 4). Thus, the illness is defined as chronic and polysymptomatic, caused by adverse reactions to inhaled, absorbed, or ingested substances, modified by individual susceptibility and specific adaptability [71].

Table 16.8. Symptoms in the girl

A. Symptoms
Asthma
Dizziness
Fatigue
Headaches
Nausea
Rashes
Sinusitis
B. Common triggers
Cleaning products
Foods
Freshly painted buildings
Home decorations
Perfumes
Pesticides
Toiletry chemicals
Off-gassing construction materials
Electromagnetic radiations

Data from [212].

A 4-year-old girl had symptoms such as CM allergy and poor weight gain as an infant, and then later developed symptoms (Table 16.8) [212] precipitated by an expanding variety of chemicals, foods, and allergens. Her mother no longer took her into public restrooms or grocery stores because the disinfectants provoked a vast array of complaints, and freshly painted walls in the hospital set off an asthma attack [212]. However, this syndrome includes a spectrum of symptoms, all with the common absence of widely accepted, standardized, clinical and epidemiological criteria, often based on unproved concepts, unproved techniques and an absence of adequately validated diagnostic laboratory tests [93]. As an example we find:

- *Stimulant reactions to foods:* aggressiveness, loquacity, depression, blushing, anxiety, inappropriate laughing, agitation, anger, panic, worrying, irrationality, muscle contractions, convulsions
- *Food deprivation reactions:* melancholy, hallucinations, torpor, apathy, amnesia, raving, paranoidism, urinary incontinence; additional affections such as headache, learning disabilities, arthritis, anaphylaxis, dermatitis, asthma, rhinitis, etc. remain indeterminate [93].

Alternative Diagnostic Tests

Clinical ecologists place special emphasis on several tests rarely employed in allergy diagnosis, including subcutaneous provocation and neutralization test, sublingual provocation and neutralization test, leukocytotoxic test, IgG concentrations, food immune complex assays, auricolocardiac reflex method, the Vega test or "black box" and hair analysis to detect possible traces of heavy metals. These approaches in addition to bioresponance, electrodermal testing, reflexology, the ALCAT

test [216] and the food allergy profile are definitely unorthodox, unreliable and expensive [71, 199]. Even if low concentrations of environmental pollutants over time are purported to damage the immune system, there is no scientific basis for environmental ecology, and as yet no DB, controlled, randomized clinical trials have demonstrated a diagnostic value for provocation and neutralization [40, 126]. In such a DB trial in 18 patients, control injection as placebo was equally as effective as active antigen (food extract) to induce either neutralization or symptoms [87], thus suggesting that neutralization may be *the effect of suggestion or chance*. Therefore, all these techniques have one thing in common: none has been validated in their application to any child affection.

Alternative Therapeutic Approaches

The overenthusiastic, uncritical and inappropriate application of unorthodox allergy treatment may be detrimental and costly. These include acupuncture, homeopathy, chiropractic and phytotherapy. Chinese medicinal plants are exposed to an alternation of conflicting communications [72, 169], such as the report of 2 of 27 children, who after a 1-year follow-up had reversible abnormalities of liver function tests [169], showing that it is insufficient that a treatment be effective to demonstrate that it works. Instead, we note a nonexistent reproducibility of the study as shown by Liu et al [114], who notwithstanding have obtained both formulas and dosages, but with negative results because six of eight patients with intractable AD dropped out of the study in the 1st week because of exacerbations of their AD, while the remaining two patients gave up the treatment after 2 weeks because their AD also worsened. These so-called remedies are apparently effective, but caution must prevail [79] since we are unaware of a reliable standardization, the precision of active ingredients, and published long-term data on the potential toxicity [72]. It is not surprising that beginning with Chinese herbal remedies [8, 79], it is pointed out that, similarly to the procedures mandatory for conventional drugs, prescription of these mixtures requires as much training as that required by governmental and/or medical regulations, they should be subjected to stringent procedures for *identification of active components* and quality control, and that medicines of any type should be combined with a careful study of the risk of *possible interactions* between different substances before their prescription. Because there is a lack of purity and consistency in dosage in their manufacture, if such products are sold without prior FDA authorization, and these materials are advertised as if they were medicines, they should be regulated as such [51]. Consumers (and pediatricians) may be misled by claims that herbal products can treat, prevent, diagnose, or cure specific diseases, despite regulations prohibiting such statements [134a].

Table 16.9. Types of alternative medicine used by children (%)

Chiropractic	36
Homeopathy	25
Naturopathy	11.5
Acupuncture	11.5
Osteopathy	9
Oligotherapy	4
Others	3

Data from [179].

Table 16.10. Clinical reasons for using alternative medicine

Clinical reason	(%)
Respiratory	27
ENT	24
Musculoskeletal	15
Skin	6
Gastrointestinal	6
Allergy	6
Prevention	5
Others	11

Data from [179].

Table 16.11. Factor influencing the choice (%)

Oral diffusion	32
Fear of side effects of conventional medicines	21
Chronic illness	19
Dissatisfaction with conventional medicine	14
More personalized attention	9
Other causes	5

Data from [179].

There is evidence that related studies are not yet supported by statistically significant results in representative samples [126] and, we add, by DBPC studies. However, the interest aroused by unconventional therapy is shown by the preference of the subjects aged 18–24 years (16%) who consulted providers for allergic disease in 6.3% of cases [59]. Tables 16.9–16.11 [179] illustrate a study reflecting an 11% incidence in a pediatric outpatient department of a Canadian university hospital. Table 16.9 shows that more than half consulted either chiropractors or homeopathists for the most frequent complaints, respiratory and/or ear, nose, and throat disorders [179]. The investigation of a cohort of 53 children aged 13.8–16.9 years affected with juvenile

Table 16.12. Clinical diagnostic criteria in children affected with multiple chemical sensitivities

Nature of incitants provoking a response
Responses to offending environmental toxicants occur at levels of exposure below the 2.5th percentile for response in the general population
Child responds to multiple substances that are unrelated chemically (namely, causes lack of specificity). The symptoms are not confined to one or several environments (namely, only sick buildings)
Biological plausibility, identifiable exposure
Symptoms are reproducible with exposure with reasonable consistency, symptoms resolve after removal of incitant exposures
An identifiable exposure preceded the onset of the problem
Topology of responses
Adverse responses affect more than one bodily system
Primary complaints include neuropsychological symptoms
The child exhibits altered sensitivity to odor
Persisting nature of perceived changes
The disorder is chronic
Differential diagnosis
No single, accepted test of physiological function correlates with the symptoms
Subjective responses and ameliorating actions of affected children
The caretakers and/or child perceive the child's response as unpleasant or disturbing
The family has sought professional advice
The individual's caretakers believe that he or she has a disorder
The family takes action to avoid exposures to symptom-inducing chemicals

Data from [132, 212].

arthritis has ascertained that the patients consulted chiropractors in 24% of cases and acupuncturists in 19% of cases. From the therapeutic point of view, 43% followed diets, 68% used copper bands or bracelets, 38% patent medicines, and 24% skin creams [179]. A child treated by unconventional therapists following a severely restricted diet experienced a remarkable growth retardation and a pattern of hypothyroidism, remitted after an appropriate dietary intervention [102]. The parents of three girls aged 17–24 months and affected with RA perhaps persuaded of the benefits of alternative medicine made this choice because they wanted to try a different approach hoping it might yield better results [179]. These are the results: one girl returned to conventional medical care with hypertensive encephalopathy at 16 years of age, a 4-year-old girl was in the 50th percentile of height for and a 6.5-year-old girl was in had the 50th percentile of weight for and a prominent cushingoid aspect. The clinical manifestations improved, but no measurable growth occurred. In a girl admitted to the hospital because of lethargy and life-threatening starvation, hyperalimentation was successful. In a third girl aged ≈7 years, the RA had deteriorated [179]. "The devices reported above are used to diagnose nonexistent health problems, select inappropriate treatment, and defraud insurance companies. The practitioners who use them are either delusional, dishonest, or both. These devices should be confiscated and the practitioners who use them should be prosecuted" (Barrett St. In: www.quackwatch.org/) which recall the words in defense of breastfeeding [29].

Tables 16.12 and 16.13 include considerations about diagnostic criteria in children affected with MCS [212].

Pediatricians, Migraine, and Other Allergies

Allergic headache is an objective reality that can no longer be ignored, because it is not surprising that growing numbers of children improve following appropriate oligoantigenic diets. Children with migraines can experience comparable or more severe impairment in school and emotional functioning as children with other serious chronic illness conditions [148]. Pediatricians therefore care for these children and when necessary they refer to specific divisions of the local university or hospital or to other specialized facilities in the city where children live. Another facet of the pediatrician's importance is with ADHD children, above all in view of the possible correlations between the ingestion of food additives and behavioral changes. In teenagers, guid-

Table 16.13. Pediatric considerations in multiple chemical sensitivities

Diagnosis
There is no agreement upon case definition of MCSs in children and little that is evidence-based in the diagnosis or treatment of children. There is even less known about children thus diagnosed than adults.
It has been proposed that children's learning disorders or ADHD might be explained by MCSs, but without any convincing scientific evidence.
Adults with MCSs have often been diagnosed with psychiatric conditions, but it is unknown whether affected children or their parents or caretakers have specific psychiatric diagnoses. However, it seems evident that they are living with considerable neurosis and pain, but there is no need for a fatalistic acceptance of the disorder.
Management
Much controversy stems from the false view that fashionable therapies may imply that the illness is all in the mind, so an eventual failure is the patient's or family's own fault. This moves parents to frequently look for a doctor and seek alternative practitioners.
It may be difficult for health care providers to communicate with parents because of their frustrations and dissatisfaction with the response of physicians to their child's MCSs.
Therapies recommended by clinical ecologists may engender additional risks if used for children. For example, severely restricted diets can interfere with the basic nutritional requirements needed for normal growth and development.
Other therapies currently in vogue, including desensitization, herbs, or vitamins, may be especially harmful to children, whose developing body systems (that is central nervous and immune systems) are especially vulnerable to injury.
The limited capacity of liver and kidneys of infants and young children to detoxify and eliminate certain herbs, hormones, minerals and dietary supplements used by clinical ecologists and other practitioners as remedies in adults may consequently provoke a higher risk of toxic reactions.
The limited detoxicating capacity may render infants and young children particularly vulnerable to fluid and electrolyte imbalances provoked by laxatives or purging, also differently suggested and employed.
Public health and psychosocial issues
The social isolation that accompanies chemical avoidance strategies is particularly disabling for children. Such isolation impairs a child's ability to make friends and otherwise interferes with normal psychosocial development. School avoidance may impair their intellectual development.
Children are entitled to the protections afforded by federal laws under the Americans with Disabilities Act. This federal law has been applied to include patients with MCSs. For children, the law could be interpreted to include prior notification of parents if pesticide spraying or other chemical applications were planned at a school or day care center. The law might mandate other accommodation of the special needs of a child with MCSs.
Children depend on adults to make responsible choices concerning their health that are in their best interests. The diagnosis of MCSs can lead to lifestyle changes potentially adding stress to family relationships, especially if parents disagree between themselves or with health care professionals on the diagnosis and management choices open to them.

Data from [132, 212].

ance should involve education about ADHD and advice on parenting principles and coping with academic problems [154]. The CFS is often a prerogative of adolescents more than of children and requires careful assessment of behavioral changes, but primarily of a psychological and existential nature. It is irrelevant to overemphasize the young victims of Münchausen syndrome by proxy, battered-child syndrome according to Rezza, or the nonmedical cures advocated by clinical ecology, since it is an ill-defined illness with no adequately validated diagnostic laboratory tests; Lessof's comment [109] applies here. It is noteworthy that >25% of parents preferred unconventional remedies to patent medicine, although 59% noted an improvement in their child's health after using alternative medicine and about 50% said they were very satisfied with it [179].

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Other Allergic Otorhinolaryngological Diseases

Von Waldeyer's Ring

The palatine and lingual tonsils, adenoids or pharyngeal tonsils and lymphoid pharyngeal tissue constitute the von Waldeyer ring [213] (Fig. 15.1) or NALT (nasal-associated lymphoid tissue) [51]. The tubal tonsils and lateral pharyngeal bands are less prominent components [85].

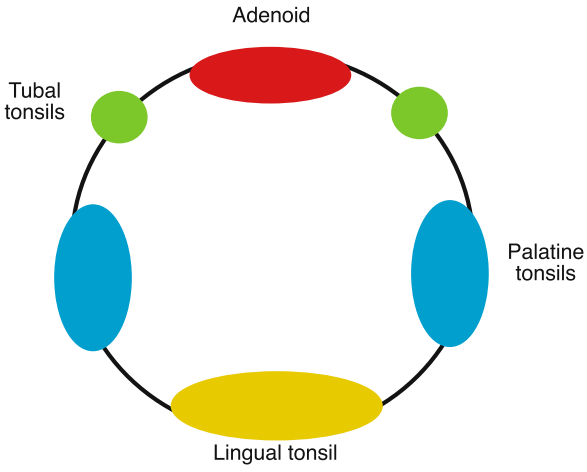

Fig. 15.1. von Waldeyer's ring. According to the original von Waldeyer report (May 5, 1884), the lymphoid pharyngeal ring was extended to the conjunctiva, cecum, ileum and uterus, with a direct and continuous pharynx–intestine passage and vice-versa [213]

Nasal-Associated Lymphoid Tissue

The NALT is located at the gateway of the respiratory (BALT, bronchus-associated lymphoid tissue) and alimentary (GALT, gut-associated lymphoid tissue) tract; thus an integral part of MALT (mucosa-associated lymphoid tissue) is similar to both BALT and GALT, because of its earlier development and location in strategic position and its antigen uptake capacity in inhaled air may be important for initiation of immunity in the upper respiratory tract [85]. Ontogenetically, there are marked similarities to Peyer's patches (PP), although these develop shortly before birth, earlier than NALT, which in rats is only present at birth, but not in human neonates [104]. This difference may reflect a more central role for PP in MALT, or may result from the gut's earlier exposure to antigens. These characteristics confirm the vital role played by these structures in defending the organs they protect [113]. Greater similarities can be found in the position occupied by macrophages in the NALT, at the border of connective tissue, which is comparable to the base of PP interfollicular area, by dendritic cells (DCs), situated in the corresponding B and T areas, and by M cells, which were present in the follicle-associated epithelium (FAE) that covers the lymphoid nodules placed above PP and on top of NALT follicular dome [194]. Histological analyses showed that after local reovirus infection, germinal centers (GCs) developed in NALT with the appearance of IgA^+ cells, whereas no GCs or isotype-switched cells were found in BALT. Thus, BALT lacks the typical lymphoid organization found in NALT and PP [239, 240]. Functionally, PPs are probably essential for the production of secretory immunoglobulins (sIg), while the NALT appear to be more involved in cellular responses. Within the respiratory apparatus, NALT is without question more active than BALT, a similarity that can be found in the T cell/B cell correlation, which in the NALT is more similar to that of BALT, while their respective functions diversify in terms of activation and number of B lymphocytes expressing membrane IgA (mIgA), rare in the NALT. In this respect BALT resembles PP more closely than does NALT [113].

Production of reovirus-specific IgA was observed in NALT and PP, but only small amounts of specific IgA were secreted by BALT [239]. The issue of antigen uptake to the NALT was addressed in Chap. 12: in particular, IgA-producing cells have been highlighted in the posterior cervical lymph nodes, indicating that these lymph nodes are involved in sIgA local synthesis, probably limited to the nasal cavity. IgA precursor cells, on the contrary, are supplied both to BALT and GALT. Instead, NALT is an important station in lymphocyte recirculation (Table 15.1) [17]. Lymphocytes migrate from this site, to return later, as well as to cervical and mesenteric lymph nodes; similar cells coming from the intestine settle in the NALT, in a definitely higher number than lymphocytes coming from PPs that return there, confirming the importance of NALT in both local and systemic immune reactions (Fig. 15.2) [113]. Signaling by lymphotoxin (LT) or TNF-α through the TNFRI

Table 15.1. Lymphocyte subpopulations in the tonsils and bloodstream

	Palatine tonsils		Adenoids		Bloodstream	
	Total cells	(%)	Total cells	(%)	Total cells	(%)
IgD	1.2±0.18	8	1.2±0.18	4	2.5±0.33	22
IgM	19.9±1.4	43	22.9±3.9	46	9.7±0.82	88
IgA	7.4±0.53	20	5.8±1.2	14	0.3±0.10	5
IgG	31.7±2.1	74	37.7±2.5	74	2.1±0.42	18
CD19	52	14				
CD3	42±2		41±5		66±8	
CD4	32±10		30±7		35±7	
CD8	8±3		7±2		29±6	

Data from [17].

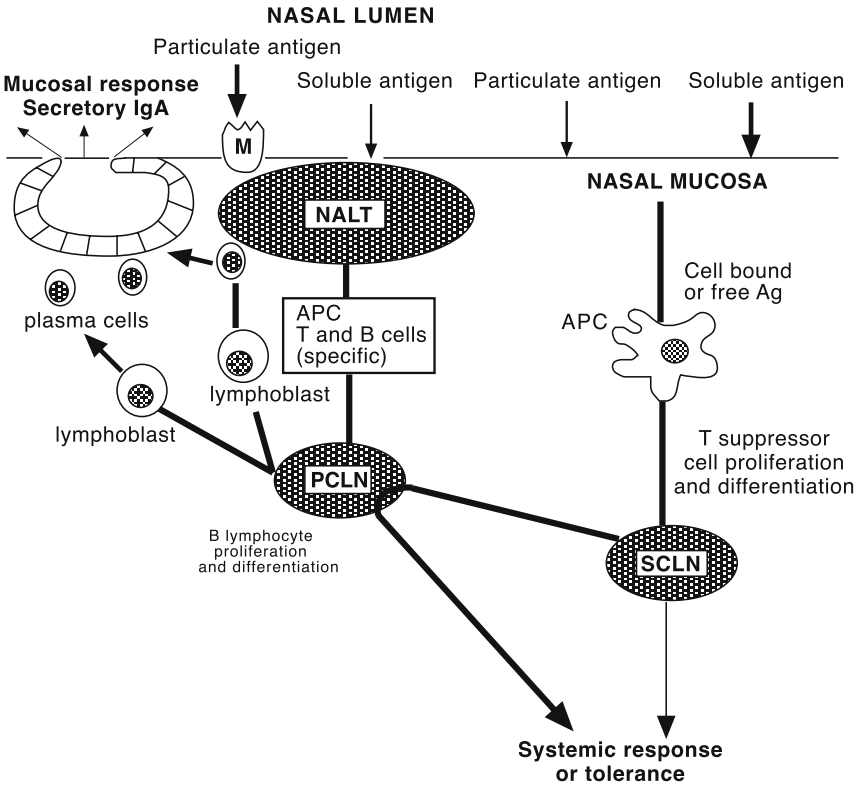

Fig. 15.2. Hypothetical scheme of the possible pathways eliciting a local mucosal response, or systemic tolerance, by NALT and nasal mucosa. *APC* antigen-presenting cell, *M* M cell, *NALT* nasal-associated lymphoid tissue

(p55) and LT heterodimers through the LTR (receptor) is of critical importance in secondary lymphoid organogenesis (NALT and lymph node [LN]). LTR is prominently expressed on LN- and NALT-HEV. Thus, there is a critical role for LT in LN and NALT development, and LTR signaling on HEV can regulate HEV-specific gene expression, via regulation of GlyGAM-1 (glycosylation-dependent cell adhesion molecule 1) [53]. Initial naive lymphocyte trafficking allowing binding to NALT HEV (high endothelial venules) (Fig. 1.3) involves predominantly CD62L and peripheral node addressin rather than $\alpha_4\beta_7$-addressin cell adhesion molecule-1 (MAdCAM-1) interactions, and that MAdCAM-1 and VCAM-1 (vascular cell adhesion molecule-1) (CD106) expressed by NALT follicular DCs (FDCs) may play an important role in lymphocyte recruitment and retention [45].

In conclusion the NALT seems to be the main lymphoid tissue for the respiratory tract mucosa, also because it develops before BALT, is more activated [113] and is chronologically the first provider of B cells for BALT, which has none during the first period of life [27].

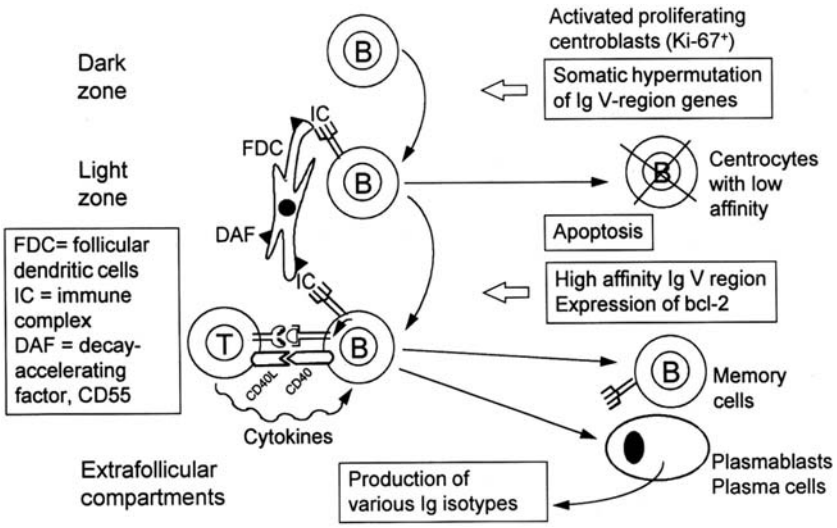

Fig. 15.3. Schematic representation of immune events occurring in the dark and light zones of germinal center (GC) of secondary tonsillar follicle. *DAF* decay accelerating factor = CD55, *FDC* follicular dendritic cells, *IC* immune complexes. (Modified from [113])

Tonsil Immunology

Although tonsils are not actually the seat of allergic diseases, they do play an important role in antigen recognition and in synthesis of IgE and other antibodies, in addition to often entering the differential diagnosis of other otorhinolaryngological (ORL) problems. A study in 88 normal human embryos has shown that palatine tonsils play a prominent role in the development of the immune system, being the first organ in the lymph system that analyses and reacts to antigenic stimulation [139]. Their ontogenesis begins *during the 14th week of gestation,* when both B and T lymphocytes invade the mesenchyme below the tonsillar epithelium. During the 16th week primary follicles are found earlier than in other secondary lymphoid organs [25]. By the 20th week, the areas surrounding the primitive crypt are richly vascularized with HEV and, similarly in the thymic medullary region, T cells are in contact with HLA-DR, IDC (interdigitating dendritic cells) and Langerhans cells (LCs) present in the crypts by the 15th week [155]. Tonsils and adenoids apparently play an important immune-inductive role as components of MALT; however, these structures also show similarities with lymph nodes and may in addition participate as effector organs of local systemic-type as well as mucosal-type of adaptive immunity [26]. *As tonsils are the first site to encounter inhaled and ingested microorganisms,* they are the first line of defense against exogenous aggressors [85]. Tonsils are active especially between 4 and 7 years of life, when they achieve their largest dimensions, then gradually atrophy in most cases, except for the lingual tonsil, which appears to remain functional until the fourth decade [155].

Antibodies and Cellular Immunity

About 2 weeks after birth, activated B lymphocytes are found in the GCs of lymphoid follicles, in T cell-dependent B-cell activation induced by exogenous antigens [26], where they differentiate and proliferate, as well as in the mantle zone and reticular epithelium [25]. T cells occupy all compartments such as the intraepithelial lymphocytes (IELs), the interfollicular regions (both provided with HEV) and the follicles [155], but CD4 are also found in GCs [231]. Two B-cell subpopulations of human tonsils were found in the follicular mantle (expressing surface CD5) and marginal zone that displayed marked differences in phenotype, in response capacity to T cell-independent antigens, and in presentation of antigens to T cells [52]. Tonsillar B cells are found in a higher number compared to those of peripheral blood, unlike T lymphocytes (CD3), while CD8 are rare (Table 15.1). Signaling lymphocytic activation molecule (SLAM, CD150) identified in vivo as a monocyte activation marker was absent in blood, but was detected in tonsils, where it could be localized to T-cell areas and GCs [63]. Tr1 and 2 (T-regulatory 1 and 2) were expressed in the MALT on human tonsillar crypt epithelium. Although Tr1- and Tr2-positive cells were in proximity to T lymphocytes in vivo, lymphocytes themselves were devoid of Tr1 and Tr2 expression [141]. Animal studies show that T cells adhere better than B cells to NALT HEV, thus more T cells enter NALT: as seen in Table 15.1, tonsillar plasma cells produce all classes of Igs (immunoglobulins) [17]. IL_{15} induces Ig proliferation and synthesis by human B cells stimulated by T-cell CD40L (Table 1.5), to prime cognate interaction in tonsillar GCs: following centrocyte activation, another crucial event is bcl-2 expression to prevent apoptosis (Fig. 15.3) [25]. IgA secretion by palatine tonsils is significantly higher than in lymph nodes, suggesting that tonsils might play a relevant role in IgA synthesis [228], thereby assuming the MALT characteristics (Fig. 15.4).

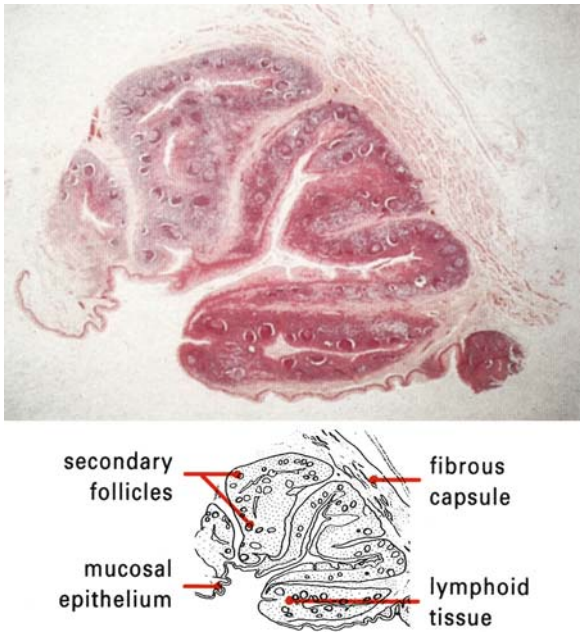

Fig. 15.4. Low-power view of human tonsil showing the MALT with several secondary follicles containing germinal centers

B lymphocytes with the J chain can differentiate into IgM or IgG plasma cells which in turn secrete Ig J (joining) [13], with differences between healthy and sick tonsils, but the isotype switching in tonsillar GC causes a high rate of extrafollicular IgA J which is reduced in recurrent tonsillitis [27] (Fig. 15.5). IgD and IgM immunocytes are found in the mantle zone, and continuing clonal differentiation both IgM and IgG antibodies are detected in the GCs [24] (Fig. 15.6) [25]. IgA and IgG are numerically correlated in the tonsils, unlike the serum; however, IgG appear to be more numerous and IgA are prevalently found in extrafollicular areas and in the crypt epithelium [231] (Fig. 15.7). Ninety-five per cent of IgA antibodies belong to IgA_1 subclasses as in peripheral lymph nodes and in the nose, but unlike the colon, IgG subclasses account in the following order

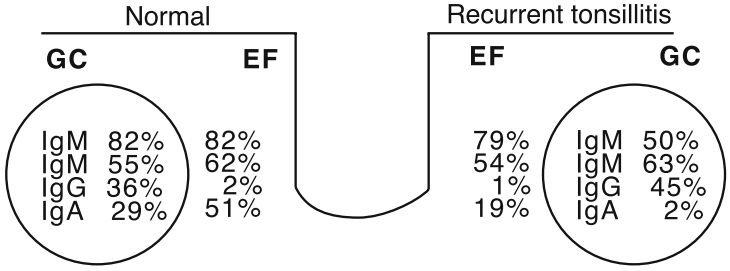

Fig. 15.5. Expression of J chain by tonsillar cells producing immunoglobulins in germinal centers (GC) and extrafollicular areas (EF) (mean values) in normal tonsils and in recurrent tonsillitis. (Modified from [27])

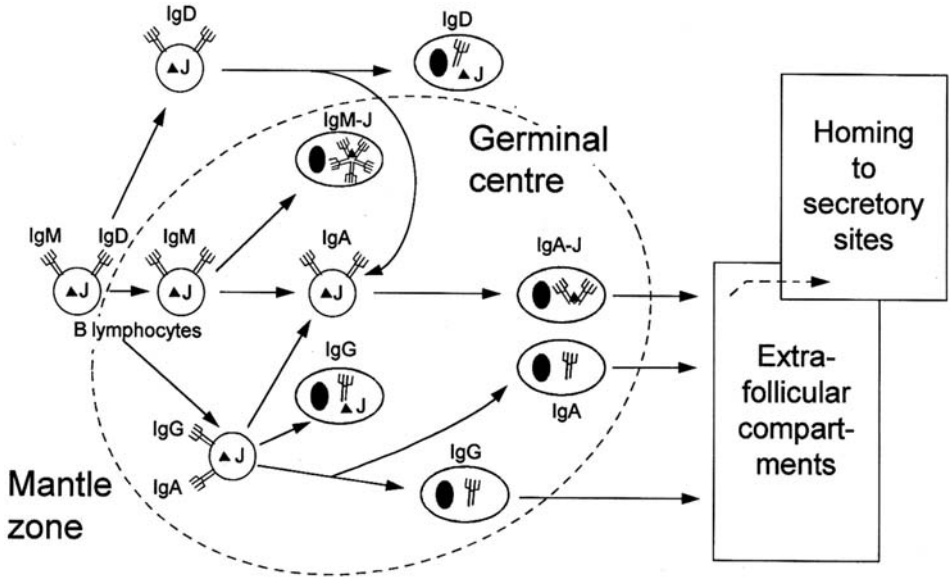

Fig. 15.6. Schematic depiction of putative B-lymphocyte developmental stages in tonsillar germinal centers (*dashed line*). The pathways to terminal plasma cell differentiation may include cytoplasmic J-chain coexpression. The J-chain-expressing plasmablasts may to some extent terminate their differentiation in extrafollicular compartments, but may also have a potential for homing to secretory sites (*dashed arrow*)

Table 15.2. Serum IgA, sIgA, IgM and IgG antibody levels (mg/dl) before and 1 and 4 months after adenotonsillectomy

Immunoglobulins	Before surgery	After surgery		*p*
		1 month	4 months	
IgA	118±22	110±23	92±33	0.02
IgM	116±26	107±21	101±28	0.01
IgG	1146±172	1036±165	902±28	0.03–0.02*
sIgA	12.07±5.89	7.9±4.59		0.01

*p=0.03 after 1 month, p=0.02 after 4 months.
Data from [35]. *sIgA* secretory IgA antibody.

for 61%, 22%, 16% and 1% [24]. The adenoids often show patches of epithelium with SC expression and have MALT characteristics [24]. The adenoids appear to be functionally comparable to NALT in rodents [26]. It is therefore understandable how upper respiratory tract chronic inflammatory processes are associated with tonsil hypertrophy, resulting in an increased antibody synthesis [113], with qualitative and quantitative differences depending on the ongoing process [111]. The abnormal reduction of lymphocyte functions, associated with structural changes correlated with hypertrophy, may establish chronicity [111]. Surgical tonsil and/or adenoid removal in children leads to a significant reduction in Ig levels and especially in sIgA levels 1–4 months after surgery (Table 15.2) [35], which becomes particularly obvious when correctly interpreting the NALT role in sIgA synthesis [32]. A Medline search (1980–2004) shows that only the studies that have measured sIgA agree with our data [98, 99, 105]. Not only salivary sIgA concentrations were decreased to the control levels at the 30th postoperative day, especially if aged 5–7 and 8–10 years, but in the tonsillectomy group the number of IgA immunocytes of the extrafollicular area and the reticular part of crypt epithelium decreased below the control group levels [99]. Other authors, who visited at variable intervals, suggest a correlation between a decline in Ig levels and a reduction in antigen stimulation [67]. A study in tonsillectomized children found high salivary Ig levels after 3–4 years [115], thus demonstrating that these antibodies may need a long time to return to normal levels. IgA total levels in parotid and whole saliva, as well as serum IgA levels, were comparable in tonsillectomized and control children 6–14 months after surgery [41]. The parotid gland is not a part of the Waldeyer ring, however [85]. Several studies measuring IgA antibodies (not sIgA) concluded that the changes do not cause significant immune deficiency [92, 109, 154], that 6 months after operation a normalization of examined immunological parameters was observed [237]. In recurring tonsillitis the J-chain production by tonsils is greatly reduced (Fig. 15.5).

In conclusion, the examinations of the parameters of the immunological system (humoral and cellular) are necessary before planned adenotonsillectomy [35, 237]. Longitudinal studies with sufficiently large numbers of children also fully responding to ethical principles appear to be necessary to evaluate the immunological effects of an adenoidectomy and/or tonsillectomy [26]. We stress that 45% of children with adenoidal hypertrophy improved after 2 weeks of steroidal therapy. Among these children, an additional 24-week treatment at a lower steroid dosage was associated with a significant 52- and 100-week clinical improvement and with reduction in adenotonsillectomy [44], thus making surgery unnecessary.

IgE plasma cells have been identified in tonsils, in adenoids and in regional lymph nodes only in atopic patients [70], while instead IgE synthesis in the nasal mucosa has not been proved, even though APCs, CD4 and CD8 T cells as well as IgA plasma cells are present [21]. It is therefore thought that in atopic patients, both uptake and processing of inhaled allergens takes place in the NALT, and that IgE synthesis takes place at the first signal in the tonsils, in parallel or alternatively to the PP [25].

Antigen Recognition

Tonsils are immunocompetent organs without lymphatic afferent vases, supporting the hypothesis that they have the duty of processing the antigens that appear on their surface [25]. The lymphoid structures are closely linked to the superficial epithelium that forms the crypts, invaginations that extend inside the tonsils, and that can have the function of taking up minute particles of foreign substances that then come into contact with posterior cervical lymph nodes [27]. This procedure is facilitated by numerous crypts, 10–20 in the human palatine tonsil, which considerably increases the surface exposure for antigen uptake. Moreover, HLA-DR-positive cells are deep in the crypts that also appear to be essential in antigen uptake [13]. Antigens enter the crypt, and taken up by reticular epithelium and transported by M cells inside the GC are later processed by APCs [17]. IDCs in the extrafollicular zone, DCs in the GCs (DCG) [13, 19] and LCs [155], found in significantly higher numbers in the adenoids of allergic children

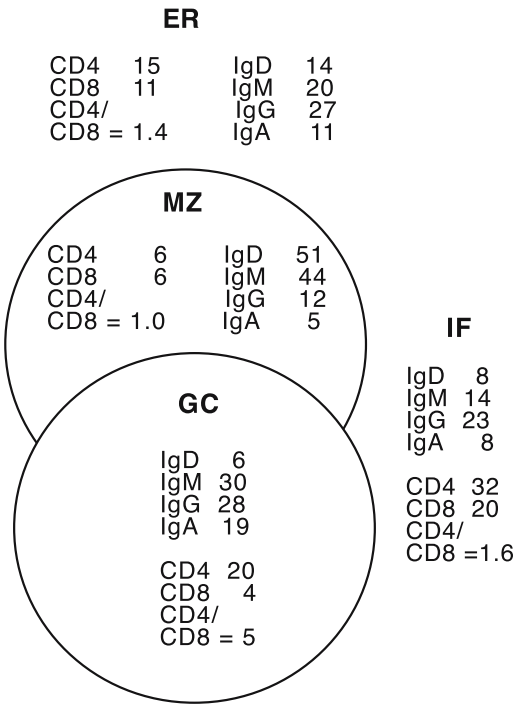

Fig. 15.7. Analysis of immunocompetent cells in a normal tonsil (see text). *ER* endothelial reticulum, *MS* mantel zone, *GC* germinal center. (From [24])

than in controls, present antigens to extrafollicular T cells [113] (Fig. 15.7 [24] and Fig. 15.8 [27]). Activated T cells (IL_2) are CD45RA and CD45RO, some expressing CD25 as a mark of recent activation and of tonsillar capacity of providing primary and secondary T reactions [24, 27] (Fig. 15.9a, b) [27] with B cells, with a high degree of final differentiation (Fig. 15.9c, d) [27]. Several adhesion molecules facilitate T-cell infiltration, first the couples CD49d/CD29-CD103 and CD34-CD62L, and CD54, thus stressing the role of integrins [155].

A facilitated route distinguishes particulate and soluble antigens: the latter may cross the nasal epithelial cells, encounter macrophages, with HLA presenting antigens and T cells, are then taken up by cells different from lymphocytes, or as free antigens seed the superficial lymph nodes, inducing more often a systemic immune reaction, or establishing tolerance. They drain into the posterior lymph nodes. Particulate antigens are cleared rapidly by the mucociliary sentinel, but because of repeated exposures, or if the ciliary apparatus is damaged (for example by the effects of passive smoking, environmental pollution or viral infection), they are taken up by M cells, thus reaching the NALT [113]. M cells in the NALT transport the Gram-positive pathogen across the epithelial layers in a manner similar to those in PPs. However, *Ulex europaeus-1,* an M cell-specific lectin, identified cells harboring streptococci at the epithelial surface of NALT and blocked bacterial colonization of this tissue [152]. In animals, an

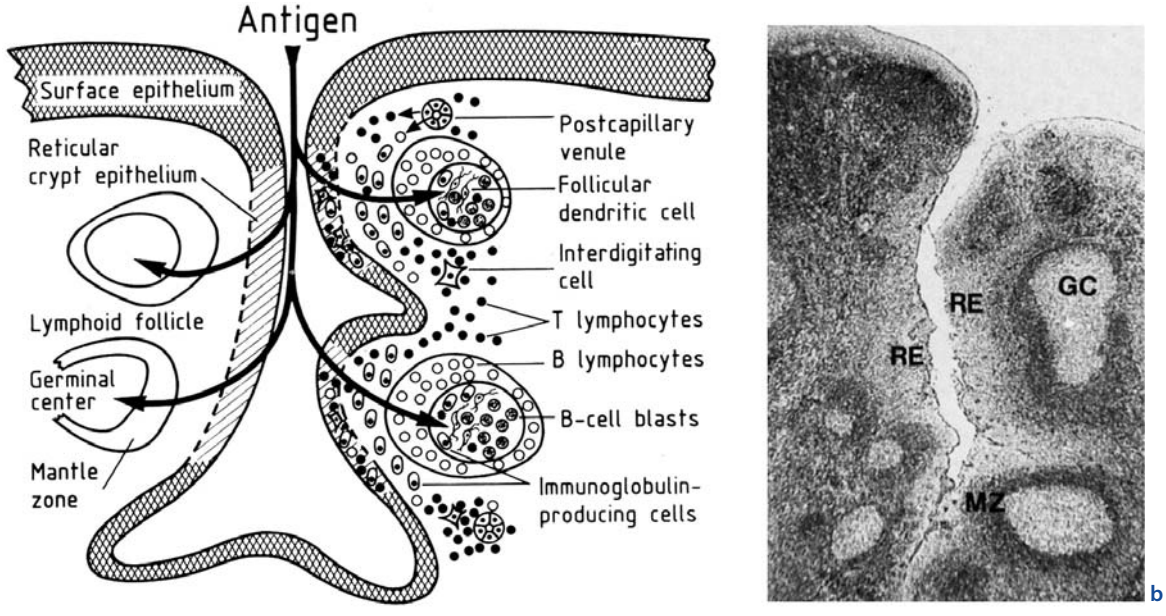

Fig. 15.8 a,b. Schematic delineation of various tissues and cells important for the immunological functions of human palatine tonsils (crypt in the center). **a** The *dark arrows* indicate the principal route of antigen uptake. The *small arrows* show the migration of B and T lymphocytes into extrafollicular region from postcapillary venules. **b** Section of human palatine tonsil including a crypt partly lined by reticular epithelium (*RE*) and secondary lymphoid follicles containing germinal centers (*GC*) and mantle zone (*MZ*). (From [27])

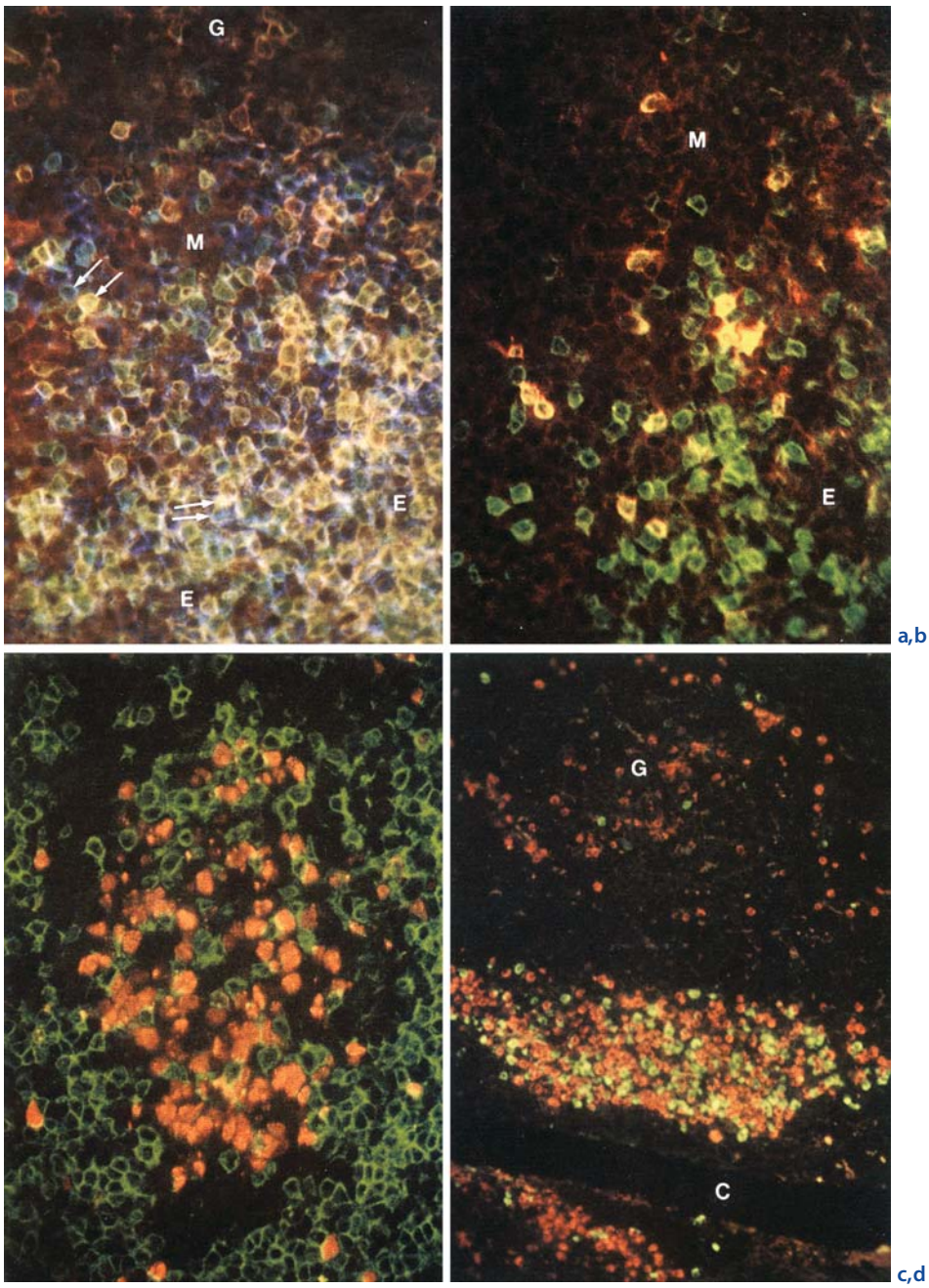

Fig. 15.9 a–d. Immune cells in the tonsils. **a** CD3 (*green*), CD45RA (*dark blue*) and CD45RO (*red*) in section of tonsil including GC (*G*), mantle zone (*M*) and extrafollicular area (*E*); many B cells are in M and a few memory T cells ($CD3^+$ $CD45RO^+$) (*yellow*) in G. Naive and memory T cells ($CD3^+$ $CD45RO^+$) (*light blue*) are intermingled in M and E (one of each phenotype shown by *arrows* in both compartments) (magnification ×280). **b** T Cells (CD3, *green*) and CD25 (IL_2-R, *red*) in section of tonsil including mantle zone (*M*) and extrafollicular area (*E*); some T cells expressing CD25 (*yellow*) are in both compartments; many follicular B cells are weakly CD25-positive (*light red*) (magnification ×280); **c** T cells (*green*, greatly proliferating cells (non-T cells) (*red*) in tonsillar GC, presumably centrocytes, centroblasts derived from activated B cells (*red*). **d** IgA (*green*) and IgG (*red*) antibodies in section of tonsil; many cells with cytoplasmic IgG and some with IgA in a large GC (*G*); dense accumulation of plasma cells are adjacent to crypt (*C*) (magnification ×111)

alternative type of presentation was proven, based on circulating immune complexes (CICs) present in DCG processes [205]. CICs meet with GC antigen-specific B cells that present the processed antigento T cells : hence specific DCGs could act as APCs for B cells [205]. Furthermore, antigen presentation can stimulate memory B lymphocytes with IgDs and IgMs (surface) to switch into very mature clones with IgA and IgG antibodies [17] (Fig. 15.9 d). To be able to present antigen directly to T cells, memory B cells must express CD80/CD86, which bind T-cell CD25. A similar expression is needed for IgD and extrafollicular IgM antibodies to work as APCs. Naive B cells cannot present antigen to T cells unless they become activated by triggering CD40, but only at lower levels [119].

Tonsils as a Source of Immunocompetent Cells

The existence of a protective local immune system, almost independent of systemic immunity, was proposed in 1919 [18]. In this perspective the palatine tonsil can act as a distribution center for B_{IgA} J^+ cells for the nasal mucosa, middle ear (ME), parotid, tear, and

mammary glands [23, 24]. Studying a particular strain of *Streptococcus mutants,* a bacterium linked to dental caries, virtually nonexistent in the nasal or ME mucosa, there was confirmation of this important acquisition: B cells can mount specific antibody levels against this organism at these sites [15]. B cells arising from the tonsils are therefore capable of migrating to an adjacent site in the upper airways such as the ME. Because B J^+ cells originate in *the tonsils,* they *are considered the upper respiratory tract PPs* [24]. The considerable development of tonsillar IgA emphasizes the palatine tonsil capacity to work as a potential source of sIgA, at the same level as GALT and BALT. By stimulating in vitro tonsillar lymphocytes with β-lactoglobulin, IgA of tonsillar origin are produced, thus proving that they belong to MALT [147]. Therefore, if IgA are provided with such a broad specificity, it follows that the NALT structure, in addition to playing an important role in immune exclusion, also appears to be a source of dimeric IgA J^+ for the upper airway mucosa and the entire organism. Finding that nasal and bronchial mucosa, along with salivary and lacrimal glands, show an IgA_1 and IgD immunocyte distribution similar to that of tonsils supports the view that such secretory effector sites are seeded chiefly by B-cell blasts generated in tonsillar CD4 and CD8 T cells [15].

NALT is thus involved in regionalized B-cell dissemination rather being solely part of a common MALT structure [25]. The NALT strategic position sanctions the important role in the defense from aeroallergens: if damaged by tonsillar disease the way is paved for pathological evolution that results in AR (allergic rhinitis) and/or asthma [27]. NALT might therefore appear as the main respiratory tract mucosal lymphoid tissue [113], its activated appearance, its function as a T-cell organ, the lymphocyte-HEV binding sites in human tonsillar tissue and its strategic position at the airway entry, which emphasizes how adenoidectomy-tonsillectomy lacks a solid scientific basis, especially given the continuous acquisition of tonsillar immunological functions [32, 155] and also because surgery is not always preceded by immunological investigations [32]. It is worth remembering that CD31 and CD54 HEV levels have increased following repeated episodes of tonsillitis, with possible autoimmune complications, and that the tonsils can represent an entrance and a site for HIV replication [155]. These observations provide indirect evidence that *both palatine tonsils and adenoids are part of an integrated MALT and represent NALT in humans* [15]. Consequently, although tonsillectomy is either ineffective or of limited efficacy, the risks of hemorrhage and additional hospitalization outweigh any potential benefits unless a distinct indication for tonsillectomy exists [170]. A considerable immunological activity persists even in diseased tonsils and adenoids of children, therefore a conservative attitude toward adenotonsillectomy appears immunologically desirable, particularly in the young age group [26].

Otitis Media with Effusion

Otitis media is anatomically and immunologically unique, because the eustachian tube (ET) under normal conditions protects the tympanic cavity, the antrum and the mastoid from bacterial invasions. These sections are aseptic, so under normal conditions only a few immunocompetent cells are found in the ME. Antigen encounter stimulates the local and systemic immune system development, parallel to age-related growth, thereby contributing to the protection of tubotympanic structures from pathogenic bacteria [22]. Otitis media with effusion (OME) is a major pediatric health care issue, and is called the *silent disease.* The role played by allergies dates back many years: along with studies confirming the close links between allergies in 80% of cases, there are controversial results, even though there are few controlled clinical studies [207]. Long-standing OME might result in the maldevelopment of communication skills, frequently causing various degrees of hearing loss [75], and is considered the *most frequent cause of deafness in children* [110], although this has recently been questioned [150].

Definition

OME, a multifactorial illness, is characterized by the presence of a ME effusion (MEE) without clinical symptoms or signs of inflammation, frequently 3–16 weeks after an initial episode of *acute* otitis media (AOM) [9], with remission of symptoms on average within 3 months, with a 95% percentile at 12 [235]. Therefore a large number of spontaneous recoveries exist, which do not protect from a relapse in 50% of cases [235]. It is labeled *chronic* when the fluid persists for >3 months or when OME recurs ≥6 times over a 12-month interval [65]. It is an important event because of a frequent change into chronic or recurrent processes, although chronic forms are not always a consequence of acute forms [186].

Epidemiology

The first episode occurs at a mean age of 4.2 [146] or 6.3 months [62], 97% with at least one episode by the 1st year and 99% by the 2nd year [146], with a mean of between 1.3 and 1.9 episodes, respectively, and a mean duration of each episode of 1.9 months [62]. The proportions developing one or more episodes of OME between age 61 days and ages 6, 12, and 24 months were 47.8%, 78.9%, and 91.1%, respectively. The mean cumulative proportion of days with OME was 20.4% in the 1st and 16.6% in the 2nd year of life [157]. The OME that persists after onset of AOM is more frequent (20% at 2 months) than the chronic OME that concerns 4.4%–

10% of the pediatric population before the age of 5 years [135]. According to others, OME mainly affects children between the ages of 0 and 3 years with a bimodal curve, with two peaks at the ages of 2 and 5 years [236]: very frequent at 6 months (85%) [146], OME prevalence increases to between 10% and 13% during the 2nd 2-year period [164] and to between 15% and 20% in the third 2-year period with a 2nd peak around the age of 5 years, to progressively decrease up to 12–14 years [65]. In Chinese children, the prevalence rates of OME in different age groups were as follows: 11.3% for 3-year-olds, 12.4% for 4-year-olds, 11.8% for 5-year-olds, and 6.1% for 6-year-olds [40].

Anatomy

The ME includes the ET divided into a cartilaginous section and a bone section, the tympanic cavity, the antrum and the mastoid cells. The nasopharynx, although not anatomically part of ME, is correlated with it from a pathophysiological point of view, because the two mucosal structures communicate via the ET.

Genetic Factors

A number of studies suggest a genetic predisposition [103, 167], based on the significant number of HLA-A2 and HLA-Cw6 antigens seen in children with OME compared to controls [103]. A heritability estimate had a correlation significantly higher in monozygotic sets (0.65–0.77) than in dizygotic sets (0.31–0.39) for each year to age 3 years [37]. In twins suffering from chronic OME, the number of positive SPTs for inhalants is very high compared to healthy twins [64]. SPTs (skin prick tests) to 7 different aeroallergens were positive in 11 atopic children [138]. Family history (FH) and SPTs were positive in 35% of 100 children [12], and in 306 children with OM or OME-positive FH was a risk factor [56].

Table 15.3. Multifactorial pathogenesis of otitis media with effusion (*OME*)

Age
Allergic rhinitis
Anatomical malformations (cleft palate)
Breast-feeding absent or short
Chronic rhinitis
Congenital or acquired ciliary dyskinesia
Down syndrome
Environmental factors
Eustachian tube dysfunction
Family history
Family size
Frequent swimming
Genetic predisposition
Gestational age
Immunological deficiency
Irritants and pollutants
Male gender
Number of siblings
Older siblings and/or with history of recurrent OME
Parent-reported ear infection
Passive smoking (especially maternal)
Public day-care attendance
Race
Recurrent otitis media in the first year of life
Season
Socioeconomic status
Viral upper respiratory infection (winter months)
Winter season

Data from [8, 58, 65, 135, 146, 149, 203, 234].

Predisposing Factors

Predisposing factors are summarized in Table 15.3 [8, 58, 65, 135, 146, 149, 203, 234], to which we add anatomical factors, adenoidal hypertrophy, breast feeding in a supine position [146], and sibling history of ear infection [203]. No significance was found for gender, passive smoking [58, 234], date of birth, FH of OME, parental socioeconomic status, histories of snoring, consultation of a physician [58], and birth weight [234]. Males are more affected than females [20, 75, 138], or males in the early stages and females at 18–24 months of age [146]. The bimodal curve of prevalence in the first part corresponds to the immune system maturation, influenced by day-care center attendance and the resulting infectious episodes, while the second part normally coincides with the 1st year of school and once again with infections [236]. Day care attendance was a significant risk factor for OME in larger cohorts described below [43, 204, 210]. Several *craniofacial malformation* syndromes are associated with OME (Table 15.4) [8]. *Anatomic factors* are less well known: the position of nasopharynx ostium related to the angle formed by ET plays an extremely important role for ensuring satisfactory drainage. In newborns and small babies, the angle of elevation is reduced to 100 (only in adults does it achieve a normal opening of 45° [22], thereby preventing adequate draining.

Table 15.4. Craniofacial malformations and syndromes associated with high incidence of OME

Syndrome	Authors
Achondroplasia	Parrot
Acrocephalosyndactyly	Apert
Craniometaphyseal dysplasia	Pyle
Gonadal dysgenesis	Turner
Mandibulofacial dysostosis	Treacher-Collins
Micrognathia, cleft palate, glossoptosis	Pierre Robin
Mucopolysaccharidoses	Many authors
Oculoauriculovertebral dysplasia	Goldenhar
Orodigitofacial dysostosis	Mohr
Osteopetrosis	Albers-Schönberg
Trisomy 13 syndrome	Patau
Trisomy 21 syndrome	Down

Data from [8].

Immunopathology

Data concerning the presence and activity of a ME mucosa immune mechanism is not entirely definite. Immunohistochemical studies have shown, in 86% of cases, *lymphatic tissue assimilable to NALT*, as results also in other structures that are part of the von Waldeyer ring [213]. Further research has confirmed that ME participates in selective antigen uptake from intraluminal spaces and can trigger immune responses following antigen exposure [173]. In the inflamed mucosa IgG, IgM and IgA are routinely found, but IgA antibody linked to bacterial antigens act as a defense [116]. *sIgA in nasopharyngeal secretions* may inhibit bacterial adherence, also *significantly reducing bacterial colonization,* the first step in the process leading to OME [114, 187]. Connective [222] or mucosal [73] *mast cells* could contribute to defense mechanisms [222] or to inflammation in its acute forms [73]. Animal studies have reported that the number of B_{IgA} cells in the inflamed mucosa is apparently lower than that observed in both GALT and BALT [162], which are sources of IgA precursors for ME mucosa, emphasizing the sharing of a common mucosal immunity (MALT) [116]. Strikingly, a cellular proliferation of MALT was found in the temporal bone of 21 neonates after the age of 1 month [104], which might reflect the activity that produces sIgA against invasion of foreign antigens [80]. Thus it may be a local response to repeated infections [104]. In specimens with purulent OM, the MALT had faint GCs that were complete in specimens with mucous OM [80]. However, while levels of adenoidal sIgA may be normal in children with OME, *SC levels are significantly lower than in healthy subjects* [68]. The role of $IL_{1\beta}$ in the pathogenesis of OME has been clarified: $IL_{1\beta}$ might be associated with endotoxin-induced inflammation in the ME and might play an important role in the induction of OME [221]. *T-lymphocyte trafficking* is reduced compared to that seen in nasal mucosal tissue, suggesting that ME is less often exposed to antigenic stimulation than both airway and alimentary tracts [91]. Otherwise it may be that the afferent limb of systemic immunity from ME operates less effectively and rapidly than that from the dermis [116]. Lymphocytes bind to epitopes released in the inflamed mucosa but not under normal conditions [134]. Concerning ME lymphocyte homing, such cells are not provided with specialized ligands or receptors that can recruit mucosal lymphocytes selectively to ME sites, since lymphocytes of different subsets show no preferential homing at this level [174]. The role played by immune mechanisms in the pathogenesis of OME follows three main routes: IgE-mediated hypersensitivity, CIC, and DTH (delayed-type hypersensitivity) [12, 207]. In particular:

- Local *IgE levels* are raised compared to plasma concentrations [153], indicating local production. The role of *IgE-mediated hypersensitivity* is not universally accepted, although there is a widespread support, varying between 20% and 93% [207]. The results of Todd's meta-analysis [207] which examined seven studies, are characteristic: the two studies confirming the association were based on SPTs and/or RAST, the five negative studies were limited to clinical diagnosis and questionnaires. The symptom frequency matches 29%, compared to 54% of FH^+ and 44.7% of OME [163]. Blood tests revealed a raised serum IgE in 28% and eosinophilia in 40% of 219 children [3]. It has been widely proved that hypersensitivity of both nasal mucosa and nasopharynx is generally able to cause ETD (ET dysfunction) [8, 12, 19, 20]. Furthermore, immediate and late reactions occurring in the ET are correlated with high levels of total and specific IgE (sIgE) in ME effusions (MEEs) [207]. Other frequently debated issues concern relations between OME and *IgE-mediated inflammatory edema* that obstructs the ET, and a possible involvement of the ME mucosa as sensitized tissue, therefore as a sort of shock organ [116]. In human beings, however, there is no convincing evidence that this immune mechanism is operative, although ETD has been demonstrated by a great deal of research on human beings and animals; following NPT (nasal provocation test) with pollens or with histamine in sensitized patients, in the aforementioned meta-analysis the correlation was present in 6/7 studies [207].
- *CIC (type III)* function is not very well documented. Findings in secretions probably represent bacterial or viral IgG complexes, which likely find access via ET, as proven by the anti-CM CIC-IgG, anti-egg and anti-wheat reported in 70% of children with OME and only in 16.6% of controls [12].

Table 15.5. Mean number (+ standard error of the mean) per field of cells in the nasal cytograms of 50 children

Disease	Eosinophils	Basophils	Neutrophils
Normal	0.11 (0.07)	0.15 (0.07)	4.87 (1.93)
OME	10.18 (5.7)*	1.42 (0.42)	6.53 (1.75)
Nonallergic otitis media	1.83 (1.04)	0.13 (0.07)	20.41 (6.78)*

*$p<0.05$ compared with other groups.
Data from [133].

- Studies suggest that *T cells (Th1) can mediate a DTH mechanism (type IV),* but there is not yet sufficient data to confirm this issue [174].

MEEs also contain higher NCF-A, PGD_2 and MBP levels derived from eosinophils, and histamine compared to serum levels. A study done on 50 children aged 2–7 (Table 15.5) [133] has highlighted a significant proportion of eosinophils in children with OME, and levels of histamine matching about 0.2 pg/mg of protein in normal and nonatopic patients and at about 5.5 (SEM about 1) in children with OME [133]. On a cellular level, neutrophils are an integral part of the inflammatory process in OME to a disproportionate degree among atopic children [89], although others have found neutrophils only in nonatopic OME sufferers [133, 138, 192]. However, the high number of neutrophils in the MEEs of these patients might represent the persistence of an infectious process during ETD [138, 192].

A possible *role played by immunodeficiency* in the pathogenesis of OME has been proposed, in particular an IgG_2 deficiency to *Pneumococcus* [46], *Streptococcus epidermidis* and other bacteria capsular antigens [12]. Furthermore, the delayed development of an antibody reaction to bacterial antigens, especially IgG reduced levels, might form the basis of OME susceptibility [159]. In this case the pathogenesis could be assigned also to a humoral immunity depression, very probably genetically controlled. Further controlled studies are needed to better consolidate this interesting hypothesis of this particular susceptibility of certain children to developing OME.

Allergen-Induced Eustachian Tube Dysfunction

The ET measures 31–38 mm, is shorter and narrower in young children and covered by a mucosal membrane, establishes communication between the tympanic cavity and the nasopharynx [22]. It consists in a bony portion lying within the temporal bone petrous portion and a fibrocartilaginous portion that communicates with the rhinopharynx via the ostium pharynx [22]. In physiological conditions its function consists in:

- Protection from nasopharyngeal sound pressure and secretions
- Drainage into the nasopharynx of secretions produced within the ME
- ME ventilation to equilibrate air pressure with atmospheric pressure
- Supplying the tympanic cavity with O_2 [22]

Although there are no definite data concerning the allergic pathogenesis of OME, there is nonetheless *a close correlation between upper airway allergy and ETD.* Prospective studies on children with chronic or recurring otitis media and ETO (Eustachian tube obstruction) of the functional type have reported that the simultaneous presence of an upper respiratory tract infection (URTI) provoked serious ETO of a mechanical type, revealed by tympanometric changes [8, 12, 19, 20]. In parallel, NPT with pollens induced OTE after 20 min (immediate reaction) and after 4–8 h (late reaction) in patients with AR and normal auricular parameters: the obstruction caused by this test is dose-dependent, correlated with serum allergen-sIgE levels and lasts longer than the AR accompanying it [65]. The phenomenon appears spontaneously during the pollen season in these patients [12]. Similar results are provoked by a NPT with histamine, but not in healthy patients: in laboratory animals histamine causes both extra- and intraluminal dose-dependent obstruction, to a greater extent in young animals than in adults [65].

It can be assumed that in children with respiratory allergy and OME, the developing pathogenetic events, based in some cases on ETD, may progress up to impairing the functions when upper airway disease is present Anatomophysiological studies have reached the conclusion that in the first period of life the ET function is compromised because of a TVP (tensor veli palatini muscle) insufficiency, or because of anatomical defects such as a different angle, by rendering the organ more sensitive to mechanical compressions and negatively influencing its functions. To understand the consequences, it is known that, unlike what occurs in the respiratory tract, the tube is usually closed, opening only when the muscle contracts. Animal experiments have confirmed this pathogenic mechanism: a tubal resection leads to the development of a condition that is similar to obstruction, with the formation of exudate in the ME after 3–4 weeks [22].

Mucociliary Dysfunction

Cilia on the respiratory tract epithelial surfaces continuously sweep mucus shed by ME (and nose, sinuses, eyes, and lungs) into the nasopharynx. Therefore, *primary ciliary dyskinesia* (mapped to chromosome *19q13.3-qter*) [130] or *secondary,* correlated either with congenital anomalies or acquired bacterial or viral infections (RSV, respiratory syncytial virus), plays a critical role in the development of nasopharyngeal viral replication. It is not clear whether the cilia function nor-

mally in the ME and ET in the chronic phase of OME. However, impaired ciliary function in primary ciliary dyskinesia is frequently associated with the development of OME. Interestingly, endotoxin in concentrations far in excess of those found in the ME with chronic OME had no effect on ciliary activity [126]. Obviously, a normal ciliary apparatus is needed for surface cleansing; a change in this delicate structure favors the establishment of allergens and/or contaminating substances of any type in the mucosa for a long period, thus reducing or blocking mucociliary transport [153] and potentially encouraging the development of infections. The defensive mechanism can be altered by viruses, because of their well-known ciliary tropism [36]. Since ciliary structures are totally correlated to function, any change that occurs during acute nasopharyngeal infections may reflect on impaired mucociliary clearance with dyskinesia and mucostasis. These events often precede the onset and perpetuation of the dysfunction that reverberates on the structures below, in turn leading to ETO and consequently to OME [19].

Eustachian Tube Dysfunction

Acute and chronic otitis media can be caused by ETO, normally of two types: mechanical or functional. The first kind can be intrinsic (caused by allergies and/or infections) or extrinsic (caused by allergies and/or enlarged adenoids), both well supported by an allergic inflammation highlighting the tubal obstruction related to edema. Functional ETO is common in infants and younger children since the cartilaginous tissue supporting ET is less scarce and less stiff than in older children, as is the case in those with palatoschisis [8, 19]. A significant role played by adenoids in OME pathogenesis has been excluded [74]. Another cause of ETD is an abnormal tubal patency, causing the air and any other possible unwanted secretion to flow freely from the nasopharynx into the ME, resulting in reflux otitis media. This excessive flux can also follow a modest positive nasopharyngeal pressure (nose blowing, crying, etc.), due to the elevated stretching capacity of tubal walls. This may also occur because of an active tubal opening (caused by TVP muscle contraction), even when the tube remains functionally closed and even if actively forced open. In parallel, nasal obstruction can cause ETD: during swallowing an initial positive nasopharyngeal air pressure is followed by a negative phase within the closed nasopharyngeal chamber [65]. The probable effect of such pressures on a yielding tube is biphasic: with positive nasopharyngeal pressure the secretions can flow toward the ME. If negative nasopharyngeal pressure dominates, the tube remains closed, because it becomes even more obstructed. This is known as the *Toynbee phenomenon:* by nose blowing, diving, swimming, or ascent in an airplane, or closed nose and mouth swallowing, the tympanic chamber is partially emptied [65]. A vicious circle is therefore established: the ME in fact, normally without secretions, contains air regulated by ET action; if the opening is reduced the air, no longer able to enter the ME, is reabsorbed. The resulting negative pressure, together with the O_2 absorbed by capillaries, causes mucosal swelling, with formation and accumulation of serous secretions. This transudate, containing sterile mucous, cellular debris and neutrophils, accumulates in the ME space in the case of prolonged ETO after rupturing the mucosal basement membrane and distorting nonciliated cells [19]. Infection transforms the transudate into mucous exudate with an increased protein content. With continuing pathological stimulation, the ME lining cells become hyperplastic; the ME mucosa changes to stratified respiratory epithelium and goblet cells, the muciparous cells proliferate, the mucosa invaginates to form submucosal cysts and the proliferation extends to glands and blood vessels with consequent hypersecretion [19]. A chronic state causes the secretion characteristics to change from yellow to grey-blue, the secretion becoming increasingly viscous, to the point of preventing, at advanced stages, small-bone and tympanic membrane movements [75, 128].

Pathogenesis

Role of Atopy

The role of atopy in OME has long been speculated but to date remains unquantified [170]. However, several studies do not examine atopy, or fail to provide a broadly accepted definition, or the diagnosis is based on variable criteria: however, *the prevalence of atopy varies from 23% to 80%* [12, 138, 192, 236] or 89% of AR, 36% of asthma and 24% of atopic dermatitis (AD) [157]. In 139 infants with recurrent otitis media atopy was associated with a relative risk (RR) of 1.9 [199]. In 8/22 (36.4%) children with recurrent OME unresponsive to drug treatment SPTs were positive for inhalant and food allergens vs 2/24 (8.3%) of the control group (p=0.032) [51]. In the MEEs of atopic children, *the percentages of eosinophils and Th2 lymphocytes are significantly higher* (7.7%±1.4% and 20.0%±3.2%, respectively) compared with those in MEEs of nonatopic children (0.9%±0.2% and 5.5%±0.6%, respectively), unlike metachromatic cells, in addition to significantly higher percentages of Th2-like IL_4, IL_5 [192], and eosinophil cationic protein (ECP) and tryptase in atopic, compared with nonatopic patients [89]. The percentage of eosinophils, T lymphocytes, and IL_4 and IL_5 cells was significantly higher in atopic children compared with nonatopic children and greater in the MEE than that seen in the torustubatis or adenoid tissue [138]. *In nonatopic children,* neutrophils and *IFN-γ positive cells were* present in *significantly higher* percentages [138, 192]. Elevation of Th2-driven IL_4, IL_6 and TNF-α in MEEs may contribute to the allergic OME persistence [97], thus evi-

dencing that allergy might play a role in OME pathogenesis [192]. Concentrations of IL_1, IL_6, and CD106 were significantly greater in the MEE and in serous (with E-selectin = CD62E) than in mucoid MEE of study children compared with those of control children [172]. Adenoidal lymphocytes appear to produce significantly less IL_2, IFN-ɣ compared to the patient's peripheral blood lymphocytes, whereas IL_4 and IL_5 appear to be synthesized slightly more in the homologous peripheral blood lymphocytes [14]. Children with recurrent OME have higher $IL_{1\beta}$ levels than the children with persistent OME; thus recurrent OME may be a stage that occurs before chronic OME [232]. These results are *consistent with the late-phase allergic response seen in other areas of the respiratory tract, which is involved in the pathogenesis of asthma, AR, and chronic sinusitis* [192]. The role played by food allergy (FA) is a recent example [140]. In 70 of 86 children (86%) with an average age of 4.6 years suffering from multiple food sensitization, elimination diets improved the clinical OME pattern, evaluated using tympanometry, audiometric and ORL examinations; in blind food challenges there was evidence of an OME relapse in 66 of 70 children (94%) [140]. Pathogenic factors such as nasal congestion and exposure to cigarette smoke increase atopy incidence nearly sixfold [20], and atopic children were reactive to dog dander (27.3%–62.3%), dust mite (50%–63.6%), cat dander (18.2%–25%), and tree mix and ragweed (13%–36.4%), with a 24.4%–30% incidence of atopy [138, 192].

The mechanisms linking atopy to OME are several [20]:

1. ME mucosa as a target for allergic reactions
2. Inflammatory edema with consequent ETO and ventilating dysfunctions
3. Inflammatory nasal and nasopharyngeal obstruction with inadequate secondary tubal functioning, thus affecting clearance
4. Seeding of bacteria-laden allergic nasopharyngeal secretions into the ME cavity

The first point has been confirmed by studies done on laboratory animals. However, allergen convergence to a relatively isolated site appears to be problematic; insufflation via ET seems to be an easily realizable method, but its accomplishment conflicts with concrete difficulties, also because the reaction appears to be limited to the allergen contact area [20]. With regard to the second and third points, untreated ETD of allergic origin can result in OME, as indirectly confirmed by ME under pressure following nasal antigen challenges in two pediatric studies [8, 142]. Persistent ETO in turn worsens with OME: in a study on >700 children suffering from AOM, the persistent effusion was highly prolonged in those with allergies compared to those without [160]. Increased mediator circulation after nasal or gastric allergic reactions has been proven as the cause of ETO and increased permeability of ME in experiments on animals: in theory these effects could alter gas exchange or increase ME vascular transudation, also contributing to OME development or persistence via this mechanism [160]. These results raise the intriguing likelihood that chronic OME and atopy development are closely interrelated, an association that warrants further investigation [157].

Role of Allergic Rhinitis

AR relations with OME are difficult to quantify: in 35 of 100 children suffering from AR, for 8% the target organ was the ME and for 27% it was the ET (Fig. 15.10) [12]. Rhinosinusitis and coincident AR were seen in 19% and 14% of 185 children with OME, respectively [143], and 21% of children aged between 5 and 19 with diagnosed ETD also suffering from AR [180]. The prevalence of nasal allergy in children >3 years with OME ranges from 35% to 50%, a 3- to 4-fold greater expression of AR among children with OME than among the general pediatric population [16]. Symptoms of AR, serum IgE lev-

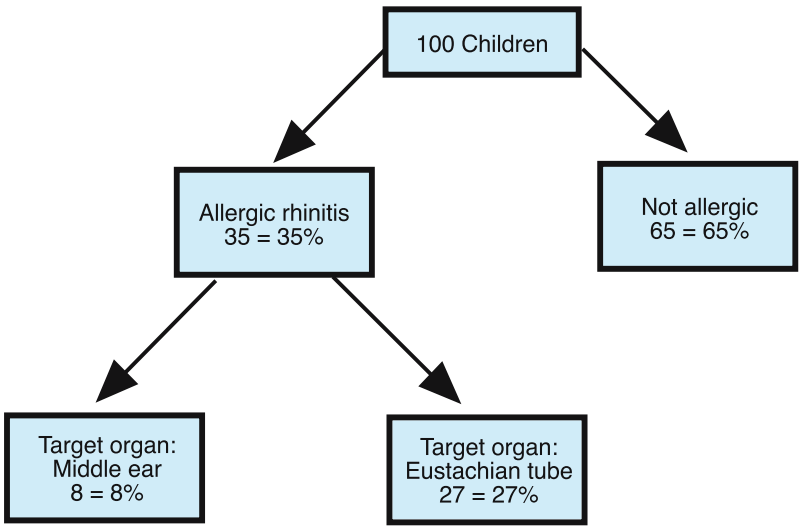

Fig. 15.10. Diagnosis of 100 children with OME: 65% had neither positive family history nor atopic symptoms. (Data from [12])

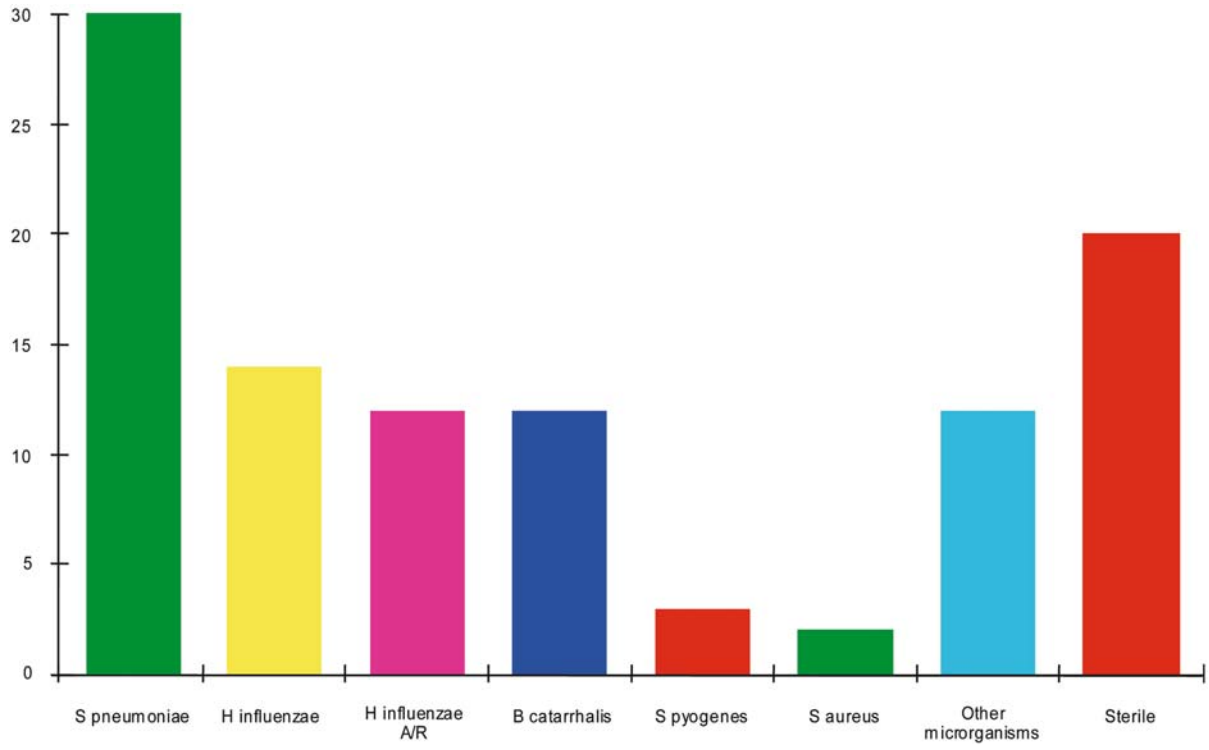

Fig. 15.11. Bacterial isolates from middle ear of children with acute otitis media. (Data from [65])

els, SPTs and nasal smear findings suggested a diagnosis of AR in 5/22 children with OME (23%), and in 1/13 children (4.8%) in the control group [106]. In 209 children, AR was found in 89%, asthma in 36%, and AD in 24%. SPTs were positive to one or more of eight common aeroallergens in 57% of children [3]. In 172 children, with OME symptoms associated with AR occurred significantly more frequently than in the control group (OR=3.34); however, the presence of AR or AD was significantly associated with OME [30]. Even without direct evidence, one can reasonably assume that seeding of nasopharyngeal secretions caused by AR can reach the ME via the Toynbee phenomenon, as for example in a crying baby. Furthermore, AR may cause a certain mechanical obstruction, either extrinsic (peritubular) or intrinsic (intratubular), but TVP muscle movement may counterbalance it. Patients with functional ETD are therefore at risk of developing a mechanical dysfunction leading to OME. If AR reduces nasal opening, a negative pressure derives from the second phase of swallowing, while a closed nose may obstruct the ET opening, and the tube itself, along with the ME negative pressure. This is characterized by ETD symptoms, fluctuating or persistent earache, hearing loss, *tinnitus* or vertigo. This occurs in AR because of swelling of the ET lining, which is similar and in continuity with that of the nose and nasopharynx, so this pathogenic theory appears to be undoubtedly plausible [65].

Role of Infections

Bacterial or viral infection of ME and nasal inflammation resulting from URTI are acknowledged as OME contributing factors. Data from epidemiology studies indicate that 25%–40% of URTIs in children <3 years are accompanied by an episode of MEE [65]. In exudates from 50%–80% of patients with OME (especially if chronic) it is possible to recover bacteria similar to those found in AOM: *Haemophilus influenzae* (20%), *Streptococcus pneumoniae* (30%), and *Moraxella catarrhalis* (20%) and *Streptococcus pyogenes* (4%) [102, 193, 206, 215], (Fig. 15.11) [65]. In another series, these pathogens accounted for 79.7% of all identifiable isolates: *S. pneumoniae* (11.3%), *H. influenzae* (21.7%), *M. catarrhalis* (28.9%), and *Staphylococcus aureus* (17.9%) [193]. Moreover, 57%–82% of MEEs do not show bacterial growth [223], and viral isolates are only present in 16%–19% of cases [185]. So, 5% of patients have positive bacterial cultures (*Chlamydia trachomatis, Mycoplasma pneumoniae*, or others), while in 15% viral antigens in secretions were noted. However, *Chlamydia pneumoniae, M. pneumoniae*, or any of the eight human herpesviruses do not seen to play a major role in the pathogenesis of OME in toddlers with OME [196]. Less common in normal children, these microorganisms adhere to the cells, damage them and affect mucociliary clearance, preventing the host defensive response and managing to replicate more freely [116]. It is obvious that *cilia dysfunction damages the mucosal membranes*

of the entire respiratory tract, with negative effects on both ET and ME, *with consequent tubal obstruction,* already physiologically *reduced in diameter early in life* [176]. It seems automatic to conclude that ETO is greatly influenced by URTIs, RSV, influenza and parainfluenza viruses, by inducing the receptors of involved bacteria, increasing their adherence to infected cells, allowing the virus to trigger bacterial ME colonization via ET. RSV-like viruses stimulate virus-sIgE synthesis, which sets off the army of mast cell inflammatory mediators, thus increasing capillary leakage, edema and OTE: RSV thereby establishes an IgE-mediated nasopharyngeal hypersensitivity [12]. It follows that viral infections constitute the highest risk factor for ETD leading to OME, which therefore is a common complication in these infectious episodes [219]. In conclusion, nasopharyngeal infection and allergy are likely related to OME development [143].

Role of Passive Smoke

Children <3 years exposed to passive smoke fall ill with OME more easily and for longer periods than their unexposed peers. Based on serum cotinine levels, smoke increases the frequency of otitis media episodes by 38%, anticipates the onset [163] and prolongs its duration (28 days vs 19), becoming responsible for 8% of all episodes and for 17.6% of persistent endotympanic effusion [60]. In young babies, the onset of symptoms was under 6 months in 51% of babies, with >6 episodes in 68% of babies and three to six episodes in 16%, with associated nasal symptoms in 59% of babies [163]. In a cohort of babies monitored by tympanometry, exposure to >30 cigarettes prolonged the illness persistence in the 2nd year of life by 38% and by 31.3% if <30 cigarettes, *also in those not exposed* (26.6%) [146], possibly due to the effect of environmental tobacco smoke (ETS). In one of two Turkish studies, 73.7% (84/114) of the study children and 55.0% (22/40) of the control children were found to be exposed (p=0.0461). The cotinine urinalysis results showed that 23.1% (9/39) of the children *without parental smoking histories* were exposed to tobacco smoke vs 84.3% (97/115) of the children with parental smoking histories [93]. In the other study children, the case group was exposed to a mean of 19.6 cigarettes per day vs 14.4 cigarettes per day for the control group (p<0.004). Maternal smoking was a significant factor [94]. Passive smoke is significantly correlated with a threefold increased risk of lower respiratory tract infections [87]. Etzel [59] studied ETS effects on OME, meta-analyzing ten studies involving a total of 4,310 children between 1–15 years of age. In seven of these studies, the damaging effect of parental smoke was noted and *in three studies neither parent smoked.* In the 2/10 studies based on cotinine levels [60, 197], 33% [197] and 8% [60] of OME cases were related to passive smoke. In a further meta-analysis of 28 studies on ETS, 53.6% showed a statistically significant risk of OME, seven more studies on children aged 6 (few) to 19 [49] were meta-analyzed, finding a RR in 2%–13% of cases, which rose to 1%–26% with tympanostomy (Table 4.25). Since sidestream smoking increases the risk of OME, legal regulations and guidelines must be established to protect these children from passive smoking [93].

Global Pathogenetic Hypothesis

It is possible that allergens interfere in OME pathogenesis (Fig. 15.12) [8], in two different ways:

- *Lymphocyte proliferation* causes ETD via lymphoid hyperplasia, with an increase of ME secretory cells.
- *IgE intervention* following mast cell mediator release results in mucosal membrane swelling. An increase in nasal secretion containing *pabulum* (where the bacteria that interact with RSV and other viruses grow) follows. Furthermore, prostaglandins may have a positive effect, decreasing vascular permeability and therefore the invasion of microorganisms.

Clinical Presentation

OME is an asymptomatic illness: it often goes unrecognized in babies before the onset of overlapping secondary infections causing an acute picture. Older children are often catarrhal (Fig. 15.13), complaining about earaches, buzzing, a feeling of ear occlusion (that can be modified with the Valsalva maneuver or by nose blowing) and transmissive hearing loss, which can be intermittent and of variable intensity, linked to swallowing and yawning, but often difficult to identify, especially in younger children. Parents notice that the child is distracted, that they are obliged to raise the voice or repeat words to be understood, teachers reproach these children because they do not follow lessons and are inattentive and sloppy [75]. This may depend on prolonged early MEE, which can result in significant sequelae, including hearing loss and lower scores on tests of cognitive ability, speech and language, and school performance at age 7 years [202]. However, language deficits are of small magnitude and may or may not be clinically significant [129], even if recently this deficit was not found in children >3 years of age [150].

A widely debated question is whether the illness lasts for >6 months, as for example, babies exposing to passive smoke can lead to a worsening of hearing loss, with a medium-moderate deficit of 25 dB conduction complicated by language and psychosocial developmental delays [110]. These complications are not confirmed [127]: in general OME may not affect later speech and language development or academic achievement [165]. Two large-scale investigations [164, 197], a very recent meta-analysis [166] and a randomized controlled trial [208] did not find any significant associations between

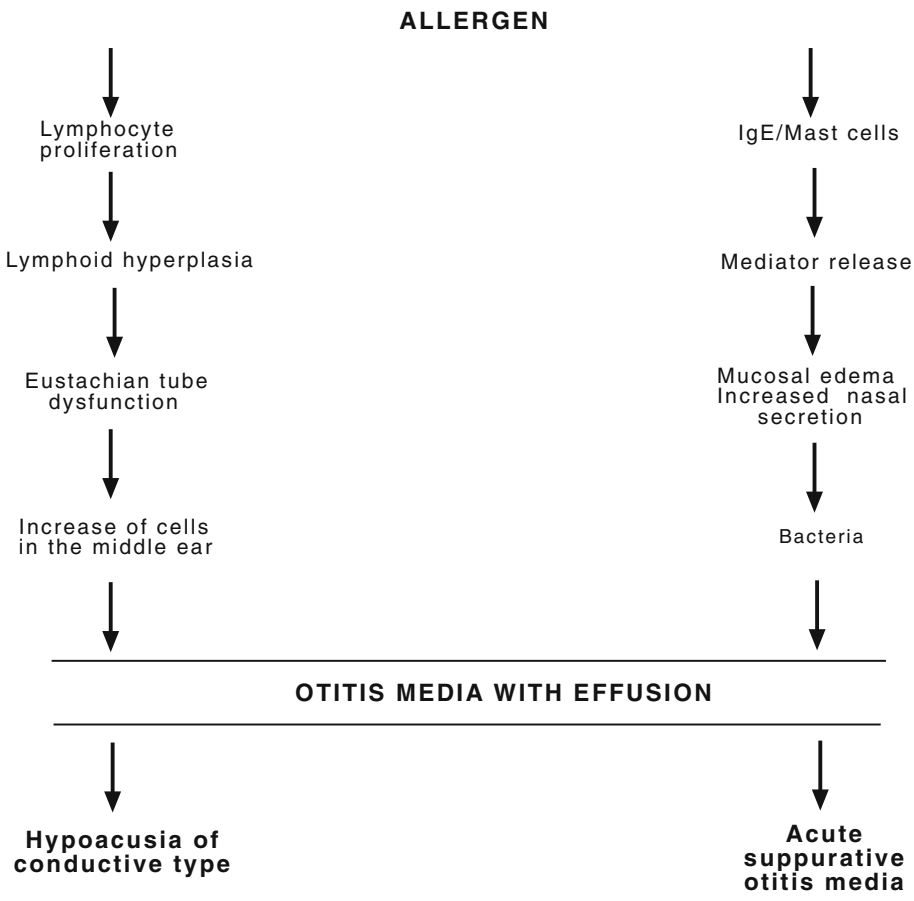

Fig. 15.12. Pathophysiology of otitis media with effusion. (Modified from [8])

OME, hearing loss [164, 166, 208], language changes and verbal expression [164, 166, 208], in children aged between 1 [164] and <4 years [197], nor in older children in language and attention progression [197]. Children with a history of OME scored lower on measures of speech and language development in some studies, compared with children who infrequently experienced OME. The data reflect outcomes for an average, otherwise healthy child; factors affecting the language development of a single child must also be considered [166]. Other results are consistent with observational studies in showing that day-care attendance increases the risk of OME, although no evidence of delay in language acquisition or behavioral problems was found [208]. It is possible that defects diagnosed in preschool age are partly correlated with an altered mother-child relation due to OME [164] and that these are overcome by the child in parallel with OME resolution [179].

Fig. 15.13. Typical catarrhal aspect: an usual appearance in children with OME

Diagnosis

Family and personal history is generally an indication; most symptoms mentioned are diagnostic. Investigations commonly start from the causes, course, and progression of recurrent ear disease, including previous ear infections, allergic reactions, especially rhinitis or rhinoconjunctivitis, and food. An environmental history should identify the child's living environment, allergic or irritating factors such as passive cigarette smoke, mites and/or molds, pets, etc., any possible change to the

face's normal conformation, the type of respiration, any possible language and/or hearing disorders, etc.

If medical history suggests an allergic cause see Chap. 6 for a careful evaluation, since FA can be a cause in subgroups of children, especially if they suffer from AR and/or are multiply sensitized [140]. Nasal examination may reveal, as commonly occurs, turbinate mucosal edema and signs of hypertrophy. For young sufferers of recurrent ME infections, see Chap. 22. Recently, the Canadian Task Force on Preventive Health Care reported that insufficient evidence was available to include or exclude routine early screening for OME in children up to 4 years of age [31]. However the American Academy of Pediatrics (AAP) Subcommittee on OME recommended distinguishing children with OME at risk for speech, language, or learning problems from children with OME without such risk and more promptly evaluate hearing, speech, language, and need for intervention and manage children with OME not at risk with watchful waiting for 3 months from the date of effusion onset (if known) or diagnosis (if onset is unknown). Hearing testing should be done when OME persists for ≥3 months or at any time that language delay, learning problems, or a significant hearing loss is suspected in at child with OME. Children with persistent OME not a risk should be reexamined at 3- to 6-month intervals, unless significant hearing loss is identified, or structural abnormalities of the eardrum or ME are suspected [170].

Diagnosis is based on finding an otorrhea, or a type B tympanogram (flat) indicating a serous effusion, or a type C tympanogram with peak compliance to negative pressure values (tubal stenosis) plus the results of tonal audiometry (any conductive hearing loss with a deficit in prevalence on medium-low frequency) [47] in children aged >5 [146]. In children aged <6 months, the tympanic membrane was not thickened, but it was immobile and the tympanogram showed a flattened tracing [55]. The following notes on additional ORL tests that are essential for diagnosis in children may be helpful for pediatricians [65].

An *otoscopic examination* shows an accumulation of mucous secretion in the ME, with increased pressure; the tympanic membrane appears to be normal or slightly retracted, or also opaque, wrinkled, thickened, and with poor mobility (Fig. 15.14). An extreme immobility with increased viscosity of the fluid present in the ME indicates a glue ear [127]. In some cases one may see, beyond the membrane, little bubbles or a fluid level [47].

With a *pneumatic otoscopy* the structure and functionality of the tympanic membranes are identifiable, as regards to coloring, position, retraction or extraversion, opacity and air-fluid levels.

Tympanometry [238] has two considerable practical advantages: it is noninvasive and does not require the child's cooperation; the only problem is that younger children do not always remain seated for the whole test duration [224]. It is an objective method for evaluating the air pressure and impedance of the tympanic membrane of ossicles, for measuring ME pressure and establishing functionality; it often reveals a lack of elasticity of the tympanic membrane [47]. This technique remains, however, useful in the diagnostic process and for screening silent diseases [65].

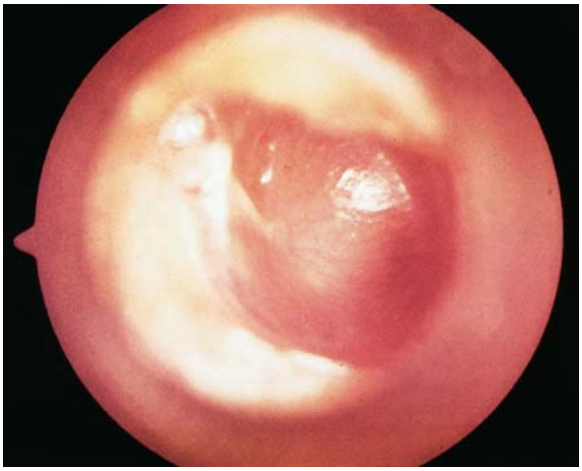

Fig. 15.14. OME in a 9-year-old girl, with no related history, but with perennial AR (PAR). Hearing impairment was noted at school. A paucity of findings is common, but OME may be demonstrated by a low level of eardrum mobility on pneumatoscopy

The tonal audiometry is a standard technique for use in children >18 months [57]. With younger children, *impedancemetry*, *behavioral audiometry*, or more sophisticated tests such as *brainstem auditory evoked response* may be used. The problem is that invasive procedures to establish any hearing loss will be impossible to carry out in children who are too young, or in older but frightened children. In this case, pediatricians can organize different tests according to age in their office with a non-quartz watch. We stress evaluating the extent of hearing deficits even in younger babies [164].

A final diagnosis is also obtained in younger children using pneumatic otoscopy and tympanometry [164], in older children with myringotomy (see below), or surgical incision followed by drainage.

Treatment (Table 15.6) [75]

General Guidelines and Allergen Avoidance

The general guidelines consist of interventions for allergen avoidance, also eliminating any possible adjuvant factor. Therefore nurslings should not be allowed the following: pacifiers, feeding bottles with too narrow openings and feeding in a supine position, associated with an early onset of the illness [146].

Environmental controls include articulated interventions for allergen avoidance: if allergy is the cause,

Table 15.6. Indications for OME therapy

No. of events per ear	Duration of events (months)	Hearing loss (months)	Behavior, language, school	History		Tympano-grams	Audio-metry	Diagnostic approach
				Personal	Family			
<4	<2	<2	Normal	–	–	–	–	Observation
>4	>2	>2	Normal	±	±	Normal	<20 dB	Observation
>4	>2	>2	Poor	±	±	Flat curve	<20 dB	Complete otological and allergic evaluation

Modified from [75].

Table 15.3 offers suggestions. Measures ensuring a good environmental humidification can help maintain the respiratory mucosa under normal physiological functions.

Therapeutic Strategies

In OME infection oral antibiotics are useful for 14–21 days [9], to be chosen on the basis of the prevalent organisms in MEEs (Fig. 15.11). It is worthwhile cultivating the drained fluid (if this does not spontaneously flow out, a myringotomy should be carried out), allowing active antibiotics to be chosen for possible resistant bacteria if the desired therapeutic effect is not achieved [65]. About 40 % of children who suffered from AOM, were left with residual MEE 1 month after onset following antibiotic treatment [96]. Whether AOM antibiotic treatment prevents OME outcome is a controversial issue [226]: antibiotic therapy is no better than placebo in OME resolution [204]. A meta-analysis has shown that efficacy of antibiotic use was limited in the short-term and non-significant in long-term use [226]. Results are definitive only when corticosteroids (CSs) are associated, as revealed by other meta-analyses [11, 168, 169]: antibiotic therapy alone is effective in 39.3 %, when associated CSs in 63.6 % of cases [11]. In children 1–9 years, after 14 days of treatment, there was a significant difference in the proportion of children who were effusion-free immediately after treatment with CSs and amoxicillin compared with those who were treated only with amoxicillin [122]. Intranasal or oral CSs can be recommended, for example, 1 mg/kg of prednisone or prednisolone in two refracted doses, for short 1-week cycles [10] or if indicated, also chromones can be used if suitable. Antimicrobials and CSs do not have long-term efficacy and should not be used for routine management [170]. Antihistamines and decongestants may reduce nasal congestion in children with AR [233]; however they are ineffective for OME, and should not be used for treatment [170].

Surgical Treatment

Surgical treatment is indicated for chronic forms:

- Clinicians should use *pneumatic otoscopy* as the primary diagnostic method and distinguish OME from AOM. It is recommended that clinicians document the laterality, duration of effusion, and presence and severity of associated symptoms at each assessment of the child with OME (Fig. 15.14). *Myringotomy with tympanostomy tube* (Fig. 15.15) is used when hearing loss ≈20 dB is present [195]; the tubes remain *in situ* for a time considered useful by ORLs, but no >6–12 months [19]. One of the main aims of treating glue ear is to improve the conductive hearing loss, and it is expected that the improved hearing will prevent problems with speech and language development, problems in the classroom, behavior, etc. [1]. The tube acts as a substitute for the blocked ET, after which tympanic membrane appearance and hearing generally return to normal [57]. *Otorrhea* is a frequent complication of this operation with a fairly high incidence (5 %–38 %) and immediate onset [74]; treatment with topical antibiotics is ineffective [74]. Young children randomized to early vs late tube insertion for persistent OME showed *no behavioral benefits from early surgery* [150, 151] and did not improve developmental outcomes at 3 years of age, regardless of baseline hearing [151].
- *Tonsillectomy* alone or myringotomy alone should not be used to treat OME. [170]. Tonsillectomy is no more effective than a simple *adenoidectomy* [104], but after a sound evaluation of the immunological data [35], it may prove beneficial in persistent forms [9] if a real obstruction is detected [19] requiring the insertion of ventilation tubes [51]. The benefit is apparent at ≥3 years of age, and independent of adenoid size [127, 148], as adenoidal tissue may regrow. As a consequence, adenoidectomy should not be performed unless a distinct indication exists (nasal obstruction, chronic adenoiditis) [170].

Further measures are for the specific competence of specialists to evaluate the role of atopy in children unresponsive to medical-surgical treatment [51]. The deficiencies mentioned up to now may appear more frequently in patients who have undergone *surgery* [179],

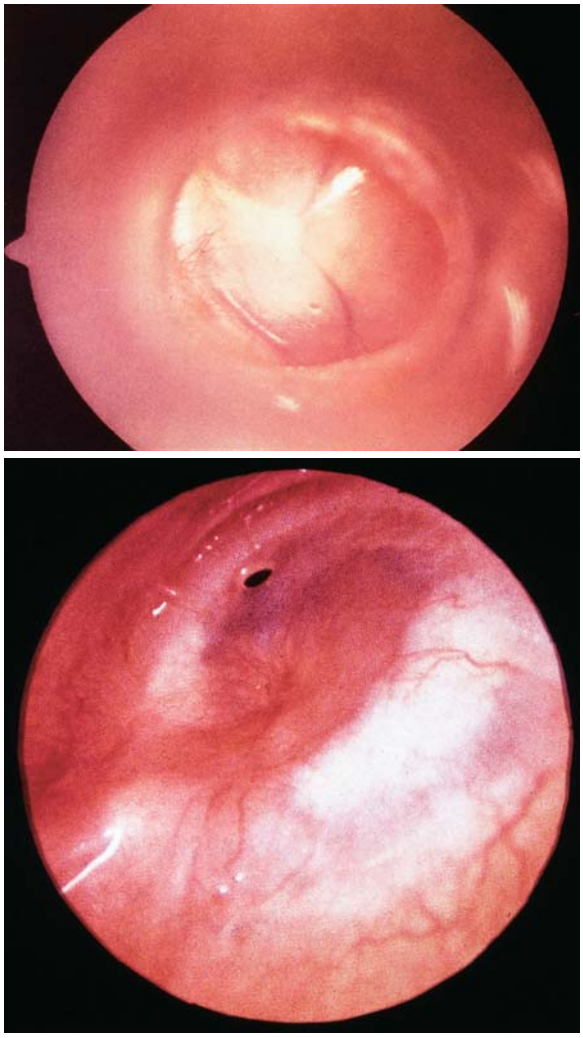

Fig. 15.15 a,b. Insertion of myringotomy tube in the tympanic membrane of a child

not resulting in improvement compared to untreated controls [235]; therefore a conservative attitude is advised [179].

Prevention

It is important to stress the protective role played by maternal milk [56, 83], rich in immunological factors (Tables 2.15, 2.16), with a sIgA defensive effect able to block microorganism adhesion (*H. influenzae*) to retropharyngeal cells and therefore their adhesion to the ME. Antigen-specific IgA are inversely proportional to OME episodes [83]. The superiority of exclusive breast-feeding affects nasopharyngeal colonization by ME bacteria, significantly reduced in 257 nurslings aged 4–6 months compared to bottlefed controls (23%–31%) vs 45%–56% breastfed babies [61]. Studies have debated the utility or not of breast-feeding; apart from the Saarinen study on 327 cases [175], some favorable [135, 153], particularly in 13,751 children [2, 55, 56, 61, 107, 146, 203, 210, 234] with significant odds ratio (OR) and confidence intervals (CI), and others not [171, 177, 199], various studies have examined different aspects: protection depends on the *duration of breast-feeding and its exclusivity*. In 1220 babies followed over 1 year, the rate of OME was 10% if the child was breast-fed for ≥6 months, 20.5% for ≥4 months [55], 19%–21% for <5–6 months [61], or 23.6% if for <3 months [146]. Infants who were breast-fed for the first 6 months of life had a 10% decrease in the amount of OME during this period compared to bottle-fed infants [146]. Among 877 children still being followed at 1 year of age, breast-feeding was found to be significantly protective for one or more episodes [203]. The risk estimates presented for bottle-feeding compared with breast-feeding are reported in several meta-analytic studies published recently [55, 204, 210]. A meta-analysis of recent follow-up studies concluded that breast-feeding even for 3 months was protective in decreasing the risk of OME [210]. These studies indicate a beneficial effect of breast-feeding (even for short durations) on reducing OME episodes in infancy [55, 204, 210].

Sinusitis

Paranasal sinuses, from an anatomohistological point of view, *are an integral part of the respiratory system.* The mucosa that covers them passes, with no breaks, in the mucosa that covers the nasal cavities, and is formed by the same type of ciliated and muciparous cells that completely cover the whole airways. Thus, processes affecting the nose would be expected to extend into the nearby sinus cavities. Therefore, sinuses are usually the target for complications in respiratory allergies; but in early infancy, a number of particular problems related to them are present, acknowledged after pioneering studies carried out by Wald [215–220] and using new sinus visualization techniques [95]. Bouts of sinusitis in early infancy are very often underestimated or labeled as banal colds that heal spontaneously. One reason for such an oversight is the only recent individuation of both maxillary and ethmoid sinuses already at birth, sinuses that are sufficiently developed to assume clinical importance before the 2nd year of life [233]. Persistent or recurrent colds depend in fact on sinus infections. Another reason for which sinusitis in this age group can be underestimated is the excessive attention paid to adenoids and tonsils in young patients, and the widespread tendency to indiscriminately blame these for all infectious episodes: there is no question that they are often diseased, but it is equally certain that the paranasal sinuses and not the tonsils or adenoids are often responsible for clinical symptoms [33]. However, an increased awareness of the magnitude of the problem of sinus disease has led to recognition that it is a major health problem in children [158].

Definition

Pediatric sinusitis is a clinical condition characterized by mucosal inflammation of the paranasal sinuses, often classified chronologically as an acute, recurrent acute, subacute, or chronic (persistent) disease process [57]. Sinusitis is defined as acute when the symptoms complex accompanying inflammation of the sinuses is present for <12 days in children (to be differentiated from viral URTI), subacute if it lasts from 2–6 weeks and chronic when it persists for 30 days [217] or >3 months [183]. For the frequent association with nasal symptoms, it is also called *rhinosinusitis,* which seems to be a more correct term, because most children begin with or have a concomitant rhinitis [76].

Prevalence

Prevalence is certainly higher in allergic children, especially in those with chronic respiratory symptoms. Sinusitis commonly has a prevalence of 5% [183]. A study based on the premise that URTI with a prolonged course corresponds to episodes of sinusitis came to the conclusion that 6%–13% of children, especially if attending day nursery, suffer from sinusitis during the first 3 years of life [219]. In children attending the primary care centers, 8.3% had a diagnosis of sinusitis: the prevalence rate was greater among children aged >5 years than among those younger (9.3% vs 7.2%) [100]. Prevalence is lower during the summer months – allergic patients are sensitive to grasses, pollinating trees and household pets – is more often prevalent in the colder seasons [90, 100] and coincides with the regional epidemiology of viral respiratory infections (VRI) common during that time of the year [90].

Genetic Factors

The role of genetic factors in sinusitis is highlighted by the association with persistent sinus disease of two well-defined genetic disorders, cystic fibrosis and primary cilia dyskinesia (Kartagener's syndrome). Immunodeficiency (ID) may be the cause of sinusitis, especially in treatment-resistant forms [158, 184]. Various primary IDs (PIDs) are also associated with recurrent chronic infections, including common variable ID, selective IgA and IgG deficiency or of subclasses [158]; exanthematous diseases such as measles and scarlet fever often cause sinusitis [162]. In class I antigens, B54 antigen is significantly increased in patients with chronic sinusitis (RR 3.23) compared with the normal control group. For class II antigens, no antigens were significantly increased [200]

Pathophysiology

Outlines of Embryology, Anatomy, and Physiopathology

The paranasal sinuses are pneumatic cavities that form as invagination of the nasal mucous membranes, apart from the sphenoid sinuses that derive from the posterior nasal fossa (Fig. 15.16) [216]. All *are lined with the same mucosa as the nose:* the sinuses are lined with ciliated pseudostratified epithelium and are covered with a mucous blanket. The epithelium is rich with mucus-producing goblet cells, but seromucous glands are sparse [145]. Both ethmoid and maxillary sinuses appear as early as the 3rd–4th month of intrauterine life, the frontal sinuses by the 6th–12th month of extrauterine life, and are recognized on imaging at age 7–8 years but are not completely developed until late adolescence, the sphenoid sinuses later, as they are generally pneumatized by 5 years of age [19, 227]. Development from birth to adult age is shown year by year in Figs. 15.17 and 15.18 [78].

The *maxillary* sinuses are the largest, beginning as little more than slits that widen progressively before taking on a quadrilateral form. Since the sinus opening, the ostium, is situated high, drainage by gravity is difficult, thus favoring mucus retention and therefore infections. The *ethmoid sinuses* are a complex system of air cells, each drained by an independent ostium. This anatomical form easily predisposes to infection, given that even a modest mucous surface inflammation is sufficient to cause its occlusion. The *frontal* sinuses, of various size and extent, are uncommon sites of infection. Sometimes they may constitute *foci* for the spread of infection to the orbit or the central nervous system. The *sphenoid* sinuses are surrounded by important anatomical structures, including the internal carotid artery, the cavernous sinus and the pterygoid canal nerve. Posterior ethmoid and sphenoid sinusitis results from obstruction of their respective ostia, which collectively drain through the sphenoethmoidal recess, while isolated infection is rare [214, 217].

The mucous covering of the walls is formed from a layer of pseudostratified columnar ciliated epithelium, interposed with caliciform muciparous cells [50]. It has a special importance:

- In cleansing entrusted to the mucociliary system
- In immune reactions to pathogen agents
- When compromised by inflammatory or hyperplastic events, etc.

In these events, ostium obstruction is a key physiopathological mechanism in sinus pathology [214]. The ostia are placed in the meatus below the three turbinate bones: inferior, middle and upper. Their small dimensions make them easily obstructed: the maxillary ostium measures 2.5–4 mm and the diameter of each ethmoidal ostium is 1–2 mm [183, 217]. When the ostium is totally obstructed because of crucial events such as mucosal edema or mechanical factors

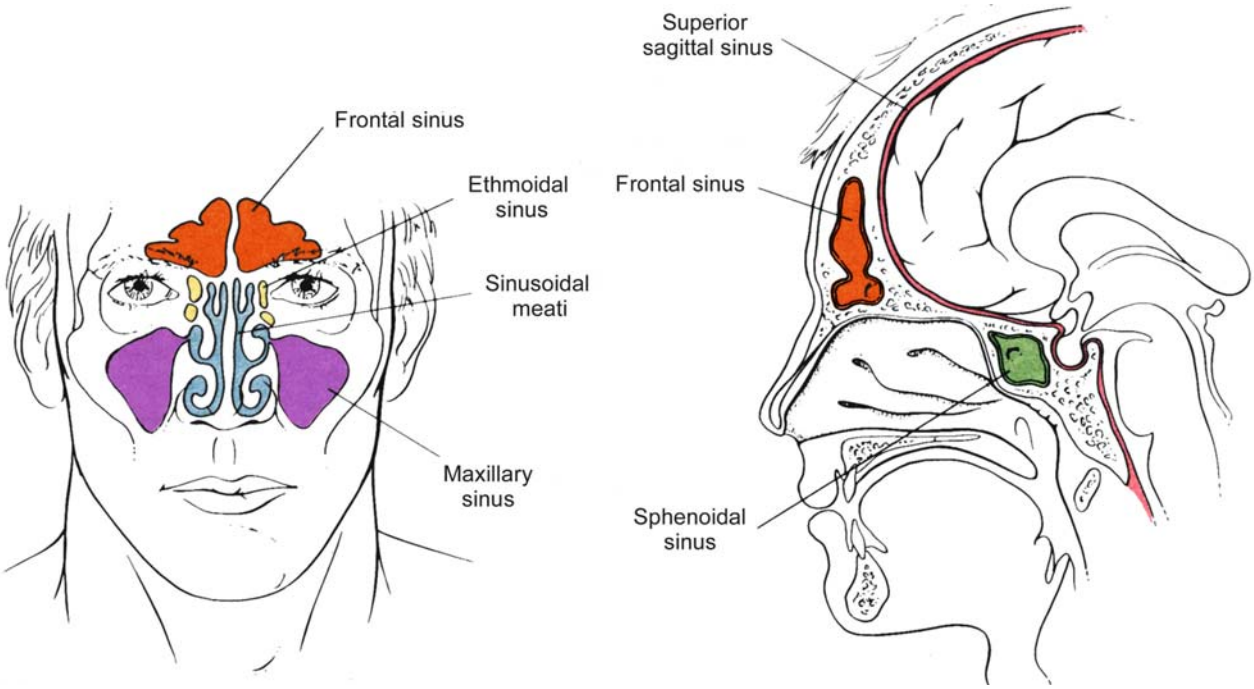

Fig. 15.16. Anatomy of the paranasal sinuses

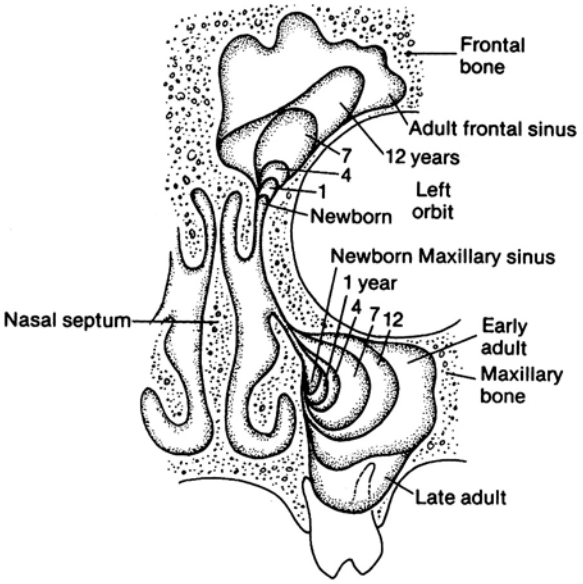

Fig. 15.17. Development and relative size of the frontal and maxillary sinus from birth to adult hood

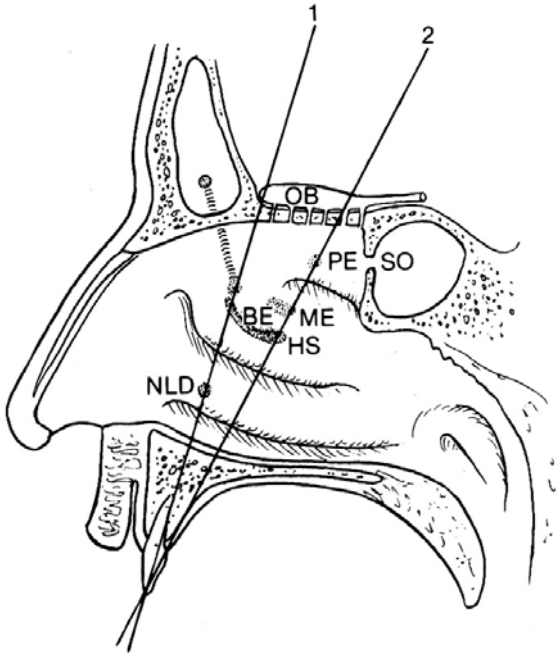

Fig. 15.18. Development and relative size of the ethmoid and sphenoid sinus from birth to adult. Note: the Fig. indicates various ages
Landmarks of the lateral wall of the nasal cavity as illustrated in sagittal view. The numbers *1* and *2* illustrate the coronal planes of section. *OB* = olfactory bulb; *PE* = posterior ethmoid ostium; *SO* = sphenoid ostium; *BE* = ethmoid bulla; *HS* = hiatus semilunaris; *ME* = middle ethmoid ostium; *NLD* = nasolacrimal duct ostium in inferior meatus. (From: Graney DO: Paranasal sinuses: Anatomy. In: Cummings CW, Fredrickson HM, Harker LA, et al (eds): Otolaryngology – Head and Neck Surgery, vol 1. St. Louis, CV Mosby, 1986, p 845; with permission.)

Table 15.7. Conditions predisposing to sinus ostial obstruction

Mucosal edema	Mechanical obstruction
Systemic diseases	**Choanal atresia**
Congenital ciliary dyskinesia	**Ethmoidal bullae**
Immune deficiencies	Foreign bodies
Allergic rhinitis	Nasal polyposis
Viral upper respiratory infection	Septal deviation
Cystic fibrosis	Tumor
Local irritants	
Diving	
Rhinitis medicamentosa	
Swimming	
Trauma, barotrauma	

Data from [183, 189, 190].

(Tables 15.7 [183, 189, 190], 15.8 [162, 189, 190]), after an endosinusal pressure transitional increase, negative pressure forms inside the cavity: when the ostium opens again, the contrast between the negative endosinusal pressure and atmospheric pressure can allow bacteria to enter the normally sterile sinus cavity [217]. In this sense, the ostium forms the gateway for sinus infections,

Table 15.9. Main functions of paranasal sinuses

Supply of warm and humid air
Aid in olfaction
Modulation of the voice thus providing voice resonance
Protection of the ear from the sound of the voice
Uptake of foreign volatile substances
Supply of protective mucus
Aid in the conditioning of inspired air (through the warm and humid air)
Protection of intracranial structures from trauma

Modified from [19].

as we have seen in the ME via the ET. In case of sinus inflammation, not only ciliary clearance is blocked, but also mucosal edema with sinus ostia closed or nearly closed with consequent interruption of sinus mucous drainage [120]. Inflammatory thickening of the mucosa and the accumulation of purulent secretions concur in blocking the ostia, *causing a dramatic O_2 reduction and a concomitant CO_2 increase,* with the consequent inhibition of the granulocytes and ciliary transport (Fig. 15.19) [50]. Because of the partial fall in O_2 pressure, further amplified by inflammation, draining the accumulated secretions seems to be impossible: they stagnate and are easily infected by anaerobic bacteria or viruses of endonasal origin, normally innocuous [120,

Table 15.8. Conditions predisposing to chronic sinusitis, or associated with sinusitis

Systemic diseases	Local factors
Viral upper respiratory infection	Adenoidal hyperplasia
Allergic diseases	Barotrauma
Allergic rhinitis	Choanal atresia
Asthma	Cigarette smoke/passive smoking
Cystic fibrosis	Cleft palate
Immune deficiencies	Dental infection
Common variable immune deficiency	Environmental pollutants
Deficiency of IgG subclasses	Foreign bodies
Combined IgA and IgG deficiency	Nasal obstruction
AIDS	Nasal polyposis
Congenital ciliary dyskinesia	Rhinitis medicamentosa
Bronchiectasis	Septal deviation
Down syndrome	Swimming
Exanthematous disease	Topical decongestant abuse
ASA intolerance or to other drugs	Trauma
	Tumors

Data from [162, 189, 190, 233].

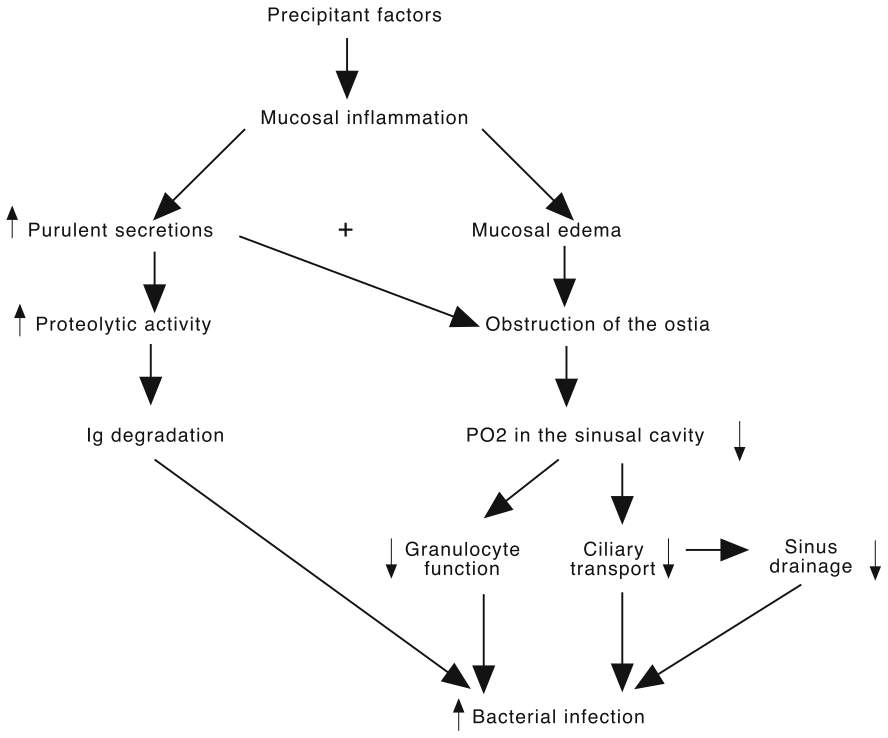

Fig. 15.19. Physiopathology of sinusitis. Modified from [50]

190]. The paranasal sinuses play an important role in various functions, as shown in Table 15.9 [19].

Immunopathology

The paranasal sinuses are equipped with efficient defense mechanisms against infections, consisting of mucociliary clearance and a local immune system at the mucosal level, which acts as a first line of defense: the sinuses in fact protect themselves from infection with an efficient apparatus [216]. The sinus lining is in part covered by a mucous layer that, with all the impurities it contains, is drained into the nose via the ostia by a coordinated ciliary movement; this self-cleansing mechanism depends on the normal ostial functioning, the correct mucous fluidity and the rhythmic ciliary movement [101] (see Chap. 12). The sinus mucosa, like mucosa in the nose and ear, contains seromucous subepithelial cells that contribute to *sIgA production;* IgG and IgM antibodies, C3 and C4 are also present [101]. Any factor capable of damaging the defensive structure fosters pathogen entry into the sinuses, automatically opening the doors to infections. The MBP (major basic protein) in particular [86] and viruses [162] have *direct cytotoxic effects on the cilia* [86, 162]. These children may have higher than average IgE levels (8%), positive SPT for aeroallergens (36%), low Ig levels (18%) and a weak response to vaccines in 10% of cases [184]. However, 14/44 children (31.8%) had extensive sinus disease, but no significant difference in the average of total and specific IgE compared to children with limited disease [117].

The immunopathology of *chronic sinusitis* is better characterized in children. Key features of this type of inflammation include the presence of chronic inflammatory cells, and large numbers of eosinophils, and in one study more lamina propria and intraepithelial eosinophils were evident in the tissue of children with chronic sinusitis, especially in those with concomitant asthma [7]. However, *the sinus mucosa of young children with chronic rhinosinusitis has less eosinophilic inflammation, basement membrane thickening, and mucus gland hyperplasia* characteristic of adult patients [39]. The densities of IL_{1b}, TNF-α, IL_{13}, and RANTES mRNA$^+$ cells were all increased in nonallergic patients compared to normal controls. Decreased expression of IL_{12} (p40) mRNA was demonstrated in sinus biopsy specimens of patients with chronic sinusitis [229]. In allergic asthmatic children with chronic sinusitis, very significant correlations were demonstrated between IL_4 and IL_{12}, IL_{12} and IFN-ɣ, IL_8 and neutrophils, and TNF-α and monocytes/macrophages. IL_4 and IL_{12} were significantly correlated as well as IL_8 and neutrophils in nonallergic children [161]. Increased levels of GM-CSF (granulocyte-macrophage colony-stimulating factor), IL_3, and IL_5 mRNA correlated with the density of tissue eosinophils, have been observed within the sinus mucosa, and up-regulation of CD106 and elaboration of RANTES may contribute to the marked accumulation of eosinophils in chronic hyperplastic sinusitis [82]. Chemokines such as MCP-3, MCP-4, and eotaxin are significantly increased within the sinus mucosa of both allergic and nonallergic chronic sinusitis and are associated with the accumulation of inflammatory cells, particularly eosinophils [230]. Like AR, the pathophysi-

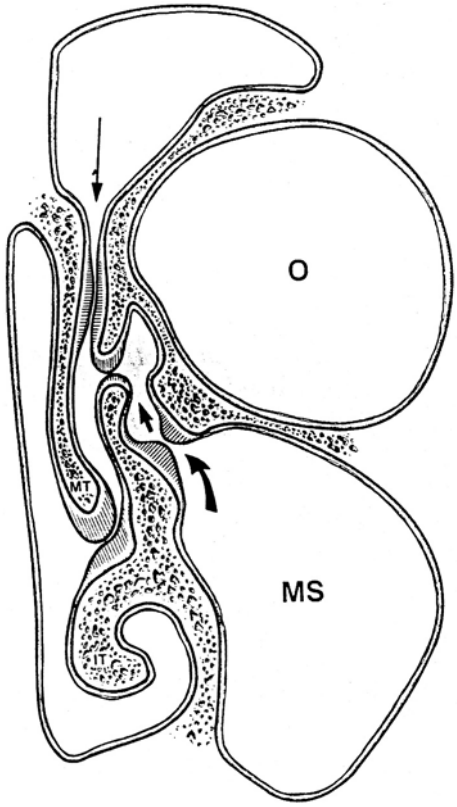

Fig. 15.20. Coronal section through the ostiomeatal complex. The *shaded area* delineates the sites of potential narrowing and obstruction. The *short arrow* indicates the infundibulum area, the *long narrow* and the *larger curved arrows* depict the drainage route of frontal and maxillary sinus, respectively. *O* orbit, *MS* maxillary sinus

ological features of chronic sinusitis has been largely attributed to the production of Th2-like T cell ILs [81]. *Allergic and nonallergic children share a typical Th2 IL pattern,* thus suggesting the existence of a common pathophysiological mechanism shared by upper and lower airways that is consistent with the concept of *united airway disease* [161].

Predisposing Factors

The conditions that more commonly act as predisposing factors, especially in chronic forms, are summarized in Tables 15.7 and 15.8. Another priority factor is the age from 2–6 years [137] (p=0.0001), as young children have not yet acquired the resistance to infections that characterizes adults and the paranasal sinus defense mechanisms that work effectively when the ostia remain open [120]. Other damaging factors are cold and/or dry air, the use of vasoconstrictors, exposure to polluting environments [162], and cigarette smoke [198]. Children exposed to passive smoking in the household had clinical sinusitis significantly more frequently than those not exposed (68.8% vs 1.2%) [100]. The importance of the so-called *ostiomeatal complex* (Fig. 15.20) has also been recognized as the area situated between the middle and lower turbinate bones, where frontal, ethmoidal and maxillary sinuses meet [214]. Two mucous surfaces come into reciprocal contact at various degrees in this complex, increasing the risk of negative effects on the delicate mechanisms of mucociliary clearance. Furthermore in younger children the duct channels are long and narrow and can easily become obstructed [120]. This is particularly evident when the defense mechanisms are damaged, causing the mucus to accumulate and stagnate, and drainage changes can be followed by bacterial infections [214]. Viral infections or abuse of nasal decongestants (rhinitis medicamentosa) often overlap allergy-caused occlusions, amplifying infectious and inflammatory assaults. It is understandable that obstruction of the ostiomeatal complex necessarily involves that of the sinuses [120], favored by anatomical causes. Oral respiration and nasal obstruction caused by adenoidal hypertrophy are often factors that contribute to lengthening the course of sinusitis. Hypertrophic adenoids could act as a foci of infections.

Allergic Sinusitis

The existence of allergic sinusitis is not certain, even though the paranasal sinuses, situated in a position of close continuity to the nasal cavities, may significantly suffer correlated anatomopathological alterations: the following data can identify the allergic pathogenesis of sinusitis [5, 162]:

- Positive family and personal history
- Signs of nasal allergies
- Frequent association with asthma
- Frequent presence of OME, up to 50%
- Positive SPT and sIgE, significant increase of total IgE, positive NPT and eosinophil count (>10%)

Figures 15.21 and 15.22 [5, 33] show the incidence of signs and symptoms in children with sinusitis associated with asthma, respectively. More than half of sinusitis is closely associated with asthma, a recognized allergy-mediated disease [138]. More than 80% of children with sinusitis have a FH of allergy (FHA), as opposed to an allergy frequency of 15%–20% in the general population [207]. A study on 413 children (aged 3–15 years) with AR provided evidence that PAR patients have a higher prevalence of sinusitis than those with seasonal allergic rhinitis (SAR). This increase was seen in children of all ages, but especially in the 1st year in children with SAR (p=0.0001) and in the 2nd and 3rd year in those with PAR (p=0.0001) [90]. In young conscripts with acute sinusitis, allergic symptoms were more frequent and positive SPTs considerably more numerous than in controls [178], up to 59% of cases [137]; furthermore atopic children present on average a higher number of sinusitis episodes compared to healthy ones

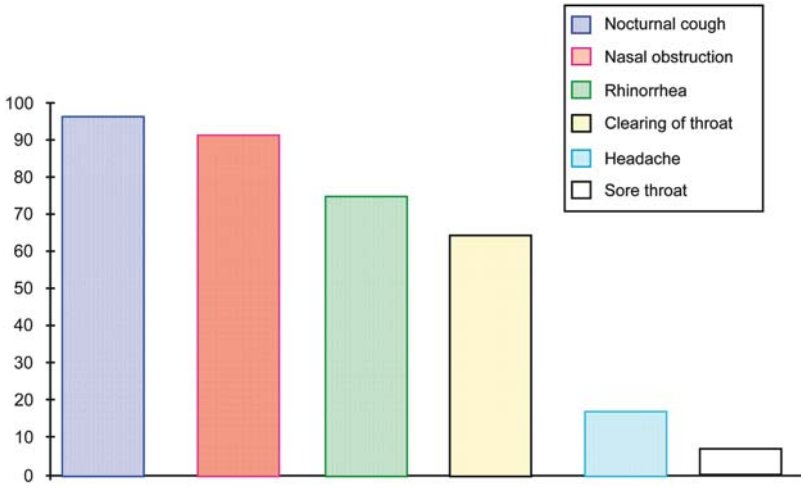

Fig. 15.21. Signs and symptoms in children with allergic sinusitis. Data from [5]

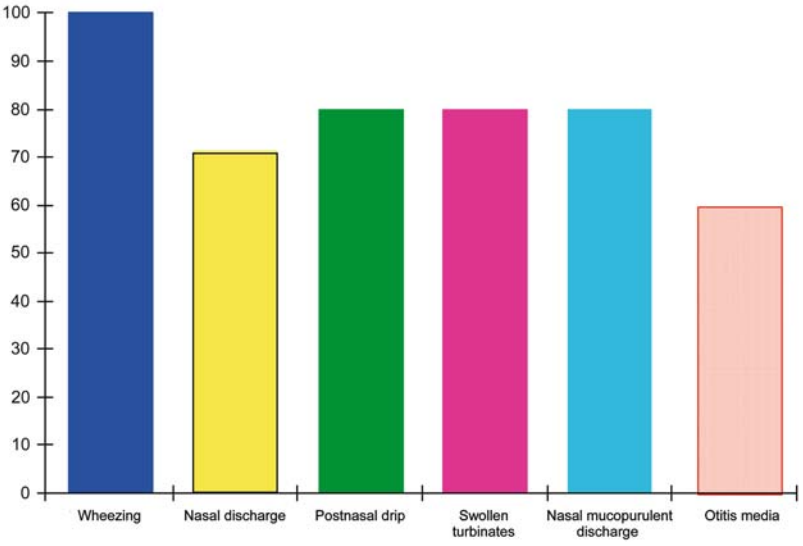

Fig. 15.22. Signs and symptoms in children with sinusitis and asthma. Data from [33]

(p=0.012) [69]. A closer link between atopy and sinusitis is proven by eosinophils as effector cells in chronic inflammation of paranasal epithelium [84].

Relationship Between Asthma, Allergic Rhinitis, and Sinusitis

Animal studies have shown that sinusitis can increase airway hyperactivity although the mechanism is not entirely clear [190]. It is known that sinusitis occurs frequently in allergic children, in whom it can be associated with asthma worsening or forms of difficult asthma [25], but above all in those with AR, which is the most important predisposing factor [69]. Mounting experimental evidence suggests that the sinusitis–AR association is an event encouraging the production of mucus and nasal mucosa swelling, with ostial obstruction that becomes an obstacle for bacteria removal [69]. The hypersecretion present during persistent and chronic AR is in fact capable of causing ostial occlusion, with a fall of sinus pressure and bacterial aggression. AR and sinusitis may coexist in 25%–70% of cases [69, 162]. In children with recalcitrant sinusitis and evaluated for allergy and ID, ≈50% were atopic, thus supporting the connection between AR and sinusitis [184]. An increased incidence of sinusitis defined as above was reported in children referred for evaluation of rhinitis, a large proportion of whom were atopic as proved by SPTs, again supporting a connection between AR and sinus disease [181]. Approximately 30%–50% of asthmatic children suffer from sinusitis, and a similar rate of

sinusitis sufferers are affected by asthma [137, 162]. In the study of 44 children, 34 (72.3%) had a diagnosis of asthma: those with extensive sinus disease had a significantly higher prevalence of moderate to severe asthma than the children with limited disease [117]. X-ray studies have emphasized significant changes in 27% of asthmatic children aged 3–16 [162], in 31% of 6- to 19-year-olds [238], and 36% of nurslings <12 months presented opacification of the maxillaries and/or inspissation of >4 mm vs only 6% of older children [112]. The correlation between these children has been confirmed by the results of a trial on entrants affected by sinusitis and asthma, in whom we have proved that treatment of sinusitis caused an amelioration of asthma [25]. Similar results were reported in a later study [190]. Chronic sinusitis in children with difficult-to-control asthma should be carefully evaluated, especially between the ages of 2 and 10 [69].

The *pathogenetic mechanisms* responsible for the triple *asthma-rhinitis-sinusitis* pathology have not yet been sufficiently explored. In his classic studies Slavin [189] has formulated various hypotheses, still a valid point of reference:

1. *Seeding into the lower airways* of bacteria originating from paranasal sinuses and containing mucopurulent material, which probably causes the tracheobronchial mucosa inflammation, a hypothesis that has recently been the object of conflicting views [6, 28]
2. *Inhibition by infections* of β-adrenergic receptors of the airways and responsible for a bronchodilator effect
3. *Reflex bronchospasm* mediated by the parasympathetic system, as nasal and sinus afferent fibers end in the mesencephalon and cause parasympathetic stimulation of airway smooth muscles
4. *Obstruction of nasal cavities* and consequent open-mouth breathing, resulting in wheezing caused by airway cooling, or bronchial mucosa exposure to allergens, or to other potentially inhaled irritating substances
5. *Bacterial toxins* causing mast cell and basophil mediator release producing a powerful bronchoconstrictor effect

The hypothesis of a reflex arc → nose → sinuses → airways is very stimulating, and could explain the association of asthmatic and rhinosinusal symptoms: chronic nasal inflammation, postnasal drip, and repeated throat clearing can trigger a parasympathetic reflex arc with consequent asthma worsening, while rhinosinusitis treatment would decrease stimuli, thus improving asthma [190].

Authors who later investigated this point did not reach unambiguous conclusions [6, 28, 43, 190]. Considering point 1, pharyngeal receptor hypersensitivity caused by local spreading of the inflammatory process provokes constrictor reflexes [28]. Points 2–5 suggest that *the nasal and upper airway filtering action protects the tracheobronchial tree from aggressive aeroallergens and noxious infectious agents,* but it is rendered inefficient by any noxa. The exclusion of this defensive mechanism added to nasal obstruction and mucosal inflammation could expose the lower airways to harmful substances which, causing inflammatory changes, may occasion bronchospasm [6].

The striking suggestion that *a single allergic stimulus* might *act simultaneously on paranasal mucosa causing sinusitis* and *on the bronchial membranes causing asthma* [189] has been corroborated by significant pathogenetic and histological acquisitions, common to patients suffering from allergic asthma and sinusitis [84, 86]. More precisely, eosinophil and MBP concentrations in the paranasal tissue appear to be significantly higher in subjects with both affections compared to patients with chronic sinusitis only [84]. In vitro it has been ascertained that MBP damages the epithelium of sinusal mucosa and causes ciliostasis, similarly to findings in vivo [86]. This data suggests that sinusitis in asthmatics is caused by the same mechanism that damages the bronchial epithelium.

Clinical Presentation

The symptoms (Fig. 15.21) are strictly related to patient age [5, 19, 162, 217]:

- *In unweaned babies* and young children (until the age of 2) slight fever, anorexia, pallor, and irritability are noted; no sign of pain or headache is apparent but when current, young children are unable to complain about their symptoms.
- *Older children* may complain of paranasal pain, moderate nasal occlusion and rhinorrhea of varying degrees, with secretion often completely retronasal as to escape observation, sore throat, voice loss, hyposmia, and breathing difficulties.
- *Sinusitis associated with AR* is characterized by a brief course, frequent attacks of nasal congestion, watery rhinorrhea, sneezing and itching with sudden onset and quick resolution; both nasal obstruction and secretion are usually of varying degrees, often accompanied by ticklish sensations in the nose, retronasal cavity or palate.
- *Chronic sinusitis* is usually a complication of AR or asthma especially chronic; persistent symptoms characterized by nasal obstruction and mouth breathing if nasal congestion prevails, or coughing especially at night if retronasal discharge is predominant, chronic rhinorrhea with little or no purulent discharge, OME and symptoms of chronic asthma with possible associated sinusitis (Fig. 15.22) [33]. Poor school results and frequent absenteeism are motivated by continual tiredness, chronic headaches, fatigue, irritability, halitosis, etc. [162].
- In some cases there are serious forms with *hyperthermia* >39 °C and purulent nasal secretion for at least 4 days, occasionally accompanied by an intense headache [217].

Table 15.10. Clinical presentation of hidden sinusitis

1.	Chronic nasal congestion
2.	Extensive school absences
3.	Tiredness
4.	Malaise
5.	Headache
6.	Perennial cough
7.	Possible coexistence of asthma
8.	Missed or delayed diagnosis
9.	Purulent nasal discharge
10.	Rx abnormalities of paranasal sinus

Modified from [182].

- Sometimes sinusitis can appear to be a *hidden disease* [182] stemming from a number of factors; the main ones are summarized in Table 15.10 [182]. The main issues are *missed diagnosis* and different symptoms compatible with chronic sinusitis that consequently is mistakenly diagnosed as a perpetual cold. Isolated sphenoid sinusitis in young children may masquerading as acute, subacute, and chronic headache thus suggesting a new aspect of sinusitis [34]. *Isolated inflammatory sphenoid sinusitis* (11 cases) should be considered in children aged ≈7 years who present with headache that does not respond to simple analgesia and visual disturbance [211].

Craniofacial Alterations

A combination of chronic nasal obstruction and mouth breathing in children can cause craniofacial modifications, not always associated with adenoidal hypertrophy; the main causes of chronic nasal obstruction in infancy are summarized in Appendix 12.2.

Complications

The principal complications are orbital cellulitis and endocranial complications, especially subdural empyema, made less frequent by medicosurgical interventions [34]. They are mentioned because they may occur *in children aged 10 months* [95], and orbital cellulitis was present in 139 children, 72% of whom presented with preseptal cellulitis, 19% with *orbital cellulitis,* and 9% with subperiosteal abscess. AR is frequently underlying the condition. Older children are more likely to develop subperiosteal abscess, while *preseptal cellulitis* is more common in younger children [88]. Preseptal complications of sinusitis can be diagnosed clinically without a CT (computerized tomography) scan and mandate an appropriate course of IV antibiotics [191].

Table 15.11. Diagnosis of hidden sinusitis based on clinical and laboratory parameters

Signs and symptoms	
A.	Major criteria
	Cough
	Purulent nasal discharge
	Purulent pharyngeal drainage
B.	Minor criteria
	Earache
	Facial pain[a]
	Fever
	Foul breath
	Headache[a]
	Periorbital swelling[b]
	Pharyngitis
	Tooth pain[a]
	Wheezing
Diagnostic tests	
A.	Major criteria
	Plain radiography in the Waters projection showing specific findings (see text)
	Coronal CT scan showing mucosal thickening and sinus opacification
B.	Minor criteria
	Nasal cytology (smear) demonstrating neutrophils and bacteria
	Sinus ultrasound demonstrating fluid levels
Assessment of diagnosis	
	Signs and symptoms: two major criteria or one major and = ≥2 minor
	Diagnostic tests: 1 major = confirmatory, 1 minor = supportive

Modified from [183].
[a] More common in adults.
[b] More common in children.

Diagnosis

The main clinical, instrumental and laboratory parameters facilitating diagnosis are exemplified in Table 15.11 [183].

Family and/or personal history is often positive and along with the typical symptoms, usually allows a correct diagnosis: sneezing, watery and/or purulent rhinorrhea, allergic salute nasal itching, pale and edematous mucosa. A *careful ORL examination* is also necessary. Coughing is a common symptom of sinusitis; the chest must be auscultated for signs of any wheezing. If the case is indicative and the child young, a *battery of specif-*

ic allergens is sufficient [69], which can be completed with total and specific IgE.

The *cytological examination* of nasal secretions shows polynucleates often containing intracellular bacteria and few eosinophils, which instead are prevalent in AR (see Appendix 12.1). Finding eosinophils and neutrophils in the secretions is distinctive, as confirmed by recent studies [54, 183], although consensus is not unanimous [238]. Significant neutrophilia (>5 neutrophils per high-power field) has been shown to be correlated with X-ray pictures and proves to be particularly useful *for the detection of hidden sinusitis* [43, 182, 183].

The diagnosis of acute bacterial sinusitis in children ≤6 years should be based on clinical criteria [108]. The *bacteriological examination* of the cultures, obtained by sinus direct puncture, emphasizes the same bacteria responsible for AOM: *H. influenzae, S. pneumoniae* and *M. catarrhalis* [193, 206, 215], especially the first two in children aged 0 to 2 years; [206]; the agents are the same in the acute and chronic forms [183]. Cultures are often negative (40%) [206], or positive in 34% of samples [193]. In the latter case, four pathogens accounted for 79.7% of all identifiable isolates: *S. pneumoniae* (11.3%), *H. influenzae* (21.7%), *M. catarrhalis* (28.9%), and *S. aureus* (17.9%) [193]. Bacterial recovery is not recommended for routine diagnosis in children [108].

Imaging studies are generally considered useless in nurslings and in a first episode, but useful in all other cases [216], especially due to the favorable cost/benefit ratio and the lower rad dosage compared to limited CT (0.6 vs 2.6) [72].

Examination of the Waters occipitomental projection shows maxillary sinus opacification, air–fluid level and evident mucosal thickening (4 mm) correlated with the invasion of noxious microorganisms, with a sensitivity of 76% and a specificity of 81% [72]. Other projections are the *occipitofrontal Caldwell projection* for ethmoidal sinuses and submentovertical for sphenoid sinuses; the first also show the frontal sinuses. The AAP [218] suggests that these studies can be safely omitted from an appropiate diagnosis because studies of children ≤6 years have shown that a positive history frequently predicts the finding of abnormal sinus X-rays. However, controversy exists about the need for X-rays to confirm acute sinusitis in children >6 years of age with persistent symptoms and for children of any age with severe symptoms [108].

CT is a great help for *visualizing the ostiomeatal complex* [120]; this is more detailed in children [72, 120] and is usually indicated for complications, recurring sinusitis and chronic treatment-resistant forms [137, 216].

Transillumination and *ultrasonography* are not of much use, also because the former is difficult to perform in young children [183] and can be used after the age of 10 [217], while the latter is not suitable for screening patients with limited lesions [120].

Sinus echography is instead useful for checking treatment progress [54].

Rhinoscopy with fiberoptic endoscope can be used for evaluating uncertain cases of sinusitis or cases that have proved to be refractory to usual medical treatment; this test permits a direct visualization of most of upper airways, often facilitating the diagnosis, unless previously confirmed, of sinusitis and adenoidal hypertrophy, in addition to nasal polyps, endonasal foreign bodies, etc. Its use in pediatrics is not recommended due to poor child cooperation [184].

Magnetic resonance imaging has made it easier to diagnose sphenoid sinusitis, a probably under-recognized disorder [137].

*Sinus aspiration*s, a definitive but not routine test [182], is carried out by an ORL specialist after sedation or a general anesthetic to obtain an adequate immobilization of young children [217]. Bacterial isolates at a concentration of at least 10^4 CFU/ml (colony forming units) is considered as representative of an ongoing infection. At least one microorganism per high-power field with Gram stain is correlated to a rate of 10^5 CFU/ml [217].

Decisions on any invasive diagnosis should be based on each individual case [123] and of cost/benefit ratio [54].

Differential Diagnosis

With acute viral URTI, consider the duration of symptoms limited to 5–10 days, the finding of nasal congestion and retronasal discharge, not accompanied by cough, that tends to recede after 10 days or little more [220]. A distinction between viral URTI and acute bacterial sinusitis is the nasal involvement that occurs in VRI. In acute bacterial sinusitis, the nose merely acts as a conduit for eliminating purulent secretions produced in the sinuses [108].

The conditions indicated in Table 15.12 must be taken into consideration for chronic forms [33]. In particular, sinusitis should be considered in *children who have severe asthma, do not respond to effective treatment,* have no obvious causes for their asthma, and have significant upper airway symptoms [144].

Particular tests can distinguish allergic children from ID children, who present frequent episodes of serious, persistent and treatment-resistant pansinusitis (>3/year), with a relapse a month after withdrawal of appropriate antibiotic therapy and after operations [158]. Diagnostic immune testing typically includes total Ig levels, IgG subclass levels, and postvaccination titers to pneumococcal and *H. influenzae* type b vaccines. Ig replacement and daily antibiotics are the basis of successful therapy [158)].

Given that children predisposed to sinusitis are mostly allergic rather than immunodeficient, it is always advisable on the basis of experience and probability, to give precedence to allergological investigations [158]. Another differential diagnosis to be carried out in sub-

Table 15.12. Differential diagnosis of chronic sinusitis

Adenoidal hyperplasia
Allergic rhinitis
Anomalies in visual refraction
Causes of persistent cough
Bronchitis by *M. pneumoniae*
Unusual diagnosis, including congenital ciliary dyskinesia
Cystic fibrosis
Gastroesophageal reflux
Chronic sinusitis associated with ASA intolerance
Dental abscesses
Foreign bodies
Idiopathic/vasomotor rhinitis
Nasal polyposis
Obstruction of sinus ostia

Modified from [33].

jects not affected by concomitant hypersensitivity to ASA concerns the rare form of sinusitis caused by fungal allergy: 141 cases in the literature in children aged 1–19 years [48, 77, 79, 121, 123, 225]. In 215 children with PAR (perennial allergic rhinitis), there was a higher risk than in those with non-mold allergy (RR, 2.49 vs 1.50, respectively) [90]. Children with allergic fungal sinusitis have symptoms of rhinosinusitis and obvious abnormalities of their facial skeleton, unilateral sinus disease, asymmetrical disease, and bony erosion, with more frequent extension of disease; findings on CT scan show bony erosion with extension of disease into surrounding structures [123].

Treatment

Treatment is based on the use of antibiotics via aerosol or nasal sprays, which has been supported by numerous controlled studies; nearly all experts advise against the use of other drugs [50, 215, 233]. The AAP recommends [218] that antibiotics be used in the treatment of sinusitis of suspected bacterial etiology. The most effective antibiotic against bacteria producing β-lactamases are the following (the percentage of children cured is shown in parentheses) [215]: amoxicillin (67%–100%), erythromycin-sulfisoxazole (95%), and cefaclor (78%–92%). In contrast to the AAP, other guidelines recommended that children with mild sinus disease and no antecedent antibiotic use in 6 weeks receive amoxicillin/clavulanate (93.5% efficacy), low-dose or high-dose amoxicillin (91% efficacy), cefpodoxime (86.7% efficacy), or cefuroxime (83.7% efficacy) [188]. The usual starting dosage of *amoxicillin* in children <2 years with mild to moderate uncomplicated acute bacterial sinusitis, who have not recently been treated with an antimicrobial (within 3 months), is 45 mg/kg/day in two divided doses or a high dose of 90 mg/kg/day in two divided doses [76, 108, 218] with clavulanic acid [233]. The broad-spectrum erythrosine-derived macrolides clarithromycin and azithromycin bind to ribosomal subunits, thereby inhibiting bacterial synthesis [95]. In acute, serious, but uncomplicated cases, 10 days of treatment are sufficient (*azithromycin,* 10 mg/kg/day on day 1, 5 mg/kg/day for 4 days as a single daily dose, or *clarithromycin,* 15 mg/kg/day in two divided doses). In children with chronic forms, it is necessary to administer them for 3–4 weeks; if the desired effect is not achieved, the cycle may be repeated [108, 218]. In a randomized single-blinded control study on 42 children aged 5 to 16 years, amoxicillin/clavulanate and azithromycin were well tolerated by 95% of children [136]. A marked improvement of symptoms was reported among children who received antibiotics (91%) compared with children who did not (21.4%) [150]. If treatment fails to eradicate the infection, the agents obtained by sinus puncture [54] or by nasal swab samples [193] should be cultivated. *S. pneumoniae* had a 32%–35% rate of resistance to erythromycin, azithromycin, and clarithromycin. *H. influenzae* showed a high rate of resistance to clarithromycin (36%). *M. catarrhalis* had a 15% rate of resistance to erythromycin and a 91.5% rate of resistance to penicillin [193]. It is also possible that *H. influenzae* and *M. catarrhalis* are resistant to β-lactam antibiotics such as ampicillin due to β-lactamase production. Instead, *S. pneumoniae* and *S. aureus* can be resistant to the penicillins and most other antibiotics by a genetic alteration in penicillin-binding proteins [76]. Children with *amoxicillin intolerance* should be treated with a cephalosporin, such as cefuroxime [76], and severely intolerant children with a macrolide as azithromycin, or clarithromycin [76]. *For children who do not respond* or who worsen while receiving amoxicillin, amoxicillin/clavulanic acid, cefuroxime axetil, and clindamycin are also recommended [102]. In children treated with antibiotics, *91% recover more quickly* and more often than do children who were not (21.4%), as early as at the 10-day follow-up visit [100], at 24–48 h after the follow-up visit, and again at 10–14 days after the office visit [1]. The inefficacy of treatment should lead the pediatrician to suspect a possible viral form [42]. The reason may be another: a recent, randomized study of 188 children with bacterial sinusitis between 1 and 18 years showed instead that antibiotic therapy is no better than placebo in sinus symptoms; however, this study included older children and *excluded sicker children* [71]. Treating sinusitis early with an antiviral may ameliorate or prevent this process: since most sinusitis is precipitated by viral infections, and because most clinicians consider sinusitis to be a bacterial infection, acute sinusitis is often overtreated with antibiotics [145]. A recent guideline suggested that children with

Table 15.13. Clinical presentation in 55 asthmatic children with or without Rx abnormalities of paranasal sinuses

Symptoms	Sinusitis	
	With (*n*=55)	Without (*n*=25)
Nocturnal cough	53	6
Nasal obstruction	51	8
Rhinorrhea	42	9
Clearing of throat	36	4
Headache	9	6
Throat ache	4	3

p=0.0001.
Data from [29].

chronic sinusitis should be treated with a combination of an antibiotic and a nasal steroid spray, without giving specific recommendations about duration of therapy and choice of drug [38]. In children with persistent sinusitis, to decongest the mucosa, CSs including beclomethasone dipropionate (BDP) or flunisolide may be administered to facilitate drainage of sinus secretions and reduce local edema [43]. A multicenter study [132] has proved that *flunisolide nasal spray* produces significantly safe positive effects on symptoms, shown on X-rays and sonographic signs, and on local inflammation, reducing in particular headache, facial pain, edema and/or turbinate obstruction, secretions, etc. [132]. The addition of *mometasone furoate nasal spray,* 400 µg twice daily (bid), to antibiotics significantly reduces symptoms of recurrent sinusitis in children ≥12 years compared with antibiotic treatment alone [131]. In acute sinusitis, do topical CSs play a role in effective management? In recurrent sinusitis, do topical CSs reduce recurrence rates? In chronic sinusitis, do CSs induce symptomatic relief? [102]. *Delayed diagnosis* and advanced disease may lead to life-threatening complications [211].

To evaluate the prevalence of sinusitis, its relationship to asthma and whether its treatment leads to an improvement of asthmatic symptoms, we conducted a trial [29] on 80 children suffering from asthma who were aged 4–14 years with positive SPT and sIgE to Der p in 95% of cases (confirming the high prevalence of atopy in infantile asthma). Of the 80 children, 55 (68.7%) presented objective symptoms (nocturnal coughing, nasal obstruction, rhinorrhea, etc.) and X-ray evidence of sinusitis (mucosal thickening, opacity, air–fluid levels) (Table 15.13) [29]. Children were evaluated 30 days following specific treatment with either inhaled nasal CS or ampicillin plus an antihistamine/decongestant; 34/55 children (62%) showed an X-ray improvement (Table 15.14) [29], while in 20 (36%) a significant improvement of asthma severity was noted (Table 15.15) [29]. Zimmerman et al failed to confirm

Table 15.14. Clinical presentation in 55 asthmatic children with Rx abnormalities of paranasal sinuses before and after therapy of sinusitis

Symptoms	Before therapy (*n*)	After therapy (*n*)
Nocturnal cough	55	14
Nasal obstruction	51	12
Rhinorrhea	42	18
Clearing of throat	40	11
Postnasal drip	41	14
Edema of turbinates	40	5
Otitis media with effusion	4	3
Rx abnormalities of paranasal sinuses	55	20

p=0.0003.
Data from [29].

Table 15.15. Severity of asthma symptoms in 55 asthmatic children with Rx abnormalities of paranasal sinuses before and after therapy of sinusitis

Asthma severity	Before therapy (*n*)	After therapy (*n*)
4+	12	2
3+	18	8
2+	17	19
1+	8	26

p=0.0001.
Data from [29].

the cause-and-effect ratio between sinusitis and asthma severity [238]. In a subsequent study, 28 children with AR and sinusitis with asthma improved their bronchial hyperresponsiveness (BHR) to methacholine and decreased their symptoms with appropriate response of their sinuses to clinical therapy compared to controls. Thus, treatment of sinusitis, which afflicts a substantial percentage of asthmatic children, can have a significant influence on the basic illness [29, 124, 125]. In another prospective, open-label study, the clinical symptoms and signs of sinusitis, but not FEV_1, showed a significant improvement after antibiotic treatment. After aggressive treatment for sinusitis, it was found that the provocative concentration of methacholine caused a 20% drop in FEV_1 of 61 children with mild asthma, and sinusitis was significantly higher after treatment [209]. The results [24, 209] suggest that every asthmatic child needs to be carefully evaluated to determine whether concomitant sinusitis is present.

Possible invasive therapies should be decided depending on each individual case [123]. *New treatments have been suggested.* Parenteral antibiotic therapy may have a role in avoiding sinus surgery in selected pediatric populations [201]. Studies have shown that adenoid removal will improve sinusitis for 70%–80% of children [212]. Nutritional supplements such as flavored cod liver oil and a multivitamin-mineral with selenium have been suggested as adjunctive therapy for children with chronic or recurrent sinusitis [118].

Pediatricians and ORL Diseases

The *Nelson Textbook of Paediatrics* (15th ed or p 1194) states that "it seems prudent to avoid surgery in most cases," hence adenotonsillectomy seems to be justified in children suffering from recurring tonsillitis (as shown in Fig. 15.4), with very significant statistical differences between healthy and sick children with regard to tonsillar GC. The operation should only be performed in infancy when the pertinent indications are accompanied by normal immunological tests and after ascertaining that the child is not allergic to anesthetics. A 7-year-old girl died in Rome on October 30th, 1998 during a tonsillectomy (we hope that this was the last death of this kind); probably her degree of reactivity to anesthetics was not tested. As highlighted by Pisacane et al, passive smoking causes surgical risks in 16%–24% of children undergoing surgery (Table 4.25), while breast-fed children appear to be significantly more protected [156]. Other points should not be disregarded. Firstly, persistent or recurring OME frequently causes different degrees of hearing loss in children, both in babies and in infants, to the point of being the most common cause of deafness in children, but it can be prevented, even in this case, by breast-feeding. Secondly, sinusitis can hide overlooked asthma. In fact we believe that paranasal sinuses should always be examined in asthmatic children or children with recurring symptoms such as nasal obstruction, rhinorrhea or nocturnal coughing to reveal any involvement.

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Anaphylaxis

Historical Outline

The first description of an event similar to anaphylaxis was made at least 4,600 years ago [179]; however, only in 1765 was a fatal case reported in Europe (Chap. 17). In 1905, the first cases (one lethal) of food anaphylaxis were reported [72, 174], the subject of a book appeared in 1919 [111], and since 1926 [214] such cases have continued with an impressive frequency. The term "anaphylaxis" (from the Greek "αναφυλαξις", away from protection) was coined by Portier and Richet in 1902 during a Mediterranean voyage [156] to define the paradoxical effect of an experimental protocol. While attempting to immunize dogs to the venom of the sea anemone, after the first nonlethal tolerated dose, they unwillingly sensitized the animals with the second dose injected after 2 weeks, either equal to or less than the previous dose, and noted that the dogs exhibited severe manifestations, even lethal. Therefore the first injection sensitizes the animal, provoking synthesis of IgE antibodies; after a latent period for sensitization, re-exposure to the inciting allergen triggers within a few minutes the very severe clinical manifestations called anaphylactic shock, the reverse result of the prophylaxis envisaged by the scientists [156]. Subsequently, reports of severe episodes occurring in humans began to appear following vaccinotherapy and serotherapy. It was thus recognized that foreign substances eliciting an anaphylactic shock include either very complex molecules, such as proteins, or simple elements. However, the inciting agent, for example any foreign protein, need not accumulate to cause its effects [129].

One of the greatest dangers of today's therapy was undoubtedly until recently the allergic shock to the ever-increasing number of chemicals and drugs, all potentially sensitizing, which have all been reported more frequently since 1902 (Chap. 19).

Definition

Anaphylaxis is a life-threatening systemic reaction resulting from a severe type I, IgE-mediated hypersensitivity reaction, triggered by allergen reintroduction, with release of chemical mediators from metachromatic cells. It is manifested in the most severe form with an *anaphylactic shock*, a sudden, systemic and potentially fatal reaction. Clinical reactions indistinguishable from true anaphylaxis, *anaphylactoid reactions*, are not IgE-mediated, thus indicating the absence of specific antigen recognition. In a certain number of cases (approximation by defect), similarly severe, the cause remains unknown: these cases are defined as *idiopathic anaphylaxis* (IA). In addition to uniphasic forms, with immediate or late onset, there are biphasic reactions with characteristic clues: acute episodes followed by asymptomatic periods, then a relapse lasting several hours, and others protracted and potentially severe [63, 194, 212].

Epidemiology

The incidence and/or prevalence rates are as high as 0.0004% [136] to 0.0032% [104] in adults, 0.0006% in English hospital discharges [181], 0.004% in hospitalized children [63], and 0.04% [54] to 0.0016% [63], in not hospitalized adults and children, respectively. Over a 6-year period, a child aged 6 months to 10 years has been admitted every year to a US allergy-immunology department, for three IA episodes (0.002%), and one by vaccination, by penicillin, or exercise-induced (0.0007%) [63]. An international study estimated that the annual incidence of the overall risk of severe anaphylaxis was 154×10^6 hospital admissions, but including the possible cases, the risk was 196×10^6 [93]. In a US county, an anaphylaxis occurrence of 30×10^5 person/year was reported over 5 years [223] and the incidencerate was 21×10^5 person/year or 10.5 episodes $\times 10^5$/person/year in 229,422 children aged <18 [28] . In a school-based study in Australia the parents of 4,173 children aged 3–17 reported a history of food-induced anaphylaxis rate of 0.59% [29]. Extrapolating for the US population, one might calculate 84,000 anaphylaxis cases and 840 annual fatalities (presumed mortality rate, 1%). In England, there were 8 deaths over an 11-year period, with an incidence per year of 0.006 deaths $\times 10^5$ children aged 0–15 years [124]. Between 1991 and 1997, 67 episodes of anaphylaxis were identified in children, resulting in 10.5 episodes $\times 10^5$ person/year [28].

In England, the hospital discharges with a primary diagnosis of anaphylaxis increased up to 47.4% between 1991/1992 and 1994/1995 [181]. However, there is no available data to exactly establish the cases of anaphylaxis or the number of fatal cases, neither in children, nor in adults. On the contrary, the incidence of severe nonfatal events is 0.2×10^5 children per year [124]. Apart

from the predisposition due to preceding exposures, there are no epidemiological characteristics that are useful for identifying subjects that should be considered at risk. Fortunately, this is an extremely uncommon event in infancy, but not unheard of [22, 172, 224]. Significantly, a voluntary registry for peanut and tree nut allergy has found that 89% of 5,149 registrants were <18 years of age; the median age was 5 years, and about 45% of the total were children aged 4 to <13 years [185]. Among 2,320 emergency admissions over a 4-year period, *infants aged <1 made up 17.9%* [2], thus moving the age index to the left.

Several studies have concluded that between atopic and nonatopic subjects, there are quantitative differences related to the risk of experiencing IgE-mediated and non-IgE-mediated anaphylaxis reactions. In the US, it has been calculated that fatalities from anaphylaxis result only from penicillin; the nearest causes are Hymenoptera stings [103].

Data on biphasic and protracted episodes vary, there being a 20%–23% and 23%–28% incidence, respectively [171, 194]. A recent trial has found a 6% incidence in 105 children. Delayed administration of SC epinephrine was associated with an increased incidence of biphasic reactions [117]. However, systemic biphasic anaphylaxis occurs in up to 7% of subjects [63]. Two cases of severe anaphylaxis by jellyfish are known, one IgE-mediated, the other fatal with a clinical and atopic pattern of the anaphylactic type [16].

Incidence of Anaphylactic Shock Episode, Responsible Agents [136, 210]

- *Penicillin:* 1:1,000–1.5:10,000 and 0.01%–0.05% of treatments, with 500 fatal cases/year or from 1–5:10,000 [183] to 1–7.5:1,000,000 [136] treatments and a risk of 0.0015%–0.002%, =1:50,000–1:75,000 treatments [183].
- *Muscle relaxants:* 1:4,500.
- *IV anesthetics:* 1:300–1:29,000; the incidence of mortality due to anesthetics in children is between 0.012 and 0.029:10,000 [90].
- *Dextran:* 1:383.
- *Hemodialysis* 1:1,000–1:5,000.
- *IV radiocontrast media:* 1:1,000–1:14,000, fatal 1:10,000–1:75,000, 1:4 if of low osmolality; the lethal cases can be lowered to 1:169,000 without differences between ionic and nonionic types [100].
- *SIT* (specific immunotherapy): one fatal case: 12×10^{18} injections (Tables 13.10, 13.11).
- *Hymenoptera venom:* 0.8%–3%.

Incidence of Reactions by Causal Factors

Food anaphylaxis has made its appearance in children in a dramatic way [170, 171]. After Hymenoptera venom, *medications* are the best known causes of anaphylaxis and anaphylactoid reactions, with a mean frequency of 1:27,000 admissions (0.00003%). Even if <10% of such reactions are fatal, they are responsible for the great majority of anaphylaxis cases (Table 19.9). The higher prevalence of the reactions to such agents is likely secondary to several factors, including above all the relative frequency of exposures and the parenteral route of administration; however, both sensitizations and systemic reactions (SRs) were also reported after oral administration. An allergic shock may occur, although sporadically, during SIT, skin prick tests (SPT), or provocation test, but in children and with appropriate emergency equipment and medications available on site, the rate approximates 0%. The repeated exposure to an anaphylactic agent may affect the incidence of anaphylaxis, but *not all persons undergo relapses:* in positive cases they always experience less severe symptoms than in the first anaphylaxis. Several factors able to influence the risk variability have been suggested, including the time interval between the original and repeat exposures, and the dose of the inciting substance [155].

IA is more unusual, to the point that few specialized journals mention it, despite being characterized by life-threatening episodes associated with urticaria and/or angioedema and possibly by upper airway obstruction [179]. However, recently a growing interest has drawn attention to this current major medical problem [83, 150, 196, 218], with reports *in three infants* [26], a child of 4 years [23], children aged 3–10 [63, 64], 2–17 [26] or 10–19 [63], but pediatric cases are on the rise [83]. Apart from preferring the female sex, relations with professional factors and medications are apparently lacking.

Physiopathology

The release of a wealth of mediators, mostly vasoactive, sets in motion a mechanism with a final outcome of the vascular collapse, related to the functional loss of intravascular blood into the interstitial spaces and this shift in the rate between extent (of the vascular bed) and volume (of the vascular mass). Hypotension and shock result from generalized arteriolar vasodilation and remarkably increased capillary permeability; the consequent transfer of 50% of intravascular fluid into the extravascular space (within 10 min) [103], via the small postcapillary venule leakage, can lead to hypovolemia and shock, with resulting edema (angioedema) in skin, pooling of venous blood in the visceral bed, hemoconcentration and an increase in blood viscosity. Low cardiac output causes inadequate coronary perfusion, and low peripheral vascular resistance causes myocardial hypoxia, dysrhythmia and subsequently cardiogenic shock [87]. In vitro studies show that histamine release provokes tachycardia and arrhythmia, while prostaglandins, leukotrienes (LTs) thromboxane A_2 and PAF (platelet activating factor) are upstream from re-

duced contractile force and coronary flow. However, as yet no direct effect of anaphylaxis on both myocardium and coronaries has been documented. In some cases the shock starts rapidly, before an extended vasodilation occurs, with an exceedingly short latency time between exposure and onset of the clinical picture, indicating a possible involvement of reflex neurogenic mechanisms [129]. If the shock has a sufficient duration, organic insufficiency may occur mainly on the renal emunctory or central nervous system. When the latency time is protracted, the shock may stem from a delayed IgE-mediated reaction or the insufficiency of other organs. Alternatively, primitive abnormalities of microcirculation may play a role in provoking severe effects, by which vicious cycles result in negative effects directed against vital organs with lesser resistance [179]. In the lungs, both histamine and LTs display bronchoconstrictor activity, the first involves the proximal airways, whereas LTs act preferentially on peripheral organs. Bronchial obstruction has an effect on gas exchanges, with resulting hypoxia, for a further aggravation of the vascular effects of anaphylaxis; pulmonary edema and intra-alveolar hemorrhages are rather frequent complications. NO_2 (N dioxide) on the one hand may decrease both signs and symptoms of anaphylaxis, and on the other aggravates associated vasodilation [103]. When anaphylactic reactions involve mucosal sites, the proteins may cross the enteric and vascular barrier, also because of increased permeability. In addition to their uptake, the lactulose/rhamnose rate augments, while histamine concentrations and mucosal enzymes and H_2O and electrolyte absorption decrease; both villi and crypts remain unhurt. *Tryptase levels,* a neutral protease found in mast cell secretory granules, is also a possible marker of intestinal anaphylaxis [176]. Unlike Hymenoptera or drug-induced anaphylaxis [229], none are diagnostic in food anaphylaxis, since the levels are detected in some patients [229] but not in others [171].

Histopathology

In the cases of fatal anaphylaxis, intense phenomena of glottis congestion have been observed, the lungs appear to be hyperinflated, with mucous plugs and focal atelectasis. The microscopic aspect shows specific findings such as edema, vascular congestion and diffused eosinophilia of the lamina propria of the larynx, trachea, epiglottis and hypopharynx. In the lower airways, there is spasm of bronchial muscles, with edema, eosinophil inflammation of the mucosa and mucus hypersecretion, undifferentiated from the findings of an acute asthmatic attack [87]. In the absence of an early and adequate treatment, a pattern of acute heart failure is caused, possibly leading to respiratory insufficiency: the *exitus* is propitiated by asphyxia, edema and upper airway congestion [129]. In the remaining organs, in several cases myocardial ischemia has been seen, most likely secondary to shock; occasionally myocardial infarct is seen. Liver, spleen and other visceral organs often appear to be macroscopically congested, microscopical investigations show hyperemia and edema. Eosinophils are detected in both sinusoids and liver [55, 177].

Pathogenesis

The list of the most common responsible agents increases uninterruptedly following the promotion of new therapeutic and diagnostic means, and of food allergens. It is possible to divide the most frequent causes of anaphylaxis into three main categories.

1. In the known mechanism (Table 20.1) [22, 113, 136, 204], the exposure of susceptible subjects to foreign proteins either in the native state or as a hapten, after a covalent binding to a carrier protein, induces an *IgE-mediated reaction*: IgE antibody rapidly associates with high-affinity receptors (FcεRI) on metachromatic cells. With subsequent allergen exposure and cross-linking of IgE antibodies, mast cell degranulation is primed, with a rapid release from storage granules of newly generated mediators, including tryptase of TC mast cells, which can cleave C3 to generate C3a anaphylotoxin [22]. The activated mast cells release a number of cytokines, including IL_6 and TNF-α, which have been implicated in the pathogenesis of hypotension in endotoxic shock [136]. It is likely that the release of mediators and/or cytokines by cells bearing low-affinity (FcεRII) receptors can induce a marked decline in pulmonary conductance and dynamic compliance, hypotension and death. The observation that in children with fatal food-induced anaphylaxis the tryptase levels were not substantially elevated [171, 229] has suggested three possible pathogenetic mechanisms:

– *Mast cells* release low amounts of tryptase and normal amounts of other mediators after receiving certain activating signals.
– *Basophils* do not release histamine in the early stages of food-induced anaphylaxis, and this could play a critical role in the later stages of anaphylaxis.
– *Additional cells,* including macrophages, monocytes, endothelial cells), may be activated by FcεRII receptors via cytokines and/or mediators released in the early stages of anaphylaxis [171].

Anaphylaxis may also depend on the exposure setting and the dose of implicated allergen; in several cases associated with drugs, foods or insect venom, it is hypothesized that *non-IgE-mediated* potentiating factors may activate mast cells, basophils, other cells via FcεRII or target organs. The physiopathological mechanisms of clinical manifestations are the result of the crucial role played by *histamine and mediators* in provoking histopathological changes and clinical manifestations upon most organ systems [22], in particular at the expense of skin, airway and circulation; relative to

Table 20.1. Pathogenetic mechanisms of causative agents of systemic anaphylactic reactions

Pathogenetic mechanisms	Causative agents	Examples
IgE-mediated reactions against native proteins	Venoms	Hymenoptera, Solenopsis, snakes, jellyfish
	Inhalant allergens	Pollens, molds, epithelia
	Foods	Cow's milk, egg, nuts, fish, shellfish, crab, peanut, etc.
	Enzymes	Trypsin, streptokinase, chymotrypsin, chymopapain, penicillinase, asparaginase
	Heterologous serum	Tetanus and diphtheria antitoxin, antilymphocyte globulin
	Human proteins	Serum and seminal proteins, ACTH, insulin, vasopressin
	Others	Protamine, latex
IgE-mediated reactions against protein–hapten conjugates	Antibiotics	Cephalosporins, aminoglycosides, penicillin, sulfamides, polymyxin B, tetracyclines
	Chemotherapeutics	Adriamycin, bleomycin, alkylating agents, cyclophosphamide, methotrexate, cisplatin
	Disinfectants	Ethylene oxide
	Others	Muscle relaxants, heparin
Complement activation and generation of anaphylotoxins	Human proteins	Gammaglobulins, other human product (transfusions in patients with IgA deficiency)
	Dialysis	Blood contact with dialysis membranes
Direct activation of mast cells and/or basophils	Hypertonic solutions	Mannitol, radiocontrast media, muscle depolarizing drugs
	Drugs	Opiates, curare, d-tubocurarine, and mediator release vancomycin
	Others	Dextran, acacia, fluorescin for angiography
Unknown mechanism	NSAIDs	ASA, indomethacin
	Anesthetics	Lidocaine, thiopental
	Preservatives	Metabisulfites, benzoates
	Steroids	Progesterone, hydrocortisone
	Exercise	
	Exercise and foods	
	Cold-induced urticaria	
	Cholinergic urticaria	
Endogenous cell stimuli	Anti-idiotype antibodies	
	Anaphylotoxins C3a, C5a	
	Chemokines[a]	
	Interleukins[b]	
	GEF	
	Neuropeptides[c]	
	MBP	
	Platelet factor 4	
	Connective tissue activating protein-III	
	Phospholipase A	
	15-HETE	
Idiopathic forms	Idiopathic anaphylaxis	

Data from [22, 113, 136, 204].
See Table 20.2.
GEF glycosylation enhancing factor, *MBP* major basic protein, *15-HETE* hydroxyeicosatetraenoic acid.
[a] By lymphocytes, macrophages, monocytes, neutrophils, platelets.
[b] IL_1, IL_3, GM-CSF, NAP-2.
[c] Gastrin, substance P, neurotensin, somatostatin, calcitonin gene-related peptide, endogenous opioid agonists such as endorphin.

other organs such as the nervous system, the mediators responsible for symptoms are unexplored [225].

2. *Anaphylactoid reactions* (Table 20.1) recognize non-IgE-mediated mechanisms: circulating immune complexes or other agents activate the complement cascade with the resulting generation of anaphylotoxins (C3a, C4a, C5a), capable of inducing mediator release from metachromatic cells; *exogenous agents* such as opioids, mannitol, morphine, and radiocontrast media, and the endogenous stimuli may stimulate mediator release directly by a pathogenetic mechanism not yet well understood, but decidedly independent of both IgE antibodies and complement [179]. Complement-mediated reactions occur instead in children with selective IgA deficiency (sIgAD) and subjects with common variable immune deficiency [204] (see "Complement Activation"). Otherwise a proper pathogenetic mechanism was not identified, for example onset of bronchospasm, urticaria and/or angioedema and systemic symptoms after taking ASA (acetylsalicylic acid) or NSAIDs (nonsteroidal anti-inflammatory drugs), muscle relaxants and radiocontrast iodinated media [204], exercise-induced anaphylaxis (EIA), anaphylactoid reactions in food pseudoallergy (Table 10.19) and the cases of IA (Tables 20.1, 20.2) [129, 204].

3. The underlying *IA physiopathology* is unknown. A pathogenetic mechanism based on non-IgE-mediated activation of mast cells (and basophils) is speculated; however, no immunological parameter appears to be compromised, apart from hyperhistaminuria reported during acute IA episodes, returning to normal values at other times [83]. Unlike systemic mastocytosis, where mast cells infiltrate the bones and skin, increasing in number remarkably, in IA no rise in mast cells has been reported, nor is the skin reactivity to bioactive mediators including histamine, PAF, and LTs increased [83]. Nevertheless, symptoms in IA may be tentatively correlated with the systemic release of mediators, although occasionally. In one report, 46% of patients were affected with urticaria and/or angioedema, which continued to be observed in 22% of 94 patients on therapy, though in the absence of other clinical manifestations [218], whereas 225 patients were all affected with urticaria and angioedema [150]. Therefore, IA might be a severe form of reaction of the immediate type caused by abnormal or inappropriate mast cell and/or basophil activation. The elevated prevalence of atopy, up to 43%–58% [150, 218], higher than that of the general population, suggests a correlation between IA and atopy, thus postulating the existence of states of IgE-dependent sensitization to barely known natural allergens. It therefore seems likely that IA results from the combination of different factors, for example, a low sensitization in itself or a high IgE affinity for mast cell receptors, which make its serum levels too low to be detected by diagnostic tests. Consequently, histamine release associated with other factors, in addition to vasodilation and mucosal increase of permeability, causes an epithelial irritation

Table 20.2. Non-food agents that most frequently cause anaphylaxis

Antibiotics
Amphotericin B
Ampicillin
Bacitracin
Cephalosporins
Chloramphenicol
Clindamycin
Dimethylchlortetracycline
Kanamycin
Lincomycin
Nalidixic acid
Neomycin
Nitrofurantoin
Penicillins[a]
Polymyxin B
Streptomycin
Tetracyclines
Vancomycin
Chemotherapeutics
Asparaginase
Bleomycin
Busulfan
Chlorambucil
Cisplatin
Cyclophosphamide
Cyclosporin
Cytarabine
Dacarbazine
Daunomycin
Doxorubicin
Etoposide
Flucytosine
Fluorouracil
Mechlorethamine
Methotrexate
Teniposide
Vincristine
Local anesthetics
Lidocaine hydrochloride
Procaine
Tetracaine
Other drugs
β-Blocking agents
β-Propiolactone
Acetaminophen
Aspirin[a]
Benzylic alcohol
Chlorhexidine
Colchicine
Dantrolene
Diphenhydramine
Ethambutol
Ethylenediamine
Folic acid
Glucocorticoid
Indomethacin
Meperidine hydrochloride
Meprobamate
NSAIDs

Table 20.2. (Continued)

Other drugs (continued)	Human gammaglobulin
Penicillinase	Pancreatic extracts
Pentamidine	Protamine
Probenecid	Whole blood
Procainamide	**Diagnostic agents**
Succinylcholine	Benzylpenicilloyl-polylysine
Thiopental sodium	Decholin
Tolmetin	Fluorescin
Triamterene	Radiocontrast media[a]
Tripelennamine	Sulfobromosulphthalein
Tubocurarine	**Seeds**
Allergen extracts[a]	Caraway
Epidermal dander	Cottonseed
Molds	Flaxseed
Pollens	Mustard
Ragweed	Sesame
Enzymes	Sunflower
Chymopapain	**Venoms**
Chymotrypsin	Hymenoptera
Penicillinase	Sarcoptes
Trypsin	Snake venom
Hormones	Triatoma
Adrenocorticotropic hormone	**Vaccines**
Estradiol	Diphtheria antitoxin
Insulin	Yellow-fever vaccine
Parathormone	Measles vaccine
Parathyroid extract	Influenza vaccine
Pituitary gland extract	Pertussis vaccine
Progesterone	Rabies antitoxin
Relaxin	Tetanus antitoxin
Vasopressin	Typhus vaccine
Macromolecules[a]	**Vitamins**
Antilymphocyte globulin	Folic acid
Blood products	Niacin
Cryoprecipitate	Thiamine
Dextran	Vitamin B_{12}
Foreign serum	
Heparin	

Data from [129, 204].
[a] More frequently incriminated; see also Table 20.1.

triggering in turn axonal reflexes, tachykinin release and in the final analysis mast cell degranulation. IgE antibodies are heterogeneous, owing to this physicochemical peculiarity, and in certain subjects could undergo a structural adaptation similar to that pertinent to mast cell activation [83].

The knowledge of new involved molecules such as the *panallergen profilin* [69] suggests that some inexplicable cases may fit cross-reactions, as shown by those between latex and fruit (Table 8.14). The food neoallergens revealed during cooking match this analysis [126], at variance with the prevalent dogma of a reduced allergenicity following warming. The report of tryptase levels that are significantly elevated in infants who died from SIDS (sudden infant death syndrome) compared to healthy controls, a proof of mast cell degranulation [89], makes us suspect that the list of cases *classified as idiopathic or by unknown causes* can be added to other risk factors *including the hidden sources of foods*, which certainly underlie even lethal cases of anaphylaxis.

Risk Factors

The elements that predispose a child to anaphylaxis are rarely acknowledged: if on the one hand there appear to be no apparent correlations with age, sex, race or nationality, on the other hand it is usually assumed that

Table 20.3. Emergency anaphylaxis admissions in 530 children by age and etiological trigger

Trigger	Children (%)	Infants (%)	Preschool (%)	Junior (%)	Adolescents (%)
Age (years)	<16	<1	1–5	6–10	11–15
Food	60 (41)	16 (62)	25 (48)	8 (32)	11 (26)
Meat/fish	1 (1)	1 (4)	–	–	–
Berries/seeds/mushrooms/plants	9 (6)	1 (4)	6 (12)	1 (4)	1 (2)
Food: other	43 (30)	12 (46)	15 (29)	6 (24)	10 (24)
Food: unspecified	7 (5)	2 (8)	4 (8)	1 (4)	–
Drug	49 (34)	7 (27)	15 (29)	9 (36)	18 (43)
Antibiotics: penicillin	4 (3)	1 (4)	1 (2)	–	2 (5)
Antibiotics: other	2 (1)	–	–	1 (4)	1 (7)
Vaccines	17 (12)	3 (12)	2 (4)	4 (16)	8 (19)
Drug: other	26 (18)	3 (12)	12 (23)	4 (16)	7 (17)
Insect venom	15 (10)	–	7 (13)	3 (12)	5 (12)
Other causes	21 (14)	3 (12)	5 (10)	5 (20)	5 (12)
Admissions (etiology recorded)	145	26	52	25	42
Admissions (etiology not recorded)	385	69	145	73	96

Data from [2].

the administration of stimulating agents by the parenteral route, the duration and frequency of exposure, and the time elapsed after the last exposure are all paramount factors in determining the risk of developing a SR: in a patient sensitized to an antibiotic, the risk of reacting to another antibacterial is increased nine-fold [224]. Recent data show that even for babies aged <1 year (17.9%), emergency anaphylaxis admissions were necessary. The preschool age is the group most at risk (35.9%) (Table 20.3) [2]. Twenty-three children with anaphylaxis (42%) were aged 1–5 years [57], more are males [57, 125, 153] and 54% are allergic [57]. Factors to be considered further are the patient's inability to identify the cause, the inexperience of those present, and the delayed recognition of case severity [224]; moreover, as regards food anaphylaxis, the public place and the lack of emergency kits are additional factors [171].

Several factors potentially causative of anaphylaxis and of anaphylactoid reactions are outlined in Table 20.2. The rates reported in children are as follows [2, 26, 57, 145]

1. Insect stings (10%–22.4%)
2. Foods (25%–57%)
3. Exercise (2.2%–9%)
4. Anesthetics
5. Muscle relaxants (1%)
6. Antibiotics (4%–9%)
7. Hormones and enzymes
8. Plasma expanders
9. Latex (1%–27%)
10. SIT and SPTs (1%)
11. Vaccines (2%–12%)
12. Age
13. Complement activation
14. Radiocontrast media (2%)
15. ASA and NSAIDs (5%)
16. Additives (1%)
17. Aeroallergens

The route of exposure to the agent was IV in 38% of the episodes, oral in 27%, and transcutaneous or subcutaneous in 20% [57].

Insect Sting Anaphylaxis

Insect stings are sufficient to produce a severe, even lethal, anaphylactic reaction in sensitized persons. Sensitization results from prior stings and patients allergic to common or cross-reacting antigen may endure another anaphylactic reaction after being re-stung by any Hymenoptera insect species [204]. More frequently, children with Hymenoptera venom anaphylaxis have a negative personal atopic history and negative SPT to inhalant and food allergens [145].

Food Anaphylaxis

The field of food anaphylaxis has recently known several new aspects related to the cross-reactivity between proteins contained in different foods such as the profilins (Table 1.72) or between foods and aeroallergens (Table 1.73). The cross-reactivity between latex and vegetables (Table 8.14) and between vegetables and pollens (Table 9.48) are paradigmatic examples of this phenomenon. Anaphylaxis is frequent in children: 27 out of 544

(4.9%) investigated children with food allergy (FA) had anaphylaxis as part of their clinical presentation [161] or FA accounted for ≈50% of severe anaphylactic episodes in children treated in an ED [145]. In 76 children 95 cases of anaphylaxis occurred, 62 of them (82%) had a *personal history of atopic symptoms;* foods were identified as causative agents in 57% of the episodes of anaphylaxis [145]. Usually symptoms are elicited between a few minutes and 1 h, on average after 15.4±27.5 min [145], sometimes after a longer interval. The reaction severity is directly proportional to the onset speed and depends on that of the stimulus (dose–response mechanism). In 1982, Fries [75] reported 61 cases of peanut allergy, including a fatal case in a child who accidentally ate a small amount of peanut butter. Peanuts hidden in a cake were responsible for one fatality in France: not even a quick injection of epinephrine saved the child's life [18]. Anaphylactic shock was triggered in 17 children and adolescents [171, 228]. The most striking pediatric case series published reviewed 13 cases of anaphylaxis, with *fatal results in six* and near-fatal results in the other seven [171]. The foods incriminated in fatal cases were peanut in three cases, cashew in two, and egg in one. In the near-fatal cases, peanuts were incriminated in one, walnuts, pecan and Brazil nuts in four, and CM and fish in two cases. All children were asthmatic and all had previously experienced anaphylactic reactions to the same food but were unaware that the allergens were contained in *common foods* such as candy, cookies, sandwiches and pastry [171]. Thus, *coexisting asthma is more strongly associated with a severe reaction* than the severity of previous reactions [13, 124, 128]. Several children and adolescents were *both atopic and asthmatic* [27, 171, 228]. Sixteen fatal cases were induced by peanuts [27, 30, 178, 228], and cod, crab and pecan nut caused one each [228]. In a report of 32 fatalities [27], 17 (53.1%) were ≤18 years old and 3 of 17 (17.6%) were aged 2–6 years, other victims were 5, 8, or 9 years old [159]. Remarkably, 9 of 17 (52.9%) died of peanut and 5 of 17 (29.4%) of tree nut anaphylaxis [27]. These fatalities occurred in association with foods from restaurants or other food establishments [27]. A severely nut-allergic 12-year-old girl perceived a nut-taste as soon as she ate a home-made cake, and soon systemic symptoms were triggered. Nobody had epirephrine at hand, and the girl despite the ED (emergency dept) died of anaphylactic shock (Italian press report, March 2004). The high prevalence of peanut allergy is caused by the marked potency of this allergen, capable of sensitizing 18.3% of children <12 months (Table 5.5), to the point that patients are positive to SPTs at a $1{:}10^7$ dilution [149]. In England, there were eight deaths over an 11-year period in 3-month- to 15-year-old children, four caused by cow's milk (CM) (50%), two by peanut, and one each by egg white and mixed food [124]. Over 2 years (1998–2000), there were six near fatal reactions (two caused by mixed food and one each by CM, egg, lentil, and walnut) and 49 severe ones (among which 13 were caused by nuts, ten by peanut, and nine by mixed food), yielding incidences of 0.02 and 0.19×10^5 at 0–15 years per year, respectively [124]. Regardless of the nature of the initial reaction in 60 children, the majority (52%) experienced potentially life-threatening symptoms to subsequent reactions [207]. CM may be a *silent killer of toddlers:* one girl aged 3 in the 32 fatalities reported (12%) [27]. Luckily enough, these are rare cases: over the past 10 years, four children have died (incidence of 0.003 deaths per 1×10^5 children aged 0–15 years per year) and 0.19 severe reactions per 1×10^5 children up to 15 years of age [124]. Near-fatal reactions to CM have been experienced by 13 children aged 9 and 12 (15.4%) [171]. CM is responsible for cases of anaphylaxis in up to 11% (Fig. 9.4), and 22% of cases [145]. To these cases we add CM-induced anaphylaxis/shock in 3 infants aged 1–15 months [23], a girl of 3 years [27] and 2 patients 2–17 years of age [26] as well as severe reactions triggered by 1–2 drops put upon the inner border of the lower lip [38], even causing anaphylactic death [27]. A CM-sensitive infant was hospitalized with systemic anaphylaxis that developed immediately after the application of a diaper rash ointment that contained 5% Ca caseinate [94]. Four babies aged 6 weeks to 6 months experienced severe life-threatening reactions, all related to unsupervised self-challenge with either a CM-based formula or a dairy product [182].

Food anaphylaxis and severe respiratory and cutaneous reactions were provoked in schoolchildren by peanuts (73%), egg (13%), CM and lupine (11%) and eight other foods [140]. Table 9.19 shows that foods may provoke respiratory reactions in 3%–46% of children (DBPCFC). Additional foods more frequently implicated are seafood (11%), nuts (13%), fish (19%), egg and fruits (11%), cereals (5%), goat milk from 4% [145] to 92% [15], nuts, figs and peas [214], squid [41], mustard and other foods and additives causing anaphylaxis or anaphylactoid reactions (Table 20.4) [6, 7, 9, 10, 11, 14, 15, 18, 22, 24, 30, 38, 41, 42, 45, 49–51, 53, 54, 65, 67, 68, 70, 71, 73, 79, 95, 97, 98, 107, 113, 122, 124, 126, 137, 140, 141, 143, 145, 147, 151, 152, 160, 161, 163, 164, 168, 170, 171, 173, 185, 196, 200, 202, 203, 209, 224, 228]. Limpet cross-reacting with Der p has induced anaphylactic reactions in five Der p-sensitized asthmatics [11]. A child we have seen experienced anaphylaxis to watermelon and peach and tested positive to a number of other foods and vegetables: his mother reacted upon seeing Italian squash and peach. Severe reactions were to skin contact with egg white and shrimp [12] as well as CM, wheat, peanut, and tomato in four children [199] and with drops of CM inadvertently spilled on the leg of a CM-allergic boy we have treated, and on the head of a girl [199]. Similarly, accidental skin contact with a drop of CM provoked systemic symptoms in a 16-year-old boy [119]. Additional severe symptoms were triggered upon trivial skin contact with minute doses of allergen in infants, children and adolescents [65, 186]. In 100 subjects aged 9 months to 19 years, skin contact reactions were present in 90%, severe in 23% and treated with epinephrine in 13% of cases [186]. Hydrolysate formulas have provoked 13 cases of anaphylaxis

Table 20.4. Foods and additives leading the list in frequency as cause of anaphylaxis (also fatal) or anaphylactoid reactions

Foods

Allspice
Almond [68, 185]
Anise seed [196]
Apple
Artichoke
Avocado [147]
Baker's yeast
Banana [76, 173]
Barley [145]
Bay leaf
Beet [24]
Black pepper
Brazil nut[a] [7, 22, 65, 68, 124, 147]
Brewer's yeast
Buckwheat [51, 140]
Cantaloupe
Carrot [122]
Cashew nut [18, 124, 140, 147, 151, 185, 200]
Castor bean
Celery [196]
Chamomile [50]
Chestnut
Chicken [152, 163, 209]
Chicken soup [168]
Chicory
Chili
Chocolate
Cinnamon
Clam[a] [42, 49]
Clove
Coconut [203]
Cod * [161, 228]
Coriander [20]
Corn [50]
Cow's milk[b] [22, 24, 38, 65, 95, 119, 140, 145, 147, 161, 182, 185]
Cow's milk in ice cream [124]
Crab [196, 228]
Crustaceans [49]
Cumin seed
Cuttlefish
Dates [145]
Egg[b] [65, 124, 126, 140, 145, 147, 152, 161, 185, 228]
Fennel seed
Fig
Filbert [147]
Fish [22, 65, 140, 145, 147, 185, 224]
Flaxseed [196]
French beans
Garlic
Ginger
Goat's milk [15, 145]
Halibut
Hazelnut [147]
Honey
Hops [196]
Horseradish
Juniper berry
Kiwi [70, 79, 140]
Lentil[b] [124]
Lima bean
Limpet [11]
Lobster[a]
Lupine [140]
Mango [6, 137]
Millet [50]
Mixed foods[b] [124]
Mixed nuts [124]
Mushrooms [196]
Mustard [141, 161, 196]
Nutmeg
Oat [145]
Orange [50, 53]
Oyster
Pea [140, 214]
Peach [202]
Peanut[b] [9, 18, 22, 24, 30, 54, 65, 67, 68, 113, 124, 140, 147, 161, 173, 185, 228]
Pecan nut[a] [22, 68, 126, 147, 228]
Pine nut [70, 107, 145]
Pineapple [97]
Pistachio [22, 71, 185]
Pomegranate [70]
Poppy seed
Potato [124]
Psyllium seed
Raspberry
Raw potato [14]
Royal jelly [164]
Sage
Salmon
Sesame seed [98, 124, 140]
Sesame seed oil [45]
Shellfish [65, 185]
Shrimp [24, 54]
Soy [50, 65, 124, 185]
Spices [24]
Squash [24]
Squid [41]
Sunflower seed [10, 196]
Sweet potato
Tangerine [54]
Tapioca
Thyme
Tree nuts [65, 185]
Turmeric
Vanilla
Walnut[a] [22, 24, 68, 124, 143, 147, 185, 196]
Watermelon [147]
Wheat [50, 124, 140, 185]
Yeast extract [124]

Continuation see next page

Modified from [196] and updated [170, 185].
[a] More frequently causative.
[b] Fatal or near fatal [124]; see Table 20.1.
See Chap. 10.

Table 20.4. (Continued)

Additives
Yoghurt [124]
Amaranth
Brilliant blue
Coloring
Dill seed
Erythrosine
Indigotin
Na benzoate
Parabens
Ponceau red
Sulfites
Sunset yellow
Tartrazine

Modified from [196] and updated.
See Chap. 10.

(5 in Table 9.28 [34]), three apparently life-threatening events, and 13 systemic reactions (Tables 9.31, 9.32) and in babies in allergy prevention (Chap. 24). Indirectly implicated are drugs [18] and baby ointments [94, 115]. Pear, potato, soy, tangerine, beet, and chocolate have yielded no reaction to SPTs in 102 patients; moreover, it is instructive that food provocation tests have confirmed only 22% of food-positive SPTs [196] (Table 20.4). The literature reports four cases of anaphylaxis to soy, one every 22.3 years [39]. However, four asthmatic children [74], all highly peanut-allergic but none soy-allergic, apparently unknowingly ate foods containing 2%–7% of soy protein and died within 10–36 min from rapidly deteriorating asthma, probably connected with a peanut-soy profilin cross-reaction (Table 1.72) (only inhaled soy causes asthma) [39], as did two more children who ate foods containing peanut [74]. Two additional children with peanut allergy but not recognized as at-risk by school staff died after eating soy-containing foods [222]. Moreover, 9 out of 17 patients have unknowingly eaten foods that contained peanut, which had previously provoked reactions to them [27].

Table 20.5 [163, 222, 230] summarizes 20 severe reactions, also fatal and provoked by hidden sources, as emphasized in Table 9.34 and Appendix 9.10. Several episodes have threatened the life of some patients in a wholly unpredictable way, including unnoticed ingestion of nuts in young allergic men [223], or a cookie containing pine nut butter, an IgE-mediated allergy to pine nuts that had never been ingested before [107], and chocolate, an unsuspected source of reactions if it contains peanut paste as a hidden allergen [86]. Children with CM allergy (CMA) have experienced acute, even severe, reactions due to the *unspecified presence of sodium caseinate in products labeled as non-CM-based*, because the FDA (Food and Drug Administration) allows labels not to specify CM, since it is considered a natural flavoring [81]. In Spain it is an authorized additive [131]: this is the cause for the unsuspected presence of CM in numerous meals [81, 131]. Fifty severe reactions, often fatal, by *allergens hidden* in foods *guarantied as allergen-free* by manufacturers or vendors, or in unknowingly eaten foods containing the allergen that had previously provoked reactions are outlined in Table 20.6 [9, 18, 22, 30, 56, 61, 69, 74, 86, 95, 124, 134, 147, 163, 195, 229]. In 1999, the death of a CM-allergic young man who ate an Easter egg made with dark chocolate was reported by the Italian daily press, as he believed that it was CM-free. In 1993, the English press reported that a peanut-allergic girl died after eating a biscuit at school, and in 1996 a 26-year-old with an acute fish allergy was assured by the manager that nothing in the appetizers had seafood in it, but on eating just three small spring rolls the man collapsed and died half an hour later in hospital [221]. In 13 cases of anaphylaxis, all were attributable to masked allergens [171] (Tables 20.5, 20.6). The case of an 18-month-old boy who experienced three systemic allergic reactions at home after eating a few spoons of mashed peas is also remarkable. But when a challenge test was performed in the hospital with the same mashed peas, although the child ate three spoonfuls willingly, he soon collapsed and died despite emergency treatment [214].

Potentially lethal food substitutions include mustard [99], buckwheat, peanuts and Atlantic pollock (sometimes used in surimi) for more expensive almonds and crab meat, respectively [36], nuts contained in foods and cakes, or unspecified sesame seeds [98]. On May 23, 2004, a 12-year-old girl died in northern Italy while eating a cake made with nuts, unknowingly to this severely nut-allergic girl. On June 12, 2004, a 9-year-old boy severely allergic to CM died in Rome after having touched drops of CM spilled from a pizza in a pizza parlor where he went with his classmates (daily press reports). Similarly, a CM allergic boy experienced systemic anaphylaxis after eating a pizza *declared as cheese-free*. Investigations showed that the pizza-maker added a small amount of CM to the pizza dough [119]. A young man allergic to chicken meat suffered from anaphylaxis when he ate white sausages made not with pork but with chicken meat: the butcher admitted the adulteration. Clearly chicken is a food and he transgressed no law; *however*, *he should have declared the change on the label* [163]. A peanut-sensitive young woman ordered a beef burger but was served a vegetable burger mistaken for a beef burger and after taking a bite she was hospitalized. Vegetable burger and beef burger have a similar appearance and presentation, but a beef burger is a kind of minced meat, whereas a vegetable burger should be prepared only with vegetables, but in this case it contained peanut seeds [61]. In 50% of cases, the food item was hidden (in sauces, dressings, egg rolls, etc.); visual identification was thus impossible [99, 138, 222]. Repeated anaphylactic episodes occurred in a child between the ages of 16 months and 4 years because of whey being mistakenly used in a *pareve cookie* instead of soy protein [73], and in a CM-allergic girl for parme-

Table 20.5. Twenty hidden allergens and foods containing them that have provoked severe reactions in sensitized children aged 3–15 years in an interval of 3 years

Food	Allergen detected	Concentration (%)	Background
Chocolate cake	Hazelnut	0.2	Undeclared
Meatballs	Egg (ovalbumin)	0.14	Undeclared on the label, recipe changed
Pasta	Egg (ovalbumin)	<0.013	Undeclared in 19/76 commercial products
Meatballs	Egg (ovalbumin)	0.16	Undeclared
	Egg (ovalbumin)	1.1	Undeclared
Ice cream (soybased)	CM (casein)	0.2	Contamination by previously processed ice cream
Plain chocolate	CM (casein)	0.8	Contamination by previously processed CM chocolate
Lollipop, strawberry	CM (casein)	0.2	Undeclared
Sausage	CM (casein)	1.0	Contamination
Sausage	CM (casein)	0.06	Contamination, fatal anaphylaxis after eating 1 kg
Hot dog	CM (casein)	0.04	Contamination
Ham	CM (casein)	2.5	Added as binder; undeclared
Meringue	CM (casein)	1.1	Undeclared
Buckwheat flour	Wheat gluten	1.3	Undeclared wheat flour added (15%–25%)
Whole pasta, wheat-free	Wheat gluten	7.8–11.9	Falsely declared as wheat-free
Corn pasta, wheat-free	Wheat gluten	8.3	Contamination
Hamburger	Soy protein	2.1	Declared but not recognized as risk by school staff (child with peanut allergy)
Kebab	Soy protein	7.0	Declared but not recognized as risk by school staff (child with peanut allergy)
Ham roll	Chicken proteins		Meat other than pork not allowed in the product; casein and soy declared additives
Cake	Peanut protein		Undeclared, believed to be almond, anaphylaxis
Crab sticks	Soy protein	0.5–0.9	Undeclared, not recognized as risk, anaphylaxis

Crab sticks (surimi) produced in China and Korea contain soy protein in the majority of cases.
Data from [163, 222, 230].

Table 20.6. Fifty additional pediatric cases reported in the literature

Food	Hidden allergen detected	References	Background
Acme tuna	Na caseinate	[69]	Undeclared, no indication
Almond flakes	Peanut protein	[74]	Replaced by peanut flakes by the baker, fatal case
Almond icing	Peanut protein	[56]	Unidentified, fatal case
Biscuit	Peanut protein	[134]	Unidentified, fatal case
Bologna	Na caseinate	[69]	Undeclared, no regulations[a]
Burrito	Peanut?	[22]	Unknown presence of allergen, fatal case
Cake	Nuts	[124]	Undeclared in a commercial food
Cake	Peanut	[22]	Unknown presence of allergen, fatal case
Cake	Peanut butter	[195]	Undeclared, fatal case
Cake	Peanut protein	[9]	Unidentified
Cake	Peanut protein	[18]	Undeclared, fatal case

Table 20.6. (Continued)

Food	Hidden allergen detected	References	Background
Cake	Pecan	[30]	Undeclared, fatal case
Cake/pastry	CM	[147]	Unknown presence of allergen
Cake/pastry	Filberts	[147]	Unknown presence of allergen
Cake/pastry	Filberts	[147]	Unknown presence of allergen
Candy	Cashews	[147]	Unknown presence of allergen, fatal case
Candy	Cashews	[147]	Unknown presence of allergen, fatal case
Candy	Peanut	[147]	Unknown presence of allergen, fatal case
Candy	Peanut	[22]	Unknown presence of allergen, fatal case
Candy	Walnut	[22]	Unknown presence of allergen, fatal case
Candy bar	Peanut	[22]	Unknown presence of allergen, fatal case
Cereal	CM	[147]	Unknown presence of allergen
Chili dish	Peanut butter	[195]	Undeclared, fatal case
Chili sauce	Peanut protein	[30]	Undeclared thickener, fatal case
Chocolate	Peanut protein	[86]	Unidentified, fatal case
Cookie	Brazil nuts	[147]	Unknown presence of allergen
Cookie	Peanut	[22]	Unknown presence of allergen, fatal case
Cookie	Peanut	[147]	Unknown presence of allergen
Cookie	Peanut protein	[195]	Unidentified, fatal case
Cookie	Walnut	[22]	Unknown presence of allergen, fatal case
Cookie	Walnut	[147]	Unknown presence of allergen
Cupcake	Peanut	[147]	Unknown presence of allergen, fatal case
Dessert	Nut	[22]	Unknown presence of allergen, fatal case
Dinner	Pecan	[22]	Unknown presence of allergen, fatal case
Easter egg	CM	[b]	Undeclared, fatal case, see the text
Egg roll	Peanut	[22]	Unknown presence of allergen, fatal case
Frozen dessert (2 cases)	Na caseinate	[69]	Undeclared, no regulations[c]
Hamburger roll	Egg	[147]	Unknown presence of allergen, fatal case
Hot dog	Na caseinate	[69]	Undeclared, no regulations[a]
Ice cream	CM	[124]	Contamination of previously processed ice cream
Ice cream	Nut	[124]	Contamination of previously processed ice cream
Lunch	Fish	[22]	Unknown presence of allergen, fatal case
Mixed nuts	Brazil nut	[22]	Unknown presence of allergen, fatal case
Nuts	Pistachio	[22]	Unknown presence of allergen, fatal case
Raspberry sorbet	CM	[95]	Labeled as "Kosher-pareve, dairy-free"
Sandwich	Peanut	[147]	Unknown presence of allergen, fatal case
Sausage pizza	Soy protein	[229]	Undeclared, fatal case[d]
Sausage roll	Chicken	[163]	Undeclared, no regulations
Snack mix	Peanut	[22]	Unknown presence of allergen, fatal case
Vegetable burger	Peanut seeds	[61]	Believed to be a beef burger

CM cow's milk.

[a] Foods labeled as "CM-free."

[b] Reported by Italian daily press in 1999.

[c] Label mentioned "natural flavorings."

[d] See text.

san cheese accidentally falling into her dish or eating a roll cut with the same knife used for butter [144]. An egg-sensitive infant developed severe generalized urticaria after playing with a pan that had just been used to prepare an egg-containing dough [12]. A child we have treated was hospitalized for anaphylactic shock after having mistakenly eaten a few drops of CM, to which he is severely allergic.

A survey on the management of anaphylactic reactions has revealed unexpected contamination during cooking (with a spoon or a spatula with food residues) and reactions occurring at schools, restaurants, or at home [65]. A CMA child was kissed by an adult who had eaten a pizza containing cheese, and another child, allergic to nuts, was kissed by another child who had eaten walnuts [65]. A girl with CMA seen by us developed angioedema where she was twice kissed by her brother, while eating a CM ice or drinking a cup of milk. An uncommon reaction happened in a playground due to contact with a football touched by another child who had just eaten fish [65]. Additional severe accidental reactions were caused by a peanut-free ice-cream stored in a container previously used for peanuts, and eating a banana that had been stored next to an open peanut bin [65]. David has reported unpredictable reactions during food reintroduction into a diet [50] and Bock several cases also in 3- to 4-year-old children due to peanut, walnut, shrimp, spices, and to sulfite-containing foods such as catsup and vinegar [24]. In Tables 20.5 and 20.6, 70 cases are summarized: 23 (32.4%) to peanuts, 17 (23.9%) to nuts, 13 (18.3%) to CM, and five to eggs (7.0%), but in the 17 children, peanut and nuts accounted for 15 (88.2%) deaths [27]. Indeed, commercial catering caused 76% of food-related reactions [159].

Unsuspected allergens are responsible for cases of anaphylaxis, including mango [6, 137], royal jelly [31, 164] with a fatal case in one girl [31], *Litchi sinensis* [69], kiwi [70, 79, 140], coconut [203], avocado [171], heated pecan nuts [126], lupine [140], raw potato [14] and anaphylaxis triggered by cream puffs contaminated by *Der farinae* in an adult [66] and a girl [193]. Three incredible cases caused by CM-inhalation occurred, once when a young woman with CMA walked into a grocery store where CM was sold, and she died after anaphylaxis by inhalation of CM protein, as reported in 1992 by the Italian daily press; an 18-year-old girl severely allergic to long-term eliminated CM inadvertently entered a dairy shop where she inhaled CM proteins and experienced a fatal reaction [13]; and a child had repeated episodes of acute bronchospasm, also by CM inhalation, that were so severe that his younger nonallergic brother had to follow the soy-based diet of his elder brother [166].

Sulfites and other food additives (Table 10.3) may induce severe anaphylaxis: the Na metabisulfite sometimes found in epinephrine formulations [123] (Table 19.32), in certain corticosteroids (CS) and other drugs is puzzling (Fig. 10.3, Table 19.32).

Exercise-Induced Anaphylaxis

EIA was first described 18 centuries ago, and recognized as a clinical entity between 1970 and 1980, and later the relationship between sporting or agonistic activity and inciting factors such as EIA occurring postprandially was soon evidenced (thus entering the *differential diagnosis with food anaphylaxis*) (Fig. 20.1). The surge in incidence is due to enhanced popular commitment to physical fitness including jogging, running, sprinting, volleyball, basketball, tennis and soccer, skiing and aerobic exercise [200]. Cases are known in 10-year-old children, but have been observed even in children of 4.5 years [112]. Predisposing factors include family history (FH) [121], atopy [91, 145], heat (64%) or cold (23%) ambient high humidity (32%), and drugs, for example ASA (13%) [91]. EIA can be associated with cold, so exercise in warm environments, or exposure to cold with no activity fail to elicit clinical reactions; some patients manifest symptoms of cholinergic urticaria,

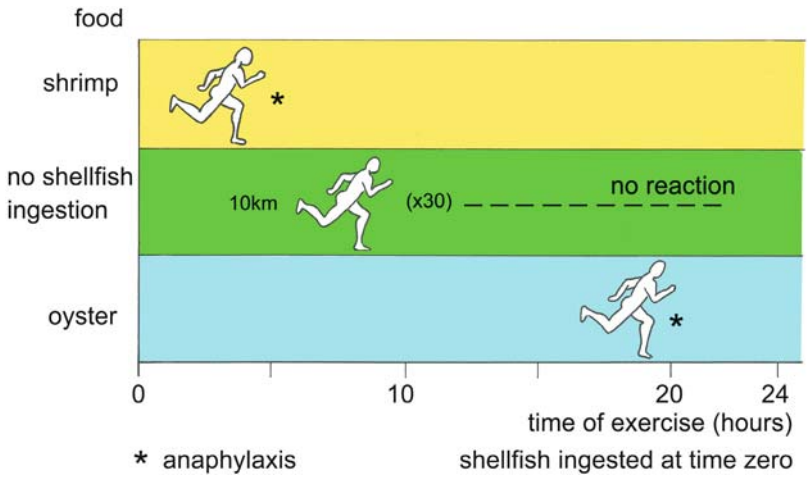

Fig. 20.1. Exercise-induced anaphylaxis associated with food ingestion. The runner with anti-shrimp IgE antibodies had severe anaphylaxis during exercise only after eating shellfish

viewed by some investigators as a less severe form than EIA [91]. EIA typically is heralded by the onset of *premonitory symptoms,* including asthenia, pruritus, warmth sensation, flushing, followed by the development of angioedema, urticaria and gastrointestinal symptoms such as abdominal cramps, nausea, vomiting, then, in the blown-up phase, progressing to wheezing, profuse sweating, vascular collapse and transient loss of consciousness [91]. In 3/4 of children aged 7–11, there was a food-associated EIA (FEIA), but a generalized pruritus was premonitory in only one [35].

Table 20.7 [43] sums up the symptom prevalence depending on the association either with exercise or with FA. Urticaria (80%–85.7%) and angioedema (48.6%–64.3%) dominate up to a real pattern of shock in 40%–43% of cases. The statistical analysis is very significant for warmth and flushing, which make cutaneous symptoms distinctive of non-FEIA, in which hypotension is significant [43]. The timing is characteristic, that is the interval between the start of exercise and the onset of anaphylaxis: the quickest onset of EIA was 5–20 min [112] and the longest 3.5 h [200], up to 25–72 h (if late reactions are operative) after eating the suspected food [91], although food ingestion not followed by exercise does not provoke any symptom (Table 20.8) [213]. The meal prerequisite may not be operative, or is operative without the food specificity, or ingestion of food(s) triggering the reaction (not followed by exercise) does not provoke any symptom; otherwise it is sufficient to *only view* the food [60]. The most common causative foods are shellfish and shrimp [60, 135, 200, 216], orange [53], chestnut [35], wheat [5, 60, 216], hazelnuts [130], tomato [35], rice [35], peach, celery, egg, grapes [43, 91] and CM derivatives [205]. The cross-reactivity between latex, fruits and foods (Table 8.14) may amplify the avoidance of foods. The incidence of FEIA has been studied in Japan in a survey of 11,647 children with 0% in kindergarten, 0.06% in elementary schools and 0.21% junior high school [200]. Among 76,229 junior high school students, the frequency was 0.017% and was significantly higher in boys than in girls. The frequency of EIA was 0.031% with no sex difference [1]. Cases precipitated by ASA [60] and NSAIDs [208] ingested before exercise are known. Related to pathogenesis, in some skin biopsies varying stages of *mast cell degranulation* were reported: ultrastructural evidence shows that before exercise mast cells are indistinguishable from those of control subjects, but immediately after exercise the loss of granule density is indicative of degranulation [180]. The data lead to speculation that a food-induced IgE-mediated reaction may be associated with specific food allergens [104]. It is likely that elevated plasma histamine [175] and tryptase levels further document that mast cells play a central role in EIA pathophysiology [175]. Several factors propitiate non-immunological mast cell degranulation or alter its threshold, namely neuropeptides, endogenous opioids, cytokines and chemokines [91]. The precise sequence of events has been less explored; however, the possible influence of genetic susceptibility to allergic disease may be an area of increasing interest in EIA pathogenesis [121]. In one family, two siblings shared an HLA haplotype with their atopic father. Both siblings developed EIA during running, but not during cycling, swimming, and on sun or cold exposure, they had no food-related symptoms. It is of note that all three individuals shared the *A3-B8-DR3* haplotype, thus suggesting heritable predisposition for EIA in some patients and families [121].

Table 20.7. Exercise anaphylaxis: clinical presentation

Symptoms	Exercise (n=35) (%)	Food-dependent (n=28) (%)
Abdominal pain	14.3	14.3
Angioedema	48.6	64.3
Asthenia	42.8	25.0
Conjunctivitis	2.8	0
Cough	5.7	10.7
Cutaneous warmth	48.6	3.6
Diarrhea	14.3	25.0
Dizziness	8.6	10.7
Flushing	68.6	35.7
Headache	11.4	7.1
Hypotension	17.1	35.7
Laryngeal stridor	40.0	28.6
Nausea	0	7.1
Pruritus	71.4	50.0
Shock	40.0	42.9
Syncope	2.8	3.6
Urticaria	80.0	85.7
Vomiting	14.3	7.1
Wheezing	34.3	17.9

Modified from [43].

Table 20.8. Exercise anaphylaxis subtypes

Subtypes	Characteristics
Classic	10–15 mm urticaria
Variant	1–3 mm urticaria
Familial	HLA haplotype correlated
Food-dependent	After any food eaten
Food-specific	After food allergen only

Modified from [213].

Anesthesia Anaphylaxis

Type I, II and IV immune mechanisms are operative. Clinically, toxic responses may be experienced by normal subjects (local, systemic) or by susceptible subjects (allergy, idiosyncrasy) [102, 210].

Anaphylactic reactions to local anesthetics have been reported. The prevalence of induced reactions follows [102, 210]:

- Barbiturates:
  Methohexital (1:700–23,000)
  Thiopental (1:1,400–30,000)
- Non-barbiturates:
  Etomidate (1:2,200–500,000)
  Propanidid (1:500–5,000)
  Propofol (rare, unknown)

The reactions are more frequent in allergic subjects than in other patients: bronchospasm, 43.5%–28.5%; cardiovascular symptoms, 82.6%–78.6%; and cutaneous reactions, 82.6%–57.1%. In allergic subjects muscle relaxants are also implicated in 75% of cases of shock. Elevated tryptase and histamine serum levels have not been considered as diagnostic [85]. Humoral immune responses are rare [78]. In some cases, vasoconstrictors and preservatives (parabens, sulfites) have been implicated [102], however not in patients subjected to challenge [78]. Local anesthetics employed in dentistry frequently provoke local and systemic reactions, rarely allergic [78], and are often complicated by latex gloves [132].

Muscle Relaxant Anaphylaxis

Muscle relaxants are widely utilized in operating rooms, making it possible to reduce the anesthetic dose, thereby facilitating surgeons' work [20]. They account for 70%–80% of all the allergic reactions occurring during anesthesia, 80%–95% of which are in females [102], also in girls of 5.5 years [133]. The prevalence of anaphylactic shock is 1:4,500 anesthesia doses given, and 6% of accidents are fatal [207]: alcuronium, 7.6%; atracurium, 6.8%; gallamine, 5.6%; pancuronium, 13%; succinylcholine, vecuronium, 37%; suxamethonium, 43%, etc. [20]. Between 1988 and 1992, suxamethonium caused 48% of severe reactions [217]. IgE-mediated reactions, positivity of Prausnitz-Küstner tests and RAST and nonimmunological release of histamine have been reported [19]. Studies with RAST inhibition have detected IgE antibodies directed to tertiary or quaternary ammonium ions present in muscle relaxants, thus a shared ammonium group may cause *cross-reactivity* among the various agents [102]. Since most of them share *two ammonium ions*, they are functionally divalent and therefore able to establish cross-linking cell-surface IgE from one side and to induce mediator release from metachromatic cells from the other [102], so binding to a carrier is not necessary to cause reactions. Cross-reactivity is common to all compounds provided with tertiary or quaternary ions such as the preservative benzalkonium chloride [192]. Muscle relaxants are widely diffused in drugs, foods, cosmetics, disinfectants and industrial materials, so patients are possibly sensitized to such compounds also via environmental contacts. Their very high prevalence in females may suggest that reactions are elicited by exposure to ammonium ions with epitopes present in cosmetics and the like [102].

Antibiotic Anaphylaxis

Antibiotics causing anaphylaxis (penicillin and β-lactam ranking first) [183] are outlined in Table 19.9, including two cases of anaphylactic shock provoked in surgical cases by local administration of rifamycin [40]. Penicillin can be detected in drinks and in frozen meat, as well as in CM as a contaminant (Table 19.36), so allergic subjects may manifest an anaphylactic shock while drinking a cup of CM containing even minimal amounts of penicillin and be labeled as CM-allergic [183]. Considering the huge number (in geometrical progression) of antibiotic prescriptions written on a yearly basis, it is understandable that the anaphylactic episodes provoked by antibiotics is a substantial challenge at a world level. In Chap. 19 we reported two fatal cases and three of anaphylaxis to ceftriaxone. Non-β-lactam antibiotics are also responsible for reactions (aminoglycosides, metronidazole, sulfamethoxazole, trimethoprim and vancomycin) [183].

Hormone and Enzyme Anaphylaxis

These substances, acting as complete antigens (Table 19.4), cause anaphylaxis and other clinical manifestations based on a mechanism that is almost always IgE-mediated.

Hormones

ACTH and Synthetic Corticotropin. The incidence of reactions (urticaria, angioedema, maculopapular erythema, wheezing, anaphylactic shock) was considerably reduced by the introduction of corticotropins. Synthetic derivatives have a different spatial configuration which makes them less allergenic.

Calcitonin. The reported incidence varies from 0.00003% to 6%, clinical manifestations are cutaneous and respiratory.

CS reactions are immediate (shock, cardiorespiratory arrest, bronchospasm, worsening of ongoing asthma, pruritus, urticaria-angioedema), and delayed (allergic contact dermatitis). The prevalence of IgE-mediated re-

actions is very low; it is more frequent to find pseudoallergic reactions by preservatives, excipients, contaminants such as succinate, with one fatal case occurred a few minutes after intramuscular injection of methylprednisolone [157], implicated also in hydrocortisone medication.

Insulin. Local reactions (immediate or delayed) may involve 5%–15% of patients, SRs (in addition to shock, mostly urticaria-angioedema and wheezing) only 0.1%–2% of patients. In the pathogenesis, immune mechanisms of all four types of reactions are implicated, in particular Arthus-like reactions by anti-insulin IgG antibodies and cell-mediated, especially to Zn, which is included in non-insulin contaminants along with protamine and proinsulin.

Enzymes

Chymopapain. Currently seldom used, chymopapain is injected intradiscally to remove the mucopolysaccharide–protein complexes of the nucleus polposus of prolapsed intervertebral discs (chemonucleolysis). Reactions upon the first injection involve 40% of cases, upon the subsequent injection 9% are involved; the incidence of severe anaphylactic reactions is 0.18% and of fatal cases is 0.01% [102]. Since this enzyme is present in several foods and various substances (Appendix 8.2), everyone is potentially at risk of sensitization from the first meal with meat or fruit juices.

Aprotinin. Aprotinin is widely used in the treatment of extensive burns. The reactions – anaphylactic, respiratory, and cutaneous – have a mean incidence of 1% (0.72% upon the first and 9.1% upon subsequent administration) [210].

Streptokinase. Streptokinase is used as a thrombolytic agent. Anaphylactic reactions, including delayed reactions, have an incidence of 1.7%–18% [210].

Plasma Expander Anaphylaxis

Use of plasma expanders has dramatically increased following the great concern brought about by HIV-infected blood. The incidence of anaphylactic and anaphylactoid reactions to dextran ranges from 0.008% to 0.069% of administrations, to fluid gelatins from 0.03% to 0.14% [162], which cross-react with food gelatins [215], to plasma protein solutions from 0.02% to 0.25% [102, 162], and to hydroxymethyl starch the incidence is 0.08% [116]. In patients with anaphylactoid reactions to dextran, the incidence of shock can be as high as 15.9%; cases of anaphylactoid reactions to serum albumin have a 0.012% incidence [162].

Latex-Associated Anaphylaxis

The cases of latex-specific IgE-mediated hypersensitivity reactions are increasing [21, 223], from 0.5% in 1989 to 12.6% in 1993 [114], mostly because of a rise in the demand for latex gloves [197] following the HIV epidemic [116]. The pertinent allergic pathogenesis is analyzed in Chap. 8. Among the several published cases of anaphylaxis chiefly in atopics [148], there are 25 episodes in children affected with spina bifida or urinary malformations exposed to latex catheters [62, 102, 197]. The risk factors are numerous: spina bifida (28%–57% of cases) [20, 62], contact with latex, FA and cross-reactions with fruits (Table 8.14), anesthetic induction [102], operations [62, 133], atopy and children, because 80% of intraoperative cases are due to latex exposure [198]. In 12 cases, anaphylaxis began 5–30 min after the start of anesthesia preparation before the operation [102]. An additional 12 cases were induced by intraoperative contact with latex gloves [62], as well as a very severe case in a 14-year-old girl [197], and in a girl who was 10 months old due to a pacifier [198]. Latex positivity has been documented by SPTs and sIgE, with the RAST better than with ELISA [102]. SPTs with pacifier eluate in a latex-sensitized adult resulted in wheals with a diameter of 5–6 mm [198]. Given the reactions induced by latex gloves, use of vinyl gloves is suggested [127]. In Chap. 8, we reported four children experiencing anaphylaxis while playing with plastic balls. In 15 of 23 (65.2%) hospitalized children [57], not only was latex the single most common causative agent, it was often overlooked in this role.

SIT and SPT Anaphylaxis

The incidence/prevalence is 0.03% in adults ($1{:}2 \times 10^6$ injections) and 0.0016% in children. The fatal cases – statistically nonexistent in children – could be $1{:}10^6$–10^7 injections in adults. Among the 47 cases reported before 1973 and up to 2001 (Tables 13.8–13.11) are seven children 7–18 years old. However, only two cases can be imputed to SIT. Two fatal cases to SPT were reported in the US in 1964, thus confirming that *children run no risk*. Two retrospective studies have been reported:

1. *SPTs:* six infants <6 months of age who suffered from generalized allergic reactions after duplicate and multiple prick tests with fresh foods whose generalized reactions responded promptly to epinephrine: the babies were breast-fed and were tested with several foods [56] and the hypersensitivity to food proteins ingested by their mothers is also known (Table 9.1). In a retrospective study on a larger series of patients (mean 30 years), six mild SRs occurred, with a total rate of 33 reactions per 10^5 SPTs, but no reactions to SPTs for food or venoms [206].
2. *SIT* (Tables 13.8–13.11). Among 95 children there was one case, or 0.3% cases/year [145] and 4 probable cases among 39 cases, or 1.3 cases/year [28].

Vaccine-Induced Anaphylaxis

Severe reactions to influenza, measles, mumps, rubella (MMR), pertussis, and tetanus vaccinations have been reported [48, 225], including in an infant of 6 months [63]. Two children aged 4.5 and 4 years had anaphylaxis after MMR vaccination but recovered promptly [145]. In Finland, for over 15 years 18 cases of suspected anaphylaxis were reported in children aged 5 years 11 months (median) [125]. Subsequently, a study was done on the serum samples of 18 vaccinees; however, for ten of the 18 patients (55.6%) with reported anaphylaxis, only three received epinephrine [152] with three more cases of anaphylaxis in non-egg-allergic children; however, one of them had IgE binding to gelatin [152], so in fact there are two cases. The anaphylaxis to MMR vaccination is caused by other components of the vaccine, including gelatin, more and more frequent in vaccinations [92, 144, 152, 169] and neomycin, but not to egg protein or to egg allergy [48].

Age-Induced Anaphylaxis

Children have a greater body surface than adults in proportion to their weight, so children disperse a higher quantity of fluids, which requires a greater administration of fluid replacement. A recent study has shown that *the 0–4 age range* suffered anaphylaxis in 20.5% of cases; however the age range of 10–14 years is more at risk (31.8%) [28]. See Table 20.3: *infants (<1)* had food-induced emergency anaphylaxis admissions in 62% and preschool (1–5) in 48% of cases [2]. Even an infant *aged 1 month* suffered from anaphylaxis [145].

Anaphylactoid Reactions

Complement Activation

The most frequent complement reactions are hemolytic ABO-incompatible blood transfusion reactions either during blood transfusions or IV immunoglobulin (Ig) administration to subjects with selective IgA deficiency or an IgG subclass, who may have pre-existing IgG anti-IgA antibodies, so IgA/IgG-anti-IgA complexes can activate complement by the alternative pathway, resulting in C3a and C5a release. These anaphylotoxins contract smooth muscles, causing alterations in vascular tone, an increase in vascular permeability and bronchial constriction [136, 204, 219].

ASA and NSAIDs

ASA and NSAIDs, inhibiting cyclooxygenase enzymes of the arachidonic acid cascade, block prostanoid formation and, acting as haptens, cause hypersensitivity reactions (Table 19.9), even systemic anaphylactoid reactions, without differences in clinical features in healthy subjects and in patients with mastocytosis, while NSAIDs cross-react with ASA [195]. ASA 30 min after being ingested by a boy, together with a cake containing peanuts, potentiated the effect of peanut allergen possibly by increasing intestinal permeability, thus aggravating the food reaction to reach anaphylaxis [37].

Iodinated Radiocontrast Media

The general incidence of iodinated radiocontrast medium reactions is ≈4.5%–8.5% of procedures [210]. It is relatively rare in pediatrics, involving above all patients aged >20 years. About 68% of reactions occur in atopic subjects and 56% react positively to SPTs vs 30% and 24% of control patients, respectively [120]. Furthermore, atopics are predisposed to experience reactions to conventional compounds of higher osmolality compared with those of lower osmolality that are nonionic with a 3.5:1 ratio [120], corresponding to 4:1 of non-atopics in a study on 337,647 cases, with a reciprocal risk (RR) of 5.5:1 [100]. The results of a meta-analysis show a RR of 6.3:1 [33]. A study in children aged >10 contests that lower osmolality radiocontrast media are better tolerated [100]; however, *a 15-month-old-girl* has experienced a reaction [17]. In asthmatic patients, 12% exhibit reactions vs 4% of controls [188]. The pathogenesis is reported in Chap. 19. These media can also contribute to anaphylaxis, via histamine and tryptase release, inhibition of platelet activity, serotonin release by platelets, enzyme inactivation, activation of the prekallikrein-kallikrein system, hypocalcemia, alterations in myocardial conductivity and contractility, and lesions of the vascular epithelium [84, 120].

Others

Additives

A severe anaphylactic reaction occurred in an 11-year-old girl with AR (allergic rhinitis) a few minutes after eating a slice of mortadella that contained Na glutamate to which the girl reacted in a DB challenge [145]. With the increasing use of additives in commercially prepared foods [81], it is likely that the prevalence of severe food-induced anaphylactic reactions will continue to rise. Chapter 10 reports the recent introduction of additives in foods for infants, toddlers and young children and three anaphylactic reactions in children to fluorescin, sunset yellow, and tartrazine.

Aeroallergens

A 6-year-old girl, with a history of atopic dermatitis and signs of respiratory allergies, presented an episode of anaphylaxis after 10 min of snow sledding. Her

symptoms included sneezing, runny nose, blushed skin, edematous lips, dyspnea. Clinically, the girl had tachypnea, tachycardia (110/min), blood pressure (BP) of 80/50 mmHg, and wheezes and crackles in the lungs. Both SPTs and RAST were positive to horse, cat and dog dander. Repeated exposure to horse allergen when the girl visited a stable elicited early asthmatic response, as confirmed by spirometry [80]. A pollen-sensitive boy we have seen experienced a severe laryngeal edema when entering a home where a dog was present or had gone outside before his entrance.

We have seen uncommon cases of anaphylaxis triggered by pollens in highly pollen-allergic children including a boy touching a blessed olive-branch on Palm Sunday: he needed an urgent ED admission. The cross-reactivity between foods and aeroallergens finds its most surprising example in the mite-snail cross-reactions (Table 1.73). Similarly, peanut- and Bet v-sensitive children should strictly avoid soy ingestion.

Clinical Presentation

Anaphylactic reactions have a broad spectrum of possible clinical pictures and are almost always alike, regardless of specific causes in the background. Characteristic symptoms may manifest in different organs or organ systems; however, the degree of involvement of target organs can vary from one child to another, from mild to moderate intensity to severe manifestations, from a simple pruritus to an irreversible shock and to fatal respiratory insufficiency [103, 188] (Table 20.9 [28, 57, 136, 185] and Table 20.10 [55, 177]). The macroscopic findings of 56 cases included signs of asthma (mucous plugging and/or hyperinflated lungs) (26.8%), petechial hemorrhages (17.9%), pharyngeal/laryngeal edema (41.1%) [160], at variance with [55]. The most common reported finding was nonspecific pulmonary congestion and edema, which was present in 41 (73%) cases [160]. Anaphylaxis often produces signs and symptoms within a few minutes of allergen exposure, but late-phase reaction can occur 2 [171], 14 [212], 24 [171], and up to 32 h after onset [103, 194]. The more rapid the onset, the more severe the episode. Once started, the reaction evolves in a dramatic way, the latency time varies from a few seconds to 1 h, commonly <10 min [157]. More precisely, the time needed was <5 min in 21.2%, 5–59 min in 37.7% and 1–4 h in 8.2% of cases [28], 15.4±27.5 min [145], 19 min [171] and 59 min [57]. All of the organs or organ systems can be involved, both singly or in combination [129].

Before the onset of full-blown features of anaphylaxis, some patients complain of *early prodromic symptoms*, which may warn of impending severe reactions, including deep asthenia, malaise, anxiety and/or psychomotor unrest, profuse sweating, etc., or warmth sensation, pruritus, tingling and a metallic taste in the mouth followed by development of rhinorrhea, hoarseness, dysphonia, dyspnea, cyanosis, beginning from the head (scalp, external auditory meatus, lips), with vertigo, headache or from the trunk, spreading centripetally down to the genital region and the tips of the toes [179]. Usually, initial symptoms progress rapidly, associated with extensive cutaneous flare, urticarial manifestations, angioedema, nasal congestion, aggravated with nausea, abdominal pain, vomiting, agitation, loss of consciousness, tachycardia, bradycardia, and hypotension. *Food anaphylaxis* manifests with oral pruritus, a sensation of swollen lips and tongue and throat constriction, urticaria, angioedema, nausea, abdominal pain, vomiting, diarrhea, laryngeal stridor, dyspnea and wheezing [28].

In terms of their spread, reactions may be local and systemic [44] (see Table 17.5):

- *Local reactions* often affect the first site of exposure to the offending antigen, or the gut with angioedema in case of food anaphylaxis.
- *Systemic reactions* affect one or more organ systems with frightening rapidity.

According to statistics, urticaria-angioedema occurs in 79% of patients, dyspnea or wheezing in 70%, and hypotension and cardiovascular reactions in 24%. Respiratory complications are associated with most fatal cases. The most frequently involved organs are the skin and respiratory and cardiovascular tracts, less often the gastrointestinal tract [57].

The clinical signs of full-blown anaphylaxis, in accordance with *severity*, can be mild or severe [129, 136, 179]:

- *Mild systemic reactions* (usually with late onset) include urticaria with or without swelling, limited to the area of antigen exposure; other manifestations such as nasal obstruction, rhinorrhea, etc., are absent or are present in mild form, which does not always remain mild [187].
- *Severe symptoms* are generalized, affect organs or systems, often accompanied by hypotension or respiratory obstruction. Such polymorphous features express the contemporaneous and tumultuous compromise of varying localizations with involvement of several target organs.

Clinical features related to the organs or organ system involved [44, 57, 106, 129, 136, 179, 185] (Table 20.9) are discussed in the following sections.

Skin and Soft Tissues

The skin is the most common target organ and usually the earliest involved. An initial pallor may become flushing (cutaneous vasodilation); often a discoloration to deep red or purplish may herald impending shock. The skin is cold with cyanosis of limbs, if respiratory obstruction coexists. Urticaria (increased vascular permeability) and pruritus affect the lips, nose, periorbital region, ears, hands and feet, whereas angioedema is common in the periorbital areas and mucosa of the oral cavity, up to glottis edema.

Table 20.9. Symptoms and signs of anaphylaxis related to target organs involved

System/target organs	Clinical manifestations
Cutaneous (93%)	Cyanosis (5.9%), pruritus (54.1%), flushing (48%), urticaria-angioedema (88%), urticaria (56.5%), angioedema (51.8%), erythema (44.7%)
Eyes	Lacrimation, periorbital pruritus (4.5%), conjunctival injection (7.1%), edema
Nasal	Sneezing (3.5%), rhinorrhea (8.2%), pruritus, congestion
Oral	Pruritus of lips, tongue, and palate, edema of lips and tongue, metallic taste
Upper airways	Pruritus, dysphagia, dysphonia, hoarseness, stridor, glottis edema (3.5%–56%)
Lower airways	Dyspnea (55.3%), wheezing (47%–48.2%), tachypnea, bronchorrhea, respiratory arrest
Cardiovascular (21.2%–26%)	Tachycardia (9.4%), arrhythmias, hypotension (9.4%–33%), chest pain, cardiac arrest
Gastrointestinal (13%–22.4%)	Nausea (9.4%), vomiting (10.6%), heartburn, cramping (7.1%), diarrhea (1.2%), abdominal pain and/or distension, fecal and urinary incontinence
Neurological (26%)	Headache (15%), dizziness (33%), seizure (1.5%), syncope (4.7%–33%)

(%) = incidence of systemic symptoms in children from [26, 57]; respiratory symptoms = 93% or 88.2%.
Data from [136, 185].

Table 20.10. Pathological features in two series of fatal cases

System/target organ	43 cases [55] (%)		113 cases [177] (%)
Lower airway		Respiratory tract	88.4
Pulmonary congestion	90		
Pulmonary edema	50	Central nervous system	59.2
Intra-alveolar hemorrhage	45		
Tracheobronchial secretions	45	Liver	53.0
Mild	12.5		
Moderate	22.5	Kidney	51.3
Severe	10		
Acute pulmonary emphysema	27.5	Spleen	41.5
Diffuse	10		
Focal	17.5	Heart	33.3
Upper airway			
Laryngeal edema	37.5	Gastrointestinal tract	26.5
Mild	12.5		
Moderate	15	Serosae	23.8
Severe	10		
Spleen		Apparent absence of lesions	4.4
Eosinophilia	22.5		

Respiratory System

Respiratory manifestations along with the skin account for an earlier onset of anaphylaxis and are more common than the gastrointestinal and cardiovascular manifestations in children (78% and 79% vs 24% and 25%, respectively) [145] and are responsible for the majority of fatal cases (≥50%) (Table 20.10). It is likely that such cases occur for both pre-existing hyperreactivity and lacking response of β-receptors. Nasal congestion caused by edema and hyperemia of nasal mucosa, watery and profuse rhinorrhea and nasal and palatal pruritus may mimic an acute episode of AR. Laryngospasm, laryngeal edema, marked bronchospasm, bronchial obstruction, or status asthmaticus can lead to hypoxemia, hypercapnia, acidosis and death. With a partial obstruc-

tion of the upper airways, the patient may complain of fits of choking, hoarseness or repeated cough, stridor and glottis edema heralded by a choking sensation, as well as of retrosternal constriction and difficulty talking with hoarseness, of such a severity that a mechanical asphyxia is envisaged, while obstruction of the lower airways is characterized by bronchospasm, dyspnea and incoercible cough simulating an asthma attack. An obstruction by mucus hyperproduction may lead to respiratory insufficiency; however, if bronchospasm is exacerbated during the reaction, reversing the episode will be more challenging [171].

Cardiovascular System

Several of the mediators of anaphylaxis can adversely affect the vascular system by dilating arterioles and capillaries and increasing vascular permeability, so that it is the second most common cause of fatal events. The drop in BP (in hypertensive patients, the values drop to normal levels) makes patients tachycardic, agitated, anxious, and even a severe collapse may follow. An exceedingly elevated tachycardia (>140 beats/min), often accompanied by dysrhythmias, is an extremely reliable sign. The pulse may be weak, rapid and very soon imperceptible (a sign of hypovolemia). Pallor is followed by intense cyanosis. Hypovolemic shock with diminished left-sided heart filling, stroke volume and cardiac output may result in a severe hypotension and thus in a further decline of coronary perfusion. Generalized arteriolar vasodilation and an increase in vascular permeability appear, with blood shifting from the circulation to extravascular spaces, a consequent decrement of peripheral resistance and reduction of blood flow. The frequent appearance of acidemia and hypoxemia aggravates the alterations already present. By activation of the intrinsic coagulation pathway, a disseminated intravascular coagulation and depletion of clotting factors may occur. Hemoconcentration and hemostasis troubles, signs of associated cardiorespiratory involvement, lead to irreversible shock and death [160].

Gastrointestinal and Urogenital System

The symptoms and signs, more frequent in children with FA [145], are less common and never occur alone, but are not life-threatening. The most common clinical manifestations depend on contraction upsurge of smooth muscles and mucosal edema. They include nausea, vomiting, abdominal or uterine cramps, colic and diarrhea, which turns bloody, or progresses to fecal and urinary incontinence. Spontaneous abortion in pregnant patients and an increase in fetal or perinatal death have been reported [204].

Nervous System

Loss of consciousness (in some instances the initial manifestation of anaphylaxis), psychological and sensorial disturbances including anxiety and motorial unrest or torpor, with hyporeflexia or areflexia, have been more frequently reported. A sense of anguish may occur, not rarely intense headache, tinnitus, vertigo, and paresis. Convulsions, with or without shock, have been sporadically reported [129]. A cohort of 44 children, aged 2 months to 15.5 years, 32% presenting hypotension, 86% severe edema, and 66% respiratory distress were admitted to a pediatric ED [128].

Idiopathic Anaphylaxis

IA is characterized by one or more severe reactions which can even be life-threatening, characterized by urticaria and/or angioedema, associated with airway obstruction, hypotension and/or bronchospasm, or with intestinal colic [83]. In six children aged 5–10 years, cutaneous, respiratory, gastroenteric symptoms and syncope were observed [63, 64], and were thus wholly polymorphic. The reported pediatric IA population, with an incidence of 6.4% [128], is made up of 12 patients aged 6-16 years (including a 24-year-old) who had urticaria, angioedema, diarrhea, and wheezing [154], ten adolescents aged 11–19 years with similar symptoms, but a low level of compliance [59] as well as eight aged 17 months to 20 years (including a 39-year-old) who had cutaneous reactions, either urticaria, angioedema, or generalized flushing, and seven were noted to have wheezing or angioedema of the airways, causing respiratory difficulties. Diarrhea was noted among five children. Clearly many had life-threatening events [88].

The manifestations of anaphylactic shock are in turn classified into [63]:

- *Life-threatening,* compromising the airways by extending to bronchi and to the cardiovascular system
- *Not life-threatening*, preferentially compromising the skin

Moreover, based on their course:

- *Monophasic or diphasic* based on the occurrence of one or more episodes
- *Protracted* based on more or less long-term course [63]

IA features are categorized as life-threatening and not life-threatening based on frequency of symptoms [64] (Table 20.11) [47, 108, 220].

Classification of Episodes

IA-generalized: systemic manifestations of wheezing, hypotension or syncope with or without upper airway obstruction.

IA-angioedema: angioedema of larynx or tongue with airway involvement as the only life-threatening feature.

Table 20.11. Features of idiopathic anaphylaxis

Disease	Clinical manifestations
1. Idiopathic anaphylaxis generalized frequent	Urticaria and/or angioedema with wheezing, hypotension, shock, gastrointestinal symptoms with or without airway compromise with frequent episodes, ≥6/year
2. Idiopathic anaphylaxis generalized infrequent	Urticaria or angioedema with upper airway compromise such as laryngeal edema, or tongue edema but with ≤6 episodes/year
3. Idiopathic anaphylaxis angioedema frequent	Urticaria and/or angioedema with upper airway compromise such as laryngeal edema, or massive tongue edema without other systemic manifestations, with frequent episodes, ≥6/year
4. Idiopathic anaphylaxis angioedema infrequent	Same manifestations as 3, but with ≤6 episodes/year
5. Undifferentiated somatoform idiopathic anaphylaxis	Patients whose history mimics idiopathic anaphylaxis but lacks correlated objective physical symptoms, shows no response to the therapeutic regimen
6. Corticosteroid-dependent idiopathic anaphylaxis	Steroid-dependent patients, but unable to completely discontinue treatment

Data from [47, 108, 220].

Frequency of Episodes

- *Frequent:* > six episodes a year or ≥ two within a 2-month period
- *Infrequent:* < six episodes a year or ≤ two within a 2-month period [64]

Symptoms may aggravate very rapidly, the patient becomes unconscious. Death occurs suddenly if treatment is not initiated immediately, but even an aggressive treatment may be unsuccessful in particularly severe cases [18, 157, 214]. We believe that the outcome can almost always be transformed into a positive one by a timely and appropriate treatment. Several factors may influence the outcome: the briefer the onset of the anaphylactic reaction, the faster death may occur; in such a case death may occur within 10–30 min of the initial exposure [55]. The nonfatal forms are self-limited, subsiding usually within 48 h [204]. The great majority of reports concern muscle relaxant and IV anesthetic-induced fatalities, with a rate varying between 4% and 6% of cases; atopics are clearly more at risk than nonatopics. The share of food-induced anaphylaxis is unknown: death is more frequent in public places than in private homes [171, 228]. Unfortunately, 5 victimes aged 2–16 died at home or at a friend's home because they received no epinephrine, or received it late [27].

In more severe forms, cardiorespiratory and neurological symptoms prevail, while in other organ systems, these symptoms are attenuated or absent, whereas severe symptoms are preponderant instead in less severe forms or with protracted progression. The initial findings in 43 cases of drug-induced anaphylactic death, including four children (Tables 20.9, 20.10) were respiratory distress in 16 cases and circulatory collapse in 15; laryngeal edema in 15 cases was considered severe enough to be the primary cause of death in 4/15 cases [55]. Some manifestations may predominate over others: more evident digestive symptoms *may mimic a surgical urgency* or acute intoxication; others simulate status asthmaticus, or epileptoid crisis, or deep coma, etc., which in certain cases appear to be neurological in nature [44].

Diagnosis

Although anaphylaxis generally causes no diagnostic problem, successful treatment of anaphylaxis emergencies requires the ready availability of a more immediate possible diagnosis, which depends upon the observation of specific signs and symptoms. History remains an indispensable tool in the diagnosis of anaphylaxis, in identifying responsible allergens and/or another related precipitating agent, factor, or event. The points able to yield valid indications are both history-based and clinical:

- History data

Past history: enquiry into previous reactions since in all fatal cases of food anaphylaxis and in half of the cases by insect sting, there is a previous reaction to the same allergen [188, 229]. Drug allergy history was significant in hospitalized children, and asthma history in outpatient children [57]. Patients with repeat episodes of unidentified etiology, despite appropriate investigations [220], may be atopic and/or asthmatic [225].

Present history: ingestion of foods or drugs, Hymenoptera sting, EIA, or parenteral administration of a drug or vaccine or biological product, have occurred ≤1 h before the onset of symptoms were fully indicative.

- *Clinical features*

– The presence of characteristic *prodromal symptoms,* hypotension, urticaria-angioedema, and laryngeal or bronchial obstruction, are symptoms indicative of initial respiratory insufficiency or of cardiovascular collapse, isolated or associated. When symptoms consist

only in hypotension and loss of consciousness, the diagnosis is more difficult.
– It is more probable that cutaneous symptoms are associated with nonlethal anaphylaxis [170, 171].

If a patient has experienced more than one episode, the responsible allergen should be unmasked: a 4-week-old baby admitted because of pallor and hypotonia lasting 25 min after breast-feeding was subjected to EEG and endoscopy and only at 8 months of age was a severe egg allergy diagnosed [52].

In IA, despite a careful history, including diary products and detailed lists of drugs, no specific agent was identifiable, nor were laboratory tests conclusive [83, 150].

Laboratory Diagnosis

Often no time is left for deeper investigations; however, a number of indications can be detailed.

- *Complete blood counts* (CBC) reveal a hematocrit rise and a neutrophil and platelet decline for a trapping phenomenon.
- *Blood exams* including glycemia, azotemia, creatinemia, uricemia increases as well as hyponatremia and hypokalemia.
- The *possible myocardial changes* are documented by an increase in ASAT (aspartate aminotransaminase), creatinine phosphokinase, SGOT (serum glutamic-oxaloacetic transaminase) and lactic dehydrogenase concentrations.
- The *increase of serum tryptase levels,* released selectively by mast cells, may be related to histamine release by degranulated mast cells and indicate either protracted anaphylaxis or mastocytosis [176, 212]; however, results are more helpful for systemic allergens [171, 229].
- A *chest roentgenogram,* in the case of respiratory tract impairment, shows hyperinflation, which may or may not be accompanied by atelectasis. In some cases, it shows pulmonary edema.
- *ECG:* unless a myocardial infarct has occurred, an ECG frequently shows an S-T segment flattening with T-wave inversion, bundle branch block, atrial fibrillation, etc.

Immune Diagnosis

As seen in Tables 20.1 and 20.2, the task is not easier, the causative agents of anaphylaxis and of anaphylactoid reactions being countless. The identification of specific allergens may become critical, because a reaction to CM may be caused by penicillin contamination [183], egg embryo-based measles vaccine [18], reactions aroused by agents with a high anaphylactic potential, or meals based on highly allergenic foods [22].

Table 20.12. Some drugs that cause anaphylactoid reactions

Aminopyrine
ASA
Codeine
Curare
Fenoprofen
Hydralazine
Ibuprofen
Meperidine
Morphine
Naproxen
Pentamidine
Polymyxin B
Tolmetin
Zomepirac

Modified from [204].

SPTs and in vitro tests may establish IgE-mediated responses to allergens. Likewise, all children with spina bifida should be investigated to uncover a latex allergy before any operation [21], a prerequisite generally not essential for the diagnosis, important for the correlation with history [227] and for prevention. In case insect stings are suspected, see Chap. 17.

SPTs are as always the most reliable procedure to establish the diagnosis; however, three children aged 7–10 had SPT+ to normally ingested foods [64] and in 78% of patients, food challenges disproved SPT+ [196]. SPTs are very effective in the diagnosis of sensitization to anesthetics and related compounds, while a negative test can exclude sensitization. SPTs to latex are unreliable when done with glove extracts, as they differ from lot to lot [227], nor are they usually recommended for certain drugs (Table 20.12) [204], which in some cases provoke anaphylactoid reactions also in healthy subjects [204]. During administration of such procedures, serially increasing extract dilutions are applied [18] since the test potentially stirs up severe reactions in hypersensitive subjects; moreover, the equipment and drug needed for office treatment of severe reactions should be available (Table 13.13).

RAST is reliable for foods and Hymenoptera stings [229]; however, false-positive results might follow nonspecific IgE bindings, such as [101] blood sampling done too early after reaction onset, unsuccessful antigen coupling, presentation of a mistaken epitope in the insoluble phase. In vitro tests give tangible positive results, but negative results fail to exclude a propensity to anaphylaxis, because the high IgE affinity to mast cell receptors may make serum IgE levels too low to be recognized by RAST; several cases of cross-reactions revealed by RAST

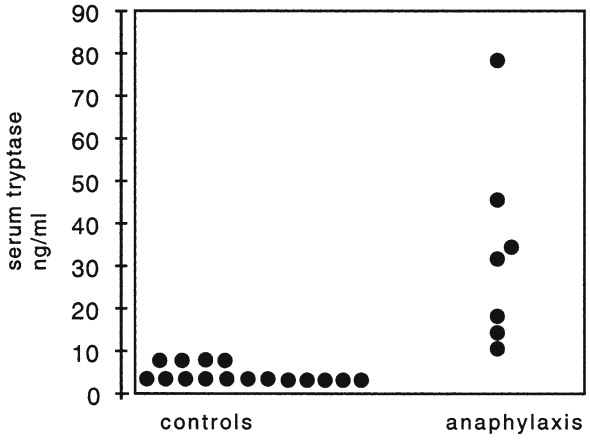

Fig. 20.2. Effect of anaphylaxis on serum tryptase levels as measured by monoclonal antibodies. (Modified from [175])

do not appear to parallel clinical conditions [192]. Positive RAST to soybean and wheat should be confirmed by a food challenge (Chap. 6).

The definitive and specific diagnosis of idiopathic anaphylaxis [201] is made by measuring serum *tryptase levels* within 60 min after anaphylactic events, which have a half-life of 1.5–2.5 h, and normal levels <1 ng/ml, which during an acute EIA are as high as 8 ng/ml [228] (Fig. 20.2) [175]; a value >10 ng/ml is considered to be high [229]. The serum mast cell tryptase, 1 h after the onset of an anaphylactic shock due to cefuroxime, was >200 µg/l (normal range, 10–16 µg/l) [158]. However, even elevated tryptase levels are inconsistent after food-induced anaphylaxis and may have no significant value when evaluating suspected food reactions [171]. Histamine plasma levels peak instead at 5–10 min after an insect sting and then decline, showing a half-life of few minutes from both immunological and non-immunological stimulations [176]. Tryptase levels may be effective, whereas histamine levels could be falsely increased by basophil stimulation during the coagulation [225]. The determination of tryptase levels allude, in case of elevation, to a near-fatal mast cell activation [225] or, as reported in a hospitalized patient with diagnosis of heart attack, who was correctly identified as a case of anaphylaxis only several days later when the blood sample taken at admission was re-examined [176]. The diagnostic complications were paradigmatic in the case of a girl who was very sensitive to peanuts and died after eating a sausage and cheese pizza and french fries; strangely enough, tryptase levels were minimally elevated. The girl had positive RAST to the soybean found in the sausage pizza but not to soybean oil used to cook the french fries; however, neither CM nor chicken proteins, frequent constituents of both cheese and sausage were tested [229].

In conclusion, as soon as a diagnostic clue arises, it is vital for the attending physician to institute the most appropriate treatment as swiftly as possible: this concept is strengthened by the disclosure that underreporting also affects these aspects: only in 4/17 patients (23.5%) was anaphylaxis diagnosed, perhaps due to imprecision of ICD9 International Classification of Disease, 9th edition, where a worldwide accepted definition appears to be lacking [105].

Differential Diagnosis

A few syndromes masquerading as anaphylaxis are considered in children and adolescents [34, 113]:

- *Vasovagal reactions* and collapse, which often occurs with injection of local anesthetics in dentistry, for example: a sudden appearance of pale, clammy skin, slow pulse, diaphoresis, nausea, vomiting, usually bradycardia (tachycardia in both anaphylaxis and insulin reactions), cutaneous symptoms and respiratory obstruction is observed.
- *Insulin reactions* have connotations such as asthenia, pallor, diaphoresis and unconsciousness, there is no occurrence of airway obstruction and respiratory distress, and BP is only somewhat depressed.
- *Reactions to other drugs* are clarified by side effects.
- *Systemic mastocytosis* is characterized by mast cell accumulation and possible presence or absence of cutaneous manifestations: sometime patients elicit anaphylaxis, but in their history there are episodes of maculopapular lesions (Chap. 8), flushing, and headaches, there is no upper airway obstruction and bronchospasm is infrequent and transitory. Diagnostic points are gastric hypersecretion and/or diarrhea, lymphadenopathy, hepatosplenomegaly, leukopenia and/or platelet dysfunction.
- *Hereditary angioedema* (Chap. 8) is considered if laryngeal edema is the prominent symptom; the diagnosis requires the demonstration of C1-INH deficiency. A rapid screening test is C4 reduction [108].
- *Cold urticaria* may occasionally occur as generalized urticaria, bronchial obstruction, cardiovascular collapse. The iced cube test in a localized and cooled room is resolvant in systemic cold urticaria (Fig. 8.8).
- *Laryngospasm* possibly due to asthma and vocal cord dysfunction.
- *Respiratory failure* can be caused by foreign body aspiration, or a severe asthma attack if the airway is obstructed, which may be mistaken for an anaphylactic reaction.
- *Epileptic seizures,* sepsis, dehydration, etc.
- *Reactions to additives* (monosodium glutamate, in other words Chinese restaurant syndrome), and/or toxins in certain fishes, etc.
- *Scombroid fish* (Chap. 10) and snake venom poisoning [32].
- *Pseudoanaphylaxis* is used to describe patients developing symptoms after injections of procaine penicillin. These reactions are of short duration and subside without the need of any intervention. Typical symptoms are

Table 20.13. Differential diagnosis of idiopathic anaphylaxis

Known causes of anaphylaxis
IgE-mediated
Non-IgE-mediated
Foods
Exercise
Postprandial exercise
Medications
Latex
Carcinoid syndrome
Chronic idiopathic urticaria and angioedema
Concomitant idiopathic anaphylaxis and anaphylaxis of defined etiology
Hereditary angioedema
Münchausen syndrome complicated by anaphylaxis
Identifiable causes (aspirin, nuts in sensitized subjects)
Factitious anaphylaxis (health professional and the like)
Simulated anaphylaxis
Undifferentiated somatoform anaphylaxis
Panic attacks
Pheochromocytoma
Severe asthma
Systemic mastocytosis

Modified from [108].

hallucinations, fear of death, anxiety, and other neurological symptoms [219].

IA should be differentiated by several the conditions mimicking it (Table 20.13) [108], including [83, 153, 217]:

- *Münchausen stridor,* a term coined after Münchausen syndrome [8], describes patients who intentionally adduct their vocal cords and present to emergency department (ED) with self-induced manifestations of dyspnea depending on laryngeal edema and with paroxysmal attacks of choking mimicking those of angioedema with glottis edema. IgE-mediated episodes have been reported after ingestion of nuts, peanuts or eggs in patients sensitized to these foods [219].
- *Anxious attacks* in children with upper airway obstruction, with anxiety, globus hystericus, in the absence of cutaneous and respiratory symptoms (except hyperventilation) and hypotension.
- *Somatoform anaphylaxis* [47], fictitious forms (such as panic attacks), however simulated [219].

Additional conditions not to be underestimated include food inhalation [13], serum sickness, hypovolemic shock and others, objectively rare in children [153].

Table 20.14. Differential diagnosis between exercise anaphylaxis and cholinergic urticaria

Exercise anaphylaxis	Cholinergic urticaria
10–15 mm urticaria	1–3 mm urticaria
Stridor with laryngeal edema	Bronchospasm and wheezing
Hypotension common	Hypotension uncommon
Occurs only with exercise	Provoked by heat, stress, exercise, anxiety, etc.

Modified from [213].

The differential diagnosis of *anaphylactoid reactions* matches that of the true anaphylaxis.

Specific Differential Diagnosis

EIA and cholinergic urticaria (Table 20.14) [213]. The ultrastructural aspect of EIA is consistent with a form of "anaphylactic" degranulation [180], which in cholinergic urticaria is instead "zonal" [91].

Immediate Treatment

The immediate treatment of anaphylaxis is the same for idiopathic and anaphylactoid forms [109, 136]. We distinguish general and specific measures related to the reaction severity [36, 87, 136, 188]: in an emergency, little time exists to consider fitting treatment, or to consult medical textbooks or other colleagues, therefore physicians, nurses and medical personnel must at all times be prepared to treat anaphylaxis [136].

The goal of initial evaluation is to assess airway patency, systolic BP, breathing, and vital signs [36]. Fatal cases occurred independently of the use [18, 214] or not [27, 171] of epinephrine. *Two out of six fatal cases* [171] received epinephrine within 1 h of the onset of symptoms also because *none of them had the emergency kit* (Table 20.15) [27, 171, 208], similarly to a 16-year-old boy [30] and two children reported in the press. The other seven, who were at home, received epinephrine within 5–55 min [171], thus proving that a medical emergency is resolved more rapidly and effectively *at home rather than in public places.* Anaphylaxis may materialize in a multiplicity of places, the most common site of occurrence of nonfatal pediatric cases was at home in 24%–57% of cases. (Table 20.16) [27, 57, 65, 145]. An analysis suggests that parents may hesitate to use the autoinjectable epinephrine (EpiPen) and seek ED treatment when indicated. The 11 (27%) children given EpiPen were taken to the ED; however, also 16 of 30 children not given EpiPen went to the ED [58]. In another cohort 32% of episodes of anaphylaxis were treated with epinephrine [187] or the first treatment was

Table 20.15. Risk factors of lethal food-induced anaphylaxis in children with food allergy

History of asthma
History of previous nearly lethal food-induced anaphylaxis
Unawareness that the eaten culprit food contained the offending food
Eating at school or in public places
Delay in administration of epinephrine
No self-injectable epinephrine on hand

Data from [27, 171, 208].

Table 20.16. Location of nonfatal anaphylactic cases in 95 [145], 35 [65], 55 [57] and of fatal cases in 17 food-allergic children [27]

Site of occurrence	(%)	(%)	(%)	(%)
References	[145]	[27]	[65]	[57]
At home	57	24	26	45
Outdoors	12			
Restaurants	5	12	4	
Doctor's office	3			
Hospital	3		4	15
Hospital, other				27
Football fields	3			
On the beach	2			
In the gym	1			
At school	1	18	16	4
Operating room	1			15
Leisure			2	
Camping		12		
Dance class		6		
Day care			6	
Friend's home		6	14	
Store		6	2	
College			6	
University (cafeteria)		6		
Church			4	

One fatality occurred in the youngest of the 17 subjects, a 2-year-old child not known to have FA, or asthma, or other atopic conditions [27].

self-administered or given by a parent (57.1%) or a physician, or an emergency team, or by a teacher, or a caregiver in *91.8% of cases* [60]. In some reports, most of 17 [27] children and adolescents did not have EpiPen [30, 171], despite a regular prescription [171], and an 11-year-old child was in such a severe condition that even 50 mg of epinephrine (and resuscitation) were insufficient [18]. Two children died despite receiving epinephrine before admission to hospital [124]. However, 4 of 17 children and adolescents died at home and *only one received epinephrine, but late,* and of the remaining 13, only one received epinephrine [27]. Of the nine children whose reactions occurred in non-hospital settings and who had had at least one prior episode of anaphylaxis, only five had EpiPen available for use, which were successfully used in three children. Of the 11 children with known FA who had reactions in a non-hospital setting, eight had known histories of asthma and only three of these children had EpiPen available for use, and only in two cases was it administered [57]. Also it has recently been reported that epinephrine for first aid use by parents and other caregivers and the EpiPen autoinjector device are infrequently used in children with recurrent episodes of anaphylaxis [82]. In a diverse field, the great spectrum of critical situations faced by parents of children with FA is illustrated by a 2-year-old peanut-allergic child who experienced a severe reaction in a store after a woman in the check-out line quickly popped a *peanut-butter candy in the child's mouth* while the mother turned her head away to pay [65].

General Treatment

In the initial phase the treatment includes the following steps [64, 103, 136, 151, 188, 219, 226, 231]:

- *Stop child exposure* to the offending allergen if present, even based on a simple suspicion.
- *With hypotension,* place child in recumbent position (Trendelenburg) and elevate lower limbs: this favors venous return to the heart; modification of the Trendelenburg position may be necessary if the child is wheezing.
- *Secure and maintain airway patent;* to secure adequate gas exchanges, in an equipped center endotracheal intubation or cricothyrotomy may be required.
- *Frequently assess vital signs,* especially at the onset of symptoms and assess level of consciousness.
- *Place a venous tourniquet* above the injection, SIT and insect sting site; this may decrease absorption of the residual allergen, which should be released every 5–10 min for 1–2 s, thus insuring tissue oxygenation.

Initial Treatment

Primary drug of choice in pediatric therapy is *epinephrine,* because it reduces mediator release by metachromatic cells and acts directly to regress vasodilation, edema and bronchoconstriction [36]. The β-adrenergic property of epinephrine produces bronchodilation and increases both inotropic and chronotropic cardiac activity. Epinephrine administration must be immediate, *at first sign of danger,* including throat pruritus or tight-

ness, lipedema, laryngeal edema, nausea cyanosis, wheezing and *before airway compromise* with stridor, dyspnea, wheezing, etc. [36, 170]. In children, epinephrine is always the drug of first choice, according to 63%–81% of cases in the US (Table 11.48), to be administered even before symptoms become aggravated [36]. In a large study of children [28] epinephrine was given in 78.8%, parenterally administered antihistamines in 50.6%, CSs in 34.1% and bronchodilators in 27.1% of cases (compare with Table 11.48) or epinephrine in 75.7% of severe cases [65] or 84% of children received epinephrine, CSs, and an H_1 blocker [57]. We stress that epinephrine often *goes unused,* even in hospitals and doctors' offices, employed only in 18% of cases of anaphylaxis [145]. However, as 87% of children had only one anaphylactic attack, parents or babysitters were unprepared to deal with this situation, in part because 57% of cases occurred at home and 62% of children were treated in an ED or hospital [145]. Pay attention to *peanut oil,* which may be used as an excipient of epinephrine [138]. Initially, epinephrine inhalation was not recommended as an alternative to the parenteral route [36], and the inhaler was withdrawn worldwide in 1998, due to the chlorofluorocarbons (CFCs) of the spray and the supplier's inability to guarantee long stability of the product [221]. This inhaler delivered 150 µg/puff: an indisputable advantage was its easier and immediate utilization, since it allowed a more rapid and constant absorption compared to the SC route [221].

Reactions of Intermediate Severity

- First of all, *administer inhaled epinephrine.* An alternative method for epinephrine inhalation consists in the administration of an aerosol including a 1:1,000 aqueous epinephrine vial/10 kg bw (body weight) and normal saline, up to a maximum of three epinephrine vials, possibly *by a three-way connection,* to administer at the same time epinephrine, O_2 and if required beclomethasone dipropionate (G. Taranto, G. Giarratana, pers. comm., Nov 5, 2002).
- Alternatively, *racemic epinephrine* (Racenephrine HCl) (doses in Table 11.16) may be administered for 5 min *by a nebulizer* with continuous flow of O_2 of 4–6 l/min (Nellcor PB Raindrop medication nebulizer). It should be stored in a cool, dark place. Discoloration is an indication for discarding epinephrine. *Additional epinephrine preparations* include:
- *Epinephrine bitartrate:* metered dose inhaler: 0.35 mg/inhalation
- *Epinephrine hydrochloride:* solution for inhalation: 1:100, 1:1,000 (Community Pediatrics Committee, Canadian Paediatric Society, May 2002)

Pay attention to the content of *metabisulfites* [123], present in levo-epinephrine preparations as a preservative (racemic), or in epinephrine acid tartrate as a vehicle,

Table 20.17. Epinephrine body weight-based dose

Body weight	Dose
3 kg	0.03 ml
5 kg	0.05 ml
10 kg	0.10 ml
15 kg	0.15 ml
20 kg	0.20 ml
30 kg	0.30 ml

The dose may be repeated q 15 min if necessary q 10–20 min ×3–4, not >0.3 mg per dose. Epinephrine acts within 5–6 s and its action persists for 30 min (ml and mg are equivalent).
Data from G. Taranto, G. Giarratana, pers. comm.

which may be the case of failure of two studies referred to in Chap. 11 in the treatment of bronchiolitis. Interestingly, the two positive studies employed epinephrine diluted in normal saline, as we do, and suggest doing, in other cases, as do G. Taranto and G. Giarratana (pers. comm.)

- *IM epinephrine.*
- *IV epinephrine.* Note that a 1:1,000 aqueous epinephrine solution in 10 ml of normal saline produces a 1:10,000 solution; in addition 0.01 ml of a 1:1,000 aqueous epinephrine solution yields 10 µg and 0.1 ml yields 100 µg; for example, adding 0.5 ml of a 1:1,000 dilution to 100 ml of normal saline at 5%, a solution at a 5 µg/ml concentration results.

A recent study stresses that the mean maximum plasma epinephrine concentration was achieved at a mean time of 34±14 min by 6/9 children and only 2/9 children achieved maximum plasma concentrations by 5 min. In 8/9 children who received IM epinephrine, the mean maximum plasma concentration was achieved at a faster mean time of 8±2 min [188]. Thus, even when SC epinephrine is injected promptly, it may not always be effective [22, 188, 228]. Therefore, *in severe cases, IV, or IM, or inhaled epinephrine should be preferred*.

It necessary, continue as follows:

- Administer SC 1:1,000 aqueous epinephrine, 0.01 ml/kg bw, repeat epinephrine twice q 10–20 min as needed (maximum dose, 0.2–0.3 ml/kg), or terbutaline in children <12 years of age, 0.005–0.01 mg/kg per dose (maximum dose, 0.4 mg) to be repeated in 15–20 min, in children ≤12 years, 0.25 mg/kg per dose to be repeated in 15–20 min, as needed. Table 20.17 shows a body-weight-based epinephrine dosage.
- In case of *injections and insect stings* in the absence of contraindications, infiltrate site of inoculation or puncture with one-half dose of epinephrine, 0.1–0.2 ml unless the puncture is not localized on the head, neck, hands or feet.
- To modulate possible *late-phase sequelae,* give diphenhydramine: 1 mg/kg per dose q 4–6 h IV or IM with maximum daily dose of 300 mg (5 mg/kg).

• All children should be monitored up to 24 h [170, 204]. However, satisfactory treatment should be continued *until symptoms improve sufficiently or subside,* so the child can be released from the hospital, with precise instructions to take the child immediately to the ED if a relapse occurs, an unexceptional event that cannot be prevented by CS administration along with the emergency treatment [136] (Tables 17.7 and 17.8).

Shock (Severe Reactions)

Anaphylaxis must be treated with IV therapy. Many children should be rehydrated due to excessive insensible loss from respiratory effort, abundant diuresis, and vomiting. Initial therapy must include intravascular volume repletion with normal saline, correction of electrolyte imbalance, and fluid and electrolyte maintenance

First Aid Treatment (ED)

[64, 103, 151, 155, 170, 188, 219, 226, 231]

1. *Immediately give IM epinephrine* into the arm every 5 min in case of hypotension. However, IM injection into the anterolateral thigh (*vastus medialis*) produces higher and more rapid peak plasma levels compared with those of IM injections administered into the arm; otherwise the absorption is too slow. The time to peak plasma epinephrine concentration (t_{max}), accompanied by prompt physiological effects, was 8±2 min after IM injection, significantly shorter than the t_{max} of 34±14 min (range, 5–120 min after SC injection [187]).
2. Alternatively, administer *inhaled, or racemic, or IV epinephrine* (1:10,000), also *by continuous flow,* or use EpiPen (0.3 mg), or EpiPen Jr (0.15 mg) may be injected *through clothing* into the anterolateral thigh.
3. *Monitor vital signs* including blood gas, BP, ECG; measure or estimate bw to calculate proper drug dosage.
4. *Start peripheral IV line.* Request CBC with differential, electrolytes, blood gas analysis, base excess (BE), etc.
5. *Place child in Trendelenburg* position (if not done previously), with head elevated in case of laryngeal edema.
6. Before any question arises about the adequacy of cardiopulmonary function, *give supplemental O_2 humidified* to 30%–40% (4–6 l/min) by nasal cannula or mask or if necessary by Ambu bag to prevent tissue hypoxia, which fosters vascular collapse; be ready for a possible intubation.
7. Secure and *maintain adequate airway* (mucus aspiration, application of Mayo tongue depressor).
8. (a) If the response is slow or inadequate, or BP is <60 mmHg, the recommended initial resuscitation dosage in children is *epinephrine 1:10,000 dilution for slow IV infusion* (over 5 min) at the dose of 0.1–0.2 ml/kg repeated every 3–5 min for ongoing arrest [103].
(b) For initial resuscitation, a commonly used sequence in children is *a continuous infusion* started without delay at the dose of 1 µg/min, with increments depending on the response; increasing to a maximum of 10.0 µg/min for adolescents.
(c) Inhaled epinephrine, repeat as above, if necessary.
9. *Check pH and BE, correct acidosis* by measuring BE: if the result is <–5 mEq/l give $NaHCO_3$ (sodium bicarbonate) 1 M ml is = to BE × kg bw/3.
10. Otherwise, *give enough IV fluid,* administer an IV solution as follows:
• *Normal saline,* 5% glucose, children should receive 20–30 ml/kg IV in the 1st h; *if hypotension persists*:
• *Plasma* or 5% human albumin at a dose of 10–15 ml/kg IV.
11. If needed, antihistamines IM *to prevent urticaria recurrence:* chlorpheniramine may be given by IM or slow (add to 5–10 ml of NaCl, sodium chloride) 0.9% and K^+ chloride (25–40 mEq/l) and give over 1 min IV injection at a dose of 2.5–5 mg to 1- to 5-year-olds, or 5–10 mg to older children [151].
12. *H_1 and H_2 antihistamines IV* such as ranitidine in 25 ml of normal saline (50 mg/2 ml) over 5 min at the dose of 12.5–50 mg (0.01 ml/kg) or 1–2 mg/kg up to 50 mg, or diphenhydramine as above [224].
13. *CS IV to prevent biphasic anaphylaxis* or late or persistent sequelae: hydrocortisone, 5–10 mg/kg (caution in subjects with past ASA-induced anaphylactic shock), up to 500 mg q 4–6 h, or methylprednisolone, 2–4 mg/kg in 25 ml of normal saline over 5 min by the peripheral IV line, max 100 mg q 4–6 h [224].
14. Continue to *administer fluids,* antihistamines, CS, etc., even once the child has sufficiently recovered.

Pay attention to the *potential relapses of anaphylactic symptoms* (cases of biphasic or persistent anaphylaxis are frequent). Because of delayed effect of CSs and the possibility that acute symptoms fail to abate wholly after ED treatment, children should be kept in an observation area up to 12–24 h before dismissal. If necessary, the child should be immediately taken to the nearest medical facility [171] and monitored over a 24-h period [22].

There is a deplorable tendency to rely only on CSs (72%) or antihistamines (20%); in anaphylactic cases noted to date, only 18% have been treated with epinephrine compared to CSs [145], also because epinephrine may be *underused by the medical staff* [128]. However, epinephrine use in the treatment of anaphylaxis should be promoted because, even among hospitalized children, the first drug used in the ED was CSs, although it is well known that epinephrine is the first choice of treatment during anaphylaxis [145]. *CSs may have no effect for 4–6 h* and *antihistamines have a much slower onset of action* compared with SC or IV epinephrine [103].
• *Angioedema and acute urticaria* generally respond to H_1 and H_2 antihistamines, which are not adequate epinephrine equivalents [36]. Systemic CSs are usually

Table 20.18. Home and out-of-hospital fixed-dose of self-injectable epinephrine by subcutaneous route

Brand name	Dose (mg)	Comments
Ana-Kit[a]	0.30	Appropriate for infants weighing less than 12 kg[b]
Ana-Guard	0.30	Syringe ready with needle dispenses 4 doses = 1 ml epinephrine for 12–25 kg and >25 kg body weight
Anahelp[a]	0.25	
Epi-Pen[a]	0.30	
Epi-Pen Jr[a]	0.15	With a shorter needle
Fastjekt junior	0.165	IM, for children weighing no more than 45 kg[c]
Fastjekt for adult use	0.330	IM
Min-I-Jet[a]	0.5[d]	IM, see notes for adult use

Data from [36, 142, 190].
[a] Kit available in two monodose preparations.
[b] Relatively low priced; however, it is more difficult to self-administer than Epi-Pen.
[c] Dose for *children weighing about 16.5 kg*.
[d] Costs only 15 % of Epi-Pen and consists of a cylinder predisposed with a needle which acts as a plunger.
Epi-pen and Fastjekt emergency kits contain a spring-activated concealed needle. Ana-Kit allows separate delivery of two doses like Ana-Guard, but contains sterilizing swabs, tourniquet and chlorpheniramine tablets (optional). Anahelp's separate plunger has four small position wings allowing four doses of 0.25 mg if broken away. Min-I-Jet consists of an injector with a mounted needle and a separate vial, which acts as the syringe plunger [142]. The preparation should be stored in dark and cool repositories. Checking the expiration date, which for Epi-Pen, for example, is 24 months. Ana-Kit may remain for 24–30 months at ambient temperature [142]. Fastjekt preparation should be checked every 15 days and discarded if the solution shows staining and/or precipitates. Ana-kit is no longer available in the USA. The Epi-Pen auto-injector is supplied in light-resistant packaging, and each 0.3 ml dose contains 0.3 mg epinephrine, 1.8 mg sodium chloride, 0.5 mg sodium metabisulfite, and hydrochloric acid to adjust the pH from 2.2 to 5.0. The Epi-Pen Jr contains epinephrine 0.15 mg and the same additional ingredients in the same amounts as in the Epi-Pen [187].

not employed acutely but may be helpful in refractory and/or prolonged reactions and in asthmatics treated with oral or topical CSs [151].

- *If wheezing does not respond* to epinephrine, add inhaled albuterol (0.1–0.15 mg/kg per dose up to 5 mg q 20 min over 1–2 h) in 3 ml of saline and/or IV theophylline (22 mg/ml, 2.5 mg/kg over 15 min) at a dose of 3–6 mg/kg depending on whether the child has received oral theophylline over the previous day (Table 11.45). Pay attention to children on current use of long-acting theophylline (the toxicity of theophylline with a level of >20 µg/ml).
- *Epinephrine is the best medication* to treat even the most severe form of anaphylaxis [221]. It is contraindicated only in coronary heart disease, an issue usually unknown in pediatric patients [104]. Self-injectable epinephrine is available in a kit with tourniquet and a preloaded syringe, containing epinephrine 1:1,000, an antihistamine tablet and a single-dose alcohol applicator. Alternatively, a spring-loaded, pressure-activated system is available [142]. A limited number of these formulations are available for out-of-hospital use [190] (Table 20.18) [142, 36, 190]. However, since the available doses vary between 0.15 and 0.30 mg, it is impossible to treat all infants, children, and adolescents from birth to the 17th birthday within this narrow range [190]. Recently, five children with a mean weight of 18 kg received the 0.15-mg dose, and five children with a mean weight of 25.4 kg received the 0.3-mg dose. In those who received 0.3 mg, systolic BP was significantly higher, but adverse effects were also more common (palpitations, headache, nausea). The authors concluded that the beneficial and adverse pharmacological affects could not be dissociated, and premeasured doses would facilitate more precise therapy for infants [191]. However, in a nonemergency setting, parents took a mean of *142 s* to draw up the medication, and half of them were >30 % off the requested dose [189].

The symptoms of *epinephrine overdose*, or inadvertent IV administration include tachycardia, vomiting, pallor, chills, cyanosis, blurred vision, irritability, sweating, ventricular arrhythmia, further pulmonary edema, respiratory failure and death. The antidote is phentolamine IM at the dose of 0.15 mg/kg; in case of no response an immediate admission is crucial. In addition to restoring the circulating cell mass, it is necessary to correct, if present, metabolic acidosis, hypoglycemia and/or hypocalcemia.

With no improvement, or subsequently to resolution of anaphylactic symptoms, the child should be admitted to an *intensive care unit (ICU),* or at best within the hospital, to prevent the risk of late forms of shock. Every effort should be made to prevent future reactions, especially to foods and drugs. Therefore first aid management should be as early and effective as possible including, if needed, *cricothyrotomy* [167], because a life-

threatening crisis may occur regardless of a pertinent and timely treatment. Since the complications of an anaphylactic shock and its management are very challenging, it should be handled in an individualized manner in accordance with the clinical features of the child and symptom severity. Management is not always appropriate: 3/7 patients were initially treated with oral drugs [228]. Dibs et al [57] point out striking differences when comparing in- and out-of-hospital sites of occurrence. In-hospital reactions were elicited by latex (n=15), IV medications (n=6), IVIg (n=1), and radiocontrast material (n=1). Surprisingly, reactions occurring out of the hospital with identifiable agents were triggered by foods (n=14), insect venom (n=8), and oral medication (n=2). Oral and SC agents were seen exclusively in the out-of-hospital group, whereas IV agents were responsible for all but two of the in-hospital reactions. The former group also had a greater prevalence of FA, atopy and asthma during their reactions.

Once emergency care has been provided, the basic management should treat the prominent symptoms (urticaria, wheezing, etc.), which is more demanding in CS-dependent children [64].

Prognosis

Prognosis varies greatly and is certainly very challenging when anaphylaxis is very severe and proteiform [225]: the briefer the interval between exposure to an allergen and onset of clinical manifestation, the more severe the reaction tends to be [55]. Therefore, prognosis depends on the route of administration, sensitivity of the recipient, duration of the latent period, time required for the recognition of an adverse reaction, and medical management within reach [179]. In the majority of cases, an allergic reaction occurs in a typically sudden and unpredictable way and with symptoms in some cases that make the earlier and more appropriate treatment ineffective [18]. Fortunately, cutaneous signs, urticaria, or angioedema are present in 80%–90% of reactions. In IA, recovery is reported in 65% of frequent and in 91% of infrequent cases [109]; two fatal cases are known [110, 153] in addition to near-fatal ones [153].

Prevention

The superlative treatment of anaphylaxis is prophylaxis, which requires first and foremost a recognition of the risk for fatality. The most pressing guideline in preventing anaphylaxis is a thorough food and drug allergy history and use of related substitutes [224]. The best control of allergic reactions should consist in their anticipation and prevention: the more severe the reactions, the more likely the attack is to be fatal or near fatal in a substantial proportion of cases. Table 20.19 [113] outlines and amalgamates the basic principles of drug anaphylaxis prevention. Specific recommendations for young victims of anaphylaxis can be summarized as follows:

Table 20.19. Prevention of drug-induced anaphylaxis in drug-allergic children

1. Identify causes of anaphylaxis and at-risk children
2. Avoid exposure to those agents whenever possible
3. Give drugs orally and slowly rather than parenterally, where appropriate
4. Ensure that children wait in office 30 min after drug administration, under observation
5. Provide instruction on proper, thorough reading of medication labels
6. Provide children at risk of anaphylaxis with warning identification
7. Provide instruction of predisposed children in proper use of self-injectable epinephrine
8. Use human serum preparations when antiserum is essential
9. Use skin tests when applicable
10. Use supervised preventative protocols for the following drugs:
Local and IV anesthetics Skin testing Occasional challenge testing
Radiographic contrast material Prophylactic pretreatment
Blood and blood products in selective IgA-deficient patients Washed erythrocytes Autotransfusion IgA-deficient blood used
Special drugs (ASA, penicillin, insulin) Short-term desensitization
Insect venom Long-term desensitization

Modified from [113].

- Allergic or intolerant children and adolescents

– Prospective and true sufferers of *past anaphylactic episodes*: a careful family and personal history is mandatory, and existing concerns on the possible association between atopy in allergic children or only with positive FH, and these children should be examined before prescribing any medication included in Tables 20.1, 20.9, and 20.10. In 46 of 76 (61%) children who had been enrolled in the previous study [145], 14 (30%) had experienced a recurrence of anaphylaxis [46]. Nine children out of 44 (20.5%) had a relapse during the following months [128]. In either case, physicians should provide these children (or their parents) with a list of foods causing anaphylaxis. The lists of incriminated foods and drugs should also include those responsible for cross-reactions.

Table 20.20. Prevention of food-induced anaphylaxis in children

1. Take a thorough food-allergy history
2. Identify cause of anaphylaxis and the children and adolescents at risk of future attacks
3. Educate children, adolescents, parents, day-care providers, caretakers, school personnel, and restaurant, pizza-shop, fast food managers, and other public institutions about potential food-induced anaphylaxis
4. Educate pediatricians, GPs and nurses on the danger of partly hydrolysate formulas in children with CMA
5. Provide emergency kits containing epinephrine in spring-loaded self-injectable syringes to at-risk children; parents, day-care and school personnel
6. Educate parents and older children on proper reading and interpreting lists of ingredients on packaged foods (careful attention to biochemical terms or technical jargon) where appropriate
7. Parents and children over 7 years old must be trained to use such kits
8. Predisposed children should carry warning identification, MedicAlert bracelets or chains at all times
9. Food manufacturers should provide unambiguous ingredient listings on prepackaged food labels, identify the potential for contamination during food processing, take steps to eliminate contamination during food processing

Data from [167, 171].

– For children *sensitized to specific foods* (Table 20.20) [167, 171], hints can be found in Tables 20.4 (foods and additives most frequently involved), 20.5 and 20.6 (hidden sources), and Appendices 9.9 (hidden sources of several foods) and 10.1 (foods and non-food products potentially containing vegetable gums). The role of ingredient labels and undeclared allergens in purchasing prepackaged foods should not be overlooked [96, 140, 211].

- Special Issues

– *Is the allergic child safe at home?* The anaphylactic event occurs at home (Table 20.14) in 25% [65], 57% [145], or 78% of cases [140], sometimes because of a lack of parental surveillance [140]. The first reactions to peanut and/or tree nut occurred primarily in the home (72%) [185]. The use of colored stickers to mark forbidden foods clearly, such as those provided by lay organizations, might diminish the risk of giving a food inadvertently [65].

– *Is the allergic child safe at school?* As discussed in Chap. 9, school personnel, the child's teachers, day-care providers [3], and all people in contact with the child should be informed.

– *FA reactions are common in schools* and preschool, as almost 20% of children experienced such reactions in school in the past 2 years [147]. The mere presence of an information pack and action plan decreases the incidence of allergen exposure in school and day-care facilities [140]. The reactions took place at school in 22% [140] or in 38% of cases [148], the meal was at school but prepared by parents in 8.8% [140], 50% [147], or 69% of cases [148].

– *Moneret-Vautrin et al encountered the refusal of a member of the teaching staff,* and of a school physician in two cases [140]. On the contrary, in the cohort of 100 subjects [186] in 55% of the teachers, in 10% of cases school nurses and even school secretary or cafeteria workers took charge of the situation. In 60% of cases parents were notified about the emergency. In one report, 66% of children with anaphylaxis did not have emergency medication available at school, an emergency action plan, or a teacher on site able to administer epinephrine for first aid use [29]. In another 77% had the medication available in school, and 81% stated that the school knew the indications for administration [184]. Otherwise, the medications were kept in the health room in 71%, the classroom in 26% [147], the nurse's office in 46%, with the teacher in 23%, in the child's bag in 18%, and in the front office in 15% of cases [148]. The emergency kit was stored in the classroom (51.3%), in the infirmary of the junior high school (23%), or in the director's office (25.7%) [140]. An emergency plan was in place for 53% of the reactions, but it was followed only 73% of the time [186]. Active parents provided schools with protocols and medications for treatment of acute allergic reactions in 91% and 93% of children, respectively, but 50% of schools utilized their routine medication forms for instructions for treatment of allergic reactions, whereas 50% used specific forms [148]. The use of Epi-pen will help to overcome the reluctance of staff members [140]. A positive prevention was accomplished by 71% of schools that made available special accommodation for children with food allergies: restricted peanuts from the classroom (25%), alternative meals (21%), separate eating areas [221], peanut-free tables in the cafeteria (8%), and other means (17%) [148].

- *Is the allergic child safe in public places?* Public venues are becoming increasingly common sites for unattended reactions. Such locations include stores, malls, sporting event sites, transportation vehicles, airlines, railways, and restaurants [185].

– *Eating away from home* (bakeries/doughnut shops, cafeteria, ice-cream and confectioner's shops, etc.) should be forbidden, parents should be instructed to request lists of ingredients from restaurant personnel [226].

– *Parents of children allergic to peanut and tree nuts* altered their approach to restaurants and other food establishments as in two CM- and nut-allergic children in a variety of ways, including always carrying medications (91%), avoiding specific types of restaurants (89%), asking more questions (84%), and 19% reported that they would reduce their frequency of eating out [77].

– In France, *a personalized care project* (PCP) has been instituted for children presenting IgE-dependent FA. One-third of children with PCP or an integrated care plan had an allergic reaction compared to five out of six children without such a plan [140].
– Epinephrine should be included in the *emergency medical treatment kits of schools, restaurants,* rail and bus carriers, airlines, other public facilities and other trained personnel in a position of responsibility for public safety, such as teachers, sport instructors, camp counselors and scout leaders [4]. We would also suggest that persons in designated position of public safety, who may be called first on the scene of accidents, including policemen, firemen and traffic policemen, etc., be correctly trained so that they can administer epinephrine as needed [226]. However, babysitters were unprepared [145].
– Not all parents were sure of entrusting the responsibility of first aid *to a nonmedical adult,* even though fully trained, who should judge the need for injection. Some teachers have argued that it is not their responsibility to make such decisions [151].

- Additional issues for children who have a history of previously suffering from reactions: objective SPT investigations should be requested, possibly with an in vitro test based on history clues (see "Diagnosis"), with the target of unmasking responsible agents, particularly the foods responsible for EIA [165], or a latent sensitization to muscle relaxants [139].
- When there is foreseeable *long-term contact* with these agents that are particularly necessary (a life-saving drug), the physician should evaluate the prospect of a desensitizing treatment [18].
- When there is foreseeable immediate or short-term contact, a prevention program with antihistamines and CSs should be started.
- In cases of *absolute urgency,* it may be medically necessary to administer *a radiocontrast agent,* particularly IV, to a child in whom it previously caused an anaphylactic reaction: the child should take as prevention prednisone (13 h, 7 h and 1 h before) + diphenhydramine (1 h before) ± epinephrine (1 h before) [119]. Alternatively the patient could be provided with a kit containing EpiPen (Table 20.18).

The Achilles' heel is that epinephrine autoinjectors are underprescribed in the pediatric population at risk of anaphylaxis [190], and many doctors provide parents with preloaded epinephrine syringes *without training parents in their use* [18]. Indeed, often patients do not receive a prescription of self-injectable epinephrine, nor are preventive measures suggested, nor are they sent to a specialist for the necessary assessment in 58.8 % of cases [105].

As has been suggested in similar cases, children at risk should permanently wear a *Medical Alert bracelet* listing the food allergens that may cause an anaphylactic reaction and carry a *cellular phone,* which for younger children will be that of the accompanying parents. This is a necessary precaution because three patients with near-fatal reactions did receive epinephrine within 15 min of developing symptoms but still went on to develop respiratory collapse requiring mechanical ventilation and vasopressor support [171].

Cold-Induced Urticaria

For patients with cold-induced urticaria, a non-stop prevention with antihistamines is suggested, as well as the above-mentioned kits [136].

Exercise-Induced Anaphylaxis

Children with EIA should avoid all activity for 2 h (even better, 6 h) after a meal, especially if consisting of shrimps and certain vegetables, stop exercise as soon as a sensation of even mild pruritus is perceived and for all eventualities, be accompanied by an *adult capable of administering epinephrine*. We also suggest that these subjects be provided with a *cellular phone.* Taking cromolyn before a meal and exercise was crucial for all parameters. In an epidemiological survey, only 31.1 % of teachers had ever heard of EIA [200].

Sulfites

Tables 10.3 and 10.4 list foods containing these additives for subjects with past *reactions to sulfites*.

Tomorrow's Treatment of Anaphylaxis

TNX-901, a humanized IgG1 anti-IgE mAb, recognizes and masks an epitope in the CH3 region responsible for binding to the high-affinity FcεRI on basophils and mast cells. A DBPC, randomized, dose escalation trial was conducted with 84 patients with a history of peanut allergy. Allergy was confirmed by a DBPC oral food challenge (OFC) and patients were randomized 3:1 in three dose groups to receive either TNX-901 (150, 300 and 450 mg) or placebo SC every 4 weeks for four doses. They underwent a final OFC within 2–4 weeks after the last dose of study medication. From mean baseline values of 178–436 mg in the three treatment groups, the significant mean increases in the OFC threshold were 710, 913, 1,650 and 2,627 mg for the placebo, 150, 300 and 450 mg for TNX-901 dose groups, respectively. The well-tolerated TNX-901 at a dosage of 450 mg significantly *increased the threshold of sensitivity to peanut* by OFC from a level of about half a peanut (178 mg) to almost nine peanuts (2,805 mg). Thus anti-IgE therapy may be an effective long-term approach for management of food-induced anaphylaxis [118].

Pediatricians and Anaphylaxis

A very challenging task is the search for the responsible food allergens, only secondary to the hunt for the hidden substances: their recognition is critical to issue specific instructions for the immediate administration of epinephrine. This should be closely considered by *pediatricians*, who should be informed of the wide spectrum of ASA or sulfite-containing medications, capable of causing or aggravating asthma, or of provoking anaphylactic reactions. High-risk groups predisposed to fatal or near-fatal food hypersensitivity reactions include children and adolescents with asthma, especially when they do not receive epinephrine immediately following onset of the reaction, and children and adolescents having presented previously with a serious anaphylactic reaction (Table 20.21). Above all pediatricians should gain the confidence of children and their parents, to be advised of all medications and over-the-counter products they take, notwithstanding the invitations to disregard them, including cold and cough preparations, eye drops, ointments, antihistamine/decongestant preparations, injectable drugs, analgesic and antipyretic preparations, etc. Families should be provided with every variation of therapeutic regimen, highlighting clearly and without fear of causing dismay that even the more severe and/or potentially fatal reactions can be successfully prevented. Only delay in spotting the signs and symptoms of anaphylaxis can result in unforeseen complications. But anaphylaxis may present without these hallmarks, especially in the perioperative circumstances where anesthesia can mask certain signs and symptoms. The task becomes hazardous when foods are on the scene (Table 20.16), above all if we consider that:

- In so many cases of *peanut-allergic children* who suffered from anaphylaxis [9, 18, 24, 27, 30, 54, 67, 68, 74, 161, 171, 185, 228], several of them were victim of hidden peanuts [9, 18, 30, 67, 74, 130, 134]. The most frightening case is that of deodorized peanuts, reodorized and sold as almonds [170].
- In 11 pediatric fatal cases, *nobody had the prescribed Epi-pen* [30, 171, 228], as is the case with most children and adolescents [27].
- The foods with *highly suspected ingredients* were regularly eaten away from home or came from uncontrolled purchases [171, 228].
- *All seven children* experienced previous allergic reaction to the incriminated food and all unknowingly ingested the food allergen [171].
- Symptoms developed *within minutes of ingestion* [171] as in the two press-reports.

In conclusion, pediatricians should inform children and adolescents at risk of anaphylaxis to food, drugs, and stings, and their parents, as soon as they are encountered for the first time, that in the office they have epinephrine and resuscitation equipment ready for use. When history is positive to such reactions, an epinephrine kit should be prescribed [36, 190].

Table 20.21. Points to cover in the medical history of anaphylaxis in infants, children, and adolescents

1. Food, drugs, and stings suspected of having provoked the reaction
2. Quantity of the ingested food or drug
3. Length of time between sting or ingestion and development of symptoms
4. Description of the symptoms
5. Similar symptoms developed on other occasions when the food or drug was taken
6. Other factors (such as EIA) inducing serious reactions
7. Length of time since the last reaction

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Autoimmune Diseases

Dysregulation of the Immune System

It is difficult to fulfill Koch's postulates when considering the etiology of autoimmune diseases (AIDs), which are multifactorial diseases where a primary role is played by genetic factors associated with environmental factors responsible for their clinical expressions [359]. Direct evidence is restricted to transplacental transmission of IgG pathogenic autoantibodies (Aab) from the affected mother to her fetus, such as in neonatal myasthenia gravis, polychondritis and Graves' disease. Indirect evidence comes mainly from AID reproduction in animal models and/or development of genetically determined AID models, and from the isolation of Aab or reactive T cells [359]. In this chapter, a multiplicity of abnormalities associated with systemic AIDs in different organs and systems will be examined.

Definitions

Autoimmunity is a condition that triggers an immune response to autologous body constituents, called autoantigens (Aag). The immune response may become manifest through humoral (Aab) or cellular mechanisms. This activity is generally prevented by control mechanisms, in particular by CD8 T cells. Normal healthy subjects possess T cells with the potential to cause organ-specific AIDs, but regulatory T cells play a dominant role in maintaining self-tolerance. Autoimmune responses are not always harmful; on the contrary, they are physiological to some extent (Table 18.1) [286, 402]. Actually, all individuals show low concentrations of Aab and autoreactive T and B lymphocytes controlled by regulatory mechanisms, which act by deleting autoreactive immature cells or inactivating peripheral mature cells [286]. When these mechanisms which safeguard the nonreactivity or tolerance to the self fail, an AID, for example, a damaging, severe and persistent autoimmune process, develops [402].

Table 18.1. Comparison between natural and disease-associated autoimmunity

Normal autoimmunity
Interaction between CD4 T cells, HLA class II molecules and APC
Interaction between CD8 T cells, HLA class I molecules and APC
Anti-idiotype antibodies
Anti-idiotype T cells
Cell–cell interactions mediated by interaction of adhesion molecules with ligands
Interaction between lymphocytes and epithelial cells
Disease-associated autoimmunity
Several factors contribute to AID pathogenesis:
a. Quantitative features of the immune response (antibody levels, size of T-cell response, IL-produced
b. Qualitative features of the immune response (antibody affinity, isotype, specificity)
c. Genetic basis (multifactorial) Genes increasing susceptibility Augmenting factors: antibody, ILs, cells of innate immunity Immunodeficiency
d. Environmental factors Infectious agents, including superantigens Physical: UV light Pollutants: cigarette smoke Iatrogenic: drug exposure Nutritional: foods, caloric intake, nucleotides, fatty acids, vitamins, minerals (Zn)
e. Random factors Underproduction of suppressive ILs Overproduction of inflammatory ILs Exposure of sequestered antigens (by trauma or infection) Somatic recombination of antibody variable regions Recombination of T-cell TcR genes Hormonal fluctuation Stress

Data from [286, 402].

Table 18.2. Organ-specific and systemic AIDs

Organ	Proven AIDs	Uncertain AIDs
Organ-specific AIDs		
Gastrointestinal tract	Ulcerative colitis Crohn's disease	
Joints	Rheumatoid arthritis	
Heart	Rheumatic fever	
Skin	Pemphigus Pemphigoid Vitiligo	Psoriasis
Liver	Primary biliary cirrhosis Acute hepatitis Chronic hepatitis	
Endocrine glands	DM Hypoparathyroidism Addison's disease Basedow's disease Hashimoto's thyroiditis	Some hypogonadisms
Eye	Sympathetic ophthalmia Uveitis	
Kidney	Goodpasture's syndrome	
Blood	Autoimmune hemolytic anemia Autoimmune neutropenia Thrombocytopenic purpura	Biermer's anemia Pernicious anemia
Brain	Myasthenia gravis Guillain-Barré syndrome	Multiple sclerosis
Spermatozoa	Some male sterility	
Systemic AID		
Dermatomyositis, polymyositis		
Lupus erythematosus, systemic		
Rheumatoid polyarthritis		
Scleroderma		
Sjögren's syndrome		
Vasculitis		

Data from [18].
DM diabetes mellitus.

Classification

AIDs include a group of diseases with different incidence, characteristics and severity, which share a common denominator: relevant immune alterations attributable to immune pathogenesis. Since AID etiology is unknown, and genetic factors are multifactorial, in most cases AIDs are preferably classified according to their tissue distribution. Those involving one or a few tissues are called organ-specific, whereas those involving many tissues are called systemic. Table 18.2 [18] summarizes the main AIDs.

Etiopathogenesis

Genetics

The role of genetic predisposition is unquestionable. Interestingly, New Zealand black mice (NZB), an AID model, develop a disease similar to systemic lupus erythematosus (SLE), whereas the white variety (NZW) remains untouched. Crossbreeding both types of mice, the hybrids (F1) develop an earlier and much more severe form of SLE. The fundamental anomaly, however, can be traced to the immune system [359]. Solid evidence has been collected by studies on family AIDs – regardless of their specificity – where, without any clinical manifestation, the proband's parents and/or siblings show higher concentration of Aab [474], and by their resolution after bone marrow transplantation (BMT) [243, 457]. Studies on homozygote (HZ) twins affected by different AIDs have shown a 15%–50% rate of concordance, thus confirming the genetic relevance in their pathogenesis, but suggesting that a large component is environmentally determined [482].

Different models have been proposed to explain the major genetic factor in AIDs [6].

Polymorphic HLA Sequences

Most AIDs show statistically significant associations with specific HLA haplotypes [359], which thus represent a relative risk (RR) for their carriers (Table 18.3) [18, 80, 125, 141, 352, 431]. Studies have also revealed associations with inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD).

Immunoglobulin Genes

Some pathogen Aabs have been shown to prefer V_H or V_K gene families. Actually, antibodies with different specificity may have identical sequences and idiotypes common to several individuals. For instance, public idiotype 16/6 is associated with anti-DNA codifying genes found in active SLE, and 20% of patients with SLE or rheumatoid arthritis (RA) show V_H gene deletion of their germinal repertoire, which could be a genetic predisposing factor [507]. Subsequent studies have not found such deficits in SLE-affected individuals [420]. Nevertheless, the HLA molecule and T-lymphocyte role in humoral AIDs remains undisputed. A major role is also played by clonotype convergence after isotype switching from IgM to IgG and by restriction of somatic mutations to complementarity determining regions (CDRs) [420].

TcR Genes

Predominant $CD8^+$ and $CD4^-$ $CD8^-$ have been seen in normal subjects, TcR-αβ/γδ have been found in the ileal mucosa of patients with CD [75], and γδ in patients with early-onset multiple sclerosis (MS) [386].

Interleukin Genes

Defects in IL gene structure, transcription and function and/or their receptors have been observed in several AIDs, for example, DM (diabetes mellitus) and IBD [465]. Studies on animal models show that ILs and/or their inhibitors may influence both positively (IFN-α and -β, TGF-β) and negatively (TNF-α, IL_2) the AID development; in particular IL_{12} [134, 225, 301, 433] especially in NOD (nonobese diabetic) mice [434]. IFN-γ has a positive action on experimental autoimmune encephalomyelitis (EAE), and a negative action on MS and DM [301].

Apoptosis Genes

Fas/FasL defects have been suggested to cause inadequate clonal deletion during B or T tolerance development, with consequent availability of a higher number of autoreactive lymphocytes [286]. In light of current knowledge, Fas/FasL deficit can be considered an autoimmune process accelerator, but not the only one [391]. Some cases of human SLE may have symptoms in common with experimental SLE in mice with lymphoproliferative mutations [469].

Complement Deficits

Especially C2, C4 or CR1 (CD35) deficits predispose to AID: a C2 heterozygous (HET) defect is shown in 0.3% of population, but 40% of these subjects are affected by systemic SLE and SLE-like syndromes. As for C4 allotypes, C4B3, C4AQ0 and C4BQ0 associations with DM, and C4B2 association with RA have been reported. The obvious association between complement defects and autoimmune manifestations in immune deficiency (ID) can explain ID development in different types of AID such as in autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura, etc. Even though the basic mechanisms of the ID/AID association are not fully understood, Aab presence in these patients seems to be relevant, despite the severe deficit of antibody synthesis against foreign antigens [357].

Table 18.3. Association of HLA alleles in autoimmune disease

Disease (reference)	HLA allele	Relative risk [18]	Relative risk [141]
Addison's disease	*DR3*	6	
Ankylosing spondylitis	*B27*	87	80
Basedow's disease	*DR3*	4	
Celiac disease	*DQ8*[a]	50	
Celiac disease	*DR3*	12	
Celiac disease	*DR3/7*	60	
Celiac disease	*DR7*	5	
Chronic active hepatitis	*DR3*	2	
Crohn's disease	*DRB1*01, 07*	NS	
Dermatitis herpetiformis	*DR3*	15	
Dermatomyositis	*DR3*	3.8	
DM	*DQ8*[b]	12	32
DM	*DR2*	4	
DM	*DR3*	4	
DM	*DR3/4*	15	
DM	*DR4*	6	
Goodpasture's syndrome	*DR2*	16	
Hashimoto's thyroiditis + goiter	*DR5*	5.6	
Hashimoto's thyroiditis	*DR3*	3	
Infantile nephrotic syndrome	*DR7*	4.4	
Juvenile rheumatoid arthritis	*DR8*	3.6	
Juvenile rheumatoid arthritis	*Dw4*		26
Juvenile rheumatoid arthritis	*Dw14*	47	
Multiple sclerosis	*DR2*	4	
Myasthenia	*DR1*	5.4	
Pemphigus	*DR4*		14
Pernicious anemia	*DR5*	5.4–7	
Primary bile cirrhosis	*DR3*	8	
Psoriasis	*Cw6*	13	
Reiter's syndrome	*B27*	37	40
Rheumatoid arthritis	*DR4*	4	
Selective IgA deficiency	*DR3*	13	
Sjögren's syndrome	*DR3*	10	6
SLE	*DR3*	16	3
Ulcerative colitis	*DRB1*15*	NS	

See also HLA antigens in disorders with ocular manifestations: Table 14.9. The most recent HLA nomenclature is in Appendix 1.1
Data on dermatomyositis from [125], on AIDs from [18, 80, 141, 352, 431].
DR8 DRB1*0801-DRB1*08032, *DR4 Dw4* DRB1*0401, *DR4 Dw14* DRB1*0404
[a] DQB1*0201.
[b] DQB1*0302 (Appendix 1.1).

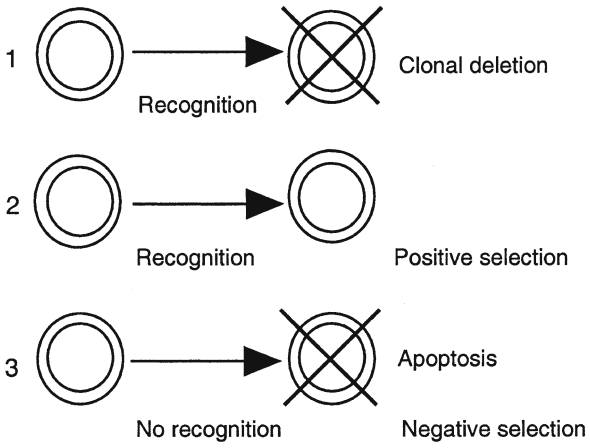

Fig. 18.1. Pathogenesis of autoimmune diseases: thymic selection and tolerance. See text for details. (Modified from [106])

Etiopathogenetic Mechanisms

Loss of Self-Tolerance

AID could be an expression of a tolerance deficit consequent to qualitative and quantitative abnormalities. Recent findings clarify the concept shown in Fig. 18.1 [106]: during lymphocyte maturation in the thymus, unlike Figs. 1.22b and 1.22c, specific T cells whose TcRs have high affinity for HLA–Aag complex are clonally deleted because they are autoreactive, whereas lymphocytes lacking this affinity fail to be rescued from apoptosis, as they are useless (anergic) [106]. However, cells with low affinity for HLA molecules do not attack the self, and populate the periphery, where they can recognize the pertinent Aag. Consequently, clonal deletion of T lymphocytes specific for high-affinity epitopes of self proteins promote low-affinity epitopes to a position of apparent immunodominance [106]. Control mechanisms can be escaped: for instance, directly activated T lymphocytes become resistant to suppression, or Aags bypass T lymphocytes and stimulate T or B effector cells (Fig. 18.2). Figure 18.3 shows additional models of T lymphocyte avoidance.

Molecular Mimicry and Shared Epitopes

Molecular mimicry [6] has been used to describe a spectrum of cross-recognition of non-self and self peptides thought to underlie AIDs [25] based on the necessity of escaping the host's specific immune responses [6]. Researchers found amino acidic sequences identical to those discovered in self antigens in various microorganisms [420]. Self and non-self antigens, for instance, can share a sequence of five amino acids, with a 3% probability that human and exogenous proteins contain this type of pentapeptide. Since there could be 20,000 human genes, the incidence of this could be higher [402]. There are numerous examples in nature. Mimicry in specialized endoparasites could facilitate invasion of host tissues or avoidance of host defense mechanisms [25]. Immunization with these peptides prevents T lymphocyte tolerance to Aags, thus eliciting an AID [25]. Most CD4 of transgenic mice have a TcR specific for the 1–11 peptide of major basic protein (MBP). This has led to the conclusion that EAEs develop only in animals bred in nonsterile environments [145]. Similarities between non-self antigens and host features can facilitate antigenic cross-reactivity supported by B or T lymphocytes, which elicit transient autoimmunity (TAI) or a chronic AID [25]. Researchers focused their interest on this subject, and recently they have proven that a single TcR can recognize both the immunodominant peptide MBP and a distinct viral peptide [484]. A TcR from an MS patient

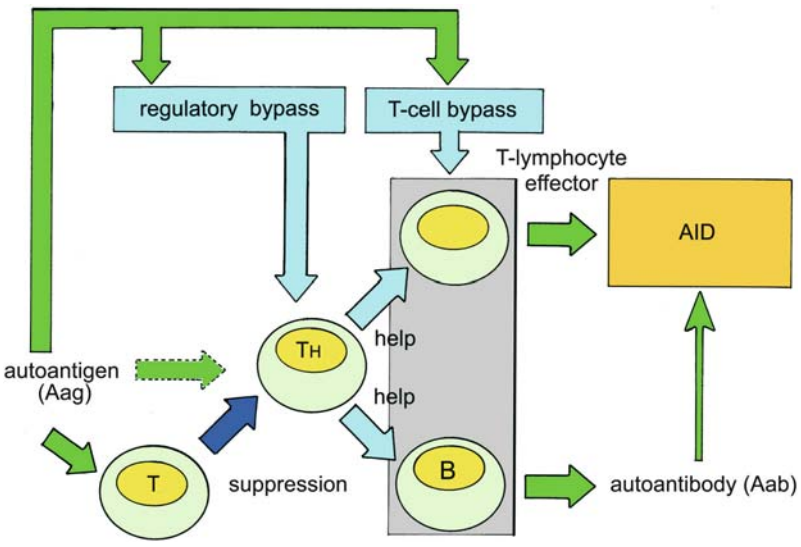

Fig. 18.2. Autoimmune disease (*AID*) may result from evasion of controls. Self-reactive B cells, effector T cells and Aag are normally present but Th cells able to induce autoimmune responses are functionally absent, because of anergy or clonal deletion, or apoptosis (Fig. 1.22), or the action of suppressor T cells. Autoimmunity may arise by a regulatory bypass, which either activates directly Th cells or indirectly T contrasuppressor, thus making T lymphocytes resistant to suppression. Aag could also bypass the Th cells by directly activating T effectors and B cells, especially if in adequate concentrations

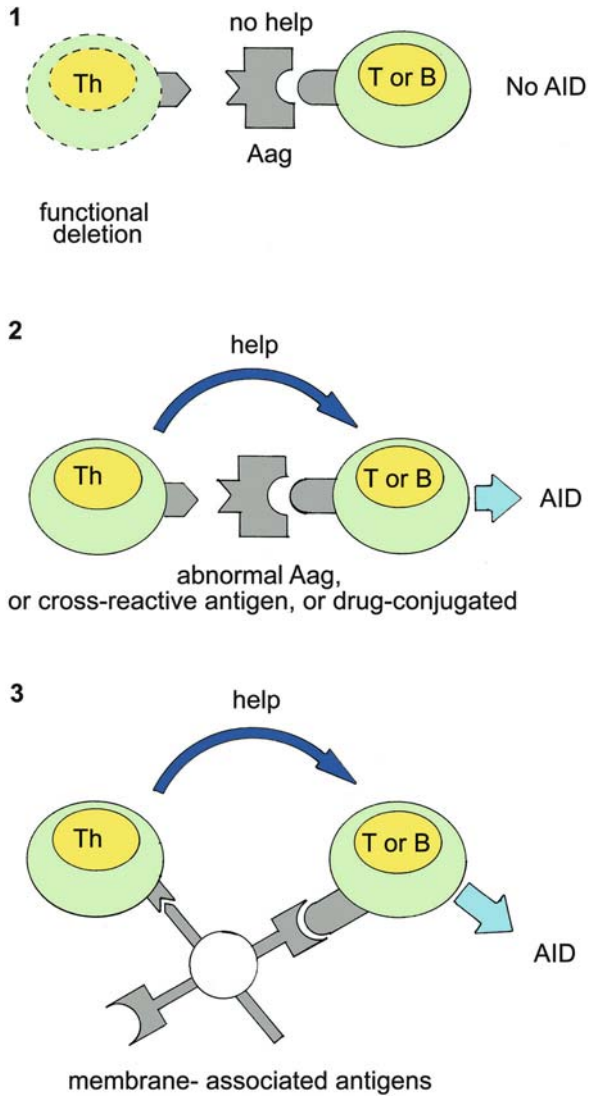

Fig. 18.3. Induction of autoimmunity by T-lymphocyte bypass. Usually AIDs do not occur because T cells reacting with Aag are functionally deleted (*1*). In the presence of cross-reactive antigens, a new T-cell subset may recognize processed antigens on the B-cell surface (*2*).The drug binding to an Aag may also be recognized by T cells as a carrier epitope, or the Aag may be structurally altered by defects in synthesis or by abnormalities in lysosomal processing. The new carrier could arise on a molecule also bearing the Aag epitope as in *2*, or may be on a different molecule, associated with Aag on cell membrane (*3*). The issue is further complicated, since the simplest viruses may express several different antigens, and each of these molecules may contain several distinct epitopes

recognized both a DRB1*1501-restricted MBP and DRB5*0101-restricted EBV (Epstein-Barr virus) peptide. The DRB5*0101–EBV peptide complex revealed a marked degree of structural equivalence to the DRB1*1501–MBP peptide complex at the surface presented for TcR recognition [219]. This provides evidence to identify homologous peptide sequences in micro-

Table 18.4. Homology between microorganisms and HLA molecules

Bacterial and viral molecules	HLA molecules
Klebsiella nitrogenase	B27
Arthritogenic *Shigella flexneri*	B27
Proteus mirabilis urease	DR4
EBV	Dw4[a]

Modified from [357].
[a] T-cell epitope.

organisms and body components as potential TcR cross-reactive epitopes, even among specific HLA proteins correlated with human AIDs (Table 18.4) [357]. Reactive arthritis following urethritis from *Chlamydia trachomatis* or gastroenteritis from *Yersinia*, *Salmonella*, etc., is an apt model for studying the molecular mimicry of these germs with HLA molecules [200]. The association between HLA-B27 and spondyloarthritis has been explained assuming that HLA-B27-derived peptides are presented by self-HLA class II to CD4 arthritogenic T cells, tolerance having been broken by mimic peptides from the triggering bacteria [388]. For instance, HLA-27 and *Klebsiella pneumoniae* share six consecutive amino acid residues (Fig. 18.4) [244]. Studies on shared epitopes fall under RA pathogenesis. In these patients, a protein from EBV shares homologous sequences with all three HLA alleles linked to juvenile rheumatoid arthritis (JRA) and uveal antigen S, and a protein of unknown function mimics part of the hypervariable third region. This would explain the frequent association between JRA and iridocyclitis [6]. A similar model may be suggested for DM [25]. These studies confirm the hypothesis that common pathogens can elicit an anti-self response, thus contributing to AID pathogenesis. However, there is still a lack of convincing evidence to extrapolate data to human pathology [474].

Cryptic Epitopes

The corollary hypothesis for the mechanism of AID infective pathogenesis is crypticism, which concerns epitopes unable to produce tolerance in conventional immune responses as they are unexpressed, expressed at a subliminal level, or hidden by dominant epitopes. This can happen because of:

- *Increased antigen degradation,* which occurs when surface receptors decrease because of high-affinity antibody or ligand inflow [369]
- *Processing modulation* when the antigen is bound to these antibodies or ligands [395]
- *Increased synthesis of HLA class II molecules* or increased expression of the adhesion and/or costimulating molecules (Fig. 18.5) [220]

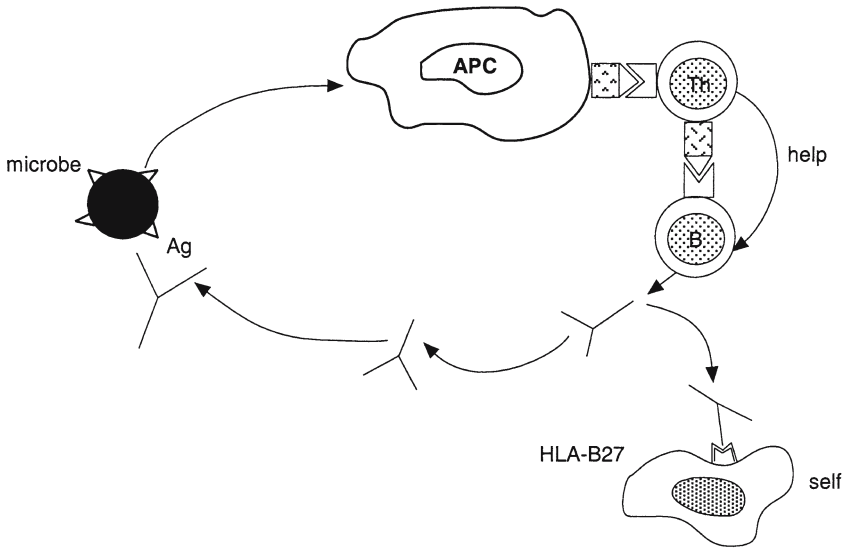

Fig. 18.4. Molecular mimicry. Microorganisms may display surface antigens very similar in structure to a particular HLA molecule. Th cells recognize processed microbial antigen and prime B cells to switch into antibody-secreting cells that react with intact antigens on the microorganisms, thus binding to HLA molecules. Following an alternative mechanism, T cells may be converted into effectors and recognize processed antigens expressed on the cell surface and complexed with HLA molecules

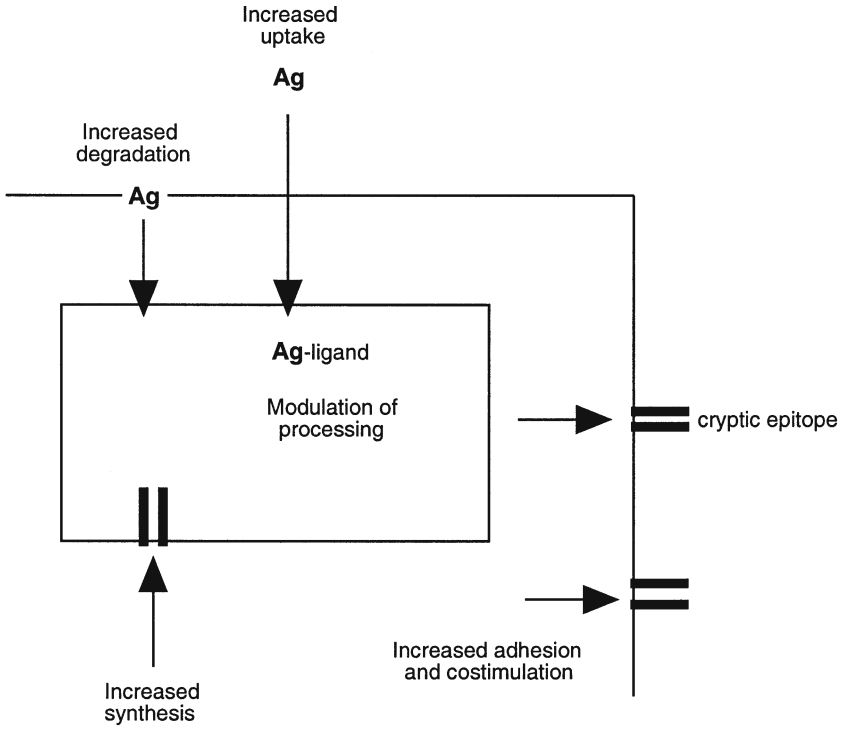

Fig. 18.5. Mechanisms possibly leading to a presentation of cryptic epitopes. *Ag* antigen. (Modified from [220])

What is the first event that activates the *autoimmune spiral?* Induction may occur through the same immune response to foreign antigens. It is also possible that cryptic epitopes are presented to nonprofessional APCs of peripheral organs such as quiescent B lymphocytes or epithelial cells [220]. Much attention has been focused on heat shock proteins (HSPs), which may provide a facilitated peptide binding to normally absent HLA molecules and an efficient presentation of cryptic epitopes [223]. In this context, T cells may also be activated by antigens presented by nonprofessional APCs or stimulated by recognized cross-reactive antigens presented by professional APCs following molecular mimicry, or by a second TcR with different antigen characteristics [220]. According to a third hypothesis, the activation of self-reactive B lymphocytes is facilitated by T-independent mechanisms, foreign cross-reacting antigens with self antigens on B lymphocytes, or by direct activation from a highly organized self antigen (Fig. 18.6) [220]. A recent study has shown that antiendomysial antibodies (EMAs) were detected in the culture supernatants of all 16 untreated celiac disease patients irrespective of gliadin challenge, as well as in 17 of the 23 biopsy samples from treated patients challenged with gliadin. The authors hypothesize that the self antigen, against which EMA are directed, are hidden, or cryptic,

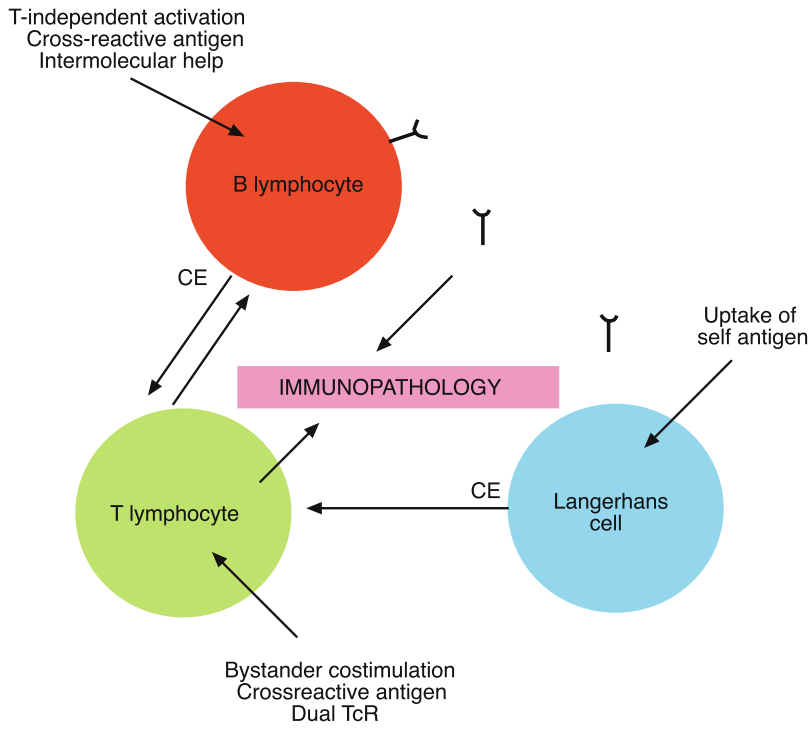

Fig. 18.6. Mechanism that may start and continue an autoimmune response to a cryptic epitope (*CE*). (Modified from [220])

and are recognized only after gluten ingestion, and preferentially recognized in the context of HLA DQ2 [330]. Celiac disease, where the trigger is well known and the pathogenic cascade is relatively well defined, is a situation that is not common in autoimmunity [239]. The relevance of crypticism appears more clearly by assuming that a first response against a cryptic epitope triggers a second wave of intramolecular and intermolecular cryptic epitopes via epitope spreading [223], which along with antigen-independent inflammatory mechanisms elicits a chronic autoimmune inflammation in RA [7] and in other chronic AIDs mediated by self-reactive T cells [455].

Modified Antigen

Sometimes, drugs induce Aab production, often persistent, even after the therapy has ended. The interaction between drug and tissue protein may induce some alterations on sites far away from the drug–protein reaction. Thus, the cells modified by the self can be recognized as non-self by the immune system and become immunogen. Antibodies or cytotoxic T cells can recognize both the native protein and the cells modified by drug–antigen action. This occurs, for instance, in autoimmune hemolytic anemia [470] AIHA. Further knowledge recently acquired in this field has shown a large number of possible applications for modified peptides, from specific immunotherapy to AID therapy. As a matter of fact, it has been observed that the replacement of just one amino acid modifies the peptide so as to prevent EAEs [297]. Studies on ligands from these modified peptides have shown new bonds to the HLA antigens headed for Th1/Th2 production [297]. It is thus possible to influence the differentiation of T cells with the most eligible HLA alleles [410] by manipulating the peptides forming complexes with HLA.

Polyclonal Activation

Recent findings observed in humans point to polyclonal activation of B and T lymphocytes as a major cause of AIDs, especially in systemic AIDs. The existence of self-reactive B cells eluding anergy and/or deletion, and thus the induction of tolerance, is confirmed by the action of a number of microbial or viral molecules (EBV; LPSs, lipopolysaccharides) (Fig. 18.7), which are polyclonal activators of B lymphocytes eliciting the Aag synthesis [420]. $HgCl_2$ injection in susceptible strains of mice and rabbits leads to polyclonal activation and an autoimmune syndrome. In the best studied model (BN rats), IL hyperproduction is induced through stimulation of CD4 Th2, whereas CD4 Th1 and CD8 are mostly stimulated in other rats. Such results could also depend on drug reactions [139]. Chronic forms of GvHDs (graft-vs-host diseases), by T lymphocyte induction in adult hybrids, are characterized by the presence of nonspecific organ Aab and the development of a syndrome similar to SLE or juvenile scleroderma (JSD). Aabs are produced by the receiver's B lymphocytes, whereas HLA class II molecules stimulate donor's Th2, leading to IL_4 and IL_5 synthesis with preferential stimulation of self-

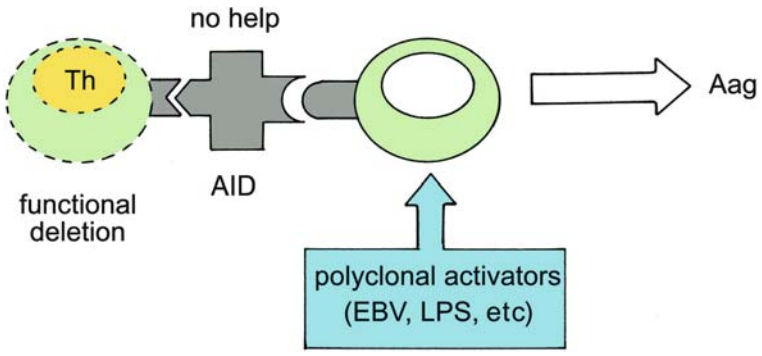

Fig. 18.7. Induction of autoimmunity by polyclonal activators

reactive B lymphocytes [139]. A similar mechanism occurs in GvHD against semiallogenic splenic cells. This protocol is used to elicit allograft tolerance in newborns. In this model, there is deletion of cytotoxic T lymphocytes (CTLs) and alloreactive Th1 T cells, while alloreactive Th2 T cells are spared, since they recognize HLA class II molecules of the receiver B cells [139]. This mechanism seems to be arguable, since Aab responses are primarily of the low-affinity IgM type, whereas tissue damage in SLE is caused by high-affinity IgG Aabs produced by T cells. In addition, IgG Aabs are antigen-dependent, though not all of them show consistent somatic mutations. Although EBV and LPS increase Aab titers in mice, either normal or SLE-susceptible, the most severe lesions are observed only in the latter. The effects of these activators can be attributed to the aggravation of SLE, but in the final analysis their impact is quite modest [420].

Idiotypic Determinants

Idiotypic determinants are seen as normal self–self interactions. In B and T lymphocytes, the idiotype consists of a set of cells with receptors that recognize only epitopes on antibody molecules produced by other B lymphocytes; T cells have similar receptors recognizing epitopes on T cells or on antibody molecules . In general, molecular mimicry may help idiotypic determinants to integrate autoimmunity mechanisms [402]. Several viruses use normal host receptors to adhere to host cells and subsequently penetrate host cells. These viruses must have equal structure, and presumably cross-react like antigens with a normal ligand for the relevant receptor. If the host develops an immune response to those viral structures, it will mount an autoimmune response to the normal ligand. Similarly, the production of anti-ligand antibodies may elicit an anti-idiotype response to that antibody; such an antibody reacts with the viral receptors [357] (Fig. 18.8). This mechanism could trigger anti-receptor conditions: myasthenia gravis, Graves' disease, etc. Broadly speaking, anti-idiotype Aabs could represent the natural outcome of a normal immune response [357]. Aab production will be constant or intermittent, depending on the infective agent consistency or intermittency. The same mechanism has been suggested for T lymphocytes [402].

Microorganisms

In principle, several mechanisms may be concerned:

- *Cross-reaction* with microbial antigens: some microorganisms have epitopes also present in self molecules. Thus, an immune reaction against these antigens could cause T lymphocytes to react with the pathogen and support antibody B production. This occurs, for instance, in RA.
- *Polyclonal activation:* some infective antigens, such as those of malaria, and EBV induce B-cell polyclonal activation, while the generated antibodies involve several self molecules.
- *Immune dysregulation:* different causes could come together, such as abnormal processing and presentation of self molecules because HLA or pathogens are more inducible, lower levels of inhibiting ILs (TGF-β), and increased sensibility and/or prolonged response to activating ILs (IL_2).
- *Possible hyperproduction of ILs:* the immune system would be by-passed by activating potentially self-reactive anergic T cells.
- *Tissue damage* can be induced via sequestered antigen release, ectopic expression of molecules, redistribution of intracellular molecules to cell surface, cryptic antigen release via increased HLA molecule expression and bystander T lymphocyte activation [420]. Bacterial DNA stimulates macrophages to release IL_{12} and TNF-α, which act in synergy to stimulate IFN-γ from T lymphocytes [286].

The predominant environmental bacterial load is demonstrated by the high variations of diabetes incidence among synergic colonies of NOD mice of both sexes, which were bred in 22 different centers [340]. On the other hand, some physiopathological conditions, especially during acute and chronic infective diseases, can cause TAI in which Aab increase is not associated with any clinical manifestation.

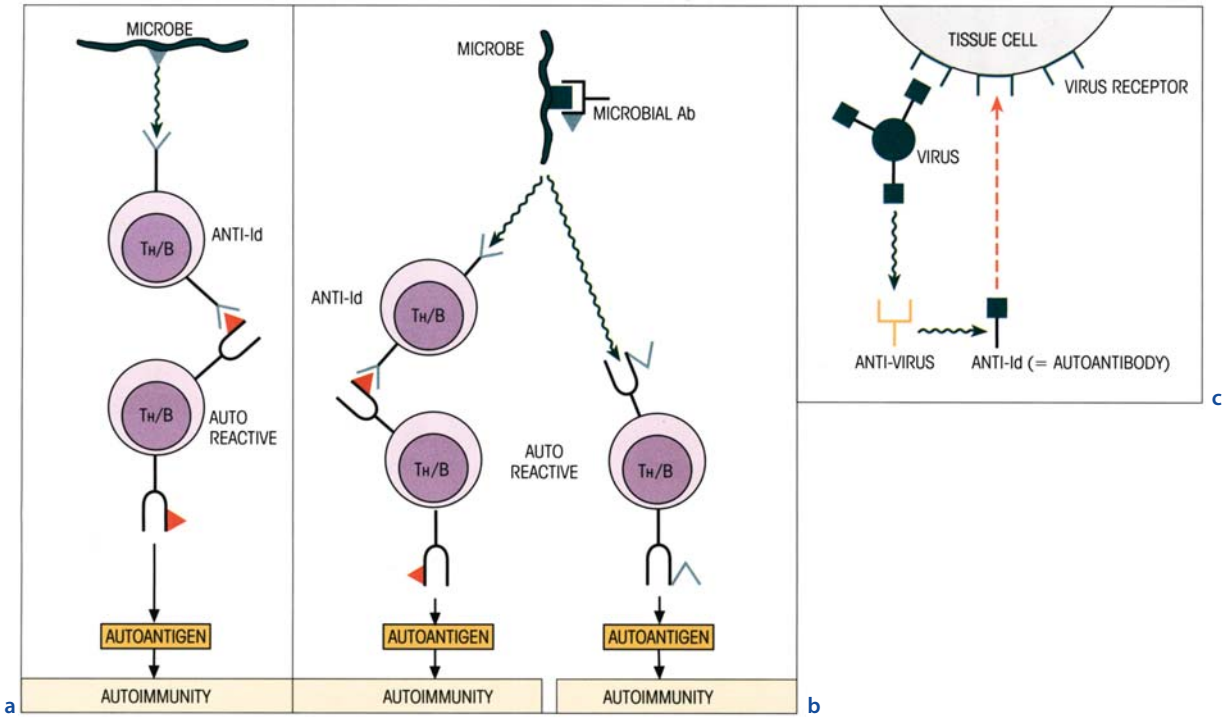

Fig. 18.8 a–c. Idiotype mechanisms leading to autoimmunity. **a** Microbial antigens cross-react with the corresponding idiotype (*Id*) on autoreactive lymphocytes. **b** Microbial antibodies either share Ids with or are anti-Id to autoreactive lymphocytes. **c** Anti-virus generates anti-id, which is Aab to viral receptors

Superantigens

Superantigens (SAs) are bacterial or viral antigens, which can activate several T and B lymphocytes and directly bind to TcRs and HLA class II molecules, without resorting to transport and intracellular processing [210]. As shown in Fig. 1.41, SAs, by binding TcR variable (v) regions and HLA class II molecules, act as a bridge between TcR-expressing T cell Vβ regions and APC HLA. In this way, T lymphocytes are activated directly without an antigen being present [210]. SAs have been suggested to intervene in the pathogenesis of Kawasaki disease (Chap. 8) and RA.

Th1-Th2 Alternation

A large body of Th1- and Th2-like T cell IL abnormalities have been associated with systemic AIDs and models; however, the Th1/Th2 paradigm (hygiene hypothesis) has no application [385]. Some occur late in illness and are probably not causal, while others may be actively involved in regulation and dysregulation of immune responses. There is not a clear demarcation line between Th1 T cells and Th2 T cells involved in cell- or antibody-mediated AIDs. However, Th1 T cells represent a significant mechanism for eliciting EAE, whereas Th2 T cells inhibit encephalithogenous T, and thus provide protection without preventing the development of EAE [231]. In view of what has been said about potential IL defects, it appears that if pro-Th2 ILs are lacking, in EAE as well as in other AIDs, the parenteral administration of IL_4 could prevent diabetes in NOD mice [231].

X Chromosome

X-chromosome inactivation and resultant tissue chimerism may explain the female predisposition to systemic autoimmunity. For example, autoreactive T cells may fail to be tolerized by self antigens encoded by one of the two X chromosomes. In the periphery, these autoreactive T cells may stimulate B cells expressing the target X-encoded antigen, so the conjecture can be extended to explain how systemic autoimmunity may be induced [406].

Hormonal Mechanism

Sex and growth hormones directly stimulate the immune response, whereas estrogens depress it. Furthermore, the close correlations between immune system and neuroendocrine system increase susceptibility to AIDs [263].

Juvenile Rheumatoid Arthritis

Definition

JRA (also called juvenile idiopathic arthritis and juvenile chronic arthritis) is characterized by chronic inflammation, synovial cell proliferation and progressive joint damage. Both terms are usually considered synonyms; however, they are characterized by arthritis persistent for at least 6 weeks in JRA. Psoriasis and ankylosing spondylitis, and other known conditions are excluded from JRA [331]. We classify RA (rheumatoid arthritis) as a general term encompassing JRA; others prefer juvenile idiopathic arthritis (JIA). According to general knowledge, JRA comprises a group of diseases of unknown etiology, which becomes full-blown before 16 years of age and peaks at 2 and 9 years in males, and at 1–5 years in females [109].

Epidemiology

Table 18.5 [112, 262] indicates frequency, male/female ratio and mean age of onset. Table 18.6 [15, 29, 30, 89, 95, 117, 158, 169, 250, 255, 323, 392, 409, 463, 483] indicates number of children, their age, subtypes of JRA, and frequency. A meta-analysis of 34 epidemiological studies of JRA since 1966 reports an annual prevalence of 0.07–4.01 × 10^3 children and an annual incidence of 0.008–0.226 × 10^3 children [254]. The mean age of onset of disease may be at 11.7±3.39 years (range, 2–15) [288], with children aged 0.2–0.7 years [250], two children aged 2.1–3 [117, 458], two girls aged 1–5 [458], and one 1.5 [95]. In Greek children, the peak ages of JCA onset were between 2 and 5 years and between 9 and 12 years, with a high female predominance in pauci articular, poly artuclar [95], and in the systemic groups [250]. Two studies [392, 483] found a male:female ratio of 1.8:1.

Classification

A classification of JRA is shown in Table 18.5.

Etiopathogenesis

Genetics

JRA, like other polygenic diseases, shows genetic anticipation, that is earlier onset and worsening conditions from one generation to another [329]. The disease gene was localized to *16q12–21* by using members of consanguineous Saudi and Arabian families for a genome-wide search for homozygous-by-descent microsatellite markers [258]. JRA-like syndrome may also be a component of the *del(22q11)* spectrum [84, 458]. In rats, RA development is linked to chromosomes 10, 12, 14 and to two *loci* at the telomeric end of chromosome 12 and APR2 (acute-phase response 2) on chromosome 5 [303], in addition to quantitative trait *loci* on chromosomes 5, 10, 17, and 18 [1].

Susceptibility to developing JRA is linked to the presence of specific immunogenetic markers such as the HLA alleles summarized in Table 18.7 [119, 343] showing that the *DQB1*0402* allele contributes most of the HLA-associated risk, although alleles at other loci contribute independently [293]. JRA is associated with both HLA class I and class II genes. Since the *E. coli* HSP DNA specifically binds within the groove of HLA class II alleles known to be associated with pauci JRA [6], the class II associations are consistently less limited than those for class I. There are some differences in the JRA

Table 18.5. Clinical forms of JRA

Clinical forms	Frequency (%)	M/F (%)	Mean age[a]
1. Systemic	34.5	19/15.5	5.0
2. Polyarticular (poly) (≥5 joints involved)			
RF (IgM) positive	3.5	3/0.5	9.6
RF (IgM) negative	12	7.5/4.5	6.8
3. Pauciarticular (pauci) (″4 joints involved)			
Type I: early onset associated with iridocyclitis and ANF	34.5	26/8.5	4.2
Type II: early onset with polyarthritis	7.5	1.5/6	10.1
Type III: with none of the above characteristics	8	3.5/4.5	6.6
4. Juvenile spondyloarthritis (see Table 18.7)			

Data related to 200 children (Department of Pediatrics, University of Pavia, Italy).
Data from [112, 262].
ANF Antinuclear factor, *M/F* male/female ratio, *RF* rheumatoid factor.
[a] Mean age at onset (years).

Table 18.6. Epidemiology of JRA in children aged 0.75–19 years

Country	Reference	No. of children	Age (years)	Subtype	Prevalence/incidence
Austria	[169]	107	<16		4.28×10^{5}[a]
Germany	[463]	457	<16		6.6 (I)$\times10^{5}$ 14.8 (P)$\times10^{5}$
Greece	[95]	80	2–12	Pauciarticular	58.7%
				Systemic	25%
Italy	[323]	42	2.5–19	Pauciarticular	87.3%
				Polyarticular	12.7%
Nordic study	[29]	315			15×10^{5}
Denmark					$9–16\times10^{5}$
Finland					21 (15–26)$\times10^{5}$
Iceland					7 (1–13)$\times10^{5}$
Norway					$19–23\times10^{5}$
Sweden					15 (12–18)$\times10^{5}$
Sweden	[30]	334	<16		64.1×10^{5}
				Pauciarticular	68.3%
				Polyarticular	21.9%
				Systemic	6.6%
UK	[409]	1,991	<16		10×10^{5}
Israel	[158]	242			8.8×10^{5}
Canada	[250]				4.08×10^{5}
USA	[89]	1,752	<16		4.0×10^{5}[b]
Costa Rica	[15]	189	<16		6.8×10^{5} (I) 31.4×10^{5} (P)
				Pauciarticular	71%
Brazil	[117]	91	10.5±4.7	Pauciarticular	31%
				Polyarticular	27%
				Systemic	42%
India	[392]	94	0.75–12	Pauciarticular	47.3%
				Polyarticular	37.8%
				Systemic	14.9%
Taiwan	[483]	228		Pauciarticular	56%
				Polyarticular	36%
				Systemic	8%
Australia	[255]	816	12		3.7×10^{3}

The incidence of spondyloarthropathy was 2.9[a] and 2.0[b]$\times10^{5}$.

subgroups (Table 18.8) [15, 46, 112, 119, 331, 343, 428, 468]: significant associations with HLA alleles can be found mostly in pauci forms, and less frequently in systemic ones [112]. Specifically, some alleles (Appendix 1.1) are shared by several forms of JRA, such as *DPB1*0201–0202* [6], *DRB1*0801* and *TAP1*0201*, whereas others are specific, such as, *DRB1*1301* and *DQA1*0101* [120]. Commonly associated with JRA are *DRB1*0404*, which implies a 47 RR, and *DRB1*0401* with a 26 RR [119]. It is significant that the alleles and *DRB1*0405* are associated with JRA, both seropositive and seronegative, in 93% of cases. However, the *DRB1*0401* allele dominates in 71% of seropositive cases, whereas one of three alleles can be equally expressed

Table 18.7. Significantly positive associations between HLA alleles and JRA subtypes

JRA subtype	HLA association	Relative risk	Confidence intervals (95 %)
Pauci	*DRB1*01*	1.41	1.06–1.90
*DRB1*08*	7.23	4.42–8.78	
*DRB1*11*	3.62	2.97–4.65	
*DRB1*13*	2.30	1.79–2.98	
*DRB1*14*	2.09	1.38–3.14	
*DPB1*0201*	2.59	1.86–3.61	
*DQA1*0401*	3.84	2.52–5.95	
*DQA1*0601*	5.13	2.02–12.9	
*DQB1*0201*	1.53	1.15–2.05	
*DQB1*0301*	8.31	7.32–10.9	
*DQB1*0302*	3.92	1.96–7.81	
*DQB1*0303*	8.10	1.63–40.2	
*DQB1*0402*	85.56	20.8–351	
*DQB1*0603*	1.85	1.29–2.67	
*DQB1*0604*	2.75	1.61–4.69	
Pauci, extended	*DRB1*08*	3.34	2.1–5.23
*DRB1*11*	1.70	1.24–2.29	
*DQA1*0401*	2.28	1.23–4.21	
DQBI-0301	2.36	1.51–3.71	
*DQB1*0603*	1.85	1.11 –3.08	
Seronegative ANA +	*DRB1*08*	3.62	1.88–7.97
polyarthritis	*DRB1*11*	1.84	1.51–2.95
*DQA1*0401*	3.67	1.70–7.92	
*DQB1*0301*	3.37	2.08–5.43	
*DQB1*0302*	4.65	1.76–12.2	
*DQB1*0402*	27.47	5.22–141	
Seronegative ANA –	*DRB1*08*	1.94	1.11–3.37
polyarthritis	*DRB1*14*	2.33	1.14–3.85
*DQA1*0401*	3.79	2.15–7.71	
*DQB1*0301*	5.42	3.66–8.04	
*DQB1*0302*	12.52	7.17–25.41	
*DQB1*0402*	48.94	10.7–222	
Seronegative polyarthritis	*DRB1*04*	2.16	1.23–3.78
*DQA1*0401*	2.89	1.17–7.13	
*DQB1*0301*	8.45	5.01–14.2	
*DQB1*0302*	19.37	8.47–44.2	
DQB1-0402	60.87	13.7–349	

The most recent HLA nomenclature is in Appendix 1.1.
Data from [119, 343].

Table 18.8. Forms of JRA

Systemic (a, b, c, d)
No gender predominance, no typical age of onset, from 6 months onward
Arthritis associated with fever, rash, serositis, hepatosplenomegaly, pleuritis, pleurocarditis (25%), neurological symptoms (20%), and abdominal pain (10%)
Variable characteristics of children:
Linear growth retardation, osteoporosis
Arthritis may develop long after systemic features with luxation of large and small joints and joint destruction
Late Rx changes may reveal fractures, marginal erosions
25% of children develop severe, chronic arthritis
Poly: type I (a, b, c, e)
Marked predominance of females aged ≈8 years
RF (IgM)+ : more unfavorable outcome (arthritis 50%)
Five or more large and small joints in upper and lower joints rapidly and severely involved
Chronic uveitis
Plane Rx show erosive changes as early as 6 months after onset of JRA
Poly: type II (a, b, c, d, e)
RF (IgM) – less unfavorable outcome (arthritis 10–15%)
Mostly young males
ANA+ in females aged <3 years, *HLAB27*+
Enthesitis, hips, knees, and ankles involved
Chronic uveitis
Pauci: type I (a, b, c, d, e)
Most common in young girls aged <5 years, with a girl-to-boy ratio of 1.5:1 [15]
≥5 involved joints
50%–90% of children ANA+
Frequency of iridocyclitis: 30%
No systemic manifestations

Pauci: type II (a, b, c, d, e)
Most common in male adolescents
May have a polyarticular course
≥5 involved joints
Clinical overlap with juvenile spondyloarthropathies
Enthesitis-related arthritis (a, d, e)
Arthritis and enthesitis, or arthritis or enthesitis with at least two of the following:
Sacroiliac joint tenderness and/or inflammatory lumbosacral pain
HLA-B27 antigen
Onset of arthritis in a male over 6 years of age
Acute (symptomatic) anterior uveitis
History of ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with IBD, Reiter's syndrome, or acute anterior uveitis in a first-degree relative
Juvenile onset spondyloarthropathy (JOSA)
Male predominance
FH+
Later onset ≥9 years
Frequent enthesitis and arthritis affecting heel, feet, knee
Urethritis, cervicitis, or acute diarrhea within 1 month before arthritis
Acute uveitis ±10% of children
High frequency of HLA
ANA and IgM RF
Clinical forms:
Arthropathy associated with AIDs
Juvenile psoriasic arthropathy
Entheso-arthropatic syndrome or undifferentiated spondyloarthropathy
Juvenile ankylosing spondylitis JAS

See also Table 18.6.
JOSA is a term for a group of HLA-B27-related disorders. The letters a–e stem from the recent ILAR (International League of Associations for Rheumatology) classification, which has introduced five variable exclusions: a) Psoriasis or a history of psoriasis in the patient or first-degree relative, b) Arthritis in an HLA-B27-positive male beginning after the 6th birthday, c) Ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with IBD, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative, d) The presence of IgM rheumatoid factor on at least two occasions at least 3 months apart, e) Children may not necessarily belong to the expected subgroup for enthesitis-related arthritis (ILAR) and juvenile ankylosing spondylitis (European League Against Rheumatism, EULAR) [29]. The ILAR classification system does define genetically distinct groups of patients [428] and has been recently updated [330].
Modified from [46, 112, 119, 331, 343, 468].
ANA antinuclear antibody, *RF* rheumatoid factor.

in 33% of seronegative cases [475]. In *DRB1*0405*-positive RA patients, type II collagen reactive T cells might play a pivotal role in the development of RA through IFN-ɣ production [301]. Studies on TcR genetic polymorphism have shown the *Vβ-6.1β* allele association with some forms of JRA, especially when it is characterized by pauci onset and poly course, and especially with the *DQA1*0101* allele, thus suggesting the existence of a genetic interaction between HLA and TcR genes in this particular form [57]. In contrast, a candidate disease-

associated TcR polymorphism was not identified in pauci-onset JRA [293].

Since *DQ/DR* haplotypes may influence the susceptibility to developing JRA, a pathogenetic role is suggested for the *HLA-DQ locus,* and a protective role for the *HLA-DRB1 locus.* Thus, JRA could be caused by *DQ* alleles presenting arthrogenous antigens, which start the autoimmune reaction, and by nonprotective *DRB1* [501]. Studies on genetic predisposition have shown that *DQB1*0301* (*DQ7*) and *DQB1*0302* (*DQ8*) are in linkage disequilibrium with several *DR4* haplotypes, and many *HLA-DR4* RA patients express one of these alleles. Therefore, *DQB1*0302* can be found in subjects both susceptible and resistant to RA [501]. In this case, the *HLA-DR* protection seems to prevail over the *HLA-DQ*-mediated susceptibility. Overall, an excess sharing of two *DR* alleles was found among affected sibling pairs with JRA; however, evidence was established for linkage between poly JRA and the *HLA-DR* region [341]. A less severe form of JRA is caused by the heterozygosity between *DRB1* alleles, both protective and nonprotective, while the homozygosity of two nonprotective *DRB1* increases the risk and severity of RA [88]. This explains the increased severity in *DRB1*0404 HZ* or *DRB1*0404/DRB1*0101* [474] HETs, and the reason why the homozygosity for shared epitopes implies the highest concordance for RA in monozygotic twins (MZ) [182]. As for the strong association with *HLA-DRB1*0401* or *DRB1*0101* molecules, the latter have a five-amino acid sequence in the 3rd hypervariable region of the β chain, QKRAA, which is found in many HLA-associated alleles (*HLA-DRB1*08*, *HLA-DRB1*11*, *HLA-DRB1*13*) correlated to RA. This shared epitope is predictive of a progressively destructive illness, which is dose-dependent and at high risk for these HZ genes [7, 474]: in fact, it is associated with *HLA-DRB1*1040* and *DRB1*0404* [474].

Interestingly, *HLA-DRB1*1104* was the allele most significantly associated with susceptibility to JRA [131], as well as three other haplotypes (*DRB1*08-DQA1*0401-DQB1*0402*; *DRB1*11-DQA1*05-DQB1*03*; *DRB1*1301-DQA1*01-DQB1*06*) [428]. Moreover, the pair of *DQA1*0501/DQB1*0301* alleles corresponds to the *DQ* molecule *DQ7* on the cell surface, which has been described to be strongly associated with JRA [114]. Alleles significantly participating in JRA protection were *DRB1*04-DQA1*03-DQB1*03* [428], *HLA-DRB1*1602* associated with a lesser degree of susceptibility [131], and *HLA-DRB1*1501* and *HLA-DRB1*1402* also with JRA protection [131]. An association between *HLA-DRB1*0701* was found in those ANA+ [131]. An interesting finding is the presence of both *DRB1*0307* and *DRB1*0308* in significant proportion in children with JRA and their absence in the controls. *DRB1*0307* was present in 15%–16% of children with pauci and poly JRA. *DRB1*0308* was only detected in children with pauci JRA, none of the children with poly JRA or the controls had this allele [10]. The most commonly expressed alleles were *HLA-DRB1*1104* in the pauci subset, *HLA-DRB1*0404* in the poly group and *HLA-DRB1*1602* in patients with systemic JRA [131]. In the early onset pauci subtype, positive-HLA associations with *DR11* and *DR8* alleles were shown. In the late onset pauci group, only the *B27* allele was increased [95]. Recent data suggests that a gene in linkage disequilibrium with *D6S265*5*, but distinct from *HLA-A*02* and a *locus* near the *D6S2447* microsatellite, which is flanked by *DQB1* and *DRB1* [363], could be markers for an additional susceptibility gene in JRA [363, 394]. *HLA-A* and *HLA-DRB1* were confirmed as independent susceptibility *loci* for pauci disease. Linkage of the HLA *loci* seemed to be attributable to maternal preferential transmission of alleles [503]. Twins show concordance for the type at onset, JRA course, etc. [179]: for instance, JRA developed in two 1-year-old MZ twins, with a lapse of 5 months between the first and the second [86]. Analyzing 71 affected sibling pairs, 63% were concordant for gender and 76% for onset type [283]. In an analysis of 118 affected sibling pairs, 14 pairs of twins were identified in which both twins had arthritis. One pair comprised a girl with poly and a boy with persistent oligo pauci. The other 13 pairs (11 MZ, two DZ and two of unknown zygosity) were concordant for gender (nine female, four male), disease onset (ten pauci, three poly) and disease course (eight pauci, five poly) [342]. In Finland, 41 JRA multicase families with 88 affected siblings have been collected over a period of 15 years. This study estimated the RR of JRA to be nearer 20 [377]. Within this set of families there were eight sets of MZ twins, two of which were concordant for JRA. Both sets of twins were concordant for disease course but were unexpectedly different for disease onset [368].

Immune Factors

JRA immune mechanisms, which are associated with HLA haplotypes (Table 18.7), require mast cell recruitment [193]. These are present in the synovia, localized on the microvascular endothelium, and are quantitatively correlated with activated CD4 [249], which under the action of IL_3 continue to grow and are activated along with B cells [126]. Mast cells degranulate and release mediators and ILs, thus contributing to clinical flare-ups [274] and, together with other inflammatory and adhesion cells, to the development and persistence of symptoms [476]. The role played by DCs in this and other AIDs is shown in Table 18.9 [423]. Early stages of JRA can be summarized in this way: T lymphocytes from thymus, which recognize self-HLA molecules, go through positive selection, whereas those unable to recognize endogenous Aags avoid deletion and enter the circulation. DCs, both at rest and circulating, in the SF (synovial fluid) express a relatively low number of HLA molecules with self peptides. They produce CD80 and CD86, localized in the SF near T cells, but they do not

Table 18.9. Features of dendritic cells (DCs) in autoimmune diseases (*AIDs*)

Features of DCs in diseased organs	AIDs
Enrichment of DCs	RA, CD, psoriasis, autoimmune thyroiditis
Infiltration of DCs early in disease	RA, autoimmune thyroiditis with perivascular distribution
Differentiation and expression of CD86	RA, psoriasis
More potent APCs than parenchymal cells of inflamed organ	RA (synoviocytes, fibroblasts, autoimmune thyroiditis (thyrocytes, psoriasis (keratinocytes), diabetes (islet cells)
Enhanced AMLR stimulation by DCs from inflamed tissue	RA (DC of SF) psoriasis (dermal DCs)

Data from [423].
AMLR autologous mixed lymphocyte reaction, *APCs* antigen-presenting cells, *CD* Crohn's disease, *DCs* = dendritic cells, *SF* synovial fluid.

express CD80 [423]. DCs are reinforced through differentiation when GM-CSF and TNF-α are present and induce autoreactive T cells that escaped thymic deletion to mature in lymph nodes so that TcRs can be primed through DC presentation of self peptides differentiated in the SF. This model suggests how an autoreactive response to self peptide can trigger JRA without the requirement for a synovial antigen that initiates JRA [423].

The pathogenic role played by *microbial SAs* is suggested by the Vβ-14 T lymphocyte decrease in the bloodstream, but they increase, both in number and in quality, in the SF of RA patients [210]. Normally, the exposure to a microbial SA can cause the deletion of most Vβ-14 T cells, whereas in susceptible subjects surviving lymphocytes can migrate to the synovial tissue, recognize an antigen *in loco,* proliferate oligo-clonally, and mediate the joint damage, which is a JRA characteristic [210]. Highly differentiated SF T cells expressed early and late markers of activation with little evidence of *in situ* proliferation. CD25 and CD71 significantly increased in the SF T-cell subset, but the differences between SF and peripheral blood were not as remarkable as for CD69 or HLA-DR. CD8 T cells expressed significantly higher levels of CD69 and HLA-DR than did CD4 T cells, whereas CD25 and CD71 expression was higher on CD4 than on CD8 T cells [34]. In addition to all activated, potentially pathological T cells, the SF from RA patients may contain $CD25^{bright}$ $CD4^{+}$ T cells with a regulatory capacity [49]. SF lymphocytes also proliferate in response to IL_{15}, showing their enrollment and activation in the synovial membrane, thus concurring to the pathogenesis [269].

T-cell activation may result in an overflow of events, including TNF-α release, possibly under the control of specific genetic markers. The frequency of polymorphisms at the –1031, –863 and –857 positions of the TNF promoter was shown to be significantly higher in a group of Japanese systemic-onset JRA patients compared with those observed in controls. [82]. Particular alleles of a microsatellite marker in the TNF-α gene were found to be strongly associated with early-onset pauci JRA in German patients [102]. In UK patients the association of several TNF single-nucleotide polymorphisms was detected and linkage of the *locus* to pauci JRA was established [504]. The TNF –308 and –238 polymorphisms were studied in Czech and Turkish JRA patients, but no association with either polymorphism was found [312]. In contrast, a positive association with TNF polymorphisms was reported in a large panel of UK Caucasian pauci JRA patients [502].

Another pathogenetic hypothesis involves *Enterobacter proteoglycans,* with the release of the relative IgA antibodies, whereas CMI responses are more difficult to prove [326]. Despite inconclusive evidence [453] and considering the large digestive system thin epithelial walls, one can hypothesize that a bacterial attack colonizes the intestine, with the microorganisms or their degradation products released into the bloodstream [326]. Recently, the use of an HSP antigen, which is equivalent to *E. coli*, has proven that sera from JRA patients challenged with the antigen, and all the more with SF cells, react significantly more than normal controls, or children suffering from rheumatic diseases different from JRA [6]. This data shows that JRA can represent a chronic form of RA, whose pathogenesis is in common with other disorders such as acute rheumatic fever, Reiter's disease and ankylosing spondylitis [6].

Recent developments of JRA pathogenesis introduce *bcl-2, Fas, NF-κB, and the status of apoptosis.* They show that a distinctive phenotype of RA fibroblast-like synoviocytes, that is persistent activation, proliferation, and resistance to apoptosis, is related to the autocrine activation of $IL_{15}R$ by fibroblast-like synoviocyte-derived IL_{15} [215]. IL_{15} levels in systemic JRA were higher than in controls. An increased apoptotic index correlated with raised IL_{15} [396]. However, blocking IL_{15} biological activities using $IL_{15}R\alpha$ chain substantially reduced endogenous expression of bcl-2 and bcl-x(L), and RA fibroblast-like synoviocyte proliferation that was reflected by increased apoptosis [215]. NF-κB appear to be activated in vivo, both Fas and FasL being expressed by synovial cells [391]. Apoptosis is ongoing in synovial tissues with significant patient variations, which could

be in part due to the level of Bax expression [393]. The role of galectin-1 (Gal-1) and galectin-3 (Gal-3) proteins with pro-apoptotic and anti-apoptotic properties, respectively, has been clarified. Thus, Gal-1 expression is down-regulated in patients with pauci JRA, accompanied by low Bcl-2 expression and low proliferation rates, and Gal-3 expression is up-regulated in synovial tissue from those with poly JRA [156].

Immunopathology of Joint Damage

The study of pathogenetic mechanisms is gathering an ever growing body of experimental evidence, with T cells, RSs (rheumatoid synoviocytes) and vitronectin (VN) as the major causes of damage. The damage worsened by the targeted migration of CD45 with all its ILs and integrins [74]. T lymphocytes have a clear dominant position in the synovial pannus, where *CD8 lymphocytes are mostly localized in perivascular areas,* while *CD4 cells infiltrate the stroma* (Fig. 18.9) along with B lymphocytes. Significantly, there are APCs, like the DCs [423], at the synovial level. The key role played by T lymphocytes in the progression of articular damage is demonstrated by the expression of activation marker cells such as the IL_2R and transferrin receptor (CD71), the RA improvement after total lymph node irradiation, the use of immunosuppressors, etc., or in patients with AIDS and cytopenia [382]. The CD4 population seems to be activated by the presence, on the cell surface, of cellular activation markers such as HLA class II and β1 integrins. T lymphocytes have the phenotype of memory cells (CD45RA and CD45RO), with CD25 and CD102, which cause their proliferation and activation. There is also prevalence of lymphocytes with Vβ TcR [210], not in serum, and γδ with preponderance of Vδ1 cells [468]. It is also possible that memory cells have greater ability in binding to endothelial cells and migrate to synovial tissues [74]. T lymphocytes activated by CD25 are thus highly responsive to IL_2, as proven by the presence of IL_2 mRNA in the SF [496]. Eventually, a wide spectrum of proinflammatory IL is generated: type A RSs express high IL_6 concentrations, whereas IL_1 and TNF-α present in the SF increase CD54 expression (ICAM-1) 496]. Inflammation is also caused by high-molecular-weight SF IgG stimulating $IL_{1\alpha}$, IL_6, IL_8, TNF-α and GM-CSF from PBMCs (peripheral blood mononuclear cells) [181]. Recent data have shown that these ILs also interact with IL_{13}, with potentially negative consequences for C3 synthesis in the inflamed joints [190]. Similarly, IL_{10} plays a significant role in immunity by regulating/inhibiting IL production by PBMCs and T lymphocytes [189]. The intra-articular production of rheumatoid factors (RFs) that are Aabs reacting with epitopes on the Fc fragment of Ig, mostly the IgM type, may reveal the *in situ* release of IL_6, a well-known primer of B plasma cells [382]. IL_6 levels, a well-known pro-inflammatory IL, were correlated with the degree of knee joint vascularity in 28 children with active JRA or JOSA, which may stress the role of IL_6 as an inducer of neoangiogenesis in JRA [383]. SF CD62E levels accurately reflected intrasynovial inflammation and soluble CD54 (sCD54) the effects of disease-modifying agents [35].

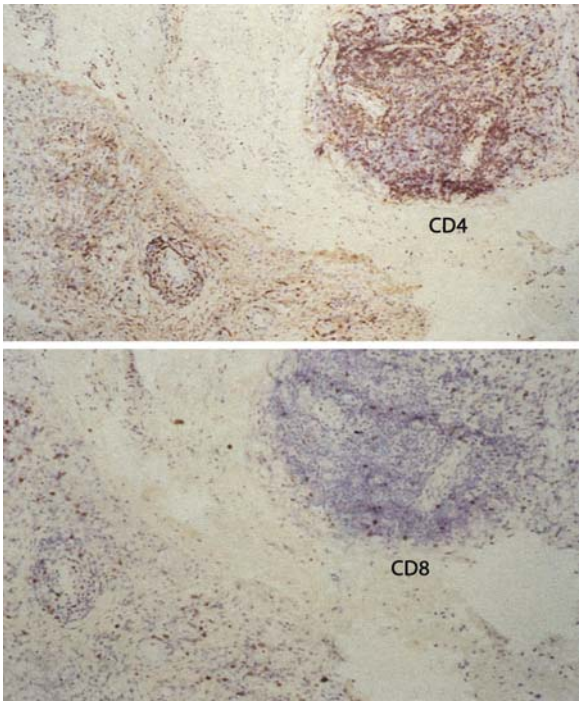

Fig. 18.9. Immunoperoxidase staining of RA synovial membrane

RSs bind to circulating immune complex (CIC) deposited on the cartilage, and since they have no FcγR (Table 1.3), they use complementary components as a go-between with CICs. The latter bind the complement and consequently VN, whose receptor is present on the RSs, which in turn can reach the cartilage surface. The following invasion front is marked by local alterations to collagen, fibronectin and other proteins from the matrix [79]. The fibronectin peptides, by interacting with synovial cell-specific receptors, can induce metalloproteinase expression in type B synoviocytes. A number of receptors for fibronectin, laminin, collagen and fibrinogen products – resulting from β1- and β3-integrin combination – have been described. The action of these superficial structures ensures a close link to the matrix. Thus, SRs are able to migrate through basal membranes and invade tissues. Cathepsins B, D and L as proteinases play a major role in destroying synovial tissue and cartilage matrix [413]. In this respect, it should be noted that SRs express large amounts of CD106, whose ligand $\alpha_4\beta_1$ is present in the rheumatoid cartilage [79]. The pathogenesis of structural modifications is summarized in Fig. 18.10. The histopathology shows the cells in action and the articular lesions: see Figs. 18.11–18.13 [357].

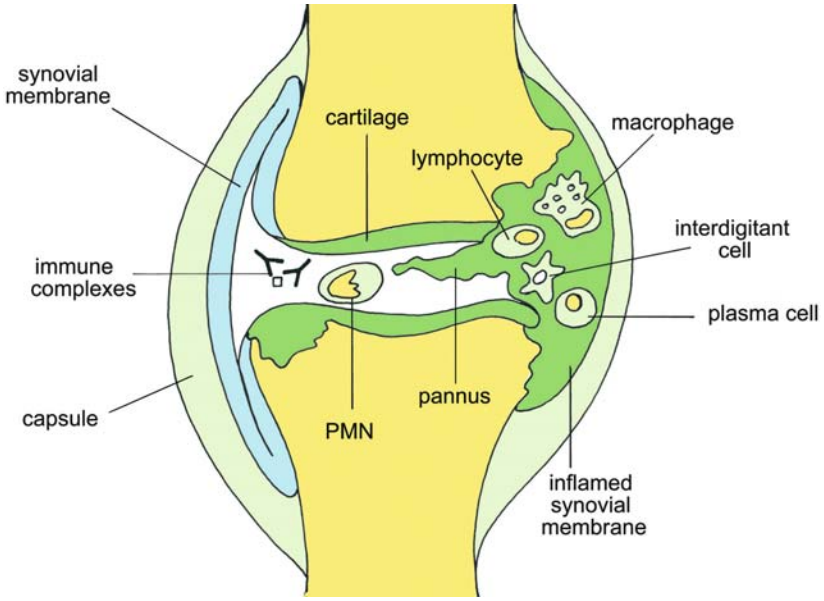

Fig. 18.10. Pathogenesis of RA. The inflammatory infiltrate, first limited to the synovial membrane, hypertrophies, covering and eventually eroding the synovial cartilage and bone

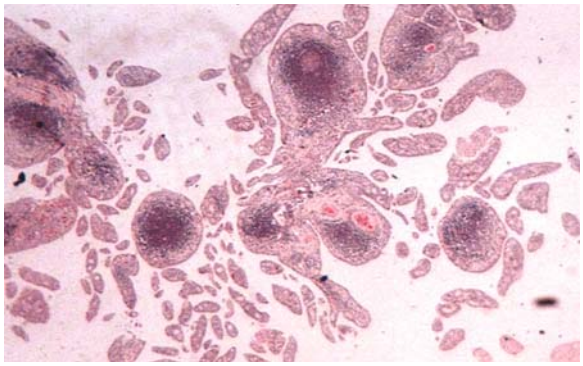

Fig. 18.11. Section of synovial membrane showing marked villous hypertrophy with cellular infiltration and aggregates organized into lymphoid follicles

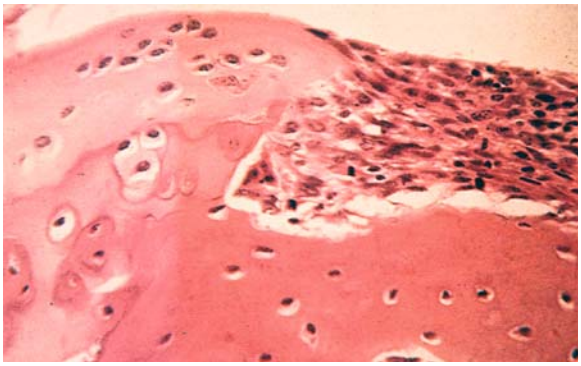

Fig. 18.12. Histology of pannus, appearing as a layer of lymphocytes, macrophages and plasma cells covering and eroding the cartilage. Note the destructive tissue changes at the pannus margin

Additional Factors

An association with food allergy (FA) has been theorized over recent years [28, 320, 321].

Changes to diet have not always improved the symptoms consistently [319]. The theory expressed by the US Arthritis Foundation, which affirmed that there was no convincing evidence on the efficacy of these manipulations, has been disproved by further research [319]. It would be strange if FA would not imply any arthritic disease such as that seen in gout and lupus [320]. As for the pediatric age, only three cases involving the connection between joint complaints and FA in children have been described in the literature [380]. This concept, however, should be further clarified in order to avoid any disappointment for families, who might sustain higher expenses only to have simple dietary changes, which would aggravate a nutritional situation already compromised.

RA can frequently be associated with IBDs: 12% in UC, and 20% in CD. The lesion severity depends on the disease activity (see below).

Clinical Presentation

Figure 18.14 shows the disease may have an early onset. Clinical presentation in JRA includes symmetrical joint inflammation early in the course of the disease and findings of joint destruction with chronic disease. Warmth, swelling, pain, reduced range of motion possibly restricted in deeper joints, and palpable effusions characterize active synovitis [41]. Musculoskeletal pain was the most common reason for referral to a pediatric rheumatology clinic [268]. Back pain in 3.1% and neck pain in 0.6% of children were not diagnosed, perhaps because they were associated with affective disorders [250]. Clinical polymorphism appears clearly from

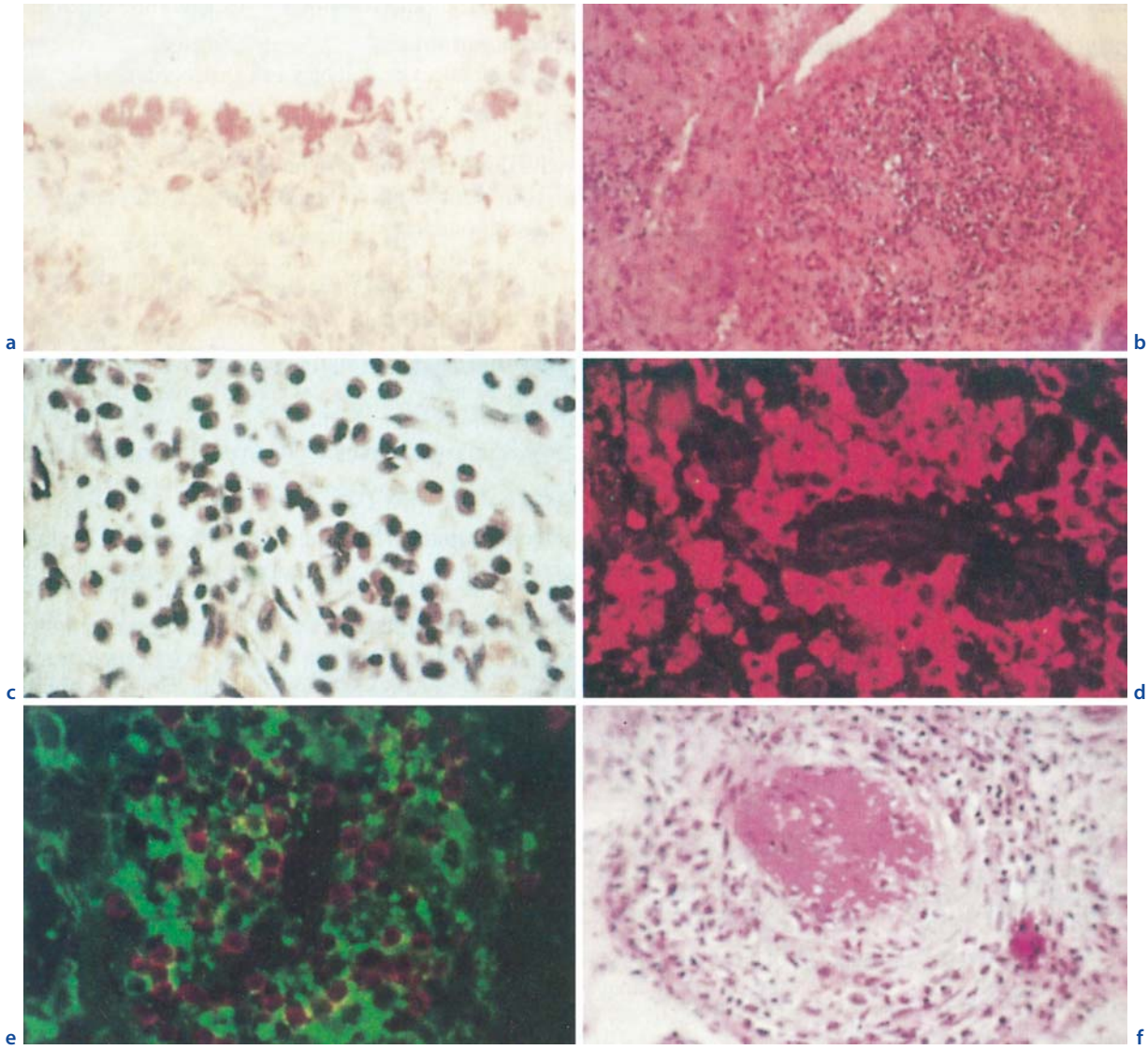

Fig. 18.13 a–f. Histopathology of RA. **a** Histology of the pannus showing long, stained dendritic processes. **b** Chronic inflammatory cells in the deeper layers of the synovia. **c** High-power view of an area of diseased synovia showing collection of plasma cells. **d** Rheumatoid synovia showing several cells stained by anti-HLA-DR (anti-class II). **e** Rheumatoid synovia showing class II-positive accessory cells (*green*) in intimate contact with $CD4^+$ T cells (*orange*). **f** Granulomatous appearance of rheumatoid noduli with central necrotic area surrounded by epithelioid cells, macrophages and scattered lymphocytes

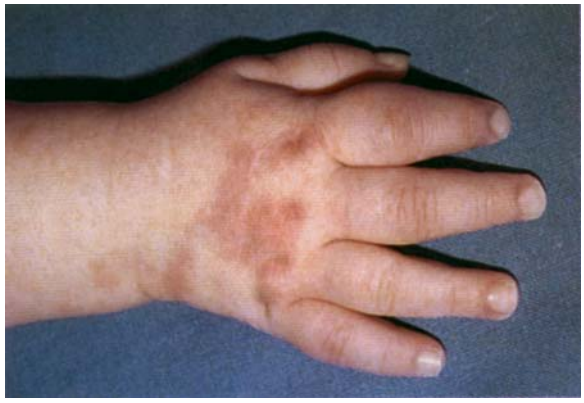

Fig. 18.14. Juvenile rheumatoid arthritis (JRA) in a child. Severe swelling of the proximal interphalangeal joints causing typical spindle-shaped fingers

Table 18.6 and Table 18.8, with relative frequencies for pediatric JRA. Systemic onset disease (subtype 1) is characterized by very high fever, which is characteristic, quotidian, with spikes to >39°, rarely intermittent, mostly at night, associated with one or more of the following: faint erythematous rash, generalized lymph node enlargement, hepatomegaly and/or splenomegaly, and serositis [330]. Arthritis is usually defined as palpable

swelling or effusion of the affected joints, with loss of range of motion, pain on palpation or mobilization in one or more joints, and loss of grip strength in the hands, leukocytosis, pleuropericarditis, and extended joint involvement [41]. JRA symmetrical presentation is often associated with a positive RF; manifestations are poly articular (≥5 inflamed joints), which are required as a criteria for classification of this type of onset, although as many as 20–40 distinct joints may be affected, and JRA may persist into adulthood (subtype 2 ≤5 inflamed joints). RF-negative children have joint involvement and probably less severe disease. An early-onset group typically occurs in young girls with a positive ANA (anti-nucleus antibodies) and an increased risk for iridocyclitis (subtype 2). Children with ≤4 joints involved may be defined as having a pauci articular disease. The joints most likely to be affected are the knees, elbows, wrists, knees, feet and ankles. The most important feature of JRA is joint erosion, which leads to deformity and, in severe cases, to severe disability [506]. In the subtype 3, arthritis affects the spine as well as other joints, although spinal involvement may not occur until children reach their late teens. Children may be HLA-B27-positive and suffer from musculoskeletal features of a JOSA [506]. The swelling associated with restricted movement, notably during or after periods of immobility, leads to the typical symptom of early-morning stiffness and to the easy fatigability after school in the early afternoon [343]. JRA also affects the cervical spine, shoulders, and hips, and hydrarthron of the knee or dactylitis of both the hand and foot may be found at onset [506]. Pain alone is not sufficient to make the diagnosis of arthritis [331]. The form characterized by early onset and poly progression is strictly connected to the JOSA, to which it belongs nosographically [262].

Uveitis (Chap. 14) complicates several forms of JRA. It was observed in 5.7% [483], 8% [506], or 8.6% of children during the first 6 months of the disease [30]. Mean age at uveitis onset was 4.39 [29] or 5.4 years [323]. In 158 children with JRA who had eye examinations, 39 (25%) developed uveitis, 16 of them (41%) had uveitis on the initial eye examination, and 23 (59%) developed uveitis within 4–81 months [205]. Pauci onset, ANA+, young age, but not gender are risk factors for developing uveitis in children with JRA [30]. Other authors observed no correlation between prognosis and sex, age at the onset of uveitis or JRA, pattern of JRA, or positivity for ANA or HLA DR11 [323]. Children with suspected uveitis should be slit-lamp examined, every 3 months if positive, and every 6 months if negative [323]. ANA should be viewed as a significant determinant for uveitis in JRA children [30].

Diagnosis

There is substantial uniformity among most major clinical variables that have been suggested for an objective definition of articular involvement, with discrepancies between subjective perception and objective assessment of joints [136]. Among 124 children with micrognathia, short stiff neck and short stature were noticed among poly and systemic onset JRA. Seventy percent of oligoarthritis group developed inflammatory low back ache. Bony ankylosis of tarsal and carpal bones were seen in eight cases [288]. Figures 18.15–18.22 show a wide range of diagnostic imaging (interphalangeal and temporomandibular joints, cervical spine, hand and wrist, knee, tibiotarsal joint and foot) in children aged 7–19 [63]. X-ray evident joint space damage was seen within 1 year of onset in poly children, and by 5 years 2/3 of poly and systemic children had damage [39], but magnetic resonance imaging (MRI) and ultrasound are now providing a more effective and safer alternative [184]. The major elements of the differential diagnosis are summarized in Table 18.10 [378]. They include the inflammatory seronegative spondyloarthropathy, or pauci onset juvenile psoriatic arthritis, occurring in symmetric distal *osteoarthritis-like* joint disease, symmetric proximal *JRA-like* joint disease, and *ankylosing spon-*

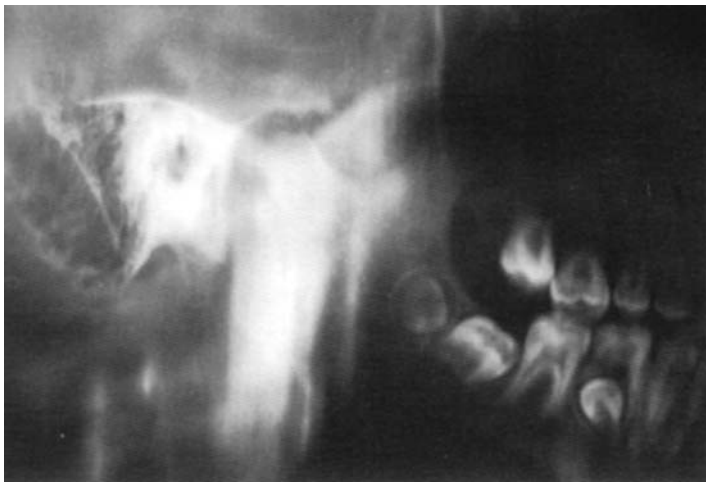

Fig. 18.15. Temporomandibular joint (TMJ). Stratigraphy in a 19-year-old boy affected with pauci JRA since the age of 18 months. Widespread ulcerations of both articular heads have provoked the destructive changes of the anterior tubercle of the temporal bone and of the proximal part of the mandibular condyle; closing the mouth, the mandibular condyle is subluxed forward and proximally

Table 18.10. Differential diagnosis of rheumatic disease

Characteristics	RA	JRA	SLE	Dermatomyositis
Sex	No predilection	Dependent on subgroup	F>M	F>M
Age at onset	≥3 years	≥1 year	Usually >8 years	≥2 years
Pathogenesis	Poststreptococcal	Unknown	ICC disease	Unknown
Manifestations				
Joint	Transient, migratory, arthritis – large joints	Pauci- or poly-articular chronic (≥6 weeks)	Arthralgia, transient or chronic arthritis	More frequent contractures occasional arthritis
Extra-articular	Fever, chorea, cardiac disease, rash, nodules	Dependent on subgroup: systemic JRA: fever, rash; pauciarticular: iridocyclitis	Occasionally systemic: nephritis, rash, CNS and hemato-logical involvement	Muscle weakness, rash, gastrointestinal pain, respiratory involvement
Diagnosis	Clinical (Jones criteria)	Clinical (JRA criteria)	Clinical (LES criteria)	Clinical: rash + myositis muscle biopsy
Laboratory	Prior streptococcal event	May have ANA, RF	ANA, low comple-ment titers, anti-DNA antibodies	Abnormal muscle enzymes, EMG, muscle biopsy
Natural history	Arthritis – transient carditis may cause permanent damage	Chronic arthritis may be destruens	Chronic or recurrent, potentially fatal	Chronic, interstitial lung disease, potentially fatal
Treatment	Prophylaxis against group A streptococcus	Anti-inflammatory, physical therapy	Anti-inflammatory, CSs cytotoxic medication	CSs, cytotoxic medication, methotrexate, alendronate

Modified from [378].
CSs corticosteroids, *EMG* electromyogram.

dylitis-like disease. Small joint disease or wrist disease within 6 months of disease onset were much more suggestive of psoriatic arthritis than those of pauci JRA [170]. Differential diagnosis of JRA also includes spondyloarthropathy, infectious arthritis, and IBD. Certain vasculitis syndromes, including Schönlein-Henoch syndrome, Kawasaki syndrome, and Takayasu arteritis (Chap. 8) should also be differentiated. Neither ANA nor RF evaluations were useful in evaluating children with musculoskeletal complaints [268]. Sex differences are not useful since girls were ANA+ in 83 of 197 (42%) and boys in 40 of 118 (34%) cases [29]. ANA should be taken into account in the diagnosis of several JRA children according to Table 18.8.

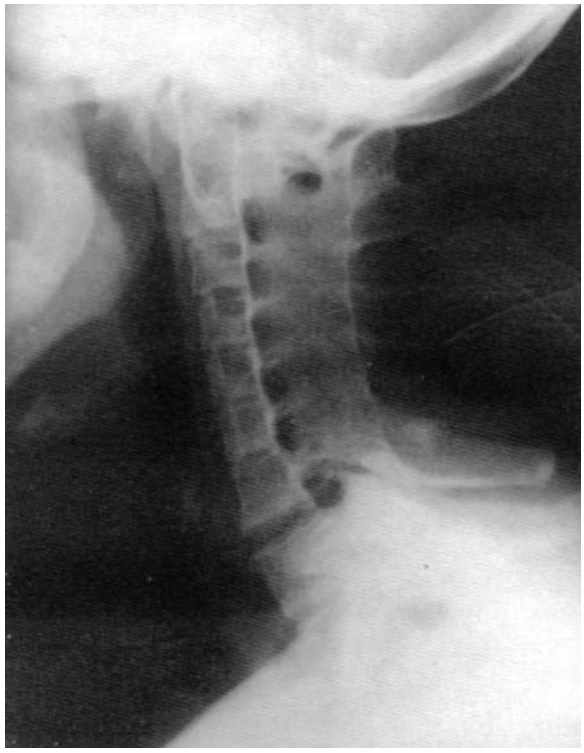

Fig. 18.16. Cervical spine. Latero-lateral projection in a 16-year-old girl affected with seronegative polyarticular JRA since the age of 12 years. C2–C7 synostosis of apophyseal spaces with consequent atrophy of intervertebral disks, hypoplasia of vertebral bodies, and loss of normal lordosis

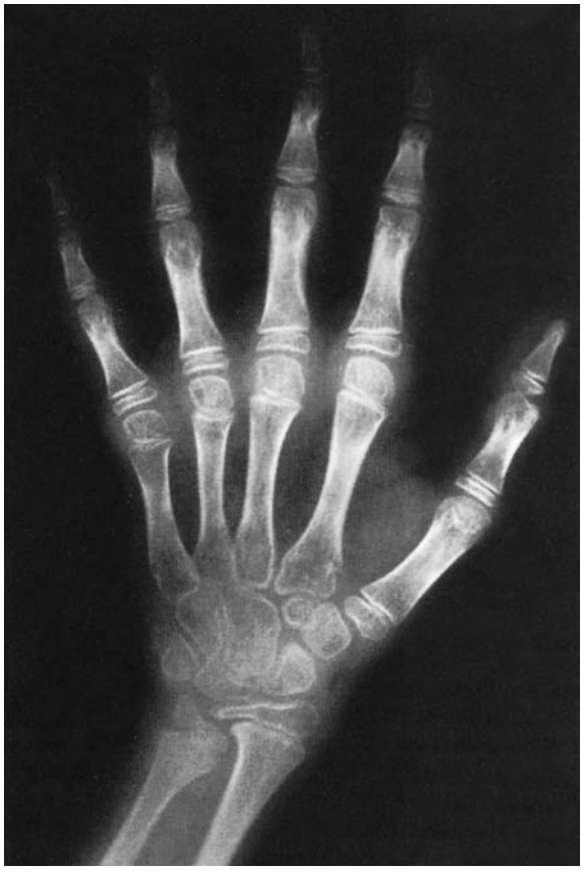

Fig. 18.17. Hand and wrist. Anteroposterior projection in a 7-year-old girl affected with pauciarticular JRA since the age of 3 years. Reduced ulnar development with ulnar subluxation of the carpal bone; fractures by compression of the radial distal epiphysis, metacarpal heads especially laterally, and phalangeal epiphyses; tumefaction of articular soft tissues; phalangeal periostitis, and destruction and fusion of wrist bones

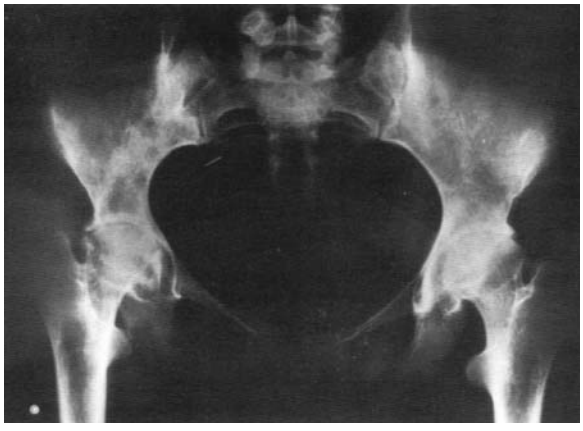

Fig. 18.18. Pelvis. Anteroposterior projection in a 12-year-old girl affected with pauci JRA since the age of 3 years. Widespread osteoporosis, cotyloid tectum thoroughly destroyed by confluent ulcerative foci; markedly hypoplastic femoral heads and with cranial intracotyloid luxation; striking narrowing of joint spaces; rough femoral heads in marked valgus by protracted load absence

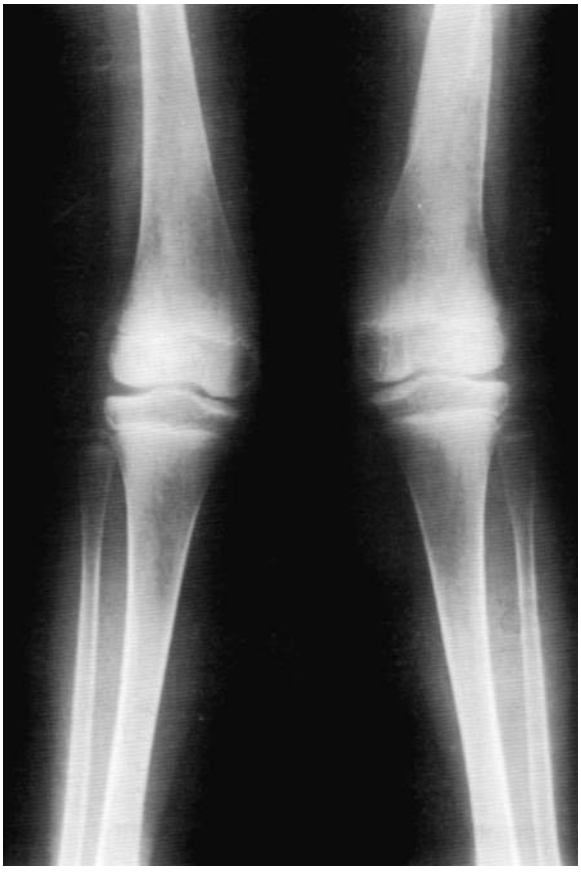

Fig. 18.19. Knee. Anteroposterior projection in an 8-year-old girl affected with systemic JRA since the age of 2 years. Symmetric metaepiphyseal hypoplasia of femoral heads with consequent genu valgus, which contrast with hypoplastic diaphyses; epiphyseal fracture by compression of internal tibial condyles; osteoporosis; result of mean diaphysis of left femur; light narrowing of joint space, negligible erosions

Treatment

The goals of treatment in JRA are to control inflammation, prevent progressive joint destruction, preserve and improve the activities of daily living, and alleviate pain [41]. Medical treatment remains a challenge [109]. The therapeutic aspects are listed in Table 18.11 [22, 137, 263, 506]. Current recommendations are to initiate treatment with disease-modifying antirheumatic drugs (DMARDs) within 3 months of diagnosis. Commonly utilized DMARDs include methotrexate (MXT), leflunomide, sulfasalazine, and hydroxychloroquine. While some children respond to treatment with NSAIDs and intra-articular or pulsed CSs, others require further treatment. There is evidence that MXT is an effective second-line drug for such children, and it is increasingly used earlier in the course of the disease, with the aim of preventing long-term joint damage. Some children, however, have disease that does not respond adequately to MXT or they cannot tolerate MXT treatment. These patients are treated with other DMARDs.

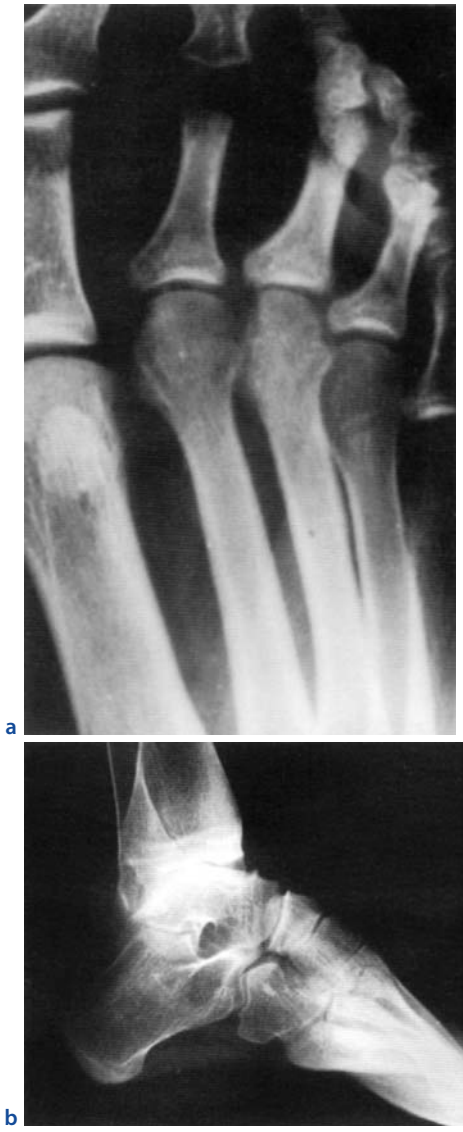

Fig. 18.20. **a** Tibiotarsal joint and foot. Latero-lateral projection in an 18-year-old girl affected with seronegative polyarticular JRA since the age of 4 years. Secondary astragalotibial arthrosis with hollow foot and dorsal subluxation of the first phalanx of toes II–V. **b** Right forefoot. Anteroposterior projection in a 14-year-old boy affected with B27-positive JRA Para-articular ulcerative changes of the third metatarsal phalanx with narrowing of joint spaces; modest osteoporosis

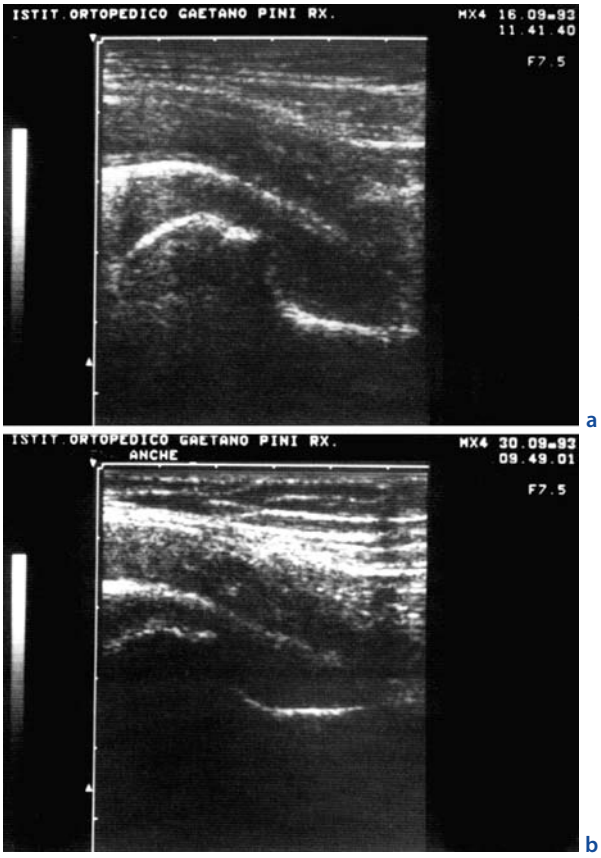

Fig. 18.21 a,b. Pelvic echography. **a** A 4-year-old boy with systemic JRA. Synovial hypertrophy and marked hydrarthrosis. **b** Same boy. Reduction of hydrarthrosis after topical corticosteroid treatment

Among US and Canadian pediatric rheumatologists, NSAIDs were the most commonly used medicines for all forms of JRA, and intra-articular CS injection was the second most common therapy for pauci JRA, but MXT was second for poly and systemic onset forms of seronegative enthesopathy JRA and arthropathy syndrome [71]. However, continuous systemic CS usage for <1 year does not affect attained adult height in JRA patients; however, prolonged CS treatment for >1 year can lead to irreversible growth impairment [464]. Chronic inflammation and prednisone therapy may adversely affect growth in patients treated with long-term CSs during childhood for systemic JRA and final height may be closely dependent both on the severity of growth retardation during JRA's active phase and on linear growth after remission [389]. It has recently reported that treatment with recombinant (r) human growth hormone (GH) restored linear growth without inducing catch-up growth, significantly improved body composition, and prevented further bone loss. Prolonged follow-up is needed to assess the benefits of GH and long-term consequences of hyperinsulinism [390]. IgG, IgA, and IgM levels fell significantly by 26%, 21%, and 17%, respectively, while on MXT, up to a in IgG to below the normal range [346]. chapter 18 reports two girls suffering from JRA who were severly worsened by unconventional remedies. It could be useful to assess whether introducing dietetic manipulations such as adequate amounts of fatty acids during the perinatal period (via breast feeding) may prevent JRA, as well as other autoimmune disease [81].

Rehabilitative cures should be provided to JRA children as soon as possible.

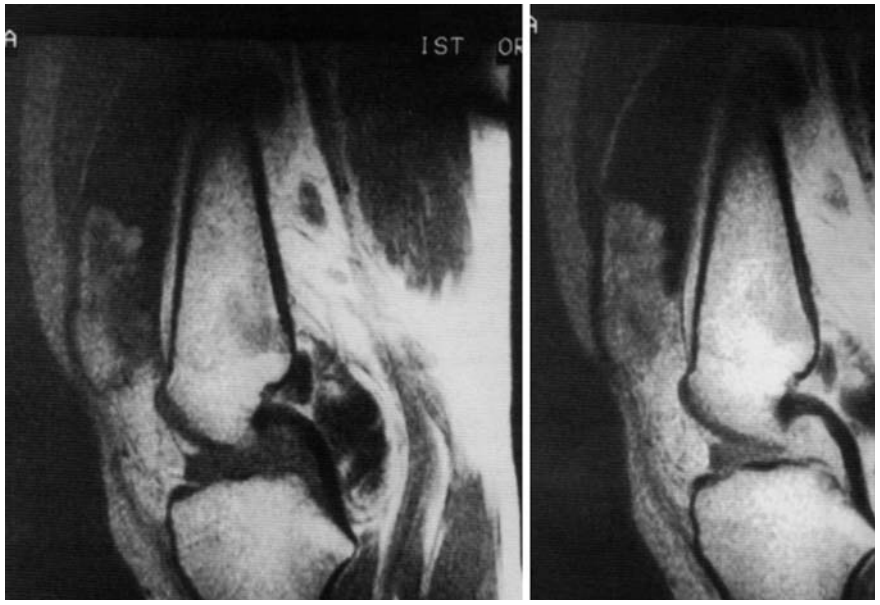

Fig. 18.22 a,b. Magnetic resonance scan of left knee in intermediary sagittal plane before IV injection of paramagnetic contrast medium. **a** A 17-year-old girl with poly JRA since the age of 5 years. Huge distention of the bursa either of quadriceps or of medial head of gastrocnemius secondary to hydrarthrosis associated with notable synovial thickening appearing as exuberant synovial pannus, and vegetating aspect with nodules. **b** After IV injection of paramagnetic contrast medium, remarkably dense signal in the synovium representing inflammation

Table 18.11. Nonsteroidal anti-inflammatory drugs (NSAIDs) and immunosuppressors for the treatment of JRA

Drug	Half-life (h)	Daily doses (mg/kg) (PO)	No. of doses	Max dose (mg daily)	Side effects
Salicylate					
Acetylsalicylic acid	9–16	<25 kg: 70–100 mg	3	1,500	Pseudoallergy, nausea, vomiting, polypnea,
		>25 kg: 2.5–3 g (Salicylemia: 20–25 mg/dl)	4		Increased transaminase levels, irritability or sleepiness, hearing disorders
Diflunisal		7–21 mg	2–3	1,500	Gastrointestinal and auditory effects
Propionic acid derivatives					
Flurbiprofen	5–6	5 mg	3–4	300	Gastrointestinal effects
Ibuprofen	2	40–50 mg	4	2,400[a]	Gastrointestinal effects
Ketoprofen	2	3–5 mg	4	300	Gastrointestinal effects
Naproxen	13–14	10–15 mg	2	1,500	Gastrointestinal effects
Indolacetic acids					
Etodolac	7	100–200 mg	2	1,500	Gastric toxicity
Indomethacin	2–5	2.5–3 mg	3–4	200	Gastrointestinal effects, headache
Sulindac	7	2–3 mg	2	400	Gastrointestinal effects. Abdominal pain
Tolmetin sodium	2–6	15–30 mg	3–4	1,800	Gastrointestinal effects
Phenacetic acid					
Diclofenac	1–2	2–3 mg	3	200	Gastrointestinal effects
Diclofenac extended release	12	2–3 mg	2		Gastrointestinal effects
Oxicam					
Meloxicam	24	7–15 mg	1		Unknown
Nabumetone	24	0.5–1 g	1		Unknown

Table 18.11. (Continued)

Drug	Half-life (h)	Daily doses (mg/kg) (PO)	No. of doses	Max dose (mg daily)	Side effects
Immunosuppressors					
Methotrexate (MXT)	6–7	10 mg/m^2 week PO, SC, IV escalate as tolerated			Hepatotoxicity, bone marrow suppression, interstitial pneumonia, decreased transaminase levels, nephrotoxicity, especially at high doses
Cyclosporine	3				
Initial dose		2 mg	2[b]		Nephrotoxicity, hypertension, hyperlipidemia
Maintenance		4–6 mg	2		Hirsutism, gingival hyperplasia
Penicillamine		5–10 mg	1		Nephrotoxicity, bone marrow suppression
Sulfasalazine[c]					
New drugs					
Etanercept		0–4 mg SC	2 weekly		Infections, use with MXT
Infliximab		3–5 mg IV	q 8 weeks		Infections, use with MXT

Data from [22, 136, 263, 506].
[a] 20–30 mg/kg daily for children >6 months. [b] Continual infusion. [c] See Table 18.16, also for azathioprine dosage.

New Drugs and Treatments

Etanercept (enbrel), a dimerized version of the soluble TNF receptor II, is a fusion protein linking the extracellular ligand-binding portion of a human TNF-αR to the Fc portion of human IgG, is a therapeutical agent related to infliximab (anti-TNF-α therapy) [251]. Etanercept was administered at a dosage of 0.4 mg/kg (maximum 25 mg) subcutaneously (SC) twice a week. Children with severe, long-standing, MXT-resistant poly JRA demonstrated sustained clinical improvement with >2 years of continuous etanercept treatment. Etanercept was generally well tolerated, a remarkable result, considering that 83% of those receiving placebo flared at a median of 28 days, whereas only 28% of those still receiving etanercept flared, and at a median of 116 days [242]. However, children taking etanercept should be monitored closely for infections [242]. The response rate to etanercept was significantly lower in patients with systemic-onset JIA than in those with pauci- or poly-onset JRA. This treatment may be associated with a wide spectrum of severe side effects in 19.75% of cases [345]. However, during etanercept treatment of systemic-onset JRA one child developed SLE and a second DM. For children with JOSA, *infliximab,* a chimeric, human-murine anti-TNF monoclonal antibody, may confer similar benefits [45]. Treatment of three children with infliximab was started at 3 mg/kg at weeks 0, 2, and 6, followed by infusion every 8 weeks, with effective and well-tolerated results in two children, but in the third child no effects were apparently noted [31]. In a nonrandomized, prospective, open-label study, 24 children (mean age, 10.2 years) with poly JRA were treated with either infliximab or etanercept, and both TNF-α inhibitors provided a significant rapid and sustained reduction in disease activity [216].

Drugs of Tomorrow

In a multicenter, blinded, placebo-controlled (PC) safety trial the addition of the rIL1 blocker *anakinra* (r-metHuIL-1ra) to a stable background regimen of RA medications introduced no important safety risk in patients with RA [417]. A multicenter randomized double-blind (DB) PC trial significantly reduced the clinical signs and symptoms of RA when used alone or in combination with weekly MTX [63]. In another trial, patients in whom RA was active despite MTX therapy were treated with a combination therapy with etanercept and anakinra with no added benefit and an increased risk compared with etanercept alone [133].

Adalimumab, another human anti-TNF monoclonal antibody, was investigated in a multicenter DBPC trial and was more effective than placebo at inhibiting the progression of structural joint damage, reducing the signs and symptoms, and improving physical function in patients with active RA unsuccessfully treated with

MTX [197]. In a DBPC phase III trial, patients with RA for whom previous antirheumatic drug treatment had failed achieved significant, rapid, and sustained improvements in disease activity and improved physical function by adalimumab monotherapy. It was safe and well tolerated [450].

Another promising strategy is to inhibit the formation of IL_6–IL_6R (receptor) complex to block the binding to gp130 receptor, a biologically active receptor for IL_6. Yokota [494] performed recent trials of anti-IL_6R antibody, temporarily called MRA, for children with acute systemic JRA intractable to long-term high-dose CS therapy.

ASCT (*autologous stem cell* [SC] *transplantation*), proposed as a possible treatment for severe AIDs such as JRA, MS or systemic, and SLE [485], has unique issues in pediatric JRA [23]. In a study started in 1997, ten children with systemic JRA and four with polyarticular JRA, all with progressive disease activity despite previous treatment with CSs, NSAIDs, MXT up to 1 mg/kg/week, and CsA (2.5 mg/kg/day), were treated with ASCT. Follow-up to 36 months showed a marked decrease in JRA severity as expressed by the core-set criteria for JRA activity [23]. During the follow-up of 3–40 months, there was a recovery of CD4 and CD8 subsets, and of CD45RO T cells, later replaced by the CD45RA phenotype. The clinical improvement was evident, with younger children reaching a catchup growth of 1–2 SDS (standard deviation score). MAS (macrophage activation syndrome) was the cause of death in two children [23]. However, these children remain at risk for severe viral infections due to a prolonged lymphopenia [485]. A third fatality resembling MAS, shortly after auto-SCT, was reported in Paris and was caused by a disseminated toxoplasmosis infection [344]. Allogenic transplantation (Chap. 22) certainly has a theoretical advantage of replacing the entire lymphocyte population, and could overcome the complications associated with the ASCT [485]. To ≥40 children treated with ASCT since 1997, an extension is reported, including 18 children with JIA treated in the Netherlands and 13 children from other European pediatric centers, with a follow-up of 8–60 months. At 5–9 months after ASCT, the numbers of circulating T cells were normal with normal in vitro mitogenic responses at 6–18 months after ASCT. In all, 17 patients showed a drug-free follow-up of 8–60 months with a marked decrease in the scores of the child health assessment questionnaire (CHAQ). During the 36 months of follow-up, the first patient showed a catch-up growth of 22 cm (and a corresponding increase in shoe size), in contrast to the minimal gain of only 2 cm in the three preceding years. The second patient also showed a rapid drug-free remission of the disease that persisted at 30 months of follow-up. Since ASCT, she has grown 18 cm in 30 months. She is on a physical therapy program to improve muscle strength after years of immobilization and prednisone-induced obesity. A relapse was noted in seven children 18 months after ASCT. These relapses have so far been mild, pauci forms and sporadic fever, which could be controlled easily with a 3-month course of low-dose prednisone and NSAIDs [486]. A US woman affected with a severe form of RA interesting 38 joints was able to discontinue all medication after having received a *stem cell transplant* from her healthy sister (Reutershealth, 9 September, 2004).

Outcome

The early onset of ankle and/or wrist disease, symmetric joint involvement, and an elevated ESR in a child with pauci JRA may be predictive of extension [9]. The prognosis varies significantly according to the different forms of JRA. More than 50% of patients, in fact, develop severe articular lesions with seropositive poly forms, 25% develop systemic forms, and 10%–20% have seronegative poly forms. In pauci forms, with adequate treatment, the remission is complete in 50% of cases. Some type I forms, however, may evolve with severe poly involvement [343]. In 703 children, at 5 years after onset >25% of poly and nearly half of systemic JRA children had functional limitations that required modifications in their school schedule [39]. Pauci JRA occurring in older boys had the best functional outcome, but growth abnormalities and Rx changes were more common in poly and systemic onset JRA [288]. However, a number of patients with type II pauci forms can develop ankylosing spondyloarthritis in adult age [263]. One should also consider the numerous problems caused by daily and/or social activities, and the psychological problems as well, especially for their impact on the child and parents and on *quality of life* [354]. Because chronic inflammatory eye disease in children in JRA is usually asymptomatic until visual loss occurs, it is vitally important that all children with JRA undergo an eye examination every 6–12 months by an ophthalmologist.

Since pain, peer rejection, and problematic social behavior are strongly associated with depressive symptoms, health care providers should assess the social functioning of children with JRA to identify those socially vulnerable who may be at increased risk of internalizing problems [372].

Inflammatory Bowel Disease

Definitions

The two major IBDs, UC [478] and CD [72] are correlated with autoimmunity (Table 18.2), UC is limited to the large bowel and pathologically is a relatively superficial, ulcerative inflammation, whereas CD can involve any part of the gastrointestinal tract and is characterized as a transmural granulomatous inflammation.

Epidemiology

These affections are characterized by an ever-growing frequency in pediatric age (data $\times 10^5$). in Great Britain, CD incidence in cases begun <16 years increased from 1.3 to 3.1, UC incidence remained at 0.7, and their prevalence was at 16.6 and 3.4, respectively [67], over the last 10 years. In a retrospective study done in Australia, the incidence of CD in children aged ≤16 rose from 0.128 to 2.0×10^5 per year over three decades, from 1971 to 2001 [331]. The incidence of IBD in Wisconsin children was 7.05×10^5, whereas the incidence for CD was 4.56, 2.13 times the rate of UC (2.14) [213]. In Norway, the rates were inverted (2.5% for CD, and 4.3% for UC) [302]. The mean annual incidence of CD in the child population was 2.5×10^5/year, whereas the incidence of UC in the child population was 4.3×10^5/year [302]. UC incidence in Spain reaches a mean value of 3.8×10^5. CD figures reach an average rate of 1.9×10^5, half of UC [318]. The average age of onset was 1.0–17.5 years in 55 German children [407]. The IBD incidence rate was 5.2 new cases $\times 10^5$ Danish children per year [451]. In other Danish children, the mean annual incidence of IBD was 4.3 (1.8 for UC, 2.3 for CD) $\times 10^5$ children <15 years. The mean annual prevalence of IBD was 15.8×10^5 children <15 years (UC 8.3; CD 6.7). A total of 152 children were diagnosed with IBD, corresponding to an overall incidence of IBD of 7.4×10^5. The incidence of CD was 4.9 and UC 2.2 The marked increase in CD incidence, while UC incidence was almost stable, led to a net increase in the overall occurrence of IBD [165]. UC has a bimodal distribution among children: its prevalence is higher between 11 and 15 years, with a peak between 16 and 20 (2.3×10^5) [201]. However, children aged <10 represent 0.5%–15% [150] of that peak. In 29% of Gryboski's cases [150], UC onset occurred during the first 2 years of life. CD is affecting a growing number of children and adolescents. According to estimates, there are 9.5–10 cases $\times 10^5$) in children <15 years, so CD is more frequent than UC among children. As a matter of fact, at least 20% of CD cases have their onset in childhood or adolescence [172]; however, CD is not rare at 6 years [208].

Genetics

The increased number of cases, at least within families, could depend on genetic anticipation. This is supported by the study of parent-son pairs. In 78.8% of cases, fathers developed the disease >5 years before their sons [374]. Genetic predisposition is likely, because these affections have higher incidence among first relatives of affected subjects [311], and among MZ twins rather than DZ twins [437]. The concordance ratio between twins seems to be more significant in CD than in UC [437]. However, no model of Mendelian inheritance has been shown in these disorders, so they could reasonably represent a heterogeneous group with multifactorial inheritance [374]. The heterogeneity of UC is confirmed by the presence of antineutrophil circulating antibodies (ANCAs) (Table 8.26). According to evidence, parents and sons, either with CD or UC, have the same disease in 100% cases, and the same symptoms in 75% cases on average [374]. First relatives are at risk of developing the same illness 5–10 times more than the general population [338]. In this respect, the familial risk is 5% for UC and 9% for CD [50]. Relatives of an individual with CD or UC have a tenfold increase in the risk of having the same disease as the patient, whereas in the relatives of an individual with UC, the risk of acquiring CD is not significant; the two diseases may occur in the same family, thus suggesting that IBDs have a genetic cause [311]. This divergent genetic behavior suggests a greater relevance for environmental factors in UC [437]. However, the development, with a lapse of a few months, of CD and UC in two sisters shows that both affections depend on the interaction of genetic factors with environmental factors [51], including smoking [374]. Some genetic markers have been studied using molecular techniques to typify DNA. Table 18.3 reports UC and CD associations with HLA molecules, UC with HLA-DR2 specificity, and CD with *HLA-DR1-DQ5* allele (more with the *DQB1*0501169*). CD has been associated with the *HLA-DRB1*07* allele as well, especially in subjects <35 years of age [352]. While the association with *HLA-DRB1*03* is significantly decreased [352] or makes CD more resistant [80], other studies suggested that UC is susceptible to *HLA-DRB1*1502* [129, 266]. These data confirm that HLA-B27 is associated with both CD and JRA. However, none of the four family members, including one daughter with CD and another with UC, presented JRA, even though all members had HLA-B27 antigen [51]. The HLA region on chromosome *6p*, referred to as IBD3, showed evidence of male-specific linkage with a max lod score of 5.9 in CD and UC male-affected families. Regions on chromosomes 11, 14 and 18 showed strong evidence of linkage also in male-affected families [121]. In UC families, the most significant lod scores were observed on chromosome *2p11* (D2S2333) and a second at proximal *12p13* score was observed, also with a high lod score [313]. A susceptibility to CD, but not to UC, was associated with homozygosity for a common haplotype, H2 ($p(c)$=0.002; RR 2.0). Genotype–phenotype analyses demonstrated that this association was very strong in patients with perianal disease ($p(c)$=0.0005; RR 1.7), mostly in individuals HZ for this haplotype ($p(c)$=0.0005; RR 3.0) [16]. Two genome-wide searches have identified suggestive linkages at chromosomes *5q33-q35 14q11.2*, *17q21–q23* [246], *2p13–11*, *11p12–q13*, *12p13–12*, *12q23*, *19q13* [313], but the linkage at 3, 7, and 12 [375] was subsequently excluded [459]. Another potential susceptibility locus for IBD2 was localized at *12q13–14* near the deoxyribonucleic acid marker D12S83 by linkage analysis [439]. *The IBD1 gene was mapped to the proximal region of the long arm of chro-*

mosome 16 (16q12) in the white population, utilizing genome-wide scan linkage strategies [171], contains the susceptibility gene encoding *NOD2*, also referred to as CARD (caspase activation and recruitment domain), has been linked to CD in different populations [37, 53, 73, 161, 313, 505] or not in French [508] and Japanese patients [490] and several more in PubMed (March 2005).

Confirming the importance of NOD2, Hampe et al have provided evidence of linkage to *loci* at chromosomes *1q*, *6p*, and *10p* and replicated linkages on chromosomes 12 and *16p* [154]. Additional *loci* with suggestive significance for IBD in a meta-analysis affected relative pairs were *2q*, *3q*, *5q*, *7q* and 16 (*CARD15* region); CD, *2q*, *3q*, *6p*, 16 (*CARD15* region), *17q*, *19p*; and UC, *2q* [454]. A group of pedigrees that contained one of the three *CARD15* variants had two suggestive linkage results occurring in *6p* (lod, 3.06 at *D6S197*, IBD phenotype) and *10p* (lod, 2.29 at *D10S197*, CD phenotype). Moreover, at *16q12* where *CARD15* is located, the original genome scan had a peak lod score of 2.18 at *D16S415* (CD phenotype) [384]. Carriage of *NOD2/CARD15 risk* alleles is associated with ileal location, earlier disease onset, and stricturing phenotype [36]. In 55 German children with CD, the genotype–phenotype analysis showed that those who had at least one of the six *CARD15* disease-associated mutations had a high risk of inflammation located in the terminal ileum and ascending colon. In ten of 19 patients with two mutations, intestinal resection surgery was necessary because of stricturing [476]. A significant association of ileal involvement was shown with *CARD15* variants, *HLA-DRB1*0701* and *DRB1*04* alleles and the capacity of combined *CARD15* and *HLA-DRB1* genotyping to predict ileal disease in CD patients was demonstrated. By contrast, the *HLA-DRB1*0103* allele was associated with later age of diagnosis and pure colonic disease [295]. Other IBD genomic regions include *IBD2* on chromosome *12q* (observed more in UC), and *IBD3*, containing the HLA region [36]. Three major coding region polymorphisms within *NOD2/CARD15* have been highly associated with CD among patients of European descent: *R702W*, *G908R* and *L1007fs* [37, 53, 161] with allelic frequencies of 0.11, 0.02 and 0.07, (or 3.3%, 0.6%, and 4.8%), respectively [53]. The *G908R*, *R702W*, and *L1007fs* variants share a common signaling defect in response to bacterial components, providing evidence for a unifying molecular mechanism whereby *NOD2* mutations contribute to disease susceptibility, while *R702W* is not associated in Ashkenazi Jews [37]. Having one copy of the risk alleles confers a two- to fourfold risk for developing CD [36], or no increased risk [505] and double-dose carriage increases the risk 20- to 40-fold [36] or 5- to 15-fold [505]. All three major CD variants exhibit a deficit in NF-κB activation in response to microbial components [36]. In CD patients, six more variants (*5 UTR-33 GT*, *S178S*, *P268S*, *R459R*, *R587R*, *V955I*) have been reported [227]. The penetrance of the *NOD2/CARD15* mutations is estimated at <1% [505]. *CARD* is expressed in macrophages and may serve as a so-called pattern-recognition receptor for bacterial LPS, perhaps regulating macrophage apoptosis [338].

Etiopathogenesis

According to new pathogenetic theories, *SAs could play an active role in cell-mediated immunity* (CMI) *for CD* by inducing autoimmune phenomena [175], while *UC could be caused by atypical type 2 helper T cells* [338]. Moreover, the mucosa in patients with UC may be dominated by $CD4^+$ lymphocytes with an atypical type Th2 phenotype, characterized by the production of TGF-β and IL_5 but not IL_4. In contrast, the mucosa of patients with established CD is dominated by $CD4^+$ lymphocytes with a Th1 phenotype, characterized by the production of IFN-γ and IL_2 [128]. In murine models, the effects of the activation of Th1 cells may be enhanced by the concomitant decrease in subgroups of suppressor T cells, which produce the down-regulatory IL_{10} and TGF-β [427].

The most reliable data to clarify the pathogenesis of chronic inflammation are as follows. *The inflammatory reaction* is triggered by macrophage activation and spread by local IL release, particularly $IL_{1\alpha}$, IL_2, IL_2R, IL_6 and IL_8, and other chemotactic factors such as LTB_4, PAF and C5a [149, 278, 304, 473]. IL_8, mediating neutrophil infiltration of the gut wall, starts and prolongs the inflammation, also correlating well with the neutrophil numbers in mucosal tissue, while IL_1 and TNF-α represent the releasing factor on the inflammatory site [278]. This interesting paradox remains to be explained, especially why serum levels are not correlated [278]. Certainly, neutrophil infiltration is a histopathological characteristic of active forms. PBMCs scattered among the crypts and interstices synthesize TNF-α and $IL_{1\alpha}$, which in turn increase their concentrations [304]. TNF-α is involved in the development of granulomas [40], which were found in 11.4% of 88 children [276].

- *Serum IgG increased* 10- to 100-fold, including IgG_1 subclasses with pro-inflammatory action and complement activation, and IgG_2 with regulatory activity, mostly in CD (Table 18.12) [28, 43, 151, 195, 196, 400]. As for IgA, the different expressions of J chains seem to have no specific meaning, nor is the secretory system involved [196]. IgG-mediated immunoinflammatory responses can damage the intestinal mucosa since they activate the complement and the cascade of inflammatory mediators [152].
- *Complement activation* is a mechanism which can induce and amplify inflammatory processes independently [474].
- *Some patients with ANCA*$^+$ CD and $ANCA^-$ UC are associated with the rare allele *R241* from CD54 [492], as well as with high levels (reduced, however, in patients with UC only) of an antierythrocyte Aab (AEA-15) in

Table 18.12. Differences in the mucosal and systemic immune response between Crohn's disease and ulcerative colitis

Immune response	Crohn's disease	Ulcerative colitis
Prevalent mucosal IgG	IgG_2	IgG_1
IgA_1 J chain	–	+
IgA_2 J chain	+	–
IL_6 serum levels	+	=
IL_1-specific mRNA	=	+
IL_8 serum levels	=	+
IL_2R serum levels	+	–
TNF-α and $IL_1β$ serum levels	+	+
p-ANCA antibody	–	+
AEA-15 levels	+	–
Antitropomyosin or P40 levels	–	+

Data from [28, 43, 151, 195, 196, 400].

the *VH3–15* [28] gene (Table 18.12). The two diseases, on the contrary, show no differences in CD44 levels [242, 272].

- *Hyperproduction of O_2 and NO radicals* is evident [38, 148]; however, antioxidant vitamins have low levels [398], with consequent oxidative stress and mucosal lesions.

The pathogenesis thus is characterized by the following major aspects:

- *Primary or secondary dysfunctions* of the immune response in IBD mucosa are caused by IL_4, able to disrupt the intestinal epithelial barrier, thus increasing intestinal permeability and macromolecular transport [92]. These changes facilitate the indiscriminate access of luminal antigens (alimentary and/or microbial and viral) [201] and the intestinal epithelial cells (IECs) ready as APC in mucosal immune responses [430]. T lymphocytes already activated and ready to react with microbial antigens reside in the lamina propria [334], which are also responsible for HLA class II antigen expression on colonic epithelial cells [473]. In addition to producing the key ILs that stimulate Th1 (IL_{12}, IL_{18}, and macrophage MIF), macrophages produce a mix of inflammatory ILs, including IL_1, IL_6, and TNF, which target a broad variety of other types of cells [338]. Increased levels of IL_4 have been shown in the early phase of relapse in the intestine of CD patients [177] and in the UC active phase [91]. T cells activated by IECs are $CD8^+$ suppressor and dependent on $CD8^-$ associated $p56l^{ck}$ activation. In this process, a 180-kD glycoprotein (gp180) has been shown to be important in $CD8^+$ T cell activation. However, gp180 expression by IBD IECs appears to be altered, and correlates with a functional alteration of $p56l^{ck}$ activation [430]. Thus, this defect may be reflected in IBD pathogenesis.
- *The inflammatory reaction* is started and propagated by the local IL release by Th1 IL_2, IL_2R, IL_6 IFN-γ [149, 473] and by PBMCs, especially TNF-α [304]. IL_2R, mostly present in CD, plays a significant role in the expansion and differentiation of CMI responses [42]. IFN-γ is the major evocator of HLA antigen expression on the epithelium, by stimulating the mucosal damage continuation and amplification [147] by an increased APC activity [357]. The damage is also caused by TNF-α, a powerful inflammatory protein activating endothelial cells, which express β2-integrins, selectins, chemokines and other molecules involved in lymphocyte and neutrophil adhesion [304]. IL_1, IL_2 and the factors activating PBMCs have a similar action on the epithelium, by inducing lymphocyte and macrophage activation and migration in the inflammatory lesions [42, 473]. When environmental antigens activate the intestinal immune system the response is modulated by the genetic makeup of the host. Usually, both IFN-γ and TNF-α inhibit the TGFβ/Smad signaling pathways. In normal individuals the TGF-β enhanced intestinal production is followed by an increase of phosphorylated Smad3 (p-Smad3) and a concomitant decrease of inhibitory Smad7 thus allowing the expression of TGF-β anti-inflammatory activity, and maintaining IFN-γ and TNF-α within the limits of a physiological intestinal inflammation. In susceptible individuals, activation of the immune system leads to increased production of TGF-β signaling, but also to inappropriately *high levels of Smad7* which inhibit p-Smad3 resulting in *defective TGF-β signaling*. Consequently, excessive amounts of IFN-γ and TNF-α are produced resulting in chronic intestinal inflammation clinically manifested as IBD [120].
- The *protective mechanism failure* consequent to inadequate metabolism of the PG/leukotriene (LT) system [400] and the generation of a legion of inflammatory and destructive molecules [473] such as IL_4, PAF, proteases, etc. underlie the chronic progression of lesions [40], facilitated by a continual involvement of inflammatory cells. Intestinal mucosa disturbance, mostly from abnormalities involved in the immune adaptation to intestinal antigens, may be explained as hyperreactivity of T lymphocytes to microbial antigens [326, 334]. Especially in CD, the mucosal destruction is similar to that caused by experimental activation of T lymphocytes in the human fetal small intestine, consequent to increased expression of HLA-DR, $IL_{2α}R$ and macrophages [247] in the lamina propria. This suggests that the primary activation of T lymphocytes is an important effector mechanism. The release of IFN-γ from T lymphocytes can lead to the expression of $IL_{2α}R$ on macrophages [247], as in CD. However, the continuing Th1 activation from bacterial microflora stimulates Th1 to release the above-mentioned ILs [326]. In UC as well, the enteric microflora can cause a major event: HLA-B27 transgenic mice do not develop UC as usual if bred in a sterile pathogen-free environment. However, when maintained in a non-

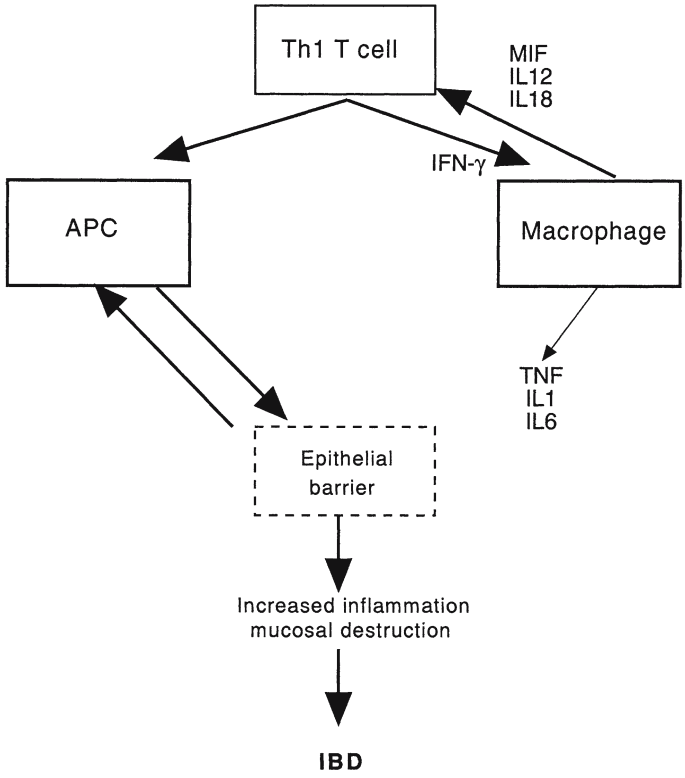

Fig. 18.23. Pathogenesis of IBD. Activation of classic APCs, such as DCs or direct stimulation through pattern-recognition receptors promotes the differentiation of Th1 T cells in CD patients or, possibly, atypical Th2 T cells in UC patients. (Modified from [338])

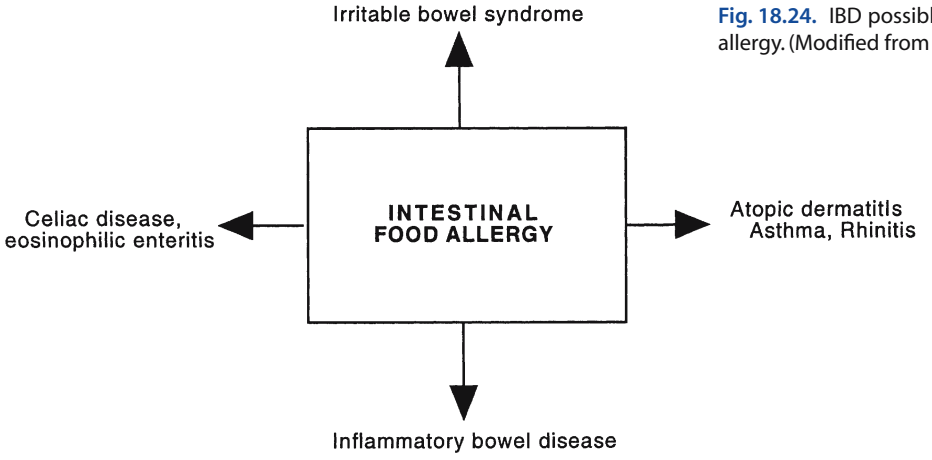

Fig. 18.24. IBD possibly related to intestinal food allergy. (Modified from [32])

sterile facility, they develop inflammatory phenomena within a few weeks [414]. Microflora have important and specific metabolic, trophic and protective functions, based on evidence obtained by the above studies [152]. Thus, the pathogenetic process should be referred to as an increased activity of the inflammatory processes and/or the absence of mucosal standard protective mechanisms, so that the inflammation goes on indefinitely [338], as shown by Fig. 18.23. Therefore, IBD patients show an impaired tolerance toward commensal bacteria of the resident flora [152]. The barrier integrity may be compromised by inappropriate and ongoing activation of the mucosal immune system, a reduced reparative response to injury, or exogenous agents, or

Table 18.13. Problems in establishing a relationship between food allergy and IBD

Unclear mechanism
Unclear definition
Lack of epidemiological data
Lack of pertinent diagnostic test
Unclear significance

Modified from [32].

products of commensal bacteria in the lumen [338]. A nonspecific repair function of the colon cells to compensate for damage to barrier function, including a reduction in the number of goblet cells and a decrease in the number of sugar residues may be present in children with both CD and UC by MUC5AC (faveolan cell mucin) and TFF1 (gastric trefoil factor) expression in goblet cells [379]. Antigens from dietary sources may also contribute [338]. The most important differences are as follows: UC DRB1*15, ANCA$^+$, AEA-15$^-$, CD DRB1*01 and DRB1*07, ANCA$^-$, AEA15$^+$.

The relationships with FA are also important for pathogenesis (Fig. 18.24) [32], though they are little known for a number of reasons (Table 18.13) [32].

Ulcerative Colitis

The major symptoms are summarized in Table 18.14 [150]. The characteristics of AID are obvious, with antibodies in the serum, activation of the complement by CICs, and lymphocytes aimed at colon epithelium. This could cause T-mediated damage to the mucosa, suggesting a type IV reaction. The presence of IgG among the complement activation products in the colon epithelium could be interpreted as an IgG-mediated autoimmune process. The accumulation of complement is directly correlated with the level of inflammation [40]. Histopathological studies have shown that the primary lesion is on the epithelium, thus the inflammation is only secondary. These phenomena, however, are not observed in all patients, and they are not correlated with clinical manifestations, whereas CS effectiveness in managing UC may be due to an immune-mediated suppression of IL production by macrophages and T cells [40]. The significant association with HLA alleles [431], with high familial prevalence of atopic diseases (22%) and minor prevalence of extraintestinal symptoms [150] (Table 18.14), supports the theory that autoimmune immunological factors could be involved in etiopathogenesis, even though it is unclear if these mechanisms play a primary or secondary role. CM has been considered the provoking factor in 14% [150] to 21% [138] of cases, since UC develops at an earlier age (6.7 years) compared to subjects with a negative history (10.6 years) [138]. It should be noted that CM ingestion causes flare-ups, while its elimination rarely leads to the resolution of stabilized forms. These data suggest that nutrition does not play a prominent role in pathogenesis. Likewise, there is no scientific evidence of a psychosomatic etiology, even though emotional and psychosocial factors can affect UC development [201]. The specific diagnosis is made based on p-ANCA (Table 18.12), which are present in 66% of children (median age 13 years), with 66% sensitivity and 84% specificity compared to CD [400]. If ANCAs are matched to a technique based on immunofluorescence and ELISA, specificity reaches 97% [480]. ANCA specificity has led to dividing patients into ANCA$^+$ and ANCA$^-$ [431] according to familial background. Treatment is essentially similar to CD therapy [400]. Experimental data show the utility of ketotifen [98]. Recently, the derivatives of 5-aminosalicylic acid (5-ASA) have been widely used, since they have both antioxidant and superoxide-dismutase-like activity [398], while sulfasalazine reduces the production of PGs/LTs [400]. Surgical therapy provides satisfactory results: the speculation that a more aggressive surgical approach to treatment could reduce the growth retardation [116] is intriguing.

Table 18.14. Symptoms in children with ulcerative colitis

Symptoms at onset	%	
Abdominal pain	92	
Diarrhea	84	
Rectal bleeding	84	
Weight loss	74	
Fever	11	
Arthralgia	8	
Arthritis	5	
Edema	5	
Failure to thrive	5	
Extracolonic manifestations	**Grybowski's data %**	**Grybowski's meta-analysis %**
Arthritis	8	13.4
Arthralgia	8	3.6
Eye lesions	3	5.4
Skin lesions	2.6	6.4
Failure to thrive	5	13.8
Colectomy	5	20.4

The extracolonic manifestations are compared with five pediatric studies (mean) reported by Grybowski, including 537 children aged 1–20 years.
Data from [150].

Crohn's Disease

CD is a painful, chronic inflammation of the GI tract, which also causes social and emotional problems in children [20]. The pathological mechanisms behind the disease are definitely different. According to the most widely supported theory, CD is a genetically determined disorder [352], immune-mediated, whose major risk factor is a positive family history (FH) [147]. The cause of immune dysregulation is still controversial, while reduced antigenic stimulation on the intestinal wall through dietotherapy appears more relevant. In CD pa-

Table 18.15. Clinical features of children with Crohn's disease

Classic presentation:	%
Abdominal pain	75
Diarrhea	65
Weight loss	65
Failure to thrive	25
Nausea/vomiting	25
Rectal bleeding	20
Extraintestinal manifestations:	
Arthritis	4.3
Relapsing fever	2.7
Recurrent oral ulcers	0.3
Cheilitis	0.3
Pyoderma gangrenosum	0.3
Recurrent acute pancreatitis	0.3
Anemia	2.7
Perianal disease	3.7
Anorexia	2.0

Data from [147, 172].

tients carrying at least one of the three *NOD2* variants, the ileum was affected more often than in noncarrier CD patients (90% vs 73%); they had stricturing or penetrating disease more often than noncarriers (88% vs 56%), and they had an increased need for bowel surgery [161].

Dietotherapy can help correct nutritional deficiencies and growth deficits, apparent both in children and teenagers [366]. *The major pediatric symptoms* are listed in Table 18.15 [147, 172]. A different case is that of a 5-year-old child with CD who developed eosinophilic gastroenteritis [415]. The pathophysiology is different, depending on the bowel region involved, the degree of inflammation, and the presence of complications such as stricture or fistula. Systemic signs and symptoms are more common in CD than in UC [172].

As a remedy to dietary factors generally considered as primary causes, consequent to unbalanced nutrition with excessive consumption of refined carbohydrates and/or sulfites – with insufficient bulk – a pediatric study has suggested reducing – during remissions – the consumption of vegetables, rich in fibers susceptible to cause postprandial pain, and increasing sweets with a higher content of refined sugars (less harmful in this regard) [422].

In some cases, a simple diet could improve symptoms, but with frequent relapses. When diet was followed by refeeding under control (one food/day, excluding food causing relapses), after 2 years remission was significantly longer (7.5 months compared to 3.8), and relapses clearly lower (62% vs 79%) compared with CS-treated controls, suggesting a new therapeutic strategy [355].

A group of children – divided into two subgroups, one treated with CSs, the other with a simple diet – has shown good results irrespective of treatment [422], whereas in 19 other groups, also randomized, the best result was obtained with medication [364]. A successful experiment was based on a 4-week discontinuation of drugs and diurnal solid feeding, which was replaced with night nasogastric feeding based on amino acids or oligopeptides. The night tube was accepted by almost all patients (7–17 years), their linear growth improved, and clinical scores significantly diminished, with no relapses during 12 months in 30% of cases. The rate reached 50% when subjects agreed to continue the night diet and reduced to 50%–60% with restoration to normal diurnal diet [479]. With severe, acute disease, children with active CD were treated for 6 weeks with exclusive enteral feeding; then treatment was cycled to overnight infusion at home. Significant improvements occurred by day 3 in inflammatory parameters and by day 7 in growth-related changes, preceding any significant changes in nutritional parameters [21]. Improved growth and development, without the side effects of CS therapy, make *enteral nutrition a better choice for first-line therapy in CD children.* Children can participate in normal daytime activities. A major disadvantage of this approach is similar to that of other therapies: early relapse on discontinuing treatment [163]. Especially in children with poor nutritional status or growth impairment, this approach may be ideal. Children and adolescents with active CD who continued nasogastric supplementary feeding after resumption of an otherwise normal diet remained well longer than those who discontinued nocturnal supplements completely. Furthermore, continued use of nasogastric supplements before completion of puberty was associated with improved linear growth [460]. However, the improvement in disease activity seems to be associated with improvement in lean body mass irrespective of the type of diet used to achieve it [198].

In children aged a mean 14.4 years, a *percutaneous endoscopic gastrostomy* was more acceptable than nasogastric feeding, with a net reduction of CS dosage and a significantly improved height score 1 year after the procedure [68].

Diagnosis

Diagnosis depends on the combination of clinical history, physical findings, and endoscopic, X-ray, and histological features, as well as the results of routine laboratory tests. Clinical, X-Ray, and endoscopic (type of lesions, distribution) examinations unequivocally confirm the diagnosis of either UC or CD [338]. CD patients positive to p-ANCA (19%) [400] are characterized by a special

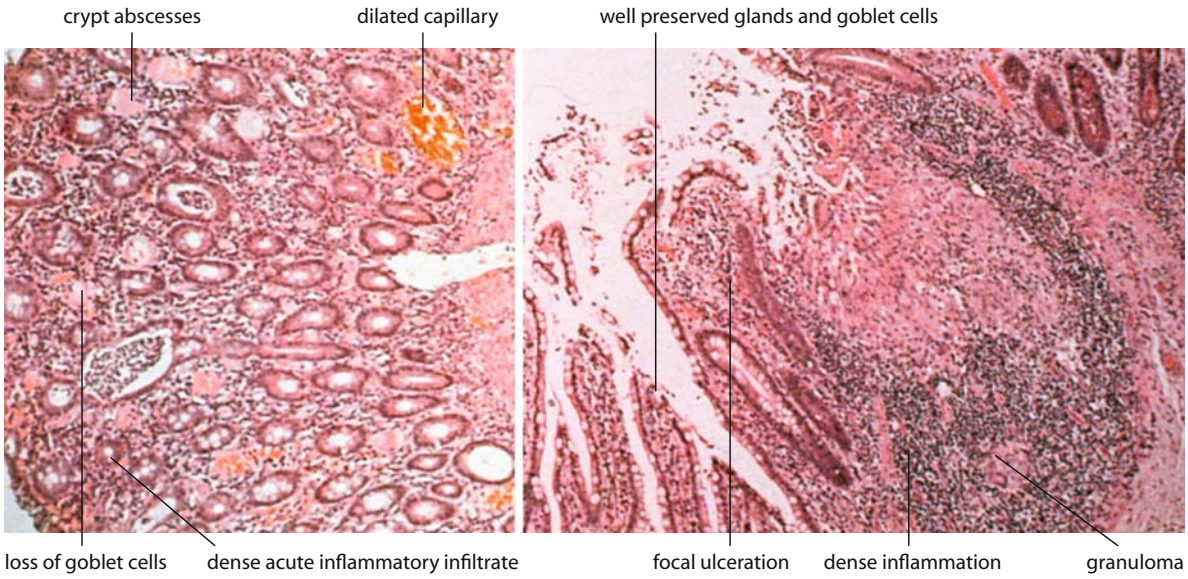

Fig. 18.25. Inflammatory bowel disease. *Left:* UC histological appearance. *Right:* CD histological appearance.

clinical phenotype [456]. The differential diagnosis of both conditions is based on the data shown in Table 18.12: IgG_1 is as specific for UC as IgG_2 is for CD, thus allowing us to make a precise diagnosis for 15% of patients (4–19 years) for whom the parameters generally used are insignificant [151]. The clinical comparison of UC (Table 18.14) and CD (Table 18.15), shows that rectal bleeding is 4.2 times more present in UC, and failure to thrive four times more present in CD. The extraintestinal manifestations, if common, are more frequent in UC; however, CD children have particular extraintestinal manifestations. Additional studies are focused on IL_6 level determination, which is useful to differentiate pediatric patients with CD [43], as well as the $CD25^+$ mucosa cells in CD, mostly T lymphocytes, whereas in CU they have the macrophage phenotype [175]. Colonoscopy in the two IBDs is highly specialized [338], and the histological test gives different results (Fig. 18.25).

Treatment

Medical treatment is summarized in Tables 18.16 [172, 201] and 18.17 [252, 371]. Pediatric CD requires prolonged pharmacological management, but the side effects of current treatments are problematic. The aim of CD therapy is to relieve symptoms and prevent complications of chronic inflammation (anemia, growth failure), prevent relapse, and, possibly, effect mucosal healing. Apart from dietetic recommendations for CD, we suggest for children special formulas that may be useful and palatable (see Appendix 9.7). The nutritional approach may be useful to reduce acute inflammation, but it rarely leads to long-term remittance; however, simple diets or, more commonly, liquid diets can give good results, especially in children [366]. Elemental diets have also been advocated as a primary treatment for CD: a nutritional approach can induce clinical remission and improve the growth failure associated with pediatric CD [362]. The best strategy is to tailor treatment to the clinical pattern [366]. Immunosuppressants have been experimented for both diseases in adults. Immunosuppressants, including cyclosporine, a powerful inhibitor of T cell activation, provide significant results in UC, but disappointing results in CD among a sample of children. Results improve with CS-based therapy [296]. The possible involvement of TNF-α and $IL_{1\alpha}$ in chronic IBD [304] has led to searching for a new therapeutic module based on the inhibitors for these ILs [232].

New Drugs and Treatments

The decisive action of anti-TNF-α has been proven on a 14-year-old girl by the total endoscopic remission of lesions for 3 months after two 10-mg/kg IV infusions of anti-TNF-α antibodies, cA2 (*infliximab*), followed by improved symptoms, a 3.2-kg weight increase in 10 weeks, and a decrease in the activity index for pediatric CD from 77.5 to 10 [90]. After a single dose of infliximab (either 1, 5, or 10 mg/kg), all 21 children (100%) aged 11–17 achieved a clinical response, and ten children (48%) achieved clinical remission. There were no infusion reactions in any child [20]. In children and adolescents, infliximab has been found to be safe and reduces the CS need, both in CD [20, 173, 404] and UC [259, 381]. The results of a two-year follow-up [253] have shown a short-term improvement in 14 of 17 (82%) patients, and sustained improvement in 10 of 16 (63%) patients followed up for >9 months. Eight additional UC children with infliximab were seen and 7/8 were consid-

Table 18.16. Medication doses for children with IBD

Drug	Daily doses (mg/kg)	No. of doses	Maximum dose	Side effects
5-ASA				
Sulfasalazine initial dose	25–40	3–4		Fever, hemolytic anemia, hepatitis, pancreatitis
Sulfasalazine maintenance	50–75	3–4	3 g	Neutropenia, rash, thrombocytopenia
Mesalamine	40–60	2–3	3 g	Nausea, dyspepsia, headache
Mesalazine	30–50	2–3	4.6 g	Headache, abdominal pain, nausea, vomiting
Corticosteroids				
Prednisone, initial dose	1/2		40–60 mg	Fluid retention, fat redistribution, hypertension,
Prednisone, maintenance[a]				Hyperglycemia, psychoneurological disturbances
Methylprednisolone, IV	1–1.5 g			Adrenal suppression, growth failure
Metronidazole	10–20	2	1 g	Nausea, metallic taste,. peripheral neuropathy
6-Mercaptopurine	1–1.5			Pancreatitis, bone marrow suppression
Azathioprine	1.5–2			Pancreatitis, bone marrow suppression
Cyclosporine (for highly selected cases)				Nephrotoxicity, hypertension, headache
Initial dose[b]	2	2		
Maintenance	4–6	2		
Methotrexate	5–25	Once weekly		Leukopenia, hypersensitivity pneumonia, hepatic fibrosis, hepatotoxicity, bone marrow suppression

Data from [172, 201].
[a] Gradual taper (alternate day vs discontinuation).
[b] Continual infusion.

ered responders. The use of infliximab for the maintenance of remission in pediatric CD and UC, as well as its long-term safety, needs to be further assessed before its extensive use can be recommended [381]. Infliximab may be safe and effective as short-term therapy of medically refractory moderate to severe CD in a pediatric population [20]. CDP571, the second anti-TNF-α, is a humanized monoclonal antibody in which only the small number of residues necessary to confer antigen specificity remain from the original murine antibody. The overall usefulness of CDP571 in a clinical trial involving adults with active CD appears to be generally similar to that observed with infliximab [399]. It is necessary to monitor the arthritic symptoms (Tables 18.14, 18.16, 18.17), especially in children who tend to develop ankylosing spondylitis with consequent disability, irrespective of the therapy for IBD [378]. Newer inhaled CS preparations offer potential for targeted therapy and fewer CS-related adverse effects [401]. Wide-spectrum antibiotic therapy reduces bacterial load and mitigates intestinal inflammation in human CD but not in UC [152]. Table 18.17 details treatment with IL_{10}, an IL that generally down-regulates the activation of Th1 cells, and IL_{11}, an IL that enhances the epithelial barrier and inhibits inflammatory ILs (Table 1.5). A large-scale study of patients with CD who were treated with a monoclonal antibody directed against the $\alpha_4\beta_7$ integrin produced promising results [142]. *Probiotics* appear to be one promising approach: *Lactobacillus GG* may improve gut barrier function and clinical status in children with mildly to moderately active CD [1531]. Other therapies have been suggested such as the psychosomatic or immune deletion therapies, but they have not been consistently confirmed by clinical studies [400]. If the method chosen is surgical resection, frequent relapses follow, especially when the involvement is widespread [147], even though more severe lesions can be prevented for at least 1 year. The clinical advantage of postsurgical prophylaxis with 5-ASA seems more permanent [366].

Drugs of Tomorrow

CDP571 binds to the free TNF-α and neutralizes it. In a 24-week PC trial CDP571 at an initial dose of 10 or 20 mg/kg was safe and effective for treatment of patients

Table 18.17. Biological agents with clinical trials in IBD

Drug	Doses × kg	Regimen	Response rate (%)
Infliximab (anti-TNF-α antibody)	8 mg	Single IV	81
	10 mg		50
	20 mg		64
CDP571 (anti-TNF-α antibody)	20+10 mg	IV at weeks 0 and 8	44
ISIS2302 (antisense oligonucleotide)	0.5–2 µg	qod for 7 days	47
Anti-α 4 integrin antibody	3	Single IV	39
	3	1 or 2 doses 4 weeks apart	46
IL_{10}	0.5–25 µg	Daily IV for 7 days	50
	5 µg	Daily SC for 28 days	29
	10 µg		14
	1 µg	Daily SC for 28 days	25
	4 µg		34
	8 µg		35
	4 µg	Daily SC for 2 weeks +	25
	8 µg	3 times/week for 26 weeks	32
IL_{11}	20 µg	SC 2 times/week	33
	3.2 µg	SC 5 times/week	42
	15 µg	SC once/week for 6 weeks	37
	15 µg	SC once/week	37

Only the most positive studies have been included.
Data from [252, 371].

with moderate-to-severe CD [370]. Also, patients with mild or moderate UC were treated openly with a single IV infusion of 5 mg/kg of CDP571. In these patients, a consistent improvement in disease activity was seen in the initial 2 weeks after infusion and the treatment was well tolerated [104]. A Cochrane Database review concludes that evidence suggests that a single infusion of infliximab may be effective for induction of remission in CD, so we can recommend a dose of 5 mg/kg. There is also some evidence that CDP571 may be effective in inducing remission in CD. However, no evidence was found that supports the use of etanercept in CD [5].

Newer biological (natalizumab) or IL-based therapies (monoclonal antibody to IL_{10} and IL_{11}) have shown preliminary evidence of efficacy in controlled trials of adults, but neither have yet been approved by the US FDA. Natalizumab, a recombinant humanized monoclonal antibody against $\alpha_4\beta_7$ integrin was effective in CD in a phase II DBPC trial. The highest remission rate was 44% and the highest response rate was 71%, after two infusions of 3 mg or 6 mg of natalizumab administered 4 weeks apart [135]. In a recent trial [371] patients were randomized to 4 weeks of SC injection with rIL_{11} 15 µg/kg or placebo weekly, or rIL_{11} 7.5 µg/kg or placebo twice weekly, which turned out to be safe and effective in inducing remission in a subset of patients with active CD.

Outcome

Reduced growth has been observed, especially in CD [164, 257], in connection with protein-energy malnutrition with multifactorial origin [400], stressing the need to guarantee early diagnosis and therapy. Despite a number of contrasting factors, the improvement of nutritional conditions, irrespective of the methods used, can foster the resolution of IBDs, especially CD. Certainly, the new diets based on amino acids with a palatable taste – as we have stressed – are useful in pediatric cases, in order to avert CS resistance or dependence, or when it is desirable to discontinue or significantly reduce CSs. An improved nutritional condition will counter the effects of malnutrition, including trace elements – especially Zn (Chap. 21) – resulting in improving the retarded growth, which is a typical aspect of pediatric IBDs.

Autoimmune Hematological Disorders

AIHA is discussed in Chap. 19.

Autoimmune Lymphoproliferative Syndrome

Genetic defects in proteins that mediate lymphocyte apoptosis caused by Fas gene mutations can result in the autoimmune lymphoproliferative syndrome (ALPS), a rare inherited illness occurring in a number of kindreds [176]. Heritable mutations in Fas or Fas ligand genes, which regulate lymphocyte survival by triggering lymphocyte apoptosis, are the most frequent causes of ALPS. In vitro studies of eight new HET *Fas mutations* suggest a second defect, because some Fas missense mutations leading to a truncated Fas product were associated with variable (instead of high) clinical penetrance [354]. Histological sections of a cervical lymph node of a 3-year-old child demonstrated a marked paracortical proliferation of occasional small and intermediate-sized lymphocytes with numerous large immunoblasts, the majority of which displayed a $CD3^+$, $CD43^+$, $CD45RO^-$, $CD4^-$, $CD8^-$ phenotype, with a $CD2^+$, $CD3^+$, $CD5^+$, $CD5^-$, Td^- ($CD4^-$, $CD8^-$) DN (double negative) profile [211]. Children develop these $CD4^-CD8^-$ DN T cells expressing the αβ TcR, hypergammaglobulinemia [310], a marked paracortical over accumulation of occasional small and intermediate-sized lymphocytes [240]. About 100-fold higher IL_{10}, but not IFN-γ or TGF-β was detected in DN than in single-positive T cells. IL_{10} was exclusively expressed in DN αβ but not γδ T cells [299]. Specifically, IL_{10} antagonizes the development of Th1 cells and indirectly enhances Th2 development. The Th2-oriented lymphocyte profile generated by IL_{10} may promote B-cell antibody production, including Aabs [240]. Currently, three genetically distinct subtypes of ALPS are recognized, caused by a mutation of the *TNFRSF6* (TNF receptor superfamily 6) gene (*ALPS type Ia*), of the FasL gene (*ALPS type Ib*), or of the caspase-10 gene (*CASP10*) (*ALPS type II*). In one subpopulation of patients, no mutations have been identified as yet (*ALPS type III*) [451]. Although these forms of ALPS differ genetically, they share defects in crucial homeostatic apoptotic mechanisms for controlling mature lymphocytes, leading to the features of ALPS [240].

Some children exhibit lymphoproliferative features during the 1st year of life and most children are well before the 5th year of life [33]. Some patients with ALPS have relatives with these same apoptotic defects who are clinically well [240]. ALPS, especially at young ages, is usually associated with lymphadenopathy, moderate to massive splenomegaly, and hepatomegaly resulting from abnormal accumulation of lymphocytes. AIHA, neutropenia, and idiopathic thrombocytopenic purpura are the most common features to diagnose autoimmunity in children with ALPS. These features have important diagnostic and prognostic value in avoiding expensive and time-consuming studies and unnecessary treatments [310]. However, the appreciation that manifestations of lymphoproliferation usually subside over time has allowed a wait-and-see approach in many patients who might previously have been treated aggressively. [33], although immunological disorders persist [354].

Autoimmune Neutropenia

Primary autoimmune neutropenia (AIN) is caused by increased peripheral destruction of neutrophils as a result of antineutrophil Aabs [47] and occurs predominantly in infants and toddlers between the ages of 6 and 24 months [435]. Primary AIN was mainly diagnosed at the average age of 5–15 months [47, 292, 412] and resolution of neutropenia was 20.4±4.9 months [412]. AIN was observed as early as day 33 of life [47], and two cases of congenital AIN in premature neonates indicate that AIN can have *a prenatal onset* [48]. Secondary AIN occurring in collagen vascular diseases such as JRA and SLE often shows more severe infectious complications [487]. In 90% of cases, AIN is associated with mild infectious symptoms (88.9% of children) [435], despite severe neutropenia, including skin abscesses, otitis media, and upper respiratory infections, only 12% have pneumonia; sepsis and especially meningitis are extremely rare in AIN [47]. One child had primary AIN associated with endocrinopathies [487]

Neutropenia is the sole abnormality, and neutrophil counts are generally <500/μl [44, 487], virtually always <10%, and usually <5%. CBC with differential usually reveals a selective, profound neutropenia with an otherwise normal CBC, often including a normal WBC. Both platelet and red cell counts are normal, although monocytosis and/or lymphocytosis may be seen. If neutrophil antibody testing in AIN is complicated, repeating testing as many as three times is recommended, to detect antibodies in clinically suspected cases if the initial test results were negative [47]. Examination of BM biopsies may reveal an increase in B lymphocytes and myeloperoxidase-positive cells with a maturation arrest at the myelocyte stage [48], with a markedly reduced number of neutrophils beyond the band stage [487]. The diagnosis of AIN depends on the demonstration of Aabs directed against neutrophil-specific antigens such as CD16 [487]. Primary AIN is usually associated with NA (neutrophil antigen)-specific antibodies, whereas secondary AIN seems to be associated with pan-FcγRIIIb antibodies [28]. Antibiotics are indicated for prophylaxis of infections. For severe infections, G-CSF, CSs, and IV IgGs are administered, resulting in increased neutrophil counts in 100%, 75%, and 50% of the children treated, respectively [47]. If the neutrophil count rises substantially to >1,000/μl (87% of children), the response con-

firms the diagnosis of AIN and provides therapeutic benefit [292]. See "Cyclic and Severe Congenital Neutropenia" in Chap. 22.

Diabetes

Definition and Classification

Diabetes is caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is an immune-mediated, but heterogeneous disease including both primitive forms: type 1 DM, and NIDDM (non-insulin-dependent diabetes mellitus) or type 2 DM, is characterized by progressive destruction of β-cells in Langerhans islands, with consequent total insulinopenia and hyperglycemia [353]. In 1%–2% of cases, NIDDM is transmitted as autosomal dominant (AD) inheritance (MODY, maturity-onset diabetes of the young), which can show itself during childhood or adolescence (<25 years) [426]. A case is transient neonatal diabetes: most newborns are growth-retarded at birth, present at a median age of 3 days, and recover at a median age of 12 weeks, and may be predisposed to diabetes later in life [416].

Epidemiology

The annual global incidence of pediatric DM ($\times 10^5$) in Europe is exceptionally high in Finland (35.2%) [441], with a high increase from 12×10^5 per year in 1953 to 45×10^5 per year in 1996) [335], and in Sardinia (33.2×10^5) [397]. In Denmark, Norway and Sweden, incidence is >20% [241]. In Poland the increase was significant in children aged 0–4 and 5–9 years in the Wroclaw province [96]. In Liguria, Italy, the incidence rate was higher in the 10- to 14-year-olds (15.01×10^5) than in 0- to 4-year-olds (9.01×10^5) and in the 5- to 9-year-olds (13.03×10^5) [69]. In Germany only 70 children were identified in a nationwide diabetes survey out of >5,000 (1.4%) young people who would meet DM diagnosis [199]. In the US, by 1994 it represented up to 16% of new cases, and by 1999, this incidence ranged between 8% and 45%, primarily in African, Asian, Mexican, and Native American children and youths [192], much less in the native population of Canada [498]. A report based on 24,423 children found during the period 1989–1998 an annual increase in incidence of 3.2%, highest for children in the 0- to 4-year-old age group: 4.8%. Central and Eastern Europe showed the highest increase, whereas Sardinia and Northern Europe (except Finland) showed no evidence of an increase [146]. In France during 1988–1997 there was an increase from 7.41×10^5 to 9.58×10^5, with a similar shift toward the same young group, higher in males than in females [56]. DM incidence has increased in Thai children and adolescents from 5% during 1986–1995 to 17.9% during 1996–1999 [233], was 0.7×10^5 per year in Peru [238], and increased in pediatric populations in several countries [307]. In a meta-analysis of 37 studies in 27 countries, the overall increase in incidence was 3.0% per year; however, the incidence of Type I DM is increasing worldwide to such an extent that by the year 2010 the incidence will be 50×10^5 per year in Finland and in several other populations it will exceed 30×10^5 per year [307]. A meta-analysis in 100 populations worldwide found that the overall age-adjusted incidence of type 1 DM varied from $<0.1 \times 10^5$ per year in China, Pakistan, Paraguay, Peru and Venezuela to the above-mentioned highest rates in Sardinia and in Finland. This represents a >350-fold variation in the incidence among the populations examined. A very high incidence ($\pm 20 \times 10^5$ per year) was also found in Sweden, Norway, Portugal, the UK, Canada, and New Zealand [188]. Among 303 diabetic twins, 81.5% had type 1 DM and 9.25% type 2 [174]. The incidence was the highest among children aged 10–14 [188]. For type 2 diabetes, recent prevalence estimates were 3.6% for Cree and Ojibwa girls aged 10–19 living in Manitoba [87] and 5% (CI 3.2–6.9) for Pima Indians aged 15–19 living in Arizona [76]. There, from 1966–1976 to 1987–1996, the prevalence increased fourfold for children aged 10–14 and sixfold for children aged 15–19 [76]. Instead, prevalence estimates were 2.7% for children aged 4–19 living in Manitoba [87]. The wide variation in geographical distribution has been related to different impacts of environmental factors [93], while its lower incidence in densely populated areas with many cohabitants in any single accommodation suggests that very early infections may play a protective role [300]. DM onset even during the neonatal period, and with 0.22×10^5 incidence [241], matches the higher number of new cases diagnosed at neonatal age [441]. There are no substantial differences between sexes as for the onset [241]. A disproportionately higher prevalence in the incidence of type 2 DM in children and adolescents has been highlighted recently, which underwent a greater than tenfold increase over the past decade [105, 361, 419]. The increase has been primarily attributed to the epidemic proportions of childhood obesity [497] in children with type 1 [192, 199, 233] and type 2 DM [105]. A study of the prevalence of childhood asthma and type 1 diabetes in 28 different countries found a strong positive correlation of the prevalence of these two conditions, suggesting that the immune processes underlying childhood asthma and autoimmune diabetes might be similar [403]. Until recently, most children with DM had type 1; however, type 2 diabetes is being reported in children from the USA, Canada, Japan, Hong Kong, Australia, New Zealand, Libya, and Bangladesh [105]. The prevalence of type 2 DM in children ranges from 4.1% of 12- to 19-year-olds in the US to 50.9% of 15- to 19-year-old American Indians [11, 105]. Between 8% and 45% of recently diagnosed cases of diabetes among children and adolescents in the US is

type 2, and the magnitude of this disease may be underestimated [11, 105].

Genetics

Twins provide a powerful tool to investigate relative importance of genetic factors on traits by comparing of concordance in MZ and DZ twins [348]. Significant deviation from 50% sharing was observed. MZ twins of patients with type 1A diabetes have a diabetes risk higher than that for HLA-identical twins ordinary siblings, suggesting that non-HLA genes contribute to diabetes risk [348]. MZ twins are also discordant (only one twin affected) for type 1 DM. More precisely, since the concordance rate among identical twins of whom one has type 1 DM is only 30%–50%, discordance for disease between such twins implies the postnatal participation of environmental triggering factors or other genetically or nongenetically determined factors, but could also be influenced by a decreased load of diabetes susceptibility genes [273]. The concordance rates for DM in MZ twins were very high: 70% in DR3/DR4 HET twins, but only 40% if they were not. If a sibling shares both HLA-D haplotypes with an index case, the risk for type 1 DM in that proband is 12%–20%; for a sibling sharing one haplotype, the risk is 5%–7%; with no haplotypes in common, the risk is only 1%–2% [357]. It can be assumed that in whites, the overall risk to cotwins is 50% within 7 years if the entrant is <10 and 23% if the he is >10 at the time of diagnosis [174]. The risk to offspring of a diabetic parent is 2%–5%, with the higher risk occurring in the offspring of a diabetic father. DM can be observed after BMT, thus confirming the immune nature of the disease [218]. The family incidence of DM (Fig. 18.26) is well known. There is a difference concerning the parents, since the number of children with DM having mothers with DM is lower than the number of children having fathers with DM. The incidence is 6% of fathers and 2.5% of mothers of children <15 years, with a total risk for children <20 years of 5.4%–7.6%, if their fathers are diabetics, and 2.1%–3.5%, if their mothers are diabetics [8, 97, 440, 466]. Of 243 children with features of type 2 DM, 76% had no known diabetic parent, 7% had a diabetic father only, 15% had a diabetic mother only, and 2.5% had two diabetic parents [308]. The risk of diabetes in offspring was significantly higher if the parent's DM was diagnosed before age 11 than if it was diagnosed later (9.3% vs 4% for the offspring of DM fathers [8]. Early onset affects the last-born child, who has a 1.75-fold risk compared to older siblings [440], who have a 10% risk before reaching 20 years of age [8]. It has been observed that DM is directly proportional to the father's age at onset, and inversely proportional to the mother's age at delivery [466]. Thus, the impact of imprinting is still unclear. Apparently, it is correlated with the maternal allele preferential expression [144]. However, if DM is correlated with the paternal allele, the neonatal DM is transitory [415]. MZ twins have a much higher risk of progressing to DM and expressing Aab than do DZ twins [348]. Studies on twins have shown a higher concordance in MZ [216], corresponding to a consistency rate of 35% in the late onset type, and 60%–100% rate in the common type [493]. In addition, carrying the high-risk insulin genotype increases the likelihood of identical twins being concordant for DM and the load of both HLA and non-HLA susceptibility genes having an impact on DM penetrance [273].

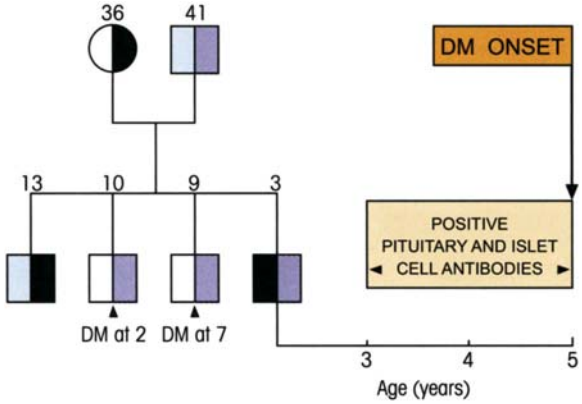

Fig. 18.26. Prospective study of a family with DM. The 3-year-old sibling had complement fixing antibodies to the islet cell surface before developing frank DM

Children at risk may develop type 1 DM through a dysfunction of HLA class II in the expression of Ii (invariant chain) p35, which may result in the impairment of antigen presentation [491]. Type 1 DM has a substantial genetic component, with consistent evidence for susceptibility *loci* in the *HLA-DR/DQ* region on chromosome *6p* and the insulin gene region (DM2) on chromosome *11p* [446]. The gene with strongest susceptibility is localized on the HLA-DQ *locus,* in linkage disequilibrium with HLA-DR [447]. Genome-wide scans for linkage have identified 18 chromosome regions showing some evidence of association with DM [83], with 10 susceptible *loci* confirmed. Each *locus* is linked to a type of DM (DM1 to DM18) [65, 118, 281, 425]. Others are correlated with MODY and type 2 DM, with late or common onset, and to neonatal DM (Table 18.18) [26, 62, 64, 65, 70, 83, 118, 144, 168, 259, 271, 280, 281, 348, 426, 446, 488, 489]. MODY 1–7 are known [280], MODY1–3 cases are transmitted by an AD trait [493], but types 1 and 3 are linked to HNF-4α and -1α mutations (4α and 1α hepatocyte nuclear factors), respectively. HNF is a transcription factor also present in Langerhans islands, which belong to the superfamily of thyroid/steroid hormones [488, 489]. Glucokinase-related MODY (MODY2) is frequent in children, mutations in the genes encoding IPD1 (insulin promoter factor 1), HNF-1β and NeuroD-1 (neurogenic differentiation factor 1) are the

Table 18.18. Chromosomal loci of DM and MODY

Type of diabetes	Chromosome
DM1 (type 1)	*6p21*
	8q24
	10p13–p11
	16q22–16q24
	17q24
DM2	*11p15.5*
DM3	*15q26*
DM4	*11q13*
DM5	*6q24–27*
DM6	*18q21*
DM7	*2q31-q33*
DM8	*6q27*
DM9	*3q21–q25*
DM10	*10p11.2–q11*
DM11	*14q24.3–q31*
DM12	*2q31*
DM13	*2q31*
DM14	Reserved but not published
DM15	*6q21*
DM16	*14q32.3*
DM17	*10q25.1*
DM18	Near the IL_{12} p40 subunit
MODY1	*20q11.2–q13.1*
MODY2	*7p13*
MODY3	*12q24*
MODY4	
MODY5	
MODY6	
MODY7	
NIDDM (type 2)	*1q21–q24*
	5q
	12q15
	14q11
Transient neonatal	*6q24*

Data from [26, 62, 64, 65, 70, 83, 118, 144, 168, 259, 271, 280, 281, 348, 426, 446, 488, 489].

rare cause of MODY4, MODY5, and MODY6, respectively [107].

Molecular biology methods have proven that the strongest relative and absolute risks were observed for *DRB1*0401*, *DRB1*0402*, *DRB1*0405*, *DQA1*0301-DQB1*0302* and *DQA1*0501-DQB1*0201* alleles [348] or for *DQB1*02-DQA1*0501/DQB1*0302-DQA1*0301* HZ, or the simultaneous presence of both *DRB1*03* and *DQB1*0302* alleles. An additive risk is suggested: *DRB1*03 is more strongly associated with DM than DQA1*0501-DQB1*02* and that *DRB1*0401* has an additive effect to *DQB1*0302*, thus the association between type 1 DM and HLA results from a complex interaction between *DR* and *DQ* haplotypes [204]. In children with type 1 DM, the frequency of risk-related alleles *DQB1*0302*, *DQA1*0201*, *DR4*, and *DR3* was more prevalent among Swedish than among Lithuanian subjects. Transmission rates of *DR4-DQB1*0302-DQA1*0301* and *DR3-DQB1*0201-DQA1*0501* alleles *from parents were higher than expected. DQB1*0302* and *DR4* were significantly more frequently transmitted *from both parents,* but *DR3* was transmitted more frequently *only from mothers* [367]. It may be related to age, since no child with early-onset DM had either of the protective *DRB1*1501* or *DQB1*0602* alleles [160]. Less common haplotypes such as *DQA1*0401-DQB1*0402* and *DQA1*0101-DQB1*0501* are also associated with high risk for DM [204]. It was hypothesized that the *DQB1*0302* either as HZ or HET encoding for aspartic acid (AA) at position 57 of the *DQ* α chain conferred protection, while the presence of arginine (Arg) at position 52 on the *DQ* β chain determined susceptibility to type IA DM, as observed in diabetic subjects and first-degree relatives as compared to controls [306], even though this predisposing factor is less relevant (Table 18.19) [348, 353]. Among the patients, 92.78% carry *DQa1* alleles that have Arg in position 52 of DQ α chain, and 78.2% are AA^- in DQβ at 57. The RR for HZ is 32.8 and 5.6, respectively [143]. A significant enrichment in *DQB1* alleles encoding for an amino acid different from AA in position 57 and *DQA1* alleles encoding for Arg in position 52 was observed in diabetic subjects and first-degree relatives as compared to controls [306]. *DRB1*1401* and *DQA1*0102-DQB1*0602* alleles [348] and *DMA*0102* and *DMB*0101* can provide protection from type 1A DM. *DQ7 is the protective allele* (OR=0.38), whereas *DQ8 is the allele conferring the susceptibility,* which is associated with an OR of 8.07 [372] and a RR of 12–32 [9, 72]. In comparison, the risk is 4 for *DR2* or *DR3* carriers alone, 6 for those carrying *DR4* only, higher for HETs on *loci DR3/DR4*, and equal to the sum of *DR2*, *DR3* and *DR4* alone; 90%–95% of diabetic patients have *DR3* and/or *DR4* haplotypes (Table 18.3), compared with 40%–50% of the general population [357]. This may be a synergistic effect of both genes, since this effect is relative, being linked to the *DQB1*0302* allele as HET in the haplotype *DR4* [353]. The observation that 20% of Americans and Europeans have *DQA1*0102-DQB1*0602*, while <1% of children with type 1A diabetes carry these alleles underlines the importance of genetics in disease development [348]. Recently, the hypothesis of non-HLA genes contributing to diabetes risk is supported by the observation that the

Table 18.19. Risk of type IA diabetes associated with DR and DQ alleles

DQAI	DQBI	DRB
High protection		
0102	0602	1501
0101	0503	1401
0201	0303	0701
Moderate protection		
0301	0302	0403
0201	0201	0701
0501	0301	1101
High risk		
0301	0302	0401
0301	0302	0402
0301	0302	0405
0501	0201	0301
Moderate risk		
0401	0402	0801
0101	0501	0101
0301	0303	0901

Modified from [348, 353].

diabetes risk for MZ twins of patients with type 1A DM is higher than the diabetes risk for HLA-identical ordinary siblings. Multiple genes, different in unrelated families probably explain this non-HLA genetic susceptibility [348]. Only two non-HLA *loci* are known. *DM2* is a polymorphic region that maps to a variable nucleotide of tandem repeat (VNTR) minisatellite, with three main VNTR classes. HZ for class I VNTR predisposes to DM1 and the longer class III alleles confer dominant protective effect. This *locus* contributes ≈10% toward disease susceptibility. Another *locus* associated with DM1 in some populations is *DM12* (Table 18.18), whose marker is the CTLA-4 (cytotoxic T lymphocyte antigen-4) = CD152 (*2q33*) (DM12). CTLA-4 and GCK may concur to the susceptibility [144] that encodes a receptor expressed by activated T cells involved in the T-cell activation. Studies in Japanese [279] and Polish [212] children have shown that a polymorphism at the *CTLA-4* gene (A → G transition at position 49 of exon 1) called the *G* allele, is preferentially transmitted to the affected siblings. Instead in nondiabetic offspring, the *CTLA-4 49 A* allele confers a protective effect in the presence of maternal *HLA-DRB1*03* and paternal *HLA-DRB1*04* alleles. Segregation analysis supports the hypothesis of a modulation by *CTLA-4 49 A → G* dimorphism of the *susceptibility conferred by maternal HLA-DRB1*03 inheritance* [108]. Recently, it has been stressed that there may be a pathogenetic relevance of genetic factors unconnected to the HLA system and particularly to the genes controlling the production of TNF-α. These are localized on human chromosome 6, and are close to class I and II genes and to the genes of some complementary components. It has been observed that this IL is one of the most important ILs produced in patients with DM; it is associated with *DR4* and is a crucial cytotoxic factor. These data cannot be applied to TGF-β [337].

Autoimmune Diabetes

Remarkable studies on transgenic mice have shown that β-cell destruction occurs via the convergent and synergic action of three genes: a HLA molecule, a TcR specific for this molecule, and increased IL local production, which lead to island inflammation and destruction; a viral infection can trigger IL production [286]. A fourth cause of β-cell destruction is reflected in increased circulating levels of adhesion molecules, supported by the observation of elevated sCD54 concentrations in 29 siblings who progressed to clinical type 1 DM [429]. The SA intervention in DM has been suggested. These SAs, by reacting with a proper TcR, signal the beginning of the destruction to T cells [286]. The most common abnormality at DM onset (60%–90% cases) is the presence of Aabs such as ICA (islet-cell antibodies) and insulin Aab (IAA), evidence of an AID, which can be found in 5% of the general population [157]. These Aabs seem to have a role not so much pathogenetic as supportive of the autoimmune activity after the initial damage, exacerbating the β-islet destruction [286]. Male gender and young age favor positive ICA reactivity, and siblings test positive for high ICA titers more frequently than parents [103]. IAAs were detected in 45% of children with an 85% positive rate in <5-year-old children, GADs (glutamic acid decarboxylases) in 66% and ICAs in 23% of children [140]. ICA and GAD-positive frequencies in type 2 DM children were 71%–76% lower than in type 1 DM children. Among children with type 2 DM, the rates were 34.8%, 30.3%, and 8.1%, respectively [159]. However, the frequency of positive ICA512 and GAD65 antibodies was significantly less in early onset DM (28.6% and 31.6%, respectively) [160]. As for GAD, there are at least two allelic forms from different genes, known as *GAD67* and *GAD65* (based on their respective MW, which might coexist in patients' sera [191], playing a preventive role against DM [472]. Conversely, anti-GAD antibodies are indicative of β-islet destruction [157]. Most peptides derived from GAD65 and islet associated-2 Aags can bind to HLA molecules predisposing to or protecting from type 1 DM [155]. In other twin studies, IL_2 and TNF-α responses to GAD were detected more frequently and at higher levels in diabetic compared to nondiabetic twins [330]. Moreover, T cell responses to a human GAD65 target epitope as a DM-associated Aag present-

Table 18.20. Major DM autoantigens (Aag) and autoantibodies (Aab)

Antigen	Localization	Clinical significance
ICA (glycolipid)	All islet cells	Main immune marker of recent-onset and preclinical DM insulin Aab in over 50% of subjects with late preclinical and recent-onset DM; especially in younger children are an index of remarkably faster β-cell destruction
IAA	β Cells	
64K/GAD	β Cells, all islet cells, neurons, ovary, testis	Aac to 64K or GAD in about 80% of subjects with preclinical DM, their titers remain more elevated than ICA during DM progression
37 kD/40 kD Tryptic	Secretory vesicles of β cells	Aab in up to 60% of preclinical fragment of 64K and recent-onset DM subjects and in 2%–3% of their relatives
38 kD (Protein from insulin secretory granule membrane)	Neuroendocrine secretory cell granule	Aab in about 30% of recent-onset DM subjects, it is identified as the target of T-cell lines from peripheral blood of recent-onset DM subjects
52 kD (PTP)	Neuroendocrine secretory cell granule	Aab in 25% preclinical and 0% control subjects. True prevalence and disease specificity unknown
Islet polar antigen	Vascular pole of β cells	Aag in up to 25% of preclinical DM subjects
GLUT-2 (glucose transporter protein)	β Cells, hepatocytes	Aag in up to 75% of recent onset DM subjects
Pro-insulin	β Cells	In preclinical and recent-onset DM subjects and in full-blown DM; usually correlated with IAA

Data from [59, 157].
GAD glutamic acid decarboxylase, *IAA* insulin autoantibodies, *ICA* islet-cell antibodies, *PTP* proteintyrosinphosphatase.

ed on HLA-DR4 molecules are experienced by a majority of DR4-positive DM patients aged 14–25, indicating that this epitope represents one of the epitopes recognized by $CD4^+$ T cells during the autoimmune events associated with DM [294]. Table 18.20 [59, 157] summarizes the main characteristics of these Aabs and Aags, and of another immunological marker, the PTP (protein tyrosine phosphatase) [59, 157]. GAD and PTP are present in 55%–60% of diabetic patients at onset and in 8%–9% and 2%–3% of relatives, respectively [59]. Glima 38 is recognized by Aabs in 20% of patients with type I DM, its biochemical properties define glima 38 as a new Aag in type I DM, able to bind *Triticum vulgare* and *Ricinus communis* I lectins [358]. Aabs to human HSP60, HSP70 and HSP90 proteins evaluated in children with newly diagnosed type 1 DM showed heightened T-cell autoimmunity to HSP70 and HSP60, but not to HSP90 [1].

Some models point to the molecular mimicry between PC-2 proteins of the *Coxsackie* B4 virus and GAD antigen [353], the enzyme catalyzing the glutamic acid conversion into GABA (γ-aminobutyric acid) [157]. Furthermore, the primary viral infection may decrease the expression of some viral genes inside β cells. It is likely, however, that cross-reactive epitopes evoke a chronic immune response specifically aimed at β cells [353].

Immune Dysfunctions

Type 1 DM is associated with immunological dysfunctions such as B-cell and related Aab dependency, and CMI deficits and the phagocytic system: type I develops after a period of autoimmunity against pancreas β cells. Multiple immunoregulatory T cell defects such as low numbers of resting $CD4^+$ $CD25^+$ T cells underlie islet-cell autoimmunity, leading to immune-mediated diabetes in humans with these lesions possibly part of a broad T cell defect. Moreover, a defective production of IFN-γ and IL_4 deficiency in NK T-enriched cells were reported [214]. T-cell recognition of insulin-secretory granule antigens is associated with the immune-mediated process of β-cell destruction, thus showing that T cells have a role during the disease process [356]. The level of lymphocytes with surface markers consistent with recent primary activation (CD45RA/CD45RO) was higher than that in the other diabetic children, who had higher levels of CD45RA/CD45RO cells than the nondiabetic controls [260]. Whether or not B cells are activated, Aabs to pancreatic β-cell antigens can be produced, such as GAD65, insulin, or the tyrosine phosphatase-like Aag IA-2, and they are able to take up and present autoantigen to T cells [351, 356]. In a 14-year-old boy with X-linked agammaglobulinemia (XLA) DM1 (immune-mediated) developed [260]. Since XLA is charac-

terized by a blocking of B-cell differentiation that results in an arrest in the progression of pre-B1a cells, it is obvious that neither Aabs nor B-cell function is critically involved in the pathogenesis of type 1 DM.

The development is also possible without HLA class I cytotoxicity, thus suggesting the pathogenic contribution of a killing factor through PBMCs, macrophages, activated CD4 and endothelial cells [207]. Secondly, the insulitis is caused by SAs or other immunostimulating products expressed by β cells. It is rather the balance between mediators and IL released by Th1 and Th2 that may cause the destruction of β cells [207]. Data obtained from studies on NOD mice stress the preferential activation of IL_{10} and IL_{12} by macrophages and resident DCs. IL_{10} plays an anti-inflammatory role by inhibiting IFN-γ and Th1, thus favoring Th2 and sparing islet cells. The same occurs with macrophages and their APC function [433]. The opposite occurs when IL_{12} prevails and Th1 are produced. Th1 along with NK cells produce high levels of IFN-γ, which in turn amplify the IL_{12}-mediated Th1 response [434]. Thus, the prevailing response is Th1, with a preventive role played by the Th2 response. This is confirmed by the Th1 of transgenic mice for diabetogenic TcR, which can transmit the disease to healthy NOD mice. Moreover, when the IFN-γ action persists, macrophages are activated to carry on cytolytic activities and secrete $IL_{1\alpha}$ and/or TNF-α, potentially cytotoxic for β cells [434]. It has been observed that IFN-γ and IL_2 cause CTLs to destroy β cells and secrete TNF-β and more IFN-γ [433]. Its damaging action is confirmed by experiments on transgenic mice developing DM when it is expressed on β cells [447]. In contrast to what has been said, NOD mice expressing IL_{10} seem to develop a severe and early form of DM instead of being protected by it [481]. This can be explained by the protective effect played by TGF-β [58]. The break in the Th1/Th2 balance is consequent to the CD28/CD80 block. This prevents the differentiation of Th2 and triggers DM [224]. The comparison of mature DCs between patients and controls showed significant differences for CD80 and CD83; no difference in T-cell stimulatory capacity was seen [500].

Recently, it has been shown that the incidence of atopic diseases in DM is not higher than the incidence among controls. There are, however, specific differences concerning late responses [405]. As for specific foods, even without direct evidence, the epidemiological data seem to corroborate the protective role of exclusive breast-feeding [462], and the close relationship between early CM ingestion and the onset of DM [78]. This hypothesis, however, is not supported by all studies [248]. Apparently, some children with DM have IgG antibodies against ABBOS, a specific peptide from bovine serum albumin (BSA), which cross-reacts with a 69-kD protein (p69), possibly induced by β-cell IFN-γ in Langerhans islands. Thus, CM ingestion, failing any symptom of CM allergy (CMA) can trigger DM if the nursing child is susceptible [186]. It is revealing that almost all diabetic patients have anti-ABBOS Aabs, triggering a T response against them, vs only 2% among normal subjects [136]. Not all researchers agree on BSA immunogenicity for subjects with DM [17]. It must be stressed, however, that in children with CMA, BSA is a powerful allergen, even though it is weaker than other CM proteins (Table 1.75), and DM seems to be linked to a cellular, not humoral, reaction. CM protein immunogenicity has been further proven in children and teenagers with early onset DM [100, 376]. It is significant that both CM and hydrolyzed casein, administered to prevent DM, induce both humoral and cellular reactions [445]. α-Casein has a five-amino acid sequence identical to the protein GLUT-2 [52], which transports glucose inside β-cells. Thus, CM exposure and/or its derivates may induce a gut cross-response to the carrier protein. This data implies inadequate regulation of oral tolerance and stresses that GALT is fundamental for immune DM pathogenesis [207]. To solve this problem, and the analogous problem with JRA, large-scale international studies, both prospective and randomized, should be elaborated.

Additional Factors

Among strictly etiological factors there is the association with viral infections [495]. The intervention of environmental factors is also possible such as ethnic and/or climatic factors, caloric excesses, social well-being, etc., but without any definite results [241]. According to some authors, all these factors rely on genetic factors [499]. As for the onset age, there are no substantial differences in a wide range of anamnestic, genetic and immunological data among children whose DM appeared at 3–6 years, except for significant differences concerning the IgA deficit and Aab presence, which are lower in younger patients [54].

Diagnosis

Since <10% of patients have previous cases of DM among relatives, screening the illness appears problematic [59], but it is possible to dose tissue transglutaminase C for diagnostic reasons, also to screen for celiac disease, found in 3%–5% of type 1 DM children [217]. ICAs and IAAs are very useful for the identification of asymptomatic subjects at the preclinical stage, especially ICAs with 100% sensitivity vs IAAs with 33% sensitivity. Above all, there is a direct correlation between HLA heterozygosity, high and persistent levels of ICAs, and a high risk (>100-fold) of contracting the disease [187]. Persistent ICA positivity associated with IAA positivity defines the actual risk (47% and 70%), while the combination of HLA and high ICA titers has the highest predictive value (77%), since it is a risk factor 73-fold higher than in general population [187]. It must be stressed that when ICAs are at low levels, high-risk HLA

markers do not lead to DM, whereas the opposite correlation is a sign of actual risk [187].

Treatment and Prevention

Insulin is the mainstay of treatment. Continuous insulin infusions administered by insulin pumps provide multiple advantages over manifold insulin pump therapy in toddlers and young children [237]. Table 18.21 shows the classification and the preventive measures [339].

Table 18.21. DM prevention

Classification
Primary prevention
Intervention at birth in at-risk children by removal of etiological factors
Diabetogenic virus or with molecular mimesis
Other environmental factors, including dietary restrictions or changes
Secondary prevention
Intervention during the latency phase in children with positive specific markers
Tertiary prevention
Protective intervention at early DM diagnosis of residual β cell mass and function
Intervention
Primary prevention
Breast-feeding
Cow's milk exclusion
Secondary prevention
Insulin
Nicotinamide

Data from [339].

Table 18.22. Therapeutic and preventive approaches for DM under investigation in the animal model of DM

Analogous of vitamin D_3
Anti-oxidants
Immune deviation using autoanalogs
Islet cell vaccination
Monocyte and lymphocyte adherence inhibition
NO inhibitors
T-cell vaccination
Thymic injection of β-cell antigens
Vaccination with specific insulin peptides

Modified from [339].

The most important measures are breast-feeding for newborns at risk [136, 498] and the use of insulin or nicotinamide in secondary prevention. The administration of low-dosage insulin to children at high risk proved to be highly effective, so much so that it could delay or prevent the DM onset [136]. It may be impossible to eliminate CM from the diet of nurslings and children at risk for DM without having more data on the possible presence of anti-ABBOS antibodies in their healthy parents as well [248]. A meta-analysis shows that there is no convincing evidence relating type or intensity of diabetic treatment to the prevention or management of cognitive impairment in MODY [14]. Therapeutic and preventive perspectives have been studied in animal models (Table 18.22)] [339]. Children with DM treated with modern regimens attain a normal height [86]. There is also the possibility of inducing tolerance by administering GAD65 to NOD mice, through the activation of Th2, which blocks autoreactive Th1 [424]. It should be noted that provoking an anti-Th1 response via Th2 may destroy the protective immunity against intracellular microorganisms, or induce allergic reactions [231]. One of the environmental factors that should be stressed is the monitoring of viruses with molecular mimicry, which can have amino acid sequences in common with GLUT-2 and/or p69 [25]. A therapeutic approach to β-cell salvage was tested in new-onset DM by means of anti-CD3 antibodies, which ensures that endogenous insulin secretion remains stable and improves metabolic control during the 1st year of treatment. Clinical responses were associated with a shift from $CD4^+$ T cells to $CD8^+$ T cells 30 and 90 days after treatment [162]. Phase 2 studies should follow.

It is also likely that supplementation of appropriate amounts of LCPUFAs (long-chain polyunsaturated fatty acids) and ω-6 and ω-3 fatty acids *in utero*, and first year of life protect against atopy, AIDs, *type 1 and type 2 DM* [81].

Systemic AIDs

Based on Table 18.2, systemic AIDs include two types of vasculitis (Table 8.17), SLE and dermatomyositis, whose characteristics are summarized in Table 18.10, and juvenile scleroderma.

Systemic Lupus Erythematosus

Definition

SLE is an autoimmune disease in which the body's own immune system is directed against the body's own tissues. SLE is a form of diffuse vasculitis caused by CICs, of which the best example is glomerulonephritis characterized by fibrinoid necrosis of vessel walls, which pro-

duce a systemic inflammatory damage to target organs. Defined by clinical criteria, SLE is a chronic disease with protean manifestations.

Epidemiology

SLE occurs in 0.6 children $\times 10^5$; the female/male ratio is 1:1 until 10 years of age, and 10:1 afterwards [13], for an incidence of 1.4% [250]. The onset of SLE before the age of 5 is uncommon [124]. Two girls aged 6 weeks and 3 months were seen with diffuse proliferative glomerulonephritis, anti-double-stranded (ds) DNA and infantile SLE [265]. Two additional infants aged 2.5 and 6 months were reported [265]. Other children may be as young as 3.2 years [250]. In 39 children with a median age at onset of 12 years, the female/male ratio was 18.5:1 [178] and 6.4:1 [250]. In 204 children, the SLE diagnosis was made before the age of 16 (19.1%) or after the age of 16 (80.9%). [438]. The trial on 1,742 children found a SLE incidence of 6% [89] and two other studies a 0.28 [250] and a 0.48 incidence $\times 10^5$ [169].

Genetics

A genome-wide linkage study of NZB x NZW F2 mice revealed 8 loci designated Lbw1–8, that were located on chromosomes 17, 4, 5, 6, 7, 18, 1, and 11, respectively. They were linked to mortality, nephritis, IgG antichromation Aab production and splenomegaly. In particular, Lbw1 was linked to mortality, nephritis and antichromatin Aabs and mapped to the HLA region. Heterozygosity at Lbw1 (H-2d/z) conferred the greatest susceptibility. Lbw2 was dominantly transmitted and associated with accelerated mortality, nephritis, and splenomegaly [421]. Four major *loci* were additionally identified, designated Lmb1–4, on chromosomes 4, 5, 7 and 10, respectively. All 4 QTL (quantitative trait loci) were linked to lymphoproliferation, Lmb1–3 were also linked to antidsDNA antibodies and Lmb4 was also linked to nephritis [461]. In a cohort of 115 multiethnic nuclear families containing 145 SLE-affected sibpairs, haplotype sharing at *1q23* increased concomitantly with increased haplotype sharing at *16q12*. Analysis of sibpairs sharing two alleles at *16q12* also showed increased allele sharing at *1q23*. No evidence supporting an interaction between *16q12* and *20p12* was observed. These data support the presence of SLE susceptibility genes at *1q23* and *16q12*, particularly in non-Caucasians [436]. In a Chinese cohort, chromosome *16q12* is a likely susceptibility gene for SLE, which was found in linkage disequilibirum with the marker D16S409 and D16S517 [115]. In 94 extended multiplex pedigrees, four genes for SLE (*20p12*, *1p13*, *1q42*, and *4q28* with lod scores ranging from 2.62 to 1.46) were identified in combined African-American and European-American families, with an effect for the FcγRIIA candidate polymorphism at *1q23* (lod, 3.37 in African-Americans) [284]. In a study in 105 SLE sibpair families, the strongest evidence for linkage was found near the HLA locus (*6p11–p21*) and at three more regions: *16q13*, *14q21–23*, and *20p12* (lod scores ranging from 2.62 to 3.90). Nine additional regions (*1p36*, *1p13*, *1q42*, *2p15*, *2q21–33*, *3cent–q11*, *4q28*, *11p15*, and *15q26*) had lod scores ranging from 1.00 to 1.68 [130] and seven additional regions had significant linkage (lod ≥3.3 or $p \geq 0.00005$) at *1q22–23*, *1q41*, *2q37*, *4p16*, *6p21–11*, *16q13* and *17p13*. Putting the pieces together, these linkages can be reproduced ranging from outright independent confirmation (*1q41*, *4p16* and *6p21*) to additional suggestive evidence in the genomic region of the purported linkage (*1q22–23* and *2q37*) [194]. Linkage analysis with covariates uncovered linkage at *13p11*, *17q11–25*, and *20q12* and greatly improved evidence for linkage at *1q22–24*, *2q37*, *12p12–11*, and *17p13* [305]. With 417 microsatellite markers, suggestive linkage in regions on chromosomes *6q* and *14q* as well as HLA on *6p* were detected, also showing evidence for excess sharing of a haplotype on *14q* and excess transmission of a haplotype on *6q* [209]. In a study on Icelandic families, five regions showed lod scores >2.0, which were located on *4p15–13*, Z=3.20; *9p22*, Z=2.27; *19q13*, Z=2.06, on *19p13* (*D19S247*, Z=2.58) and on *2q37* (*D2S125*, Z=2.06), while in Swedish families two regions showed lod scores >2.0: on chromosome *2q11* (*D2S436*, Z=2.13) and *2q37* (*D2S125*, Z=2.18). Notably, both family sets gave a highly significant lod score at *D2S125* of Z=4.24 in favor of linkage for *2q37* [236]. Recently the Oklahoma group has found evidence ($p<0.004$) of linkage at *16p13* and *16q12–13* with SLE in a genome scan based on 37 Hispanic families. [289], linkage at *11p13* for SLE identified in 27 African-American families [290] and linkage at *12q24* that may cause SLE in Hispanic and European American families [291]. In Chinese children, the haplotypes *HLA-A9B40/DRB1*15* and genotypes *HLA DRB1*09/DRB1*15*, *HLA-DRB1*03/DRB1*15* were correlated with SLE [228]. *The HLA-DRB1*1501* (protective for JRA and DM) *greatly enhanced the risk of developing lupus nephritis* conferred by the *DQA1*0101* allele (OR, 65.96), whereas *DQA1*0102 demonstrated a significant protective effect* (OR, 0.31) by suppressing the nephritogenic effect of *DRB1*1501* [256]. *HLA-A24* may confer additional risk of more severe disease expression in female patients with SLE and SLE patients carrying 39-kD *sHLA-I* have increased risk of developing renal disease [2]. *HLA-DMA*0104* may be *a novel allele of susceptibility* to SLE [282] and *TAP2*Bky2 may be a susceptible gene* not only to the disease of SS but also to SS-A/Ro Aab production [185]. Infants of SS-A/Ro- and SS-B/La-positive mothers bearing *HLA-A1*, *B8*, *DR3*, *DQ2,* and *DR52* are at greater risk of having neonatal lupus [221]. Thus multiple genes, including one in the *HLA* region, may influence susceptibility to human SLE [130]. Amazingly, mannose-binding lectin (MBL) variant alleles may confer a 1.6-fold overall increased risk of severity and infection for definite SLE [132].

Classification

The presence of a wide spectrum of clinical forms, from the most benign to the most severe, has triggered the hypothesis that SLE could be a syndrome rather than a well-defined illness [13]. Its major characteristics are summarized in Table 18.10.

Pathogenesis

SLE pathogenesis is not completely clear. However, a number of different factors seem to influence the expression of SLE. In 12% of patients, close relatives are affected, showing a *strong hereditary component* [378]. Studies on *HLA* haplotypes show a significant association with *DR3* (Table 18.3), clinically linked to subacute cutaneous SLE, neonatal SLE, and Sjögren syndrome. MZ twins show higher SLE concordances than HETs. The level of genetic contribution can be measured by comparing the concordance rate between MZ and DZ twins. MZ twins show higher SLE concordances than HETs. The MZ twin concordance rate for the clinical expression of SLE has been found to be 25%–69%, whereas that of the presence of serum Aabs can be as high as 92%. For comparison, only 1%–2% of sibs and DZ twins are concordant for SLE [180, 235]. Children show a relatively higher incidence of familial cases than adults. Furthermore, some children's relatives or family members are affected by other immune diseases, which suggests that SLE develops mostly in patients whose families are susceptible to immune-mediated diseases [18]. In 10%–15% of cases (vs 2% in the general population), there is HZ deletion of the *C4A* allele, which can reach 50%–80% peaks, almost doubling the normal value, when it is HET [378]. Complement deficits are most often associated with SLE-like syndromes [420] as well.

Immune dysregulation appears to underlie the development of SLE, where dysregulated $CCR2^+$ T cells and the CD4/CD8 ratio are also switched to the left [12], with clear stimulation of T-like ILs. Such ILs participate in the activation and differentiation of B cells into Aab-producing cells: as both purified T and B cells express FasL as well as several foreign infectious agent-derived proteins may share the epitope (aa – aminoacids 161–170) with human FasL [275]. This *aberrantly expressed FasL* may facilitate escape of the autoreactive B cells from the immune tolerance system and may contribute to the sustained Aab secretion in patients with SLE [287]. A major cause of Aab production is molecular mimicry which stimulates the development of Aab secreting lymphocytes [275]. Increased apoptosis and decreased clearance of apoptotic cells as observed in SLE might be a contributory factor in systemic autoimmunity [4]. Dysregulation of cell death rates in SLE may lead to self-reactive lymphocytes that normally undergo apoptosis before birth, which suggests that the aa 162–169 region is important for Fas/FasL interaction [275]. A synergistic effect between susceptibility alleles of both *bcl-2* and IL_{10} genes increases the odds of developing SLE by >40-fold [205]. Not only *bcl-2* [110, 277] and IL_{10} [77, 277], but also Fas and TNF-α [277] may interfere with the apoptotic process, promoting lymphocyte hyperactivity secondary to increased IL levels, thus denoting their role in disease activity. IL_{10} was a genetic susceptibility factor significantly associated with SLE in Italian patients [77]. Overexpression of bcl-2 was strongly correlated with SLE activity, thus suggesting an alteration of apoptotic regulation in patients with juvenile onset SLE [110]. Fas levels possibly secondary to TNF-α action, bcl-2 antigen expression and IL_{10} serum levels were shown to be related to the maintenance and progression of SLE [277]. The overexpression of Fas antigen may be associated with the reduction of bcl-2 expression in the basal cells, which correlates directly with the extent of apoptosis in the epidermis [19]. Efforts to characterize SLE as a Th2 or Th1 disease based on CD4 T-cell phenotype have met with difficulty, but IL_{10} seems to be increased in human and murine SLE, along with IL_6. The ratio of IL_{10} to IFN-γ-secreting cells in SLE peripheral blood is increased, thus implying *a Th2 cell predominance* in the circulation [62]. B lymphocytes are stimulated by T cells, with consequent polyclonal hypergammaglobulinemia, possibly caused by alteration of regulating mechanisms, or exogenous factors such as viral infections producing IFN-γ or bacterial infections stimulating the expression of HLA-DR on macrophages, etc. [13]. The marked Aab production leads to CIC formation with antigens and complement, CICs bind to C3b receptors, CR1 (CD11a), which, by interacting with erythrocytes, carry them to RES, in particular to the hepatic sinusoids, for the transfer to CD11b–CD11c-positive cells, mostly macrophages, which removes them from circulation [18]. Consequently, conditions like SLE with high CIC levels are characterized by a CD11a deficit [378]. Similarly, the lack of C1, C2 or C4 markedly reduces the link between complement and CICs, which become larger and less soluble, and, moving in peripheral plasma, deposit in tissues [378, 420]. The correlation of sCD62L with C4 in SLE may indicate that down-regulation of shedding of cell surface CD62L is involved in continued leukocyte adherence to endothelium, probably causing supplemental damage and CIC deposition in SLE children [35]. Clinically, Aab titers are closely related to the degree of inflammation. Anti-DNA antibodies may combine with circulating antigen and contribute to CIC deposition in renal glomeruli [7]. CIC-derived DNA of SLE patients reinforces the evidence that reactive O_2 species may be involved in its pathogenesis [7].

Clinical Presentation

The most frequent clinical manifestation of pediatric SLE, summarized in Table 18.23 [55, 178, 261, 378] and

Table 18.23. Main clinical manifestations of pediatric SLE (%)

Reference	[378]	[55]	[178]
Malaise, weight loss, failure to thrive	96		67
Skin changes	91		72
Blood abnormalities	91		
Fever	84	61	
Renal changes	84	46	28
Musculoskeletal involvement	82	87	78
Pulmonary/pleural disease	67	7	5
Hepatosplenomegaly or lymphadenopathy	58		8
CNS lupus	49	39	28
Heart disease	38		
Hypertension	33		
Vasculitis	33	20	2.5
Eye abnormalities	31		
Gastrointestinal symptoms	27		8
Lymphadenopathy			15
Main cutaneous manifestations of pediatric SLE (%)	**[261]**	**[55]**	**[178]**
Alopecia	44		23
Butterfly rash	66	79	44
Morbilliform rash	37		
Mucocutaneous ulcerations	22	34	36
Photosensitivity	11	47	18
Raynaud's phenomenon	7	29	10

Table 18.24. Common SLE cardiac, renal and CNS manifestations

Cardiac manifestations
Pericarditis
Valvular vegetations (Libman-Sacks endocarditis) most commonly in the mitral valve
Myocardial dysfunctions, including heart infarction
Subacute infectious endocarditis
Lupus nephritis
Class 1: normal
Class 2: mesangial
2a: minimal lesions
2b: mesangial glomerulonephritis
Class 3: focal segmental proliferative glomerulonephritis
Class 4: diffuse proliferative glomerulonephritis
Class 5: membranous glomerulonephritis
(WHO classification)
Neurological disorder
Aseptic meningitis
Behavioral changes
Chorea
Coma
Cranial nerve palsy
Headache
Infarction of nervous tissue
Mental deterioration
Peripheral neuropathies
Pseudo-tumor cerebri
Psychosis
Seizures
Transverse myelitis

Data from [222, 468].
WHO classification, accessed at http://www.mcl.tulane.edu/classware/pathology/medical×pathology/New×for×2004/renal/who.html. The WHO classification has recently been modified as follows: **class I**, mesangial immune deposits without mesangial hypercellularity; **class II**, mesangial immune deposits with mesangial hypercellularity); **class III** for focal glomerulonephritis (involving <50% of total number of glomeruli) with subdivisions for active and sclerotic lesions; **class IV** for diffuse glomerulonephritis (involving ×50% of total number of glomeruli); **class V** for membranous lupus nephritis; **class VI** for advanced sclerosing lesions. Data from [471].

Table 18.24 [222, 468], are not significantly different from the diagnostic criteria defined by ARA [411]. Symptoms are typically variable, as is their natural history (Table 18.10), with alterations in different organs (Table 18.24; Fig. 18.27). The clinical features are atypical, the symptoms are aspecific and heterogeneous. From Table 18.23 we see that malaise, weight loss, failure to thrive, and skin changes are present in 81.5% of cases, and fever in 72.5% of cases [55, 178, 378]. In addition, children exhibit nephropathy (20%), and lymphadenopathy (6%) as presenting clinical features. During the progression of SLE, the children had an increased prevalence of malar rash (79%) and chorea [124]. Arthralgia and arthritis are also present [178]. *The butterfly rash* (Fig. 18.28) is characteristic, whereas the discoid rash is rare in children, lesions that are scarring and occur either alone or in association with SLE. The malar rash is a fixed or raised erythema, over the malar eminences, tending to spare the nasolabial folds, which may be the initial presentation and must be distinguished from other causes of a so-called red face. *The discoid rash*

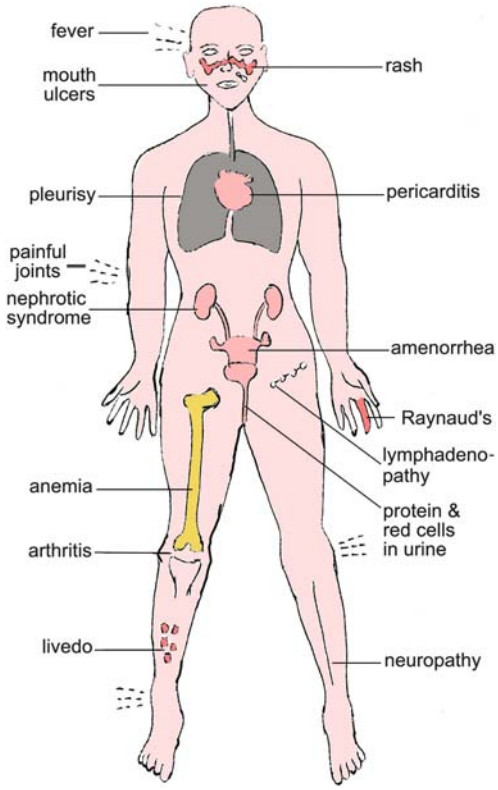

Fig. 18.27. The classical SLE patient

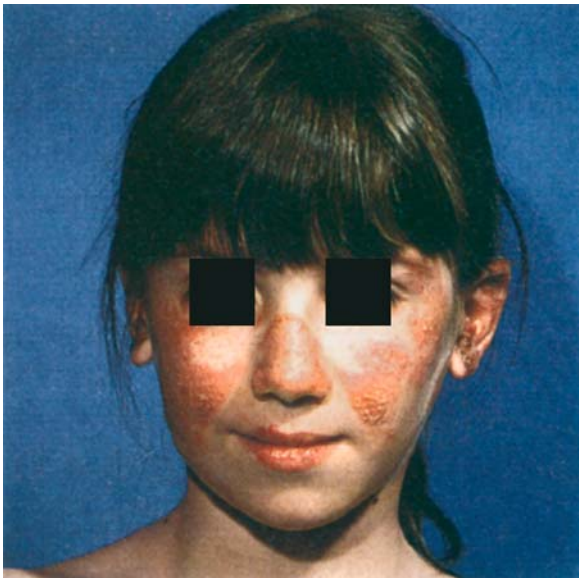

Fig. 18.28. SLE: "butterfly" rash

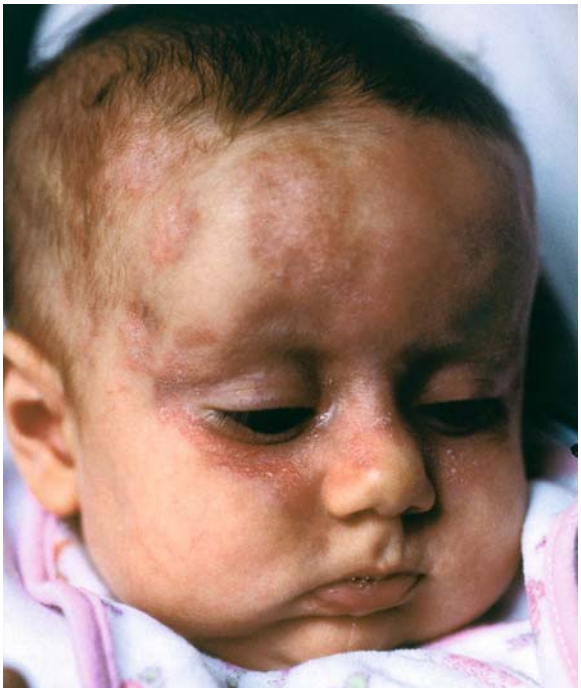

Fig. 18.29. Neonatal lupus

consists in erythematous, hyperkeratosic, scaly atrophic telangiectatic plaques on sun-exposed skin, with adherent keratotic scaling and follicular plugging, which remain unchanged for months, in regions such as the face, especially if exposed to sunlight, which frequently heal with scarring and dyspigmentation. Alopecia, despite being present in 34% of cases, is not part of the diagnostic criteria, in view of the obviously poor reliability and quantification of observations [178, 261]. Photosensitivity, causing oral and nasopharyngeal ulcers, Raynaud's phenomenon, and systemic complaints, is important. *Multiorgan involvement* is particularly characteristic, probably because of diffuse CIC deposition in vascular structures [41]. Hypocomplementemic urticarial vasculitis may be frequent [85]. A hematological disorder may include pancytopenia, leukopenia (<4,000/ml), lymphopenia (<1,500/ml), or a platelet disorder. A possible *neurologic disorder* (Table 18.24) may include behavioral changes, seizures, psychosis resistant to treatment, hallucination, autolesionism, possibly caused by a cerebral vasculitis (S. Cioschi, personal communication). There are also cases of *neonatal lupus* (Fig. 18.29) caused by transplacental passage of Aabs from mother to fetus between the 12th and 16th week of gestation, explaining the infant disease, as well as its spontaneous resolution, which is coincident with the disappearance of maternal antibody. Cutaneous lesions are rare features (annular, erythematous, scaly plaques typically on the head, neck, and upper trunk) (16%) often associated with thrombocytopenia (27%), hepatosplenomegaly, mild elevation of liver enzymes (26%), and atrioventricular block (1.6%) [61], and wane by 6 months, generally resulting in clearance of the rash. An incidence of 0.3% was found [250]. Since 50% of the mothers of babies with neonatal lupus do not clinically have SLE but carry the Aabs, it is not uniformly manifested in all offspring

of SS-A and SS-B Aab$^+$ mothers. In addition, other contributing etiological factors are being explored given the wide range of clinical manifestations associated with it [41, 221].

SLE course should be followed with particular care, as the progression is often unpredictable. The outcome is potentially dangerous because of sudden relapses, including frequent infections to which these children seem susceptible. Renal involvement should also be taken into account. Renal involvement (proliferative mesangial glomerulonephritis) is present in up to 75% of patients with other SLE features, which, if not aggressively treated, progresses to renal failure. This is a most common complication of proliferative glomerulonephritis.

Diagnosis

The diagnosis is based on the data from Tables 18.25 [166, 412] and 18.26 [178]. Table 18.24 [222, 468, 471] completes the list of symptoms.

The Major Laboratory Abnormalities

The excretion of >500 mg of urinary protein/24 h (>0.5 g/day) or >3+ proteinuria on dipstick testing), the presence of casts (including RBCs, hemoglobin, granular, tubular, or mixed), hematuria (>5 RBCs/hpf) or pyuria (>5 WBCs/hpf), or a protein-to-creatinine ratio >2.0 is evidence of renal disease and should prompt the

Table 18.25. SLE diagnosis, 1982 revised ARA criteria for clinical and laboratory SLE diagnosis

Criterion	Definition
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions.
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
5. Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a. Pleuritis – convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion OR
	b. Pericarditis – documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a. Persistent proteinuria >0.5 g/day or greater than 3+ if quantitation not performed OR
	b. Cellular casts – may be red cell, hemoglobin, granular, tubular or mixed
8. Neurological disorder	a. Seizures in the absence of offending drugs or known metabolic derangements, eg, uremia, ketoacidosis, or electrolyte imbalance OR
	b. Psychosis – in the absence of offending drugs or known metabolic derangements, eg, uremia, ketoacidosis or electrolyte imbalance
9. Hematological disorder	a. Hemolytic anemia – with reticulocytosis OR
	b. Leukopenia– <4,000/mm^3 total on two or more occasions OR
	c. Lymphopenia– <1,500/mm^3 on two or more occasions
10. Immunological disorder	a. Anti-DNA: antibody to native DNA in abnormal titer OR
	b. Anti-Sm: presence of antibody to Sm nuclear antigen OR
	c. Positive finding of antiphospholipid antibodies based on (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) false-positive serological test result for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear	An abnormal titer of antinuclear antibody by immune fluorescence, antibody or an equivalent assay at any point in the time and in the absence of drugs known to be associated with drug-induced lupus syndrome

Note the classification is based on 11 criteria: if any four or more of the 11 criteria are present simultaneously or progressively during the course of disease, the diagnosis of SLE is made with 98% specificity and 97% sensitivity.
Data from [166, 412].

Table 18.26. Laboratory diagnosis (including immune ARA criteria)

ESR	Elevated
Hemoglobin	Low
	a. Chronic inflammatory anemia
	b. Autoimmune hemolytic anemia in some patients
Leukopenia	In particular lymphocytopenia
Thrombocytopenia	In some patients
CRP	Often normal unless in case of infection
C3, C4 and CH50	Commonly decreased
IgG	Often elevated
IgM	Can be elevated
IgA	Sometimes absent
Rheumatoid factor (IgM)	Can be positive
Anticardiolipin antibody, lupus anticoagulant	Sometimes present
ANA	≥1:80 in 97% of children
	≥1:640 in 70% of children
Anti-Sm antibodies	in 45% of children
Anti-Ro (SSA)	In 33% of children
Anti-La (SSB)	In 18% of children
Anti-dsDNA	In 95% of children
Double helix anti-DNA antibodies (native DNA)	Often present
Evaluation of renal involvement	
Azotemia	
Creatininemia	
Creatinine clearance	
Quantitative proteinuria (24 h)	
Cellular casts	
Urine culture	
Renal biopsy (where suitable)	

Data from [178].

clinician to a more thorough investigation of renal status and referral to a specialist [222]. CPK (creatinine phosphokinase) levels should be monitored: a constant increase is an unfavorable sign. In addition to urinalysis, blood urea, nitrogen, creatinine, CBC (complete blood count), and complement levels (CH 50, C3, and C4), specific assays for the presence of SLE are anti-Sm, anti-SSA, and anti-SSB. Only 40%–50% of patients with SLE have anti-dsDNA antibodies, although 99% are ANA-positive (see the pediatric data in the Tables 18.23, 18.25, 18.26).

Treatment

Treatment is multifactorial, thus it cannot follow strict protocols, but it should be adapted to each patient, paying special care to the disease and its course. The drugs of choice are CSs, their doses should be gradually tapered once partial remission is reached, with normalization of both blood complement and levels of anti-DNA antibodies [13]. Low CS doses (<10 mg/day) did not affect FasL expression of lymphocytes, whereas in patients taking high CS doses the circulating lymphocytes were dramatically reduced [287]. If necessary, CS can be alternated with NSAIDs in milder forms without renal involvement [378]. Hydroxyquinoline as well as other DMARDs are successfully used in forms with prevalent cutaneous and arthritic manifestations, but it could be effective for preventing relapses in patients with latent disease, or scarcely reactive to treatment [468]. The initial dosage is 6–7 mg/kg/day for almost 3 months, to be reduced to 5 mg/kg/day. If therapy continues >6 months, periodic ophthalmological tests [13] are required. Cytostatic drugs are used when high CS dosage fails to control the disease. They are potentially useful, even when IV administered, if renal involvement is particularly severe [468]. Always monitor the drugs administered in order to prevent any possible adverse effect. Adequate measures for seriously ill children are controlled nutrition, hydrosaline balance, early diagnosis and treatment of infections, and congestive heart failure and arterial hypertension therapy [13]. We suggest avoiding excessive exposure to UV rays during central hours of the day, adopting convenient measures (sun glasses, barrier creams, hats, etc.). Children should have a normal life and take measures for physical fitness. A leg ulcer was treated with a skin graft (S. Cioschi, personal comunication). In severe, life-threatening cases of renal insufficiency, dialysis may be necessary. SLE neonates may need blood transfusion, and IV immunoglobulin (IVIg) (dose of 1 g/kg for 1–2 days), especially when CS is contraindicated [60].

New Treatments

Etanercept is absolutely not recommended in children with SLE. *ASCT for SLE is recommended* [183], but not yet applied in children. In a first study, the conditioning regimen used was cyclophosphamide, antithymocyte globulin, and methylprednisolone. The median follow-up of 15 patients with severe SLE after ASCT was 36 months (range 12–66 months). All patients demonstrated a gradual, but marked, improvement. Of the 12

patients followed up for >1 year after HSCT, ten have discontinued immunosuppressive medications, only two patients have demonstrated clinical evidence of recurrence of active lupus. No deaths occurred following treatment; however, of 20 originally screened for ASCT, three died after being deemed unsuitable for ASCT and two died after stem cell harvesting [432]. Data collected from 53 patients with SLE treated by ASCT in 23 centers show that disease duration before ASCT was a median of 59 (range, 2–155) months, and median age was 29 (range, 9–52) years. At the time of ASCT, a median of 33 (62%) had nephritis. *Ex vivo* CD34 SC selection was performed in 42% of patients. Conditioning regimens employed cyclophosphamide in 84%, anti-thymocyte globulin in 76% and lymphoid irradiation in 22%. The mean duration of follow-up after ASCT was 26 (range, 0–78) months. In conclusion, this registry study demonstrates the efficacy of ASCT for remission induction of refractory SLE in 33/50 (66%) evaluable patients by 6 months, of whom 10/31 (32%) subsequently had a relapse after 6 (range, 3–40) months, which was associated with negative anti-dsDNA antibodies before ASCT. Mortality appeared high: there were 12 deaths after 1.5 (range, 0–48) months associated with a longer disease course before ASCT, of which seven (12%) were probably influenced by the procedure. The safety of this procedure is likely to be improved by patient selection and choice of conditioning regimen. The return of disease activity in 33% of patients might be reduced by long-term immunosuppressive therapy after ASCT [183]. LCPUFAs modulate Th1 and Th2 cell generation, their IL production, and suppress the production of TNF-α and of OX40 (CD134) that belongs to the TNFR superfamily and the expression of bcl-2, suggesting that these fatty acids introduced *in utero* have the ability to *prevent or suppress SLE* [81].

Prognosis

Prognosis depends not only on symptoms and response to treatment, but also on the compliance to treatment [18].

Juvenile Dermatomyositis

Definition

Juvenile dermatomyositis (JDM), polymyositis and inclusion body myositis are the major forms of the idiopathic inflammatory myopathies (IIM) [41], which affect skin and striated muscles. If the lesion is localized to the muscles, the disease is called polymyositis. JDM is by far the most common inflammatory myopathy in children. The central lesion is a vasculitis affecting capillaries, venules and small cutaneous and vascular arteries [314].

Epidemiology

Incidence is 1×10^6 year, onset is after 2 years of age, with a peak at 10 years, and females are more affected than males: the initial ratio 1.7:1 increases to 6:0.7 in the second decade [468]. For children aged 2–17, recent US data report that the 4-year average annual rate (1995–1998) ranged from 2.5 to 4.1 JDM cases $\times 10^6$ children, and the 4-year average annual rate was 3.2×10^6 children. Estimated annual incidence rates by race were 3.4 for white non-Hispanics, 3.3 for African American non-Hispanics, and 2.7 for Hispanics $\times 10^6$ [272]. The female to male ratio was 2.3:1 [272] or 1.5:1 [250]. Two nationwide trials estimated the JDM incidence of 0.8% [250], or as 1.9×10^5 children aged <16 years, with a median age at onset of 6.8 years and a median delay in diagnosis of 4 months. The female to male ratio was higher 5.0:1 [408]. A trial on 1,742 children found a 0.4 JDM incidence $\times 10^5$ children at risk [89] and another study a 0.15 incidence $\times 10^5$ [250].

Pathogenesis

HLA class I overexpression is an early event in JDM and may occur in the absence of lymphocytic infiltration and muscle damage [229]. The familial occurrence was shown in two sisters, one with classic JDM and the other with amyopathic JDM, who shared the *HLA-DQA1*0501* allele [336]. Among 16 families there were three pairs of MZ twins [354]. Pathogenesis is strictly linked to *HLA-DR3* [97] (Table 18.3) and *HLA-DQA1*0501* haplotypes as in DM, whose incidence is significantly increased compared to controls [349]. The *HLA-DQA1*0501* allele in the mother was associated with the presence of chimerism more often in JDM children than in their unaffected siblings or in healthy controls. These chimeric cells play a direct role in the JDM disease process and the mother's HLA genotype facilitates the transfer and/or persistence of maternal cells in the *fetal circulation* [350]. IgA selective deficit is relatively common. The involvement of a provoking factor such as a virus, especially *Coxsackie* B, is not well supported by experimental data. Recently, a patient with dermatomyositis, polyarteritis nodosa, and recurring infections sustained by *Streptococcus* presented molecular mimicry between skeletal myosin and streptococcal type 5 M protein [264]. Thus, a model of antigen (perhaps viral) induction of an apparent AID based on dynamic interaction between muscular, vascular, and immune systems in the genetically susceptible (*DQA1*0501*$^+$) child could be hypothesized [387]. It is also speculated that *DQA1*0501* is associated with JDM susceptibility to an infectious process, eliciting and activating NK cells early in the JDM course [317]. In addition, the increased circulating concentrations of thrombospondin-1 (TSP-1), a potent antiangiogenic factor associated with the *TNF-α-308A* allele, suggest that TSP-1 may play a

significant role in the augmented vascular occlusion observed in JDM children with this genetic marker [245, 330]. A chronic JDM course appears to be associated with the *TNF-α-308G* allele. Untreated children with JDM who had the TNF-α-308A allele had an increased number of TNF-α stained muscle fibers than children with the *TNF-α-308G* allele, thus muscle fiber production of TNF-α could provide a microenvironment in which TNF-α acts synergistically with other mediators to prolong muscle fiber damage [113]. Moreover, new-onset, untreated JDM has four times as many CD56 NK cells in the muscle as in matched peripheral blood, which is associated with a relative increase in $CD8^+$ T cells [316]. Untreated JDM children had a significantly high increase in the proportion of lymphocytes expressing CD19 (B cells) and decreases in the percentage of lymphocytes that were $CD3(^-)$ $CD16(^+)$ and/or $CD56(^+)$ (NK cells) and $CD3(^+)$ $CD8(^+)$. Supposedly, $CD54(^+)$ non-B cells, $CD8(^+)$ T cells, and NK cells are removed from circulation and may participat in the pathophysiology of the disease [298].

The *immune pathogenesis* is still unclear. Cell-mediated functions are abnormal and include circulatory lymphopenia, significant reduction of CD3/CD8, increased CD4/CD8 ratio and CD19 (B lymphocytes) increment [314]. CD4 and B lymphocytes prevail in tissue infiltrates, suggesting that Aabs play an important role in tissue damage. The major role played by monocyte chemoattractant protein-1 (MCP-1) in the pathophysiology is demonstrated by CCR2 isoform expression by different cell subsets in both normal and JDM muscle. CCR2A, a main MCP-1 receptor, was detected in vessel walls and by some mononuclear cells, especially in cells involved in partial invasion in JDM. CCR2B expression was observed in all satellite cells, in the muscular domain of neuromuscular junctions and in some regenerative fibers, but not in inflammatory exudates [24]. Macrophages, IL_6, IL_1, and TNF were found in milk of calcium developed in two children [285]. Serologic studies have disclosed circulating ANAs with different frequencies, up to 50%, but no other Aab [264]. IgG, IgM and C3 in CICs have been shown to deposit in muscles, veins and capillaries. Complement deposition in these microscopic vessels is apparently the earliest pathological alteration. Consequently, vascular obstruction provoking perifascicular destruction, which is the typical pathological lesion of the disease, can be observed [468].

Clinical Presentation

Onset may be acute, with aspecific symptoms, including hyperthermia, asthenia, rash, muscle weakness, etc. More often, however, onset is insidious, with general symptoms like hyperthermia, malaise, irritability, and alternating rash. Symptoms observed in 79 children are reported in Tables 18.27 and 18.28 [261, 315]. An ill-defined, erythematous to violaceous scaly, minimally pruritic eruption occurs in photo-distributed areas such as the face (heliotrope eyelids), upper trunk, and extensor extremities. It resembles the butterfly rash in SLE (Fig. 18.30), a telangiectasia on eyelid borders, and erythematous maculopapular eruptions spread to large joints and over knuckles (Gottron sign) (Fig. 18.31). The periocular involvement of the heliotrope rash may cause the appearance of half-closed eyes, particularly in young children (Fig. 18.32). Papules are present on the extensor surfaces of metacarpophalangeal and interphalangeal joints and become whitish, atrophic, and scaly. Lesions can also extend to neck and torso as a consequence of heliotrope rash. Periungual telangiectasias (dilated capillary loops at the fingernail base) can be visualized with a handheld ophthalmoscope set at +40 diopters [41]; they are not included in criteria, but are present in 48% of children [261]. Skin manifestations are frequent, are associated with the severity of the disease, and are included in JDM diagnostic criteria. However, cutaneous features may precede the systemic illness, reflecting disease activity. New associations of JDM include the findings of pruritus (38%) and a psoriasiform scalp dermatitis (25%) [328]. Muscle weakness is always symmetrical and mostly proximal: it becomes

Table 18.27. Most common symptoms in 79 new cases of juvenile dermatomyositis

Symptoms	%
Muscle weakness	100
Rash	100
Muscle pain	72
Fever	65
Difficulty swallowing	45
Abdominal pain	37
Arthritis	36
Calcifications	22

Data from [261].

Table 18.28. Main cutaneous manifestations in juvenile dermatomyositis

Symptoms	%
Gottron sign	87
Heliotropic rash	74
Eyelid capillary telangiectasia	52
Telangiectasia	48
Calcinosis	35
Photosensitivity	22
Vasculitis	17

Data from [315].

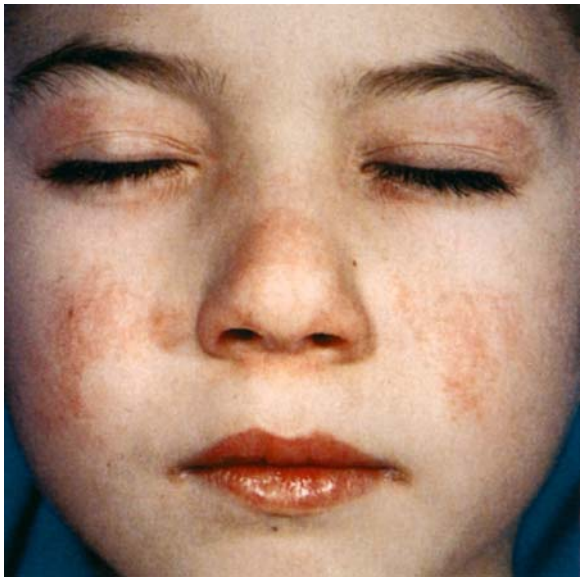

Fig. 18.30. Juvenile dermatomyositis: palpebral rash and butterfly rash

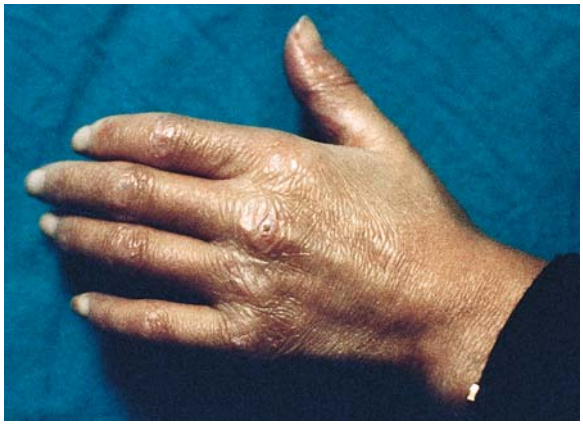

Fig. 18.31. Juvenile dermatomyositis: Gottron's sign

apparent when children climb the stairs, stand up from a chair, comb their hair, or use their hands trying to stand up from stretched position (Gowers sign) [314]. Arthralgia or full-blown arthritis is observed in 36% of pediatric cases: usually, they are transient and resolve after treatment of primary disease. In early stages, ulcers may be present on oral mucosa, with painful mastication, but they are less extended than those observed in SLE. The medical course of 39 children with JDM followed up for 3–22 years was complicated by respiratory diseases (20%), gastrointestinal diseases (24%), and calcinosis (30%) [276]. Calcinosis is found in 23%–70% of children [122], which despite treatment has remained around 30%. Calcinosis can localize on the cutis, subcutaneous layer, muscular fascia, but not in muscles, and mostly in regions subject to pressure (elbow, olecranon, knee). This is a late complication that becomes evident 3 years after disease onset and tends to spontaneously

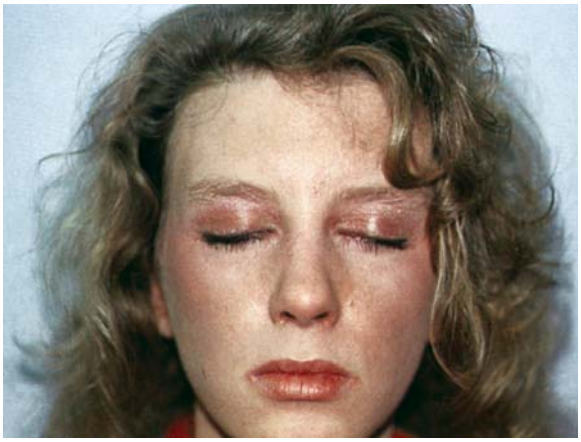

Fig. 18.32. Juvenile dermatomyositis: typical heliotrope rash on the face, with a rose-white aspect of eyelashes

regress [314, 361]. Onset of calcinosis is associated with significantly prolonged time to diagnosis and treatment, a longer duration of elevated muscle enzymes, and disease duration [122]. Pathological calcifications are associated with the *TNF*-α-*308A* allele and with increased TNF-α production, which may prolong the inflammatory response [316]. Cutaneous vasculitis, with eschar formation, is a very rare complication (17%). Interstitial lung disease may develop, which is detected by KL-6, a mucin-like high-MW glycoprotein [203]. All segments of the enteric tract may be involved via intestinal vessels, with consequent ulcers, perforations, bleeding, and necrosis [314]. Mesenteric vasculitis may provoke an intestinal perforation: a child from the evening to the night developed this type of perforation, which required an intestinal resection (S. Cioschi, personal communication). The JDM course is shown on Table 18.10.

Diagnosis

Diagnostic criteria, which closely follow the mixed skin manifestations and major laboratory data, are shown in Table 18.29 [468]. Calcinosis is detected by plain X-rays showing the pouring Ca phosphate. Differential diagnosis is mostly with polymyositis, 10–20 times less frequent and without rash [314] and the other IIMs.

Treatment

Treatment is focused on CSs and immunosuppressants (Tables 18.16, 18.17). The former especially have dramatically changed the prognosis: in the US, CSs have reduced mortality rates from 33% in the 1960s to 3% at

Table 18.29. Criteria suggested for the diagnosis of dermatomyositis

1. Proximal, symmetric muscle weakness
2. Typical dermatomyositis rash, also photosensitive (see text for details)
3. Increase of skeletal muscle enzymes, including creatinine phosphokinase (CPK), aldolase, lactate-dehydrogenase (LDH) and SGOT/SGPT
4. EMG results typical of myopathy; the changes of myogenic type are often nonspecific, with denervation potentials characterized by brief polyphasic motor units of decreased amplitude, insertional irritability, spontaneous fibrillations
5. Muscle biopsy may show necrosis of type I and II fibers, with small blood vessels resembling a necrotizing vasculitis

The diagnosis is posed when three or four criteria (plus the rash) are positive, probable when three criteria (plus the rash) are positive, possible when one criterion (plus the rash) is positive. Muscle biopsy is recommended in children with a doubtful diagnosis; a negative result does not skip the diagnosis, since the result may be false-negative in at least 10% of cases. Modified from [468].
EMG electromyography.

present [314]. Pulse CSs should be administered IV, to avoid scarce absorption. With minimal muscle damage, oral CSs (prednisone, 1–2 mg/kg/24 h) may suffice [122]. However, prompt institution of high-dose intermittent IV methylprednisolone therapy may rapidly normalize the muscle enzymes, and additional therapy is used if the indicators of inflammation remain elevated. Pulse IV CS therapy (methylprednisolone 30 mg/kg/daily for 3 days) usually is given initially; thereafter, the frequency of methylprednisolone administration ranges from three times per week to once each week until muscle enzymes or the indicators of inflammation normalize. Low-dose oral prednisone (0.5 mg/kg daily) is given on non-IV methylprednisolone days [105, 122, 314]. MXT can be added if laboratory values fail to normalize as rapidly as expected, but should be given in conjunction with folic acid (1 mg/24 h). Cyclophosphamide, given with bladder protection, is considered for children unresponsive to IV methylprednisolone and MXT. Reports note improved outcome with high-dose CSs, yet the incidence of calcinosis has remained between 14% and 30% [122]. Children who fail to respond within 6 weeks were started on a MXT regimen (65.7%) [122], or alendronate can be an effective treatment [111, 285]. Stepwise, aggressive CS [122], or MXT treatment directed at achieving rapid and complete control of muscle inflammation is highly successful in minimizing the long-range sequelae of JDM, including calcinosis [96]. CSs can cause osteopenia with consequent compression fractures. Thus, bone density should be monitored. If it decreases, vitamin D and a Ca-rich diet should be administered [314]. These patients should also be protected against sun rays by a sunscreen (free of *p*-aminobenzoic acid, PABA) that provides maximal protection against ultraviolet (UV) rays.

Juvenile Scleroderma

Definition

The element in common for many forms classified as JSD is collagen accumulation in the skin and subcutaneous layers, which makes the areas concerned hardened and rigid (scleroderma), with a wide range of lesions.

Epidemiology

The incidence among the general population is 1×10^5, with 3% of all cases in pediatric age; 1.5% of these cases have onset before 10 years of age, and 7% between 10 and 19 years [261, 378]. A trial on 1,742 children found a JSC incidence of 2% [89] and the Canadian trial found a 0.2% frequency of both JSD and morphea [250]. Otherwise the incidence of localized JSD is $0.2–0.4 \times 10^5$ [123]. Approximately 10% of patients with systemic JSD evolve to the disease before the age of 18 years [123]. The female-to-male ratio is between 1.5:1 and 4:1 [443], or 1.3:1 [127].

Classification

JSD is subdivided into a localized form, separated into three major subtypes, morphea, generalized morphea and linear scleroderma, and a systemic or limited form (Table 18.30) [226, 378].

Etiology and Pathogenesis

HLA studies did not reveal either a clear increase of any haplotype or a significantly higher familial incidence. The prevalent hypothesis is based on multifactorial pathogenesis. Similarly to other AIDs, an unidentified antigen, either environmental or self, could interact with the immune system of a genetically susceptible host and activate monocytes and lymphocytes, which in turn release ILs. These agents injure endothelial cells, causing abnormalities in extracellular matrix (EM) and vessels, due in particular to fibroblast proliferation and increased collagen deposit (Fig. 18.33). As shown in Fig. 18.34 [443], the endothelium reacts to these changes by expressing adhesion molecules which entrap platelets and inflammatory cells, which in turn bind integrins to their receptors. Polyclonal hypergammaglobulinemia is common, and ANA and antiendothelial

Table 18.30. Pediatric scleroderma

Characteristics	Localized scleroderma	Systemic scleroderma
Sex	F>M	F>M
Age at onset	≥2 years	≥4 years
Manifestations	Cutaneous fibrosis; patchy (morphea) or linearis, no systemic involvement	Raynaud's phenomenon, diffuse cutaneous fibrosis: face, trunk, extremities, or absent; arthritis; fibrosis of internal organs: heart, lung, kidney, gastrointestinal tract; hypertension
Laboratory findings	Possibly ANA increase, FR increased Ig concentrations	Possibly ANA increase, FR increased Ig concentrations, capillary nailbed changes, cutaneous and systemic fibrosis
Diagnosis	Clinical, biopsy	Clinical
Natural history	Possible progression or remission, occasional crippling	Possible progression or remission, poor, may be fatal
Therapy	No specific medications	No specific medications

Raynaud's phenomenon is practically absent in localized scleroderma, whereas in the systemic scleroderma it arises within 1 year of the onset of cutaneous manifestations and persists for years or decades.
Data from [226, 378].

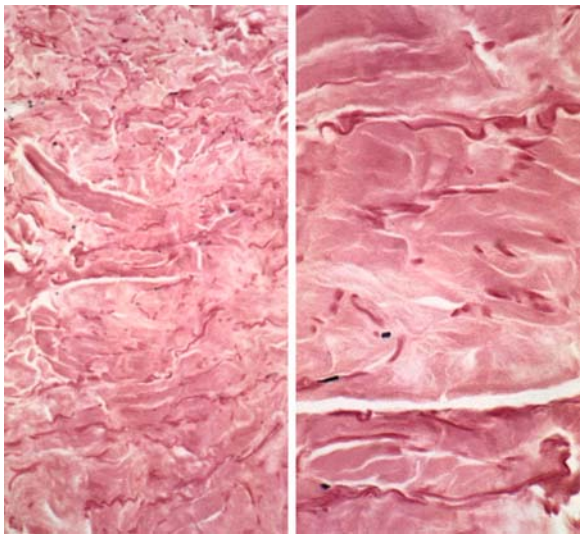

Fig. 18.33. Progressive systemic scleroderma: swollen and eosinophilic dermal collagen fibers

antibodies occur. A high proportion of children have ANA (see "Diagnosis"). The ANA specific for topoisomerase 1 (SCL70) and the centromeres are found in 50% of children with JSD at the onset and during the course of childhood [127], which suggests that autoimmune processes play a role in pathogenesis. ACA (anti-centromere antibodies) positivity was related to the presence of Raynaud's phenomenon in the studied sample, as 86% of children suffering from the phenomenon were ACA-positive, in particular 60% of children with JSD [167]. The presence of vascular lesions is proved by the finding of high levels of von Willebrand factor, which is a marker of endothelial damage, and platelet aggregates. Platelets and inflammatory cells adhere to endothelium and infiltrate tissues. Here they find IL_1-secreting macrophages, which induce platelets to produce PDGF (platelet-derived growth factor). These two ILs stimulate the proliferation of fibroblasts, which react to signals coming from a wide variety of cells and the EM ILs. The presence of IL_2 in the sera may be indicative of disease activity. The prominently frequent incidence of IL_6 in the early stage of disease and in patients with severe systemic involvement indicate a potential role of IL_6 as a parameter of disease activity [230]. The fibroblasts also express integrins, which reach the inflammatory infiltrate already deposited near the small vessels since the first stages [443]. The hyperproduction of connective proteins, especially collagen, generate interstitial and vascular fibrosis associated with widespread obliteration of small vessels (Fig. 18.35) – typical of JSD – along with a relatively modest inflammatory reaction, which leads to the irreversible involvement of many organs [443]. Consequently, one can observe the PDGF in vessels and fibroblasts in skin biopsies of patients with JSD, both localized and systemic, as well as increased CD45RA and reduced CD8, in connection with the stages previously described. This data confirms the activation of T cells, which in turn activate some B lymphocytes. The final result of these signals is Aab production.

It is still unclear if the pathogenesis is infectious. One of the many sclerodermic syndromes reported is the syndrome caused by toxic oil, which is described on Chap. 16.

Clinical Presentation

The mean age of onset of symptoms was 8.0±2.8 years and the age at diagnosis 10.1±3.0 years [127], but onset may be much earlier (Table 18.30). Morphea's early stages are characterized by erythematous and edema-

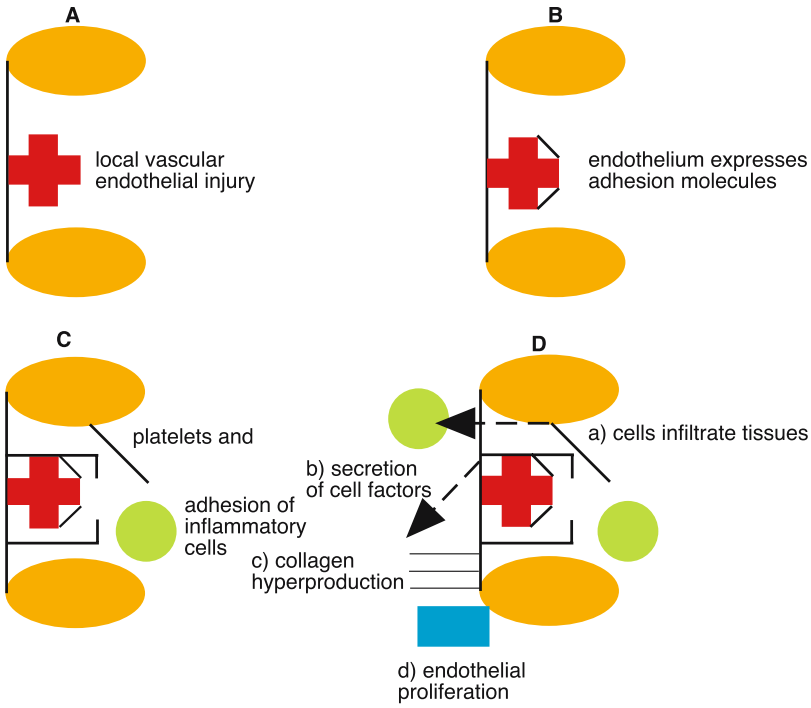

Fig. 18.34. Possible pathophysiological mechanisms in scleroderma (see text). (Modified from [443])

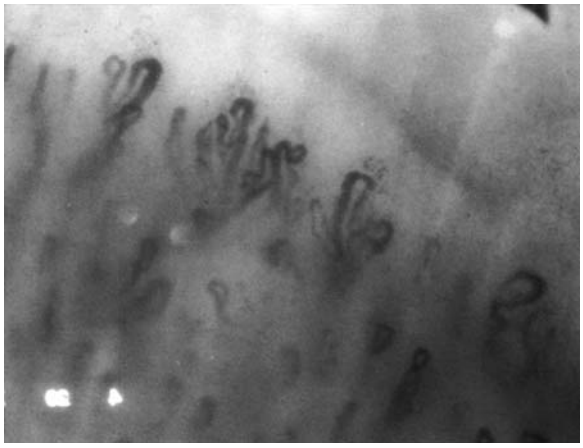

Fig. 18.35. Systemic scleroderma: capillary microscopy delivers dramatic evidence of vasculopathy

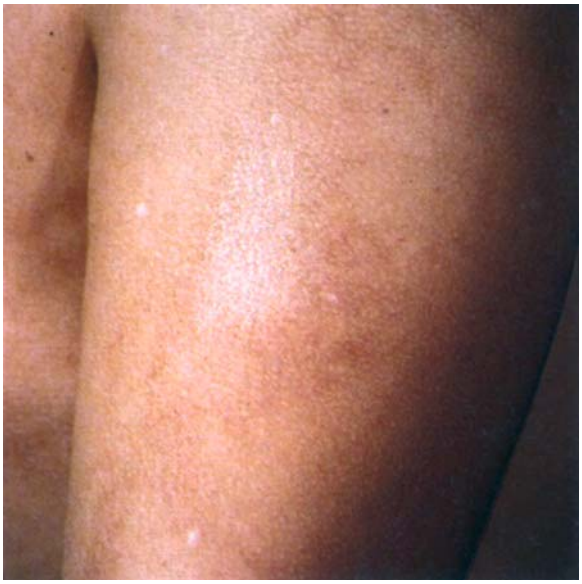

Fig. 18.36. Localized scleroderma: typical aspect of the lesions

tous lesions becoming purplish, with skin thickening and single or multiple patches, and puffiness of the fingers. Later they evolve into a thickened, smooth, waxy, tight lesion, which adheres to underlying strata and cannot be raised in folds (Fig. 18.36). Then the skin tends to soften and becomes atrophic with hypo- or hyperpigmented areas. Linear scleroderma thickening, instead, appears as a linear band, called *coup de sabre* when front and scalp are involved (Fig. 18.37). The changes in a boy with JSD from 10 to 16 years included an expressionless appearance of the face, small mouth with puckering of the lips, pinched nose, and hyperpigmentation of the neck [443]. In some cases, localized scleroderma can gradually involve larger and/or deeper areas, causing relevant atrophy of subcutaneous tissue, striated muscle, periosteum, and bone, along with vascular abnormalities in the head. Out of 30 children, 26 had linear scleroderma, 19 on an extremity and seven on the face, three had morphea, and one had generalized morphea [442]. Thus the two forms largely overlap, and often morphea and linear lesions coexist and appear as a growth deficit, with or without contractures [261]. Among 168 patients were noted 193 extracutaneous manifestations, as follows: articular (47.2%), neurologic (17.1%), vascular (9.3%), ocular (8.3%), gastrointestinal (6.2%), respiratory (2.6%), cardiac (1%), and renal (1%). Other autoimmune conditions were present in 7.3% of patients [509].

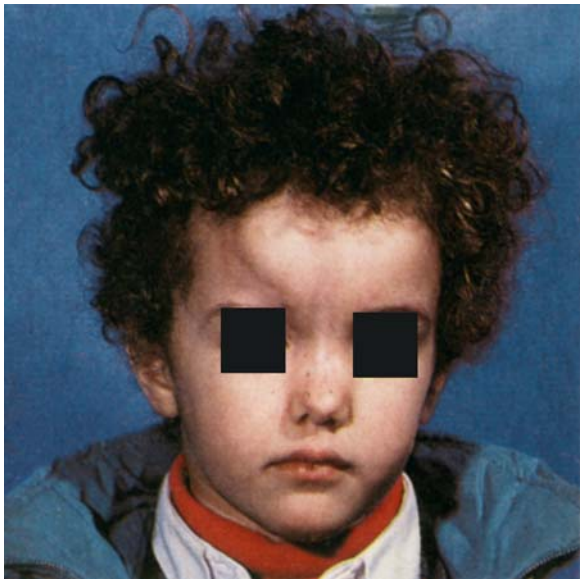

Fig. 18.37. Localized scleroderma: linear scleroderma, sabre-cut type

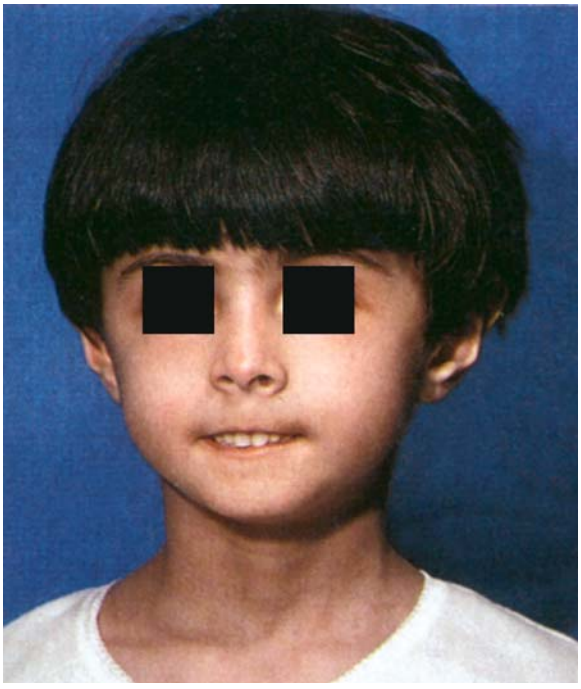

Fig. 18.39. Systemic scleroderma: sclerotic changes of the face and around the mouth associated with severe involvement elsewhere

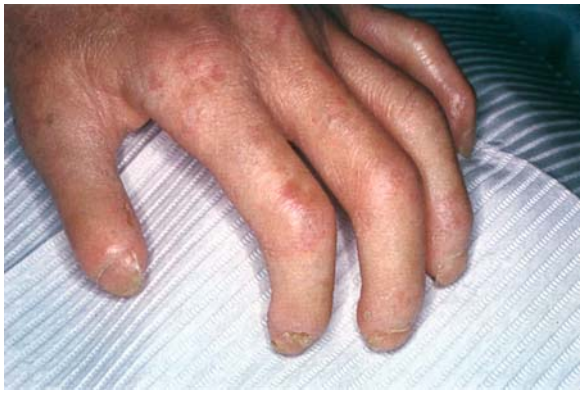

Fig. 18.38. Calcinosis cutis and sclerodactyly associated with systemic scleroderma

Systemic or progressive JSD may be restricted to the skin, which presents a tense, symmetrical, subcutaneous edema, localized to the fingers and the back of hands and feet, mostly evident at awakening. These stages, often asymptomatic, may persist for some time, and could be followed by a sclerotic stage, with hard, tense, translucent, inelastic and atrophic skin (sclerodactyly), and restricted joint movement [443] (Fig. 18.38). The involved face takes on a typical amimic expression, with loss of normal skin wrinkles, and a reduced opening of the mouth (Fig. 18.39). Atrophy of the frenulum linguae and lung fibrosis are commonly seen [127]. A child with a limited type of systemic sclerosis presented with erythema, tight skin over his face and digits, subcutaneous calcification, telangiectasias, and Raynaud's phenomenon [234]. Raynaud's phenomenon is present in 90% of children and is the presenting symptom in 70% [443] or 89% of cases [127]. One possible variant is known as CREST [calcinosis-Raynaud-esophageal (motility disorders)-sclerodactyly-telangiectasia].

Fibrosis, with evident signs of inflammation, is much more exceptional in pediatric patients with diffuse skin involvement. Fibrosis develops on proximal portions of limbs and trunk, along with vascular alterations and symptoms referable to different organs (Table 18.30). Telangiectasias may be localized on the skin, especially in periungual areas, while the subcutaneous calcifications that develop on fingers, elbows and knees could limit joint mobility. Renal involvement is present in up to 60% of children, and nephropathy develops on average 2–3 years after diagnosis [443]. With time, ischemia may provoke ulcerative and/or necrotic lesions on digital pulp skin and bone reabsorption from ungual phalanges [261]. The gastroenteric tract, which is the third major site of alterations, can be involved in all its segments, from mouth to anus [443].

Diagnosis

The diagnostic work-up of these children should include the several organ systems involved. The immune status investigation should encompass anti-topoisomerase antibodies, ANA, and ACA. Related specialists

should monitor cardiac, respiratory, gastrointestinal, and renal status [41]. Muscle enzymes should also assessed; a muscle biopsy may be necessary [443]. Otherwise it is essentially clinical (Table 18.30). ANA were present in 23%–80% [123, 127, 442] and RF in 25%–40% of children [123, 442] and RF in 39% of 30 children [442]. Approximately 50% of children have anti-Scl-70 (topoisomelase I) antibodies at the onset and during the course of childhood. The clinical symptoms and examination of serological autoimmune antibodies were supportive of an early diagnosis [127]. Differential diagnosis is with the scleroderma overlap syndromes including polymyositis, Sjögren syndrome, scleromyositis and synthetase syndrome [41].

Treatment

It is rarely possible to contain the development of the disease because the disease has various subtypes, each with a different course [443]. Approaches must take into account the therapeutic effect on the child (growth failure and osteoporosis from CSs) as well as the psychosocial impact of chronic illness and physical deformity on children and their family [101]. MXT, 0.3–0.6 mg/kg per week, was given to children with localized JSD. Pulse IV methylprednisolone, 30 mg/kg for 3 days monthly for 3 months, was given to children at the start of therapy, a treatment that appears to be effective and usually well tolerated. Discontinuation causes flare-ups of the lesions [443]. No specific treatment can be suggested. For example, out of 67 children, 27 were taking only NSAIDs, 11 were given NSAIDs and MTX, 15 were also receiving prednisone and 14 were given CSs and alendronate [111]. Dipyridamole, d-penicillamine, colchicine, and nifedipine may be taken into consideration [233]. Combined UVA/UVB phototherapy and PUVA bath are valuable and safe therapeutic options for selected children who do not respond to other treatments [324].

New Treatments

Experience with ASCT for childhood JDM or JSD is very limited [485]. Professor Wulffraat is preparing a transplant for JSC. They will try IV cyclophosphamide pulse therapy first. In London, Great Ormond Street, a girl with JDM has received a transplant, she did well initially but died of respiratory infection. Probably she received ASCT too late (L. Wedderburn, pers. comm., July 3, 2005). As yet the islet of Langerhans transplantation for type 1 DM has not been done in children (M. Markmann, pers. comm., April 19, 2005). A revolutionary and noninvasive cure for JDM could be based in the near future on the transplant of a novel population of fetal cells, the pregnancy-associated progenitor cell (PAPC), which remains in maternal blood and tissue for decades following delivery (D. Bianchi, pers. comm., Mrch 8, 2007). This recalls the sister-to-sister transplant.

Pediatricians and Autoimmune Diseases

AIDs have protean manifestations, and reports of physicians' clinical skills are tested by the assessment of whether or not a patient has this diagnosis [41, 385]. AID therapy is projected into the future. In addition to what has been said on DM, some gene therapies are aimed at treating AIDs characterized by demyelinization with autoreactive T cells that can stimulate tissue regeneration by expressing NGF (nerve growth factor) or producing immunoregulating factors. Examples of this are the primers for MBP or proteolipid protein (PLP), to direct the transgenic expression toward oligodendrocytes, and the primers for insulin, to affect the β-pancreatic cells [267]. Infliximab has been widely used for CD therapy and in JRA as well [99]. In the meantime, pediatric rheumatology centers and pediatric rheumatologists should care for JRA children, since at diagnosis 78% of the polyarticular patients required medication, 98% of the patients functioned in Steinbrocker classes I and II, and 6% of pauciarticular, 27% of polyarticular, and 11% of systemic patients had limitations in school function. Nearly 1/3 of poly patients already had joint space narrowing on X-rays. By 5 years after diagnosis, all pauci, 88% of poly, and 70% of systemic JRA patients were in Steinbrocker classes I and II; but 6% of pauci, 28% of poly, and 44% of systemic JRA patients had limitations in school function. Nearly two-thirds of poly and systemic patients had joint space narrowing [39].

Children with uveitis refractory to other therapies received *infliximab* at doses between 5 and 10 mg/kg at 2- to 4-week intervals, and then were maintained at 4- to 8-week intervals at doses of 5 to 18 mg/kg. After a follow-up of 48 weeks, all children showed reduction in their intraocular inflammation after infliximab therapy was initiated. Only two children reported transient adverse reactions; however, no one has had to discontinue treatment [345].

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Malnutrition and the Immune System

Immunological Effects of Child Malnutrition

Although it has long been known that malnutrition, immunity and infection are intricately linked to one another, the study of malnutrition and immunology has been progressing only relatively recently [97]. However, malnutrition and infections in children is a destructive interplay with global dimensions. Human studies and laboratory experiments have confirmed that the alterations of nutritional thymectomy, correlated with kwashiorkor, correspond to deep immune deficiencies [52]. The pioneering studies by Suskind [108] and Chandra [22, 23] have established the relationships between immunity and malnutrition, stemming from a profound knowledge of the endangered functions and the employment of more reliable technologies [52]. It is now common understanding that nutritional deficiencies, either quantitative or qualitative, also during pregnancy, may alter to different degrees humoral and/or cell-mediated immune (CMI) responses [28], thereby representing the most common cause of secondary immunodeficiency (ID) [9] after AIDS [23]. Primitive malnutrition is associated with atrophy of lymphoid organs, with a consequent increase in the susceptibility to pathogens, reactivation of viral infections and the advance of opportunistic infections [22–24, 52, 76]. In the first few days of life, neonates must be prepared to deal with bacterial colonization of the gut and allergenic macromolecules, so malnutrition may reduce mucus secretion and *debilitate the defensive function of the mucosal barrier* (Chap. 2). Until now, in addition to hospitalization (prevalence of 5.1%–6.6%) [82] and prolonged total parenteral nutrition (TPN), and peritoneal dialysis [12], inappropriate elimination diets both for the treatment or prevention of allergic disease may also be considered as causes of malnutrition in children [122]. The connection between the nutritional state and the Münchausen syndrome by proxy has promoted large-scale investigations on iatrogenic causes [52]. A pertinent issue is the recent access of unconventional remedies infantile affections, not prescribed by medical doctors, as far as can be deduced from the report [105].

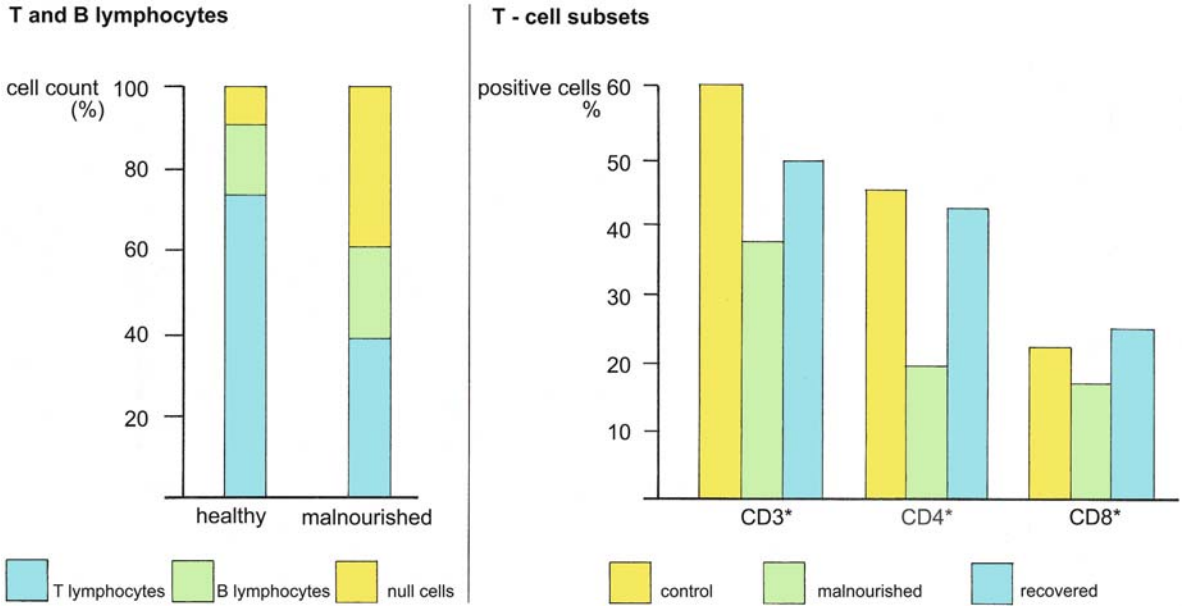

Fig. 21.1. Lymphocyte counts in malnutrition. *Left*: the proportion of circulating T cells is decreased in malnourished patients compared to healthy subjects, the B cell is commonly unaffected and null cells increase. *Right*: profound reduction of CD4 cells, and less of CD8 cells, the CD4:CD8 ratio returns to normal after 4–8 weeks of appropriate nutritional therapy

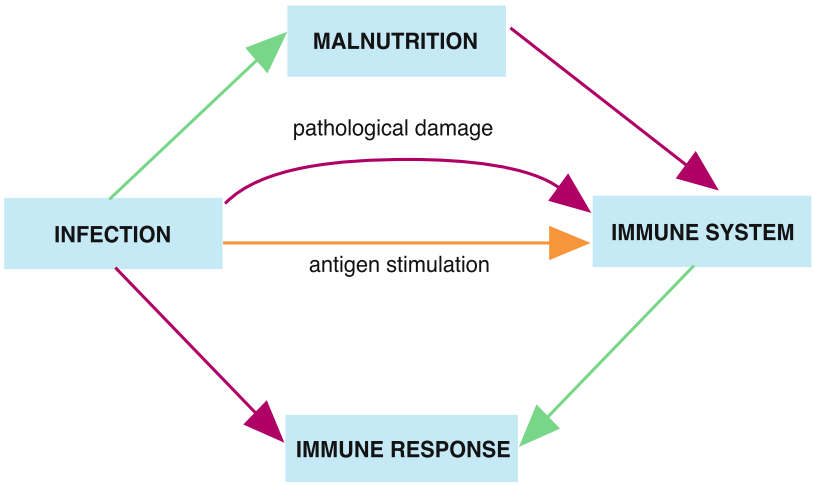

Fig. 21.2. Interactions between nutrition, immunity and infections. (Modified from [23])

Alterations of the functional activity of the immune system are usually caused by protein-energy malnutrition (PEM), but often the type of effect depends on vitamins, trace elements and other nutrient deficiency. Nutritional deterioration is frequently aggravated by a concomitant deficiency in necessary elements such as Zn (zinc) and other trace elements, vitamin B_{12}, folic acid, etc. [34, 42]. Recent research has demonstrated that nutrition is an essential prerequisite of the immune system functionality, which is reflected even in moderate forms of PEM and in apparently healthy individuals, as a consequence of nutritional deficiencies not always pointed out [84]. *Immunotoxic substances* in the diet may in turn impair host defenses, ultimately resulting in a pseudo-ID with a consequent uprising susceptibility to infections [18]. Several studies highlight that infection is the prime mover, and results in the host's nutritional impairment [35, 69], eliciting a closed cycle where infections have the ability to worsen the malnutrition-induced immune alterations (Fig. 21.1) [23], which consequently augments the risk of acquiring infections in undernourished children (Fig. 21.2) [23].

Protein-Energy Malnutrition

The WHO (Word Health Organization) defines PEM as a wide spectrum of pathological conditions ensuing from coincident repeated and prolonged lack of proteins and calories in varying proportions, occurring more often in infants and young children, commonly associated with infections, which find them unprepared, especially if they *were deprived of colostrum* [28]. The terms "primary" and "secondary PEM" refer to malnutrition resulting either from inadequate food intake or from increased nutrient needs, decreased nutrient absorption, and/or increased nutrient losses. Currently, a third disorder, *marasmic kwashiorkor,* which has a number of overlapping features, is recognized [58]. Although dietary factors are known to affect the structure and function of all cells of the body, lymphoid

Table 21.1. Effects of protein-calorie malnutrition on immune function

Cell-mediated immunity
Reductions:
Delayed cutaneous hypersensitivity responses (recall, new antigens)
Skin homograph injection
Thymic size
Thymic hormone activity
Thymic T cells and T cell-dependent zones in lymph nodes and spleen
Lymphocyte proliferation and DNA synthesis
Circulating CD3 subsets
Circulating CD4 subsets
Mixed lymphocyte reaction (MLR)
IFN-γ, IL_1, IL_2 and IL_2R generation
Moreover:
Increased relative proportion of TdT immature T cells
Potential increase of suppressor T cells and/or circulating suppressor factors
Anergy
Response to mitogens (PHA, Con-A)
Humoral immunity
Decreased:
Immunoglobulin production
sIgA
T cell-independent antibody production
Bactericidal power
Nonspecific immune function
Decreased:
Complement components
Phagocytosis, metabolic activation and destruction of bacteria
Macrophages and neutrophils, neutrophil chemotaxis
Lysozyme levels in secretions
Moreover:
Skin and mucosal dysfunctions
Alteration of intestinal flora
Rise in gastric pH

Modified from [24, 111, 125].

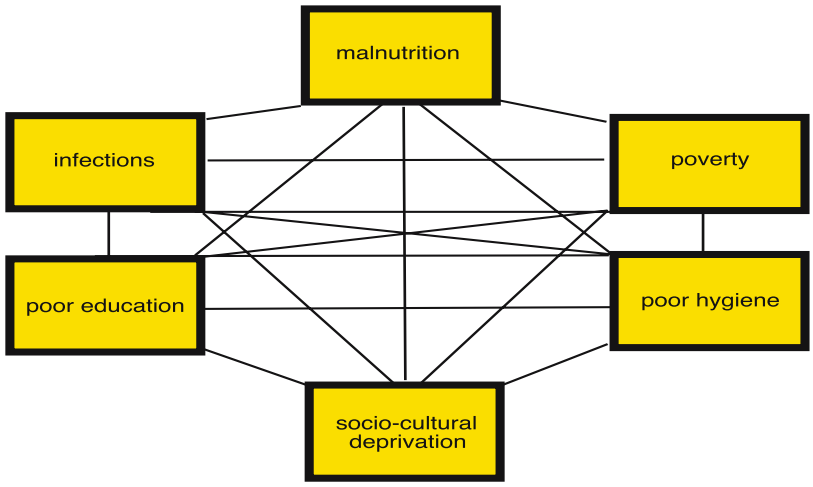

Fig. 21.3. Multifactorial interactions causing malnutrition. (Modified from [23])

Table 21.2. Specific effects of dietary deficiencies on cell-mediated immunity

Food deficiencies
Blunted CD4 levels and not CD8 levels
B cell increase in the circulation
Decreased concentrations of complement components
Calorie deficiency
Decreased Th1 numbers?
Increase in PGE_2
In mice Decreased IL_6 and TNF-α levels Increased TGF-β levels Increased T cell response to mitogens Decreased PGE_2 release by splenic cells
Protein deficiency
Decreased Th1-mediated oral tolerance
Increased Th2-mediated oral tolerance

Data from [97, 111, 125].

tissues are particularly susceptible to their rate of turnover and synthesis of immunomodulating cells. The pioneering studies go back to about 70 years ago, when it was observed that in persons who died of starvation, the thymus underwent an involution, with reduction in both size and number of lymphoid cells. Cell depletion was also found in thymus-dependent zones of the spleen and lymph nodes [42], changes observed in children hospitalized for severe PEM [78] (Fig. 21.3) [23].

Several distinct responses have been observed in the first term of fetal development. In addition to genetic factors, environmental influences such as nutrition play an important role in influencing the developing human immune system [23]. Studies have shown that infants and children with PEM have a *profound depression of several aspects of immunity the world over.* CMI shows the most evident alterations, as indicated in Tables 21.1 [97, 111, 125] and Tables 21.2 and 21.3 [24, 39], and best documented by Fig. 21.4 as compared to Fig. 1.1. Systemic CMI shows varying degrees of impairment: in more severe cases, a thymic hypotrophy, with gross structural alterations on histological examination up to an evident involution characterized by lobular atrophy,

Table 21.3. Humoral and cellular quantitative reduction

	IgA	IgM	IgG	TD lymphocytes	IgA-containing (%)	IEL
Children						
Malnourished	57±6	33±11	10±3			7.4%
Well-nourished	80±5	18±4	4±3			9.5%–40%
Animals						
Malnourished				18.4	0.46	4.3±0.7
Well-nourished				44.5	0.84	18.8±1.8

IgA, IgM, and IgG, mean ± SD; TD lymphocytes and IgA-containing, geometric mean.
Data from [24, 39].
TD, thoracic duct, *IEL* intraepithelial lymphocytes.

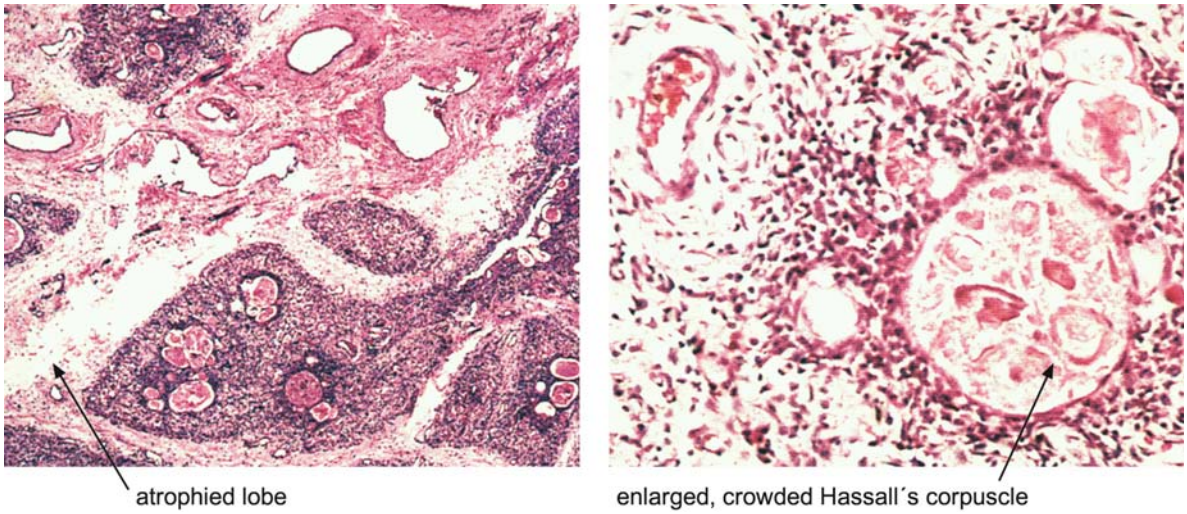

Fig. 21.4. Thymic histology in protein-calorie malnourished (PCM) children. Compared with normal thymus, in PCM there is a tangible involution, characterized by lobular atrophy, loss of distinction between cortex and medulla and lymphocyte depletion, particularly in the cortex. Lower Hassall's corpuscles appear degenerated, hypertrophied, crowded with cells and often calcified

loss of corticomedullary distinction, reduction in the numbers and function of lymphocytes in the cortical zones, depletion of varying degrees of lymph node germinal centers (GCs) and paracortical cells in peripheral lymphoid tissues, whose alterations are proportional to the duration and severity of nutritional deficiency [22, 23, 52]. Significant variations in the number of B and T lymphocytes are reported, associated with distorted proportions of B- and T-cell subpopulations. Malnourished bacterium-infected (BI) children showed significantly lower B lymphocyte values of $CD20^+$ in relation to the results seen with healthy children [72]. The number of total circulating T cells was reduced in PEM children, as well as the CD4:CD8 T cell ratio [23]. Lymph nodes are hypotrophic, the responses to PHA (phytohemagglutinin) and ILs are decreased, NK-cell activity is reduced, as well as delayed-type hypersensitivity (DTH) and macrophage functions [111]. Peritoneal macrophages from PEM mice exhibit significantly *greater levels of apoptosis* at baseline and when stimulated with pro-apoptotic agents compared with controls, a propensity that may be attributable in part to decreased bcl-2 protein expression [87]. CD3, CD4, CD8, the CD4:CD8 ratio, CD45RO, IL_1, IL_2, IFN-γ, lymphocyte circulation and homing (Chap. 1) are altered in number and function [22–24, 84, 111], paralleling the alterations of T lymphocytes with immature phenotype. IFN-γ production is markedly decreased, IL_1 production and functional activity on target cells is depressed, IL_2 production and IL_2R binding are altered [24], the whole proportional to the duration and severity of the nutritional insult. In vitro production of IL_1 and IL_2 was significantly lower in patients when compared to normal controls. The total leukocyte count was significantly higher than in the noninfected controls, thus indicating a *functional rather than numerical* cellular impairment [63].

Similar changes are seen in regions populated by lymphocytes of the spleen, tonsils and lymph nodes, including paracortical and periarteriolar regions, but with preservation of B lymphocyte-rich GC and primary follicles [52]. Underfeeding reduces IL_{10} secretion by anti-CD3-treated cells. Lymphocyte proliferative responses to anti-CD3 ± anti-CD28 antibodies were lower in iron-deficient mice than in controls, IL_{10} levels negatively correlated with lymphocyte proliferation. In addition, iron deficiency has a generalized deleterious effect on cells that secrete IL_{10} and IFNγ [57]. On humoral immunity, T cells do not provide adequate help to B-cell production of immunoglobulins (Ig), so Ig concentrations are reduced compared to a significant increase in IgM levels in well-nourished controls, and there are fewer intraepithelial lymphocytes (IELs) [24]. The mucosal immune deficiency may be more severe, characterized by MALT (mucosa-associated lymphoid tissue) hypotrophy, Peyer's plaques and sIgA level reduction, and secretory component synthesis by epithelial cells, but levels were restored with return to a normal diet [107]; *mucosal IgA levels are remarkably reduced* (Table 21.3), (Fig. 21.5) [23]. Alterations in certain nutrients may have widespread and/or localized effects on the lymphocyte network (Fig. 21.6) [52]. *IEL counts under the lower limit* of normal in malnourished children [39] and animals [24] (Table 21.3) may be a sign of PEM-induced immunological deficiency. PEM also impairs *innate immunity* (Tables 21.1, 21.2), phagocyte functions and neutrophil activity, and the oxidative and glycolytic activity of these cells is quantitatively reduced,

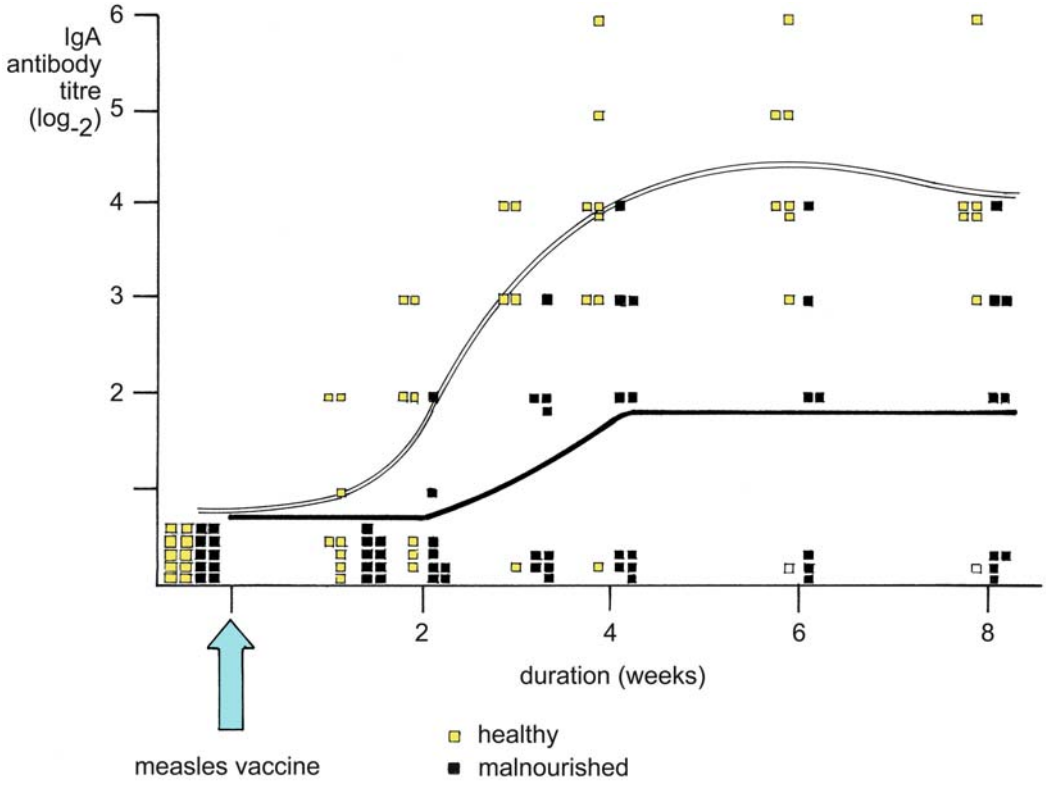

Fig. 21.5. Secretory IgA (sIgA) response in PCM. Two groups of children, one of children who were malnourished with PCM and one group of age-matched healthy children, were given a single dose of live attenuated measles virus vaccine; the sIgA level in nasopharyngeal secretions was measured for 8 weeks. Antibody levels were lower in malnourished children and its appearance was delayed

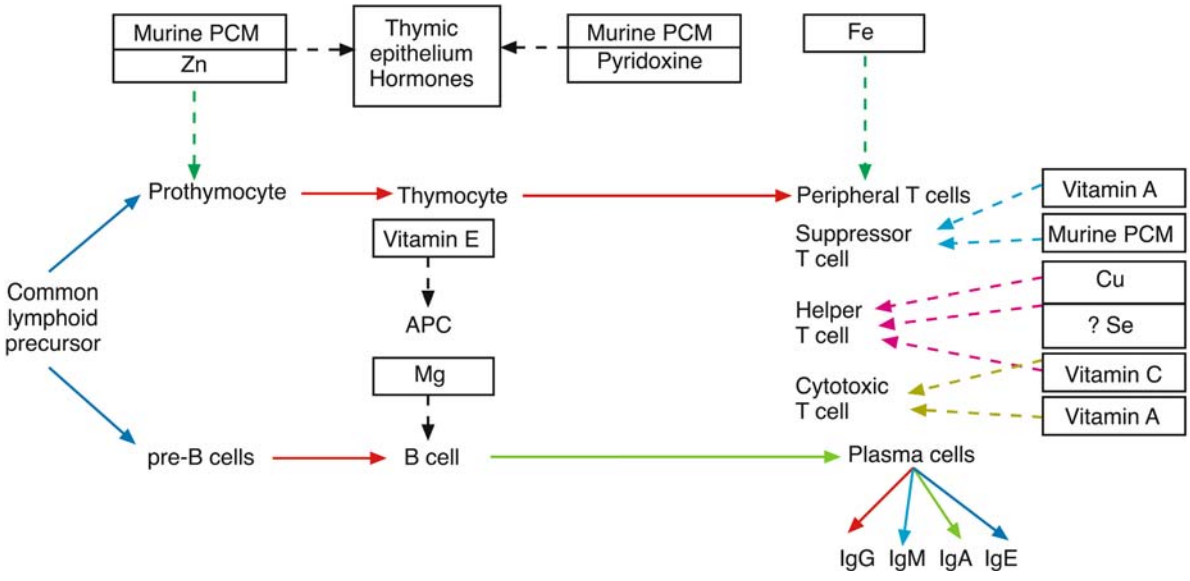

Fig. 21.6. Localization of specific effects of nutrients on the immune system. *Dashed lines* show sites or specific cells affected by deprivation or, in certain cases, by excess of nutrients. *APC* antigen-presenting cells, *PCM* protein-calorie malnutrition. (Modified from [52])

but processing by macrophages is normal [111]. The levels of complement components, chiefly of C3, are very reduced in PEM acute phases [99], but may return to normal following an adequate alimentation (Table 21.4) [52]. Infections occur as a consequence and are facilitated by the network of interacting factors also causing

Table 21.4. Effects of protein-calorie malnutrition on complement system

Decrease in total hemolytic complement activity of both classic and alternative pathways
No apparent alterations of regulatory proteins
Possibly reduced synthesis of complement proteins and weakened acute-phase proteins
In vivo complement consumption, with complement breakdown products in the bloodstream
Serum concentration decrease of individual complement components
Depression of complement-mediated functions such as opsonization

Modified from [52].

Table 21.5. Identifying infants and children at risk for protein-calorie malnutrition

Reduced nutrient intake
Intolerance
Vomiting
Gastroenteric disorders
Anorexia
Iatrogenic
Too restrictive and prolonged elimination diets Chronic parenteral nutrition Inadequate re-alimentation Therapy-related anorexia Pre-, postoperative fasting Staff unpreparedness on nutritional assessment and needs
Abnormal nutrient losses
Regurgitation
Malabsorption
Pancreatic insufficiency
Luminal disorders (altered absorption, etc.)
Damaged skin barrier
Burns and trauma
Increased energy expenditure
Hypermetabolic states (burns, trauma, fever, sepsis, etc.)

Modified from [11, 38, 112].

Table 21.6. Nutritional risks associated with elimination diets

1. Children with recent anaphylactic episodes
2. Infants and children with significant adverse response to a food or foods
3. Infants and children recently diagnosed with a food allergy risk
4. Children avoiding multiple foods due to an unconvincing diagnosis
5. Older children or adolescents on self-imposed food-avoidance diet
6. Anyone avoiding food(s) due to an unconvincing diagnosis
Moreover
a. Unbalanced diets for religious or vegetarian causes
b. Münchausen syndrome by proxy
c. Unconventional therapies

Modified from [122].

Table 21.7. Disease states often associated with protein-calorie malnutrition in children

Cystic fibrosis
Short bowel syndrome
Low-birth-weight infants
Mucosal disease
Intestinal disease Celiac disease Cow's milk allergy Cow's milk enterocolitis Immunodeficiencies Infectious enteritis Intractable diarrhea Soy enterocolitis Tropical sprue
Anorexia nervosa
Burns and trauma
Chronic liver disease
Chronic renal disease
Congenital or chronic heart disease
Crohn's disease
Intestinal neoplasms
Ulcerative colitis

Modified from [38].

malnutrition. In BI children who do not have PEM, a significant decrease in the proportion of T $CD3^+$, $CD4^+$, and $CD8^+$ lymphocyte subsets was seen compared to the results seen with healthy children [72]. Intestinal infections, possibly occurring because of a reduction in IgA levels as seen in cow's milk allergy (CMA), are frequently associated with malnutrition, which causes mucosal dysfunction, *impairment of digestive function and absorption,* thinning of the gut wall and flattening of villi [109]. Similarly, dysfunctions of both the B and T lymphocyte network have been shown to contribute to *severe and protracted diarrheal disease* in infants with

malnutrition, also enhancing intestinal permeability, as noted in PEM [109]. *Macronutrient titers are also reduced,* for example arginine deficiency, a nonessential amino acid, capable of inducing thymic involution with numeric reduction of thymocytes, a decreased response of lymphocytes to mitogens and poor IL_2 production. Glutamine deficiency, necessary to RNA and DNA synthesis and to cells in the replicating stage [60], involves instead both forms of immunity [3], thus paralleling the characteristics of arginine deficiency [23]. However, glutamine expresses defensive properties: it plays an important role in the gastroenteric tract by improving gut trophism and enhancing local immunity, which reduces the risks of bacterial translocation [3]. In addition, tissues are damaged but inflammatory reactions may be in need of repair, thereby consuming available amino acid substrates. Further studies indicate that PEM-negative effects are also substantiated by micronutrient deficiency such as vitamins, trace elements [24, 80, 89] and nucleotides.

Numerous are the causes of secondary PEM, often enlivened in infancy by alimentary causes (Tables 21.5, 21.6) [11, 38, 112, 122]**;** therefore the issue may have a dramatic corollary [73, 122]. Secondary PEM is often associated with the principal growth disturbances in children. Malnutrition at age 3 years is associated with poor cognition at age 11, independent of psychosocial calamity [62] (Table 21.7) [38].

Micronutrient Deficiency

Dietary micronutrients encompass vitamins and trace minerals that are known modulators of host immune responses against common pathogens. Micronutrients are required for the integrity and optimal functioning of the immune system. The full genetic potential of the child for physical growth and mental development may be compromised as a result of subclinical deficiencies of micronutrients. These children are more vulnerable to developing frequent and more severe common infections, thus triggering *a vicious cycle of undernutrition and recurrent infections* [100].

Vitamin Deficiency

Vitamins have many relevant functions: vitamins A and C increase immune responses, vitamin E increases both humoral and cell-mediated types of immunity if present in abundant quantities in the diet, and B_6 increases the numbers of T lymphocytes [4, 7, 8, 110].

Isolated vitamin deficiencies are rare nowadays; however, studies on experimental animals show that deficiencies of vitamins A and C, pyridoxine and folic acid have deleterious effects on CMI and T-cell-dependent humoral responses [42]. In particular, vitamin deficiencies (Table 21.8) [76, 111, 118, 125] are associated with

Table 21.8. Effects of vitamin deficiencies on immune function

Vitamin A deficiency
Atrophy of thymus and spleen
High infection susceptibility
Higher bacterial adhesion to respiratory epithelium
Decreased: Delayed hypersensitivity (Candida, tuberculin) Number and function of circulating T cells, CD4 and CD45 Number of NK and Th1 cells T- and B-cell responses to mitogens and antigens Antigen-specific antibody response sIgA, IgG_1 and IgG_3 titers
Increased: Th2 cells and IFN-ɣ
Vitamin B_6 deficiency
Prolonged skin homograft survival
Decreased: Thymic epithelial cells Delayed hypersensitivity (Candida, tuberculin) T- and B-cell numbers Antibody-forming cells Neutrophil phagocytic activity
NB: Fetuses of B_6-deficient mothers have hypoplastic thymus and spleen and their neonates have decreased humoral and cell-mediated immunity
Vitamin B_{12} deficiency
Decreased: Delayed hypersensitivity (Candida, tuberculin) T- and B-cell responses to mitogens and antigens Neutrophil phagocytic and bactericidal activity
NB: Pernicious anemia may be associated with autoimmune phenomena
Vitamin C deficiency
Transient decrease of: Antibody and cell-mediated immunity Phagocyte activity Macrophage and neutrophil activity Resistance to infections
Vitamin E deficiency
Decreased: Lymphocyte proliferation DTH reaction T- and B-cell responses to mitogens Immunoglobulin synthesis PGE_2 production Phagocyte function
Increased: Th1 activity
Folic acid deficiency
T-cell responses to mitogens
Reduction of: Delayed hypersensitivity (Candida, tuberculin) Antibody synthesis

Data from [76, 111, 118, 125].

Table 21.9. Pediatric prevalence of dietary vitamin deficiency syndromes

Vitamins	Children at highest risk	Symptoms
Fat-soluble vitamins		
A	Preschool children	Keratomalacia
D	School children	Rickets
E	(Preterm infants)	(E-responsive anemia, lung disease)
Water-soluble vitamins		
B_1	Infants, preschool children	Beriberi
B_6	(CM-formula-fed children)	(B_6-responsive seizures)
Niacin	School children	Pellagra
Folic acid	Young infants	Growth retardation
B_{12}	(Young infants)	(Failure to thrive)
	All ages	Pernicious anemia
Biotin	(Infants)	(Seborrheic dermatitis)
Carnitine	(Infants)	(Carnitine-responsive inborn errors)
C	Infants	Infantile scurvy

The terms in parentheses show associations relatively uncommon, or controversial, or insufficiently investigated.
Data from [6].

quantitative and especially qualitative deficiencies of B and T lymphocytes, and with impairment of antibody production, sIgA and resistance to infections in particular [24, 96]. In vitro studies have demonstrated that vitamin deficiency may be negatively involved in DNA synthesis, regulation of cell proliferation and maturation of immune cells [111]. In this context, a CD8 deficiency correlated with an increase in CD4 T cells with Th2 phenotype may be mirrored by IgE level growth in sensitized subjects, stimulating or aggravating the clinical expression of atopy [111].

Low *vitamin A* titers in mothers with AIDS increase virus transmission from 7% to 32% of cases [96], and in children this causes ID that significantly affects immune functions [71], reduction of $CD4^+$ CD45RA and to a lesser extent CD45RO, reversed along with the CD4:CD8 ratio when subjects were given a dose of 200,000 IU of vitamin A [97]. Such children are more susceptible to acquiring diarrheal disease and acute respiratory infections (decreased sIgA levels) [42]. Studies in vitamin A-deficient animals show enteric mucosal antibody reduction up to 90% in response to cholera toxin [119], aberrant T-cell function and impaired T-cell-dependent antibody response, which could be postulated as both priming of Th1 cells and inhibition of Th2-like ILs [121]. Infection of vitamin A-deficient animals with *E. coli* induced diarrhea with diffusion of the infection to the gut and various peripheral organs, and a greater IgE response compared to deficient animals that were not deficient, even though vitamin A deficiency is normally associated with suppressed antibody production [120]. Vitamin A is a growth factor for Th2 lymphocytes, vitamin D_3 selectively suppresses Th1 functions and related IL_2 and IFN-γ synthesis, but not Th2 and CD8 T cells, at variance with vitamin E, which stimulates Th1.

Vitamin C deficiency inhibits both humoral and CMI responses [125]. *Vitamin D* plays an important role in the impaired functions of T lymphocytes, which may be frequently associated with infection episodes, as in a group of infants with vitamin D-induced rickets: the CD4:CD8 ratios were significantly higher due to both an increase in $CD4^+$ cells and a decrease in $CD8^+$ cells [15]. Table 21.9 summarizes age-related dysfunctions: several variables may influence the peak age of each one for association with deficiency signs and symptoms [6]. An excess of vitamin C may interact with Fe (iron) metabolism, by increasing the production of oxygen-free radicals (FR). *Vitamin K* is most likely the prime example of a nutrient with a peak vulnerability to deficiency materializing at birth [6]. Studies on FR reveal the effective *antioxidant activity* elicited by natural vitamins A, C, E, and enzymes associated with trace elements such as superoxide-dismutase (SOD) (Cu = copper, Mn = manganese; Zn), glutathione-peroxidase (Fe, Se = selenium) [8, 36]: the not unknown pertinent deficiency [9, 33, 71] may foster the imbalance of the oxidant–antioxidant ratio, thus making the cells of the immune system potentially more vulnerable to xenobiotics that may be present in the diet, or environmental contaminants [18], concurrent with increased asthma and rhinitis incidence [95].

Table 21.10. Effects of trace elements and mineral deficiency on immune function

Iron deficiency
Decreased: Total Th1 lymphocytes but not Th2 lymphocytes DTH reaction and graft rejection T cell response to mitogens Activated T-cell cytokine production Cytotoxic activity of phagocytes Antibody production Neutrophil bactericidal power and killing NK cell cytotoxicity
Zinc deficiency
Atrophy of lymphoid organs
Homograft rejection
Decreased: Serum thymulin level Thymocyte count in thymus DTH reaction T lymphocyte development and response to mitogens Macrophage function (phagocytosis, intracellular killing) Monocyte and neutrophil chemotaxis NK cell activity Number and function of T cells, CD4 and CD45 Antibody production
Increased: Monocyte chemotaxis Number of CD8 cells Number of null cells Antibody-dependent cell-mediated cytotoxicity

Manganese deficiency
Depression of cell-mediated immunity
Reduced serum levels of immunoglobulins
Reduced antibody-forming cells
Selenium deficiency
Suppression of T cell response to mitogens and antigens
Decreased: Antibody production Antioxidant activity CD8 T cells, CD4:CD8 ratio (thymocytes) Cell-mediated cytotoxicity IgG and IgM titers Monocyte and macrophage phagocytosis Neutrophil chemotaxis T cell response to mitogens and antigens
Increased: CD4 T cells (thymocytes) Platelet aggregation and leukotriene synthesis Virulence of Coxsackie virus
Copper deficiency
Decreased: Thymus weight Cell-mediated and humoral immunity CD4 T lymphocytes, IL_2 and NK cells DTH reaction Integrity of cell membranes Number of antibody-producing cells Neutrophil counts
Bactericidal power and phagocytosis
Increased: B-lymphocyte numbers

Data from [60, 68, 100, 111, 118, 125].

Trace Element Deficiency

The role played by minerals both in the nutrition and the regulation of immune responses is widely known, as well as the role of their deficiency (Cr, Cu, Fe, Mn, Mo, Se, Zn), which is a source of negative effects on immunocompetence, readily individualized (Table 21.10) [60, 68, 100, 111, 118, 125]. The deficiency is caused by an inadequate supply, as established in the following occurrences [1, 31, 43, 65, 68, 90]:

- Chronic TPN: Fe, Zn, Cu, Se, Cr (chromium), Mo (molybdenum)
- Chronic TPN: Fe, Zn, Cu, Se, Cr (chromium), Mo (molybdenum)
- Congenital deficiency: Fe, Zn, Cu, Mo
- Excessive losses (malabsorption, diarrhea, etc): Zn, Cu, Cr
- PEM and multiple nutritional deficiencies in general, inappropriate diets, diets that are too restricted or restricted for too long [122]: Fe, Cu, Zn, Cr, Se
- Prematurity: Fe, Zn, Cu, Se, Cr
- Synthetic/semisynthetic formulas: Zn, Se
- Unnatural feeding with diets not supplemented with trace elements: Fe, Zn, Cu
- Vegetarian diets: Zn, Cu

Table 21.11 outlines the clinical effects of the trace element deficiency [1], frequent in children aged >6 months, especially of Fe and Zn [65]. Deficiency of only one trace element is sufficient to lead to quantitative and qualitative alterations of the immune system [99].

Fe is transported from extracellular fluids bound to transferrin. This complex, by binding to transferrin receptors, enters the immune cells, and is transported inside the cells via a process of endocytosis, and is critical for important functions in cell division, electron transport and oxidoreductive reactions [65]. Fe is a component of numerous metalloenzymes involved in protein synthesis, including NO synthetase, cyclooxygenase, lipoxygenase and catalase [125]. Fe deficiency

Table 21.11. Clinical manifestations of trace element deficiency

Clinical manifestations	Zn	Cu	Se	Cr
Anemia	±	+	+	–
Cardiac changes	–	+	+	–
Growth retardation	+	+	±	±
Hepatic necrosis	–	±	±	–
Impaired immunity	+	+	+	–
Integumental lesions	+	+	±	–
Intestinal pancreatic "atrophy"	+	+	+	–
Metabolic alterations				
Carbohydrate/energy	+	+	–	+
Nitrogen and proteins	+	–	–	+
Lipids	+	+	±	±

Modified from [1].

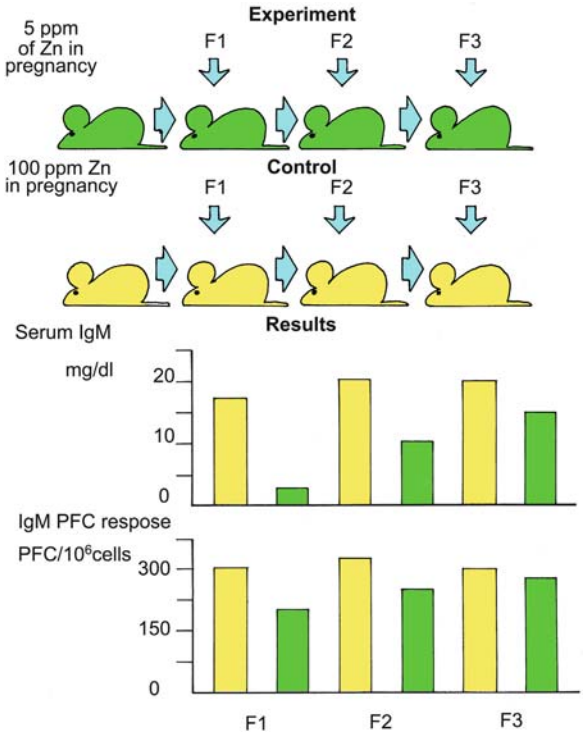

Fig. 21.7. Effects of Zn deficiency on immunity studied in two generations. Experimental animals were fed a Zn-deficient diet (5 ppm Zn) during the later two-thirds of pregnancy and a control was fed a Zn-adequate diet (100 ppm Zn) during the same period. Antenatal Zn-deficiency has severe and prolonged effects on immune functions. *PFC* plaque-forming cells, *SRBC* sheep red blood cells

reduces the bactericidal capacity, since it is necessary for lymphocyte, neutrophil and NK cell function, which is associated with an altered lymphocyte response to mitogens, reduced DTH reaction [84] and production of cytokines, IL_1 or IL_2 [29]. Fe deficiency provokes immune alterations well before the anemia is uncovered [31]. Indeed, patients with PEM-induced Fe deficiency suffer from negative immune responses in addition to having a high incidence of infections [1].

Cu deficiency associated with PEM emphasizes the vulnerability to several microorganisms [81]. The role played as a cofactor of both cytochrome-C-oxydase involved in the electron transport system and SOD, a FR scavenger, may explain in part copper's impact on immunity and the risk of FR aggressions in case of deficiency [45]. Cu deficiency impairs complement functions and Ig structure and has interactions with Fe, thus playing a key role in Fe metabolism and consequently in Hb biosynthesis [125].

Zn is an indispensable trace element, and the effects of its deficiency, both acquired and inherited [118], are impressive. Zn influences all cell subsets of the immune response [118], because it is essential for the stabilization of more than 300 metalloenzymes, and for protein catabolism and energy metabolism, as well as being an important cofactor of a wealth of enzymes, including RNA- and DNA-polymerase and thymidine kinase, which participate in cell replicative processes such as the synthesis of nucleic acids [111, 118]. Zn is determinant for a normal immune system evolution, critical to the biological activity of thymulin and therefore for T cell maturation [30]. The significant role in the activation of immunoregulatory genes is exemplified by production of IL_1, IL_2, IFN-ɣ and related receptors [65], thus elucidating why the cells of the immune system with an intensive replicative activity may negatively react to blunted Zn levels [24], which also lower thymic hormone activity [41] and jeopardize vitamin A transport [24]. As seen from Table 21.10, primitive Zn deficiency is implicated in the pathogenesis of profound ID, by impairing humoral and CMI, especially antenatally (Fig. 21.7), and lymphocyte response to mitogens, and of the inborn disorder *acrodermatitis enteropathica* with an autosomal recessive inheritance, characterized by signs of severe Zn deficiency when weaned, due to a defective intestinal Zn retention [14, 41] (Fig. 21.8). Clinically, Zn deficiency may also lead to a loss of smell [115]. In Egyptian children with atopic dermatitis (AD) and asthma, Zn concentrations were significantly lower than in control children [35], in agreement with possible Zn deficiencies during long-term elimination diets (Table 9.21). Similar alterations of the immune function are recorded in patients assuming excessive Zn doses continued for too long (Fig. 21.9). Human studies have shown that Zn bioavailability in breast milk is significantly higher than in CM and in soy protein formulas. In casein infant formulas, Zn bioavailability is lower than in whey infant formulas [65].

Secondary Zn deficiencies have been reported in [65, 111]:

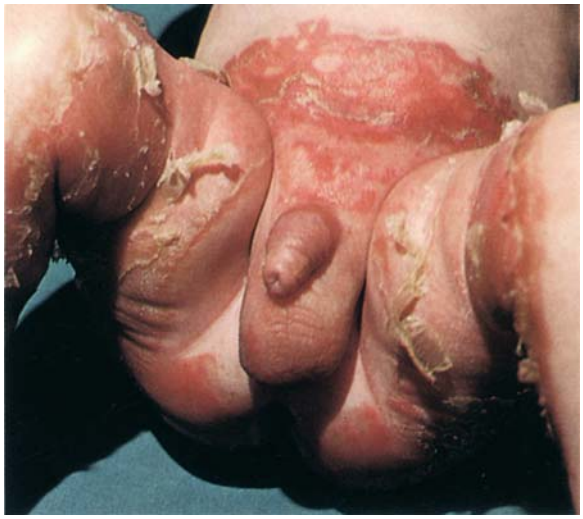

Fig. 21.8. Acrodermatitis enteropathica with typical combustiform lesions

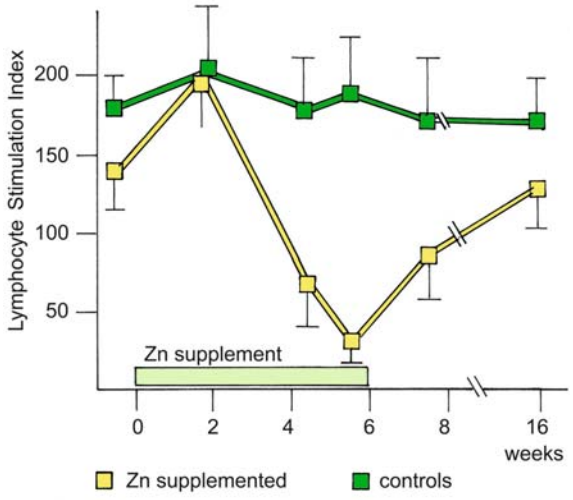

Fig. 21.9. Results of lymphocyte transformation test in subjects fed 300 mg elemental Zn daily for 6 weeks

- Children who are long-term TPN-fed and severely malnourished children living in the Andean regions, which commonly have volcanic soil with low Se content [98], may have dietary Zn deficiency, probably as a consequence of enteric disorders. Zn concentrations not covering basal requirements in the solutions are determinant for TPN.
- Surgical conditions, for example, after jejunoileal bypass.
- Disorders such as cystic fibrosis, essential fatty acid (EFA) deficiency and kwashiorkor.
- Metabolic disorders, such as methylmalonic acidemia, biotin-dependent multiple carboxylase deficiency, MSUD (maple syrup urine disease) especially in infants fed isoleucine-deficient diets and/or branched amino acids [40].
- Intestinal disorders such as IgA selective deficiency, ulcerative colitis, Crohn's disease, celiac disease, diarrheal syndromes [52], etc.
- Acute lower respiratory tract infections [52].

Mn deficiency does not occur in infants and toddlers, Fe deficiency enhances Mn absorption, but Fe medications inhibit Mn [65]. Studies show that Mn deficiency causes depression of CMI (as well as Se deficiency) and decreased serum Ig levels.

Se deficiency is accompanied by suppression of T cell response to mitogens and antigens and by antioxidant effects of the Se-dependent glutathione peroxidase enzyme [103, 106], and clinically is correlated to atopic asthma and to rapid AIDS progression [68]. Children who are TPN-fed over the long term may have extremely low Se levels, and in infants and children the Se requirement is increased to meet growth demands [55].

Essential Fatty Acid Deficiency or Excess

Essential fatty acids (EFAs) represent the major components of cell membrane phospholipids, where they regulate fluidity, conformational changes, enzyme functions and transport and are found in triglycerides as an energy supply. Moreover, being eicosanoid precursors, EFAs may cause immune system dysfunctions: their deficiency induces depression and excess immunosuppression (Table 21.12) [111]. Both heighten the susceptibility to infections, and change humoral and CMI; the reticuloendothelial system functions as well as the neutrophil capacity of migration and phagocytosis [5]. The metabolic cycle of arachidonic acid (AA) is shown in Fig. 1.57; however, the immunosuppressive effects are inadequately known, including the cell membrane fluidity alterations, which are negatively reflected in the distribution and functions of surface receptors. In addition, there is a prostaglandin (PG) excess, whose precursors, AA and linolenic acids, may suppress T lymphocytes and NK cells, as well as their IL_2 primed proliferative phase that follows T cell activation [77]. Various effects of EFA deficiency (Fig. 4.30) have been associated with either exogenous or endogenous causes and show some characteristics of patients with AD [46]. Although in children a severe EFA deficiency is unlikely, subdeficiency states may also occur in this case, following an inappropriate diet. In EFA-deficient, severe PEM children, δ-6 desaturase activity was impaired, and there was a nonsignificant AA decrease. EFA deficiency is a type of malnutrition and is associated with an aggravation of all parameters in severe PEM [104]. In the lung, EFA deficiency has negative effects on O_2 diffusion, surfactant production and PG metabolism [127]. Recently, a new chapter has opened up on trans fatty acids (by a rotation of the molecule across its double-bond, the

Table 21.12. Effect of fatty acid deficiency or excess on immune function

Fatty acids	Effect of deficiency	Effect of excess
Polyunsaturated	Reduced humoral response for both T cell-dependent and T cell-independent antigens	Immunosuppression, delayed rejection of skin grafts, suppressed DTH, reduced T-cell response to mitogens and antigens and reduced neutrophil chemotactic and phagocytic activity
Saturated	Rare	In vitro inhibition of T lymphocyte response to mitogens and antigens
Cholesterol	Rare	Inhibition of humoral and cutaneous hypersensitivity to antigens, reduced lymphocyte and macrophage function

Modified from [111].

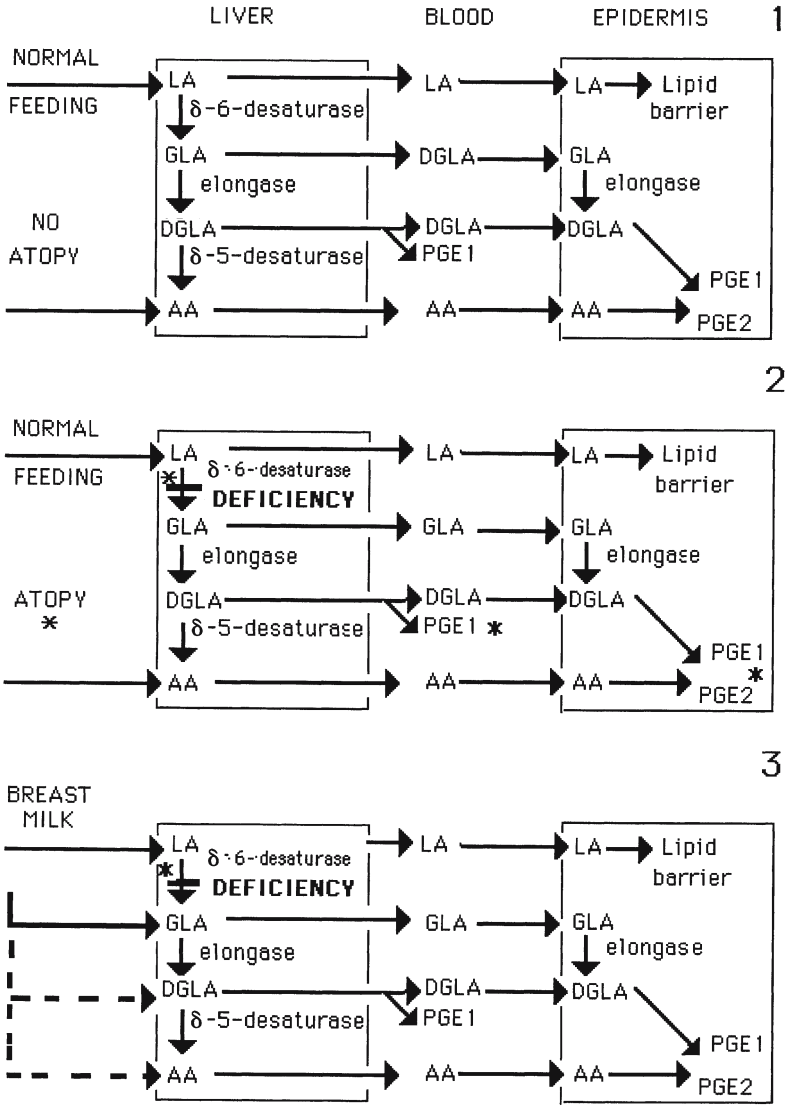

Fig. 21.10. EFA metabolism and effects on the epidermis. *1* Normal child; *2* atopy associated with δ-desaturase deficiency and potential PG decreased development; *3* effect of breast milk administration, which may restore the missing DGLA. *AA* arachidonic acid, *LA* linolenic acid; *GLA* γ-linolenic acid; *DGLA* dihomo-γ-linolenic acid, *PG* prostaglandins. (Modified from [69])

–COOH group is on the opposite side of the $–CH_3$ group. So hydrogenated vegetable oils are solid at room temperature), contained in variable concentrations in several kinds of foods [68, 70], which could alter PUFA (polyunsaturated fatty acid) metabolism in infants, by modifying PG and cell membrane function [27]. However, the pertinent problem is at a close end due to clear indications related to infantile food composition [27]. In Denmark, trans acid utilization has been reduced to 5% and will be reduced to 1% [50].

Table 21.13. Immune response modulation in humans via food lipids

Lipid supply	Food	Effects
Eicosapentaenoic acid	Fish oil	Reduction of LTB_4 and free radicals, reduced chemotaxis
α-Linolenic acid	Vegetable oils	None

Modified from [5, 51].

The consequences of EFA deficiency on the infantile immune system are to be evaluated: dihomo-γ-linolenic acid is a PGE_1 precursor and AA of PGE_2, both potentially playing a relevant role in immunoregulation, in particular PGE_1, active in the thymus has elevated concentrations, and thymus-like effects on CD8 maturation (Chap. 7). The PG activity on the immune system might be mediated in part by their capacity to stimulate cAMP synthesis, whose reduced levels are a marker of atopic constitution. Clinically, dietary *linoleic acid deficiency* (Table 21.12) results in failure to thrive, scaly dermatitis, amplified susceptibility to infections, electrocardiographic changes, decreased muscle tone, degenerative changes in kidney, lung and liver tissues, impaired water balance, increased fragility and permeability of cell membrane. *Linolenic acid deficiency* may cause 50% loss of visual acuity due to reduction of the electroretinographic wave primarily related to photoreceptor function [37, 46, 113]. Figure 21.10 [69] shows the consequences of EFA deficiency and that breast milk prevents it [69]. When a δ-6-desaturase deficiency is suspected, the administration of evening primrose oil [124] has yielded controversial results in breast-fed children with AD [94, 124]. On the other hand, EFA (see Table 7.21) or nucleotide balanced supplements may be added to the diets if necessary should breast milk not be available (Chap. 24).

Recent data have emphasized salient characteristics of *EFA excess*, especially of long-chain PUFA, and a dietary excess of these proteins is highly immunosuppressive [37]. PUFA double bonds are the primary target of *FRs*, which start the chain reactions leading to formation of lipid peroxides, able to alter cell functions up to cell lysis [101]. Since EFA is an integral part of cell membranes, and certain ones are PG and LT precursors [127], changes in membrane fluidity caused by lipid peroxides influence immune responses negatively [102], likely reducing lymphocyte ability to respond to immune system solicitations [18]. Furthermore, the EFAs stemming from linoleic acid (ω-6 series) (Fig. 4.30) are metabolized into more immunosuppressant products than those stemming from eicosapentaenoic acid (EPA) (ω-3 series); PGs and LCTs originating from the ω-6 series have an inflammatory effect that mediators from the ω-3 series do not have [42].

PEM directly provokes a reduction in PUFA levels [59], including AA, docosahexanoic (DHA) and EPA acids, proportional to the degree of malnutrition [32], present also in pediatric AIDS [16], with deleterious effects induced by PUFA ω-3 and ω-6 deficiency [32]. Table 21.13 shows examples of immune modulation brought about by alimentation under EFA influence [5, 51].

Other Causes of Malnutrition

Protein and Amino Acid Deficiency

Both lipids and protein are required for cell growth, and a balance between protein and fat is present in all basic foods; thus foods poor in protein are also poor in fat and EFA. Protein deficiency may impair purine, pyrimidine and creatinine synthesis, as well as enzyme metabolism and nitrogen balance; hypoprotidinemia is associated with hypoalbuminemia, and often with hypergammaglobulinemia. Mice experiments indicate that chronic protein deprivation has selective effects on immune functions, qualitatively similar to PEM-induced functions, with negative consequences above all on cutaneous and mucosal lesion repair, lymphocyte proliferation, humoral and CMI, and decreased IgG levels, DTH responses and *oral tolerance* [49]. When protein dietary deficiency was accompanied by induction of ovalbumin tolerance, there was an increase in humoral tolerance, but associated with a parallel increase in DTH responses [49], thus suggesting a prevalent alteration of CMI, without involving Th1 cells priming DTH reactions (Tables 21.1, 21.2). Infections and a protein-deficient diet affect the branched chain amino acids in the same way, but both glycine and alanine have opposite effects [116, 117, 126]: a protein-deficient diet in healthy young adults has no effect on phenylalanine [126], infection brought about an increase in infected individuals [116, 117]. Table 21.14 [116, 117, 126] shows the relative contribution of each influence to the plasma concentration

Table 21.14. Effect of a protein-free diet, infection, or malnutrition on the plasma concentration of selected amino acids

Amino acid	Protein-free	Infection	Malnutrition
Valine	×	×	×
Leucine	×	×	×
Isoleucine	×	×	×
Phenylalanine		×	×
Alanine	×	×	×
Glycine	×	×	×

Data from [116, 117, 126].

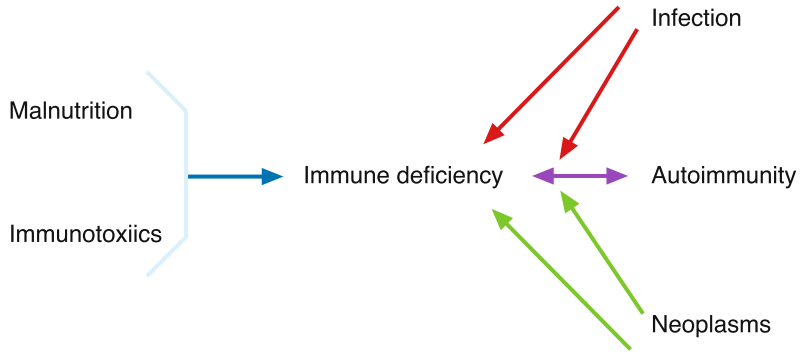

Fig. 21.11. Interactions between malnutrition and xenobiotics. (Modified from [18])

Table 21.15. Effects of nucleotide-free formulas

Normal children fed nucleotide-free formulas compared to breast-fed or nucleotide-supplemented fed children show significantly lesser levels of: T lymphocytes NK cells IL_2
Experimental animals fed nucleotide-free formulas show: Protein malnutrition Hepatic lipid accumulation Gut wall thickness Reduced mucosal height Reduced survival Reduced macrophage phagocytic activity Reduced lymphoid cells in thymus, bone marrow, and spleen Reduced T lymphocytes in popliteal lymph nodes Reduced T lymphocyte proliferation and differentiation Reduced production of NK cells and IL_2

Modified from [20, 83, 125].

Table 21.16. Nontoxic levels of vitamins and minerals (see text)

Vitamins	New limit	Recommend dose/day
A	1 mg	1 mg
B_6	5 mg	2.5 mg
C	1 g	83 mg
D	25 µg	25 µg
E	40 mg	10 mg
Niacin	33 mg	16.5 mg
Folic acid	600 µg	300 µg
Minerals		
Fl	0.04 mg[a]	0.02 mg[a]
Se	150 µg	75 µg
Zn	15 mg	15 mg

Source: French Supreme Council of Public Health.
[a] /kg bw.

of individual amino acids. Clearly, there are major changes to be characterized in protein and amino acid metabolism in malnourished individuals [49].

Nucleotide Deficiency

Ribonucleotides or nucleotides are the basic units of the nucleic acids DNA and RNA, because they can be synthesized endogenously (Fig. 2.20). They are usually labeled as nonessential nutrients, becoming semiessential or essential when the endogenous supply is insufficient for normal function, as during the rapid cell proliferation occurring in newborns [21]. Recent studies show that bone marrow, lymphocytes and red cells are unable to synthesize purines *de novo* and intestinal cells have low, inadequate rates, so these tissues utilize liver-supplied preformed nucleotides. The pertinent deficiency produces in children [20] and experimental animals [83] immune dysfunctions analogous to those described in PEM sufferers (Table 21.15) [20, 83, 125]. Dietary deficiency restrains more immune functions such as resistance to infections and macrophage activation and alters the intestinal architecture [20].

Malnutrition and Immunotoxicants

Recent data have disclosed a relationship between generalized, marginal, or single nutrient malnutrition and the effects of *dietary xenobiotics:* these interactions are schematized in Fig. 21.11 [18], which shows that dietary immunotoxicants and/or malnutrition may cause an ID complicated by susceptibility to infections, autoimmunity and neoplasms, which in turn worsen the initial ID. The French Supreme Council of Public Health has recently revised the studies on the potential toxicity of Se, Zn and Fl and vitamins A, C, D, E, B_6, niacin and folic acid, fixing their limits to a tenth of the dose demonstrated as toxic (Table 21.16).

Immunological Effects of Neonatal Malnutrition

The development of the immune system begins in early intrauterine life (Table 2.4) and continues into the first few months after birth. Malnutrition occurring in early gestation, as epitomized by small-for-gestational age (GA) and low-birth-weight (LBW) infants, is also reflected on fetal ontogeny, all the more severe if PEM interferes with a developing immature immune system (Table 2.1); the effects on CMI are very severe and long-lasting [24]. Infants suffering from malnutrition during fetal life suffer subsequently from numerous and quantitative cellular deficiency, persisting for several months or years; unlike LBW of appropriate GA, who also have depressed immunocompetence, but recover within a few months after birth [23]. Fetuses of B_6-deficient mothers have a dysfunction of both the thymus and spleen, and neonates have comparable CMI deficiencies [111], especially when several immune functions are immature or unprepared (Chap. 2); moreover pyridoxine deficiency causes a reduced lymphocyte response to mitogens [23]. The human fetus is ill-suited to synthesize EFAs, which therefore must be derived from the maternal bloodstream and reach the baby [37]. In the very first days of life, neonates should count on breast milk; however the issue may be more complex.

Malnutrition and Breast Milk

Several foreign substances, especially pollutants, may pass from mother to fetus through the placenta and be found in breast milk, potentially leading to malnutrition [17]. Infants are at great risk from breast milk pesticides because their intake relative to body weight is high [85]. In the study of Koppe et al, 4/14 neonates (28.6%) exhibited hemorrhagic manifestations in the first 4 weeks of life: in their mother's milk, the TCDD (tetrachlorodibenzo-*p*-dioxin) level was significantly higher than in the milk of the other 10 mothers [54]. Besides TCDD, a wide spectrum of halogenated pesticides and industrial chemicals are present in the milk of nursing mothers, such as β-hexachlorocyclohexane (β-HCH) (banned in the majority of industrialized countries), persistent organic pollutants (POPs), polychlorinated biphenyl (PCB), etc. [88] (Table 4.21). Six studies done between 1978 and 2001 [64] in breast-fed children aged 0–12 years have shown high levels of POP, including DDE (dichlorodiphenyl dichloroethylene), DDT, PCB, β-HCH, and β-hexachlorobenzene (β-HCB).

Thirty-two percent of countries report breast milk contamination from chemical exposure, thus raising critical concerns about breast milk safety for nursing infants. In China, breast milk samples from 35 cities were found to have traces of DDT 5–10 times, and in Delhi 12 times higher than permissible limits. While the WHO tolerable daily intake of dioxin is 1–4 pg/kg,

Table 21.17. Breast milk/plasma rate of some drugs in breast milk

Drugs (Data from [88])	**Milk:plasma ratio**
Acetaminophen	0.76–0.92
Alcohol (ethanol)	0.9–0.95
Amiodarone	1.75–10.25
Amoxicillin	0.014–0.043
Aspirin	0.6–1
Atenolol	1.5–6.8
Captopril	<0.01
Carbamazepine	0.6–0.7
Cefotaxime	0.029–0.16
Cephalexin	0.008–0.14
Cephalothin	0.073–0.5
Cephapirin	0.068–0.48
Chloramphenicol	0.5–0.6
Chlorpromazine	0.3–0.5
Chlorthalidone	0.03
Cimetidine	4.6–11.76
Clonidine	1.5
Codeine	2.16
Diazepam	0.08–0.13
Dicoumarol	0.01–0.02
Digoxin	0.6–0.8
Disopyramide	0.9
Doxycycline	0.3–0.4
Erythromycin	2.5–3
Ethambutol	1
Ethosuximide	0.8
Ethyl-biscoumacetate	0.6–0.8
Haloperidol	0.5–1
Heparin	0.01
Hydralazine	1.4
Imipramine	0.08–0.5
Isoniazid	1
Kanamycin	0.05–0.4
Lincomycin	0.13–0.17
Lithium	0.25–0.77
Meperidine	1.1–1.2
Meprobamate	2–4
Methadone	0.83
Methotrexate	0.1
Methyldopa	0.35

Table 21.17. (Continued)

Drugs (Data from [88])	**Milk:plasma ratio**
Metoprolol	2.6–3.7
Metronidazole	0.6–1.4
Mexiletine	2
Nalidixic acid	0.08–0.13
Nitrofurantoin	0.3
Norgestrel	0.2
Novobiocin	0.1–0.25
Oxazepam	0.1
Penicillin	0.02–0.2
Pentobarbital	1
Phenindione	0.012–0.06
Phenobarbital	0.21–0.71
Phenylbutazone	0.1–0.3
Phenytoin	0.12–0.24
Primidone	0.81
Procainamide	1–5.7
Propranolol	0.5
Propylthiouracil	12
Quinidine	0.71
Ranitidine	6.8–23.8
Rifampicin	0.2–0.6
Streptomycin	0.5–1
Sulfanilamide	1
Sulfapyridine	1
Sulfathiazole	0.33–0.5
Terbutaline	1.4–2.9

Drugs (Data from [11])	**Milk:plasma ratio**
Tetracycline	0.62–0.81
Theophylline	0.7
Thiopental	1
Thiouracil	3
Tolbutamide	0.09–0.4
Trimethoprim	3.7
Valproic acid	0.01–0.1
Verapamil	0.54–0.94
Warfarin	0.01

Xenobiotics (Data from [11])	**Milk:plasma ratio**
Benzene	2.30
Carbon tetrachloride	3.26
Chlorobenzene	2.19
Chloroform	1.52
Halothane	2.19
Lead	0.2
Mercury	0.8–0.9
Methyl chloroform	3.12
Methylene chloride	1.63
N-benzene	2.10
Styrene	2.17
Tetrachloroethane	3.55
1,1,1,2 Tetrachloroethane	3.18
Toluene	2.68
Xylenes	2.98

US nursing infants receive 35–53 pg/kg/day and in Japan 100–530 pg/kg/day. In Guatemala, pesticide residues in breast milk are reported to be 250-fold the amounts allowed in CM. Breast milk from Inuit women in Canada had concentrations of PCBs, a fourfold higher metabolite of DDT and tenfold higher metabolite for the pesticide mirex than women in control groups (The '103 Report). Studies in Zimbabwe found that almost all of the breast milk samples taken in some regions showed contamination with DDT [26].

In general, the pollutant levels that pass into breast milk are lower than the levels occurring in maternal plasma, but breast-fed atopic children can have concentrations of immunotoxic compounds 321% higher than nonatopic children [123]. The reported amounts are normally very low and quite distant from the doses considered as harmful and depend on several parameters, such as the POP amount ingested by the mother, its absorption, distribution, metabolism and the elimination of individual substances, either in the mother or the nursling neonate [86]. There is absolutely no positive evidence of hazards posed to infants and of possible toxic effects, although sometimes there were *prenatal exposures* [67, 123], but *breast milk benefits outweigh any possible disadvantages* [53], also because concentrations of chemicals found in breast milk are unquestionably lower than the concentrations detected in maternal plasma [86, 93]. Estimates of prenatal and perinatal PCB exposure of newborn babies in cord blood (CB) and breast milk-negative associations between breast milk PCB and mental and motor development were significant from 30 months onwards. However, prenatal PCB exposure at current background levels inhibits mental and motor development and spreads its effect up to

Table 21.18. Minimal dose of xenobiotics allowance for a nursling

Xenobiotics	Daily consumption by a 5-kg baby (mg/kg)
DDE	0.0084
PCB (12 peaks)	0.0035
PCB (2 peaks)	0.0061
Dieldrin	0.0000070
Heptachlor epoxide	0.000039
Oxychlordane	0.000098
TCDD	0.000000070
PCDD	0.0000018
PCDF	0.00000032

Data from [88].

42 months of age [114]. Table 21.17 [11, 88] details the milk:plasma ratio, which is reassuring, but Table 21.18 shows how minimal the dose for a nursling infant may be [88]. Maternal vitamin and trace metal deficiency may be reversed by supplements during lactation on human milk [2].

Table 21.19. Effects of malnutrition on lung defense

Children
↓ sIgA in bronchial secretions
↓ Complement levels
Infant rats
Thymic atrophy
↓ T lymphocyte transformation

Modified from [47].

Table 21.20. Pulmonary changes from rat food deprivation

Short-term deprivation
Insignificant surfactant decrease
Normal air volume/pressure curves
No elastic recoil changes
Long-term deprivation
Significant surfactant decrease
Decreased lamellar bodies in granular pneumocytes
Abnormal air volume/pressure curves
Decreased elastic recoil

Modified from [47].

Clinical Presentation

It is known that infections that interfere with numerous physiological and metabolic alterations reflect, although transiently, upon the host nutritional status [52]. Detrimental effects on nutritional status are summarized as follows: anorexia, decreased absorption of nutrients, usual food replacement with a hypoproteic and low-energy diet and increased urinary losses of N, K, Mg, Zn, S, P and vitamins A, C and B_2; the loss of trace elements and vitamins further complicate the pattern [1, 6]. Additional effects of malnutrition include increased basal energy expenditure, marked muscle catabolism and depletion of N, glycogen, lipid and EFA deposits [112]. To face the lack of protein substrates, the organism resorts to an increased compensatory uptake of amino acids from the muscles, accompanied by a significant insulin hypersecretion, coming from the dietary carbohydrate intake [52, 112]. The EFA deficiency promotes important metabolic alterations, heralded by hepatic steatosis derangement [49]. When the intestine is involved, it is sufficient to imagine that in CMA with intestinal manifestations, protein absorption can be reduced by 20%–33% and fecal fat loss can approach 40% of fat intake [52]. In the lung mucosa, the reduction of sIgA in bronchial secretions and thymic atrophy in rats are crucial (Table 21.19) [47], with more severe and lasting effects (Table 21.20) [47].

Without taking into account starvation types such as kwashiorkor, the infectious episodes, the inappropriate diet, sometimes with dramatic effects [74] (Table 9.23), and the effects of unconventional remedies are fitting examples [49, 105]. The subject of inappropriate diets has recently been stressed [19]. A 22-month-old male child was started on a rice beverage after weaning because of a history of chronic AD and suspected CMA, while the intake of solid foods was very poor. This rice beverage, fallaciously referred to as rice milk, is extremely low in protein content, thus the resulting daily protein intake was only 25% of the RDA (recommended daily allowance). Consequently the baby developed severe kwashiorkor. A 17-month-old black male was breast-fed until 10 months of age, when he was weaned to a soy health food beverage, which was not fortified with vitamin D or calcium. Intake of solid foods was good, but included no animal products. Thus the child was diagnosed with rickets [19]. In Holland, 24% of 200 neonates and 93 children aged 1.2 admitted to an ICU (intensive care unit) showed signs of acute and/or chronic malnutrition [48]. Allergic children who are the victim of parental attitudes appear to be pale, irritable, with thin and dry hair, succulent subcutaneous edema of lower limbs, hepatosplenomegaly, and weight and height in the 3rd–10th percentile for age, a typical dystrophic aspect explained by unbalanced dietary manipulations with protein deficiency [17, 74, 105].

Table 21.21. Recommended micronutrient daily allowances (LARN 1996)

Category	Age Years	Fe mg	Zn mg	Cu mg	Se µg	Vit C mg	Vit A RE µg
Infants	0.5–1	7	4	0.3	8	35	350
Children	1–3	7	4	0.4	10	40	400
	4–6	9	6	0.6	15	45	400
	7–10	9	7	0.7	25	45	500
Males	11–14	12	9	0.8	35	50	600
	15–17	12	9	1	45	60	700
Females	11–14	12–18	9	0.8	35	50	600
	15–17	12–18	7	1	45	60	600

The Fe daily allowance is of 18 mg for menstruated adolescents, of 12 mg in non-menstruated adolescents. Vitamin A is expressed in µg of RE (retinol equivalent), = to 1 µg of retinol, = to 6 µg of β-carotene, = to 12 µg of other active carotenoids.

Diagnosis

A high priority should be given to the clinical history of admitted children with PEM: documenting multiple deficiencies, investigations to evaluate nutritional parameters (Appendix 9.11) as well as immune background and concentrations of trace elements, vitamins [82], EFA and nucleotides are sometimes needed. A fitting dietary history is necessary and should include inquiry into the use of CM alternatives [17]. The histological examination of intestinal lesions may reveal changes resembling those of celiac disease [13]. The increase in the C18:1/C18:0 δ9 desaturase activity and enhanced lipid peroxidation without any EFA deficiency could be early markers of PEM [104].

Treatment

A large body of epidemiological evidence shows that the often interacting effects of malnutrition (and excess EFA intake) accentuate the need for regular check-ups in case of protracted therapeutic elimination diets [49], either of child growth based on national standards (Appendices 6.1–6.4) or early signs of malnutrition (Appendix 9.12). The management of chronic malnutrition, as seen in pediatric AIDS (Chap. 23) is more demanding and complex. The basic management with Zn (5–10 mg/kg of Zn as Zn sulfate) and breast milk can resolve acrodermatitis enteropathica [14], Zn administration in children with PCM may prevent the related deficiency and improve the immune function [91], and in patients with HIV infection it has the ability to stimulate a CD4 increase, thus reducing the incidence of bacterial infections [118]. When the dosage calculated based on RDA is continued for too long, a reduction in monocyte phagocytosis and an interference in Cu and Fe absorption can result [92]. Similarly, Zn prescription to not Zn-deficient subjects as a nonspecific immunostimulant should be discouraged [118]. In children belonging to disadvantaged population groups, a 2-month period was required for complete immunologic recovery [25]. Six months after the ICU admission, almost all children showed complete recovery [48]. Zn supplements should be prescribed to children with AD undergoing long-term elimination diets or with atopic asthma [35]. However elevating Zn intake from 0.3 to 0.6 mg/kg/d in very LBW infants did not influence serum Zn concentration [66]. If possible, babies with AD should be breast-fed, because Zn absorption is higher from breast milk than from CM [65]. Children with atopic asthma should receive Se supplements, since they are low in platelet glutathione-peroxidase [76]. We believe that formula-fed babies are not as well-provided as breast-fed babies in terms of Se nutrition [55].

Table 21.21 outlines recommended vitamin and mineral dosages according to age and sex. Normally, a well-balanced and carefully prepared diet can ensure an excellent, rapid result as regards micronutrients [41, 43], including vitamins [61] and nucleotides [79] in abundance in breast milk (Tables 2.16, 2.17); a few days are sufficient to normalize liver histology [112]. As regards vitamins, it has recently been ascertained that assuming commercial preparations for preventive or protective purposes has no effect [44, 56, 75], at variance with the vitamins contained in fresh fruits and vegetables [56]. The necessary EFA supplements for the nutrition of term and preterm neonates [37] are to be found in both colostrum and breast milk (Tables 2.14, 2.15); Appendix 7.3 shows the recommended doses. With a rich nucleotide diet, as well as with breast milk (Table 2.16) both the number and functions of intestinal T cells are reversible [79], with positive effects on both humoral and CMI (increase in IgA, CD3, CD4, CD8 levels and CD4:CD8 ratio at 3–3.5 months) [73] and long-chain PUFA levels [21].

Pediatricians, Malnutrition and the Immune System

Assessment of nutritional status and the immune response is new, and our understanding of the mechanisms involved is far from exhaustive. For each of the nutritional deficiencies so far analyzed, such as PEM, vitamin, and trace metal deficiencies, and excess fatty acids, evidence has accumulated that dietary dysfunctions in the nutrients alter immunity and/or susceptibility to infections in infants, children, and adolescents. Some of these deficiencies are reversible with nutritional supplements. In particular, we have included
malnutrition in Table 9.22 among the possible consequences of inappropriate diets. That nutritional deficiencies can provoke alterations of the immune response at various degrees, indirectly aggravating the pediatric allergic affections, is widely understood, without neglecting in this context, trace element deficiency, with Zn in the first place. Pediatricians should thereby ensure that allergic children/adolescents submitted to restricted diets are monitored often, especially regarding their pondostatural growth, to avoid nutritional deficiencies. It is also known that human organisms are unable to accumulate Zn supplies, hence a regular introduction into the diet is needed. To their youngest patients, pediatricians should advise breast milk, in which Zn bioavailability is first-class compared to CM and CM-derived formulas.

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Pediatric AIDS

Twenty-Five Years of Science

Never before in the history of medicine have the nosology and etiology of a new disease been defined as quickly as in the case of AIDS (acquired immune deficiency syndrome). The worldwide dissemination of human immunodeficiency virus (HIV) over the past 47 years is one of the most catastrophic examples of the emergence, transmission, and propagation of a virus [248]. AIDS was first observed in the USA in 1981 through the study of young homosexual males demonstrating a high rate of *Pneumocystis carinii* pneumonia (PCP) associated with Kaposi's sarcoma (KS), also at a visceral level [222]. At the time, KS was considered a rare form of cancer that mainly affected subjects over the age of 60. Reports of cases of pneumocystosis and/or KS, often associated with other severe opportunistic infections, rose to such an extent that 159 cases were reported between June and November 1981 [222]. It was soon discovered that HIV infection had spread above all among young adults from already identified risk categories: homosexuals, drug addicts, hemophiliacs, recipients of transfusions of blood or blood products, or natives of Haiti and Zaire, Rwanda and Burundi [61, 87, 182, 455, 555]. The early cases of HIV infection originated in Central Africa, where it was identified in Kinshasa, Democratic Republic of Congo, [555] in a *sample of serum stored since 1959* [599] and then spread outwards to neighboring Tanzania and Uganda in the east, and Congo-Kinshasa in the west [555]. On the basis of the HIV-1 (HIV type 1) sequences obtained from a sample of serum that was stored in 1959 and more recent isolates, it has been estimated that the date of the common ancestor of the main (M) group of HIV-1 is 1931 (1915–1941) [292]. The most recent common isolates of HIV type 2 (HIV-2) have been dated to the 1940s [326]. *As early as in 1983* [477] *several investigators reported AIDS in children* [17, 61, 182, 477], *and especially in infants* [182]. In addition to HIV-1, a second virus, HIV-2, was subsequently identified in West Africa [107, 108], causing a syndrome that was fully analogous to HIV-1 but was different from an antigenic standpoint [82]. HIV infection is characterized by virus replication in a limited number of cells, including T lymphocytes and macrophages, leading to a CD4 depletion and a profound immune deficiency (ID) [31, 202, 205, 442]. In children, the disease is marked by a short incubation period of even <1 year [494], while in adults it can develop in HIV-infected subjects within a decade (or up to even 19 years following HIV-2 infection) [158]; many of these patients remain asymptomatic for long periods of time, with normal CD4 counts [85] and normal-looking lymph nodes, despite persistent virus replication [423].

The WHO estimated that $>40\times10^6$ people worldwide are currently infected, and AIDS has caused $>20\times10^6$ deaths. In the developing world, where the epidemic is most prevailing, the disease's adverse social and economic impact should not be underestimated [438]. Sixty-six percent of infected persons are in Africa, and 20 % are in Asia, where the epidemic has been growing rapidly in recent years. Global statistics have made it clear that at the end of 2003, an estimated 34.6×10^6 to 42.3×10^6 people throughout the world were living with HIV infection, and the AIDS epidemic has claimed the lives of >20 million people [523, 549]. In addition, because HIV-infected mothers are likely to die of AIDS, 10 million children have been orphaned thus far and an estimated 20 million will be orphaned by 2010 [577]. The WHO now recommends quality-assured, fixed-dose combinations of lamivudine (3TC), stavudine (d4T) and nevapirine (NVP) in a single pill as first-line treatment [576] (see "Treatment"). There, when parents die, they leave orphans, most of whom are not HIV infected, thus HIV infection has a disproportionate impact on children, resulting in the loss through illness or death of those persons who can make the greatest contribution to the social support systems and economic vitality of their regions [191, 208].

HIV

HIV-1 and HIV-2 belong to the subfamily of *lentiviruses* (Table 23.1) [139] from the family of retroviruses (Table 23.2) [139], which also includes simian ID virus (SIV), which is most similar to the two HIV types [82]. In contrast to HIV-1 infection, which is spread through all continents, HIV-2 is primarily restricted to West Africa and to population movements from or through this region [326]. HIV-1 is undergoing rapid mutations and recombinations that contribute to its genetic diversity. Three genetic groups, designated M and O (outlier), and N (non-M/non-O), have been identified. M, which

Table 23.1. Main lentiviruses

Classification	Host	Associate pathology	Name
Ungulates	Ram, goat	Encephalitis, interstitial pneumonia	Virus visna
	Goat	Encephalitis, arthritis	CIEV (caprine infectious encephalitis virus)
	Horse	Infectious anemia	EIAV (equine infectious anemia virus)
	Bovine	Bovine lymphocytosis, ID?	BIV (bovine ID virus)
Felines	Cat	Immunodeficiency	FIV (feline ID virus)
Primates	Macaque	Immunodeficiency	SIV_{mac} (simian ID virus)
	African green monkey	–	SIV_{agm}
	Mangabey (cercocebus)	–	SIV_{smm}
	Man	Immunodeficiency	HIV

Data from [139].
ID immunodeficiency.

Table 23.2. Retrovirus classification

Subclass	Natural host	Human retroviruses
Spumavirus	Man	Foamy virus
	Other mammals	
Oncovirus	Man	HTLV I, HTLV II
	Other mammals	
	Birds	
	Reptiles	
Lentivirus	Man	HIV-1, HIV-2
	Other primates	
	Sheep	
	Equines	

Data from [139].

is highly prevalent, is further classified into ten established envelope (env) subtypes designated clades, A through J according to their degree of genetic similarity [326]. Subtypes A, B, C and D are known, and they are distinguishable by their genome, while E and G are recombinants with subtype A, and the sequences of subtypes F, H, I and J are not available [367]. Clade B is the most common in the US and Western Europe; clades A, C, D, and E are most common in the developing world [253]. A high heterogeneity of HIV variants is also possible, as seen in Lebanon [435]. Differences in vertical transmission and progression rates have also been reported among these strains [253]. As in other retroviruses, there are cell proteins in the viral preparations, such as the HLA-DR and β chains, β_2-microglobulin, HLA class I and adhesion molecules CD11a/CD18 and CD44, suggesting selective incorporation of these human proteins in the potential host genome [24, 413].

As shown in Fig. 23.1, like other retroviruses HIV is composed of two identical copies of RNA, arranged inside a core of viral proteins [301]. The gene structures in the 9 kb that form the RNA strand are reported in Table 23.3 [214, 456]. The genome includes at least nine separate genes with sequences that partially overlap: these chains are flanked on both sides by long terminal repeats (3′ and 5′ LTR), which contain the set of essential signals to regulate the provirus transcription and become integrated in the cell genes. It must be noted that some LTR regulatory sequences share cell sequences [214]. For a better understanding of the role of these genes, we can theorize that when the cell encounters activation phenomena, this also involves LTR, whose signals include a promoting part activated by cell RNA, an enhancing part, another with a negative regulatory activity and the tat sequence, a positive HIV replicator that is capable of 1,000-fold acceleration of the production of viral proteins [132].

Schematically, the HIV virion is spherical in shape, has a diameter of 100–120 nm, and a basic structure similar to that of other retroviruses [103, 301, 467, 509]:

- *The viral env,* a lipid bilayered membrane, is studded with various host cells (including HLA class I and II) and contains 72 external spikes formed by the two major viral-env proteins gp120 and gp41 [100] (Fig. 23.1). These originate from enzymatic splitting of the larger viral pre-protein gp160; they elicit viral entry and syncytium formation. Gp120 has a variable protein domain containing the V3 loop, which triggers a strong immune response [194, 584]. The cell shows mature and budding particles (Fig. 23.2). Within the virus, structural proteins surround an inner viral core that contains enzymes and proteins required for viral replication including protein p24. In addition to p24, the HIV-1 core contains three nucleocapsid proteins, p17, p9 and p7 [226]. These proteins are proteolytically cleaved from a

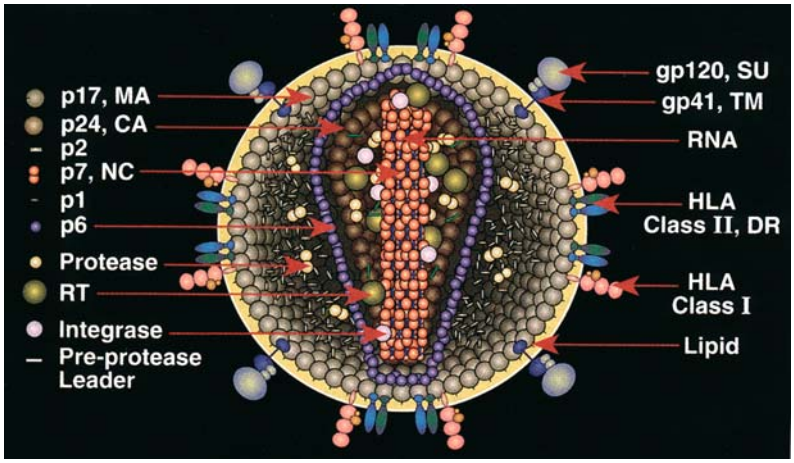


Fig. 23.1. Schematic cross-sectional view of the mature HIV particle. The viral particle is composed of mature gag and pol products. The viral products, two identical RNA molecules, are surrounded by a lipid bilayer with membrane-bound surface proteins of viral (gp 41 and gp 120) and cellular (HLA class I and II) origin. *p* Protein, *gp* glycoprotein. (Adapted from NIH Publication 94–1536)

Table 23.3. HIV genes

Genes	Name, size (kD), functions, localization
HIV structural components	
gag (Group-specific antigen)	p17 Membrane anchoring (MA), envelope interaction, nuclear transport of viral core, virion
	p24 Core capsid (CA), virion
	p6 Binds Vpr, virion
	p7 Nucleocapsid (NC), binds RNA, virion
	p9 RNA-associated nucleoprotein
pol (Polymerase)	p11 Protease (PR), (gag-pol-env cleavage and maturation), virion
	p66/51 Reverse transcriptase (RT), reverse transcription, RNase H activity, virion
	p32 Integrase (IN), DNA provirus integration, virion
env (Envelope)	gp120 External viral glycoproteins, binds to CD4 and coreceptor, plasma membrane, virion envelope
	gp 41 Transmembrane glycoprotein or fusin, fusion protein
Regulator proteins	
tat (Trans-activant)	p16/p14 Viral transcriptional transactivator, activates indispensable transcription for viral replication
rev (Regulator of viral expression)	Codes a 20-kD protein for RNA transport, primarily in nucleolus–nucleus, shuttles between nucleolus and cytoplasm
nef (Negative factor)	p27/25 CD4 down-regulation (myristylated protein), plasma membrane, cytoplasm, virion
vif (Viral infective factor)	p23 Promotes virion maturation and infectivity, cytoplasm (cytosol, membranes), virion
vpu (Viral protein U)	p16 Promotes extracellular release of viral particles, degrades CD4 in the endoplasmic reticulum, integral membrane protein
vpx (Viral protein X)	Encoded only by HIV-2 and SIV appears to be required for viral replication in peripheral monocytes and macrophages
vpr (Viral protein R)	p10–15 Promotes nuclear localization of preintegration complex, inhibits cell division, arrests infected cells at G2/M, virion, nucleus

Data from [214, 456].
gp glycoprotein.

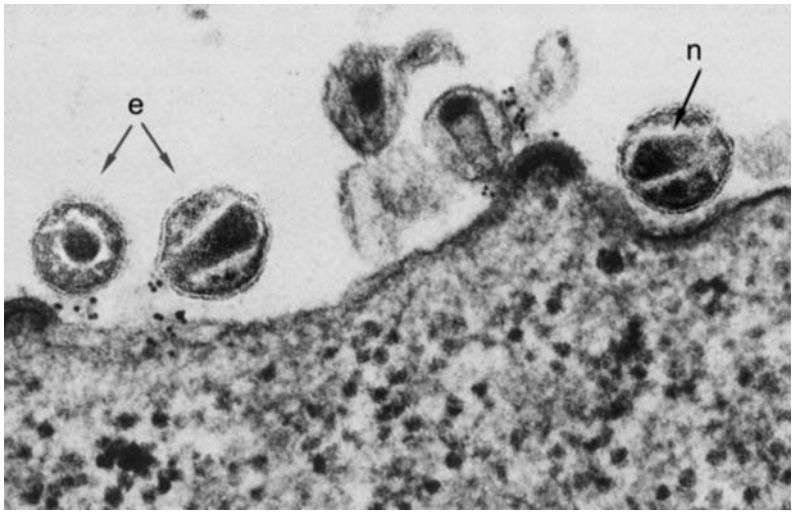


Fig. 23.2. HIV appearance at electron microscopy. At the surface of an infected cell the HIV eccentric nucleus (*n*) appears [p24, p13, reverse transcriptase (RT) and diploid genome], and *e* (*two arrows*) shows the envelope with two small spinous projections (glycoproteins) and internal membrane (p17). In the middle appears a budding viral particle, and others that are mature produced by the same cell: the free HIV-1 virion thus produced from the host cell can initiate the retroviral life cycle by infecting other $CD4^+$ target cells

common 53-kD (p53) gag precursor by the virion protease [226]. The phosphorylated p24 polypeptide forms in vivo the chief component of the inner shelf of the nucleocapsid that encloses two genomic RNA strands and the viral enzymes. The myristoylated p17 (matrix protein) is associated with the inner surface of the lipid bilayer. p9 is a nucleocapsid protein not covalently attached to the viral RNA [343].

- *After attachment,* the next step in the infectious process is *viral penetration.* The viral life cycle begins with the gp120 glycoprotein protruding from the surface, anchored by the transmembrane *env* molecule gp41 (fusin). The viral lipid *env* fuses with the lipid membrane of the target cell, which occurs by a disulfide bridge (S-S-bond): gp120 has a binding site for CD4 and associates noncovalently with gp41 to form a peplomer on the virus surface [58] (Table 23.3). They mediate viral entry and syncytium formation, one way in which infection spreads [459].
- *The main nucleus and virion protein enter the cell* and the single-stranded viral genomic RNA is transcribed into double-stranded DNA by reverse transcriptase (RT): double-stranded HIV DNA, complexed with the viral enzyme integrase, a product of the *pol* gene, travels to the nucleus and is inserted into chromosomal DNA [72]. As HIV virus particles bud from the surface of infected cells, are enveloped by the host cell membrane, and incorporate portions of the membrane and cellular proteins of the infected cell, HIV acquires its outer membrane as the virion exits the cell [23].
- *Binding to CD4* is necessary, but not sufficient for HIV entry into the cell. *CD26 is also necessary,* since it is the enzyme dipeptidyl-peptidase IV (substrate is the V3 loop of HIV-1 and HIV-2). Cell susceptibility to HIV infection is correlated with CD26 expression, and HIV transactivator *tat* and envelope protein gp120 are reported to interact with CD26 [410]. HIV-1 uses two different types of receptors for cellular attachment and viral entry into the target cell. Initial viral attachment occurs through cognate recognition of the env protein gp120 with the cell surface CD4 and a chemokine co-receptor, either CXCR4 or CCR5 (CXC chemokine receptor 4 and 5, respectively) [39]; especially CCR5 serves as the major portal of entry for HIV-1 [103]. Thus HIV enters the CD4 and exposes gp120 on the CD4 surface. As a result, HIV can be transmitted from one cell to another, as gp120 is the key for accessing CD4 [63, 340].

Like all retroviruses, the HIV genome consists of *gag*, *pol* and *env* genes, which code the main structural proteins and virus enzyme activities, and two LTRs at the chain's ends, on position 5′ and 3′ of the viral genome (Fig. 23.3). These gene sequences, which are common to all retroviruses, are implicated in controlling the transcription process [226]. In addition to these essential genes, HIV also contains seven more regulatory proteins: *tat, rev, nef, vif, vpr, vpu, and vpx* that control the proteins with regulatory functions, given that they intervene positively or negatively in its replication and maturation [546]. In the early formation phases of the transcription complex at 5′-LTR, the primary RNA molecule undergoes multiple splicing events that yield the synthesis of these regulatory proteins (Table 23.3). *Both tat and rev* regulate viral gene transcription and *are essential for HIV replication. tat* is a powerful transactivator that binds the TAR (transactivation responsive sequence) region contained in 5′-LTR and amplifies the viral replication transcription phase. Another indispensable gene for replication is the *rev* gene because, by continuing the splicing events, it transmits mRNA from the nucleus to the cytoplasm [467]; the *rev* gene reaches threshold concentrations and codes specific response sequences present in the viral genome [546]. The precise function of the *nef* gene is being debated, as it is required for the dissociation of CD4 from $p56^{lck}$, degradation in cytosol [458] and the progression of AIDS in monkeys with SIV [282]. Its function as a negative regulator in HIV replication has not been confirmed [458] since *nef* favors HIV entry into target cells by the

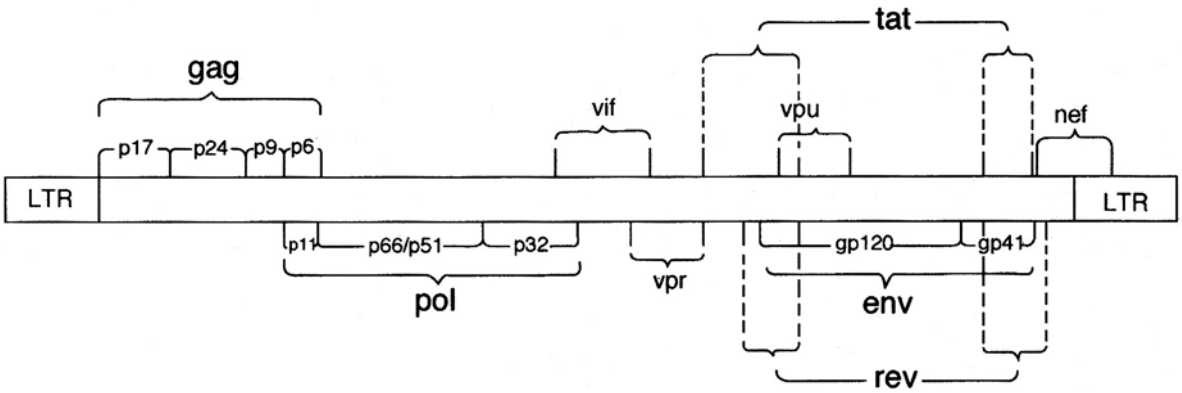


Fig. 23.3. Molecular structure of HIV. The 10-kb RNA genome has two LTRs (long terminal regions) on either end. These LTRs are important for regulating expression of viral genes, and for integrating within the host genome. The HIV genome has three structural genes: *gag* and *env* coding production of constituent proteins, the nucleus and viral envelope, respectively, and *pol* cleaving the precursor *gag-pol*. RT and other enzymes catalyze both transcription and dimension of nucleus and viral envelope proteins. There are seven regulatory genes, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu* (unique to HIV-1) and *vpx*. Several coding regions are discontinuous and overlap with each other and with other regulatory proteins

CD4 and chemokine-dependent route [491]. In fact, *nef* enhances HIV in unstimulated human lymphocytes [517], while *nef* absence can be associated with the non-progression of HIV infection [287]. The other genes, *vif*, *vpu*, *vpr* and *vpx* [58], could increase replication speed, fostering the spread and cytopathic effect of HIV [456]. *Vpr* and *vif* might participate in this nuclear transport. *Vpr* is thought to enhance the HIV-1 preintegration complex transport to the nucleus [592]. *Vif* increases the efficiency of HIV infection in vitro. *Vpu* intervenes in the assembly process and *vpr* in the nuclear transport of the viral genome [491]. Specifically, the lack of *vif* protein entails the inability to complete the synthesis of proviral HIV, and therefore it seems to be necessary for the viral initial cycle once it has entered the host cell [564]. *Vpr* appears to favor the replication of HIV, transporting it from the cytoplasm of the infected cell nucleus [331].

Etiopathogenesis

Genetic Factors

Genetic susceptibility involves *several HLA genes associated with HIV progression.* HLA genes are divided into three groups: those associated with a rapid progression, those with a slow progression/non-progression over the long term, and those with frequently exposed HIV-negative subjects. HIV-infected patients have a higher percentage of circulating lymphocytes expressing *HLA-DR that, if associated with CD8, are a sign of a poor prognosis* [298]. The class I genes that are considered protective include *HLA-A25*, *A32*, *B18*, *B27*, *B51* and *B57*, which are quite rare in various populations [575]. *HLA-B35* has a direct role in rapid progression, whereas *HLA-A29* and *HLA-B57* are associated with slow progression to AIDS [190]. In American HIV$^+$ children, the *HLA-DR3* haplotype (*DRB1*0301-DQA1*0501-DQB1*0201*) is a risk factor associated with a faster decline of CD4 levels and an increased rate of encephalopathy and death prior to the child's second birthday, whereas *DQB1*0101* is *highly protective* and has an equally marked effect [275]. Instead, in Spanish children, *DQB1*0301 is protective* and *DQB1*0201 is a tenfold risk* factor [276]. The pattern of allelic involvement has been shown to be distinct from that influencing HIV-1 infection outcome. The mothers who transmitted despite low viral loads (48%) had HLA-B1302, B3501, B3503, B4402, or B5001 alleles, vs 8% of nontransmitting mothers. Notably, the expression of *HLA-B4901* and *B3501* alleles inhibited mother-to-infant HIV transmission despite high maternal viral loads [579]. The concept that a person's haplotype plays an important role in determining the susceptibility to HIV infection and clinical outcome may appear incompatible with the characteristics of chimpanzees, which are 98% identical to *Homo sapiens* on a genetic level yet are resistant to HIV infection [242]. Moreover, while HLA class II genes appear to be virtually homologous in both species, there are subtle differences in the HLA-B and HLA-III regions. In particular, chimpanzees lack the homologs for the HLA genes associated with rapid progression, such as *HLA-B8*, as if this absence were due to a prior HIV infection [575]. HZ (homozygotes) carriers of variant mannose-binding lectin (MBL) alleles are at increased risk of HIV infection, also associated with a significantly shorter survival time after a diagnosis of AIDS [204, 351].

Viral Factors

The main receptor of HIV-1 and HIV-2 is the CD4 molecule: the high-affinity interaction takes place between the N-terminal domain D1 of CD4 and a region composed of four constant parts of gp120, situated in areas that are quite far from the primary structure. Infection

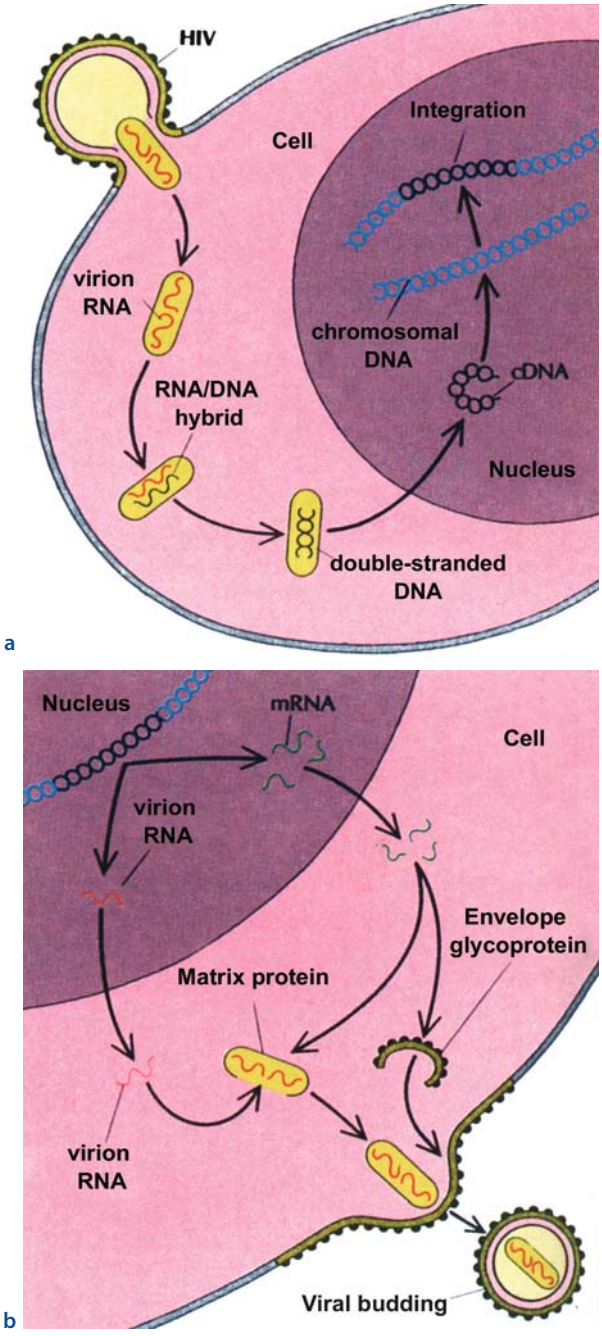


Fig. 23.4 a,b. HIV life cycle. After internalizing and uncoating, HIV fuses with cell membrane (**a**). HIV loses the nucleus and because of interaction of viral gp120 with CD4, enters the cytoplasm, where RT initiates processes inducing production of RNA/DNA hybrids, double-stranded DNA and chromosomal DNA, which integrates as provirus into the host genome. Cell activation (**b**) leads viral DNA to transcriptional production of viral mRNA. Structural proteins regulated by additional genes lead to subsequent rounds of HIV replication, and viral proteins are assembled with virionic RNA to form the viral genome. Free HIV virions are produced by viral budding from the host cell, as depicted in Fig. 23.2

by HIV starts because of this affinity: two gp120 regions show an evident homology with HLA-DR and another homology with the immunoglobulin (Ig) V_H domain [529, 575]. The gp120–CD4 interaction allows the hydrophobic end of gp41 to be inserted in the cell membrane phospholipid *env*. Virus-cell fusion then occurs, allowing the virus to penetrate the cell and start its replication cycle (Fig. 23.4). After penetration, the next step is the viral RNA transcription into a double-helix DNA copy, which is performed by viral RT [420]. More precisely, this enzyme is a dimer of two proteins (p66/51) (Table 23.3). According to some authors, a key that allows HIV to be transmitted from cell to cell is integrin CD11a/CD18 (LFA-1) [421], although others disagree and attribute a marginal role to it [218]. *CD30 and CD8 favor replication* [147]; 88-kD CD30 soluble form (sCD30) is higher in the serum of infected patients [6], whereas CD8 express CD30L (ligand) [147]. HIV can thus start its *reproductive cycle in young children* via other viral enzymes, particularly integrase p32, and the provirus is incorporated into the host cell DNA, where it can remain latent for months or even years [71], probably as a result of regulatory genes [467]. Indubitably, the signals that trigger the physiological T lymphocyte activation also mediate the transcription of the provirus, for example, by promoting the IL_2 (interleukin) gene transcriptions that have not been transcribed yet (Chap. 1); NF-κB (nuclear factor κB) also activates the provirus transcription. This occurs because the LTR and the regions regulating the IL_2 genes share certain nucleotide sequences [420].

Several key conceptual issues regarding the role of host factors in the pathogenesis of HIV-1 infection remain unresolved, in particular the relationship between expression levels of CCR5, the major coreceptor for HIV entry into cells, and HIV-1 pathogenesis [39]. CCR5 is a G-protein-coupled chemokine receptor that is used as a cofactor by macrophage-tropic (M-tropic) isolates of HIV-1 to gain entry into host cells [283]. M-tropic strains (R5) seem to preferentially use the CCR5 coreceptor, whereas T-tropic strains (X4) preferentially use CXCR4, although several dual-tropic strains have been described [102]. Recent studies have demonstrated that CD4 cannot act alone, and neither the galactosylceramide of cerebral and enteric cells nor FcR are adequate alternatives, although this bond can intensify the infectivity of HIV [330]. This is confirmed by studies on genetically manipulated mice that resist HIV, although they express CD4 [331]. This is the reason why a research trend has focused on identifying other coreceptors that permit HIV fusion [64, 136, 444]. These findings have been confirmed with the CD4 of several individuals that were exposed to HIV but were not infected [429]. As shown in Fig. 23.5 [330], the interactions of HIV before it replicates are quite complex, and thus the approach is different. If cell penetration of *T-tropic strains is mediated by CXCR4* [185] (Table 1.57) *that of M-tropic strains is mediated by CCR5* [394]; the

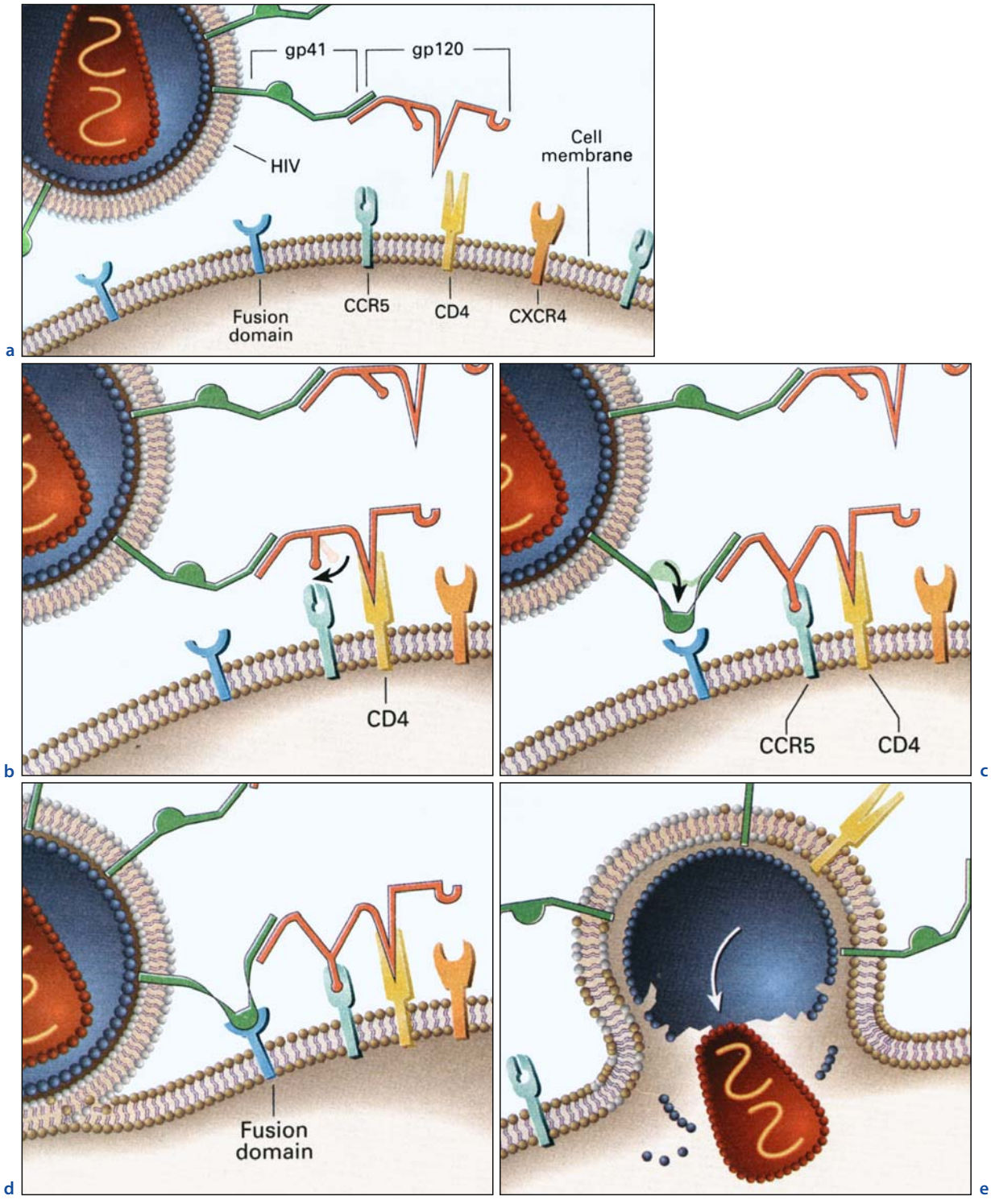


Fig. 23.5 a–e. Interactions between HIV and the cell surface. HIV interacts with a cell-surface receptor, primarily CD4, and through conformational changes becomes more closely associated with the cell through interactions with other cell-surface molecules such as the chemokine receptors CXCR4 and CCR5 (**a**). Alternatively, some viruses, such as certain strains of HIV-2, could attach to CXCR4 directly. The likely steps in HIV infection are as follows. The CD4-binding site on HIV-1 gp120 interacts with the CD4 molecule on the cell surface (**b**). Conformational changes in both the viral envelope and the CD4 receptor permit the binding of gp120 to another cell-surface receptor, such as CCR5 (**c**). This second attachment brings the viral env closer to the cell surface, allowing interaction between gp41 on the viral env and a fusion domain on the cell surface. HIV fuses with the cell (**d**). Subsequently, the viral nucleoid enters the cell, most likely by means of other cellular events (**e**). Once this stage is achieved, the cycle of viral replication starts

β-chemokines MIP-1α, MIP-1β and RANTES (new terminology CCL3, CCL4 and CCL5, respectively) secreted by CD8 were shown to prevent infection with primary, M-tropic viruses by blocking the replication of M-tropic HIV strains but not of T-tropic HIV strains [9, 114]. It may also be true that CCL3, CCL4 and CCL5 are inactive, whereas SDF-1 is a powerful inhibitor of infection by T-tropic HIV-1 strains [407]. See "Future Perspectives". *CCR5 haplotype pairs* associated with enhanced risk of transmission *were the chief predictors of a faster disease course in 649 Argentinean children* exposed perinatally to HIV-1 [351].

Pathophysiology of HIV Infection

It is clear at this point that whatever analytical approach we use, *all HIV-1 strains infect primary CD4 T lymphocytes,* that is, CD4 T lymphocytes directly isolated from the peripheral blood, *and replicate in activated CD4 T lymphocytes.* The predominant consequence is progressive CD4 inactivation, thus *inverting the CD4/CD8 ratio* and inevitably causing ID: to understand this, we simply need to recall that bone marrow can replace only 50% of these lymphocytes [498] and that HIV demonstrates tropism for all cells that express CD4, which the virus sees as physiological receptors [132]. Thus, above all HIV infection reflects an immune microenvironment inability to renew the competent antigen-specific T cells [242]. Moreover, based on more in-depth knowledge of the role played by HIV, we have been able to define the broad range of its detrimental actions: as opposed to what was previously thought, research into CD4 subsets positioned in the lymph nodes has established that *up to 25% of CD4 cells are infected by the virus* [463]. Other studies have made it possible to understand the large number of cells that carry mRNA for CD4: monocytes/macrophages, dendritic cells (DCs) and follicular dendritic cells (FDCs), Langerhans' cells (LCs) and microglial cells. The mRNA for CD4 is also found in certain B-cell clones, in the CNS (central nervous system) capillary endothelium in enterocytes and probably also in retinal cells (Table 23.4) [132]. These cells also appear to act as an HIV reservoir, permitting its spread throughout the body. It has been theorized that HIV can instead dysregulate the FCD precursors, germinal centers (GCs), DCs [230], and mucosal LCs [593]. HIV gp120 produced by infected DCs impairs $CD4^+$ T cell-mediated immune responses in vivo [280].

Another area of increasing interest is *CD8 T-cell depletion,* which is another hallmark of HIV infection. Younger age or a $CD4^+$ T-cell count under 400 cells/μl was associated with poor $CD8^+$ T cell responses and high HIV loads. In contrast, vigorous, broad $CD8^+$ T cell responses against HIV Ags were frequently observed in children >3 years and maintained $CD4^+$ T-cell counts >400 cells/μl [488]. Infants with median $CD4^+$ or $CD8^+$ T cells <25% had a relative risk (RR) of progression to AIDS that was 3.35- or 4.95-fold higher than those with $CD4^+$ or $CD8^+$ above this level, respectively [453]. A greater number of children (81%) from the $CD8^+$ >25% group than from the $CD8^+$ <25% (40%) presented significantly undetectable viral load levels and showed a 4.5-fold higher relative proportion for achieving a viral load <400 copies/ml than the $CD8^+$ <25% group [454].

Table 23.4. Human cells sensitive to HIV-1 infection

Hemopoietic cells	CD4 lymphocytes
	B lymphocytes
	Monocyte-macrophages
	Promyelocytes
	Follicular dendritic cells
	Stem cells
Gastrointestinal cells	Colonic mucosal cells
	Epithelial cells
	Enterochromaffin cells
	Stromal lymphocytes and macrophages
Cutaneous cells	Langerhans' cells
	Fibroblasts
Brain cells	Astrocytes
	Oligodendrocytes
	Macrophages (microglial cells)
	Capillary endothelial cells
Additional cells	Kupffer cells
	Epithelial cells of hepatic sinusoids
	Fetal chorionic villi

Modified from [132].

Depletion of the Immune System

The immunopathogenic mechanism by which lymphocytes and various other cells are infected, eventually culminating in severe ID, is largely unknown to date. *The depletion of CD4 T cells and disease progression occur more rapidly in children than in adults* and viral replication plays an important role in the depletion of CD4 T cells and disease progression [247, 346]. Moreover, the viral replication dynamics in children may be similar to those in adults, although a large and renewable pool of permissive host cells may contribute to persistently high plasma HIV-1 RNA levels in infancy and early childhood. CD4 T cells and thymocytes might be particularly important substrates in young infants [578]. *Disruption of thymopoiesis occurs during fetal life*; thus the lymphocyte reservoirs never build up to a normal level

Table 23.5. Potential mechanisms responsible for the functional and qualitative depletion of CD4 lymphocytes

Direct HIV-mediated cytopathic effect
HIV-mediated formation of syncytia
Virus-specific immune response
HIV-specific cytolytic T lymphocytes
ADCC
NK cells
Autoimmune mechanisms
Anergy caused by inappropriate cell signaling
Anergy caused by gp-120-CD4 interaction
Superantigen-mediated deletion of T-cell subsets
Programmed cell death (apoptosis)

Modified from [422].
ADCC antibody-dependent cell-mediated cytotoxicity.

Table 23.6. The cytopathic effect is not the sole mechanism underlying HIV infection

Human herpesvirus 6 (HHV6) kills CD4 T cells in vitro; however, an infection with this virus is not AIDS-associated
Direct cytopathicity is incompatible with the long latency period observed in AIDS
Noncytopathic HIV strains induce a rapid loss of CD4 T cells in severe combined immunodeficiency mice grafted with human peripheral blood lymphocytes
No correlation exists between cytopathicity of HIV strains in vitro and AIDS pathogenesis in vivo

Modified from [529].

for age [296, 402]. Moreover, HIV infection produces an inhibition of the thymus function in children [128] depending on the ability of T-tropic viruses to infect T-cell precursors using CXCR4 receptors, usually highly expressed in immature thymocytes [127]. Thymus from children progressing to accelerated disease showed severe thymocyte depletion along with a profound disorganization of the thymic epithelial network [48, 272]. An increased influx of $CD34^+$ precursors was demonstrated during primary infection and was followed at a later stage by thymus damage, with DP $CD4^+CD8^+$ thymocytes particularly sensitive to apoptosis [470]. Late thymic failure is reminiscent of the fact that, among patients with advanced disease administered ARTs (antiretroviral therapy), CD4 cell counts often remain below normal, despite long-term suppression of viral load [325]. However, the main hallmark is the progressive CD4 depletion: these cells are destroyed by direct lysis and the formation of multicellular syncytia, preventing lymphocytes from performing their functions (Table 23.5) [422]. Above all, this extends the cytopathogenic effect to the normal cells that have been trapped there. These cells are defined as *dormant cells,* as they do not reproduce [588]. What is certain, however, is that HIV is directly cytopathic for the infected cells and is responsible for their lysis when it is activated and starts to replicate. Moreover, the cytopathic effect cannot be the sole immune mechanism underlying HIV infection (Table 23.6) [529]. High serum concentrations of gp120 and anti-CD4 autoantibodies (Aab) may operate *an autoimmune T-cell response* against CD4 cells. Thus, gp120 (a non-self-antigen), by altering CD4 processing (a self-antigen), can unveil hidden immunogenic self peptides, thus awakening specific autoreactive T and B cells from their lethargy [485]. Therefore more immune pathological mechanisms may be associated. This led researchers to theorize that during the initial stages of HIV infection, there is a strong anti-HIV-1 response and a weak anti-Fab response, pointing to a shift toward anti-HLA class II [485].

The next phase can involve the anti-anti-CD8 Aab, anti-anti-gp120 and anti-CD4 recombinants and, above all, interactions of substances that mimic anti-HLA class I and II, triggering an increase in immune complexes [529]. *Apoptosis* may be a mechanism for the observed depletion of T lymphocytes [77] (Fig. 23.6) [422]. TcR binding by gp120, alone or with an antigen, may set off a series of intracellular events leading to apoptosis, as the HLA class II^+ antigen complex binds to the CD4-TcR complex [382]. In this scenario, the simple activation of a cell that has been prepared for apoptosis by the antigen involved would set off the cascade of programmed death: this would thus explain the destruction of CD4, such as, CD4 lymphopenia, decreased naive phenotype and lymphoproliferation, thus excluding the requirement that a substantial number of these lymphocytes actually be infected by HIV [422]. When apoptosis has been initiated in CD4 subsets by interactions with gp120 and TcR, it can be avoided through degradation and internalization [458]. Infected thymocytes and lymphocytes are protected from apoptosis because of their high capacity to respond to IL_7 and the subsequent high level of bcl-2 [231]. By inhibiting apoptosis, IL_7 favors HIV persistence, converting thymocytes and lymphocytes in viral reservoir [231]. Productive HIV infection kills infected cells but is not sufficient to cause the death of a significant number of uninfected $CD4^+$ T cells, which are by definition bystanders [228]. A mechanism for HIV to directly participate to maintain its production from this CD4 reservoir derives from the HIV *env* ability to induce virus expression from HIV-infected resting $CD4^+$ T cells without eliciting apoptosis [286].

Another possible mechanism is *anergy,* which could be what underlies the functions specific to CD4 and induced by gp120 in humans but not in chimpanzees [242]. In vivo and in vitro studies have led researchers to speculate that a negative signal reaches the CD4 cells following the interaction of their surface receptors with gp120 or with gp120-anti-gp120 complexes. Anti-gp120

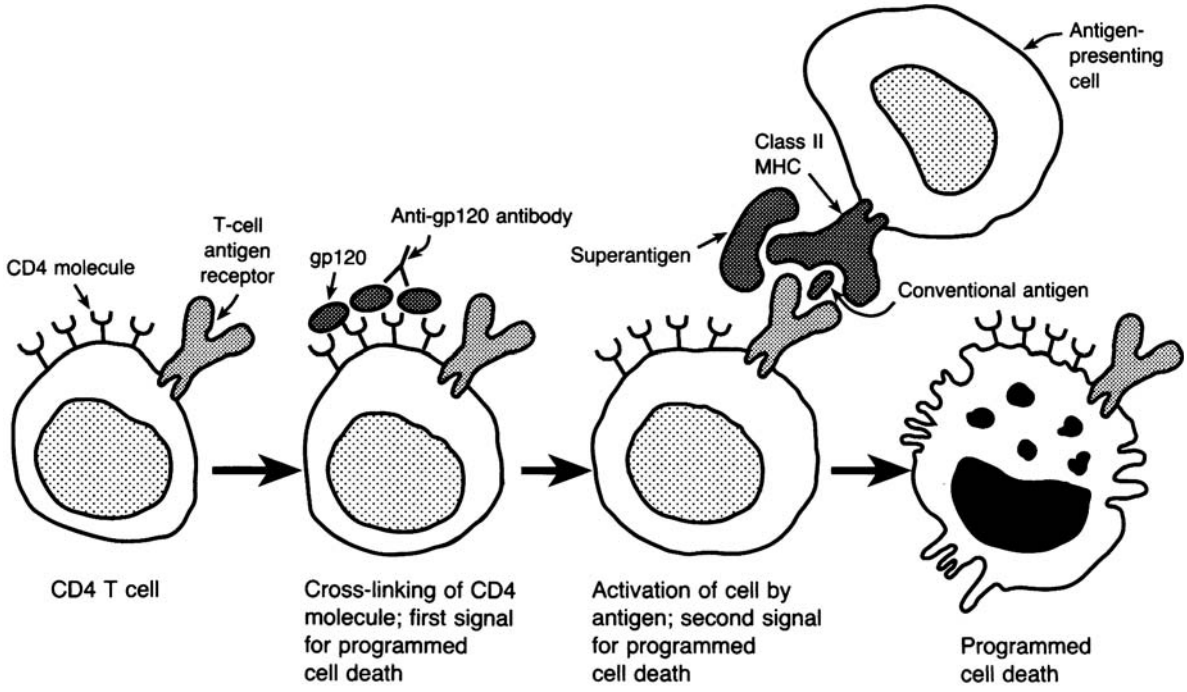


Fig. 23.6. Apoptosis (PCD) in HIV infection. Cross-linking of CD4 molecules to one another by gp120 alone or gp120 complexed with anti-gp120 antibodies provides the first signal required for PCD. Cell activation via TcR by other conventional antigen or superantigen is the second signal

antibodies have indeed been found with the CD4 of HIV-infected patients [12]. In effect, the CD4 lymphocyte count is considered a marker of HIV infection progression or outcome [522], but a marked lymphocyte depletion may also occur late, for example, in children after years of retroviral therapy [510].

It has been postulated that *microbial superantigens* (Table 1.29) can play a significant role in the immunopathogenesis of HIV infection: although there is no direct proof in vivo and preliminary data are available from test animals, it has been observed that CD4 stimulation in mice leads to anergy or to the deletion of a substantial amount of CD4 that have the specific Vβ region of TcR [513]. In keeping with this theory, there have been reports of patients presenting deletions of CD4 subpopulations that have the same Vβ region [513]. However, it seems more likely that if CD4 are active in the HIV infection, the superantigens would activate the CD4 – making them more open to infection – instead of damaging them [422]. We cannot overlook the influence of certain ILs on HIV replication; in particular, a rise in IL_{16} serum levels correlates with progression to AIDS and vice versa (Table 23.7) [97, 331, 422]. ELISPOT (enzyme-linked immunospot) assays suggest that only a fraction of HIV-specific $CD8^+$ T cells are able to produce IFN-γ, which are positively correlated with plasma viral load [75]. Importantly, the serum IL profile at baseline in the progressor group showed *a Th2-like IL profile* (elevation of IL_4 and IL_{10} levels with decreased IL_2 and IFN-γ levels) and inversely correlated in the non-progressor group with a Th1-like IL profile (elevation of IL_1 and IFN-γ levels with decreased IL_4 and IL_{10} levels) [101]. IFN-γ production was impaired in children with a severely depleted and phenotypically altered $CD4^+$ T cell compartment and circulating gag-specific $CD8^+$ T cells [488]. As seen in mice thymus/liver implants, HIV-1 infection can greatly distort the T-cell IL profiles [290]. Thus, HIV progression leads to a progressive decline in the production of several ILs, including TNF-α, IFN-γ, $IL_1Rα$, IL_2, IL_{10}, IL_{12} and $IL_1Rβ$, whose low production was shown to be associated with increased mortality risk in the patients [414].

Table 23.7. Cytokines influencing HIV replication

Stimulator	Inhibitor	No effect
IL_{2-6}, IL_9, IL_{18}	IL_{13}, IL_{16}	IL_1, IL_7, IL_8, IL_{10}
TNF	IFN-α, IFN-β	
IFN-γ	TGF-β	
GM-CSF	CCL3, CCL4	

Data from [97, 331, 422].

Table 23.8 [331] summarizes the immune system alterations that occur during HIV infection. The infection seems to attack preferably the CD4 CD45RO memory lymphocytes; the next subpopulation is that of virgin CD4 cells that express CD45RA, followed by IL_2, critical for T-cell responses. High virus load and advanced

Table 23.8. Cellular immune perturbations during HIV infection

T cells
Defects in IL proliferation and production in response to antigens or mitogens, also due to ineffective IL_2 expression
Inadequate expression of effector functions (reduced intervention of CD4 and CD8 T cells in antibody production and CTL/DTH induction)
Skewed TcR repertoire
Reduced CTL cytotoxicity
Selective loss of memory cells
Transient oligoclonal expansion
Chronic partial activation
B cells
Increased polyclonal activation and antibody synthesis
Increased response to stimulating ILs
Reduced response to antigens and mitogens
Reduced isotype switching
Immature cells in the bloodstream
Autoantibody production
Thrombocytopenia
NK cells
Reduced cytotoxicity
Monocyte-macrophages
Spontaneous IL_1, IL_6, TNF and PGE_2 production
Reduced response to activating signals
Defective phagocyte functions
Reduced ADCC
Reduced expression of HLA class II molecules
Poor APC function
Conclusion
Random depletion
Anergy of non-HIV-infected cells
Immune system breakdown

Modified from [331].
ADCC antibody-dependent cell-mediated cytotoxicity, *APC* antigen-presenting cells, *CTL* cytotoxic T lymphocytes, *DTH* delayed-type hypersensitivity, *IL* interleukins.

immunosuppression correlate with increased perturbations within CD45RA but not CD45RO CD8 T cells: HIV-1-induced disruption of TcR diversity within CD45RA CD8 T cells correlates with disease progression [295]. A selective CD45RO cell loss may be due to their activation following infection, making them a very appealing site for viral replication. Characteristically, HIV preferentially infects memory $CD4^+$ T cells ($CD45RO^+$), whereas naive cells ($CD45RA^+CD62L^+$) are infected at a lower frequency [59]. By this means, HIV-specific memory $CD4^+$ T cells contain more HIV DNA than memory $CD4^+$ T cells of other specificities: thus, by inciting a response in HIV-specific $CD4^+$ T cells, HIV can infect the cells displayed against it [521]. Memory T-cell counts are presumably preserved as a result of chronic stimulation [366]. However, if HIV infects monocytes and other accessory cells, it can further alter the generation of memory cells [331]. The function of cytotoxic T lymphocytes (CTL) is relatively preserved during asymptomatic infection, but is seriously affected in symptomatic patients, due above all to the CD4 cell limited function which has largely been deprived of IL_2. Given that CD8 cells derive from double positives (DP), $CD4^+CD8^+$ (Fig. 2.1), their development can be affected as early as the precursor stage. As HIV^+ children move through stages of HIV disease progression, their $CD8^+$ T cells acquire activation markers (dual expression of DR and CD38), lose CD28, and acquire CD57, in relation to *rapidity of disease progression in pediatric HIV infection* [428]. A maximal $CD8^+$ cell suppression of HIV replication is achieved when the infected $CD4^+$ target cells and the effector $CD8^+$ T cells are syngenic, and thus share the same HLA class I and II genotype [350]. The outcome is a partially activated but chronically aberrant state that prevents these cells from functioning normally [263], ultimately lysing uninfected CD4 cells [594]. Other studies have instead cited a positive albeit incomplete function against HIV and secondary infections [556, 597]. However, these positive effects do not translate into a direct AIDS therapy, as the most effective control of HIV is achieved using noncytotoxic responses that suppress HIV-1 replication (Table 23.9) [332], possibly aided by CAF (CD8 T-cell antiviral factor) (Table 23.10) [332, 590]. Recent data indicate that α-defensins 1, 2, and 3 collectively account for much of the anti-HIV-1 activity previously attributed to CAF [591].

NK, K and LAK cells (lymphokine-activated NK cells), involved as a front-line defense against viral infections, are generally directed against viral antigens gp41 and gp120, and seem to be well preserved during the initial phases: in fact, HIV patients can produce LAK cells *ex vivo*, although less efficiently than normal subjects. It is likely that the ability to participate in ADCC functions gradually becomes altered and reduced, and that the LAK cell defect contributes significantly to AIDS progression. The functional defect can be reduced only in part by adding IL_2 and, consequently, the NK/K lymphocytes of IL_2-deficient patients are able to bind the target cells normally, but not kill them. In addition, the intracellular mechanisms that lead to a NK/K cell dysfunction fail to have a single pathogenic ground and could instead work synergistically with the reduced IL_2 production [507]. HIV infection of primary $CD4^+$ T cells results in a 61%–68% reduction in surface expression of HLA class I molecules, but despite this drastic decrease

Table 23.9. Characteristics of the noncytotoxic anti-HIV response of CD8 T cells

Only observed with $CD8^+$ T cells, not in other cells
Observed with $CD8^+$ T cells, from either lymph nodes or peripheral blood
Dose-dependent
Killing is not involved
HLA compatibility is not required
Exhibited preponderantly by $CD8^+$HLA-DR and $CD8^+CD28^+$ cells
Inhibit HIV replication at the level of transcription
Can prevent HIV replication by a million-fold in vitro and likely by 1,000-fold or greater in vivo
Can suppress HIV replication at low $CD8^+$:$CD4^+$ ratios
Correlates directly with clinical state and the number of $CD4^+$ in peripheral blood
Mediated at least in part by a soluble factor (see Table 23.10)
$CD4^+$ activation and proliferation are not affected
Active against HIV-1, HIV-2 and SIV strains, including cytopathic and noncytopathic variants

Modified from [332].

Table 23.10. CAF (α-defensin 1, 2, and 3) characteristics

Trypsin-resistant
Staphylococcus Vβ protease-sensitive
Different from other known cytokines
HIV replication in naturally and acutely infected $CD4^+$ cells is blocked
HIV replication after virus integration is blocked by inhibiting LTR-driven transcription
$CD4^+$ activation or proliferation is not affected
Stable at high T (30 min at 56 °C, 10 min at 100 °C)
Stable at low pH

Data from [332, 590].
CAF CD8 antiviral factor, *LTR* long terminal sequences. *T* temperature.

the NK cells were unable to destroy autologous HIV-infected T-cell blasts [53]. HIV-induced NK deficiency could be partly mediated by a defect in perforin and granzyme A expression, which could be restored by IFN-α [443].

The deficiency and dysfunction of B lymphocytes (Table 23.8) is particularly evident in pediatric infection. Because *the youngest patients* have not yet encountered a large number of environmental antigens, they have few B- and T-memory cells. As a result, their humoral immunity is still poor in many respects [10]. The result is that these children *are infected by pyogenic bacteria* rather than opportunistic infections. Moreover, in certain settings strong B-cell hyperactivation can be harmful rather than protective [10]. Along with *nef* [463], gp120 acts as a B lymphocyte polyclonal activator, contributing mainly to IgA and IgG isotype hypergammaglobulinemia, and this virtually becomes prominent in HIV-infected children [11], generally preceding the numeric T cell decline [463]. Likewise, IgD levels are high, sometimes as high or higher than IgG and IgA levels, while IgM concentrations remain within normal limits. IgA titers tend to be correlated with the progression of HIV infection [28]. Epstein-Barr virus (EBV) infection is a mechanism underlying hypergammaglobulinemia; EBV rapidly infects B lymphocytes, which, thus altered, escape the T-cell immunoregulatory control and move toward an increased cycle of spontaneous proliferation [28]. B-cell hyperactivity is characteristic of *neonatal HIV infection:* the spontaneous in vitro production of direct antibodies against viral antigens, mainly anti-gp120, is used as a surrogate marker of HIV infection [11]. The dysfunction of B lymphocytes and of humoral immunity plays a key role in pediatric HIV for another reason as well. Two studies [149, 471] have demonstrated that the serum levels of IgG-neutralizing antibodies are inversely correlated with mother-to-fetus transmission: these data suggest that *antibody deficiency* is a factor permitting perinatal infection.

As opposed to the T cells, monocytes/macrophages (Table 23.8) are unresponsive to the cytopathic effects of HIV and can, in turn, become part of the HIV reservoir, disseminating the infection throughout the body. In addition, in their APC function they can present HIV to CD4 lymphocytes, promoting their destruction [378]. However, HIV alters the immune functions of these cells, including APC and IL production, ultimately interfering with antimicrobial activity [378]. Considering that these cells are infected very early in the contagion, during the latency phase of HIV multiplication in their cytoplasm, the latent condition of HIV in these sanctuaries allows them to escape the host's immune system and thus persist in different organs for any length of time [258]. This pathogenic theory does not exclude that syncytial cells are the main cause of AIDS progression, but it seems certain that M-tropic HIV strains are transmitted more readily *from mother to child* [396], especially because IP-10 (interferon-inducible protein-10) may stimulate HIV-1 replication in monocyte-derived macrophages and peripheral blood lymphocytes [317].

In HIV infections, a leading role is played by lymphoid organs, which act as sanctuaries, or the preferential anatomical sites for the propagation of the infection. As we noted, these organs are the CD4 lymphocytes, FDC and monocytes/macrophages, as well as the lymphoid organs and the brain. Persistent generalized lymphadenopathy (PGL) is an early manifestation that can be observed in both children and adults. The main histopathological alterations involve the lymphoid folli-

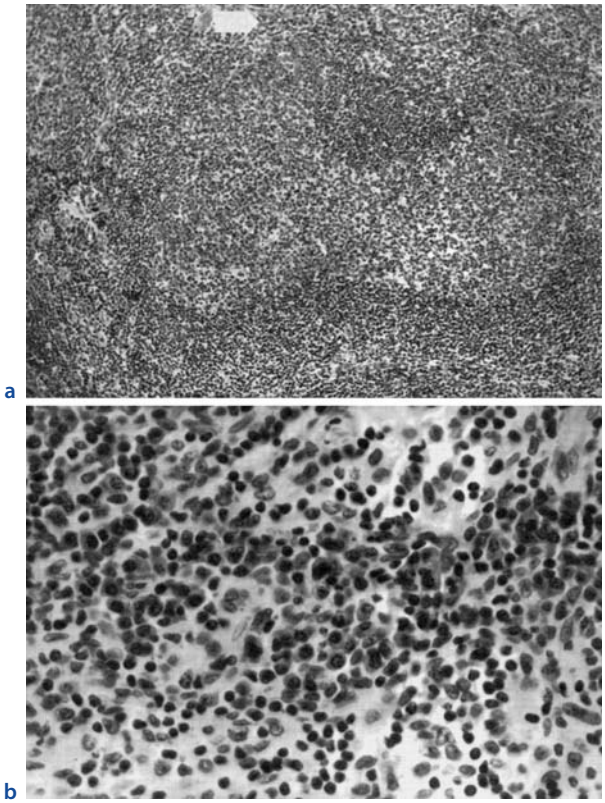


Fig. 23.7 a,b. Cervical lymph node of a child with persistent generalized lymphadenopathy (for details see text)

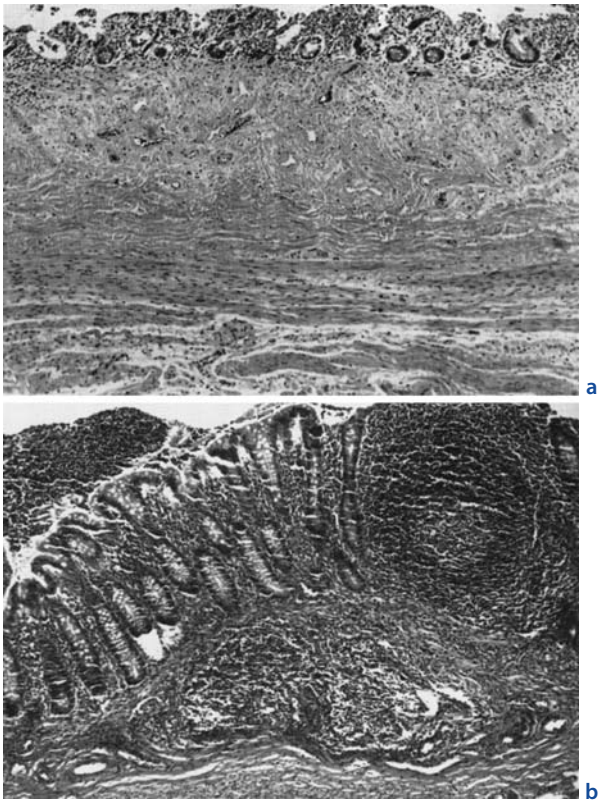


Fig. 23.8. a Normal appendix with an active follicle, and lymphocytes and plasma cells in the lamina propria and submucosa (magnification ×20). b Appendix with severe lymphoid depletion and lack of follicle formation; a few plasma cells and an occasional eosinophil are seen. There is a hypertrophy of fibrous tissue in the submucosa (magnification ×20)

cles: both the primary and secondary are enlarged and are increased in number (Fig. 23.7). In Fig. 23.7, we can see irregular shapes in the paracortical and medullary zones, perhaps the fusion of two follicles. Hyperplasia and the increased cell turnover in the GC are indicated by the increase in cells and mitoses, as well as the presence of numerous "tingible" bodies. The mantle zone, composed above all of CD4, CD8 and B lymphocytes, shows reduced cellularity. In the lower part of the figure, plasma cells are relatively increased in the two indicated zones. The intestinal tract acts as the reservoir for most lymphocytes: Figure 23.8 compares a normal appendix with that of an HIV-infected child.

Pediatric HIV Infection

Children with symptoms reminiscent of those caused by HIV infection have been reported since 1979 [477]. Initially, the possibility that they could have been infected by HIV seemed remote, but starting in 1983–1984 a number of studies have described HIV infection in children, particularly in infants [17, 182, 411, 477]. Doctors took note of this and by the end of 1985, several Centers for Disease Control (CDCs) reported 307 cases in children <13 years old [464]. The main differences between HIV infection in children and adults are summarized in Tables 23.11 [240, 494] and 23.12 [240, 525]. HIV-1 infection in children shows distinctive characteristics from the adult features [453], and these differences may have important consequences for the management of these children. What is striking about these young patients is the greater severity of clinical manifestations, including neurological findings [35, 48, 66, 169, 172, 174, 249, 446, 503, 535, 544]. Before the use of highly active antiretroviral therapy (HAART), distinct patterns of disease progression were seen in perinatally infected children. Some infants rapidly progressed to full-blown AIDS, whereas others maintained immunological control of their infection and had years of slow decline before the development of symptoms indicative of AIDS [41, 265]. The infection in newborns and infants had a bimodal expression of clinical and biological symptoms [41], thus being significantly different than in adults, because after the initial peak at about 2 months of age, a long persistence of viremia was seen prior to attenuation,

Table 23.11. Differences between pediatric and adult HIV infection: clinical differences

	Pediatric HIV infection	Adult HIV infection
Incubation and latency	Shorter (<12 months)	Longer >10 years
Immune dysfunction	Greater and earlier	Reduced, declines slower
Encapsulated bacterial infections	Greater number	Less common
Opportunistic infections	Unusual	Later
Lymphoid interstitial pneumonia	Present	Absent
Loss or lack of gain of developmental milestones	Present, common	Absent
Failure to thrive	Marked, wasting	Less
Lymphoma	Rare	KS, CNS lymphoma
Hepatosplenomegaly	Not in neonates	Yes
Encephalopathy	More frequent	Less frequent
Viral reservoirs in macrophages	Yes	Yes
Dendritic cells and microglia	Yes	Less important

Data from [240, 494].

Table 23.12. Immune differences[a]

	Children <1 year	Children of 1–13 years	Adolescents/adults
Lymphopenia	+	++	+++
CD4 depletion	–	++	+++
Hypergammaglobulinemia	+++	+++	++
Reduced antibody response to bacterial antigens	+++	++	+
Cutaneous anergy	ND	++	++
Reduced response to mitogens	+	++	++
Reduced response to antigens	++	++	++

Data from [240, 525].
ND not done.
[a] According to the Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children [240], there are unique considerations needed for HIV-infected infants, children, and adolescents, including:
1. Acquisition of infection through perinatal exposure for many infected children
2. *In utero,* intrapartum, and/or postpartum neonatal exposure to zidovudine (ZDV) and other AR medications in most perinatally infected children
3. Requirement for use of HIV virological tests to diagnose perinatal HIV infection in infants under 15–18 months old
4. Age-specific differences in immunological markers (i.e., CD4 + T cell count)
5. Changes in pharmacokinetic parameters with age caused by the continuing development and maturation of organ systems involved in drug metabolism and clearance
6. Differences in the clinical and virological manifestations of perinatal HIV infection secondary to the occurrence of primary infection in growing, immunologically immature children
7. Special considerations associated with adherence to ARTs for infants, children and adolescents

occurring gradually by the age of 2 [504], as opposed to the rapid drop usually observed in adults [246]. This persistence may partially explain the more rapid progression of HIV infection observed in the youngest patients [504]. In the first few weeks following pediatric infection, rapid increases in plasma HIV-1 load are common, such as plasma HIV-1 RNA copy numbers of 10^3–10^7/ml of plasma [1, 419, 504]. Virus levels may be correlated to the infection severity in the subsequent period: children with mean levels of HIV-1 RNA copies exceeding 299,000/ml of blood showed a 44% probability of progressing toward full-blown disease or death <2 years, but this forecast came true in only 15% of cases if the levels were below this titer (Fig. 23.9) [504]. The progression of HIV infection in women of childbearing age [91, 102, 319] (7% in 1985, 13% in 1993, and

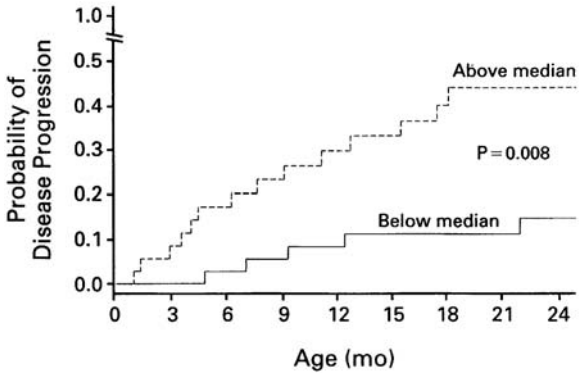


Fig. 23.9. Kaplan-Meier estimates of the probability of progression of HIV infection according to the median number of HIV-1 RNA copies during the first 2 months of life. The median value of 299,000 RNA copies/ml was used as the threshold to predict a rapid progression of disease

23% in 1999) [91] has led to a constant increase in new pediatric cases. Studies of the prevalence of positive blood samples drawn from newborns throughout Italy have made it possible to confirm the rise in births by HIV-1+ mothers, going from 0.085% in 1990 to 0.096% in 1992, reflecting an increase of 89.5% [262]. Considering that the expected annual birthrate is about 560,000, we can estimate that an average of 515 will be at risk: applying an 18% transmission quotient, we can consider that approximately 80–100 infected children are born in Italy every year [262]. All these data contribute to making pediatric HIV infection a priority problem for the public health system.

Definition

AIDS is a lethal multisystem disease that has become a major public health problem since its first description [222]. CD4 cells become depleted since they are the major receptor involved in the binding and entry of HIV into target cells, whereas B cells are polyclonally activated with increased Ig production, but the infection progressively causes overall defects that damage the immune system's two arms. Pathogenesis is almost exclusively by vertical transmission in the perinatal period. Clinically, there is a bimodal curve in which infants infected early have a high mortality rate, within 10–12 months after birth, similar to what is seen with opportunistic infections and encephalopathies. A meta-analysis of individual longitudinal data for 3,941 children from eight cohort studies and nine randomized trials in Europe and the USA concluded that in children >2 years of age, receiving no ART or AZT monotherapy only, the risk of death increased sharply when the CD4 percentage was <than 10%, or 15% for risk of AIDS, with a low and fairly stable risk at a greater CD4 percentage. The risk of progression to AIDS or death for a stated value of CD4 percentage was highest in young children – such as, an approximately threefold higher risk of AIDS in a 1-year-old child than in a 5-year-old child, and a difference of about sixfold for death [161]. Independently of treatment, children with low CD4 cell counts (<15%) and high viral loads (≥250,000 copies/ml median value) had the worst survival rate; children with high CD4 cell counts (≥15%) and low viral loads (<250,000 copies/ml) had the best survival rate [531]. Among 122 infected children tested, 33% were severely immunosuppressed ($CD4^+$ cells <15%), 42% were moderately immunosuppressed ($CD4^+$ cells 15%–24%), and 25% had no immunosuppression ($CD4^+$ cells 25%) [531]. Other children in whom the infection appears later have a higher survival rate >18 months [89], up to 6–10 years of age [265], but there are perinatally infected children who may progress as slowly as many adults [355].

The term *"infected"* refers to people who have been confirmed as HIV-infected, as demonstrated by a direct viral assay and/or by the development of a clinical picture of AIDS. The term *"antibody-positive"* can indicate a person who has tested positive to an HIV antibody assay, bearing in mind that this test does not demonstrate that a newborn is infected but that the baby *may* be infected [324]. Infection is defined as *in utero* when there is a positive HIV-1 culture in the first 48 h, and as *intrapartum* if virus cultures are negative during the 1st week and positive afterwards [504].

Epidemiology

Since 1983–1984, when the first cases were described in children through receipt of contaminated blood transfusions [17, 182, 222, 477], the number of reports grew exponentially until 1986, after which the upward trend leveled off. Above all, this increase involved children infected by vertical transmission in the perinatal period, accounting for >90% of cumulative pediatric cases [15] and virtually 100% of new cases [34, 169]. However, the development of effective preventive and therapeutic strategies may have reduced the number of newly infected infants, therefore changing clinical outcomes in infected children [528]. The number of children in whom AIDS attributed to perinatal HIV transmission that was diagnosed peaked in 1992 at 954 and declined 89% to 101 in 2001 [94]. At the same time, Italian cases (0–19 years) were 0.057% of the total [98]. It was foreseen that by the year 2000 there would be 6×10^6 pregnant women and from 5 to 10×10^6 children infected with HIV [490]. Figure 5.26 shows the current rates of newly infected children <15 years of age around the world; Fig. 23.10 shows the number of children <15 years estimated to be living with HIV as of the end of 2003, with a total of $2.1–2.9 \times 10^6$ children involved while 500,000 children had died [549].

It has been calculated that the rate of HIV-2 infections accounts for 1%–2% of all cases of pediatric HIV infec-

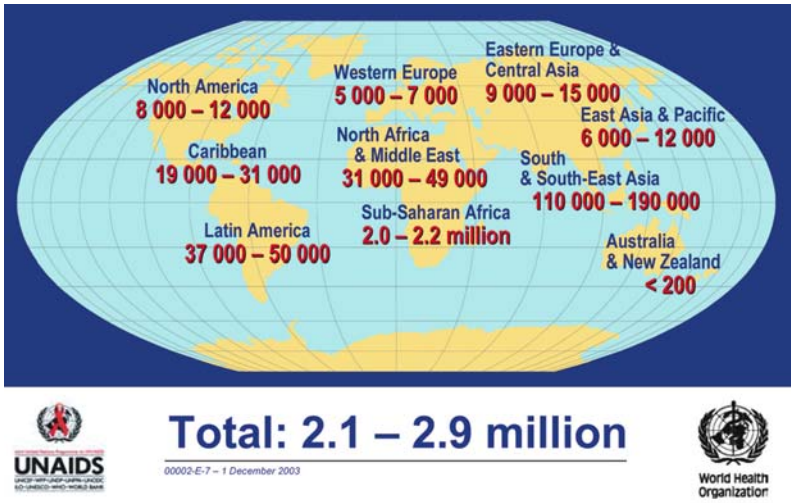


Fig. 23.10. Number of children (<15 years) estimated to be living with HIV/AIDS as of the end of 2003

tion [183]. During the period 1985–1993, pediatric AIDS incidence rates increased in most European countries and leveled off thereafter [138], but perinatal AIDS cases decreased by 75% between 1992 and 1998 [336], and in Italy between 1994 and 2003, the rates dropped to 2.9% [98]. In a few countries (Germany, Greece, Denmark, Austria, and The Netherlands), AIDS incidence rates were $<2 \times 10^6$ children in 1994–1996 [138]. In Romania AIDS has been diagnosed in 40% of enrolled children (a high rate due to hospital HIV transmission) and 52% are receiving antiretroviral therapy (ART) [299]. In Western Europe, where effective treatment is widely available, only 6,000 people died of AIDS in 2003 [549].

Since the mid- to late-1990s, the pediatric HIV epidemic in industrialized countries has witnessed a dramatic decrease in the frequency of mother-to-child HIV transmission, alongside that development and implementation of guidelines for PCP prophylaxis and availability of highly active ART. Fewer children are at risk for clinical progression of HIV disease and other severe manifestations of HIV infection, and most opportunistic infections in children now have lower rates [528]. In children with HIV infection in the UK and Ireland, mortality, AIDS, and hospital admission rates have declined substantially since the introduction of three- or four-drug ART in 1997 [528].

As infected children are living longer, there is an increasing need to address their medical, social, and psychological needs as they enter adolescence [212]. In Italy, in 2004 there were 18 pediatric cases, 12 by vertical transmission and six by undetermined causes [98]. The burden remains greatest in Africa, although it is home to only 11% of the world's population. Eight of the nine countries that have the most HIV-infected people in Africa are South Africa (5.3×10^6), Nigeria (3.6), Zimbabwe (1.8), the United Republic of Tanzania (1.6), and the Democratic Republic of Congo, Ethiopia, Kenya, Malawi and Mozambique (>1). Of an estimated 2.1–2.9 million infected children <15 years of age at the end of 2003 [215], 90% were in Africa, where Nigeria alone had 290,000 infected children and South Africa 230,000. As of the end of 2003, 700,000 (590,000–810,000) children <15 years of age were newly infected with HIV, about 2.5 million (2.1–2.9 million) were living with HIV/AIDS, and 500,000 (420,000–580,000) had died [215], including 440,000 in sub-Saharan Africa. Moreover, despite the availability of effective ART, about 630,000 infants contract HIV infection from their mothers each year, including 550,000 in sub-Saharan Africa [549]. In other parts of the world, the general incidence of AIDS is spreading rapidly in New Zealand [427], Asia [42, 281, 284, 307, 480], Africa [167, 362, 469, 572], Central America [357, 358, 432, 447] and South America [79, 179, 557]. In Brazil the AIDS incidence declined from 14.7×10^5 in 1996–1997 to 9×10^5 in 1998–2000 [376]. The incidence of HIV infection is increasing at an alarming rate in southern and eastern Asia, where large *numbers of children are orphans of parents who died of AIDS* [255] and more than 1 million new infections were expected [191]. The prevalence of HIV-1 is increasing most rapidly in sub-Saharan Africa, where an estimated 4×10^6 new infections occurred in 2001, especially in children [192]. About 2,000 new HIV infections a day in 2002 are in children <15 years of age, 95% of them live in developing countries [549].

Natural History

The information available is scarce: 50% of infected newborns develop symptoms within the first 5 months after birth [122]. Of these, 75% survive to the age of 5 and 6% have not yet developed symptoms at this age [265]. According to other studies, 15%–31% or 29%–59% develop the infection before their first birthday, 79% by 23 months and 27%–40% by the age of 4; 5%–16% die before they are 1 year old and 16%–41%

before the age of 5 [177, 229]. According to CDCs [92], among perinatally infected children aged 0–2 years diagnosed between 1982 and 1977, 60.7% were aged 0–5 months. Of 1,064 pediatric admissions in March 2000, 187 (18.9%) were HIV-infected, and most common in children aged <6 months, 53 of 166 (32%). Almost 30% of HIV-infected children died, compared with 8.9% of uninfected children [466]. Over a 10-year period, 42 children with HIV infection were admitted to an English ICU, mainly for respiratory failure, due either to PCP (45% of cases) or to other respiratory pathogens (32%). Half of the children died, and over 80% of the survivors had good outcomes in terms of growth and development [126]. Recent data demonstrate that Italian children aged 0–19 years totaled 3.7% in 1989, 1.9% in 1993, 0.2% in 2002 and 0.003% in 2004; these decreases, recorded above all in 2004–2005 for both genders, could be attributed to the effectiveness of information and prevention campaigns and, at least in part, to an improved application of guidelines for treating pregnant women [98]. We can also view the reduction in PCP diagnosis along these lines, ascending from 16% in 1982–1992 to 42.9% in 2004–2005 [98]. With regard to HIV-2 infection, 5–7 children reached the age of 8–13 years [183].

Pathogenesis of HIV Transmission in Childhood

Although mother-to-child transmission (MTCT) is the leading source of infection for children (vertical transmission), we cannot omit the fact that the primary modes of transmission of HIV have changed little over the years: unprotected intercourse, unprotected penetrative sex between men, injection-drug use, unsafe injections and blood transfusions, and transmission from mother to child during pregnancy, labor and delivery. Direct blood contact, such as the sharing of drug-injection equipment, is a particularly efficient means of transmitting the virus [523], and a small, residual number is caused by blood transfusions (2%) of the cumulative total from July 1999 to June 2000 [93], or 0 cases since 1998 [98]. As far as MTCT is concerned, HIV-positive mothers who already have an infected child are at a higher risk of transmitting AIDS, and the pregnancy itself seems to aggravate the mother's infection [104].

Vertical Transmission

The possibility of vertical transmission was first reported in 1984 and was confirmed by isolating the virus in cervicovaginal secretions [111, 243], the placenta [244], amniotic fluid [400] and the uterine cervix [441] of infected mothers, and in the gastric aspirates of their children [406] and, as we will see in the next section, in abortuses of different ages. An extreme case is the transmission of *P. carinii* infection to the fetus [395]. The risk of vertical transmission has been calculated to be between 15.3% and 19% in Europe [177, 229, 360], rising to 21% in Africa [195]. Rates of 25.5% [121] involve a particularly selected population, but in the US this is a normal rate [15, 140, 306]. Higher figures can come from samples that are not uniform in age, along the lines of the different sensitivity to the tests (HIV DNA PCR = polymerase chain reaction) [306]. In a US study, 35.7% of vertically infected children contracted AIDS, with a 50.3% mortality rate [140]. Recent Italian data is summarized in Table 23.13 [98], corresponding to CDC data for the cumulative period of 1982–1998 and for 1998 in particular [92]. In the case of HIV-2, the percentage totals 1.2% [183] or a maximum of 2%±4% [195].

Intrauterine Transmission

HIV-1 vertical transmission is thought to take place in utero, at childbirth or thereafter via breast-feeding (BF). We will examine these cases (Table 23.14) [67] (see also "Differential Diagnosis").

The mechanism of intrauterine transmission is assumed to be transplacental: cited as evidence are studies on tissues of fetuses aborted in the first trimester, between the 10th week and the 2nd trimester [274, 319, 518], in which HIV was identified by means of cultures and PCR, which amplifies it and shows the proviral sequences in the DNA of the peripheral blood mononu-

Table 23.13. Cumulative pediatric AIDS cases (<1994–2005) by mode of transmission, diagnosis and sex in Italy

Type	2004–2005[a]	Total (%)	Males (%)	Females (%)
Vertical transmission	3	690 (93.0)	332 (90.2)	358 (95.7)
Hemophilia	0	15 (2.2)	15 (4.0)	0
Transfusion	0	12 (1.8)	5 (1.4)	7 (1.9)
Undetermined	1	25 (3.4)	26 (4.3)	9 (2.4)
Total	4	742	368	374

[a] Total number of cases on June 30, 2005.
Data from [98].

Table 23.14. Potential factors influencing mother-to-child HIV transmission

Fetal/placental	Prematurity
	Chorioamnionitis
	Infant host-immune response
Maternal	Clinical advanced AIDS
	Primary HIV infection
	Coinfection
	First-born of twins
	Obstetric events
	Timing of infection
Viral	Viral load
	Cell-associated
	Cell-free
	Phenotype
	Syncytium-inducing
	Tropism
	Genotype
Immune	Decreased CD4 count
	Humoral
	Neutralizing antibody
	ADCC
	gp120 V3 loop antibody
	Other
	Cell-mediated
	Cytotoxic T lymphocytes
	CD8 suppression
	Mucosal immunity

Modified from [67].

clear cells (PBMCs) [129, 348, 511], even in tissues without material contamination [129]. Studies provide an explanation of the virus passing through the placental barrier during *in utero* HIV-1 vertical transmission, showing that placental trophoblasts could be infected by HIV-1 by a mechanism involving T cells in placental contact. Moreover, placental infection enhanced CD54 (ICAM-1, intracellular adhesion molecule 1) expression and leukocyte adherence, an event which was required to transfer HIV-1 infection to T cells [22]. HIV has also been detected in the fetal thymus [129, 379], where at about the 22nd week proviral DNA was found in 2% of fetuses [65]. Given that there is no entry of HIV-1 in the thymus before the 13th week and that gp120 must bind the CD4 molecule before entering the cell, transplacental transmission does not occur before this period [587]. Further evidence in favor of this theory stems from identification of the virus in blood samples collected at birth from the newborn [73]. The absence of direct infection markers (PCR) in 62% of 271 newborns within 48 h of birth [162], the 4 weeks required for sIgA to develop in 60%–92% of infants (Chap. 2) and the seroconversion with IgG that occurs in 65% of cases at about 2 months of age lead to the conclusion that *60% of newborns acquire the infection at birth and that in the remaining 40% transmission takes place during the last 2 months of intrauterine life* [476]. The observation of infants with severe forms of AIDS in the first few months after birth is assumed to be indirect proof of sensitization during pregnancy [387].

Perinatal Transmission

These data complete the discussion on vertical transmission.

The literature now contains extensive evidence indicating that in a large proportion of children, the infection is acquired at delivery [73, 179, 216, 303], Perinatal transmission was and is *the predominant mode of HIV acquisition for an uncountable number of children,* 649 in one report [351], 944 in another [212], and up to 94.9% of children [179]. A study on twins at risk further demonstrated *intrapartum transmission.* In this case, the infection shows an 80% concordance [141, 159], but the outcome is different: for example, one twin is infected and the other is not, and of them the firstborn is more likely to be infected (Fig. 23.11) [159], regardless of whether delivery is vaginal or by cesarean delivery, as the newborn is the first to encounter the infected barriers formed by contaminated maternal blood and genital secretions [159, 216, 377]. Focus is on preventing virus transmission from an HIV mother to her newborn during the prenatal, intrapartum, and postnatal periods [377]. The differences can be prenatal with 25%–35% of total transmission, occurring mainly in the late pregnancy, or intrapartum: 70%–75% of total transmission. The postulated mechanisms are microtransfusion from the constant massage the placental bed gets from uterine contractions, or exposure of the baby's mucocutaneous surface to maternal blood and cervical secretions [277].

Research has been conducted on the behavior of high-affinity/avidity maternal antibodies directed to neutralizing gp120 or gp41 V3 hypervariable region epitopes [149, 217, 237, 550], but the results differed. Other authors have investigated the mother's condition, documented by immunological impairment, viral load, immune response to the virus, viral phenotype, integrity/non-integrity of the placental barrier and other factors [74, 175, 199, 256, 309, 337, 472, 478, 519], but without reaching definitive conclusions. However, it seems likely that a *high viral load during pregnancy,* regardless of the stage of the expectant mother's illness, *increases the risk of a rapidly progressive AIDS in her children* [312]. It must be pointed out that high autologous levels associated with a very low viral load are also found in

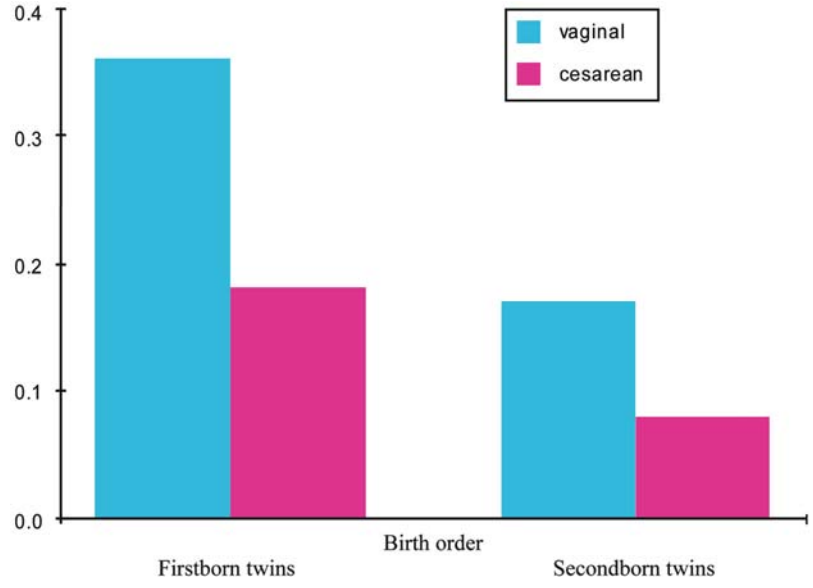


Fig. 23.11. Prevalence of HIV-infected newborns by birth order and delivery route (vaginal or cesarean). (Data from [159])

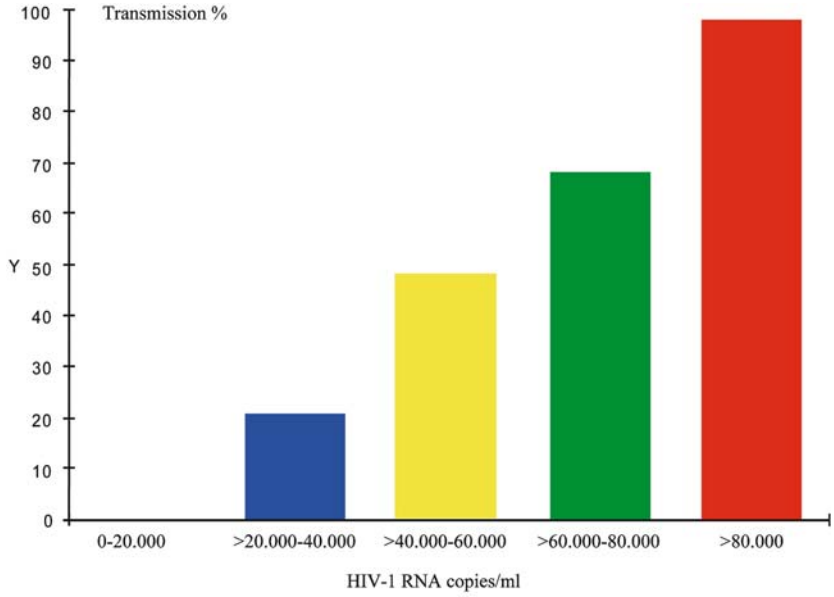


Fig. 23.12. Perinatal transmission rate as based on HIV-1 RNA number of copies at delivery. (Data from [152]

asymptomatic subjects for long periods of time [85, 423]. Relatively few studies have examined the role of factors more closely bound to delivery [74, 176, 305, 315, 383, 519]. The parameters of 525 mother–child pairs significantly correlated with HIV-1 transmission are summarized in Table 23.15, specifically the chronology of membrane rupture [315], which has arisen as a critical factor for mother-to-infant HIV transmission [306]. Based on previous discussions, it seems obvious that the less time it takes to deliver the child, the more difficult it is for the virus to infect the baby. Consequently, *cesarean delivery* should be favored [229]. However, the duration of labor is not correlated with the risk of transmission [315]. There was no correlation in a retrospective study concerning maternal HIV risk factors, mode of delivery, the child's gender, birthweight, gestational age and age at symptom onset [200]. In a prospective study, transmission was lower with ART, cesarean section, greater birthweight, and higher CD4 cell count [260]. What emerges upon examining maternal factors more strictly is the association with an RR of 1.5–1.6 even if the mother's age at delivery is ≥30 years, as opposed to data on the use of alcohol or tobacco [315]. The increased risk of MTCT depends not only on the viral load and its characteristics, but also on the maternal clinical conditions declining during pregnancy, the reduced number of circulating $CD4^+$ cells (RR between 1.6 and 2.1) [315, 519, 541] and malnutrition, including vitamin A deficiency (Chap. 21). It is the increased viral load during pregnancy and/or at delivery that is significantly associated with the risk of transmission [55, 152, 180, 516] (Fig. 23.12) [152], also because people who have recent-

Table 23.15. Analysis of 525 mother–infant pair variables significantly related to HIV-1 transmission

Variable	RR (CI 95%)	*p*
Gestational age >37 weeks	1.0	0.02
Gestational age ≤37 weeks	1.61 (0.74–1.52)	
Birth weight ≥2,500 g	1.0	<0.001
Birth weight <2,500 g	1.98 (1.32–2.85)	
Rupture of membranes (spontaneous)	1.0	0.01
Rupture of membranes (artificial)	2.13 (1.29–3.51)	
Duration (hours)		
<4	1.0	0.002
″4	1.82 (1.25–2.64)	
<1	1.0	<0.001
>24	3.40 (0.20–5.72)	
No hard-drug use during pregnancy	1.0	0.008
Hard-drug use during pregnancy	1.62 (1.15–2.20)	
Mean CD4 level during pregnancy >29%	1.0	<0.001
Mean CD4 level during pregnancy <29%	2.15 (1.47–3.14)	
Mean CD8 level during pregnancy <50%	1.0	<0.001
Mean CD8 level during pregnancy ≥50%	1.89 (1.31–2.74)	
Negative HIV culture at delivery	1.0	0.005
Positive HIV culture at delivery	2.13 (1.20–3.78)	
Maternal age at delivery <30 years	1.0	0.02
Maternal age at delivery ≥30 years	1.51 (1.07–2.13)	

Data from [315].
RR relative risk, *CI* confidence intervals.

ly been infected have high titers of circulating HIV and can be highly contagious [7, 271]. Although there is no minimum threshold below which one is safe [516], perinatal HIV-1 transmission occurs in only 1% of treated women with RNA virus loads <1,000 copies/ml and may be almost eliminated with ART prophylaxis accompanied by suppression of maternal viremia [260].

Postpartum Transmission

It has been repeatedly hypothesized that HIV can be transmitted from mother to child *through breast milk*. This disregards that BM of both HIV-positive and -negative mothers contains factors that inhibit CD4 binding to gp120 in the HIV envelope [404]. According to data found in the literature, HIV has been isolated from human milk [539] and postnatal transmission of infection has been suggested by two clinical cases [473], but this was not documented [78], since in several cases the mother apparently acquired AIDS from a postpartum blood transfusion [118, 327, 524, 574, 596]. In five other cases, two had the last PCR^+ at birth or at 12 months and another child became positive at the age of 30 months [553]. Aside from this, the low percentage of cases in other studies [25 (1%), 47, 173 (5%), 199 (3.9%), 264 (9.5%)] can lead to a type 2 error. Recently, several studies on larger cohorts have concluded pro [130] or against BM [164, 168, 328, 384, 474], including breastfeeding >15 months [168], mostly in non-European children. Nearly all infants in developing countries are initially breast-fed, and most children continue to receive some breast-feeding until at least 6 months of age but frequently into the 2nd year of life, especially in sub-Saharian Africa and Asia [137]. Recent papers have summarized the current state of knowledge on breast-feeding transmitting HIV infection [270], and highlighted the outstanding issues that will need to be addressed in the very near future before research advances can be translated into public health practice [137], by modifying infant feeding practices [468] and by preventing mother-to-child transmission by passive and active immunization strategies [484], or by means of vaccines studied on newborn and infant macaques [201].

A multicountry African perinatal trial (PETRA) among breast-feeding HIV-1-infected women has shown

that AZT and 3TC administered starting at 36 weeks' gestation, given orally intra- and post-partum for 1 week to both mother and infant, reduced transmission at age 6 weeks by 42% and by 18% at 18 months compared with placebo [433]. In the HIVNET 012 randomized trial with 99% of babies breast-fed (median duration, 9 months), intrapartum/neonatal NVP (200 mg at labor onset and 2 mg/kg for babies within 72 h of birth) significantly lowered HIV-1 transmission risk in a BF population in Uganda compared with a short intrapartum/neonatal AZT regimen (600 mg orally at labor onset and 300 mg every 3 h until delivery, and 4 mg/kg orally bid for babies for 7 days). The absolute 8.2% reduction in transmission at 6–8 weeks was sustained at age 18 months (10.1%) [268]. BM-associated risk may also be of late postnatal transmission [164, 328]. Prolonged breast-feeding by an HIV-infected mother doubles the overall risk of mother-to-child transmission of HIV from <20% to as much as 40% [137]. Exclusive BF carried a significantly lower risk of HIV-1 transmission than mixed feeding (hazard ratio [HR], 0.52 [0.28–0.98]) and a similar risk to no BM (HR, 0.85 [0.51–1.42]) [130]. At 3 months, the difference in HIV transmission between all BM fed or never BM fed was 2.5% [130]. The risk for transmission predicted by the Malawi study was *higher within the first 6 months of life than later:* 0.7% during age 1–5 months, 0.6% during age 6–11 months, and 0.3% during age 12–17 months [384].

We underline that in addition to high viral titers in recently infected mothers [7, 46], following desquamation that starts 24 h after birth, the newborn's *stratum corneum* is just 0.01–0.05 mm thick, as opposed to 0.1–0.9 mm in adults, thus it is easily vulnerable to the virus found not only in BM but also in the blood and secretions of an HIV^+ mother in close contact with her child [112]. If the mother contracted HIV infection during delivery or in the perinatal period, BM can create a higher risk for the newborn compared to the risk yielded by mothers who have had the infection for some time [135, 553], as confirmed by a meta-analysis [387]. Consequently, the risk of infant infection from BF is influenced by an elevated BM HIV-1 RNA, which is highest early after delivery: in BF women, a twofold-increased risk of transmission was associated with every tenfold increase in BM virus load [475]. This is the dilemma to solve, as randomized double-blind (DB) studies are excluded for obvious ethical reasons [324]. Nor can it be settled by a serious statistical analysis, since it is based on an odds ratio (OR) over 1 (1.19, CI 95% 1.10–1.28) [142]. Therefore, we feel that the problem is limited, given that the vertically infected newborns account for only 0.4% in 2005 [98]. However, before current BF recommendations can be changed, the assertion that exclusive BF is associated with a lower rate of perinatal HIV transmission compared to bottle-feeding [130] should be further analyzed. If the test for viral RNA is already positive at birth (39% of newborns), it is clear that infection must have occurred *in utero* (61%) [152]. $HIV\text{-}1^+$ mothers have been advised against BM in the so-called industrialized countries [324], and the CDC, WHO and UNICEF will invest major resources in formula feeding and little in heat treatment of expressed BM to kill the virus, wet nursing, and donation and even sales of BM [320]. More precisely, while <5% of the 2,807 children in four studies from industrialized countries were breast-fed and no HIV-1 infection was diagnosed, by contrast, late postnatal transmission occurred in 49 (5%) of 902 children in four cohorts from developing countries, in which BF was the norm, with an overall estimated risk of 3.2 per 100 child-years of BF follow-up (95% CI 3.1–3.8) [328]. However, this is a massive burden: of the estimated 700,000 children who were infected with HIV in 2003, about *315,000 (45%) were infected through breast-feeding* [549]. Current UN recommendations state that "When replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all BF by HIV-infected mothers is recommended. Otherwise, exclusive BF is recommended during the first months of life and should then be discontinued as soon as it is feasible." HIV-1-infected women should therefore make a decision regarding formula feeding on the basis of safety, feasibility, and affordability of formula [577]. In South Africa, commercial infant formula is the only replacement milk that meets all nutritional needs of infants aged <6 months. Revisions of WHO/UNAIDS/UNICEF HIV and infant feeding course replacement milk options are necessary [424].

Other Pathogenic Factors

In Italy, the problem of drug addicts continues to be an open issue, as they account for 57.2% of AIDS patients [98]. The impact of this factor is demonstrated by rates of 75% of vertically infected pediatric cases, 52.9% of children of female drug users and 45.5% of children of mothers who acquired the infection heterosexually; 37.8% of the women had a drug-using partner, who may or may not have been infected [98]. Sex was engaged in with an injecting-drug user in 14% of cases [93]. No hemophiliac children were included in 2002, the last of whom were in 1992, as well as one from unspecified causes (0.13%) [98]. In the US, the rates in the 1995–1997 period were 20% for drug use and 37% through heterosexual contact [92]; in the 1998–2000 period, they were 30.5% and 1.8%, respectively [93]. Other data on related problems are summarized in Table 23.16 [46, 179, 225, 411, 416, 464, 540, 545]. Drugs are essentially destructive: in vitro they increase viral replication, can modify the immune response, foster premature birth and can cause placental damage [462]. If drug use continues throughout the pregnancy, the woman presents a higher viral load (expressed as the greater risk to test positive), and she demonstrates a higher probability of transmitting the infection to her child, associated with an RR of 1.5–1.9 [315, 462]. Interestingly, individuals

Table 23.16. Impact of drug addiction in different countries

References	[411]	[540]	[46]	[416]	[464]	[225]	[545]	[179]
Features (%)								
Drug addicted father/mother	75	37		59	48	85	72	73
Maternal promiscuity	25						26	
Mother with AIDS		6	43					
Heterosexual father					3	3		
Father with AIDS		3	29					

Table 23.17. Transfusion-associated risk of HIV transmission

Risk	Notes	References
1:360,000	95% CI, 210,000–1,140,000	[308]
1:450,000	In the window period	[308]
1:450,000–1:660,000	Including Red Cross data	[308]
1:493,000	95% CI 202,000–2,778,000	[497]
1:1370,000	French Society of Blood Transfusion	[437]
1: 2,135,000	RR estimates in donations from repeat donors after NAT Red Cross data	[153]

CI confidence intervals.
One child (1%) was reported by the CDC in 2000 [59].

having multiple sexual partners (78.2%), drug addicts (38.8%), homosexuals (39.2%), commercial sex workers (52.2%) and health care workers (16.2%) were identified as high-risk groups among 600 female college students [181].

Another way of contracting HIV infection, via a blood transfusion, was responsible for a considerable number of cases of AIDS in the early years of this pandemic [80]. Several newborns contracted HIV through a single transfusion of infected blood [318, 489]. It is rare that a child who received a blood transfusion has instead infected the mother [87]. A possible blood transmission has been set mainly in relation to the so-called window period: in fact, it is not always possible to confirm that a subject has recently been contaminated by HIV, such as when the person is viremic but not HIV-positive. The danger dropped substantially following the introduction of more specific measures to reveal the presence of anti-HIV antibodies in donors and in blood derivatives [308, 497], but it may be found in HIV-2 infected children [183]. This transmission rate ranged from 1:450,000 to 1:660,000 according to extensive studies [308, 497], but it can also be 1:2,135000 [153] (Table 23.17) [59, 153, 308, 437, 497]. Italian figures from the end of 1994 indicate that the number of pediatric cases caused by blood transfusions or blood derivatives was over 5% [265], and it is currently zero (the last case was in 1996) [98]. It was estimated that there were 2% [393] or 3% [363] of transfusions of contaminated blood-transmitted HIV, and five cases were reported during a 4-year period [304]. The introduction of NAT (nucleic acid amplification technology) screening [153] and HIV-1 inactivator PEN110, which reduces HIV-1 to the limit of detection by targeting the viral nucleic acids [405], attests that the remaining risk of transfusion-related transmission of HIV could be greatly reduced.

Other potential causative factors demonstrated by multiple logistical regression analysis include birth in regions characterized by a high cumulative rate of AIDS and in metropolitan areas [262]. HIV has also been isolated several times in the tears, saliva, feces and cerebrospinal fluid (CSF) of subjects at risk, even though no association was documented with the horizontal transmission of AIDS [123, 197, 533, 539]. The horizontal route has been suggested by rare pediatric cases, which are not well documented, on transmission, for example, after being bitten – without bleeding – by a sibling living in the same home [567]: to avoid any perplexities, we must note that the history was reconstructed retrospectively and it is not at all certain if the child was HIV-positive before biting his brother.

Several authors have studied IgE concentrations in HIV^+ children, noting a possible protective role [500] or, more frequently, a marker associated with PCP and infections spread by CMV (cytomegalovirus) [165] or with the progression of AIDS revealed by the increase in bacterial infections and the vertical drop in CD4 levels [562]. Instead, atopy, evaluated by family history, PTC or RAST, has no correlation with IgE titers [165, 297, 562].

Clinical Presentation

In vertically infected infants, ID manifests itself very early, and the disease has characteristics which markedly differentiate it from adult AIDS, with severity inversely proportional to the age when symptoms begin and with rapid progression [46, 177, 360, 540], accentuated by high levels of p24 antigen [23, 54, 360]. In addition to what we noted previously, it is thought that *the average age at onset is 5.2 months* [200]; 15%–20% contracted a

Table 23.18. First symptoms in infants with AIDS which should draw the attention of parents and pediatricians

References	[81, 83][a]	[177]	[225]	[331]	[267]
Mean age (months)	6.5	1–11	⩽12	9.3	0–12
Hepatosplenomegaly	87				
Hepatomegaly		59			58
Splenomegaly		88			62
Failure to thrive	82	82	57		39
Lymphadenopathy	72	82	61		69
Fever	62	70	54		29
Lymphoid interstitial pneumonia	69	29	63	30	
P. carinii pneumonia	40	44	43	36	
Widespread CMV infections	28		25	18	
Recurrent bacterial infections					5
Opportunistic infections					11
Recurrent oral candidiasis	62	76	68	34	
Persistent and/or recurrent diarrhea	50	53	32		23
Neurological disease	44	47	7	9	18

[a] Meta-analysis of previous data.

severe form of AIDS in the first 12 months of life [49], and 32.4% of these infants died at an average age of 4.9 months [177], 9%–17% at an average of 12 months [177, 499, 544] and 29.5% at 13 months [200]. The course of disease in infected children enrolled in two European prospective studies of infants born to HIV-infected women has been studied: 20% of these children progress rapidly to AIDS and die between the ages of 2 and 4 years, whereas the majority progress more slowly, with a median survival time of 8 years. Progression to CDC group C disease or HIV-related death is an estimated 20% during the 1st year of life, and 4.7% per year thereafter, giving a cumulative incidence of 36% by 6 years [50]. In 11 European centers >15% of infected children will progress to category C or death by age 1 year and nearly 50% by 10 years, and <20% of children will show severe ID by age 1 and 75% by 10 years. However, the prognosis has improved with more widespread availability and use of combination ART [224].

Pediatric infection has characteristics that clearly distinguish it from the disease in adults (Tables 23.11, 23.12): a more severe progression and a wide variety of significantly different associated pathologies. The frequency of initial symptoms, in infants aged 2.5–12 months, that should arouse the attention of parents and attending physicians is summarized in Table 23.18 [81, 83, 177, 225, 267, 331]. In infants, the pleomorphic clinical manifestations are initially predominated by polyadenopathy and hepatosplenomegaly and by delayed growth. Between 6 and 12 months, infected children grew on average 1.6% less in height and 6.2% in weight than uninfected children, who by 10 years were on average an estimated 7 kg heavier and 7.5 cm taller than infected children, with no difference before and after use of ART prophylaxis [405] (Table 23.18). In 10% of cases, the onset is associated with gastroenteric manifestations such as abdominal distension, diarrhea, malabsorption and malnutrition [81]. This picture evokes a subpopulation of children with an immune phenotype characterized by evident thymic dysfunction (Fig. 23.13) such as what is seen in DiGeorge syndrome [296]. On the one hand, this recalls the thymic lesions observed in the fetus [425]: in infants

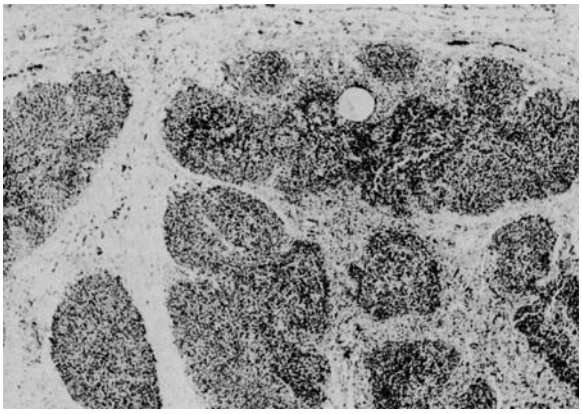


Fig. 23.13. Early involution of thymus. The loss of corticomedullary demarcation should be noted, as should the virtual absence of lymphocytes, particularly in the cortex and marked quantitative reduction in the Hassall's corpuscles: only one dilated is seen (magnification ×100)

infected in the perinatal period, the virus damages the immune cells involved in ontogenesis and the immune mechanisms, which are devoid of previous antigenic experiences and are instead physiologically oriented toward tolerance (Chap. 2). In older children, the common clinical manifestations at presentation included oral candidiasis (43%), pulmonary tuberculosis (35%), recurrent respiratory infections (RRI) (26%), bacterial skin infection (21%), papulopruritic dermatitis (19%), hepatosplenomegaly and lymphadenopathy (14%) each and chronic diarrhea (7%) [349], as well as mucocutaneous findings such as oral candidiasis (33%), *herpes zoster* (6%), chronic *herpes simplex virus* (HSV) (3%) related to a degree of immunosuppression [569]. In a group including children as young as <15 months, the clinical signs were predominantly cutaneous lesions (59%), diarrhea (41%), fever and malnutrition (35%) each, as well as oral moniliasis, sepsis, esophageal candidiasis, otitis, and varicella in different patients [133]. In 187 children, clinical symptoms and signs were not adequately sensitive or specific. HIV was common in children with malnutrition (40%), lower respiratory tract infection (LRTI) (29%) and sepsis (28%) [466].

Respiratory Infections

Recurrent infectious episodes do not differ much from the ones that characterize primary immune deficiencies (PID) (Chap. 22). Typically, the subjects are infants and small children with serious infections borne by agents that are rarely (or never) very pathogenic such as *Candida albicans*, *Pneumocystis carinii*, *Toxoplasma gondii*, CMV, HSV, *varicella zoster virus* (VZV), EBV, atypical mycobacteria [*Mycobacterium avium-intracellulare* (MAIC)], *Streptococcus pneumoniae*, *Streptococcus aureus*, etc. [411]. Respiratory infection is the leading cause of death, accounting for 29 of 35 (83%) deaths among HIV^+ and 8 of 12 (67%) deaths among HIV^- children [21]. Over half of cases start with respiratory manifestations, in 53% of cases with PCP [464], among the leading causes of morbidity and mortality in infants and young children with AIDS [21, 46, 81, 83, 182, 225, 411, 464, 477, 540, 545]. Children with PCP died 1 month after diagnosis [499] or at the age of 3–4 months [177], and in 59% of them HIV was identified about 30 days prior to PCP diagnosis [505] (Fig. 23.14). PCP is the most severe pulmonary manifestation (Fig. 23.15), observed earliest and most frequently, is marked by an acute onset, thus responsible for 48% of deaths in infants ≤1 year [21]. It is characterized by fever, cough, tachypnea, dyspnea, hypoxia, blood gas analysis indicative of alveolocapillary block, subcrepitant rales and prolonged exhaling [91]. Thirty children with a median age of 10.4 months experienced respiratory insufficiency, in 13 (43.3%) due to PCP, in six (20%) to CMV and in five (16.6%) to *Pseudomonas aeruginosa* [559].

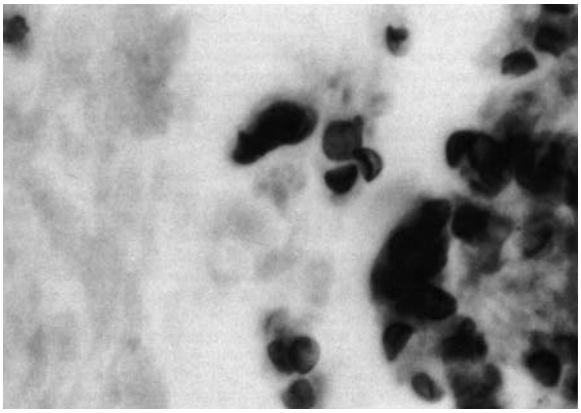


Fig. 23.14. *Pneumocystis carinii* pneumonia characterized by the organism in the alveolar lumen (magnification ×400)

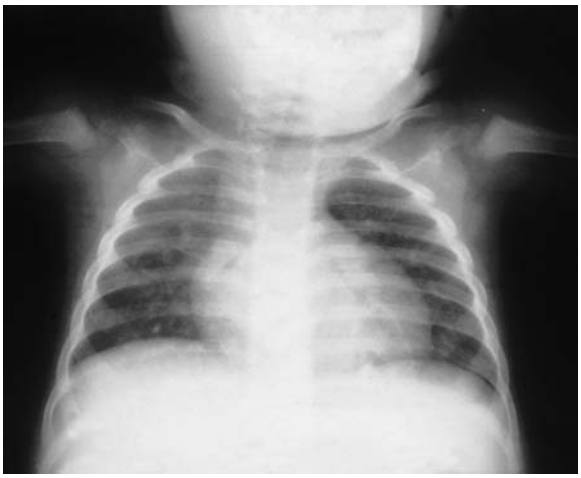


Fig. 23.15. Chest radiograph of HIV-infected infant with *Pneumocystis carinii* pneumonia

The frequency of infections from capsulated bacteria may depend on the scarce repertory of specific antibodies established by young children to these organisms present in most adults when they become HIV-infected [496]. CMV infection is often the first manifestation, because virus exposure can occur during delivery, with disseminated forms in 18%–28% of cases (Table 23.18), substantially shortening the survival in these infants, who can even be 2–5 months old [174]. In addition, it can lead to interstitial pneumopathies of unknown origin, lymphocyte interstitial pneumonia (LIP), enteritis and, more rarely, hepatitis, chorioretinitis and encephalitis [56]. MAIC infections, more common among children with a CD4 count <50/mm^3, initially begin with gastroenteric or pulmonary locations, which precede the septic and disseminated forms, with a clinical picture of weight loss, anorexia, lymphadenopathies and splenomegaly [333].

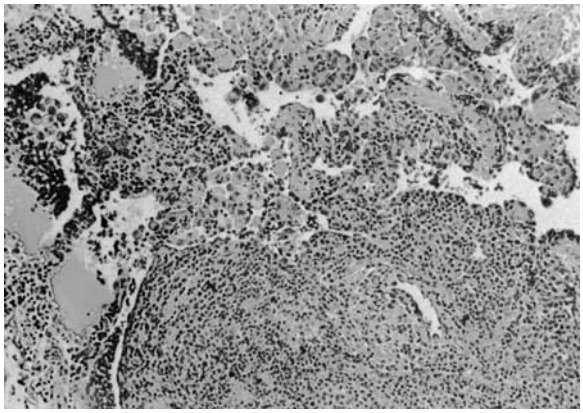


Fig. 23.16. Lung biopsy specimen showing pulmonary lymphoid hyperplasia (PLH)/LIP, with a lymphoid nodule and lymphoid infiltrate extending into alveolar septa. Alveolar PBMCs represent a desquamative interstitial pneumonia-like reaction (magnification ×200)

Among mycotic infections, the earliest one in the child is recurrent oral candidiasis in 34%–76% of cases (Table 23.18), while the invasive forms, also from catheters, are more rare. Other mycoses such as cryptococcosis, cryptosporidiosis and strongyloidiosis are observed more and more often in these young AIDS patients, with proteiform pictures in which meningitis stands out [566]. Cryptococcal meningoencephalitis in African children presents acutely or subacutely, can have a fulminant picture and is consistent with progressive meningoencephalitis. Affected children presented with headache (85%), nuchal rigidity (69%), vomiting (46%), impaired mental status (38%), convulsions (38%) and focal neurological signs (23%) and were significantly more likely than adults to have seizures (38% vs 11%) and normal cerebrospinal fluid protein (67% vs 10%). Convulsions and impaired mental status were associated with increased mortality The in-hospital mortality was 43% [233]. A number of authors have pointed out the incidence of interstitial lung diseases caused by unknown germs, including LIP, as observed in certain PIDs. LIP, which strikes 30%–70% of infants (Table 23.18) and is probably associated with EBV infection [279], is defined as a reticular-nodular pulmonary infiltrate that persists ≥2 months with or without hilar adenopathy, and it does not respond to the usual antibacterial treatments [120]. The initial symptoms are tachypnea (88%), exertional dyspnea (88%), persistent cough (86%) and fever (79%) [120]. From a histological standpoint, it is characterized by interstitial infiltrates of PBMCs and plasma cells distributed around the bronchioles and between the interalveolar septa (Fig. 23.16); these nodules, which can vary in size, tend to be located at the hilar site and extend peripherally [587]. According to CDC, this form is not a cause for concern but it is reported nevertheless.

Neurological Manifestations

As occurs during infection by other *lentiviruses* [82], HIV infection is often distinguished in children – as in adults – by numerous neurological findings, which range from peripheral forms to cortical atrophy that occur early and frequently, similar to the respiratory manifestations [35, 113, 300, 342, 359, 379, 403, 446, 451, 455, 502, 503, 535]. HIV-infected children have an increased incidence of cerebrovascular disease that is associated with severe immune suppression and with vertically or perinatally acquired HIV infection [426]. In infants and children with perinatal infections, neurological locations may be frequent and represent another sign of a poor prognosis. In the youngest, in addition to slow postnatal growth and the arrested acquisition of psychomotor skills (followed by regression), hypotonia is also observed, with bilateral pathological reflexes (foot clonus, persistence of Moro reflex and of the neck's tonic reflexes > the 4th month) [403]. In older children, in addition to foot symmetrical clonus the extensor plantar reflex also persists [379], correlated with the results of EEG examination (diffuse background slowing) [291]. Ensuing microcephaly is associated with pyramidal disorders that become increasingly evident [379], while histological tests show a reduced volume in both hemispheres, the cortex, the white and gray matter, and the basal ganglia [300]. Neuroimaging studies showed cortical atrophy, calcification of the basal ganglia, and toxoplasma abscesses [291], aneurysms, infarctions, and fusiform aneurysms of the cerebral arteries [426]. In most cases, progressive encephalopathy (PE) is observed [359, 451], with mononuclear cells and astrocyte infection [502], associated clinically with apathy, lack of acquisition of motor and intellectual skills, which can be followed by spastic diplegia [35].

The great prevalence of severe neurological dysfunctions and the very early onset of symptoms, starting at age 2 months, in children infected during the perinatal period suggest that *HIV-1 is more neurovirulent in the immature host* [35, 535]. As we noted, the brain can be a site of fetal infection, and thus the damaging mechanism involves structures that are in a differentiating and developing process. As a result, this completes and expands the lesions following birth [587]. If these infants have been infected in utero, it is unclear whether HIV-1 invasion occurs throughout the entire pregnancy (and in which trimester) or in the neonatal period or even later. In other words, does the CNS act as another sanctuary for HIV-1? [35]. Since encephalopathy was not prevented by AZT treatment during gestation [535], this indirectly contrasts the fetal origin. The CNS represents a unique reservoir site for HIV-1 [595]. HIV has been cultivated positively in fetal cerebral cells [274, 518], thus confirming that, in addition to lymphocytes, the brain can also be a primary site for HIV infection *and replication* (Fig. 23.17). Astrocytes can be infected by primary

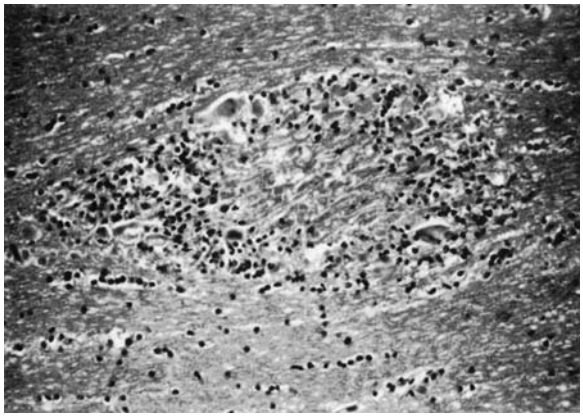


Fig. 23.17. Focus of cerebral necrosis surrounded by inflammatory cell infiltrate (magnification ×100)

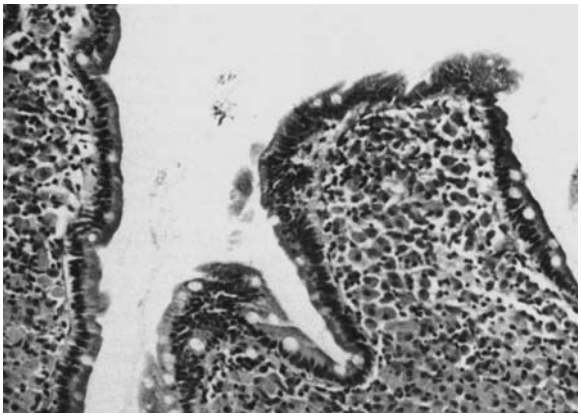


Fig. 23.18. Jejunal biopsy specimen showing diffuse dense infiltration of the lamina propria with pale staining, PBMCs resembling the lesion of Whipple's disease (magnification ×200)

HIV-1 isolates and be potential carriers of latent HIV-1: activated cells may spread the infection to other neighboring cells such as microglia or macrophages [479]. HIV or viral DNA and RNA sequences have been observed in the brain of three infants [503] and nine children [249, 446, 535] with PE, who later died of AIDS. The risk of HIV-mediated PE in children infected in the perinatal period is 4% by age 1 year and 13.9% by age 4 [342]. The comparison with adults shows another difference (Tables 23.11, 23.12). The incidence of encephalopathy was higher in children than in adults during the 1st year (9.9% vs 0.3%) and the 2nd year (4.2% vs 0%) after infection but was similar thereafter (<1%/year in each group). However, the resulting cumulative incidence at 7 years postinfection reached 16% in children and 5% in adults [535], a 3.2-fold difference. Mortality of 58 HIV-1-infected children who developed PE was significantly higher than of children who did not develop PE. Blood $CD8^+$ T-lymphocytes <25% in the first months of life suggest a RR of progressing to PE fourfold higher than those with $CD8^+$ >25% [487]. PE is aggravated by its potential association with cardiomyopathy [342]. These data confirm the *HIV neurotropism* [35, 359, 446, 503], although it is still debated how the virus penetrates the CNS (the HIV-infected macrophage is probably the infection vehicle), and which pathogenic mechanism may underlie the neurological symptoms [250]. The genetic and morphological correlation between HIV and the visna virus, a lentivirus that causes chronic degenerative neuropathy in sheep, certainly suggests that the virus is neurotropic [219]. However, proof comes from the observation of apoptotic neurons in the cortex and basal ganglia of children with HIV-induced PE, associated with inflammatory infiltrates containing HIV-infected macrophages and polynuclear giant cells [209]. Recent studies have investigated the interactions between the immune system and the CNS and have identified the potential role of depression, anxiety and stress in modulating immune responses [533]. This might suggest starting HAART regimens for HIV-1 infection of the CNS early and the need for new therapies either to preserve or to augment an adequate $CD8^+$ T-lymphocyte immune response [487].

Gastroenteric Manifestations

In children with HIV infection, gastroenteric symptoms include poor growth, diarrhea, anorexia, abdominal pain, malabsorption, weight loss and, less frequently, vomiting, dysphagia and death [386]. Diarrhea is reported in 13%–53% children <1 year (Table 23.18). As for pathogenesis, the virus's broad range of damaging actions involves the mucosal CD4 and even nonspecific defenses such as the secretion of gastric acid and peristalsis. Many enteric pathogens, which are often opportunistic, have been implicated in the enteric forms of pediatric AIDS (Fig. 23.18), including infections from *Cryptosporidium, Lamblia, Salmonellae, Isospora belli, Candida albicans* [236] and CMV [272]. CMV causes ulceration, hemorrhages, perforations, intestinal blockage and a high rate of morbidity (particularly because of associated arteriopathies) and mortality [272]. The infections occur by fecal-oral transmission, via contaminated water or food or interpersonal contagion, while the amount needed to produce them varies, but is lower in children with ID; the clinical manifestations triggered by different agents are essentially similar [386]. When the reaction late phase occurs, the functional alterations caused by infections on a cellular level lower the immune system response: these functional losses play a role in the appearance of opportunistic infections [586]. In a cohort of infants born to HIV^+ mothers, the infected infants had 3.2 episodes of diarrhea per year, as opposed to only 1.5 for noninfected infants. Moreover, the symptom incidence peaked in the first 5 months of

life and diarrhea often lasted >14 days, while its persistence was *a marker of HIV rapid progression* [294]. In a study conducted in Zaire among HIV^+ infants, the risk of dying following complications from diarrhea was 11-fold higher than in HIV^- babies [537].

Renal Disorders

In five children infected in the perinatal period, progressive nephropathy associated with HIV was observed between the ages of 6 months and 9 years. From a histological standpoint, focal glomerulonephrosis and mesangial proliferative glomerulonephritis were noted [119]. The prevalence of nephropathy diagnosed via biopsy was 10%, but the renal complications of pediatric AIDS are probably far more prevalent than has been thought to date [119, 499, 527]. The mean age at diagnosis was about 3 years [499, 527], but nephropathy can also be the first symptom [499].

Hematological Disorders

Thrombocytopenia ($<10^5$/µl) is an important blood complication and it can be part of a fulminant clinical picture accompanied by opportunistic infections. However, it can also mark HIV infection onset in infants and children [460]; it is observed in 10.7% of children aged 0–12 months [200]. Anemia (Hb <8 g/dl) is seen in up to 94% of children and can be so severe that it becomes an indicator of AIDS progression [166]. Likewise, *neutropenia* (<1,000/µl), reported in 3.7%–5.4% of cases [200, 544], is *a negative prognostic factor* and, like anemia, it arises regardless of the mechanisms responsible for it. It is notable that the high incidence of blood disorders observed in these children is caused not only by HIV-1 infection, which also precedes the impoverishment of the related precursors, although not everyone is in agreement [390], but also by the production of antibodies and the factors suppressing hematopoiesis that specifically accompany cases of immune deficits.

Cancer

In children with HIV infection, >60 cases of cancer have been reported, two-thirds of which are lymphoid in origin, 90% being non-Hodgkin's lymphoma, with an incidence of 7% in one cohort [272]. Age at diagnosis is between 5 months and 6.5 years, with a median of 31 months [163]. The association with KS has been found in 15 children aged 6 days to 11 years (reviewed in [163]) (Fig. 23.19), and with malignant lymphoma, even in the case of two children dying at age 14 months or 2 years [113, 451]: compared to adults, these complications are quite rare in children. Primary cerebral lymphoma should be suspected if there are convulsions, sensory alterations or focal signs [163]. In addition, smooth muscle tumors can also develop (leiomyomas, leiomyosarcomas) [234, 371], as seen in 14 subjects aged 3–17 (reviewed in [163]), and EBV infection of these cells can contribute to the pathogenesis of these tumors [163].

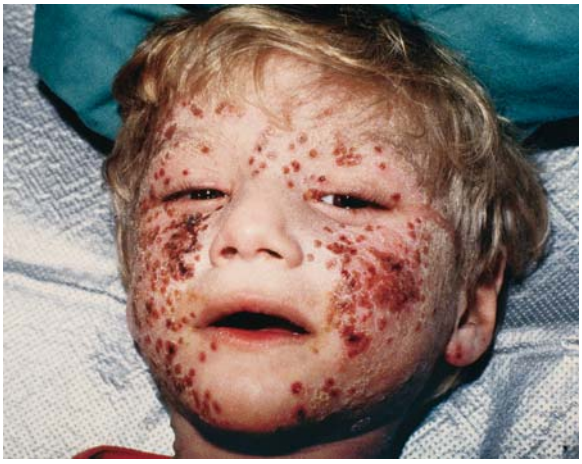


Fig. 23.19. Child with Kaposi's sarcoma

Miscellaneous

We must also mention skin infections caused by *Herpes simplex* (Fig. 23.20) and *zoster* (Fig. 23.21) and *Molluscum contagiosum* (Fig. 23.22). Ocular lesions in pediatric AIDS patients are frequent, including anterior uveitis and CMV retinitis (Fig. 23.23) (33%) followed by retinal detachment (16.7%) and vitreous hemorrhage (16.7%) [43]. Manifestations in children infected by transfusions (Fig. 23.24) or vertical transmission (Figs. 23.20, 23.25) are also characteristic.

As the life expectancy of children with HIV/AIDS increases, *quality-of-life* outcomes are of increasing concern. The use of analgesia in neonates undergoing repeated venipuncture or other medical procedures associated with pain was formally recommended [16]. Increasing age is significantly associated with increased reports of pain. The OR of a report of pain for children aged 5–11 increased 69% compared with those <5 years, that for females was 66% greater than for males, and for adolescents (≥ age 12) was doubled compared with children ≥5 years. Finally, the OR of a report of pain for black, non-Hispanic children was 37% lower than for white, non-Hispanic participants. As the percentage of CD4 T lymphocyte cells decreased, reports of pain increased [207].

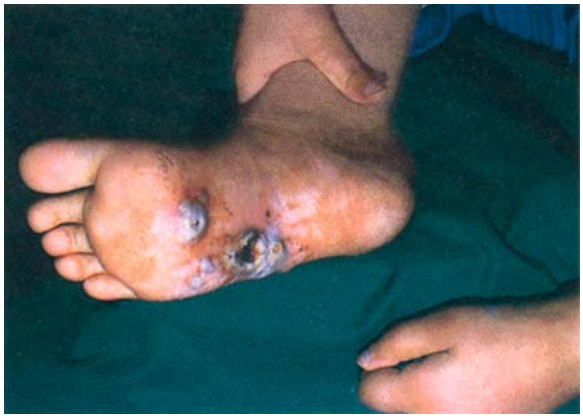


Fig. 23.20. Hyperkeratosis foot sole by mucocutaneous *Herpes simplex* infection in a 5-year-old child with vertically transmitted HIV infection

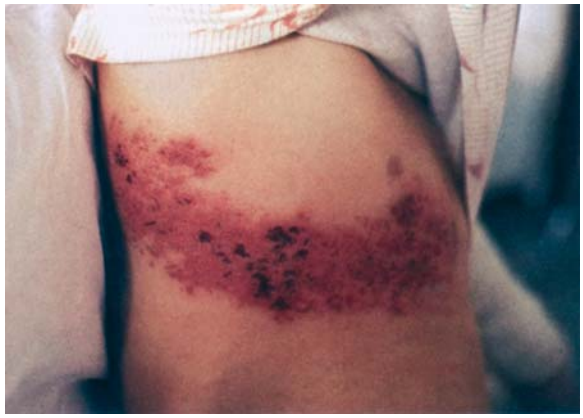


Fig. 23.21. Severe skin infection by *Herpes zoster* in a 10-year-old child with HIV infection

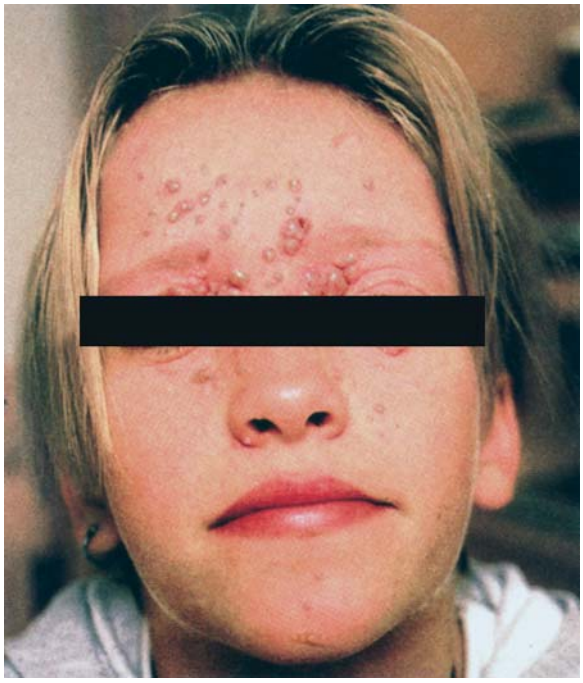


Fig. 23.22. Severe *Molluscum contagiosum* in a 12-year-old girl with HIV infection

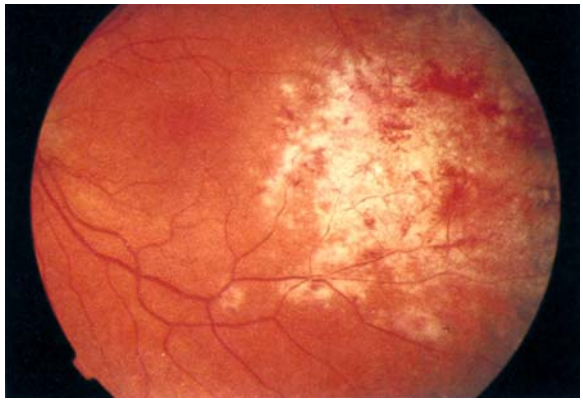


Fig. 23.23. Severe CMV retinitis, exudate, hemorrhage, necrosis and edema in a 16-year-old girl with HIV infection

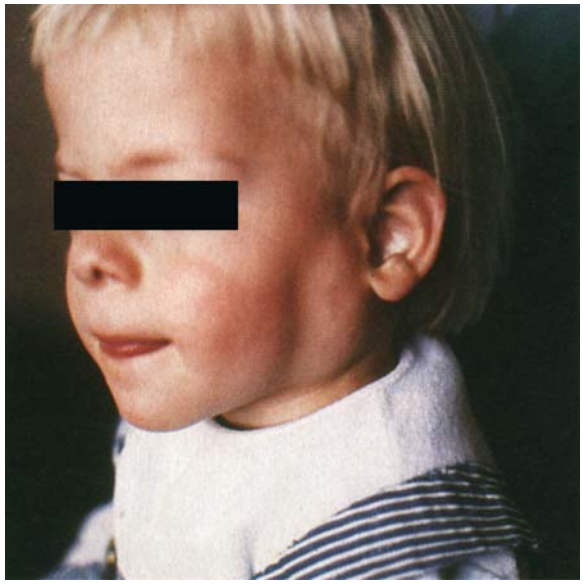


Fig. 23.24. Bilateral parotid swelling in a 3-year-old child with HIV infection by blood transfusion

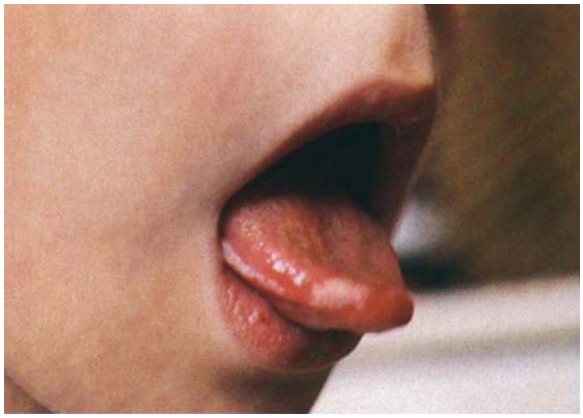


Fig. 23.25. Oral leukoplakia at the lingual border in a 5-year-old child with vertically transmitted HIV infection

Immunological Aspects

Immunological aspects are summarized in Table 23.19 [81, 132, 401, 461]. The typical picture is one of persistent T-lymphocyte reduction (quantitative and qualitative), associated with B-lymphocyte hyperactivity. The outcome is hypergammaglobulinemia, often quite noteworthy, stemming from B-cell hyperactivity. This principally involves IgG and/or IgM (91% of cases) and, due to the selective CD4 reduction (20.4% between 3 months and 18 years) [169], a rightward shift of the CD4/CD8 ratio (between 0.28 and 0.88). In chronic viral infections, such as those caused by CMV or EBV, a reduced CD4/CD8 ratio is also observed, but in this case the decrease is due to a rise in CD8 suppressors.

Table 23.19. AIDS ongoing immune dysfunction in infants and children

Cell-mediated immunity
Absolute lymphopenia
Increased counts of CD8 lymphocytes
Marked depletion of CD4 lymphocytes
Reduction/inversion of CD4/CD8 ratio (unstable)
Decreased proliferative response in vitro to mitogens and antigens
Cutaneous anergy to different antigens
Reduced IL_2 production in vitro
Decreased proportion of naive T cells
Reduced direct cytotoxicity of NK cells
Thymic dysplasia/hypoplasia
Disruption of thymopoiesis
Humoral immunity
Increased counts of B lymphocytes
Lower antibody titers
Hypergammaglobulinemia (IgG and/or IgM) or hypogammaglobulinemia
Defective IgM–IgG switch
Defect of IgG subclasses
Elevated autoantibody levels
Increased circulating immune complexes (CIC), decreased in vitro proliferative response to B mitogens and antigens
Decreased in vitro primary and secondary antibody response

Data from [81, 132, 401, 461].

Diagnosis

Early in life, diagnosing HIV infection can be a difficult problem (Table 23.18), but early diagnosis is important in order to start treatment immediately and offer a reliable prognosis [95]. A New York State program of expedited HIV testing (48-h turnaround results) of pregnant women and newborns (unknown HIV status at the time of delivery) found that 39 (37.5%) of the newborns were unanticipated, and 9 (70%) of HIV-infected babies were also born to mothers whose HIV status was unknown at the time of delivery [373].

For the clinical diagnosis, since 1985 CDCs have regularly updated the diagnostic criteria: the updated pediatric staging of HIV infection can be found in Tables 23.20 and 23.21 [89], the CDC criteria for the diagnosis of HIV infection are in Table 23.22 [89], and the

Table 23.20. Staging of pediatric HIV infection: clinical categories

	HIV infection symptoms			
Evidence of immune suppression[a]	N: none [b]	A: mild	B: moderate	C: severe
1. None	N1	A1	B1	C1
2. Moderate	N2	A2	B2	C2
3. Severe	N3	A3	B3	C3

Data from [89].

[a] Evaluated in function of the number of CD4 cells/μl (Table 23.21).

[b] Children whose HIV infection status is not confirmed are classified by using the above grid with a letter E (for perinatally exposed) placed before the appropriate classification code. For more details, see Table 23.23.

Table 23.21. Staging of pediatric HIV infection: immunological categories based on age-specific $CD4^+$ T-lymphocyte counts and percentage of total lymphocytes

Child age	<12 months		1–5 years		6–12 years	
Evidence of immune suppression	**CD4/μl**	**%**	**CD4/μl**	**%**	**CD4/μl**	**%**
1. None	≥1,500	≥25	≥1000	≥25	≥ 500	≥25
2. Moderate	750–1,499	15–24	500–999	15–24	200–499	15–24
3. Severe	<750	<15	<500	<15	<200	<15

Data from [89].

Table 23.22. CDC classification: diagnosis of pediatric HIV infection

Diagnosis: child with HIV-1 infection
Category N: not symptomatic. Children who have no signs or symptoms considered to be the result of HIV infection or who have only one of the conditions listed in category A
1. A child <18 months of age who is known to be HIV seropositive or born to an HIV-infected mother or the source of HIV transmission is unknown:
Has positive results on two separate determinations (excluding cord blood) from one or more of the following HIV detection tests:
a. HIV culture
b. HIV PCR
c. HIV antigen p24
Or
Meets criteria for AIDS diagnosis based on the 1987 AIDS surveillance case definition
2. An infant or child >15 months of age born to an HIV-infected mother or any child infected by blood, blood products, or other known modes of transmission (eg, sexual contact) who:
Is HIV-antibody positive by repeatedly reactive EIA and confirmatory test (eg, WB or IFA)
Or
Meets none of the criteria in 1) above.
Diagnosis: perinatally HIV-1 exposed
Infants and children who meet any of the criteria above:
1. An infant or child who does not meet the criteria above who: is HIV seropositive by EIA and confirmatory test (eg, Western blot or IFA) and is <18 months of age at the time of test
Or
2. Has unknown antibody status, but was born to a mother known to be infected with HIV-1
Diagnosis: seroreverter
Infants born to HIV-1-infected mothers
1. A child who is born to an HIV-infected mother and who has been documented as HIV-antibody negative (ie, two or more negative EIA tests performed at 6–18 months of age or one negative EIA test after 18 months of age)
And
2. Has had no other laboratory evidence of infection (has not had two positive viral detection tests, if performed)
And
3. Has not had an AIDS-defining condition

Modified from [89].
CDC Centers for Disease Control, Atlanta, USA, *EIA* enzyme immunoassay, *IFA* immunofluorescence assay, *WB* Western blot.

Table 23.23. 1994 Revised Human Immunodeficiency Virus Pediatric Classification System: clinical categories

Category N: asymptomatic
Category A: mildly symptomatic, two or more of the conditions listed below but none of the conditions listed in Categories B and C
Lymphadenopathy (≥0.5 cm at more than two sites; bilateral = one site[a])
Hepatomegaly
Splenomegaly
Dermatitis
Parotitis
Recurrent or persistent upper respiratory infection, sinusitis, or otitis media
Category B: moderately symptomatic
Children who have symptomatic conditions other than those listed for Category A or C that are attributed to HIV infection
Examples of conditions in clinical Category B include but are not limited to:
Anemia (<8 g/dl), neutropenia (<1,000/mm^3), or thrombocytopenia (<100,000/mm^3) persisting ≥30 days
Bacterial meningitis, pneumonia, or sepsis (single episode)
Candidiasis, oropharyngeal (thrush), persisting >2 months in children >6 months of age
Cardiomyopathy
Complicated chickenpox
Cytomegalovirus infection (onset <1 month of age)
Diarrhea, recurrent or chronic
Hepatitis
Herpes simplex virus (HSV) stomatitis, recurrent (more than two episodes per year)
HSV bronchitis, pneumonia, or esophagitis with onset <1 month of age
Leiomyosarcoma
Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex
Nephropathy
Nocardiosis
Persistent fever >1 month
Shingles (*Herpes zoster*) involving at least two distinct episodes
Toxoplasmosis of the brain, onset *before* 1 month of age
Category C: severely symptomatic (see Table 23.24)

Modified from [89].
CDC Centers for Disease Control, Atlanta, USA.
[a] Bilateral lymphadenopathy at the same site is considered as belonging to the same site; development of any of the conditions listed in categories B and C excludes the above diagnosis.

CDC criteria for classification into clinical categories are shown in Tables 23.23 and 23.24 [89]. The CDC diagnosis is based on the positivity of one or more parameters. For the most seriously affected children, they involve an ID that cannot be explained otherwise, indicated by the presence of infections caused by opportunistic germs (particularly PC) or, more rarely, the presence of KS, non-Hodgkin's lymphoma or primary cerebral lymphoma. Invasive diagnostic procedures are required to demonstrate infections caused by the opportunistic germs observed most frequently (PC, *Candida*, disseminated CMV), such as the isolation of PC during bronchoscopy, but this exam can be quite invasive and is not very suitable for small children who are seriously ill [436]. Otherwise, in the first episode of PCP a diagnosis can be made using the bronchoalveolar lavage fluid (BALF), showing lymphocytosis [227]. On the other hand, infections such as cryptococcosis, cryptosporidiosis and strongyloidoisis are also observed in young children with AIDS. Since the association with KS or

Table 23.24. CDC classification: clinical categories of pediatric HIV infection. Category C

Category C: severely symptomatic
Children who have any condition listed in the 1987 surveillance case definition for HIV infection, with the exception of LIP
Severe bacterial infections, recurrent or multiple (ie, any combination of at least two culture-confirmed infections within a 2-year period), of the following types: septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media, superficial skin or mucosal abscesses, and indwelling catheter-related infections)
Candidiasis, esophageal or pulmonary (bronchi, trachea, lungs)
Coccidioidomycosis, disseminated (at site other than or in addition to lungs or cervical or hilar lymph nodes)
Cryptococcosis, extrapulmonary
Cryptosporidiosis or isosporiasis with diarrhea persisting >1 month
Cytomegalovirus disease with onset of symptoms at age >1 month (at a site other than liver, spleen, or lymph nodes)
Encephalopathy (at least one of the following progressive findings present for at least 2 months in the absence of a concurrent illness other than HIV infection that could explain the findings):
a. Failure to attain or loss of developmental milestones or loss of intellectual ability, verified by standard developmental scale or neuropsychological tests
b. Impaired brain growth or acquired microcephaly demonstrated by HC measurements or brain atrophy demonstrated by CT or MR imaging (serial imaging is required for children <2 years of age)
c. Acquired
Symmetric motor deficit manifested by two or more of the following: paresis, pathological reflexes, ataxia, or gait disturbance
HSV infection causing a mucocutaneous ulcer that persists for >1 month; or bronchitis, pneumonia, or esophagitis for any duration affecting a child >1 month of age
Histoplasmosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
Kaposi's sarcoma
Lymphoma, primary, in brain
Lymphoma, small, noncleaved cell (Burkitt's), or immunoblastic or large cell lymphoma of B-cell or unknown immunological phenotype
Mycobacterium tuberculosis, disseminated or extrapulmonary
Mycobacterium, other species or unidentified species, disseminated (at a site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
Mycobacterium avium complex or *Mycobacterium kansasii*, disseminated (at site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
Pneumocystis carinii pneumonia
Progressive multifocal leukoencephalopathy
Salmonella (nontyphoid) septicemia, recurrent
Toxoplasmosis of the brain with onset at >1 month of age
Wasting syndrome in the absence of a concurrent illness other than HIV infection

Modified from [89].
CDC Centers for Disease Control, Atlanta, USA, *HC* head circumference, *MR* magnetic resonance, *CT* computerized tomography.

cerebral lymphoma is exceptional, if not virtually nonexistent [113, 163, 451], the diagnostic parameters listed in Table 23.24 are applied to older children. These children should be divided into two arbitrarily defined clinical categories: asymptomatic infection or symptomatic infection (wasting, thrush or unexplained fever for >2 weeks). All children in the second category should be offered ART (see "Treatment").

Table 23.25. Characteristics of virological assays for early diagnosis of pediatric HIV infection

Test	Sensitivity	Specificity	Days to results	Cost
HIV culture	Good	Excellent	15–35	High
ELISPOT[a]	Good	Moderate[d]	1–2	Moderate
IgA	Good	Excellent[d]	1–2	Moderate
IVAP[a]	Good	Moderate[d]	7–10	Moderate
p24	Moderate	Excellent	1–2	Low
HIV DNA PCR[b]	Excellent	Excellent	1–2	High
HIV RNA PCR	26.7%	100%	At birth	Unknown
OraQuick[c]	Excellent	99.6%	20 min	Unknown

Data from [162, 313, 452, 473 and FDA press release, March 5, 2003].
[a] According to CDC [60], anti-HIV IgA and ELISPOT/IVAP are less commonly used.
[b] A branched DNA (bDNA) assay yielding an overall specificity of 97.2% (95% CI, 92.0–99.4) (timing unspecified) [473].
[c] Complete name: OraQuick Rapid HIV-1 Antibody Test; can be stored at room temperature, requires no special equipment, and can be performed outside clinical settings.
[d] Only after 2–3 months of life.

Table 23.26. Sensitivity of virological assays for early diagnosis of pediatric HIV infection

	Timing of testing				
Test	1[a] week	2–4 weeks	1–2 months	3–6 months	>6 months
HIV culture	30–50	50	70–90	>95	>95
HIV DNA PCR[a]	30–50	93	96	>96	>96
p24[a]	10–25	20–50	30–60	30–50	20–40
p24 (–IC)	30	NQ	NQ	>90	>90
ELISPOT	Low, NA	Low, NA	Low, NA	>95	>95
IgA	<10	10–30	20–50	50–80	90
IVAP	Low, NA	Low, NA	Low, NA	>95	>95

See Table 23.25.
Data from [162, 303, 452].
– *IC* less immune complexes, *NA* not applicable, *NQ* not quantitated.
[a] A 100% sensitivity was obtained at 4–9 weeks for both PCR and p24 antigen [303].

Laboratory Diagnosis

In a newborn or nursing infant with anomalous results from immunological tests showing CMI or humoral ID (Table 23.19), the diagnosis – though based on the presence of characteristic symptoms – is confirmed by tests for early diagnosis. Table 23.25 [162, 313, 452, 473] indicates the characteristics of these tests and Table 23.26 [162, 303, 452] their sensitivity in the period ranging from 7 days to 6 months. None of these assays can give certain results in the 1st week after birth (for treatment purposes), and above all their positive predictive value is poor. Viral culture is the gold standard test but is costly and takes 2–4 weeks for the result to be available, and sensitivity varies with the time. It has 33% sensitivity in the first 2 weeks, 70% between 1 and 2 months and 100% at 5–7 months. The HIV DNA PCR test is used for the early diagnosis of HIV infection in the newborn with a sensitivity of 30%–35% within the 1st week after birth, which increases to 100% by 1 month of age [452]. In a meta-analysis of published data, the HIV RNA PCR test was positive in 38% of 271 HIV-infected newborns *by age 48 h* [162]. These data suggest that a positive PCR assay within 72 h after birth may reflect *not only intrauterine transmission* but also intrapartum transmission of actively replicating HIV-1 virus. At birth and age 6 weeks, a significantly larger proportion of infected infants were identified by means of the RNA PCR assay than by the other assays. RNA assays require less blood volume and yield rapid results (the sensitivity for the RNA PCR assay was 94.7% at 6 weeks of age and 85.7% at 24 weeks of age); thus RNA PCR assays may be used

Table 23.27. Differential diagnosis between bimodal forms of pediatric HIV infection: with significant differences at birth in infected neonates: mean (+ SD)

Characteristics	PCR or viral culture				*p*
	Positive		Negative		
Head circumference (cm)	33.2	(2.2)	34.0	(1.7)	<0.03
Adenopathy and/or splenomegaly and/or hepatomegaly (%)	15		3		<0.0001[a]
CD4 (cells×10^9/l), *n*=54	2.57	(1.46)	3.49	(1.71)	<0.04
CD8 (cells×10^9/l), *n*=54	1.67	(8.18)	2.45	(2.03)	<0.08
Platelets (cells×10^9/l), *n*=139	267.0	(104.1)	302.1	(91.7)	<0.05
IgM (g/l), *n*=90	0.85	(0.83)	0.39	(0.45)	<0.01
PCR or positive culture (%)	26.4		9.3		<0.006
Positive p24 antigenemia (%), no. 140	22		2		<0.001[b]

Data from [360].
RR relative risk.
[a] RR=2.5 (CI 95%, 1.4–6.0), PCR or culture; RR=2.8 (CI 95%, 1.3–6.1), p24.
[b] RR=3.5 (CI 95%, 1.9–6.2).

Table 23.28. Differential diagnosis between bimodal forms of pediatric HIV infection: other variables

Clinical features	Early/severe HIV infection	Evolutive HIV infection
Frequency	15%–25%	75%–85%
Asymptomatic period	Short	From 2 to >10 years
Opportunistic infections	Severe <18 months of age	After 2 years
Severe encephalopathy	Yes	No
Other manifestations	Hepatosplenomegaly, adenomegaly	LIP, parotitis, recurrent bacterial infections
High mortality rate	Within 18 months	After 4–5 years and longer
Laboratory data	Early/severe HIV infection	Evolutive HIV infection
Viral charge in the neonatal period	Elevated	Low or undetectable
Lymphopenia CD4	With rapid progression	Stable from 2 to >10 years, then progressive

Data from [45].

for early diagnosis of HIV-1 infection in infants [313]. The OraQuick test gives results *within 20 min with 99.6% specificity (100% for the RNA PCR assay)* [313] and facilitates receipt of test results. HIV-positive test results will require confirmation by Western blot or immunofluorescence assays [95].

In the neonatal period, virus isolation in the circulating blood or *in a culture* by *in situ* hybridization is the test of choice to diagnose an infection that is underway, although a negative result cannot totally exclude possible infection [150]. In this case, a PBMC culture lasting at least 1 month is used, subsequently examining the culture supernatant for antigen p24 or RT, which will reveal the presence of HIV in ≤50% of infected infants [150]. Alternatively, the viral genome can be assayed via PCR, amplifying the provirus DNA >10^6 times: this test is as sensitive as a viral culture and *takes only 1–2 days to complete,* but it requires two successive concordant determinations [145]. The results are contrasting in newborns: PCR may be not positive in the neonatal period [553] or be positive at the age of 2–3 days, as opposed to other tests [322], or in 38% [162] or in 62% of newborns at birth [380], or in 67%–83% between the ages of 1 and 3 months [380]. In conclusion, PCR was positive at birth in 26.4% of newborns at risk and in 9.3% of those with negative tests and a high RR, and a sensitivity of 35% in the 1st week of life (Tables 23.27, 23.28) [45, 360]. In the first few months, *antigen p24* cannot be used for this type of assay, as the child must be 3–6 months old, although as shown in Table 23.26, the immune complexes in serum samples can be dissociated from p24 by acid hydrolysis, improving sensitivity [417]. However, as we can see from Tables 23.27, 23.28, in 1-week-old newborns p24 levels were also significantly higher in the newborns who would later suffer from the most severe form of AIDS: 50% vs 14.4%, with a high

RR. The task of IVAP (in vitro antibody production) is to identify the B lymphocytes that secrete anti-HIV antibodies, whereas ELISPOT targets other cells with similar characteristics [145]: these assays likewise guarantee identification of >95% of cases only after the 3rd month (Table 23.26). False-positive results during the neonatal period are caused by maternal B-cell spread in the fetal circulation, due to the mother-to-fetus transfusion that can be noted occasionally during labor. The false-negatives must instead be attributed to the short duration of infection, since a sufficient number of B lymphocytes is not circulating yet [548]. US guidelines state that since plasma HIV RNA levels are higher in HIV-infected infants than in older infected children and adults, it may be difficult to interpret HIV RNA levels in infants <12 months of age, since the levels are overall high and there may be overlap in HIV RNA levels between infants who have and those who do not have rapid disease progression [240].

Specific IgA will point to infection: this is a very sensitive and specific test, particularly starting 3–6 months after birth (Tables 23.25, 23.26), since their levels are low in the first few months (Table 1.15), thus leading to false-negative results [356]. In an 8-week-old infant who was apparently infected *in utero,* a characteristic pattern was observed in the development of specific anti-HIV IgG subclasses: IgG_1 and IgG_3 were present, and IgG_2 and IgG_4 absent [445], indicating HIV infection and permitting diagnosis when total IgG and anti-HIV subclasses decrease [302].

If the culture or PCR is positive, for a firm diagnosis they must be repeated after the 1st month [68] or at about the 3rd month [15, 150]. Instead, if these tests performed at birth and at 1–2 months are negative and the infant is asymptomatic, they should be repeated at age 4 months [15]. However, the positivity of these tests in the 1st week after birth *is 3-fold more likely to be associated with the severe form of pediatric AIDS* in the child's 1st year. Consequently, despite their low diagnostic value, it is best to perform them routinely at birth in newborns who are at risk [360]. An infant about 4 months old *in whom neither the viral culture nor PCR are positive* (despite assays performed at the age of 1 and 4 months) [68] has a >95% probability of not being infected. Follow-up is indicated for infants of about 12 months exposed to HIV who, if they test negative and have no indicative symptoms, *are considered uninfected,* with the recommendation of repeating serum assays at the age of 2 years [15].

Beyond the neonatal period and the need for early diagnosis, other methods include ELISA (enzyme-linked immunosorbent assay), WB immunoprecipitation on gel, and virus load. ELISA has a sensitivity of 99.5% and a specificity of 99.8%, and can be considered a good screening test, due also to its low cost. In WB, electrophoresis in polyacrylamide gel separates the viral proteins by molecular weight (MW), and they are then transferred to nitrocellulose strips. Incubation with the serum being examined (thus with anti-HIV antibodies) causes the formation of antigen–antibody complexes directed against viral antigens such as p24 and gp120: the minimum criterion for a positive WB requires the presence of at least two bands among those of p24, gp41 and gp120/160 molecules, possibly with the addition of p31 [302]. ELISA is believed to require confirmation by other tests such as WB, to avoid diagnosing false-positive results as positives and vice-versa, although WB effectiveness can also be poor [257]. Measures of viral load, except for values from the 1st week of life were significantly associated with imminent vulnerability to CDC-C or death during the first 18 months of life [4].

In addition, even if many laboratories have requested adequate funding to perfect these techniques, they remain the prerogative of specialized centers and, for the time being, they cannot be used for routine diagnosis without turning to nonoriginal tests because of the high cost of WB [536]. Given what we noted above, any expenditure should be considered appropriate (or even mandatory) if it serves the purpose of an early and certain diagnosis of AIDS in a newborn or nursling. In conclusion, a child who is not HIV-infected could have a negative PCR and WB after 2–3 months and after 9 months, respectively, or an ELISA that is also negative at ±18 months, while in an infected child the results should essentially be the opposite, that is, two positive PCRs at any age, one confirming the other, and a positive WB or ELISA at ±15–18 months.

Diagnosis of HIV-2 is essentially made after 18 months. Viral cultures and DNA PCR are used, or modified versions of ELISA (HIV-1+2 test), which are highly sensitive but not always specific and must thus be confirmed with HIV-2-specific WB. Other tests reveal both HIV-1 infection and HIV-1/HIV-2 coinfection [183].

Differential Diagnosis

- *The French register* has proposed an important differentiation among children from the groups with characteristics that do not differentiate between early and severe phases, based on the positivity of viral culture or PCR [360]. Tables 23.27, 23.28 report the pertinent significant differences and RR. It has thus been confirmed that the incubation period follows a bimodal curve [464].
- The difference between intrauterine and subsequent forms lies on the *positivity of viral culture and PCR* [67], possibly broadened to include the other parameters in Tables 23.27, 23.28.
- The type with different PID (Chap. 22) indicates that there is ID in the parents and a positive anti-HIV serum assay only in pediatric AIDS [83].

For the time being, *prenatal diagnosis* cannot be proposed for a number of reasons: 1. The serum negativity of the fetus does not mean that there will not be infec-

tion later. 2. There is a risk of contaminating an uninfected fetus. 3. The virological diagnosis may not be reliable. 4. There is the possibility of mother-to-fetus transmission at a later stage in the pregnancy [84].

Treatment

Guidelines for ART use in pediatric patients are rapidly evolving [240]. Current US pediatric guidelines thus recommend therapy for all infected infants <12 months of age in clinical stage A, B, or C or with CD4+ T-cell values <25% and recommend consideration of therapy for asymptomatic infected infants <12 months of age who have normal immune status [240]. The European guidelines recommend initiating therapy in infants only if they have any of the following findings: clinical stage C, <20% CD4+ T cells, or HIV-1 RNA levels that persistently exceed 10^6 copies/mm. All infants presenting with clinical stage C disease should start HAART as soon as treatment of their AIDS-defining illness permits [454, 492]. However, HIV infection progresses more rapidly in infants than in older children or adults; therefore some experts would treat all HIV-infected infants <6 or <12 months of age, regardless of clinical, immunological or virological parameters [240]. In general, treatment should be offered to children with <350 CD4+ T cells/mm^3 or plasma HIV RNA levels >55,000 copies/ml (by RT-PCR or bDNA assay). Treatment is divided into various approaches, such as ART, treatment of infective complications including PCP prophylaxis, other forms of treatment, appropriate nutritional support and psychological support. Before starting therapy or prophylaxis, the levels of CD4 with prognostic value must be checked (Table 23.29) [89, 91 240]. From this table, we can see that in infants and children the percentage values (above) or the CD4 count (below) at which ART or PCP prophylaxis should be started vary based on age (Tables 1.34–1.38, for comparison). In infants, the values must be checked at the ages of 1 and 3 months, proceeding at 3-month intervals up to the age of 2 years. Subsequently, controls must be performed every 6 months, unless the percentage or CD4 count reaches threshold values correlated with age: in this case, the assays must be repeated every month. Instead, infants and children *with episodes of PCP must receive prophylaxis regardless of their CD4 values* [89, 91]. The death of some infants at 10 (in 1990–1995) and at 29 months (in 1996–1997) was related to age <12 months, low CD4+ count, severe bacterial infection and PCP, although PCP prophylaxis and combined ART ensured *increased survival and lower mortality rates* [179]. In HIV-infected children, mutations associated with resistance after nucleoside ART and syncytium-inducing phenotype, higher HIV-1 RNA load and lower CD4+ cell count were significantly correlated with increased risk of HIV clinical disease progression [171]. In HIV-1-infected children, mainly African-American or Hispanic, total lymphocyte count and serum albumin independently predicted mortality, an assessment warranted in resource-poor settings [389].

Prevention of HIV-1 Mother-to-Child Transmission

Several articles illustrate this relevant aspect. Researchers are testing to see whether drugs such as NVP could protect babies during breast-feeding [20].

A two-dose intrapartum/newborn NVP regimen reduced perinatal HIV transmission in Ugandan women not receiving antenatal ART. Detection of HIV infection was very low since it occurred in 9/631 (1.4%) NVP group deliveries and 10/617 (1.6%) placebo group deliveries, thus with no difference [154].

A subsequent report demonstrates the astonishing efficacy of dual ARTs in reducing HIV-1 MCTC, which

Table 23.29. CD4 counts suggested to start antiretroviral therapy or PCP prophylaxis in children under 13 years of age

Age at CD4+ measurement	Values suggested for antiretroviral therapy	Age at CD4+ measurement	Values suggested for PCP prophylaxis
CD4 (%)			
<1 year	<25	<1 year	None
1–5 years	<25	1–5 years	<15
6–12 years	<25	6–12 years	<15
CD4 (cells/mm^3)			
<1 year	<1,500	<1 year	None
1–5 years	<1,000	1–5 years	<500
6–12 years	<500	6–12 years	<200

Factors to be considered in decisions about initiation of therapy include the risk of disease progression as determined by CD4+ percentage, the potential benefits and risks of therapy, and the ability of the caregiver to adhere to administration of the therapeutic regimen.
Data from [89, 91, 240].

showed that adding single-dose NVP to an AZT prophylaxis beginning at 28 weeks of pregnancy, in women not breast-feeding, and with viral loads of at least 2,500 HIV-1 RNA copies/ml and CD4 cell counts of ≤200/mm^2 or less, can achieve effective and safe results. The rate was reduced from 6.3% among 348 women who, like their infants, received the placebo to 1.1% among 353 women who received the standard regimen plus a single intrapartum dose of NVP, with a single dose of NVP also given to their infants soon after birth [311].

In a study done in Malawi, breast-feeding mothers received a 200-mg single oral dose of NVP intrapartum (n=448) and infants received either a 2-mg/kg oral dose of NVP or NVP (same dose) plus 4 mg/kg of AZT twice a day for 1 week (n=446). The MCTC of HIV at birth was 8.1% in infants administered NVP only and 10.1% in those administered NVP plus AZT. A life table estimate of transmission at 6–8 weeks was 14.1% in infants who received NVP and 16.3% in those who received NVP plus AZT. For infants not infected at birth and retested at 6–8 weeks, transmission was 6.5% in those who received NVP only and 6.9% in those who received NVP plus AZT [532].

A similar short course of AZT had previously been established as the standard regimen in Thailand, reducing the rate of HIV-1 MCTC from 25% to 6.5%. [275], a 3.8-fold difference.

Of 8,221 deliveries in a routine service setting, 1,234 (15%) occurred in women known to be HIV-infected. HIV transmission rates of 8.7% at 6 weeks and 8.9% at 3 months of age in the study population verify the high rate of NVP administration. The authors underline the ability of women to formula-feed their babies and abstain from breast-feeding [493].

In Cameroon, HIV-1-positive pregnant women were given a single dose of NVP at the onset of labor, and babies were given 2 mg/kg NVP syrup within the first 72 h of life. NVP-treated children were regularly followed up and examined for HIV-1 infection at 6–8 weeks and 5–6 months: out of 123 children, 13 (10.6%) were infected and presented with high viral loads, in general >500,000 copies/ml [27].

A recent Cochrane review concluded that short-course AZT and single-dose NVP are effective therapies for reducing HIV MTCT. The potential value of NVP used for longer durations in breast-fed populations should be considered, as it may further reduce the MTCT risk, particularly if combined with early weaning [62].

Antiretroviral Therapy

The ART effect was demonstrated in the 1996–1999 period, when the transmission rate for mother–infant pairs not breast-feeding was 8.6% with elective cesarean delivery, 4.4% with any ART, and 2.4% with these interventions combined [266]. The drugs that are currently used are analogs of 2'-3'-dideoxynucleosides that can inhibit RT, which, as we noted, enable the transcription of viral RNA into DNA. They include:

- *Nucleoside RT inhibitors* (NRTI): zidovudine (ZDV or AZT), dideoxyinosine (ddI) or didanosine, dideoxycytosine or dideoxycytidine or zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), adefovir
- *Non-nucleoside RT inhibitors* (NNRTIs): delavirdine (DLV), nevirapine (NVP) and efavirenz (EFV)
- *Protease inhibitors* (PI): saquinavir (SQV), ritonavir (RTV), nelfinavir (NFV), indinavir (IDV) and amprenavir (APV)
- *Entry inhibitors:* T-20

Drug dosages are listed in Table 23.30 [19, 106, 121, 143, 254, 288, 345, 398, 418, 450, 481, 482, 560, 580]. Table 23.31 deals with the management of *in utero* exposed babies [15, 418].

The CDC suggests that when initiating therapy in children naive to ART, one should begin with a regimen that is expected to achieve sustained suppression of plasma HIV RNA, a sustained increase in CD4$^+$ T-cell count, and a favorable clinical outcome. Strongly recommended regimens include either IDV, NFV, RTV + SQV, RTV + IDV, RTV + lopinavir or EFV in combination with one of several dual NRTI combinations [88]. WHO/UNAIDS now recommend quality-assured, *fixed-dose combinations of 3TC, d4T and NVP in a single pill* as first-line treatment of MCTC for 3 million mothers, making it happen by 2005 (*the 3-by-5 strategy*) [576]. This seems to be unlikely. However, there is reason to be hopeful that growth rates will continue to increase in the three-year period 2005–2007 [583]. The Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection [240] strongly recommend for children ≤3 years old or who cannot swallow capsules two NRTIs + NVP, and for children >3 years two NRTIs + EFV (with or without NFV), and as an alternative for children >3 years, two NRTIs + NVP. These guidelines recommend therapy for all infected infants aged <12 months who have symptoms or immune suppression and recommend consideration of therapy for asymptomatic infected infants aged <12 months who have normal immune status [240]. However, a study in HIV-1-infected children aged 0.5–3.0 months (median, 2.0) (*early therapy*), or 3.5–24 months (median, 7.6) (*delayed-therapy*) found no significant differences among the treatment regimens in the rate of viral suppression at week 16, but the regimen of d4T, 3TC, NVP and NFV appeared to be superior to the two regimens of RT inhibitors in suppressing viral replication at weeks 48 and 200 (>50 copies/mm in 85% of 26 children). *Early initiation of therapy (at ≤3 months of age) also appeared to be associated with improved long-term suppression of viral replication.* Treatment-related adverse clinical or laboratory events were infrequent, and only one of the 52 children discontinued therapy because of drug-related adverse effects [347] (see the doses in Table 23.30). In four infants with vertically acquired HIV-1 infection, plasma HIV-1 RNA

Table 23.30. Antiretroviral agents in infants/children

Agents	Age	Oral dosage	Reference
Nucleoside reverse transcriptase inhibitors (NRTI)			
Abacavir (ABC)	Infants aged <90 days	50 mg/m^2 q 12 h	[19]
	>1–3 months	8 mg/kg q 6–12 h	[450]
	Adolescents	300 mg q 12 h	[19]
Adefovir	≥3 months	1.5 mg/kg bw per day	
	≥1 year	3.0 mg/kg bw per day	[254]
Didanosine (ddI)	2–16 months	120 mg/m^2 q 12 h	[345]
	4–10 years	180 mg/m^2 q 12 h	[289]
Lamivudine (3TC)	Neonate	2 mg/kg q 12 h	
	Children	4 mg/kg q 12 h	
	Adolescents	150 mg q 12 h	[19]
Stavudine (d4T)	0.8–15 years	2–4 mg/kg/die q 12 h	[288]
	0.4–6 years	1 mg/m^2 q 12 h	[289]
Zalcitabine (ddC)	Children	0.01 mg/kg q 8 h	
	Adolescents	0.75 mg q 8 h	[19]
Zidovudine (AZT)	0–2 weeks	2 mg/kg/dose q 6 h	
	2–4 weeks	3 mg/kg/dose q 6 h	
	4 weeks to 13 years	90–180 mg/m^2 q 6–12 h	[121]
Non-nucleoside reverse transcriptase inhibitors (NNRTI)			
Delavirdine (DLV)	5–18 years	40 mg/kg q 12 h	[579]
	Adolescents	400 mg q 8 h / 600 mg q 12 h	[19]
Efavirenz (EFV)	5–18 years	9–13.5 mg/kg/day	[579]
Nevirapine (NVP)[a]	2–16 months	5 mg/kg or 120 mg/m^2 for 14 or 28 days, then 200 mg/m^2 q 12 h	[345]
	Pediatric	120–200 mg/m^2 q 12 h	[19]
	Adolescents	200 mg q 12 h	[143]
Protease inhibitors (PI)			
Amprenavir	Children 5–18 years	20–40 mg/kg q 12 h	[579]
Indinavir	Children	300–600 mg/m^2 q 8 h	
	Adolescents	800 mg q 8 h	[19]
Nelfinavir (NFV)	Pediatric age	30–50 mg/kg q 8 h	[418]
Ritonavir (RTV)[b]	6 months to 14 years	250–400 mg/m^2 q 12 h	[398]
	6 months to 12 years	230/57.5–300/75 mg/m^2 q 12h	[481]
Saquinavir (SQV)	NS	300 mg/m^2 q 8 h	[143]
	Adolescents	600 mg q 12 h	[143]
Entry inhibitors			
T-20	Children	30 or 60 mg/m^2 q 12 h sc	[106]

Footnote see next page.

Table 23.31. Specific management of babies exposed to HIV in utero

Age	Management
Birth	HIV DNA PCR, start AZT
2 weeks	HIV DNA PCR, CBC (monitor for anemia)
6 weeks	HIV DNA PCR, CBC, discontinue AZT, start PCP prophylaxis
4 months	Continue PCP prophylaxis
6 months	Discontinue PCP prophylaxis if HIV tests remain negative
12–18 months	HIV-1 ELISA

Data from [15, 418].
CBC complete blood count.

levels ranged from 230,000 to 1,000,000 copies/ml before onset of triple therapy (<2 months of age) and fell <50 copies/ml at 12–33 weeks of life in three infants [235]. In a meta-analysis of eight cohort studies and nine clinical trials in the US and Europe that included nearly 4,000 untreated, infected children, the 1-year risk of AIDS or death was substantially higher in younger than older children at any given level of $CD4^+$ percentage, particularly for infants aged <12 months [245]. Therefore, ARTs are safe, effective, and well tolerated during years of administration and should be started in infancy [512], as early as possible [347].

NRTI

- *ZDV* is phosphorylated inside the cells to arrive at the triphosphate form, which is active in inhibiting retroviral RT, as it competes with the normal thymidine triphosphate nucleoside, thereby blocking the synthesis of viral DNA. It is absorbed well when administered orally, with a bioavailability of 60%–65%; following ingestion, it reaches a peak concentration in 30–60 min. Concentrations of >1 µmol are preferable, as they can inhibit viral replication. ZDV serum half-life is 1 h and the intracellular half-life is 3–4 h; as a result, its rapid elimination suggests a dose every 6 h. However, two doses a day seem to be equally sufficient for maintaining satisfactory levels [45]. In children with mild to moderate disease, low-dose (90 mg/m^2 per dose) ZDV will result in substantial cost savings and should be the recommended dose. ZDV's distinctive features are good distribution in the tissues, with a volume equal to 1.4 l/kg, and its ability to cross the blood–brain barrier, reaching the CSF with a CSF/plasma ratio of 0.24 [370]. ZDV is metabolized by the liver and cleared through the kidneys. However, these favorable results are offset by its myelotoxicity, particularly cytopenia [227, 439]. Thus, ZDV toxicity may restrict its extended use in pediatrics [151]. A recent report suggested that mitochondrial dysfunction in eight of 1,754 children (0.46%) and symptomatic hypokinetic hypertrophic cardiomyopathy in one child may have been caused by ZDV alone or in combination with 3TC, but two infants previously exposed to ZDV–3TC contracted severe neurological disease and died [51]. No significant differences in ventricular function were observed between infants born to HIV-infected women and exposed to ZDV in the perinatal period and controls not exposed to ZDV during the first 14 months of life [338]. Studies conducted in France and the USA have indicated that ZDV may also be used in infants and young children at a daily dosage of 90–100 mg/m^2 qid with equivalent results [57, 227], or by continuous IV infusion at a dose of 0.5–1.8 mg/kg/h [439]. These studies noted an increase in weight and cognitive capacity, as well as a drop in p24 levels in the blood and CSF, parallel to an increased CD4 number, but the effect was limited to 3 months [368]. This may be *the*

[a] Nevirapine doses may be greater than 300 mg/m^2/day, which is higher than currently recommended [560].
[b] Pediatric oral solution: 80 mg lopinavir and 20 mg RTV per ml; capsules: 133.3 mg lopinavir/33.3 mg RTV [482].
The drugs and dosages received by 52 children [345] were as follows: *For infants <29 days of age,* Zidovudine (ZDV) was dosed as 4 mg/kg tid; Lamivudine (3TC) as 2 mg/kg bid; Stavudine (d4T) as 0.5 mg/kg bid; Abacavir (ABV) as 8 mg/kg bid; NLF as 30 mg/kg tid; and NVP as 5 mg/kg qd for 14 days, followed by 120 mg/m^2 bid for 14 days, followed by 200 mg/m^2 bid. *For infants 30 days of age,* ZDV was given as 180 mg/m^2 bid; 3TC as 4 mg/kg bid; d4T as 1 mg/kg bid; ABV as 8 mg/kg bid; NLF as 30 mg/kg tid; and NVP as 120 mg/m^2 qd for 14 days, followed by 200 mg/m^2 bid (K. Luzuriaga, pers. comm., 26 July 2004). Except for efavirenz, delavirdine, indinavir, ritonavir, and saquinavir available in tablets or capsules, all other agents are in oral solution formulations [418]. *ddI* is a dry powder that should be reconstituted with water to reach a concentration of 20 mg/ml but requires antiacid formulation to reach a final concentration of 10 mg/ml. The stomach should be empty 1 h before and 1 h after each administration. A combination ZDV (300 mg) and 3TC (150 mg) called *Combivir* is available [418]. d4T should be diluted until 0.2–1 mg/ml, without antiacid coadministration; it is available in gel capsules of 1, 5, 20 and 50 mg [288]. *Indinavir* is administered on an empty stomach and adequate hydration is a must to avoid the risk of nephrolithiasis. *Saquinavir* is instead administered on a full stomach [143]. *Neonatal dose: under evaluation* in Pediatric AIDS Clinical Trial Group protocol 332 [19]. *Nevirapine:* initiate therapy with 120 mg/m^2 (maximum 200 mg) administered once daily for 14 or 28 days. Increase to full dose (120–200 mg/m^2) administered every 12 h (maximum 200 mg every 12 h) if no rash or other untoward effects. A problem not always taken into account is the possible lack of compliance of young children due to the necessary administration of a high number of tablets daily, since better adapted liquid formulations are not available. Some children and adolescents may be unable to adhere to rigid protocols. The *poor knowledge of the minimal effective dose of these agents in the pediatric age is significant.*

reason why ZDV use was limited to initial treatment. In a 26-month-old child with encephalopathy, ZDV was used in association with intravenous Ig (IVIg) [359]. Moreover, it is unclear whether positive results noted following a 9-week cycle are attributable to ZDV, Ig, or both. A targeted study (Pediatric AIDS Clinical Trials Group, PACTG 076) concluded that among mothers who had not been treated previously, administering ZDV before (beginning anytime between the 14th and 34th week of gestation until the end of pregnancy) and after delivery, followed by an IV infusion during labor (2 mg/kg in 1 h), plus administration to the newborns within 8–12 h of birth (Tables 23.30, 23.31) for the first 6 weeks of life, reduced the incidence of HIV infection by 67.5% [121] or, according to a similar study, by 78% [8]. In France, a trial similar to PACTG 076 achieved a 66.6% reduction, uninfluenced by maternal CD4 levels or p24 antigenemia during delivery [361]. An eightfold reduction in maternal viral load was also noted [152], but this has not been confirmed [8]. Subsequently, it was clarified that ZDV must be started during pregnancy or even earlier [361], regardless of virus or CD4 serum levels [516], thus reducing HIV transmission from 20% to 5% [361]. Four additional studies have demonstrated dramatic decreases in perinatal transmission with incorporation of the PACTG 076 ZDV regimen into general clinical practice [124, 187, 188, 238]. In the setting of maternal transmission, ZDV effectively *protects fetal cells from infection* even when maternal viral burden is high [8]. Despite these therapeutic efforts, the protocol of PACTG 076 cannot be applied fully. Several mothers are not informed in time and/or exhaustively of the protocols, they refuse to undergo diagnostic testing for HIV even after being advised to do so, or they refuse ZDV treatment even though they are aware of being HIV^+ [565]. Combination therapy was received by only 323 (8%) women studied [178] vs 1,150 (78%) of 1,472 women (PACTG 367) [551]. A large meta-analysis of seven clinical studies that included 2,123 HIV-infected pregnant women who delivered infants during 1990–1998 and had received prenatal ART and 1,143 women who did not receive prenatal ART demonstrated that the use of multiple ART as compared with no therapy or treatment with one medication was not associated with increased rates of preterm labor, low birth weight, low Apgar scores, or stillbirth [547]. A significant contribution to overcoming this difficulty is the demonstration that ZDV is effective even if *first administered to the mother intrapartum* and *to newborns during their first 48 h of life* [565]. A randomized, DB equivalence trial comprised four regimens of ZDV: started in the mother at 28 weeks' gestation, with 6 weeks of treatment in the infant (the longer regimen), which is longer than protocol 076 [516]; ZDV starting at 35 weeks' gestation, with 3 days of treatment in the infant (the shorter regimen); and two intermediate regimens. The rate of *in utero* transmission was significantly higher with the two regimens with shorter maternal treatment (5.1%) than with the two with *longer maternal treatment* (1.6%) [310]. Infants who became HIV-infected despite maternal ZDV monotherapy were observed to *experience more rapid disease progression,* more severe immune suppression, and a lower survival rate than infants born to mothers not receiving treatment [267].

- *Dideoxyinosine or ddI,* converted inside the cell into dideoxyadenine, is phosphorylated to ddATP, the active substrate that inhibits RT. It is given to children suffering from intolerance or with virus strains that are resistant to ZDV. Pharmacokinetic studies have shown that in children, there is a marked inter-individual variability in the absorption of ddI, so it is wise to monitor its plasma levels to arrive at the optimum dosage [151]. The safety and tolerability of ddI given once daily are substantially similar to those of the traditionally recommended schedule of two divided doses [354]. Given its acid lability, when administered orally it must be taken on an empty stomach, together with an antacid. It has a plasma peak of approximately 0.5 h and its intracellular half-life is >12 h; 33% of the drug reaches the renal emunctory unchanged [151]. In children, it has shown *clinical improvement* and, less constantly, an improvement in the neuropsychological picture, with a reduction of adenomegaly and splenomegaly, an increase in body weight, and a parallel *positive progression of the immunological parameters,* with a significant increase in CD4 lymphocytes and a decrease in p24 antigenemia, both of which are correlated with *the dosage of ddI that is absorbed* and not how it is administered. In terms of *side effects,* pancreatitis is reported in children in up to 7% of cases, more frequently in those who received the highest dosage, with prompt regression when treatment is discontinued. Other side effects include diarrhea, increased transaminase, uricemia and triglyceridemia, which are generally reversible following reduction or discontinued treatment. It is not myelotoxic [151].
- A 70%–90% bioavailability has been attributed to *ddC,* but its half-life in children is 1.4 h, as opposed to 1.9 h in adults, leading to the presumption of a lower level of bioavailability or faster clearance. It is not present extensively in the CSF (20% of plasma concentrations) and is cleared through the kidneys. If ddC is administered at mealtimes, its plasma level drops by 39%, also prolonging the time required to reach maximum concentration in the plasma. There is little myelosuppressive effect in ddC, but it can cause dose-dependent *peripheral neuropathy* that regresses when the treatment is discontinued. However, the incidence of this disorder is low in the pediatric field [151]. Administration may be followed by skin rash, aphthous stomatitis, headache, hyperglycemia and, rarely, pancreatitis [585]. The ddC anti-HIV activity has been reported in treated children, demonstrated by *p24 antigen reduction and CD4 lymphocyte numerical increase,* along with a better appetite and growth increase, as well as a reduced adenopathy and hepatosplenomegaly [440].

• *Stavudine or d4T* has the same characteristics and mechanism of action as ZDV; its bioavailability following oral administration is 90%, while peak levels are reached rapidly, in 0.5–1.5 h, dropping off just as fast, as its mean half-life is 1.2 h [151]. In CSF, it reaches 46.5% compared to serum levels and is cleared through the urine. On a clinical level, it shows *lower cytotoxicity than ZDV* [288]. Twenty percent of children experienced nonrelevant side effects such as rhinitis, cough, diarrhea, vomiting, nausea and skin rash in 51%–76% of cases, peripheral neuropathy in 22% and a decline in neurological tests in 11% of cases [288]. Neutropenia occurs less commonly among children receiving d4T than ZDV [288], and doses of dT4 can be halved [57]. From an immunological standpoint, a *significant increase in CD4* is observed starting in the 12th week, remaining constant for at least 24 weeks in 44.4% of children, with a parallel *drop in p24 antigenemia* below basal values [288].

• *Lamivudine or 3TC* is a ddC analog and it is less toxic in vitro than other NRTIs; bioavailability is 62% following oral administration, and it goes into the CSF. Preliminary results show that the drug is safe and well-tolerated, and an increase in appetite and weight gain have been observed [151]. A phase I/II study of 3TC showed that 3TC could decrease viral burden by 0.77 logs when used as monotherapy; 3TC has activity against HIV-1, HIV-2 as well as hepatitis B virus. The CSF/plasma ratio in children is relatively low (0.11) compared with that of ZDV (0.25), but higher than that of ddI (0.05) [334]. In PACTG 300, *children receiving ZDV and 3TC had a lower risk of HIV disease progression* or death than those receiving ddI alone [369]. In a trial of q12h- vs q24h 3TC and ABC, 80% of children had a viral load <100 copies/ml at baseline compared with 89.5% at week 24. There was no marked difference in antiviral activity between q12h and q24h regimens [36].

• *Abacavir (ABC)* can induce a 1.5–2 $\log_{10}$ decrease in plasma RNA, but in a randomized study comparing ABC/ZDV/3TC with ZDV/3TC alone, four of 146 (2.7%) children receiving ABC and two of 44 (4.5%) children in the ZDV/3TC group who switched to open-label ABC therapy developed a *hypersensitivity reaction,* which resolved upon discontinuation of therapy [480]. Common toxicities are rash, nausea, vomiting and fever, which are difficult to diagnose and can even culminate in shock, in which case ABC treatment must be stopped definitively [19].

• *Adefovir,* unlike some antiviral nucleosides, does not require the initial phosphorylation step for its activity [254]. It can induce a 0.5–0.7 $\log_{10}$ decrease in plasma RNA and be administered in a single dose together with L-carnitine [450].

• *Tenofovir is not recommended by the CDC* [88], although serious adverse events leading to discontinuation of tenofovir DF were infrequent (5%), occurring with an incidence similar to that with placebo (8%) [198]. Patients receiving tenofovir must be monitored closely for early signs of tubulopathy since renal failure, proximal tubular dysfunction and nephrogenic diabetes insipidus have been reported in such patients [278]. Elevated *hepatic transaminase levels* during tenofovir monotherapy developed in 2/18 HIV-infected children aged 8.3–16.2 years who had progressive disease with ≥2 prior ART regimes. The monotherapy for 6 days was followed by the addition of individualized ART regimens. Children were monitored through 48 weeks. The remaining 16 children had a median of *4 ART agents* added to tenofovir. HIV plasma RNA levels decreased from a median pretreatment level of 5.4 $\log_{10}$ copies/ml to 4.21 $\log_{10}$ copies/ml at week 48, with 6 children having <400 copies/ml, including 4 with <50 copies/ml. $CD4^+$ cell responses among the responders were high and sustained. An additional toxicity attributed to tenofovir was a >6% *decrease in bone mineral density* for 5/15 children evaluated at week 48, necessitating the therapy discontinuation for 2; all 5 children had >2 $\log_{10}$ copies/ml decreases in HIV plasma RNA levels [239].

NNRTI

• *Delavirdine (DLV)* is metabolized in part by the hepatic cytochrome P450 3A (CYP3A) enzyme system, and in contrast to EBV inhibits P450 isoenzymes and may decrease the metabolism of certain drugs resulting in increased APV levels. Alternatively, DLV may be administered with APV in children [580].

• *Efavirenz (EFV)*, switching from PI to an EFV-containing regimen in 17 HIV-infected children, 24–160 months of age, elicited a significant improvement in fasting total cholesterol, low-density lipoprotein cholesterol, triglycerides, and the cholesterol:HDL ratio. The EFV-containing regimen was well tolerated and successfully maintained virological suppression in all children [365].

• *Nevirapine (NVP)* (dipyridodiazepinone) is used in association with ZDV and/or ddI, and has proven to be safe and effective. NVP is metabolized by the liver and the hepatic enzymes induce a twofold increase in its clearance, so that the dose must be reduced in the first 2 weeks [345]. NVP has various properties that make it an ideal candidate *for interrupting the HIV-1 transmission during childbirth and in the early postpartum phases,* given that it is absorbed rapidly and transferred to the newborn through the placenta if given to the mother during labor or delivery. In PACTG 316, a single-dose NVP administered during labor and to the newborn was added to the woman's existing ART regimen, but newly detectable NVP-resistance mutations were detected at 6 weeks postpartum in 14 (15%) of 95 women who received single-dose intrapartum NVP and had detectable HIV-1 RNA at delivery: the *overall transmission rate was 1.4* [134]. Resistance mutations to NRTIs (which include ZDV) were detected 10 days after delivery in 5% of mothers and in blood samples obtained 10 days postpartum from 32% of the women who

had received intrapartum NVP and who had at least one mutation that conferred resistance to NNRTIs [273]. In an open-label short course ART regimen of either NVP or multiple-dose ZDV/3TC, the overall estimated HIV-1 infection rates in 1,307 infants by 8 weeks were *12.3% for NVP and 9.3% for ZDV/3TC.* There were no drug-related serious maternal or pediatric adverse events [392]. However, children in the NVP-containing arms experienced moderate or worse skin rash more frequently than those not receiving NVP [581].

PI

- *Amprenavir (APV)* is a substrate for and inhibitor of the cytochrome P450 isoenzyme CYP3A4. Like other agents in this class, there could potentially be multiple drug interactions [19]. So far it has not been used *widely for children* (>4 years), and its dosage is limited to bid [580]. Children receiving APV should be advised not to take supplemental vitamin E [19].
- *Indinavir (IDV)* tends to precipitate in the renal tubules, causing hematuria and nephrolithiasis, thus requiring adequate hydration. Headache, nausea and abdominal pain were reported by 10% of patients [418]. In 33 infected children who had received >96 weeks of IDV/ZDV/3TC treatment (with an initial 16 weeks of IDV monotherapy), a median *increase in CD4$^+$ cell* count of 199/mm^3 and a median *decrease in HIV RNA* of 0.74 log was observed at 96 weeks [269].
- *Nelfinavir (NFV)* can cause headache, nausea, diarrhea and vomiting, and must be administered with food [450]. PACTG 382 studied 57 ART-experienced, PI and NNRTI-naive children given a combination of NFV, EFV and at least one NRTI. The combination was well tolerated by most children in the study. Viral suppression to <50 copies/ml was seen in 53% of the children studied at 48 weeks of treatment [520]. In a cohort of 22 HIV-infected infants aged 15 days–2 years in PACTG 356 given the recommended NFV dose of 20–30 mg/kg tid, clearance was significantly higher than in older children (2.7 l/h/kg vs 1.2 l/h/kg in older children), and the peak NFV levels were less than half those reported in older children [86]. As a consequence, doses of 55–65 mg/kg bid are currently under study in the young children in this protocol. Since NFV shows high interindividual variability in children, children <2 years tend to be at increased risk for low NFV levels. These data show that the NFV suggested dose is inadequate in most children. Also, these data suggest that pediatric dosing of NFV based on body surface area should be considered [38]. NFV in the absence of NVP resulted in less than half the drug exposure in children who weighed <25 kg compared with children who weighed >25 kg. An NFV dose of 50–55 mg/kg bid, to a maximum of 1,500 mg per dose, in children who weighed >30 kg resulted in equivalent or superior drug exposure [189]. NFV, 45 mg/kg was given bid to 25/39 children [33].
- *Ritonavir (RTV)* is a coformulation with lopinavir and has a notable antiretroviral activity; peak concentrations are reached in 2–4 h. In PACTG 338, children receiving RTV and one or two NRTIs had a mean decrease of >1.5 log in viral RNA levels after 12 weeks of therapy. After 48 weeks of RTV plus two NRTIs, 42% of children had an undetectable viral load (<400 CD4$^+$ cells/mm^3) compared with 27% of children receiving RTV plus one NRTI [401]. After 12 months of therapy, PI-naive children receiving RTV with two NRTIs *had <400 CD4$^+$ cells/mm^3* [542]. The increase in CD4 cell numbers is independent of dose level and is also observed even in children with low counts at study entry [398]. Early increases in lymphocytes after RTV therapy are a result of recirculation, as shown by increases in B cells and CD4$^+$ CD45RO and CD8$^+$ T cells [508]. PACTG 377 randomized ART-experienced, PI- and NNRTI-naive children into four different treatment regimens including RTV/d4T/NVP. They had a median *increase in CD4$^+$ cell count* of 254 cells/mm^3 and 41% of children had *HIV RNA <400 copies/ml* at 24 weeks of treatment [580]. In 31 children with advanced HIV infection who were receiving a triple therapy with RTV as PI, no correlation could be demonstrated between elevated plasma drug concentrations and abnormal cholesterol or triglycerides values [160].
- *Saquinavir (SQV)*, used in adults, is a highly specific inhibitor of HIV proteases, which are essential for final assembly of the viral particles for HIV replication. In combination with NRTIs, it further reduces viral load with respect to other monotherapies [151]. SQV can cause headache, nausea, diarrhea and abdominal pain, and must be administered within 2 h after a large meal [450]. In *a trial of 13 children,* adding NFV to a regimen of SQV with one or two NRTIs significantly increased SQV concentrations, and median change in HIV RNA levels was 2.58 log, with 62% of children having HIV RNA levels <50 copies/ml at 48 weeks [290]. In another cohort of *11 HIV-infected children* with intensive prior therapy, salvage therapy with combination SQV/RTV and SQV/NFV with at least one NRTI was well tolerated; reduction in viral load and increase in CD4$^+$ cell count were more pronounced in the group receiving SQV/RTV combination [251]. An advantage of PI-based combination therapy was *the largest CD4% increase among the youngest participants* (9.2% and 8.0%, for ages <5 years, and 5–9 years). Moreover, 16% of significantly immunocompromised entrants achieved CD4% values >25%, whereas children with pre-PI values >25% maintained such values. Although PI-based therapy was associated with substantial improvements in CD4%, initiation before severe immunosuppression and *at younger ages* may be more effective for recovery or maintenance of normal CD4% [512].

All PIs interact with the hepatic cytochrome P450, giving rise to potential interactions with other antiretrovirals and commonly used drugs, some of which are frequent and sometimes dangerous. Doctors and

family members should be given lists of these interactions in order to prevent them [418]. The use of combination therapy including PIs has markedly reduced mortality from 5.3% in 1996 to 0.7% in 1999 (<4.6%) among HIV-1-infected children and adolescents [221]. Their use was not associated with retardation of growth in height or weight, but with small annual increments in height and weight growth in HIV-infected children [70].

Recent studies have shown that long-term HAART therapy induces in HIV-infected patients an increased cell count of CD4 lymphocytes from year 3 to 6, whereas 55% of subjects may demonstrate decreases in CD4 cells counts from year 5 to year 6. A significant difference was noted when comparing naive CD4 cell increases from baseline to year 6 with year 1 *thymus thickness* [510]. Also in vertically HIV-infected children treated with potent ART regimens the rise in CD4 cells and in $CD4^+$ $CD45RA^+$ $62L^+$ T cells was statistically associated with *changes in thymus size* observed over time [563].

Entry Inhibitors

Entry inhibitors, a new class of antiretroviral agents, interfere with the attachment, coreceptor interaction or fusion of HIV-1 with host target cells. The fusion inhibitor *T-20 (enfuvirtide),* the first in this new class, is associated with suppression of HIV-1 replication during 24 weeks of administration. T-20 has been studied *in 14 children,* 4–12 years of age, with incompletely suppressed HIV-1. In ten subjects (71%) virologic suppression of ≥1.0 $\log_{10}$ was achieved at 24 weeks; six subjects (43%) had viral loads >400 copies/ml and three (21%) had <50 copies/ml at 24 weeks. No child discontinued because of adverse events [106]. In 491 patients at 24 weeks, there was a decrease of 1.696 $\log_{10}$ copies/ml in the T-20 group, and a decrease of 0.764 $\log_{10}$ copies/ml in the control group, but more cases of pneumonia (5.6%) were seen in the T-20 group than in the control group (0.3%). The addition of *enfuvirtide* to an optimized ART provided significant immunological benefit through 24 weeks in patients who had previously received multiple ART drugs and had multidrug-resistant HIV-1 infection [323]. In addition to T-20, investigational entry inhibitors include *PRO 542* (a CD4 attachment inhibitor) and *T-1249,* 5 Helv and IQN-17, other inhibitors of membrane fusion [232].

Anti-HIV Drug Updates

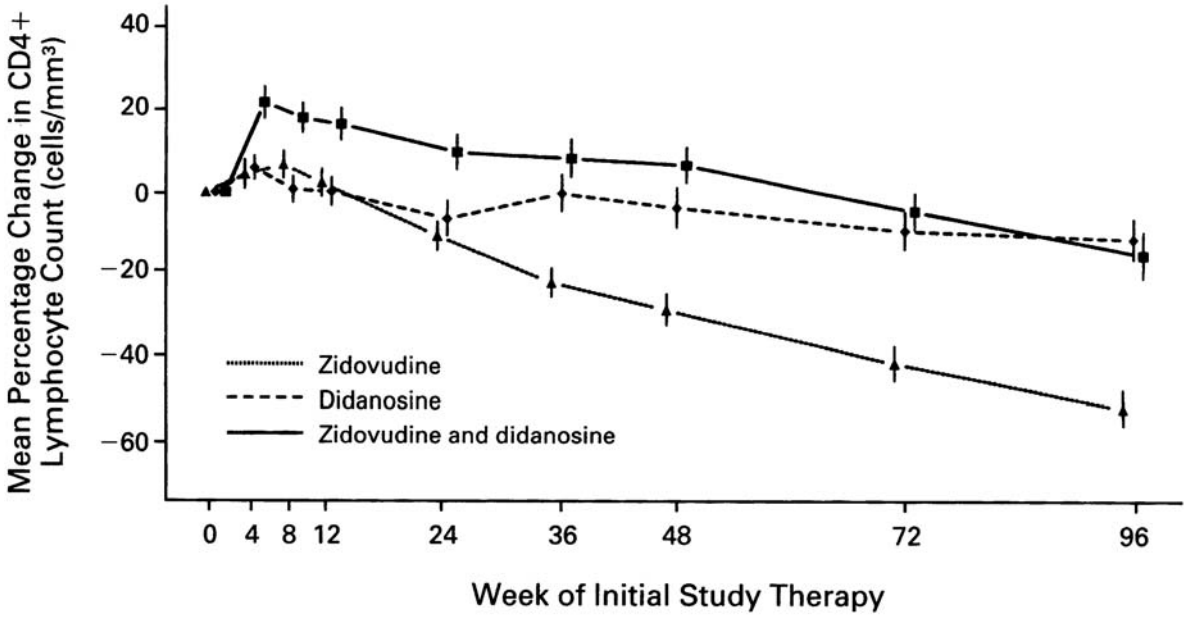
Despite the availability of 17 ART drugs approved for the treatment of HIV infection, nine candidate drugs with novel properties are in development. Investigational NRTIs include *emtricitabine* (FTC) and *amdoxovir* (DAPD). FTC, a potent deoxycytidine NRTI recently approved by the USFDA for the treatment of HIV infection proved to be effective and safe in *25 children* 22 months to 17 years of age receiving two single oral doses of FTC (60 and 120 mg/m^2, up to a maximum of 200 mg, in solutions) [570].

Investigational NNRTIs include DPC 083 and TMC 125. Investigational PIs include *atazanavir*, *tipranavir* (TPV), *rever set* (RVT) [259] and *TMC 114* [232].

Studies with Associated Therapies

So far, we have examined the properties of different drugs. However, anti-HIV-1 monotherapy has now been replaced by multitherapy associations, which have proven to be more effective. Two studies have reported results: one treated eight 2- to 16-month-old children with a combination of *ZDV, ddI and NVP,* which was very effective and well tolerated [345], while in PACTG 152, a multicenter study, 831 children aged >30 months were given ZDV or ddI or both drugs [170]. It was clear that ddI as well as the ddI-ZDV combination were more effective than ZDV alone (Fig. 23.26) [170]. It was found that *ddI alone had an efficacy* similar to that of ZDV plus ddI (RR of disease progression or death, 0.98), and a significantly lower risk of anemia or neutropenia was seen in children receiving ddI alone [170]. In a further pediatric study comparing therapy with ZDV alone or associated with ddC, the combination was well tolerated, apart from an increase in neutropenia (14%) compared to children treated with monotherapy (5%). However, the decreased number of CD4 was greater with monotherapy (25% per year) vs 13% per year [29]. In turn, d4T was well tolerated, without showing symptoms of myelosuppression and alterations of the blood parameters, probably due to the incremental dosage that was used [288]. These objectives were extended to PACTG 327, a randomized trial to evaluate the safety, tolerance and antiviral activity of ddI and d4T in combination or d4T alone, which found the combination to be superior to d4T monotherapy in 108 ART-experienced children previously enrolled in PACTG 240 (d4T monotherapy vs ZDV monotherapy) or who had received ZDV monotherapy for at least 6 months, with a greater safety from a clinical standpoint and a favorable effect on viral load [288].

Triple therapies, as we have prviously seen, are effective in view of the increasingly frequent isolation in HIV^+ subjects of HIV strains that are resistant not only to ARTs but also to PIs [241]. For example, the therapy referred to as *HAART relies on two NNRTIs and a viral-PI.* Administered twice a day, it also permits better compliance. Long-term (>96 weeks) immunological improvement and reconstitution with a naive T-cell phenotype ($CD4^+$ $CD45RA^+$) have been seen in some children receiving the combination ZDV, 3TC and the PI indinavir [269]. ZDV has been studied as part of a PI-sparing, three-drug nucleoside analog regimen (ZDV, 3TC and ABC) in ART-experienced children. Increased viro-



number of patients

Zidovudine	273	235	232	241	237	225	210	165	107
Didanosine	278	242	245	245	231	216	211	174	115
Zidovudine and didanosine	269	237	226	236	233	219	212	166	117

Fig. 23.26. Mean (±SE) percent change from baseline in $CD4^+$ lymphocyte counts at interim analysis

logical benefit was found in those children who had two new NRTIs added to their regimen [481]. Combination triple therapy with NVP, ZDV and ddI in eight infected infants 2–16 months of age with HIV-1 MCTC has been associated with sustained viral suppression [345]. PACTG 377 randomized 181 mild to moderately suppressed children to one of four combination treatment regimens. Interestingly, those children receiving a *quadruple regimen* containing both NVP and a PI had a significantly greater increase in $CD4^+$ cell count from baseline to week 24 than those receiving other regimens [581]. In two pediatric open-label studies, 70% of children receiving IDV/ZDV/3TC had HIV RNA levels of <500 copies/ml after 6 months of therapy [554], or HIV RNA levels were maintained at <400 copies/ml after 18 months of IDV/d4T/3TC therapy in 87% of children who entered the study with $CD4^+$ cell counts in CDC immune class 2 and 72% of those who entered with CDC immune class 3 [561]. Early treatment with d4T, ddI and NFV was well tolerated and associated with good clinical and immunological outcomes at week 72. However, a high rate of virological failure with emergence of genotypic resistance is of great concern. More palatable drug combinations for infants are required [1]. In a phase II, randomized, multicenter study, 41 children aged 5 months–21 years with prior NRTI and no prior NNRTI or PI experience received either NFV 30 mg/kg bid, RTV 400 mg/m bid and ddI 240 mg/m/day (arm A) or NFV 50–55 mg/kg bid, NVP 120 mg/m bid and d4T 1 mg/kg bid (arm B). The proportion of children with HIV-1 RNA ≤400 copies/ml at 48 weeks was 65% among children in arm A vs 28 in arm B ($p = 0.039$). No significant difference in median CD4% change from baseline to week 48 was found (3% vs 1%) although a trend toward a higher rate of permanent discontinuation of study treatment was noted among children in arm A compared with those in arm B (35% vs 10%), $p = 0.12$ [285]. NRTI-sparing regimens have not yet been systematically evaluated in children. To take advantage of NVP and EFV lowering PI plasma levels, coadministration of *lopinavir*/RTV with NVP and EFV necessitates an increased dose of 300/75 mg/m^2 tid lopinavir/RTV to compensate for the EFV-induced drug–drug interaction in HIV-infected children [37].

The PACTG studies also examined *quadruple therapies:* PACTG 152 demonstrated that ddI or ZDV + ddI treatments were superior to ZDV alone [170]; PACTG 356 in children aged 2 weeks to 2 years includes a triple regimen with ZDV, 3TC and NVP, and two quadruple ones, one with ZDV, 3TC, NVP and ABC and the other with the three previous ones + NFV, while PACTG 345 involving children aged 4 weeks to 2 years includes another triple therapy with ZDV, 3TC and RTV. The US-based Pediatric AIDS Foundation has instead promoted a trial with ZDV, 3TC and ABC, and combinations with adefovir and APV are also being studied [344, 345]. The

RH (relative hazard) of death in children receiving monotherapy or double or triple combination therapy was 0.77, 0.70, and 0.29, respectively, vs no ART [144].

Results of epidemiological and clinical trials suggest that women receiving HAART regimens that effectively reduce HIV-1 RNA to <1,000 copies/ml or undetectable levels *have very low rates of perinatal transmission* [125, 154]. Children receiving HAART achieved a plasma HIV-1 RNA level <400 copies/ml by a median of 4 weeks after initiation of therapy and a decline to <50 copies/ml by 20 weeks. Children whose CD4 lymphocyte counts increased >70 cells/μl by 20 weeks on therapy were more likely to achieve durable virological and immunological benefit [515]. Among 57 children treated with EFV, NVP, and NRTI, 76% had plasma HIV-1 RNA levels >400 copies/ml at 48 weeks of therapy and 63% had levels <50 copies/ml. A high plasma HIV-1 RNA level at baseline significantly decreased the likelihood that plasma HIV-1 RNA levels would become undetectable during treatment [520]. Because transmission can occur even at low or undetectable HIV-1 RNA copy numbers, RNA levels should not be a determining factor when deciding whether or not to use ZDV for chemoprophylaxis [388]. Use of combinations of RT drugs should not overlook the fact that these can act together to further increase HIV-1 mutant frequencies, which could have important implications for virus population dynamics and could compromise drug therapy regimens [353].

To conclude, the virological effectiveness of multidrug ART in previously drug-naive individuals appears to be such that viral suppression, once achieved, can be maintained for several years in patients not interrupting therapy. Clearly, it is necessary to develop regimens that can be followed consistently and safely for such long periods of time [434].

There are data on the decline of CD4 cells or percentage during *structured* [99] (STI) *or unplanned* [213] *treatment interruptions* in HIV-infected children. It is suggested that when designing pediatric trials of planned treatment interruptions, although the average CD4 decline after stopping HAART appears similar to that in adults, the length of interruption should be determined by the time taken for CD4 to decline below a threshold, rather than by imposing interruptions of fixed duration [213]. An STI HAART regimen that consisted of dosing every other week AZT, 3TC, and NFV was started in three chronically HIV-1-infected, ART-naive African children with advanced disease. After 14 months, *a sustained increase of CD4$^+$ T lymphocytes was observed.* At 26 months after the initiation of the STI regimen of HAART, the three children *attended school regularly* and rarely missed days because of AIDS [99]. This approach may increase HIV-1-specific cellular immunity by intermittently exposing the patient to autologous virus, which results in the control of HIV-1 replication [391], particularly during acute primary HIV-1 infection, since treatment interruption has been shown to boost HIV-1-specific immunity in some cases. [381]. Although virus recrudescence was noted during the treatment interruptions, and the off-treatment viral rebound rate [415], *failure to reach full viral suppression rarely occurs,* and the development of resistance after reinitiation of treatment has been infrequently described [381].

Drug Resistance

The issue of drug resistance is not new, the evolution of ART phenotypic and genotypic drug resistance to ZDV, 3TC, ABC and NFV was studied in 113 prior untreated children in the PENTA 5 trial, which showed that selection of 3TC-resistant virus was most frequent, followed by NFV and/or ABC; selection of ZDV-resistant virus was rare. The most sustained HIV-1 RNA response was in the 3TC+ABC arm, but mutations conferring reduced susceptibility to 3TC and/or ABC evolved more frequently if virological failure occurred with 3TC+ABC than with ZDV+ABC [211]. *Genotypic resistance was common in children* treated with 3TC (91%), NVP (75%), and ZDV (64%). The prevalence of mutations was lower among those receiving other NRTIs and PIs. It is noteworthy that ART resistance was common among children failing treatment: as adherence to treatment was low in 50%, this was likely to be an important contributory factor [399]. Following the progress of children who had HIV genotyping performed after HAART failure, resistance mutations to antiretrovirals was detected in two-thirds of children without prior exposure to that drug [116]. *HIV-1 resistance to T-20 emerged in 2/16 HIV-1 infected adults* enrolled in the phase I T-20 clinical trial during 14 days of treatment [573]. There is increasing evidence that resistant strains of HIV are being transmitted in North America with an estimated prevalence of 1%–11% [336], in Europe [60], Ivory Coast [5], Gabon [558], and in infants [184] as a result of the difficulty of completely suppressing viral replication [457]. Resistance to one drug often confers some level of reduced sensitivity to other drugs of the same class, which dictates that genotypic resistance be evaluated before switching therapies [501].

In conclusion, we should disclaim early expectations that combination ART would eradicate infection, since virus can persist in latent reservoirs for many years despite effective ART [186, 431]

Immunological Rationale

We must consider that newborns physiologically have a relative lymphocytosis, with a characteristic profile of lymphocyte subpopulations (Tables 2.5, 2.6). The relative increase in CD4 lymphocytes depends, in normal T cell ontogenesis, on a stage where CD4 and CD8 membrane proteins are coexpressed by the same cell, so that these lymphocytes are present in the newborn's circula-

Table 23.32. Recommendations for PCP prophylaxis and CD4+ monitoring for HIV-exposed infants and HIV-infected children, by age and HIV infection status[a]

Age/HIV infection status	PCP prophylaxis	CD4+ monitoring
Birth to 4–6 weeks, HIV exposed	Prophylaxis	1 month
4–6 weeks to 4 months, HIV exposed	Prophylaxis	3 months
4–12 months HIV infected or indeterminate	Prophylaxis	6, 9, and 12 months
HIV infection reasonably excluded	No prophylaxis	None
1–2 years, HIV-infected	CD4+ counts <750 cells/μl in first 12 months	
	Or <500 cells/μl at 12 or 24 months, or CD4<15%	Every 3–4 months
2–5 years, HIV-infected	CD4+ counts <500 cells/μl or percentage <15%	Every 3–4 months
6–12 years, HIV-infected	CD4+ counts <200 cells/μl or percentage <15%	Every 3–4 months
All ages, HIV-infected, at risk of PCP		Every 3–4 months

See text related to children with negative tests.
Data from [15, 60].
[a] If CD4 counts or percentages (Table 23.29) are approaching the age-adjusted threshold level for initiation of prophylaxis, the CD4+ lymphocyte count should be assessed more frequently (at 1-month intervals) to evaluate the rate of decline and the need to initiate prophylaxis. PCP prophylaxis may be considered independently of T-cell count, as follows: (a) All children previously suffering from PCP reasonably ascertained; (b) All HIV-infected children in the first 2 years of life; (c) All children aged >2 years with severe clinical manifestations or severe immunodeficiency. Prophylaxis should be considered on a case-by-case basis for children who might otherwise be at risk for PCP, such as children with rapidly declining CD4+ counts or percentages or children with Category C conditions (severely symptomatic). Children who have had PCP should receive lifelong PCP prophylaxis.

tion, thus inducing a relative increase in CD4 lymphocytes. As compared to adults, children have a much higher proportion of virgin T cells that are initially undifferentiated and pluripotent, differentiating into CD45RO only after their encounter with the antigen. As long as the thymus continues to function, children may greatly recover T lymphocyte number and function via their reserve of virgin cells. This also explains why, in *HIV-positive newborns and infants, the lymphocyte percentage and absolute decrease is more difficult to detect* and, in any event this is a more laborious parameter to identify than in adults [46, 83, 169, 174, 182, 225, 416, 464]. Since it is impossible to preserve the immune infrastructures, this prevents the renewal of impaired T lymphocytes and complete immune reconstruction [242]. However, adequate suppression of viral replication by combination ART results in the rapid correction of TcR diversity within CD8 subsets because of the emergence of new T cells from the thymus [295]. Moreover, an increase in naive CD4 T cells in treated patients was shown to occur more precociously and at a higher rate in young children than in adults [117, 193]. Resting or naive cells contributed significantly to the rise in CD4 T cells in infants and young children [52]. Additionally, in children with CD4 rates <20%, usually associated with higher viral load, the decreased viral load after ART *allows the generation of new CD4 T cells by the thymus,* thus recovering their normal number [128]. Suppression of viral replication in HIV-infected children particularly occasions a recovery of thymic function that should help to reestablish diversity within the T-cell repertoire and correct T-cell function [156]. Significantly, at 96 weeks, CD4+ cell counts were 85% higher in the children receiving ddI and 76% higher in the children receiving combination therapy than in the children receiving ZDV alone [345].

Treatment of Infective Complications

PCP Prophylaxis

PCP was reported as a disease marker in 33% of affected children in 1991 [174] and in 39% in 1994 [177], and only in 2.4% in 1996 [169], showing that many children are identified earlier as being at risk for HIV infection and thus receive PCP prophylaxis [15]. For this purpose, the infants who were not given prophylaxis prior to PCP diagnosis have also been screened, thus allowing their identification at the time of diagnosis [506]: prophylaxis must therefore be initiated in more children than recommended by guidelines [505], expanding this prophylaxis to all newborns of HIV+ mothers. Since PC can also be transmitted to the fetus [395], this prophylaxis should also be necessary for pregnant mothers.

Therapy and prophylaxis for PCP, the most common and highly lethal opportunistic infection, is based on CD4 values according to age, as noted (Table 23.29). Data on PCP prophylaxis and CD4 monitoring are listed in Table 23.32 [15, 60].

Table 23.33. CDC recommended drug regimens to prevent first PCP episode in children ≥4 weeks of age

Drug	Preventive regimen		
Trimethoprim	150 mg/m^2/day		
+ Sulfamethoxazole	750 mg/m^2/day administered orally in two divided doses three times a week on consecutive days		
	Or		
Trimethoprim	5 mg/kg/day		
+ Sulfamethoxazole	25 mg/kg/day administered orally in two divided doses three times a week on consecutive days		
Alternative regimens	As above, administered orally as a single dose three times a week on consecutive days, or in two doses administered orally on three alternate days, or bid/day		
Alternative regimens if trimethoprim + sulfamethoxazole is not tolerated			
Dapsone	Children aged ≥1 month, 2 mg/kg, max 100 mg, orally q day, or 4 mg (max 200 mg) q week		
Pentamidine	Aerosolized in children aged ≥5 years, 300 mg administered q month or pentamidine IV, 4 mg/kg, every 2–4 weeks[a]		
Or			
Atovaquone	Weight (kg)	Daily dose	Dosage regimen
/proguanil HCl	11–20	62.5 mg/25 mg	1 pediatric tablet daily
(Malarone)[b]	21–30	125 mg/50 mg	2 pediatric tablets daily
	31–40	187.5 mg/75 mg	3 pediatric tablets daily

Data from [1, 90, 96].

[a] see Chap. 19 for details.

[b] At the time of writing no data are available on safety and efficacy of Malarone for PCP prophylaxis in children weighing <11 kg, although studies are in progress.

Start of PCP Prophylaxis

In all infants born to HIV-infected mothers, PCP prophylaxis must be started at the age of 4–6 weeks, regardless of CD4 values indicated in the table. If PCP is identified after the 6th week, prophylaxis must begin at the time of diagnosis. Prophylaxis must not be moved up, due to the immaturity of bilirubin metabolism, which can lead to side effects. Especially among children being treated with ZDV, it can potentially exacerbate ZDV-induced anemia: in this case, prophylaxis should be started at the 6th month, when ZDV is discontinued [15, 91].

Prophylaxis in 4- to 12-Month-Old Infants

PCP prophylaxis must be continued until the child is at least 1 year old, unless the two subsequent tests (culture, PCR) are negative at the ages of 1 and 4 months, or at 6 months if these tests are not available. The drugs and their dosages are listed in Table 23.33 [1, 90, 96], and it must be remembered that co-trimoxazole triggers several reactions, which are documented in Chap. 19. Nevertheless, this therapy substantially lowers the risk of PCP in HIV-infected infants <18 months old [538], although certain children may experience PCP despite regular prophylaxis [397]. *Atovaquone* is a medication employed for malaria treatment that can be employed in children who cannot tolerate the other medications [1].

Prophylaxis in Children 12 Months and Older

All children of ≥12 months with HIV infection must have CD4 monitored based on the indications in Table 23.32; those who have not undergone prophylaxis due to the lack of an initial diagnosis or because the prophylaxis has been interrupted must start it based on the data listed in Table 23.29. On the other hand, children who have received prophylaxis regularly between the ages of 12 and 24 months must be retested [91]. A hospital-based study in Malawi described the clinical presentation and outcome of PCP in young children. Among 150 children with Rx-confirmed severe pneumonia, 16 cases of PCP were identified and 10 of these children died. All cases of PCP were <6 months of age [223].

Other Infections Caused by Opportunistic Agents

There are several active infections in pediatric AIDS, each of which must be treated aggressively and in a targeted manner, according to specific protocols. Again, the main viral infections are caused by CMV, EBV, HSV, and

Table 23.34. Antiviral agents and related dosages for treating opportunistic infections in HIV-infected infants and children

Virus	Agent	Dose/day	Route
CMV			
Severe, life-threatening disease	Ganciclovir		
	Induction	5 mg/kg × 3	IV
	Maintenance	6 mg/kg	IV
	Or		
	Foscarnet		
	Induction	60 mg/kg × 3	IV
	Maintenance	120–150 mg/kg	IV
HSV			
Mucocutaneous			
Mild	Acyclovir	10–20 mg/kg × 4	PO
Generalized	Acyclovir	500 mg/m^2	IV
Encephalitis	Acyclovir	10–15 mg/kg × 3	IV
Acyclovir-resistant	Foscarnet	40 mg/kg	IV
Measles			
Severe disease	Ribavirin	5 mg/kg× 3	IV
Pneumonia	Ribavirin	6 g	Aerosol
VZV			
Chickenpox	Acyclovir	500 mg/m^2	IV
	Or		
	Acyclovir	20 mg/kg	IV
Zoster	Acyclovir	20 mg/kg	IV
		Or	
	Acyclovir	500 mg/m^2	IV
Acyclovir-resistant	Foscarnet	40 mg/kg	IV

From [56, 566].

VZV. Table 23.34 [56, 566] summarizes the main drugs that can be used to treat these infections. For CMV, initial therapy with ganciclovir or foscarnet must continue for 14–21 days and be followed by IV maintenance therapy indefinitely, with the likely association of anti-CMV hyperimmune Ig [56]. It should be noted that, in children, *ganciclovir* provokes reversible neutropenia, thrombocytopenia and exanthema, while *foscarnet* triggers tubulopathy, hypocalcemia and alteration of liver function tests [566]. Treatment with acyclovir must be continued for at least 10–14 days. For *ribavirin,* we refer to the comments concerning the treatment of bronchiolitis. Among infections caused by atypical mycobacteria, MAIC ranks first. The spread of these infections has also fostered a new propagation of *M. tuberculosis*. Pediatric therapy relies on drug combinations: Tables 23.35, 23.36 [1, 14, 56, 252] indicate the most effective ones also for infections caused by *M. tuberculosis*. Other opportunistic infections include candidiasis, which is responsible for systemic forms, and *cryptococcosis, cryptosporidiosis and strongyloidiosis,* which are increasingly prevalent in the pediatric age range (Table 23.37) [56, 448, 566]. By incorporating amphotericin B in liposomes, higher dosages of the drug can be administered without increasing the side effects [352]. For infections caused by parasites, the therapy for toxoplasmosis is summarized in Table 23.38 [1, 56, 552, 566]. Given the surge in tuberculosis (TB) infections, a frequent and important coinfection of HIV, it has quite recently been observed that the risk in children of HIV-infected mothers can increase, regardless of the child's specific status. Therefore, before the child is allowed to leave the maternity ward or, better yet, during pregnancy, it is advisable to ascertain the mother's TB status, giving due consideration to the anergy that frequently accompanies HIV infection and can yield a negative Mantoux test (MT),

Table 23.35. Treatment of tubercular infection in HIV-infected infants and children[a]

Treatment	Dosage/day (per os)
Isoniazid	10–20 mg/kg/day, max 300 mg/day
	Or 20–30 mg/kg (max 900 mg) bis in week
Isoniazid-resistant	
Rifampin	10–20 mg/kg/day, max 600 mg/day
Multidrug-resistant	
Ethambutol	15 or 25 mg/kg day, max 2.5 g/day
Pyrazinamide	20–40 mg/kg/day, max 2 g/day

[a] Usually a two- to three-drug regimen is recommended, and a four-drug regimen for disseminated infections: in this case isoniazid and rifampin are coadministered as a single medication, which is coadministered with pyrazinamide as a second medication.
Data from [1, 14, 56, 252].

Table 23.36. Treatment of intracellular *Mycobacterium avium* infection in HIV-infected infants and children

Treatment	Dosage/day (per os)
Amikacin	15–22.5 mg/kg in 2–3 doses
Azithromycin	5–10 mg/kg once a day
Ciprofloxacin	10–15 mg/kg × 2
Clarithromycin	7.5 mg/kg × 2
Ethambutol	15–25 mg/kg/day
Rifabutin	<6 years 5 mg/kg once a day
	>6 years 300 mg once a day

Amikacin and ciprofloxacin have been used above all in adults.
Data from [1, 14, 56, 252].

making a chest X-ray necessary [14, 56]. We recommend obtaining information about the TB status of other persons living in the same household and keeping the child away from anyone who is contagious. Every child exposed to these people, even if the MT and X-ray are negative, must follow *preventive treatment with isoniazid* (Tables 23.35, 23.36) in a single dose for 3 months, then repeating the MT: if it is positive, specific therapy must be started [14].

Other Forms of Treatment

Immune-modulating therapies with IL_2 have been partially ineffective in adults [298], as well as with IL_{15} [259]. In the presence of antibody defects, IVIg is used, and this has yielded encouraging results so far: both the clinical progression and several immunological parameters have shown improvement in children aged <2 years, with significant differences with regard to the rate of infections in those not being treated with TMP-SMZ (trimethoprim-sulfamethoxazole) [514]. The administration of Ig at high doses is thus justified in pediatric AIDS based on the following considerations:

- Replacement of the insufficient formation of antibodies against new antigens, which is extremely important in pediatric AIDS
- Prevention of infections caused by concomitant viruses (particularly EBV)
- Favorable effect of Ig on autoimmune phenomena, which indubitably participate in the pathogenesis of AIDS [83]

However, in 30 HIV-infected children aged 2–11 years who received 6 monthly IVIg infusions, plasma p24 levels decreased, but CD4 cell levels, plasma RNA copy number, cellular virus, Ig levels, and neutralizing antibody titers were minimally affected by the infusions [526].

Future Perspectives

Mother-to-fetus transfer can be used to prevent the vertical transmission of AIDS, if the possibility to compensate for the deficient anti-gp120 antibody transmission is confirmed [316]. Following this line of research, HIVIg (anti-HIV Ig) for the prevention of viral transmission was transfused to pregnant mothers and their babies, and this led to a reduction in p24 levels, an effect that was not achieved with standard IVIg [314]. Data regarding currently or imminently available immunoprophylaxis to prevent MTCT of HIV-1 indicate a potential use in neonatal trials within the coming 1–2 years [484].

Perhaps, as previously mentioned, the *defensins* inhibited the replication of HIV-1 isolates in vitro (Tables 23.9, 23.10). *Soluble CD4* (a known ligand for HIV gp120) completely reverses HIV gp120-mediated immunosuppression and may be a potential immune adjuvant in HIV-infected individuals [280]. Another option is the non-expression of CCR5, which translates into a reduced disease progression; thus subjects HZ for a mutant allele of CCR5 (*CCR5D32*) are safe from sexually transmitted infection [341]. The genetic variants that cause *coreceptor deletion cause AIDS resistance in HIV-exposed* high-risk individuals, or they can delay its progression [146, 341, 486]. Therefore, taking a cue from CCR5, *CCR5D32* mutant alleles valid for HZs [341] and CCR2, CCR2-641 that can delay AIDS onset in HZs and heterozygous (HET) by 2–4 years, exogenous or mutated chemokines could be administered, stimulating the host to produce them in greater quantities, or their actions could be mimicked with different substances [407, 444].

Caucasians in North America or Europe who are HZ for a 32-bp (base pair) deletion in the coding region of the *CCR5* gene, which results in a truncated, nonfunc-

Table 23.37. Antifungal agents and related dosages for treating mycotic infections in HIV-infected infants and children

Infection	Agent	Dose/day	Route
Aspergillosis	Amphotericin B	0.5–1.5 mg/kg	IV
Blastomycosis	Amphotericin B	0.5–1 mg/kg	IV
Candida albicans			
Oral thrush	Nystatin	200,000–500,000 µg × 4	PO
	Or		
	Ketoconazole	5–10 mg/kg	PO
	Or		
	Fluconazole	2–6 mg/kg	PO
Invasive	Fluconazole	3–6 mg/kg	PO or IV
	Or		
	Amphotericin B	0.5–1 mg/kg	IV
Metastatic	Amphotericin B	0.5–1 mg/kg	IV
	±		
	Flucytosine	37.5 mg/kg × 4	PO
Coccidiomycosis			
Non-CNS	Amphotericin B	0.5–1 mg/kg[b]	IV
Meningitis			
Initial	Amphotericin B	0.5–1 mg/kg[b]	IV
	±		
	Amphotericin B	0.1–0.3 mg/kg	IT
Maintenance	Amphotericin B	1 mg/kg	IV[c]
	Or		
	Fluconazole	3–6 mg/kg	PO
Cryptococcosis meningitis			
Initial	Amphotericin b	0.5–1 mg/kg	IV
	±		
	Flucytosine	37.5 mg/kg × 4	PO
Maintenance	Fluconazole	3–6 mg/kg	PO
Histoplasmosis			
Initial	Amphotericin b	0.5–1 mg/kg[a]	IV
	Or		
	Intraconazole	2–5 mg/kg q 12–24 h	PO
Maintenance	Amphotericin B	1 mg/kg	IV
	Or		
	Intraconazole	3 mg/kg	PO
Strongyloidiosis	Azithromycin	30–40 mg/kg	PO[d]

Data from [56, 448, 566].
[a] Total dose = 15–20 mg/daily.
[b] Total dose = 30 mg/daily.
[c] Every 7 days.
[d] For 2–4 weeks.

Table 23.38. Treatment of toxoplasmosis in HIV-infected infants and children

Treatment	Dose/die	Route
Clindamycin	20–30 mg/kg in four doses	PO
	Plus	
Folinic acid	5–10 mg every 3 days	PO
Leukovorin	5 mg every 3 days	PO
Pyrimethamine	1 mg/kg/day[a]	PO
	Plus	
Sulfadiazine maintenance or (for nonresponders)	50 mg/kg q 6 h 80–120 mg/kg q 6–12 h	PO

Data from [1, 56, 552, 566].
The daily dose of TMP-SMZ recommended as the preferred regimen for PCP prophylaxis appears to be effective against toxoplasmosis encephalitis. If children cannot tolerate TMP-SMZ, the recommended alternative is dapsone-pyrimethamine, which is also effective against PCP. Atovaquone might also provide protection [552] (Table 23.33).
[a] Maximum, 25 mg/daily.

tional receptor in CCR5, lack expression of this receptor and thereby resist infection [18, 283, 341]. HET mutations are associated with lower pre-AIDS viral loads and delayed progression to AIDS [203], but at least four HIV-1-infected individuals HZ for the 32 allele deletion in the *CCR5* gene have been described [44]. This hypothesis is not supported in patients of other races or continents [374, 408, 589]. The participation of other receptors, such as CCR2b and CCR3, has also been suggested [105, 148]. An association between CCR5 promoter polymorphisms and long-term asymptomatic HIV-1 infection with individuals lacking the *CCR5 59029A/CCR5 59353C* HZ genotype is likely to progress more slowly towards AIDS [109]. *Two pediatric studies* have observed a benefit of the *CCR5D32* deletion on disease progression [32, 385]. *CCR5D32* HET exerted a protective effect against perinatal transmission in 181 infants exposed to a low maternal viral burden [412]. However, there is discrepancy as to the impact of the *CCR532* allele on pediatric HIV-1-related disease progression. A meta-analysis *on 1,317 HIV-1-infected children* showed that no deaths occurred among *CCR5D32* carriers in the first 3 years of life, and for *CCR2-64I*, the HR for death was 0.69 (OR) in the first 6 years of life and 2.56 (OR) in subsequent years. *CCR5532* and *CCR2-64I* offered *no clear protection after clinical AIDS had developed* [261].

Another option of CCR5 deletion is the anti-CCR5 which blocks the coreceptor binding to HIV env. Blocking the HIV-CD4 fusion means blocking the virus entering the cell, and thus HIV replication [259].

Another option could be to block the interaction between IP-10 and CXCR3: this may represent a possible new target for ART [317].

Gene therapy instead entails a head-on fight against HIV. Modification of vesicular stomatitis virus (VSV) genome, by replacing the genes coding its env proteins with those of human CD4 and fusin proteins, leads to HIV fusion with VSV, as HIV thinks it is binding to lymphocytes. Instead, once inside, VSV will ultimately destroy it. Moreover HIV loses its ability to infect the host, but continues to replicate indefinitely. This allows VSV to enter HIV-infected cells, multiplying inside the cells and making them unable to spread AIDS, almost as if it were a targeted drug released on a cell level. In fact, a few days after VSV was added in vitro to a mixture of lymphocytes and HIV, levels decreased by 300–10,000-fold [495]. Another study reached similar results using the rabies virus that was unable to replicate [375].

As regards *the vaccines,* studies gave rise to the hope that, once they leave the limbo of research in vitro and on animals, vaccines can be applied to numerous other human disorders. Vaccines in monkeys with *inactivated viruses* or with *live attenuated viruses* have not yielded positive results in humans so far, but current promising study results need a period of 5 years in order to be turned into an effective vaccine for humans. Currently, several proteins from the HIV env are being used, such as gp120, or structural ones from the env, such as inactivated tat protein, or the natural and active type. The inactivated (or dead) HIV virus or gene therapy could also be used. One of the challenges faced by an HIV vaccine is the different HIV clades and recombinants of these clades. Therefore, an HIV-1 vaccine will need to be made for each cluster or cross-clade protection will be sufficient [461]. An infection can escape a T-cell vaccine by a single nucleotide mutation within its epitopes [30], or exhaust its T-cell response by the persistence of activated virus-specific T cells without effector function [590], or by suboptimal T-cell help due to progressively impaired function of HIV-specific $CD8^+$ T cells [293]. A recently tested HIV vaccine is composed of recombinant forms of gp120 that resemble two gp120 proteins found on the HIV strain subtype B. This vaccine was shown to be protective only in non-white people. Considering the high prevalence of AIDS in children outside Europe (Fig. 23.10), this vaccine could be a notable advancement in the anti-HIV fight [364]. A series of therapeutic RNA-based inhibitors of HIV-1 infection may suppress HIV infection by targeting a different gene product including a U6 Pol III promoter-driven short hairpin RNA targeting the *rev* and *tat* mRNAs of HIV-1, a U6 transcribed nucleolar-localizing TAR RNA decoy, and a VA1-derived Pol III cassette that expresses an anti-CCR5 ribozyme [335]. The HIV infection could be blocked by the gene encoding CCL3L1 (MIP-1αP), a potent HIV-1-suppressive chemokine and ligand for the HIV coreceptor CCR5. Protection from HIV infection could depend on a high CCL3L1 copy number. These findings could provide key insights into the immune correlates of an effective vaccine [220].

Outcome

A number of children have presented transitory forms of AIDS [33, 54, 69, 145, 175, 210, 544], which in some large studies reached a figure of 2.5%–4.7% [175, 544]. These were children diagnosed at birth by isolating the virus from PBMCs [69, 210], and they subsequently proved to be HIV-negative in all specific analyses after 5 [69] and 9 years [210]. To avoid any uncertainty, guidelines have been proposed for diagnosis [372]. An extensive European study has verified that the incidence drops with cesarean delivery [430]: if it is *associated with ZDV therapy, the rate drops to 1%* [42].

Ever since it became clear that HIV infection is not an illness circumscribed to small groups but that it strikes growing numbers of patients around the world, in many countries AIDS has become public enemy number one. Critically important is the fact that >90% of the estimated 2,000 new pediatric infections that occur each day around the world occur in developing countries [528] and hundreds of thousands continue to become infected annually [3], even in those settings where concerted efforts are being made to prevent HIV MCTC with proven interventions that reduce, but do not completely eliminate, new infections [268]. It is even more important that during an overwhelming epidemic among adults, many countries may opt to neglect the specific needs of infected children and treatment programs sometimes appear to *include children only as an afterthought* [3]. Systematic sidetracking of mothers at risk could ensure the most appropriate and timely treatment of their infected children (Table 23.39) [84, 565]. In a trial including 826 *pregnant women interviewed, 8% (n=65) refused HIV testing*. Independent predictors of HIV testing refusal were being foreign-born, not receiving general information about HIV, and not receiving specific information about HIV and pregnancy (OR between 2.11 and 7.48 with related CI). The most common reasons for testing refusal were being in a monogamous relationship for foreign-born women (41%) and already being tested for US-born women (65%) [26]. The prognosis for infants with early forms is very reserved, survival is low and the mortality rate is high in 50% of cases at 3–4 months after the onset of PCP [161]. *The analogy with the Slaughter of Innocents by a new Herod* is bewildering but by no means unjustified: however, those little, blameless victims died at the age of 2 and in a matter of minutes, not after months or years of unspeakable and inhuman suffering [79, 83].

Confronted with the unprecedented development of effective prophylactic and therapeutic strategies, everyday complications are extensive. Children with neurological and psychological disorders may suffer from problems and difficulties of socialization since their families' lives were damaged by isolation and rejection from the community [291]. The psychiatric manifestations in the general pediatric population <15 years of age in 2002 had an incidence of 6.17 cases per 1,000 person-years, significantly higher than the incidence of 1.70 cases per 1,000 person-years, as reported in the 2000 National Hospital Discharge Survey, yielding a 3.62-fold increase [206].

Table 23.39. Prenatal care in HIV-infected women encouraging positive thinking

1.	Identification of HIV infection should start early in pregnant women, especially in those with unknown serology: awareness of HIV status of pregnant women identifies HIV-exposed infants.
2.	Recommend to HIV+ women of child-bearing age, whether symptomatic or not, to voluntarily accept HIV counseling and specific prenatal HIV testing.
3.	If HIV+ mothers prefer not to get pregnant, inform them about the pharmacological and therapeutical progresses, both in HIV infection prevention and management.
4.	Expectant mothers who decide to give birth should be assisted with particular care, allowing mothers to become informed about the effectiveness of a treatment that can also be delayed.
5.	Advise against prenatal diagnosis.
6.	Weigh the prospect of anticipating the start of treatment in pregnant women as well as of multiple chemotherapy and prophylaxis.
7.	Reduce to a minimum the risk of intrapartum HIV transmission by programmed cesarean sections.
8.	Select an economic, rapid and reliable HIV testing to ensure an early diagnosis of neonatal HIV infection, thus differentiating the infant carrier of maternal antibodies from the truly infected ones.
9.	Consider unborn babies at risk and initiate without delay a preventative/therapeutic program starting zidovudine at birth.
10.	Educate and motivate prospective mothers to take care of their general health, avoid infecting others by abstinence or engaging in safer sex (must use condom for every sexual act).

Data from [84, 565].
OraQuick test facilitates routine HIV testing of all pregnant women, and, as a safety net, the routine screening of any infant whose mother was not screened.

Current Implications

Prevention

The primary prevention is that of MTCT. The first step is the primary prevention of HIV among parents to be, then the prevention of unwanted pregnancy among HIV-positive females, and, moreover, prevention of HIV transmission from HIV-infected females to their in-

Table 23.40. HIV infection prevention in healthy children

What is not infectious
Indoor contacts with HIV+ children
Haphazard use of toys, combs, coffee cups, dishes, flatware, glasses, bed and bath linen, toiletries
Sleeping and bathing together
Hugging and kissing on the lips
Outdoor contacts with potentially HIV+ children
Saliva (do not indulge in prolonged kissing)[a]
Tears
Droplets (cough, sneeze)
Direct contact, sweating (contact with body parts, handshake, caressing, gymnastics, etc.)
Use of sanitary facilities (check that no blood is on the toilet bowl)
Promiscuous use of bar and restaurant coffee cups, serving in general, glasses, flatware, napkins and the like
Bathing with a lot of water (shower, swimming-pool)
Domestic pets, mosquitos and other stinging insects
The above suggestions are no longer valid with bleeding lesions
Particular issues
Vaccinations, blood sampling, injections:
Always use only disposable syringes or droppers
Medical consultations:
Use only disposable tongue depressor, other instruments (gynecological speculum, hammer for reflexes, etc.) should be disinfected after a single use
Dental visits and cures:
All metallic instruments should be disinfected after their use
Nonmedical use of metallic instruments and utensils or of sharp and/or cutting materials capable of initiating bleeding:
Razors, combs, scissors, other instruments used by barbers, beauticians, manicurists and chiropodists, needles or sharp or cutting instruments for whatever use (acupuncturists, tattooing, including ear/nose piercing, re-use of depilatory wax, etc. should be disinfected after their use with common disinfectants) (1:10 or 1:100 Na hypochloride, chlorhexidine, etc.)
Toiletries
Avoid sharing personal objects outdoors, of whatever type, including toothbrushes and other items that may be contaminated by blood

Data from [80, 123, 196, 465].

[a] Saliva could contain HIV-inhibitors such as lysozyme and ribonuclease.

fants, through ART to pregnant females and infants, and where applicable, replacement feeding for the infant. Primary prevention of HIV infection in prospective parents is the only 100% effective method in preventing HIV transmission to infants [277]. It includes HIV education, safe sex practices, avoidance of infected syringes and introduction of infected blood into the body by any route [80]. Subsequently, three main mechanisms are essential for achieving maximum effective reduction of MTCT: 1. Reduce maternal viral load with ART drugs. 2. Prevent avoidable exposure to maternal virus at birth through improved obstetric practices. 3. Reduce exposure to HIV through "3 by 5" [259].

Those most at risk are teenagers who are unwilling to use condoms (in 27%–56% of cases) [110]. Unprotected sexual intercourse between men and women is the predominant mode of transmission of the virus. In addition, it must be pointed out that even if the sexual act is interrupted, it is nevertheless possible to become infected, because the initial secretions contain the virus thus endangering drug addicts who postpone ART [241]. However, many studies have reported positive results with the measures taken among teenagers.

HIV infection is a disease that can be prevented very effectively. The virus is not transmitted by air, water or food, nor by 99% of human relations. As we can see from Table 23.40 [80, 123, 196, 465], all commonplace items can be used without any danger and all normal situations can be faced unreservedly. These data come from specific studies [465] and have been checked with observations that have cumulatively lasted several decades [196]. The preventive measures listed in Table 23.41 [80, 505] are not associated with any type of risk, in the sense that in school workers the possibility of contracting AIDS by assisting an HIV+ student is no different than the risk of health workers in the broadest sense. The risk is virtually wiped out by applying minor precautions that are universal and are simply common sense, to be adopted in all cases in which assistance implicates potential contact with contaminated blood. Breast milk is expressed manually or by a breast-pump and subjected to pasteurization by boiling and inactivating by heat (over 60 °C) for 30 min, which kills the virus. This cannot be used at the individual level, but it is important for breast milk banks to avoid HIV infection [80, 277].

Nutritional Support

A priority aspect we previously mentioned is *malnutrition*: the digestive symptoms aggravate the nutritional disorders, which reflect negatively on HIV infection [84]. This can be prevented with complex intervention using food supplements, to be established in the hospital and continued at home.

PI-treated children should be given supplemental vitamin D preparations because PI medications may markedly suppress the activities of 25- and 1α-hydroxy-

Table 23.41. Seropositive children: possible HIV infection transmission to schoolmates

Schools, daycare centers etc.
There is no risk for teachers, school staff and other children. Several studies have demonstrated that no community runs risks by adopting the well-known preventive hygienic measures.
In particular, children may touch their schoolmates, exchange effusions, and play with them, but if children play with bodily fluids, they should wash the hands immediately with soap and warm water for 10 s (same principles universally valid).
During campaign of dental prevention, each child should use an individual toothbrush.
Precautionary measures to be adopted in daycare centers and/or school
Open cuts and sores of whatever type should be completely covered with waterproof medications.
The school should be given vinyl/silicon gloves and disinfectants (1:10 or 1:100 NaCl, chlorhexidine, etc.).
School personnel caring for children should wash thoroughly after having changed or washed the child with gloves and should be preventively trained in proper disinfecting techniques to be utilized in case of wounds and/or spontaneous hemorrhages.
Avoid the promiscuous use of blood-contaminated objects of whatever type.
Thorough prevention against exposure to blood should be followed.
Specific attention should be paid to protect young children, who may be unable to prevent such exposures on their own.
Areas in the daycare center should be selected for the temporary or ongoing care of young victims of injury or illness.
When a seropositive child requires emergency medical care, personnel should know to which hospital he could be admitted.
General hygienic measures
Dishes and flatware can by cleaned with warm water or dishwasher by using a common detergent.
Laundry can usually be washed with warm water (60 °C) or in a washing machine.
Daily cleaning of the rooms does not require special precautions: walls, floors and other surfaces alike are not associated with HIV infection transmission; if surfaces appear to be contaminated by blood or other biological fluids, diluted NaCl is sufficient, as above.

Immunosuppressed children may be at increased risk of having serious complications of infectious diseases, even trivial. Data from [80, 505].

lase, which are critical in 1,25(OH) D synthesis, while exerting mild inhibition of 24-hydroxylase, responsible for 1,25(OH) D catabolism. Defective 1,25(OH) D production could contribute to the bone demineralization in HIV children [131].

Social Considerations

In disseminating information, the preferable course of action is one that is widespread at various levels, to be handled by qualified experts. This should involve the establishment of centers to provide information and advice to users from different extractions (but organized with a DB method to guarantee maximum privacy), as well as equally anonymous centers that test the samples of anyone who contacts them. In these cases, *discretion is a top priority* and it has been observed that patients prefer to receive delicate information in neutral places like the office or private places like their own home, rather than at the doctor's office [571]. In 73.5% of cases, young people aged 14–20 have obtained helpful information from television and/or radio, and in 73.3% of cases they feel no doubts and/or fears of becoming infected after sex. The majority of female college students (71.5%) have *discussed AIDS with their friends,* while discussion with siblings, parents and teachers was not common [181]. Adolescent females were found less knowledgeable about HIV [449] (Tables 23.42, 23.43). In 67.5% of cases, they are faithful to their partner (Tables 23.44, 23.45). However, Table 23.45 highlights a serious aspect of the falseness factor: a study conducted on 665 subjects of both genders revealed that 34% of men and 10% of women lie to ensure sexual relations [115].

Management of the HIV^+ child is important, and as always, the pediatrician plays a leading role. We have demonstrated that these children, for whom survival studies suggest that a large percentage also reach the age of socialization and schooling, are youngsters who are the same as others on the outside and are not a danger for anyone. Instead, there is a higher risk of exposing the immunodepressed child to infectious diseases, even commonplace ones (Table 23.41). Among persons living with HIV/AIDS individuals, the impact is particularly hard on girls and young women: the burden of care usually falls on them. *Girls drop out of school to care for sick parents or for younger siblings.* Young women often take on the burden of caring for ailing parents and later, when they die, adopt the parental role for the orphaned children. They are often also responsible for producing an income or food crops. In addition, young women caring for orphans and sick children may be isolated socially because of AIDS-related stigma and discrimination [259].

Table 23.42. Sources of information about HIV infection: results of a study in 7,632 14- to 20-year-old students (43% males, 57% females) (%)

Age (years)	14–15		16–17		18–20		Total	
Source	M	F	M	F	M	F	M	F
Television	25.1	23.4	23.2	23.5	26.2	25.8	74.5	72.7
Books	4.1	3.1	5.3	3.2	4	4	13.4	10.3
Pamphlets	5.2	7.3	6.1	8.1	8.2	9.2	19.5	24.6
Children's journals	8	6.4	12.2	10.3	14.1	15.1	34.3	31.8
Publications	4.3	5.3	5.1	4.3	5	6	14.4	15.6
School	4.2	5.3	5.1	4.2	4.2	5.2	13.5	14.7
Friends	10.2	11	7.1	5.3	6.3	5.7	23.6	22
Family	7.2	8.4	6.1	7.8	4.2	4.2	17.5	20.4
Meetings with interviewers	3.2	4.3	2.1	3.1	4.2	5.2	9.5	12.6

More than one answer was sometimes given.
Data from Rapporto AIED. Italian students and AIDS: Knowledge and prejudices. Press conference. Rome, May 25, 1997.

Table 23.43. Doubt/fear of being infected after sexual intercourse: results of a study in 7,632 14- to 20-year-old students (43% males, 57% females)

Age (years)	14–15		16–17		18–20		Total	
	M	F	M	F	M	F	M	F
Yes	5.8	4.9	9.1	7.3	14.9	13.5	29.8	25.7
No	17.6	20.3	24.5	23.8	28.1	30.2	70.2	74.3

Data from Rapporto AIED. Italian students and AIDS: Knowledge and prejudices. Press conference. Rome, May 25, 1997.

Table 23.44. The fidelity factor: results of a study on 7,632 14- to 20-year-old students (43% males, 57% females) asked if they were faithful to their fiancé(e)

Age (years)	14–15		16–17		18–20		Total	
	M	F	M	F	M	F	M	F
Yes	23.5	24.3	20.6	21.5	21.8	23.8	66	69.1
No	11.4	12.5	10.3	9.5	12.3	8.9	34	30.9

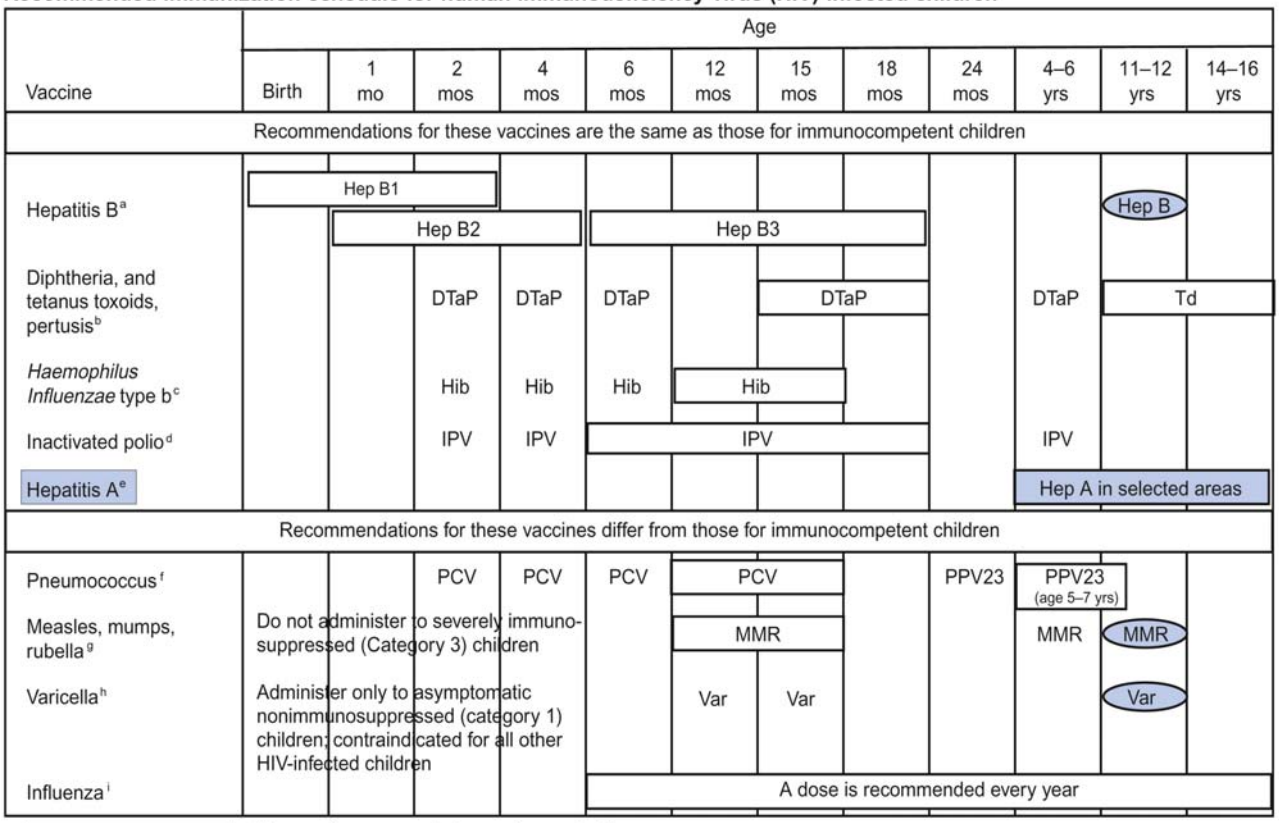
Data from Rapporto AIED. Italian students and AIDS: Knowledge and prejudices. Press conference. Rome, May 25, 1997.

Table 23.45. The dishonesty factor: results of a study on 18- to-25-year-old students of both sexes

	Males (%)	Females (%)
Has told a lie in order to have sex	34	10
Sexually involved with more than one partner	32	23
Partner did not know	68	59
Has been lied to for purposes of sex	47	60
Would reduce the number of previous partners	47	42
Would never disclose a single case of infidelity to the partner	43	34

Data from [115].

Recommended immunization schedule for human immunodeficiency virus (HIV)-infected children*



Range of recommended ages for vaccination

Vaccines to be administered if previously recommended doses were missed or were administered at other than the recommended minimum age

Recommended in selected states or regions

* This schedule indicates the recommended ages for routine administration of licensed childhood vaccines as of November 1, 2000, for children aged birth–18 years. Additional vaccines might be licensed and recommended during the year. Licensed combination vaccines might be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturer's package inserts for detailed recommendations.

[a] Infants born to hepatitis B surface antigen (HBsAg)-negative mothers should receive the first dose of hepatitis B vaccine (Hep B) at birth and no later than age 2 months. The second dose should be administered ≥1 months after the first dose. The third dose should be administered ≥4 months after the first dose and ≥2 months after the second dose, but not before age 6 months. Infants born to HBsAg-positive mothers should receive Hep B and 0.5 mL hepatitis B immune globulin (HBIG) ≤12 hours after birth at separate sites. The second dose is recommended at age 1–2 months and the third dose at age 6 months. Infants born to mothers whose HBsAg status is unknown should receive Hep B ≤12 hours after birth. Maternal blood should be drawn at delivery to determine the mother's HBsAg status; if the HBsAg test is positive, the infant should receive HBIG as soon as possible (no later than age 1 week). All children and adolescents (through age 18 years) who have not been immunized against hepatitis B should begin the series during any visit. Providers should make special efforts to immunize children who were born in, or whose parents were born in, areas of the world where hepatitis B virus infection is moderately or highly endemic.

[b] The fourth dose of diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP) can be administered as early as age 12 months, provided 6 months have elapsed since the third dose and the child is unlikely to return at age 15–18 months. Vaccination with tetanus and diphtheria toxoids (Td) is recommended at age 11–12 years if ≥5 years have elapsed since the last dose of diphtheria and tetanus toxoids and pertussis vaccine (DTP), DTaP, or diphtheria and tetanus toxoids (DT). Subsequent routine Td boosters are recommended every 10 years.

[c] Three *Haemophilus influenzae* type b (Hib) conjugate vaccines are licensed for infant use. If Hib conjugate vaccine (polyribosylribitol phosphate-meningococcal outer membrane protein [PRP-OMP]) (PedvaxHIB® or ComVax™ [Merck and Company, Inc., Whitehouse Station, New Jersey]) is administered at ages 2 and 4 months, a dose at age 6 months is not required. Because clinical studies among infants have demonstrated that using certain combination products might induce a lower immune response to the Hib vaccine component, DTaP/Hib combination products should not be used for primary immunization among infants at ages 2, 4, or 6 months, unless approved by the Food and Drug Administration for these ages.

[d] An all-inactivated poliovirus vaccine (IPV) schedule is recommended for routine childhood polio vaccination in the United States. All children should receive four doses of IPV at age 2 months, age 4 months, ages 6–18 months, and ages 4–6 years. Oral poliovirus vaccine should not be administered to HIV-infected persons or their household contacts.

[e] Hepatitis A vaccine (Hep A) is recommended for use in selected states or regions and for certain persons at high risk (e.g., those with Hepatitis B or C infection). Information is available from local public health authorities.

[f] Heptavalent pneumococcal conjugate vaccine (PCV) is recommended for all HIV-infected children aged 2–59 months. Children aged ≥2 years should also receive the 23-valent pneumococcal polysaccharide vaccine; a single revaccination with the 23-valent vaccine should be offered to children after 3–5 years. Refer to the Advisory Committee on Immunization Practices recommendations (see CDC. Preventing pneumococcal disease among infants and young children: recommendations of the Advisory Committee on Immunization Practices [ACIP]. MMWR 2000;49[No. RR-9]:1–38) for dosing intervals for children starting the vaccination schedule after age 2 months.

[g] Measles, mumps, and rubella (MMR) should not be administered to severely immunocompromised (category 3) children. HIV-infected children without severe immunosuppression would routinely receive their first dose of MMR as soon as possible after reaching their first birthdays. Consideration should be given to administering the second dose of MMR at age 1 month (i.e., a minimum of 28 days) after the first dose rather then waiting until school entry.

[h] Varicella-zoster virus vaccine should be administered only to asymptomatic, nonimmunosuppressed children. Eligible children should receive two doses of vaccine with a ≥3-month interval between doses. The first dose can be administered at age 12 months.

[i] Inactivated split influenza virus vaccine should be administered to all HIV-infected children aged ≥6 months each year. For children aged 6 months–<9 years who are receiving influenza vaccine for the first time, two doses administered 1 month apart are recommended. For specific recommendations, see CDC. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2002;51(No. RR-4):1–32.

Fig. 23.27. Recommended immunization schedule for human immunodeficiency virus (HIV)-infected children

Vaccinations

The opinion on whether or not it is appropriate to vaccinate children with AIDS does not diverge from the criteria commonly applied in the active immunoprophylaxis for the pediatric population at large [13] (Fig. 23.27). Children in whom the infection is asymptomatic must be vaccinated according to the usual calendar. For the others, including symptomatic ones, as a rule the recommendation is to *avoid using vaccines composed of viruses or attenuated live bacteria* (antipolio, BCG) and to prefer the inactivated antipolio vaccine, such as the Salk vaccine [321]. Triple vaccines against diphtheria-pertussis-tetanus and measles-mumps-rubella (MMR) can be administered according to the usual schedule (12–15 months) to HIV-infected children without any risk of complications, with a booster dose at 4–6 years [321]. MMR can be anticipated (before 15 months) in the event of exposure to the measles virus: in this case, the vaccine must be repeated at 15 months. The measles vaccination is recommended in HIV-infected children despite the concern about administering a vaccine with live viruses to an immunocompromised host [56]. In children with a symptomatic HIV infection, the antibody titer to the above vaccines generally tends to drop as the infection continues, until it no longer reaches protective levels. In the severely immunocompromised child (based on CD4 percentage counts) and also in HIV-exposed or HIV-infected children, in case of contact immediate prophylaxis is advised with hyperimmune γ-globulins, unless IVIg was administered in the previous 3 months. In the case of measles, if exposed the HIV^+ child may benefit from immunization if administered within 72 h of exposure, or from γ-globulins even within 6 days following exposure [56]. In the event of contact with subjects affected with other infective diseases, the child with symptomatic HIV infection, even if vaccinated, can be given immediate prophylaxis for whooping cough, diphtheria and *Haemophilus influenzae* with macrolides, penicillin and rifampicin, respectively [56]. The influenza vaccine must be administered regularly to HIV-infected children who are at least 6 months old or who live with infected people; the antipneumococcal vaccination must be given at the age of 2, while the *chicken pox vaccine is not recommended for these children*. Notably, HIV-infected children undergoing stable HAART with $CD4^+$ cell counts of 25% or higher and lower viral loads at the time of vaccination developed a higher additional clinical protection gain from 23PSV vaccination than did children with a lower percentage of $CD4^+$ cells [534]. If one person in the household has been vaccinated and develops a rash, contact must be avoided until the rash disappears. In a study to determine the safety and immunogenicity of varicella vaccine in 41 children with HIV infection who were mildly affected by HIV (CDC stage N1 or A1) and had no history of prior varicella infection and had negative titers, they were immunized with two doses of live attenuated varicella vaccine separated by 3 months [329]. Only a minority of the vaccine recipients had mild local or systemic reactions. In HIV-exposed or HIV-infected children, or in not definitively diagnosed children, it is advisable to utilize anti-varicella hyperimmune γ-globulins within 72 h of exposure, unless IVIg has been administered in the previous 3 months [13].

Medical and Legal Aspects

Table 23.46 [80] summarizes the child's position, a lawful subject/object with regard to AIDS. We must recall that the law bans any type of third-party ascertainment without the child's knowledge and, therefore, many medical actions can be performed with informed consent, as we clarified in Chap. 6. As with all other cases, information about case history must be requested from the parents or legal guardians, who should be informed of diagnoses and/or the need to proceed with additional testing. Naturally, what we have pointed out in the preceding section indicates that there is no risk to health personnel and nurses as a result of tests performed on an HIV-positive child and these checks do not differ from normal medical services. If necessary, all health personnel should wear vinyl gloves or masks and so on for normal procedures. Likewise, they should disinfect surfaces contaminated with blood and/or body fluids. Only two activities are prohibited by law: refusal to assist the patient and the disclosure of patient information to third parties.

Table 23.46. The child subject/object of rights and HIV infection

The child, at birth, is a citizen (with full rights) and a minor (object of protection)
The neonate-citizen benefits from the right to live
The HIV-infected neonate/child-citizen benefits from a limited right to live
The neonate/child-citizen cannot come to conscious anti-AIDS decisions
HIV exposure is damage from a third party:
In utero transmission
Perinatal transmission
Postnatal transmission
Others
Specific juvenile legislation regarding infantile HIV infection is lacking
The HIV-infected/seropositive minor may be the innocent recipient of prejudice deriving from the disease

Data from [80].

Pediatricians and HIV-Positive Children

Pediatricians who must face emerging clinical problems and everything they entail such as the need to establish specific ART and promptly prevent complications using the appropriate instruments, or who must urgently step in to handle current complications, must cope with the need to ensure a satisfactory *quality of life* as long as possible. We are the advocates of simple and basic measures, and we believe that the best actions for prevention and information begin at the base, for example, at home, at day-care centers, at school, at work, etc. History has taught us that important battles are won not so much for the general ability, soldiers' bravery, or effective weapons, as for the importance given to preparation, equipment, and field knowledge. In this sense, everything possible must be tried for these toddlers, children and adolescents, *whose label as pariahs is palpable*. At the same time, we should reflect on the outstanding results that have been achieved in Ireland, where for 4 years, not one HIV-infected child has been born to an HIV^+ mother treated during pregnancy and childbirth, to the point that a number of HIV^+ women want to have more children. And yet recent data [565] offer the option of stepping in even at the last minute, a first task reinforcing the pediatrician's functions. AIDS has also taught us that fixed concepts can be subjected to a 360° revolution. During the early 1980s, when the first perinatally acquired AIDS cases were documented, HIV infection in the greater part of children progressed rapidly to death. Now, the number of HIV-infected females who are becoming both sexually active and pregnant is increasing [181]. This underscores the need for early disclosure of HIV status to infected adolescents of both sexes and for increased discussions about sexual risk reduction among all perinatally infected adolescents [598], especially adolescent females [449], a second task reinforcing the pediatrician's functions.

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Primary Immunodeficiencies

A World in Motion

Primary immunodeficiencies (PIDs), once considered to be very rare, are now increasingly recognized because of growing knowledge in the immunological field and the availability of more sophisticated diagnostic techniques and therapeutic modalities [161]. However in a database of >120,000 inpatients of a general hospital for conditions suggestive of ID 59 patients were tested, and an undiagnosed PID was found in 17 (29%) of the subjects tested [107]. The publication of the first case of agammaglobulinemia by Bruton in 1952 [60] demonstrated that the PID diagnosis is first done *in the laboratory*. However, PIDs require specialized immunological centers for diagnosis and management [33]. A large body of epidemiological evidence supports the hypothesis of the existence of a close etiopathogenetic relation between PID and atopy [73]. In particular, an elevated frequency of asthma, food allergy (FA), atopic dermatitis and enteric pathologies can be found in various PIDs. In addition we will discuss another subject that is certainly of interest: the pseudo-immunodepressed child with recurrent respiratory infections (RRIs), an event that often requires medical intervention and that very often leads to the suspicion that it involves antibody deficiencies [149].

Immunodeficiency and Atopy

In several PIDs (Table 22.1) [61, 65, 76, 101, 162, 351, 414, 480, 514, 543], atopic symptoms are present: gastroenteric and rhinitis in selective IgA deficiency (SIgAD), severe AD in Wiskott-Aldrich syndrome (WAS) and hyper-IgE syndrome (HIgES), in which it spreads over the entire body, and to which other allergic manifestations can also be associated such as asthma, rhinitis and angioedema. Various acquisitions indicate that PID is also an *opsonization deficiency*, observed in 5% of the normal population [469]. In this disease, microorganism phagocytosis by polymorphonuclear (PMN) leukocytes appears annulled, and the patient is subject to severe infections supported by capsular bacteria: the deficiency, described in association with severe and recurrent infantile infections [175, 485, 487], depends on the lack of mannose-binding lectin (MBL) [487], its structural genes, and also perhaps on the lack of 2/4 C4 genes [506]. MBL deficiency is due to one of three point mutations in the gene for MBL, each of which reduces levels of the lectin by interfering with the protein oligomerization [351]. In children with this kind of deficiency, the level of MBL is 4.9 µg/l compared to the 143 µg/l in controls [487]. Regardless of whether the children are homozygote (HZ) or heterozygote (HET) in relation to a given mutation, the defect appears to be more consistent in small babies aged 6–18 months [487], who show an immaturity in providing immune response to capsular bacteria and in whom low levels of opsonin are incapable of compensating for this [506]. The risk of contracting infections is similar in HZs [175] and HETs [486], though it persists throughout life in HZs because of an abnormal allele, while it exhausts itself in the HETs, where the frequency of abnormality is similar to that of the general population [175]. Anomalies in immunoglobulins (Ig) and in opsonization have been observed, respectively in 13%–20% of children suffering from frequent asthma and IgG subclass deficiencies. Children suffering from *cystic fibrosis* also present an elevated prevalence of immediate cutaneous reactions to aeroallergens, and although without primary defects of adoptive immunity, they are susceptible to severe RRIs; therefore it may be possible that they suffer from the mucosal antigenic exclusion [61]. Unlike asthmatic children, in whom a relatively high concentration of IgE for respiratory viruses was observed [172, 173, 457], positive skin prick tests (SPTs) are more common for *Aspergillus fumigatus* [61].

The hypothesis suggested by these observations is that atopy derives from an unbalanced immune response to foreign antigens, with a consequent lack of their early identification or the capacity to neutralize or eliminate them. This hypothesis is based on the evidence that ID precedes the development of atopy: in the Taylor et al studies, 22 newborn babies, the children and/or siblings of atopic patients, presented a significant reduction in serum concentrations of IgA when aged 3 months: this was *transient hypogammaglobulinemia (hgG) of infancy* (THI). The association of very low IgA levels with atopy has been proposed again in the classic prospective study on the association of viral respiratory infections (VRI) and the onset of allergic manifestations, which proved serum IgA levels at the lowest normal levels in the children studied [173]. This data has been confirmed within

Table 22.1. Primary immunodeficiency diseases

Classification and inheritance	Chromosome	Gene defect
A. Predominantly B-cell deficiency		
1. Agammaglobulinemia or Bruton's tyrosine kinase deficiency, XL	*Xq21.3–22*	Btk
a. Pre-BcR		
b. AR		
c. Surrogate light chain	*22q11.22*	
d. μ heavy (H) chain	*14q32*	
2. Gene deletion for H chains, AR	*14q32.3*	
3. κ-chain deficiency, AR	*2p12*	IGKG
4. Selective Ig deficiency		
a. IgG subclass deficiency with or without IgA deficiency	*14qx32.33*	IGHG
b. Selective IgA deficiency, AR	*6p21.3*	IGAD
5. Selective antibody deficiency with normal Ig isotypes (SADNI)		
6. Selective deficiency of other Ig		
7. Common variable ID, AR, AD associated with antibody deficiency (IgA)	*6p21.3*	
8. Transient hypogammaglobulinemia of infancy (THI)		
B. Combined T-cell and B-cell deficiency		
1. T^-B^+ SCID		
a. X-linked (SCID-X1)	*Xq13–21.1*	$IL_2R\gamma$
γc Gene mutations	*Xq13.1*	
γc Gene mutations with an atypical NK phenotype		
b. Autosomal recessive		
JAK3 gene mutations	*19p13.1*	JAK3
$IL_7R\alpha$ deficiency	*5p13*	$IL_7R\alpha$ mutation
CD45 deficiency	*1q31-32*	
2. T^-B^- SCID		
a. RAG1 or RAG2 deficiency	*11p13*	RAG1 or RAG2
b. ADA (adenosine-deaminase) deficiency, AR	*20q13.11*	ADA
c. Reticular dysgenesis, AR		
d. Radiation sensitive, AR	*10p13*	Artemis
3. T^+B^- SCID		
Omenn syndrome, AR		
$IL_2R\alpha$ deficiency, AR ($IL_2R\alpha$-chain gene mutations)	*11p13*	$IL_2R\alpha$
4. Hyper-IgM or CD154 deficiency, XL, AR	*Xq26.3–q27.1*	CD154
Non-X-linked hyper-IgM (or hyper-IgD) syndrome		
5. Purine-nucleoside-phosphorylase (PNP), AR	*14q13.1*	PNP
6. HLA (major histocompatibility complex)		
a. HLA class II antigen deficiency, AR	*16p13.3*	CIITA
b. HLA class II antigen deficiency	*1q21*	RFX5
Deficit in RFXAP	*13q14*	RFXAP
Deficit in RFXANK	*19p12*	RFXANK
c. HLA class I antigen deficiency	*13q*	

Table 22.1. (Continued)

Classification and inheritance	Chromosome	Gene defect
7. CD3γ, or CD3ε, or CD3 deficiency, AR	*11q23*	CD3γ/ε
8. CD3δ		
9. ZAP-70 or CD8 deficiency, AR	*2q12*	ZAP-70
10. TAP-2 deficiency	*6p21.3*	
11. NFAT deficiency, AR		
12. NK-cells deficiency		
13. Undifferentiated SCID		
Human nude SCID		
p56lck SCID, AR		whn
C. Predominantly T-cell defects		
1. Primary CD4 T cell deficiency		
2. Primary CD7 T cell deficiency		
3. Multiple cytokine deficiency		NFAT
4. Nezelof syndrome		
5. Fas (CD95) deficiency		
D. Other well-defined immunodeficiency syndromes		
1. Wiskott-Aldrich syndrome, XL	*Xp11.22–11.3*	WASp
2. Ataxia telangiectasia, AR	*11q22.23*	ATM
a. Nijmegen breakage syndrome, AR	*8q21*	NBS
3. DiGeorge syndrome	*22q11.2*	DGCR
a. DiGeorge and Del *22q11.2* syndromes	*10p13*	
4. X-linked lymphoproliferative syndrome, XLP	*Xq24–26*	*SH2DIA* gene
5. Hyper-IgE syndrome	*4q*	
6. Chédiak-Higashi syndrome	*1q41.1–1q42.2*	LYST
7. Cartilage hair hyperplasia, AR	*9p13*	*RMRI* mutation
E. Phagocyte deficiency		
1. Chronic granulomatous disease, XL		
a. X-linked (deficiency of 91-kD binding chain of cytochrome b)	*Xp21.1*	gp91phox
b. Autosomal recessive (deficiency of cytosol factors)		
p22phox	*16q24*	CIBA
p47phox	*7q11.23*	NCF1
p67phox	*1q25*	NCF2
2. Leukocyte adhesion deficiency (LAD)		
a. LAD type I, AR	*21q22.3*	CD18
b. LAD type II, AR	*11*	CD15s
c. LAD type III		CD63E
d. LAD type IV, AD		
e. LAD type V, AR	*22q12.3*	Rac
3. Deficiency of multiple leukocyte integrins		
4. Glucose-6-phosphate-dehydrogenase (G6PD) deficiency, XL	*Xp28*	
5. Myeloperoxidase deficiency, AR	*17q21.3–q23*	

Table 22.1. (Continued)

Classification and inheritance	Chromosome	Gene defect
6. Specific granule deficiency, AR	*Xq28*	CEBPE
7. Neutropenia		
a. Cyclic neutropenia	*19p13.3*	ELA2
b. Congenital neutropenia (Kostmann syndrome)		CSF3R
8. Shwachman-Diamond syndrome, AR	*7q1.1*	
9. Leukocyte mycobactericidal defect		
a. IFN-γR1 deficiency	*6q23.q24*	IFN-γR1
b. IFN-γR2 deficiency	*21q22.1q22.2*	IFN-γR2
c. $IL_{12}R$ p40 deficiency	*5q31.1-33.1*	IL_{12}Rp40
d. $IL_{12}R\beta1$ deficiency	*19p13.1*	$IL_{12}R\beta1$
$IL_{12}R\beta1/IL_{23}R\beta1$ associated deficiency		$IL_{12}R\beta1/IL_{23}\beta1$
STAT deficiency AD		STAT
STAT deficiency AR		STAT
F. Complement deficiency		
1. C1q deficiency, AR	*1p34*	C1q
2. C1q/r deficiency, AR	*12p13*	C1q/2
3. C4 deficiency, AR	*6p21.3*	C4
4. C2 deficiency, AR	*6p21.3*	C2
5. C3 deficiency, AR	*19p21*	C3
6. C5 deficiency, AR	*9q32.1*	C5
7. C6 deficiency, AR	*5q13*	C6
8. C7 deficiency, AR	*5q13*	C7
9. C8 deficiency, AR	*1p32*	C8
C8α + C8γ deficiency, AR	*1p34*	Cα/γ
C8λ deficiency, AR	*9q34*	Cλ
10. C9 deficiency, AR	*5p13*	C9
11. C1 inhibitor deficiency, AD	*1q p11*	
12. Factor I deficiency, AR	*4q25*	
13. Factor H deficiency, AR	*1q3.2*	
14. Factor D deficiency, AR	*19*	
15. Properdin deficiency, XL	*Xp11.4–p11.2*	PPC

We follow the WHO nomenclature [455], recently updated [351].
Data from [61, 65, 76, 101, 162, 351, 414, 480, 514, 543].
AD autosomal-dominant, *ADA* adenosine deaminase, *ATM* ataxia-telangiectasia mutated, AR autosomal-recessive, *Btk* Bruton's tyrosine kinase, *CIITA* class II transactivator, *ELA* elastase, *ID* immunodeficiency, *JAK* Janus-family kinase, *PNP* purine nucleoside phosphorylase, *RAG1* and *RAG2* recombination-activating gene-1 and -2, *RFX5* regulatory factor X5, *TAP-1* and *TAP-2* Transporter associated with antigen presentation 1 and 2, *WASp* Wiskott-Aldrich syndrome protein, *XL* X linked.

a possible atopy dependence on IgA underproduction rather than on IgE hyperproduction (Fig. 4.1): in children with levels of IgA at the minimum normal level, and followed from birth until the age of 18–23 months, a greater severity of atopic manifestations and an increased cumulative incidence of asthma, AD and otitis media with effusion (OME) were observed compared to controls.

The close links between ID and atopy are confirmed by *symptoms similar to AD* present in some forms of WAS (70%), HIgES (85%), XLA (X-linked agammaglobulinemia) or autosomal recessive (AR), ataxia-telang-

Table 22.2. Serum IgE concentrations (U/ml) in patients with PID

PID	No.	Age	Range (U/ml)	GM
Ataxia-telangiectasia	7	5–14 y	<1–54	7
Chronic granulomatous disease	10	6 m–17 y	<1–3,160	88
Hyper-IgE syndrome	11	3–31 y	3150–40,000	11,305
Nezelof syndrome	3	8 m–3 y	5–7,000	55
Non-X-linked agammaglobulinemia	15	6–35 y	1–10	3
Other variable immunodeficiency	6	1–14 y	11–2,880	142
Selective IgA deficiency	74	5 m–50 y	3–3,800	124
Severe combined immunodeficiency	9	3–17 m	<1–82	2
Transient hypogammaglobulinemia of infancy	8	3–20 m	2–31	6
Wiskott-Aldrich syndrome	4	8 m–12 y	135–720	381
X-linked agammaglobulinemia	10	3–16 y	<1–9	2
X-linked immunodeficiency with hyper-IgM	3	7 m–2 y	<1–2	1
Normal infants	12	2–19 m	3–81	18
Normal infants and adults	106	2–55 y	2–549	55

Data from reference [62].
m Months, *y* years, *GM* geometric mean.

iectasia (ATA), thymic alymphoplasia, SCID (severe combined ID) (48%) [44] and, occasionally, by DiGeorge syndrome (DGS), ID with hyper-IgM (HIgMS) now CD154/CD40L deficiency, selective IgM deficiency, biotin-dependent carboxylase deficiency, CGD (chronic granulomatous disease), primary neutropenia, and in Netherton, Nezelof, Omenn and Shwachman syndromes [434]. Other forms, in addition to those discussed, are associated with *gastrointestinal symptoms*: diarrhea and malabsorption of XLA and THI, diarrhea in WAS and DGS, food-related allergies (43%) in SIgAD and also an elevated frequency of asthma [36]. Among *secondary ID*, only AIDS is associated with AD (Chap. 23).

Immunodeficiencies Associated with Hyper-IgE

The association between a deficiency of T cells and high levels of IgE, observed in patients with HIgES, Nezelof syndrome, ATA, WAS and other diseases, has been known for some time (Table 22.2) [62]. Experimental studies on animals indicate that there may be an inverse correlation between serum IgE levels and T-cell functions: this could be attributed to a T-lymphocyte deficiency in atopics, genetically determined, which makes them more vulnerable to the cAMP inhibiting activity, and consequently causing an imbalance between the two subclasses of T cells, which could lead *to IgE hyperproduction and atopy development;* however, in no case is there evidence of a relationship between CD8 deficiency, IgE levels and allergic symptoms. It has been proposed that in these patients CD4-Th2 levels are sufficient for modulating IgE synthesis, but CD8 T-cell levels are inadequate for inhibiting IgE synthesis, which results in increased IgE synthesis. This hypothesis is supported by the observation that Omenn syndrome, WAS and especially HIgES, with an immunological phenotype characterized by a quantitative and qualitative reduction of CD8 T cells, are accompanied by extremely high levels of serum IgE [61, 162, 196]. Lymphocytes in subjects with normal levels of IgE are incapable of producing them, not even after stimulation with polyclonal activators such as PWM (pokeweed mitogen) or EBV (Epstein-Barr virus), while patients with high antibody levels spontaneously synthesize in culture sIgE (specific) levels between 200 and 2,000 pg/ml, also releasing factors capable of increasing IgE secretion (IgE-PF) [287]. Supernatant derivatives from the T cells of patients with HIgES are in fact capable of inducing in vitro the pre-B cells to increase IgE production; furthermore, when the T lymphocytes in these patients are isolated on the basis of receptors for the IgE Fc fragment, the remaining cells release IgE-PF [287]. Considering the suppressive activity of human lymphocytes with CD8 phenotype on sIgE, it has been observed that these lymphocytes are able to suppress sIgE synthesis in patients with high antibody levels; similarly $CD8^+$ cells from a bone marrow transplant (BMT) can suppress IgE production in the HLA-compatible recipient [478]. The study of patients with ID associated with hyper-IgE has supplied useful information concerning IgE system biology, although the immune defect essentially responsible for IgE increased production and for severe atopic

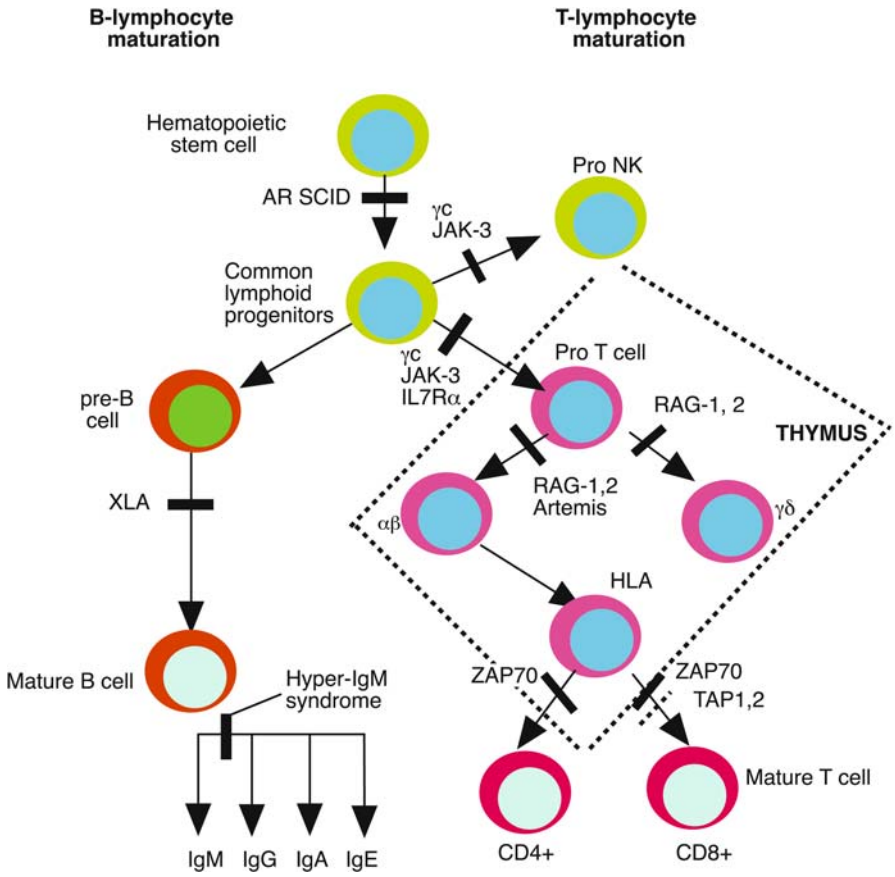


Fig. 22.1. Pathogenesis of some PIDs based on B and T lymphocyte maturation (fully analyzed in Chap. 2).The *bars* indicate where maturation is blocked, thus evidencing the main molecular defects of the related PID. (Modified from [413])

manifestations has not yet been identified. The most interesting syndromes from this point of view are the three syndromes analyzed above, characterized by common clinical indications such as early AD onset, increased susceptibility to all varieties of pathogens, as well as an exceptionally high IgE serum level [162, 394] (Table 22.2).

Immunodeficiency with Autoimmunity

Several PIDs have autoimmune features, including Chédiak-Higashi syndrome, CGD, complement deficiencies C1q, C1r, C1s, C2, C4, Griscelli syndrome, HIgMS (CD154 deficiency), LAD (leukocyte adhesion deficiency), HLA class I deficiency, HLA class II deficiency, Omenn syndrome, WAS, and XLP (17), which will be dealt with subsequently. In 25 children with a mean age of 44 months, autoimmunity was chronic and severe requiring prolonged immunosuppression, however with no spontaneous remission of such manifestations [44].

Primary Immunodeficiencies

Definition

PIDs (Fig. 22.1) [413] consist of a heterogeneous spectrum of congenital, individual and combined anomalies of the immune system (humoral deficiencies, combined deficiency of B and T cells, the complement, phagocytes, neutrophils, etc.), as well as syndromes and diseases associated with ID that are traditionally classified as PIDs. The updated classification (Table 22.1) has divided ≈100 PIDs into six main groups, also including secondary ID with infections (first among them all AIDS) that cause deficiency and immunosuppression [414]. The classifications of PIDs is based on characteristic clinical features and specific alterations in immune status. Advances in molecular genetics now make it possible to complete the table according to the types of genetically altered molecules involved [63]. To complete this data, see Table 22.1 and Table 22.3 [236, 246, 407, 453] showing the behavior of antibodies and circulating B and T cells.

Table 22.3. Characteristics of B and T lymphocytes and serum Ig in PID

Disorder	Antibody	B lymphocytes	T lymphocytes
A. Primary antibody deficiencies			
1. X-linked agammaglobulinemia	All isotypes ↓	–	
2. Hypogammaglobulinemia with hyper-IgM	N ↑ IgM, other isotypes ↓	+ (B_{IgM} and B_{IgD})	
3. Gene deletion for H chains	Various subclasses ↓	N	
4. κ/λ chain deficiency			
5. Selective IgG subclass deficiency	Various subclasses ↓	N or immature	
6. Antibody deficiency with normal Ig levels	N	N	
7. Common variable ID	Various or all isotypes ↓	N/↓	
8. Selective IgA deficiency	IgA ↓	+ or immature	
9. Transient hypogammaglobulinemia of infancy	IgG, IgA ↓	+ or immature	
B. Combined T-cell and B-cell deficiency			
10. Severe combined ID (SCID)			
a. Autosomal recessive	↓↓	↓/N	↓↓
b. X-linked	↓	N/↑	↓↓
JAK3 gene mutations	↓	+	↓↓, NK
Omenn syndrome	↓	↓	N
SCID-ADA deficiency	↓	↓[a]	↓[a]
11. PNP deficiency	N/↓	N/↓	↓[a]
12. HLA class I antigen deficiency	NN	↓ CD8, NK	
13. HLA class II antigen deficiency	N/↓	N	N, ↓ CD4
14. Reticular dysgenesis	↓ (Maternal)	↓↓	↓
15. CD3γ, CD3δ, CD3ε deficiency	N	N	↓↓
ZAP-70 deficiency	N	N	N/↓[b]
NFAT deficiency	N	N	N/↓
16. CD8 deficiency	N	N	↓ CD8
T-linked lymphoproliferative syndrome	↓	↓	↓
C. Other well-defined immunodeficiency syndromes			
17. Wiskott-Aldrich syndrome	IgM↓ IgE↑	N	↓
18. Ataxia telangiectasia	↓/variable	N	↓
19. DiGeorge syndrome	N/↓	N	N/↓

Some PIDs in the literature are indicated without number.
Data from [236, 453]; other data from [246] (Omenn syndrome) and [408] (JAK3).
ADA adenosine deaminase, *ID* immunodeficiencies, *JAK* Janus-family kinase, *PNP* purine nucleoside phosphorylase, ↓ decreased, ↓↓ markedly decreased, ↑ increased, – absent, + present, *N* normal.
[a] Progressive.
[b] Not functional.

Epidemiology

Data concerning incidence has increased considerably thanks to a greater availability of specific tests and more widespread knowledge in the medical profession related to these PIDs, including ATA [94]. However, because PIDs occur infrequently and are highly heterogeneous in nature, relatively few centers gain extensive experience in the diagnosis, so it is difficult to estimate the prevalence of these disorders from routinely collected health statistics [33]. Studies in 13 countries on all continents have included 10,895 patients: Tables 22.4 and

Table 22.4. Comparison of ID registries on the incidence of major antibody and cellular PID in different countries

Country	Italy	Japan	CH	Sweden	Japan	US	France	Australia	CZ	Tunisia	Austria	Spain	USA
References	[299]	[425]	[425]	[151]	[214]	[472]	[41]	[525][a]	[297]	[35]	[33]	[321]	[236]
Selective IgA deficiency	354	80	79	75	27	29	–	24	74	7	26	764	5
CVID	117	111	49	19	5	19	25	12	20	5	27	389	9
THI	–	–	0	3	33	16	–	61	–	1	1	15	4
XLA	33	72	15	12	13	8	30	7	3	8	8	84	2
SCID (all types)	113	60	31	17	4	6	58	14	0	13	5	87	8
ATA	50	58	4	8	7	8	42	–	0	53	2	48	1
WAS	14	46	14	8	4	4	24	–	0	4	2	29	1
DiGeorge syndrome	8	36	6	5	4	2	–	–	–	–	3	52	7
Complement deficiency	13	–	–	11	0	0	4	–	–	3	7	207	2
CGD	12	–	–	10	–	–	42	–	2	7	1	64	1
Hyper-IgE	12	–	–	1	–	–	29	–	1	4	–	25	1
Total PID	706	525	123	150	628	3356	399		99	152	500	2,050	91
Population of country $\times 10^6$	55	111	6.6	8.3	111	248	59		2	9	18.5	39	248
Incidence % $\times 10^5$	1.29	0.5	1.85	0.19	0.56	1.4	0.67		4.9	1.8	2.7	5.25	0.36

The total may not correspond to the sum of the cases because it may include some PID with very low incidences. The figures should be divided into the years that were considered.

[a] Incidence $\times 10^6$ live births; the THI figure includes probable cases.

Table 22.5. Extended number of registered cases of PID on the incidence of major antibody and cellular PID in different countries

Country	Brazil	Latin America	South Africa
Reference	[200]	[550]	[141]
Primary specific ID			101
Combined immunodeficiency			12
SCID		65	
$T^- B^-$ SCID			4
$T^- B^+$ SCID			5
CD40 ligand deficiency			3
X-SCID	4		
AR-SCID	3		
ADA-SCID	1		
Omenn syndrome		2	–
Reticular dysgenesis		1	–
Primary CD4 + T-cell deficiency			2
T-cell activation defects			1
Predominantly T-cell defects			3

Table 22.5. (Continued)

Country	Brazil	Latin America	South Africa
Predominantly antibody deficiencies			66
IgA deficiency	60	413	10
CVI D	5	154	23
XLA	9	109	9
THI	14	60	11
Selective IgG subclass deficiency	10	39	
Autosomal hyper-IgM syndrome	3	34	2
Selective antibody deficiency with normal Igs	4	20	10
Cellular and antibody ID syndromes associated with other major defects			20
ATA	7	149	12
WASp syndrome	31	34	2
DiGeorge anomaly	1	18	6
HIE	4	63	6
Nijmegen anomaly		1	
Immunodeficiency associated with or secondary granulocyte dysfunctions			9
LAD		43	
Defects of phagocyte number and function			
CGD	14	85	3
Cyclic neutropenia	1	11	1
Kostmann's syndrome	4	14	
Schwachman syndrome		1	
Complement deficiencies	10		6
C1-esterase deficiency	4	12	1
C3 deficiency	1	4	
C4 deficiency	1	3	
Factor 1 deficiency	1	2	
Properdin deficiency	1	1	
C2 deficiency	1	1	
C6 deficiency		29	4
Complement deficiency – undefined			1
Total PID	166	1,428	122
Time period	15 years	20 years	11/1983–12/1999

Latin America includes eight countries.
XLA X-linked agammaglobulinemia, *CVID* common variable immune deficiency, *THI* transient hypogammaglobulinemia of infancy, *SCID* severe combined ID, *WAS* Wiskott-Aldrich syndrome, *ATA* ataxia-telangiectasia, *CGD* chronic granulomatous disease.

22.5 report the incidence of the main PIDs in 13 countries (the US and Japan twice) and all continents [33, 35, 41, 141, 151, 200, 214, 236, 297, 299, 321, 425, 472, 525, 550]. High incidences were also found in Colombia [352] and Singapore [292], especially of antibody (IgA) deficiency. Among 172 infants with SCID, consecutively seen, 45.9% had X-linked SCID with mutations of γc receptor, 16.3% ADA deficiency, 9.9% AR, 9.9% IL_7R deficiency, 6.4% Jak3 (Janus kinase 3) deficiency 0.6% reticular dysgenesis, 0.6% cartilage hair hypoplasia,

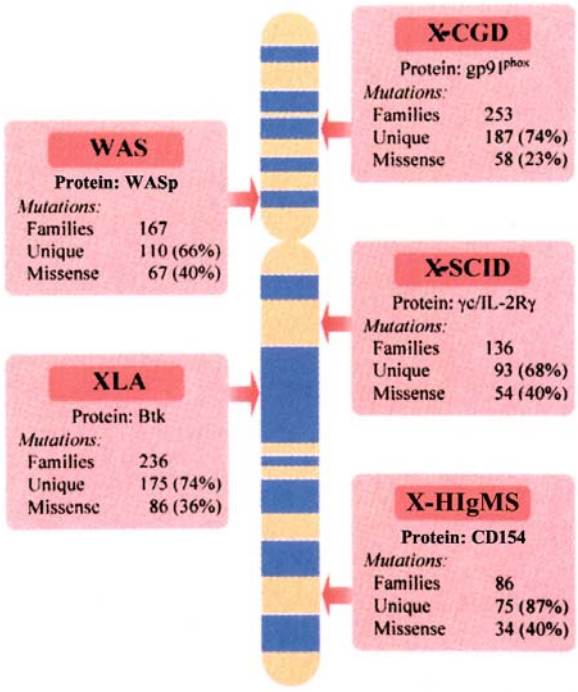


Fig. 22.2. Schematic representation of chromosome X with five X-linked PIDs. *phox* Phagocyte oxidase, *Btk* Bruton's tyrosine kinase, *SCID* severe combined immunodeficiency, *WAS* Wiskott-Aldrich syndrome, *WASp* Wiskott-Aldrich syndrome protein, *X-CGD* X-linked chronic granulomatous disease, *X-HIgMS* X-linked hyper-IgM syndrome, *XLA* X-linked agammaglobulinemia

11% unknown mutations, 2.9% RAG deficiency, 1.2% Artemis [67], 11.1% SCID of undetermined type [68] and 2.2% IL_7R deficiency [69]. Over 20 years, 400 cases were found in Iran, a country with 62.3 million inhabitants, with an incidence of 3.3×10^5 [1]. Predominantly antibody deficiency were found in 45.9% of patients, phagocytic disorders in 29.09%, T-cell disorders in 24.31%, and complement deficiencies in 0.68% [1]. The comparison with Norway data is interesting: antibody deficiencies total 50.8%, combined deficiencies including other ID syndromes 12.4%, complement deficiencies 21.0%, phagocyte disorders 6.7%, and ID associated with other congenital diseases 9.1%. With a population of 4.45 million people, the total prevalence of 302 PID-affected in Norway in 1999 is 6.7×10^5 inhabitants [482]. Not all studies report the range of years during which the patients were found. However, an average incidence of 1×10^5 is seen as acceptable [101], with the exception of SIgAD, in which it varies between 1×300 and $1 \times 20{,}000$ [367]. SCID has an incidence of 1×10^6 [462] or 1.5×10^6 [514]. *The age of onset at diagnosis* is classified as follows: 40% during the 1st year, 40% by the 5th year, 15% by the age of 16 and 5% in adults [101]. In a retrospective study during a 20-year period, antibody deficiencies were found in 52.6%, T-cell disorders in 24.69%, phagocytic disorders in 22.2% and complement deficiencies in 0.4% of 130 children. Common variable immunodeficiency (CVID) was found in 50%, ATA in 30%, XLA in 25.3%, CGD in 22.3% and SIgAD in 15.4% of children [149].

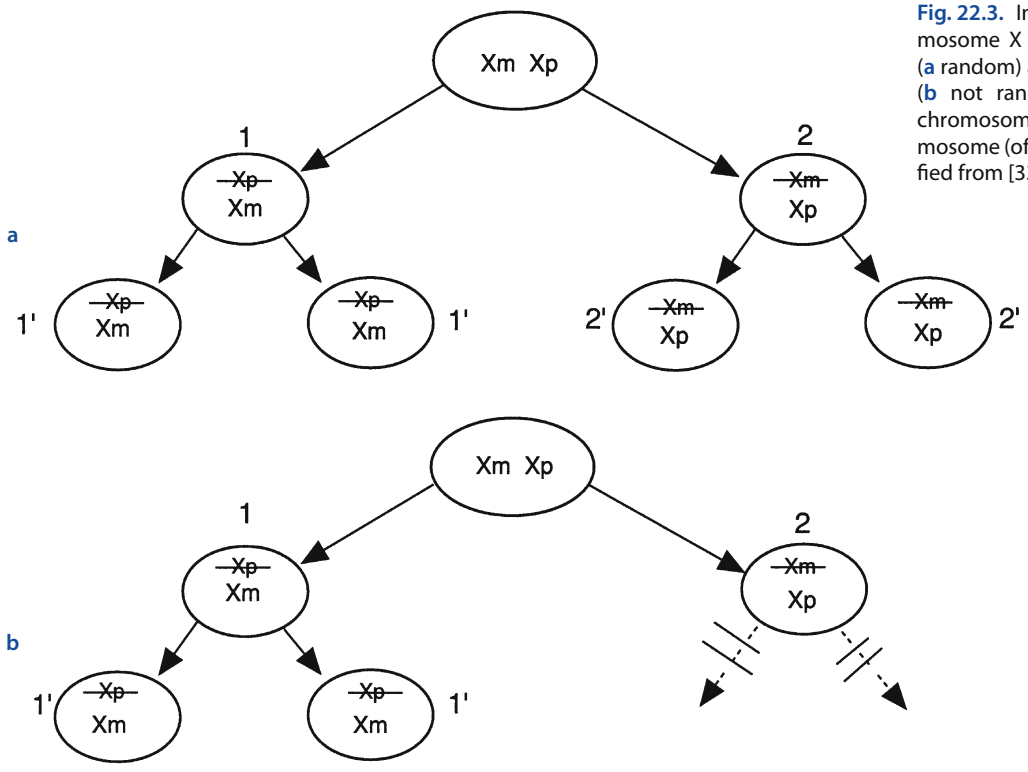


Fig. 22.3. Inactivation of chromosome X in normal women (**a** random) and carrier women (**b** not random). *Xm* normal chromosome, *Xp* carrier chromosome (of mutations). (Modified from [335])

General Characteristics

The genes responsible for ID linked to chromosome X have been recently mapped on the respective chromosome bands (Fig. 22.2): the bands on the short limb are designated "p" and those on the long limb "q" (Table 22.1). This was possible thanks to the refinement of DNA recombinant technology (rDNA), including DNA probes (sequences of radio-marked DNA) and restriction fragment length polymorphism (RFLP). The closer the gene segregates to RFLP, the lower the chance that they might be separated by recombination phenomena when meiosis occurs: the identification of deficient genes allows early diagnosis, even prenatal, and if necessary gene therapy or BMTs [76]. Furthermore, the observation that numerous PIDs are transmitted with an X-linked modality allows a relatively simple diagnosis of males with a positive family history (FH); if FH is negative (40%–50% of XLA cases) or there are females presenting a clinical pattern of PID, or when sporadic cases are caused by a new mutation, carrier identification is based on the study of immunologically normal female carriers, with two populations of B precursors, using X-chromosome inactivation analysis. This test does not take into account the existence of possible gene mutations and the availability of already affected relatives, and it is also relatively simple and fast [537]. Molecular studies follow the hypothesis that, at an early stage during embryogenesis, one of the two X chromosomes is randomly inactivated in the cells of all tissues of female embryos (persisting as Barr's chromatin) [300]. Therefore in normal conditions, one has a cell mosaic that actively expresses for 50% the paternal X chromosome and for the remaining 50% the maternal X chromosome (lionization) [300] (Fig. 22.3a) [355]. In female carriers of XLA, the cell mosaic expresses 50% for an X chromosome with Btk in an active form and the remaining 50% for an X with a mutated Btk (Bruton tyrosine kinase). This means that in the carrier mother it is inactivated in preference to the X chromosome carrier of the defective gene in B, which matures therefore in an unbalanced manner (not randomly), while in all other cells activation occurs randomly. It follows that in fixed carriers only the B lymphocytes that have the X carrier of a normal gene complete the differentiating route, while the precursors that express the X chromosome with a mutated Btk do not mature into B cells, but remain blocked [100, 413] (Fig. 22.3b). In X-SCID, the study of fixed carriers follows the corrected Lyon hypothesis, because the cells with a normal active X develop into normal T lymphocytes; however, when T precursors with a mutant X reach the stage where the X is needed, they do not find it and consequently do not develop: thus female carriers have only one normal and active X, instead of the random mixture of cells with one of the two active X [389]. The inactivation test appears to be reliable and can also be used for other recessive X-linked PIDs to identify cell lines with genetic defects, as is the case of WAS [537]. One must, however, properly consider the phenomenon of mutations, that can render useless the inactivation method, as has been proved in WAS, in XLA and also in SCID, in which the mutation is not in the maternal T cells but in the germlines [389]. The main clinical aspects of humoral and cellular PID are schematized in Table 22.6 [80]; further in numerous PIDs there is a deficiency of chemotaxis (Table 1.65) as in CGD [416]. In antibody deficiencies, current treatment, while waiting for genetic treatment to become available, complicated in XLA by several Btk mutations, is based on the prophylactic administration of IVIg, combined with quick antibiotic treatment during infectious episodes.

Table 22.6. Clinical data of humoral and cellular ID

Clinical data of humoral ID
Chronic sinusitis, otitis, enteritis
Chronic sinopulmonary infection leading to bronchiectasis and respiratory insufficiency
Recurrent infection with high-grade extracellular encapsulated pathogens
Growth retardation not evident
Palpable lymphoid and nasopharyngeal tissue is scarce in X-linked agammaglobulinemia
Increased incidence of autoimmune disorders and malignancies
Survival to adulthood or for several years after onset of the condition may occur[a]
Clinical data of cellular ID
Intractable diarrhea, pneumonia, thrush, growth retardation, failure to thrive
Severe, recurrent infections with low-grade or opportunistic infectious agents such as fungi, viruses, or *Pneumocystis carinii*
Sepsis, meningitis, mastoiditis, otitis, and abscesses
Absence of lymph nodes and tonsil tissue
High incidence of malignancies
Short life span[a]
Susceptibility to GvH disease caused by maternofetal transfusion or if given fresh blood or plasma, or from allogeneic cell transfusion
Fatal reaction following live virus or bacteria (BCG) vaccination

Modified from [79].
BCG Bacillus Calmette-Guérin.
[a] Dependent on treatment (for details see text).

Predominantly B-Cell Immunodeficiency

X-Linked Agammaglobulinemia or Bruton Tyrosine Kinase Deficiency

Inherited in an X-linked trait, only 50% of males have a FH positive for PID; female cases are also known, supporting an AR trait [100]. Classically affected subjects present levels of IgG at <100 mg/dl, with very low circulating IgA, IgM and B cells (Table 22.3), in which are found, in addition to BM, pre-B lymphocytes in an almost normal quantity [100]. XLA is characterized by a *blocking of B-cell differentiation* that results in an arrest of the evolution of *pre-B1a cells*: low levels of cytoplasmic IgM and high levels of *surrogate light (L) chains* (CD179b) into later-stage B cells [348].The B-cell differentiation arrest in the majority of XLA patients appears to be homogeneous, with approximately 80% of the pro B-cell compartment being negative for cytoplasmic Igμ expression [349]. The size and nature of the residual more mature B-cell population (leakiness) varied among patients, independent of the type of Btk mutation. Further, it appears that the pro B-cell compartment composition in bone marrow (BM) of some XLA patients can be influenced by low levels of wild-type Btk mRNA [349]. On the contrary, T cells are normal both in function and in number, as is thymus architecture, including Hassall's corpuscles and thymus-dependent areas of spleen and lymph nodes. B lymphocyte zones are typically depleted, with an absence of GCs, plasma cells, and cortical and medullar differentiation compared to normal (Figs. 22.4 and 22.5) and an absence of adenoidal tissue (Fig. 22.6). The intestinal lamina shows a similar deficiency [227], even if both B and T cells use the same recombination (Chap. 1). In the BM, increased pre-B lymphocytes without CD19 and CD21 can be observed. The pre-B cells are capable of transcribing and translating microgram intracytoplasmic (IC) H chains, but not the L chains [453], thus pre-B only form microgram chains not associated with V_H, while only 5% of normal cells produce incomplete chains [278]. Experimental data currently indicate that the defect lies in the XLA gene mutations that codify for btk [505, 513]. The *XLA* gene is expressed by B cells during differentiation, but is not transcribed in the T cells, thereby explaining the B lymphocyte maturative block at the pre-B level [61] (Fig. 2.6), immediately after their appearance in the BM [302]. XLA, however, presents a genetic heterogeneity, explained by mutations in the 5 btk domain (PH, TH, SH3, SH2, kinase), with a frequency proportional to the pertinent domain dimensions [514]. The mutation size was ascertained by finding 175 mutations in 236 patients (Fig. 22.7). Equally, genes codifying for marker proteins and receptors that are essential for B-cell maturation and development are also involved: in fact many of these proteins, including btk, Hμ chains and surface proteins are crucial for B-cell differentiation [395]. Studying children of both sexes with XLA, various mutations of Hμ germline have been identified, in addition to deletions affecting the D, J_H and Cμ genes and other gene alterations capable of blocking H chain synthesis on B lymphocytes [548]. An equivalent molecular defect was observed in an infant girl with XLA, with differentiation block preceding the Ig gene rearrangements by early pre-B cells [316].

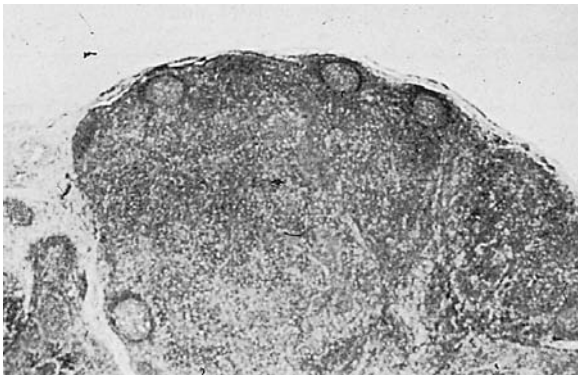


Fig. 22.4. Normal lymph node from a healthy 4-month-old boy, showing differentiation into cortex and medulla, with well-formed germinal centers (*GC*) and groups of lymphocytes

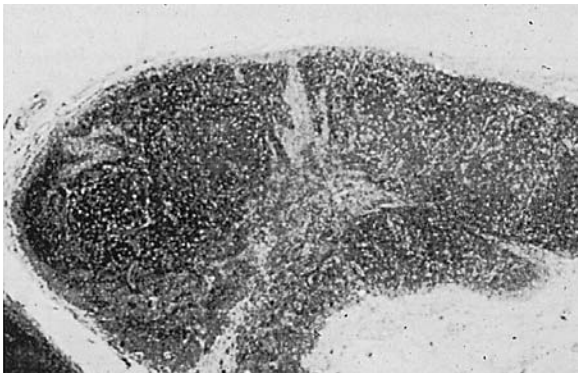


Fig. 22.5. Lymph node from a 10-year-old boy with XLA showing absence of differentiation into cortex and medulla, no GCs or lymphoid follicles and no plasma cells

There are also the so-called leaky forms, with absent or few B cells and various antibody deficiencies [240], which can be attributed to individual mutations of btk [267], for example in the non-kinase domain, which permits the expression of normal btk levels [427]. However, btk mutations can be even more detrimental for B lymphocyte proliferation compared to the total kinase absence [370]. XLA is *clinically characterized* from its onset in male babies, at 5–6 months of life (but also at the end of the 1st year), when the maternal IgG passive protection ceases. It usually attracts attention due to delayed growth and mostly recurrent and severe bacterial infections dominate (sinusitis, otitis, bronchitis,

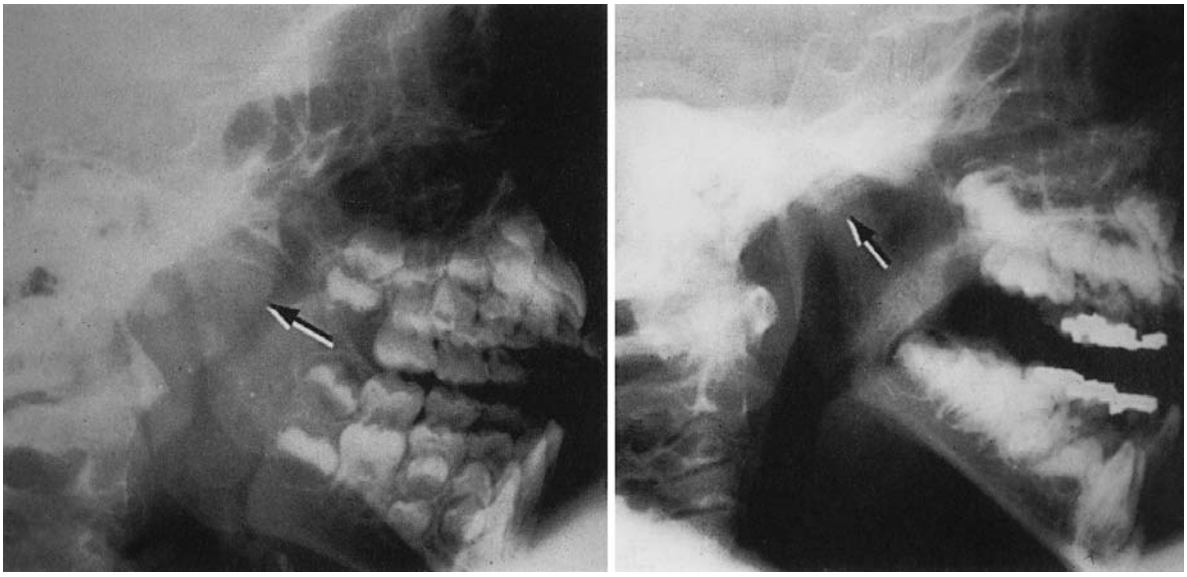


Fig. 22.6. Lateral roentgenograms of the nasopharynx: Adenoidal tissue is present in a normal 10-year-old boy (*left, arrow*), but is absent in a 10-year-old boy with XLA (*right, arrow*)

pneumonia), caused by pyogen bacteria such as streptococci, pneumococci, staphylococci and *Haemophilus influenzae*, typically capsulated and Gram+ (not by fungi, which are a T-cell competence), chronic diarrhea with malabsorption caused by *Giardia lamblia* and *Campylobacter jejuni* [61, 79, 302]. *Rotavirus* and *ECHO viruses* also cause severe meningoencephalitis in 5%–15% of patients [100]. Phenotypic variability may occasionally be present, as in a family spanning three generations [332]. In 33 patients with a median age of 9.4 years the median age at the XLA onset was 8 months and the median age of diagnosis was 4 years, with a median diagnosis delay of 33 months. The common infectious diseases were pneumonia, otitis, diarrhea, sinusitis, and arthritis. The most common chronic infections were seen in 75.8% of the patients: in the respiratory tract in 93.9%, in the gastrointestinal tract in 75.8%, in the central nervous system (CNS) in 33.3%, and in the musculoskeletal system in 21.2% of patients [324]. Bronchiectasis, malabsorption, arthritis, autoimmune and tumor-related diseases are the most common complications, as well as edema, contractures, etc. (Fig. 22.8). One must predict the onset of bronchiectasis and intervene quickly with specific physiotherapy, because forms that are initially localized later spread, causing respiratory failure in older children and adolescents. One-third of all cases start with mono- or rheumatoid arthritis (RA) caused by *Ureaplasma urealyticum* with a sterile exudate, which usually regresses following treatment with IVIg [355]. Anti-polio vaccinations with live attenuated viruses should be forbidden, because they can cause very severe pneumonia [278].

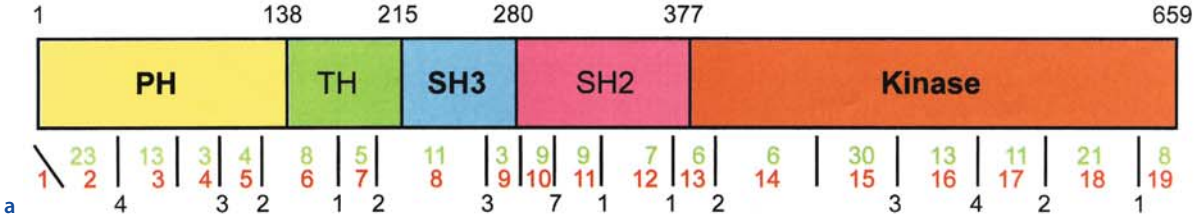
About ten cases of XLA associated with GHD are known. In addition to reduced growth, clinical symptoms are typical of XLA, though it is not a variant, because a molecular study reveals the absence of detectable mutations [476]. Although representing the phenotypic picture of humoral ID, confirmed as a separate deficiency [459], XLA associated with GHD is mapped in the same region as the X chromosome of the isolated XLA.

Gene Deletion for H Chains

The observation of HZ deletion of one or more C_H genes for the H chains of Ig in 5%–10% of normal patients has led to the identification of various polygenic deletions concerning the genes of one or more isotypes and subclasses [282, 414]. Some subjects are lacking in genes of all or some IgG subclasses, associated with IgA_1 and IgE deficiency, with no clinical symptoms in 94% of cases [282, 414]. In Italy these deletions have a frequency of 2.7% and the expected frequency in HZs is of 1:1,400 [385].

κ and λ Chain Deficiency

Only some of the families that produce λ chains and not the κ chains are known. In one family the molecular bases of the deficiency were ascribable to two different punctiform mutations, one in each *Cκ* allele that prevented the formation of -S-S bridges between the κ and H chains. The κ:λ ratio in human Ig is 2:1, and the relative alterations can be observed in numerous primary or secondary IDs [414]. Only one patient is known with an λ chain deficiency, hgG and RRI (upper and lower respiratory tract) [508]. Table 22.1 indicates the pertinent loci.



a

SH2 DOMAIN

281 WYSKHMTRSQAEQLLKQEGKEGGFIVRDSSKAGKYTVSVFAKSTGDPQGVIRHYVVCSTPQSQYYLAEKH 350

351 LFSTIPELINYHQHNSAGLISRLKYPV 377

KINASE DOMAIN

378 SQQNKNAPSTAGLGYGSWEIDPKDLTFLKELGTGQFGVVKYGKWRGQYDVAIKMIKEGSMSEDEFIEEAK 447

448 VMMNLSHEKLVQLYGVCTKQRPIFIITEYMANGCLLNYLREMRHRFQTQQLLEMCKDVCEAMEYLESKQF 517

518 LHRDLAARNCLVNDQGVVKVSDFGLSRYVLDDEYTSSVGSKFPVRWSPPEVLMYSKFSSKSDIWAFGVLM 587

588 WEIYSLGKMPYERFTNSETAEHIAQGLRLYRPHLASEKVYTIMYSCWHEKADERPTFKILLSNILDVMDEES 659

PH DOMAIN

1 MAAVILESIFLKRSQQKKKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEKITCVETVVPE 70

71 KNPPPERQIPRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTEELRKRWIHQLKNVIRYNSDL 138

TH DOMAIN

139 VQKYHPCFWIDGQYLCCSQTAKNAMGCQILENRNGSLKPGSSHRKTKKPLPPTPEEDQILKKPLPPEPAA 208

209 APVSTSE 215

SH3 DOMAIN

b 216 LKKVVALYDYMPMNANDLQLRKGDEYFILEESNLPWWRARDKNGQEGYIPSNYVTEAEDSIEMYE 280

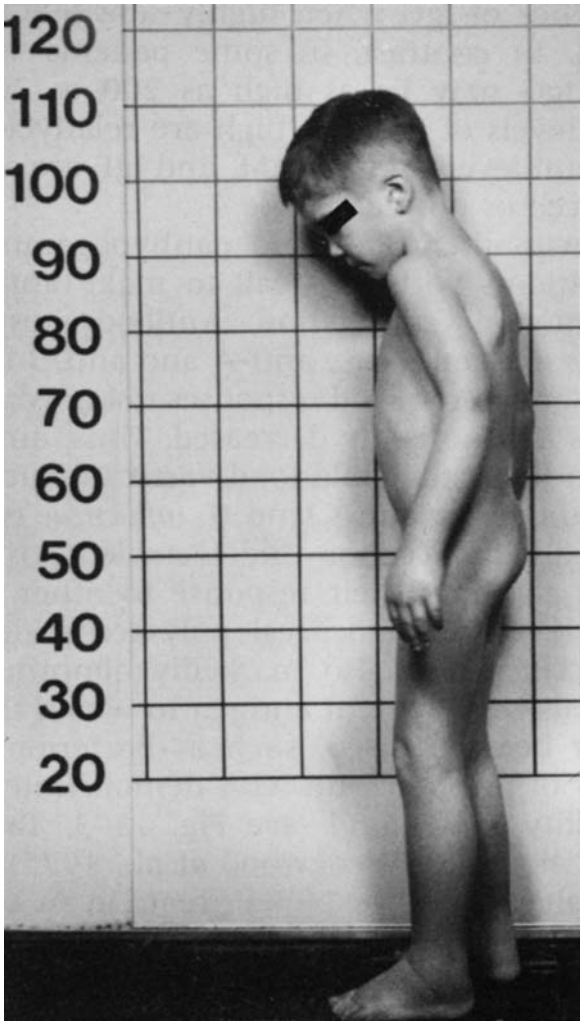


Fig. 22.8. A 6-year-old boy with XLA and a dermatomyositis-like syndrome caused by disseminated Echovirus 24 infection. Note the limb edema, especially the hands and feet, the gluteal wasting and the flexion contractures of his arms and legs

Selective Ig Deficiency

IgG Subclass Deficiency with or Without SIgAD

Sporadic cases have been described, occasionally associated with SIgAD (10%–20%) and more often with ATA (80%) and susceptibility to infections [227] or without RRIs [385], differentiating patients with probable PID from those with low levels of IgG_2 (Table 22.7) [387], in whom it may represent delayed maturation [450]. Selective deficiency of IgG subclasses presents three different aspects:

- *Total lack* of a subclass
- *Two SD levels* below average
- *Inability to produce antibodies* relative to the subclass in question, even when hematic concentrations are normal [465]

The following selective deficiencies are present [283, 465]:

- *Isolated IgG_1:* deficiency in only a few cases has been described, also because this subclass represents 60%–70% of all IgGs (the others account for 25% [IgG_2], 6% [IgG_3] and 3% [IgG_4]), its absence is very probably an indication of an evident HgG; these patients usually have a reduced level of total IgGs and react normally to antigens with a polysaccharide capsule.

Table 22.7. Age-related reference values of IgG subclasses (M + 2 SD) mg/ml in normal subjects

Age (years)	IgG_1	IgG_2	IgG_3	IgG_4
<1	2.7 (1.6–5.4)	0.83 (0.2–1.8)	0.25 (0.11–0.37)	0.05 (0.01–0.45)
1–2	3.3 (1.8–5.1)	1.12 (0.4–2.2)	0.3 (0.24–0.64)	0.12 (0.04–0.70)
3–5	4.4 (1.5–13.6)	1.5 (0.7–4.1)	0.28 (0.19–0.75)	0.28 (0.06–1.24)
6–9	4.0 (2.4–9.6)	1.8 (0.6–4.5)	0.38 (0.20–0.60)	0.35 (0.05–0.87)
10–14	5.3 (2.1–12.6)	2.8 (1.0–7.2)	0.50 (0.15–1.06)	0.51 (0.11–1.43)
≥15	7.1 (3.8–14.6)	3.8 (1.5–8.2)	0.51 (0.28–0.96)	0.40 (0.09–1.76)

Data from [387].

←

Fig. 22.7 a,b. Domain organization of Btk. **a** From the N-terminus the protein contains pleckstrin homology (*PH*), tec-homology (*TH*), src-homology 3 (*SH3*), SH2 and kinase domains. The exon boundaries are shown by vertical lines. The *green numbers* specify the number of families having mutations in the exons that are numbered in *red*. The number of families having intron mutations is shown in *black* below the exon-intron boundaries. **b** Mutations causing XLA. The sequences are arranged according to domains. The mutations shown *above* the sequence cause either severe (classic) or moderate XLA, whereas those *denoted* below the sequence cause clinically mild disease. The number of affected families is color coded: from *black* (one family), *blue*, *green*, *magenta*, to *red* (five or more families). Insertions are shown with @, deletions with #, and stop codons with ∗

- *Isolated IgG_2* deficiency: often associated with an IgG_4, IgA and IgE deficiency.
- *Isolated IgG_3* deficiency: often occurs with a selective IgG_1 deficiency.
- *Isolated IgG_4* deficiency: often linked to an IgG_2 deficiency.
- *Other combined ID* (CID): for example IgG_2+IgG_4, IgG_2+IgG_3, IgG_1+IgG_2+IgG_4, IgG_1+IgG_2, IgG_2+IgG_3+IgG_4, some of which are associated with a deficiency of IgA or its subclasses [153, 465].

The association of SIgAD and defects of IgG subclasses is explainable in view of Ig production ontogenesis by B cells: one starts with IgM, moving on to IgD, and then to IgG ending up with IgA passing by IgE [296]; therefore the deficiency could originate with an immunological defect involving the T lymphocyte regulating work or B cells secreting Ig, with an effect on the final stages of their production. In rare cases, more or less extended deletions in chromosome 14 have been observed, in the region that codifies the H chains; in most cases the genome is instead intact, confirming a possible defect in B lymphocyte switching [367]. Very rarely this deficiency depends on gene HZ deletions [227].

Selective IgA Deficiency

This PID is probably the most common of all (Tables 22.4, 22.5), especially in nonselected populations of Caucasian origin [367]. It is defined by the presence of a serum level of IgA <5 mg/dl and the absence of sIgA in the total deficiency; in the partial SIgAD levels are <5 mg/dl but <2 SD compared to normal levels for age, with measurable sIgA [367]. In total deficiencies, IgM and IgG levels can be normal [414] (Table 1.15), but IgG_2 and IgG_4 levels are low [153]. In several cases, the partial deficiency is transient [383], returning to normal levels of IgA in 50% of cases by the age of 14 and in 80% by 18 (Fig. 22.9) [383]. The SIgAD is transmitted sporadically; however, cases of multifactorial and dominant AR, with a variable or incomplete expression [105, 536] transmitted within the same family have been reported. Functional alterations reflect on the final maturing process of B lymphocytes, given that about 80% of B_{IgA}+ lymphocytes show an IgM+IgD+IgA+ membrane phenotype, a normal aspect only in newborn babies [103].

Studies on chromosome 18 have not led to conclusive results, because deletions in children with SIgAD are associated with mental retardation, facial dysmorphisms, failure to thrive, etc. An association with HLA haplotypes situated on chromosome 6 is instead more consistent, and common in patients with CVID. Interesting indications for understanding the pathogenesis come from molecular genetic studies that have allowed the formulation of a hypothesis of multifactorial origin, given that the combinations of more widely involved HLA haplotypes and extended haplotypes involve the class I–III genes, to the extent that they are more often

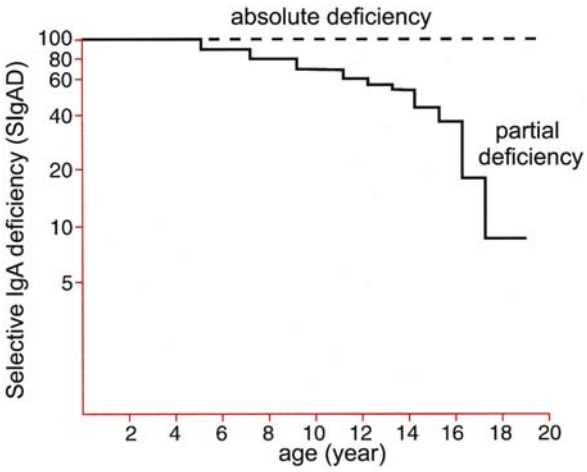


Fig. 22.9. Selective IgA deficiency (SIgAD). SIgAD natural history, its persistence in 40 SIgAD-affected children (*dashed line*) and 40 affected with partial IgA deficiency (*solid line*), the complete deficiency is irreversible. Serum IgA levels normalized in 40% of children with partial deficiency at about 14 years. (Modified from [383])

encountered in the general population [105]. Among the class III alleles the most studied is the gene that dictates the C4A, among class I and II the most common are *A1*, *A28*, *B8*, *B13*, *B40*, *CW6*, *DR1*, *DR3*, *DR7*, *DQW1*, associated with haplotypes such as *A1*, *B8*, *DR3*; *B13*, *DR7*; *A1*, *B14* or *A28*, *B14*. Other haplotypes are extended, such as *B8/SCO1/DR3*, *Bw65/SC2[1,2]/DR1*, *Bw57/SC61/DR7* and *B44F/FC31/DR7*, the first of which is increased in patients with a combined IgA, IgG_2 and IgE deficiency [170, 313, 536]. The association with *DR3* gives SIgAD a risk factor of 13 (Table 18.3). One HLA supertype is also found in deficiencies of 21-hydroxylase with a late onset, suggesting an important locus for IgA differentiation close to class III HLA genes [99]. It has been thought that to induce SIgAD a non-HLA gene or an environmental penetration factor might be necessary, due to the possible SIgAD discordant expression in HLA-identical twins [536]. However, the analyzed sequence of involved alleles showed a significant SIgAD correlation with some alleles belonging to HLA-DQ locus, composed of a protective allele with aspartic acid and a susceptible one with valine or alanine in the β chain in position 57 [358]. We have observed that the presence of aspartic acid ensures protection in DM.

Furthermore, the immunological deregulation extends to the typical formation of auto-antibodies and characterized by the presence of IgG anti-IgA [61, 196]. In these subjects, there is a wide symptom range, mostly represented by RRIs, allergic and autoimmune diseases (AIDs), among which is diabetes. Allergic diseases are twice as common in partial deficiencies, unlike AIDs [507]. From a clinical point of view, the frequency of chronic diarrhea and malabsorption, associated with celiac disorders and *Giardia lamblia* infestation are not surprising, considering sIgA's prominent

role in the formation of a barrier against the penetration of polypeptidic macromolecules through the intestinal mucosa. SIgAD therefore facilitates the penetration of food antigens through the mucosa followed by the formation of specific antibodies. For example, 50% of patients present CICs and precipitins to CM, 23% to bovine anti-serum and 13% to anti-serum of calf fetus [106]. Symptoms affecting the respiratory tract are also caused by the absence of sIgA, as in 30/36 children aged 1–15 with increased susceptibility to RRIs [327]. Patients balance the sIgA deficiency with the sIgM, but in some cases the compensation is insufficient for exempting them from RRIs and asthma [227]. SIgAD should be diagnosed on the basis of both serum and secretory IgA, because normal levels in adults are achieved at different times (Table 1.15). Some drugs such as phenytoin (an anticonvulsant) can determine SIgAD, sometimes persisting in time after the drug has been discontinued. The clinical symptoms in these cases are not different from those of patients with SIgAD [507]. There is no random treatment; these patients do not benefit from therapy with IVIg, even when enriched in IgA. There are no counter-indications for obligatory and optional vaccinations [507]. Whole blood or plasma transfusions containing IgA can sensitize patients or cause anaphylactic shock in those already sensitized [105]. *Life expectancy is excellent;* however, the random discovery of SIgAD in asymptomatic children should not be underestimated. They should in fact undergo periodic clinical and laboratory controls so as to identify as early as possible any possible pertinent symptoms. At the same time, there is the need to ensure a *good life quality* with adequate prevention of RRIs in those patients whose respiratory tract is affected [383].

Selective Antibody Deficiency with Normal Ig Isotypes

SADNI translates into the inability to respond to certain antigens, especially if polysaccharide. While some individuals are normal, others contract sinopulmonary infections. The reduction of IgG_2 levels is more of an associative relationship than a random one; IgG_2 levels, on the other hand, do not predict antibody responses. Subjects who do not respond to anti-hepatitis vaccination may fall into this category [414]. In one retrospective survey at a pediatric tertiary care center, SADNI was the most frequent diagnosis, accounting for 23% of ID diagnoses [236].

Selective Deficiency of Other Igs

Selective IgE Deficiency

There are cases of patients in good health, without IgE due to gene deletion [62]. In two siblings, deficiency of IgA_1, IgG_2, IgG_4 and IgE due to deletion of Ig H chain constant region genes were associated with undue susceptibility to infection [384] (see "RRI").

Selective IgM Deficiency

A few cases of selective IgM deficiency are known, associated with RRIs and various other symptoms [414]. IgM deficiency was detected in four children with RRIs. Isolated IgM defect was present in two children, and two more children had an associated IgG_3 subclass deficiency [160].

Common Variable ID

CVID includes a heterogeneous group of unhealthy conditions that have in common hgG and RRIs; it has an incidence of between 1:50,000 and 1:200,000 [414]. The inheritance of two susceptibility genes within the HLA on the short arm of chromosome 6: one located near the class II region and the other near the junction between the class III and class I regions is a serious risk for the development of CVID [441]. There are autosomal dominant or AR forms also linked to sex; sporadic cases are the most common [413]. The molecular bases are not totally clarified as yet: the pathogenetic mechanisms may depend on B lymphocyte (80% of patients) and T lymphocyte (20%) defects [227]. The B-cell intrinsic defect is attributable to an alteration of the differentiating line at different stages of maturation, resulting in a poor formation of antibodies, with hgG of variable degrees, while in patients with XLA the circulating B lymphocytes are virtually absent. The IgGs are <500 mg/dl (with a reduction in all the subclasses: a normal phenotype is observed in only 14% of patients) [367]. More often there is a hierarchical order in the shortage: $IgG_3 < IgG_1 < IgG_2 < IgG_4$ [528]. IgA and IgM antibodies are <5–50 mg/dl [102], reflecting the potential CD154 underexpression, implying an activation deficiency [150] or a T–B cooperation defect [232].

A study of T lymphocyte subpopulations indicates various subgroups of patients: 60% have T cells with scarce IL_2, IL_4 and IL_5 levels, while 30% have a reduced CD4:CD8 ratio, with an increase in CD8 bearing the CD57 marker, which suppresses IgG production, elaborates normal IL_2 levels and increases IFN-γ production [232]. It can also accompany a deficiency of interleukins (ILs: IL_{10}, IFN-γ), suggesting a defect in the signaling mechanisms based on the TcR/CD3 [191]. A T-lymphocyte deficiency is therefore difficult to evaluate [527], also because this could be a VRI effect [232]. CVID can also be observed following congenital rubella or EBV infections; it can also be induced by some drugs such as phenytoin [355]. In 2/9 CVID families, 5 subjects were identified with identical large mutations in the ICOS (inducible costimulator) gene, expressed on the surface

of activated T cells, which interacts with the ICOS ligand gene expressed on B cells. An additional 181 patients with sporadic CVID were examined, and no mutations were found. Only 9 in 226 patients with CVID screened thus far (<4%) have been found to have ICOS mutations. One unexplained feature of CVID is that the onset of clinical symptoms does not occur until late childhood or adulthood [429].

PID is variable either in the clinical and immunological pattern, or in the onset period, more common during the school years or in adults, but also between the ages of 1 and 3 [102]. The acute bacterial recurrent and/or severe *lower respiratory tract infections* (LRTI) are characteristic: sinusitis (60% of pediatric cases), otitis media (47%), bronchitis, pneumonia (87%) and/or digestive tract infections (diarrhea 57%) [213]. The prevalence of infections caused by mycetes has increased as well as cases of pneumonia caused by *Pneumocystis carinii*, a signal for cell-mediated immunity (CMI) [152]. The *gastroenteric tract* is dominated by symptoms similar to those seen in celiac disease, with generalized malabsorption, steatorrhea, lactose intolerance, protein-losing enteropathy, inflammatory bowel disease (IBD), saccharidase deficiency and malabsorption of vitamin B_{12} and folic acid, supported also in this case by intestinal infestation caused by *Giardia lamblia* [527]. The tumor necrosis factor receptor family (TNFR) member TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor) mediates isotype switching in B cells. In 4/19 unrelated subjects with CVID and 1/16 subjects with SIgAD there was a missense mutation in one allele of TNFRSF13B (encoding TACI). None of these mutations were present in 50 healthy subjects. TNFRSF13B mutations cosegregated with the phenotype of CVID or SIgAD in family members of the 4 index subjects. B cells from subjects with TACI mutations expressed TACI but did not produce IgG and IgA in response to the TACI ligand APRIL (a proliferation-inducing ligand), probably reflecting impaired isotype switching [87]. Other characteristics are hemopathies, hepatosplenomegaly, autoimmune hemolytic anemia (AIHA) and X-linked lymphoproliferative disease (XLP), and cutaneous and internal organ granulomas (which differentiate it from XLA), in particular RA, thrombocytopenia, and neutropenia [102]. Offspring of CVID patients are at risk throughout their lives for CVID development and should be monitored with a high index of suspicion [441].

IgA- and CVID-Associated Deficiency

Based on experimental evidence, it has been hypothesized that IgA- and CVID-associated deficiencies may be the extreme opposites of one clinical spectrum: there is a block of B-cell differentiation, different only in the isotype involved. Both defects often appear in different members of the same family groups and more or less the same alleles are present [313]. The most accredited hypothesis is that a number of extended haplotypes of the HLA system are shared, to which gene duplications, deletions and polymorphisms codifying for some class II and III alleles correspond [22]. In fact, a number of common HLA haplotypes, especially belonging to class III, are observed in patients, and at least two haplotypes in 77% of cases [518], such as *HLA-DQB1*0201*, *HLA-DR3*, *C4B-Sf*, *C4A*, *G11–15*, *Bf-0.4*, *C2a*, *HSP-70–7.5*, *TNFa-5*, *HLA-B8* and *HLA-A1*, postulating therefore the existence of a common genetic basis [22], with a susceptible gene (*6p21.3*) possibly the association marker [61]. For example in five members of a large family with one of the two PIDs, duplications of the *C4* genes were associated with a selected group of HLA class II and III genes [22]. The fact that four members without PID also had these haplotypes indicates that their presence alone is not sufficient for expressing PID, leaving room therefore for other factors [22] such as overlapping relations with celiac disease. The analysis of linked genes has confirmed a strong association with locus 4A, suggesting that an important role in both PID is played by the gene codifying C4A or an adjacent one [162].

Not-X-Linked Hyper-IgM

See "X-linked Hyper-IgM (or Hyper-IgD) or CD154 Deficiency (XHIgMS)" for further discussion.

Transient Hypogammaglobulinemia of Infancy

Unlike transient hgG that occurs when the maternal IgGs gradually disappear from circulation (Table 1.15), in the original study by Taylor et al IgA levels had fallen, becoming regular in newborn babies with THI after 1 year, corresponding to the nonatopic levels [493]. It consists therefore of a pathological delay in the normal antibody production maturing process. Walker et al have calculated that the prevalence of *THI* is 23×10^6 in children, equal to the prevalence of symptomatic SIgAD (24×10^6) [525]. In all 15 children the IgG and in 12/15 (80%) the IgA were <5th percentile, 9/15 (60%) had IgM levels <20th percentile resolved around the 22nd month; further confirmation consisted in the fact that the 12 children had symptoms either of AD or of FA or food intolerance [525]. From Table 22.4 the mean incidence is from 1 to 61×10^6. During 7 years 30 children aged 6–46 months were diagnosed with THI and an incidence of 4.3/year [124]. In other studies the main defect was in the IgG: in one it had normalized between 18 and 40 months [129], in another trial 13/247 babies (5.3%) exhibited at the age of 10 months an absence of serum IgG levels and of specific antibodies to viral agents, which in eight children were detected before the serum IgG levels returned to normal, whereas in

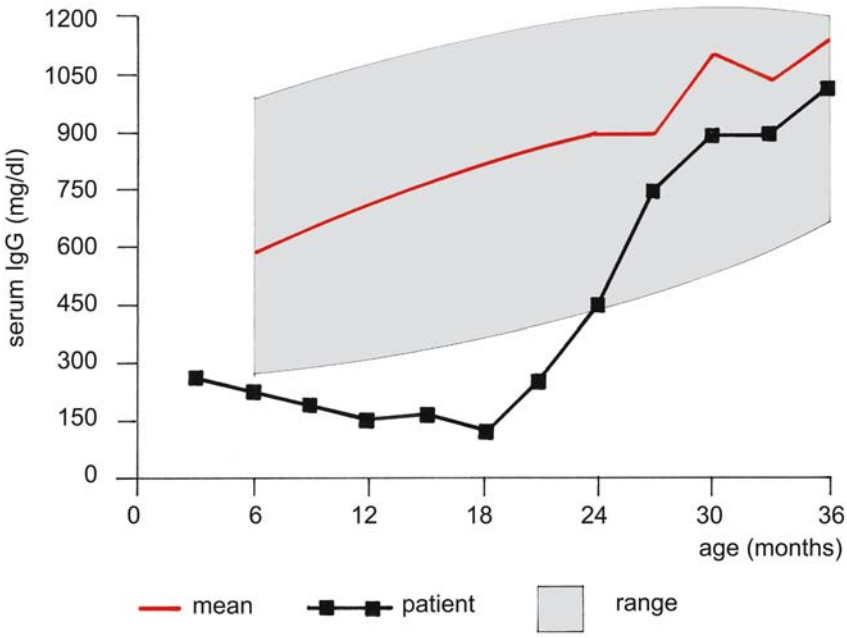


Fig. 22.10. IgG levels in transient hypogammaglobulinemia of infancy (THI). IgG levels normalized at age 2 years

two children normal IgG levels were detected even before the appearance of specific viral antibodies. IgG levels usually normalize at between 15 and 36 months [78], at the age of 2 years (Fig. 22.10) or before 36 months of age in 33/40 children; however, 7/40 still had low Ig levels at 40–57 months of age [253]. At 27 was in 9/30 children Ig levels were still <2 SD for age and in 5/9 various IgG subclass deficiencies were detected [124].

A prospective study with an 8-year follow-up found that IgG and IgA deficiency is normalized by the age of 6, but in a minority of cases this may be a prodrome of SIgAD or another humoral deficiency [315]. A study with a 10-year follow-up of 35 children with IgG deficiency as well as IgA deficiency in 34% of the cases, observed multiform clinical symptoms. Since THI can gradually normalize, some children have low antibody titers, and others low IgG levels. However, both groups experienced significant infections [110]. In some cases, THI is asymptomatic; in others infections, especially of the respiratory tract, are present. The designation of THI may be a misnomer, and an alternative designation could be added to THI such as "with recovery" or "with development of other dysgammaglobulinemia" [315].

Combined T-Cell and B-Cell Deficiency

General characteristics of combined T-cell/B-cell immunodeficiency are summarized in Table 22.8 [453].

T^-B^+ SCID

T^-B^+ SCID is a heterogeneous group with an incidence of between 1:50,000 and 1:75,000 livebirths [509]. *X-linked* FH is positive in 53% of cases [68]. The genetic basis is an IL_2R deficiency [529], more precisely of the γ receptor mapped on chromosome *Xq13* [347]. The sole deficiency of IL_2R is not sufficient for producing an immunological phenotype as devastating as SCID [529]. Because the γ chain of IL_2R ($IL_2Rγ$), a shared component of IL_4R, IL_7R, IL_9R, $IL_{13}R$, $IL_{15}R$, $IL_{21}R$ and $IL_{23}R$ [351], γc mutations interfering with its link to the ILs deprive the lymphoid progenitor cells of the crucial signals for normal lymphocyte intrathymic development [424]. Mutations in any of the genes: *$IL_2Rγ$*, *$IL_7Rα$*, *JAK3*, *ARTEMIS*, *RAG1*, *RAG2*, *CD38*, *ADA*, *CD45* cause SCID [68, 69, 211, 247, 272, 347, 350, 365, 391, 424, 443].

A total of *264 $IL_2Rγ$ gene mutations* have been sequenced, of which 169 are unique [341]. Each of these mutations has resulted in *γc deficiency* with varying degrees of ID. The mutations are distributed throughout the eight exons of the gene, as well as in the regions necessary for proper transcription and translation. The penetrance of each of the above $IL_2Rγ$ mutations is unknown. Exons 5 and 7 have mutation hot spots. The types of mutations identified include missense, nonsense, insertions, deletions, splice mutations, and mutations that affect RNA processing and translation [341]. Among 93 mutations in 136 patients, the most numerous (67%) are punctiform mutations (Fig. 22.11) plus one missense related to amino acid residues [390], with a lack of JAK3 and γc interactions [424].

In an atypical form, the substitution with residual cysteine of the arginine at position 115 appears to be decisive for γc chain expression, probably a mutation reversion at the basis of the molecular defect, with a numeric and functional T-cell normalization [475]. The mutations, by inactivating the common γ chain, render the T cells of boys with SCID-X1 unresponsive to several ILs. The result is a block in T-cell development and a severe deficiency of mature T cells. B cells, although pre-

Table 22.8. Characteristics of T-cell and combined T- and B-cell PID

PID	Thymic shadow	DHST	Mature T cells	Lymphocyte proliferation
	Combined B and T cell ID			
SCID	A	A	A	A
X-linked	A	A	A	A
Autosomal recessive	A	A	A	A
ADA deficiency	D	A	D	D
Omenn's syndrome	V	A	V	V
PNP deficiency	D	A	V	D
HLA class I deficiency	D	A	D	V
HLA class II deficiency	D	A	D	V
TcR deficiency	D	A	V	V
	Primarily T-cell ID			
DiGeorge syndrome	A	A	D	A
Wiskott-Aldrich syndrome	D	A	D	D
Ataxia telangiectasia	D	A	D	D
Cellular ID with Immunoglobulins	D	A	D	D

Modified from [453].
A absent, *D* decreased, *V* variable, *DHST* delayed hypersensitivity skin test, *NPD* nucleoside phosphorylase deficiency.

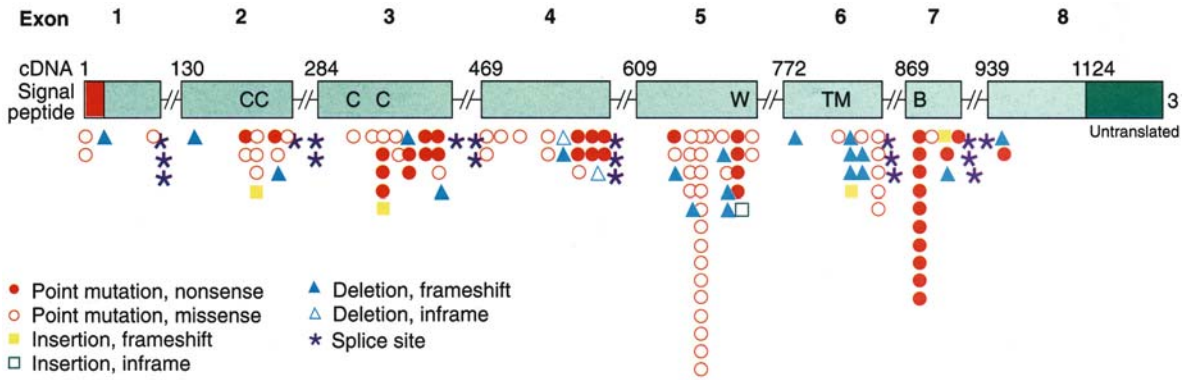


Fig. 22.11. $IL_2R\gamma$ exons and intervening sequences. *$IL_2R\gamma$* IL_2 receptor gene

sent in normal or even increased numbers than in other forms of SCID, are dysfunctional [347]. B cells do not mature or produce antibodies due to a complete B-cell differentiation arrest at the pre-BcR checkpoint, showing the absence of complete *VDJ* recombination [350]. Other forms are also known with an attenuated phenotype and a partial T-cell function [162]. Typical SCID-X1 represents the most common form, with 45.5% of cases [68] (5.5% in Tables 22.4, 22.5).

In the thymus, there is a severe hypocellularity, without lymphocytes and Hassall's bodies where thymic epithelial cells predominate without grossly evident corticomedullary differentiation (Fig. 22.12). Severe lymphopenia is often associated with eosinophilia; NK cells are within the norm or rare. The majority of infants with SCID-X1 lack both T and NK cells (T^- B^+ NK^- phenotype) [68, 391]. CD3 T cells, if present, are of maternal origin, because the block, as also in SCID AR, occurs at the level of $CD4^-$, $CD8^-$, $CD44^-$, $CD3^+$ and $CD1a^+$; developing T cells and $CD83^+$ thymic DC are reduced >50-fold when compared to age- and gender-matched control thymus [209] (Fig. 2.2, pre-T, TN), thus SCID T^- B^+.

The study of other subpopulations distinguishes the SCID subtypes: T cells are reduced in all variants, the absolute cord blood (CB) number is 158–2,400 lymphocytes/mm^3 (Tables 1.34, 1.35, so that any count below 4,000/mm^3 is lymphopenic). Moreover, in ADA deficiency (adenosine-deaminase) there is a maximum reduction in total lymphocytes, in SCID-X1 and in JAK3 defi-

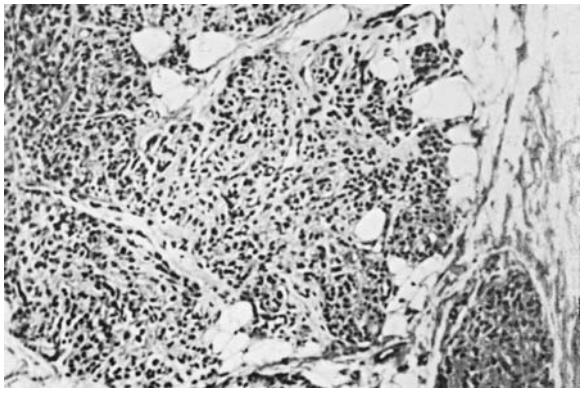


Fig. 22.12. Histological section of the thymus

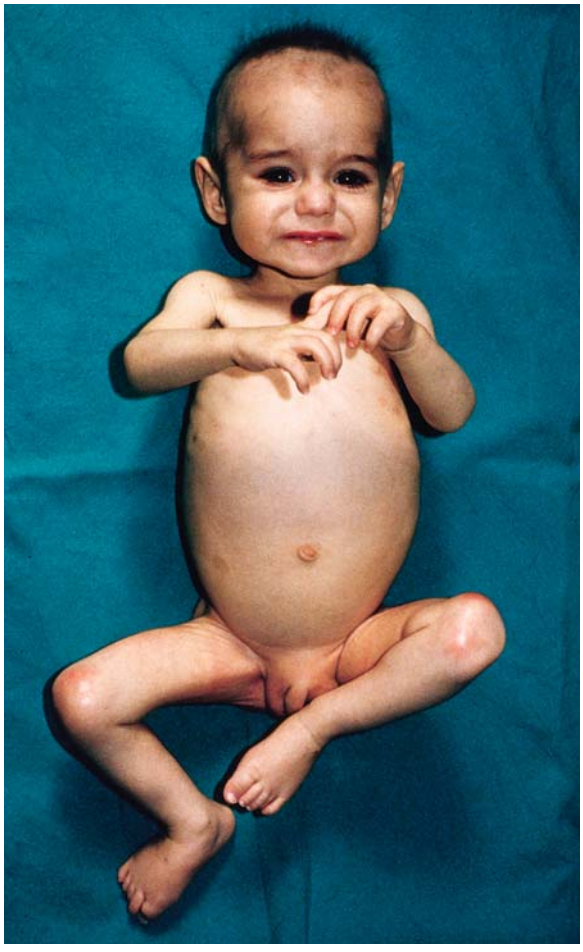


Fig. 22.13. Infant with SCID

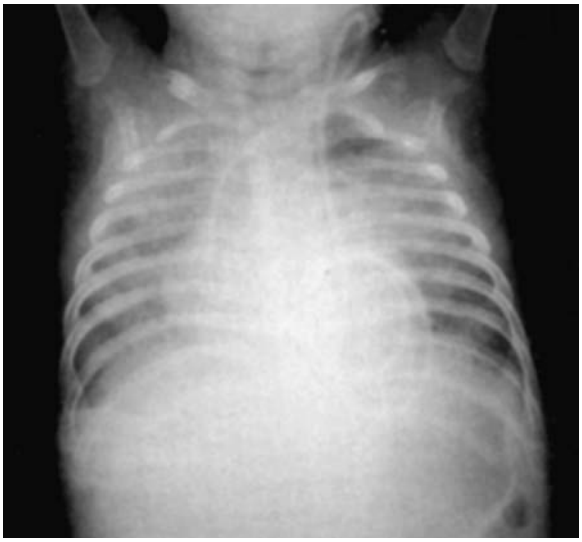


Fig. 22.14. SCID with B lymphocytes. Four-month-old baby with interstitial pneumonia; at 5 months haploidentical BMT; in good condition after 1 year

ciency, the number of B cells is the highest and that of NK cells is the lowest ($B^+ > T^- > NK^-$); NK cells on the contrary reach their highest levels in the AR form [68]. In SCID there can also be B alymphocytosis [474]. This divergent data is, however, characteristic of $T^- B^+ NK^-$ molecular defects (γc, JAK3 defects), $B^+ T^- NK^-$ (ADA deficiency), or T and B (possible recombination anomalies) and against a common T and NK cell drop [68]. Furthermore, in two cohorts [68, 474] the affected females had the same phenotype, indicating a possible complex molecular defect [68]. There is no response to delayed SPTs, and there is an absence of lymphocyte proliferative responses to mitogens and to a specific antigen such as tetanic toxoid [61, 189, 474].

The *average age at diagnosis* was 4.4 months in 31 children [21], similar to the ages reported in >200 children with SCID from all causes [68, 474]. The male:female ratio is 3:1 and diagnosis is often missed or occurs too late to save the lives of those infected infants who may manifest GvHD early on with morbilliform eruption in the first few days of life due to the transplacentally acquired maternal T cells, *intractable diarrhea* resulting in a *severe malabsorption* (Fig. 22.13), *severe interstitial pneumonia* (Fig. 22.14), or giant cells caused by anti-measles vaccination or BCG (Bacillus Calmette Guérin), with death caused by chickenpox or infections caused by *Pneumocystis carinii*, herpes, adenovirus, CMV (cytomegalovirus), etc. [63]. The absence of tonsils is observed and also lymphoid tissue [508] and thymus [453] hypoplasia. These children must be transferred urgently to a specialized center and be placed in a sterile room to receive a BMT [474].

γc Gene Mutations

On rare occasions, *$IL_2R\gamma$ mutations* have caused an atypical mild SCID that presented beyond infancy [390]. Up-regulation of bcl-2 by an IL_2R lacking $IL_2R\beta$ tyrosine residues leads to increased cell survival after IL deprivation; astonishingly, this survival signal does not occur when γc tyrosine residues are absent. Thus, if

γc-dependent signals are revealed only in the absence of $IL_2Rβ$ tyrosine, IL_2R engages at least two distinct signaling pathways to regulate apoptosis and ccl-2 expression [293]. In two clinical series, patients with mutations in $IL_2Rγ$ represent 28%–45% of all SCID cases [68, 474]. Children with $IL_2Rγ$ mutations have lymphopenia in 95% of cases, with total lymphocyte counts <2,000/mm^3 (normal levels, 4,000–13,500/mm^3), based on clinical case series [68, 474]. All patients have *very low or absent T cells,* and approximately 88% have *low or absent NK cells* [390].

AR SCID

AR mutated genes on autosomal chromosomes have been identified in ADA deficiency, Jak3 deficiency, and RAG1 or RAG 2 deficiency [65]. The existence of B- and T-lymphocyte lymphoid precursor differentiated defect is particular, in some cases a *RAG1* and *RAG2* mutation, the two genes that activate VDJ recombination [443] was observed; however, this *RAG2* gene function has been questioned [411] since a RAG defect is more present in T^-B^- SCID and the Omenn syndrome [491]. In babies suffering from SCID, there is a marked reduction of T and B lymphocytes (Table 22.3) and all in vivo and in vitro responses are absent. Onset and clinical and histological pattern is similar to that of X-SCID.

JAK3 ID

The *JAK3 gene mutation* (Tables 1.31–1.33) variant has a frequency of 5.9%–7.4% [68, 408] among babies affected by SCID and in the absence of T (3±2%) and NK cells (1±1%) [407]. The molecular base is the mutation affecting the JAK3, which prevents it from associating with the γc chain and from sending signals to the above-mentioned ILs [354] and to other marker proteins belonging to the JAK–Stat complex [301]. At the origin is a lack of T lymphocytes that transform into the SCID phenotype [424]. These patients present B^+ T^- NK^-: the B (70±12%) with IgA equal to 2±2% [407], and those with X-SCID present a defective differentiation, but are capable of producing elevated levels of IgE in the absence of other isotypes [354]. This data indicates that γc and JAK3 are essential for T- and NK-cell development [407]. The clinical characteristics are identical to those in X-SCID, with the difference that the SCID-JAK3 phenotype is also observed in females (50%) [408]. Furthermore, a JAK3 deficiency could be an important cause of SCID AR and should be considered in all patients with the B^+ T^- NK^- phenotype, without an X-recessive heritage [68]. In a 6-month SCID-X1 infant presenting with a history of recurrent infection and failure to thrive, a novel splice mutation, γc-dependent, was described, characterized by near-normal count of functionally deficient NK cells (B^+ T^- NK^- cell phenotype). Cell surface γc expression was undetectable on NK cells and in trace amounts in the minority of B cells. T cells were absent, IgG and IgA undetectable, and IgM were within the normal range [183]. BMT is not a perfect therapy, because B-cell function developed in 3/9 children, and NK functions normalized in 2/9 children after BMT [408].

IL_7R Deficiency

The family pedigree shows an inbred family with consanguinity across five generations. Two brothers were diagnosed with SCID. One, at the age of 4 months, presented with persistent oral thrush, oral ulcers, and failure to thrive. He had no palpable lymph nodes and no thymus shadow on a chest X-ray film. The second was diagnosed soon after birth and the third brother has always been healthy. Three other male cousins died in infancy from severe infections consistent with SCID; a 4th cousin presented with oral candidiasis at the age of 2 weeks and failure to thrive. No thymic shadow was detected on chest X-ray film and peripheral blood lymphocytes showed persistent lymphopenia. He had no lymph nodes, failed to reject a skin allograft and did not show an increase in the blood IgG and IgM antibodies for DTP after three vaccinations. The three affected patients were HZ for a C→T transition at nucleotide 394 in exon 4, leading to a proline to serine substitution (P132S) in the extracellular domain of IL_7R. The cousins and their parents harbored both wild and mutant alleles. This partial deficiency is sufficient to block T-cell development and lead to a SCID phenotype. The FH of severe PID with multiple affected male infants strongly suggested an X-linked inheritance. Nevertheless, this family consanguinity is in favor of an AR inheritance [410]. Defective IL_7R expression caused in three patients a T^- B^+ NK^+ SCID, indicating that the T-cell defect in SCID-X1 resulted from inactivation of $IL_7Rα$ signaling. Thus IL_7R-mediated signaling is required for T cells but not for NK ontogenes. Mutations in the gene for the IL_7R chain on chromosome *5p13* were found in all three patients [391].

T^-B^- SCID

RAG1/RAG2 Deficiency

These infants resemble those with other types of SCID with respect to their susceptibility to infection and the absence of functional T cells and B cells. However, they differ in that their circulating lymphocytes *are primarily NK cells.* RAG1 and RAG2 are required for the rearrangement of *TcR* and *BcR* genes [343]. Half of the patients with T^-B^- SCID had mutations in their RAG1 or RAG2 genes, thus highlighting the crucial role of these genes in normal V(D)J recombination machinery [443]. RAG-thymocytes lack a functional pre-TcR and hence arrest at the CD44-/CD 25+ stage of differentation [491]: without RAG1 and RAG2, mature *Ig* and *TcR* genes cannot be assembled, and lymphocyte development is arrested at very early stages [53].

Table 22.9. Biochemical features associated with ADA deficiency

1. ADA undetectable in red cells, lymphocytes and in all tissues
2. Marked elevation in red cell deoxyadenosine triphosphate (ATP) (>200- to 1000-fold increase)
3. Total deoxyadenosine nucleotides (AXT) also very high
4. Increased plasma concentrations of adenosine and deoxyadenosine (adenosine >deoxyadenosine)
5. Excretion of adenosine, deoxyadenosine and methylated compounds (deoxyadenosine >>adenosine)
5. Secondary inhibition of SAH hydrolase

Modified from [219].

ADA Deficiency

The lack of ADA is observed in 14.8% of patients with SCID [68]. The ADA enzyme catalyzes the conversion of adenosine and deoxyadenosine into inosine and deoxynosine; although ADA is found in all cells (CD26 anchors ADA to the lymphocyte cell surface (Table 1.2), the deficiency damages above all the immune system [219]. Table 22.9 [219] summarizes the biochemical foundations of this PID.

More than 50 ADA mutations are known, including >30 amino acid substitutions, deletions and punctiform mutations or anomalies of the gene itself, such as exon 1 deletion and exons 4, 5, 7 and 9–11 mutations with a total of 15, nine of these in patients with ADA-SCID and six in those with a partial deficiency [219]. An additional 29 mutant alleles have been found (28 missense and 1 single-codon deletion) [21].

Adenosine and deoxyadenosine are also apparent suicide inactivators of the enzyme S-adenosylhomocysteine (SAH) hydrolase, with consequent accumulation of SAH, a powerful inhibitor of virtually all cellular methylation reactions [61]. The accumulation of metabolites, including cAMP, deoxy-ATP and 2'-*O*-methyladenosine, has a toxic effect on the cells by blocking DNA synthesis and dividing and resting T lymphocyte proliferation [61] (Fig. 22.15).

Four different clinical phenotypes have been described for ADA-deficient subjects (Table 22.10) [219], which cover a broad spectrum of immunological aberrations, from the complete absence of B and T immunity, indicating SCID (85%–90% of patients) (the thymus in Fig. 22.16) to forms with a delayed onset or partial deficiency (10%–15%) [219]. In children, the delay between onset of symptoms and diagnosis has been estimated to average 2 months [474]. If clinical symptoms indicate an *early onset,* in addition to typical SCID symptoms, there are also X-ray pathognomonic skeletal abnormalities of the chondrodysplasia type, especially

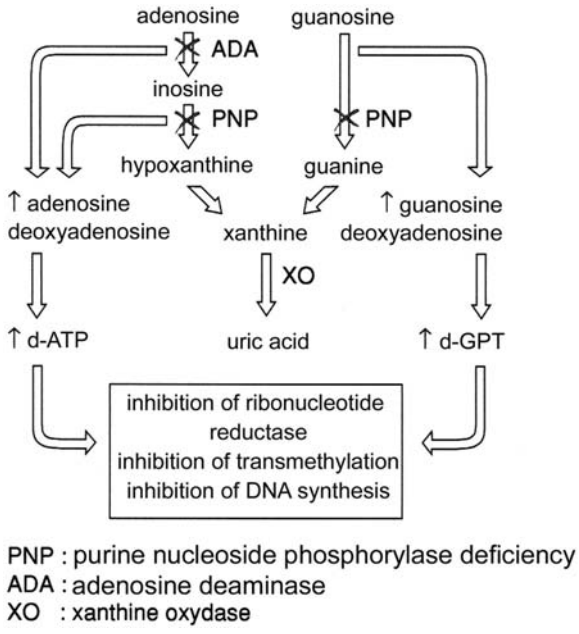


Fig. 22.15. Abnormalities of purine metabolism associated with ADA and PNP deficiencies. *XO* xanthine oxydase

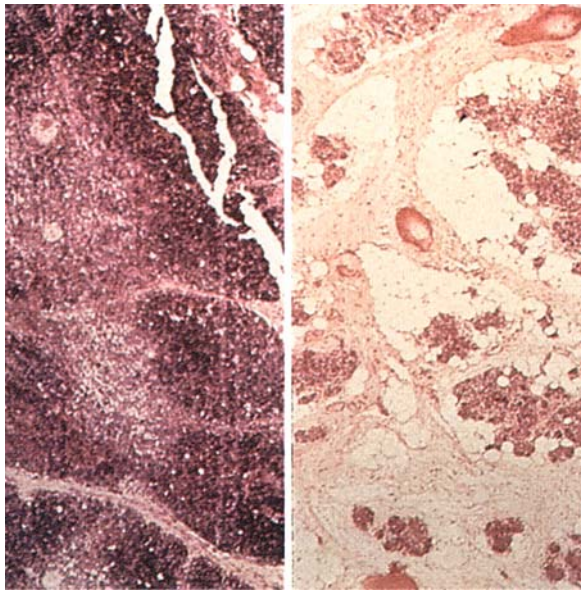


Fig. 22.16. Thymus in SCID. *Left*: normal pattern; *right*: thymic dysplasia

abnormalities of the chest, scapula and iliac bones and short and stumpy limbs [219, 224]. X-ray abnormalities are documented in Fig. 22.17: the absent thymic shadow and a notable cupping and flaring of the ribs' ends (arrows) can be observed, while histological studies of the chondrocostal junctions document their *total cellular disorganization* (Fig. 22.18). This deficiency is the object of a great deal of attention because it was the first to be treated using gene therapy [313].

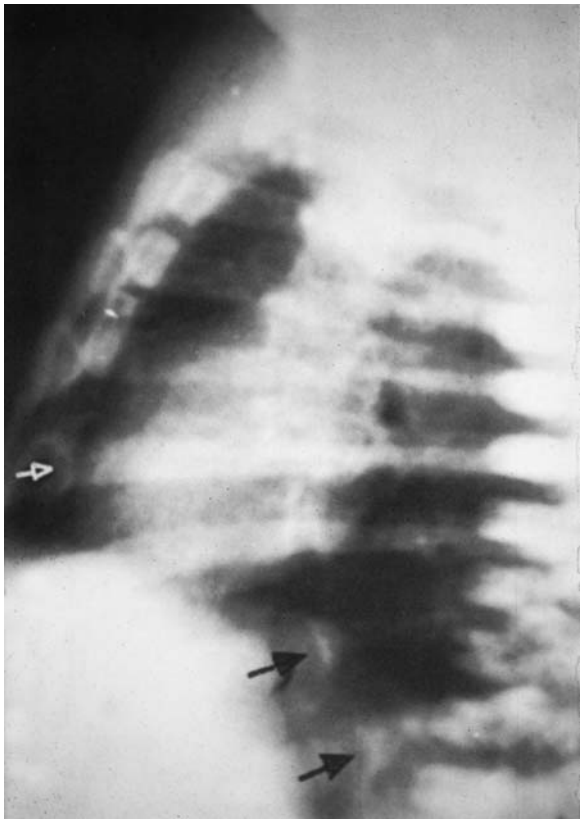


Fig. 22.17. Lateral view in ADA deficiency (for details see text)

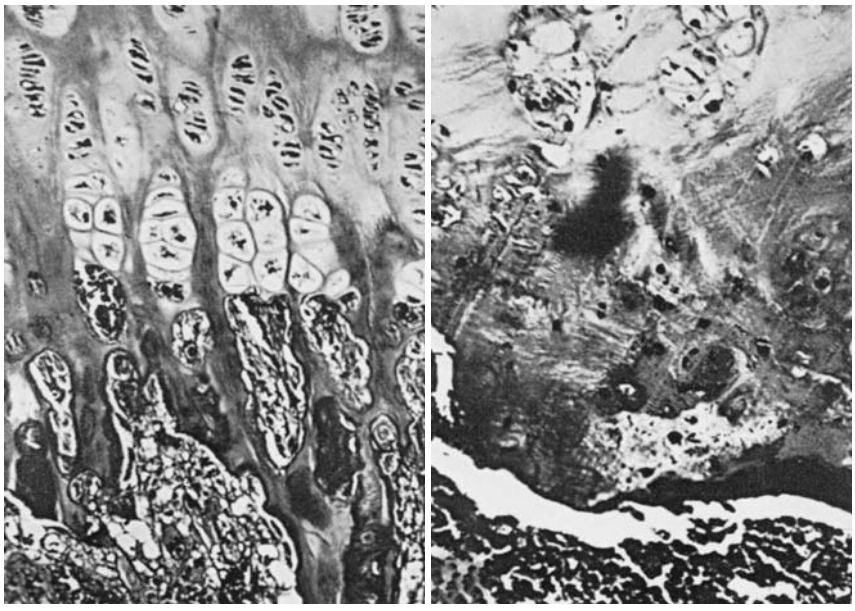
Table 22.10. Phenotypes of ADA deficiency

1.	SCID (85%–90%). Closely resembling SCID without ADA deficiency, except for bony lesions
2.	Delayed-onset (10%–15%). Predominant cellular ID with markedly decreased antibodies and absent over time
3.	Late-onset. Diagnosis not before 5–8 years of age with predominant cellular ID and antibody assays reveal no clear-cut deterioration
4.	Partial deficiency. Immunologically normal? Found as a result of normal infant screening

Modified from [219].

Reticular Dysgenesis

This very rare AR type of SCID was observed for the first time in 1959 in identical twin male infants who exhibited a total lack of both lymphocytes and granulocytes in their peripheral blood and bone marrow. It has a frequency of 1% in cases of SCID [68]. The children are symptomatic in 90% of cases within the first days after birth [46] and is usually fatal within the 3rd month of life without a BMT [224]. Due to the common stem cell (SC) non-maturation [224], it is characterized by total block in lymphoid and myeloid precursor differentiation, therefore not only by an extraordinary lymphopenia, but also by a *marked cytopenia in all sections* (Table 22.11) [474], in the spleen, in the lymph nodes and in the gastroenteric tract, and a high frequency of severe successive infections [474]. The thymus is always much reduced in volume, no Hassall's bodies are seen [224]. Seven of the eight infants reported by WHO with this defect died between 3 and 119 days of age from overwhelming infections; the eighth underwent complete immunological reconstitution from a BMT [543]. An additional three of five children who required two HSCTs (hematopoietic SCT stem-cell transplantation) and received intensive conditioning therapy before haploidentical HSCT (matched for 3 of the 6 HLA loci) are alive and well



a b

Fig. 22.18 a,b. Photomicrographs of costochondral junctions. **a** Normal child. Note the normal linear columns of proliferating cells and hypertrophic cells in the growth plate. **b** Child with ADA deficiency: in comparison to **a** there is no cell organization, no transition of proliferating to hypertrophic cells, only scattered hypertrophic cells, with uninterrupted calcified cartilage formation

Table 22.11. Immunological characteristics of a child with reticular dysgenesis

Immune cells	Child's values	Laboratory ranges
Leukocytes (cells/µl)	300	5,000–19,600
Lymphocytes (cells/µl)	100	4,000–13,500
CD3 (cells/µl)	75	1,800–3,000
CD4 (cells/µl)	60	1,000–18,500
CD8 (cells/µl)	15	800–1,600
B lymphocytes (cells/µl)	10	700–1,300
IgG (g/l)	7	2.28–6.16
IgA (g/l)	0	0.27–0.63
IgM (g/l)	0	
Neutrophils (cells/µl)	200	1,000–8,500

Data from [474].

with myeloid and T- and B-cell lymphoid reconstitution [46]; another child is alive and well after 32 months [13].

Radiation-sensitive SCID

B-SCID, characterized by increased cell sensitivity to radiation secondary to mutations of the *ARTEMIS* gene, could carry a poorer prognosis because of defective repair of DNA breaks [406], occurring around the time of BMT, from the effects of chemotherapy, infections, and GvHD [206, 247, 332]. One group of patients with SCID with an additional sensitivity to radiation was found to harbor large deletions or truncation mutations in the *ARTEMIS* gene mapped on *chromosome 10p* [65], implying a role for Artemis in DNA double-strand break repair, which is mutated in human SCID [350].

T^+B^- SCID

Omenn Syndrome

Omenn syndrome is classified as a SCID because newborn babies exhibit symptoms similar to a GvHD, due to 1a antigen expression and CD1a absence [241], and because it can coexist in families with alymphocytosis [117]. This is an AR syndrome with an unknown pathogenesis, sharing characteristic clinical and immunological abnormalities with T^+B^- SCID [515]. Severe cutaneous lesions with hyperkeratosis, apoptotic Malpighian necrosis and basal membrane destruction can be associated [241]. *No lymphoid cells or Hassall bodies are found in the thymus* [241]. The immunological structure reveals histiocyte infiltration of the skin, BM and lymph nodes, with proliferation of T infiltrating the epidermis and the enteric mucosa, increased T cells with an activated phenotype and poor functional capacity [117]. Studies involving HLA typification and DNA polymorphism show that T cells belong to the host, ruling out, therefore, the etiology of maternal cell engraftment [117], unlike other types of SCID [474]. The *absence of circulating B* is also characteristic [117], equal to 3.8%–7.1% of normal levels [474], reaching 0% [520], *high IgE levels* (526 UI/l), hypereosinophilia reaching $3{,}000\times10^9$ cells/l (normal, 0–0.5 cells/l), and low Ig levels at the beginning [246] then declining to the point of agammaglobulinemia [520], comparable to that in reticular digenesis (Table 22.11) [474]. The marked B-cell depletion can also bear *RAG1* and *RAG2* gene missense mutations that decrease the efficiency of VDJ recombination, which results in impaired but not absent rearrangement of both BcR and TcR. Four missense mutations were detected in the RAG-2 in 6/8 patients [491]. In 13/16 patients (81%) the mutations affected the RAG1 gene, and in 3/16 (19%) the RAG2 gene [515]. Increased IgE is linked to Th2 primary infiltration, with spontaneous production of IL_2 IFN-γ, IL_4, IL_5 and IL_{10}, which is down-regulated by IFN-γ therapy [437]. Clonal expansion of $V\beta14^+$ $CD3^+$, $CD4^-$ $CD8^-$ secreting high IL_5 levels and low IL_4 and IFN-γ levels [318] could indicate an analogy with the Fas (CD95) defect. T lymphocytes show an activated phenotype and a spontaneous apoptosis associated with reduced expression of bcl-2 gene product, and a higher cell death of $CD4^+$ $CD45R0^+$ cells [59]. Given that high CD30 levels in the lymph nodes, skin and serum of three children generated Th2 lymphocytes [95], a Th2-mediated pathogenesis is possible: the CD30 are Th2 markers (Chap. 1). As in human SCID, B and T cells are found in mice with SCID, but with a final repertoire that is decidedly oligoclonal and lacks the heterogeneity characteristic of a normal immune system, so lymphopenic SCID and Omenn syndrome could be two aspects of the same disease with different clinical expressions, especially of time [224]. Clinically, young babies *soon after birth* show a generalized exudative erythroderma and desquamation, often mistaken as AD, alopecia, widespread lymphadenopathy, hepatosplenomegaly, persistent and profuse diarrhea, failure to thrive with malnutrition (Fig. 22.19), AIHA, recurrent infections caused by common and opportunistic germs (Fig. 22.20), and markedly elevated serum IgE levels [136, 241, 474, 520]. This outline included four babies from the same family with the same symptoms until death occurred at 10–19 months, but who did not present hypereosinophilia and were diagnosed as DM [375]. *Differential diagnosis* may be challenging since Omenn syndrome and GvHD show dyskeratosis and basal vacuolation, but the first always shows acanthosis and usually parakeratosis. GvHD shows a flat epidermis and rarely parakeratosis. Both can be distinguished after immunohistochemical staining for CD45 and CD68, which shows predominantly lymphocytes in the dermal infiltrate in Omenn syndrome, and relatively more macrophages in GvHD [438].

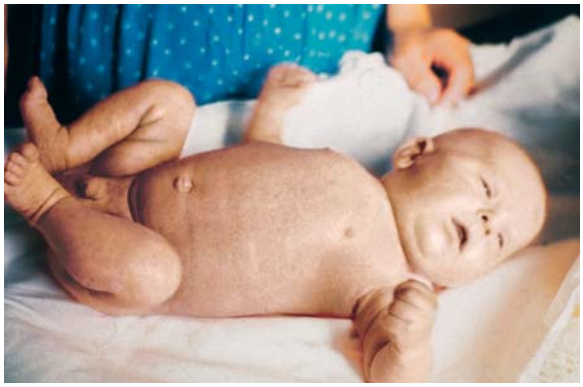


Fig. 22.19. Infant with Omenn's syndrome. The infant shows erythroderma, alopecia and edema

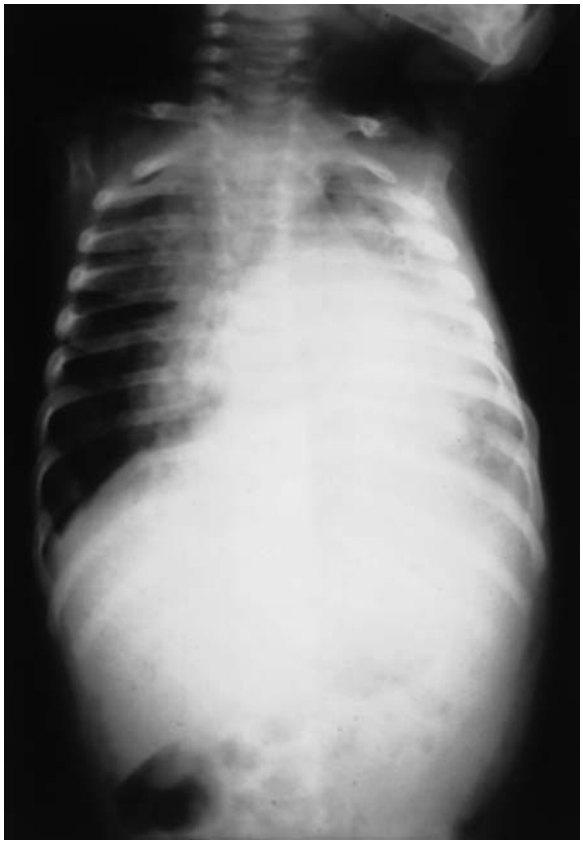


Fig. 22.20. Infant with Omenn's syndrome and *Pneumocystis carinii* pneumonia

IL_2 Deficiency ($IL_2R\alpha$-Chain Gene Mutations)

In a child with SCID and circulating T cells within the norm, a gene transcription deficiency was ascertained [414]. A male infant of first cousin parentage presented at the age of 6 months with CMV pneumonia, persistent oral and esophageal candidiasis, adenovirus gastroenteritis, and failure to thrive. He developed lymphadenopathy, hepatosplenomegaly, iron deficiency anemia with no evidence of hemolytic anemia, and chronic inflammation of his lungs and mandible. Biopsies showed extensive lymphocytic infiltration of his lung, liver, gut, and bone. Serum IgG and IgM were elevated, but IgA was low. He had T-cell lymphocytopenia, with an abnormal CD4:CD8 ratio of 1:1. The T cells responded poorly to anti-CD3, phytohemagglutinin and other mitogens, and to IL_2. He was found to have a truncated mutation of the $IL_2R\alpha$ chain (CD25). He was given a successful allogeneic BMT after cytoreduction [452].

X-Linked Hyper-IgM (or Hyper-IgD) or CD154 Deficiency Syndrome (XHIgMS)

About 225 cases have been reported, [75, 290, 539], 78% [75] or 100% [539] of which have the X-linked form of CD154 deficiency [75]. Patients are generally male, but there can be a non-X-linked form [224], in which 22% of patients are females [75]. An estimated minimal incidence was calculated of 1 in 1,030,000 live births. Over half of 79 patients developed ID symptoms and were diagnosed by 1 year of age, and over 90% by 4 years of age [539]. Although carriers of XHIgMS are considered to be asymptomatic, an extreme lyonization of the normal X can lead to a mild expression of the XHIgMS which is similar to CVID [118]. It can be secondarily caused by environmental factors and also stem from congenital rubella [278]: this indicates its heterogeneity.

Mutations in the *TNFRSF5* encoding CD154 in XHIgM patients result in a lack of B-cell signaling by activated T cells [53]. However, 21 boys out of 56 failed to express CD154, and *TNFRSF5* mutations were found in 20 of these boys, whereas no *TNFRSF5* mutations were found in 16 boys with weak expression of CD154 [184]. As a result, XMIgM B cells fail to undergo isotype switching and produce only IgM due to a defect in the RNA editing enzyme, activation-induced cytidine deaminase (AICDA), an enzyme expressed only in B cells and required for the processes of class-switching and somatic hypermutation of Ig genes [357]. The marked reduction of IgG (<150 mg/dl), IgE and IgA is accompanied by a sharp increase in mature IgM and circulating IgD, but B cells do not express other Ig [227]. Interestingly, 25% of patients with confirmed XHIgMS who had *TNFRSF5* mutations had low concentrations of IgG, IgA, and IgM. Most of the remaining patients with XHIgMS had the classic pattern of normal or raised IgM with low concentrations of IgA and IgG [184]. The *CD154* gene defect is usually expressed on the membrane by activated T lymphocytes, which therefore cannot bind B-cell CD40 [266, 346]. Figure 22.21 shows *75 CD154 localizations and mutation frequencies,* in 39.5% of cases mistakenly. For example, a sense codon substitutes for a missense one, creates a premature stop signal: therefore specific pertinent mutations, such as *G144E*,

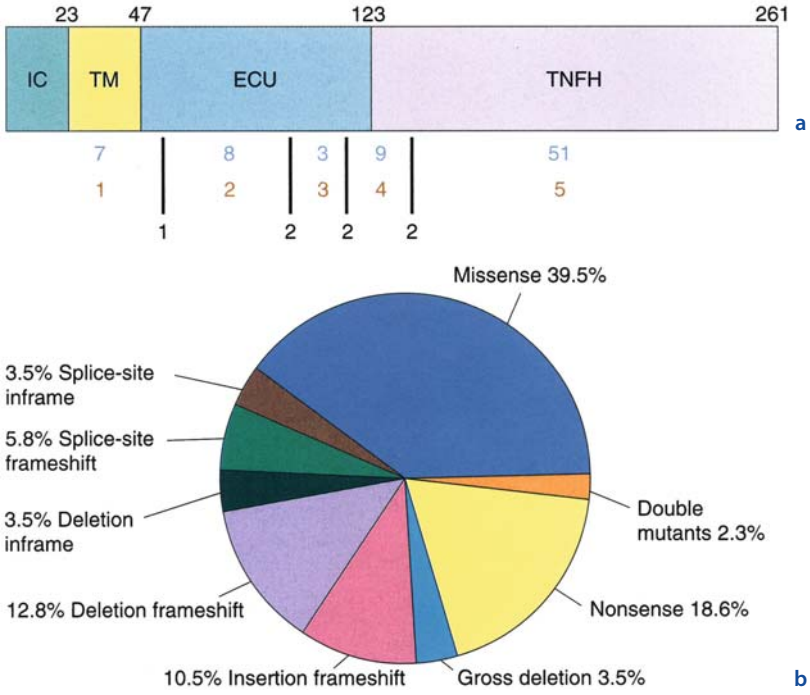


Fig. 22.21 a,b. CD40L (CD154) structure and mutation distribution. **a** *Vertical bars below* the figures indicate the exon boundaries. The *blue numbers* represent the number of families with mutations in the respective red-numbered exons. The sum of families with splice-site mutation is shown in *black below the vertical bars*. **b** Distribution of the type of mutations in CD40L gene identified in families with X-HIgM. *ECU* extracellular unique, *IC* intracytoplasmic, *TM* transmembrane. *TNFH* Tumor necrosis factor homology

can interfere directly with the link site for CD40 (Fig. 22.22). Consequently the signal which indicates that B cells should begin isotype switching, limited to the production of low-affinity IgM, is missing [346]. Without isotype switching, GC formation is minimal [508] (Fig. 22.23) and follicular dendritic cells (FDCs) are reduced in number, also having an abnormal phenotype [147]. As shown by Figs. 1.31–1.33, the lack of cross-linking of CD40 by CD154 results in B-cell failure to up-regulate CD80 and CD86, important costimulatory molecules that interact with immunoregulatory molecules on T cells such as CD28 and CTLA-4.

Two patients with normal levels of CD154 have also been described [359]. As in males with XLA, infections start during the 12th month, those most often observed are otitis, pneumonia or sepsis cased by pyogenic bacteria, opportunistic infections, in particular caused by *Pneumocystis carinii* [290], and also ulcerative stomatitis, RA, neutropenia, AIHA, lymphoproliferating complications and type B gastroenteric lymphomas with IgM [224, 413]. The most prominent clinical infections were pneumonia (81% of patients), upper respiratory infections (URTI) (49%–87%) including sinusitis (43%) and recurrent otitis (43%), LRTI (82.1%) recurrent/protracted diarrhea (34%–55.3%), CNS infections (12.5%–14%), sepsis (13%–14.3%), cellulitis (13%), hepatitis (9%–16.3%), and osteomyelitis (1%) [290, 539]. Lymphoid tissues are normal or hyperplastic [75].

Recently, a rare form of HIgMS associated with hypohydrotic ectodermal dysplasia (EDA) characterized by the absence or hypoplasia of hair, teeth, and sweat glands has been described. Unlike patients with HIgMS, these patients failed to have a history of opportunistic infections. This disorder is related to mutations in the gene that encodes the nuclear factor κB (NF-κB), which is required for activation of the transcription factor NF-κB, or NEMO (NF-κB essential modifier), also known as IKK (inhibitor of B kinase). The phenotype observed in X-HIgMS-EDA patients shows that the putative zinc-finger domain of NEMO has a regulatory function and demonstrates the definite requirement of CD40-mediated NF-κB activation for B cell Ig class-switching [233]. Three other genes, expressed by B cells, have been associated with the HIGM phenotype giving place to HIGM 2–4. Mutations of activation-induced cytidine deaminase (AICDA) (HIGM2) and uracil glycosylase (UNG) (HIGM4), both expressed by follicular B lymphocytes, lead to defective class switch recombination and somatic hypermutation. Mutations of CD40, the CD154 receptor, cause a rare autosomal form with a clinical phenotype similar to CD154 deficiency (HIGM3). These rare PIDs may shed light on the complex events leading to the production of high-affinity, antigen-specific antibodies of different isotypes [146, 355].

Early treatment with IVIg associated with antibiotic prophylaxis have reduced the incidence of life-threatening infections and improved the growth of children with HIgMS [290]. Cycles of G-CSF (granulocytecolony stimulating factor) in the presence of severe neutropenia are advised [290]. Substitute therapies with soluble forms of recombinant or gene type CD154 [76] are being studied. A recent review of CD154-deficient patients showed that 75% develop liver disease and only 20% survive into the third decade of life [290]. BMT has a *successful outcome in young children* (65%); older patients with more ad-

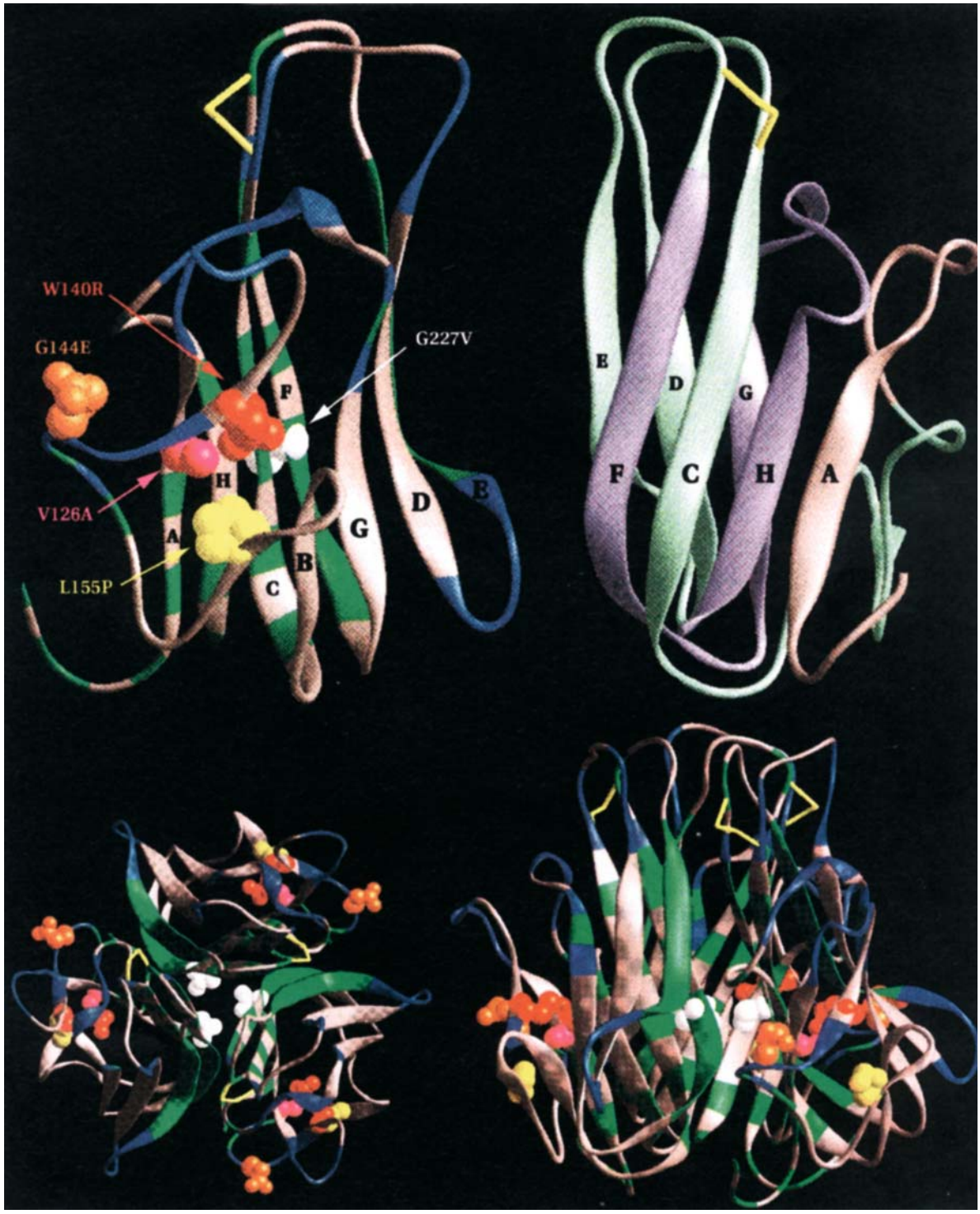
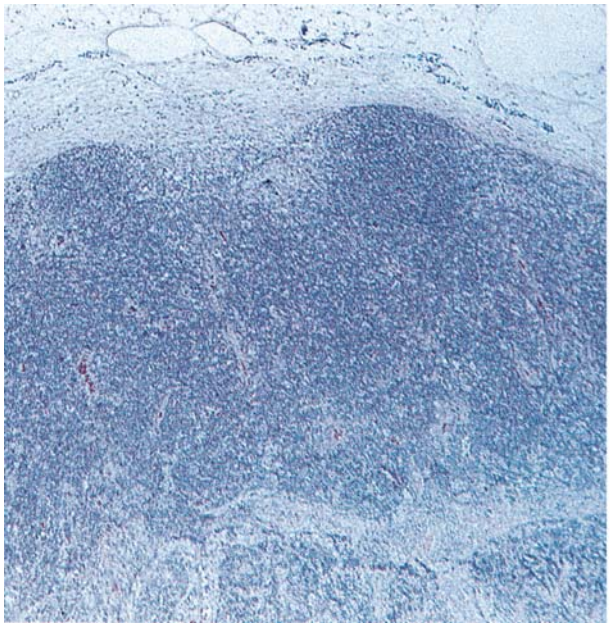


Fig. 22.22 a–d. Ribbon representation of the human CD40L (CD154) models. In **a** and **b** there is a monomer structure and in **c** and **d** a trimer structure. The *ribbon color code* in **a**, **c** and **d** indicates loops potentially interacting with the receptor (*blue*) and residues involved in subunit contacts leading to trimer formation (*green*). In **b** the *ribbon color* indicates the remainder of CD40L structure in two identified truncated mutations in the TNFH domain. The five point-mutations of TNFH domain are shown in **a**, **c** and **d**, regarding mutants affecting either monomer folding, trimer formation, or the CD154 contact sites with CD40. The β-strands forming the CD140 monomer are indicated by *black letters* on the monomer structures in **a** and **b**. The disulfide bond linking the C-strand end with the loop connecting E- and F-strands is depicted by *yellow bars*



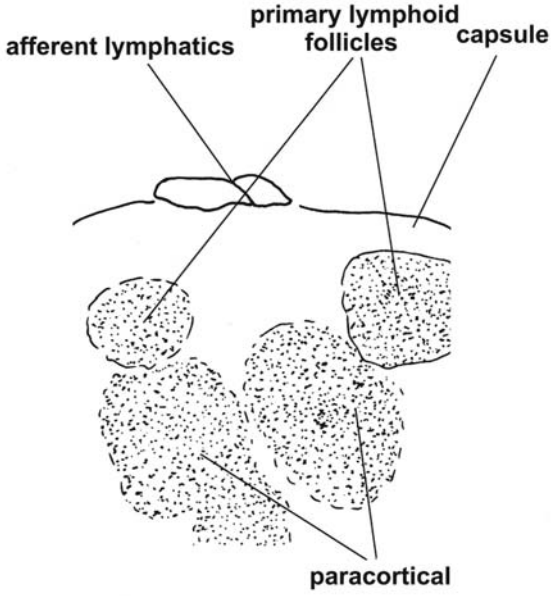


Fig. 22.23. X-HIgM. Histology of a lymph node obtained after vaccine stimulation. Despite the stimulation, the cortical follicles are not transformed into GCs, but maintain the characteristics of primary lymphoid follicles; the paracortical is markedly hypertrophied

vanced liver disease may die because cryptosporidia infection that has progressed rapidly following pretransplantation cytotoxic conditioning therapy [252]. Therefore, a patient with end-stage liver disease related to CD154 deficiency first received a liver graft, and as soon as liver-graft function was satisfactory, BMT was performed with a nonmyeloablative conditioning protocol of fludarabine and melphalan [208].

The screening for CD154 deficiency should include children with severe RRI, and with dysgammaglobulinemia with a normal or increased IgM level [197]. Conventional allogeneic HSCT from an HLA-matched or a matched unrelated donor (MUD) is curative and feasible, if performed before significant infections and organ damage occur [503]. An approach for high-risk patients including nonmyeloablative HSCT was workable in a retrospective analysis of 38 European patients undergoing HSCT for CD154 deficiency in eight European countries between 1993 and 2002. The donor SC source included 14 HLA-identical siblings, 22 MUDs, and two phenotypically matched parental stem cells (SCs) (12 TCD [T-cell depleted]). Of these patients, 12 (32%) died from infection-related complications, with a positive result in 68.4% of patients [180]. Carriers can be detected, and this is useful for making *a prenatal diagnosis* [407].

Purine-Nucleoside Phosphorylase Deficiency

Purine-nucleoside phosphorylase (PNP) deficiency, AR, for which 35 patients have been reported [53], is characterized by the absence of an enzyme necessary for the catabolism of purines, which converts inosine, deoxynosine, guanosine and deoxyguanosine into hypoxanthine and guanine (Fig. 22.15); the responsible gene has been mapped to chromosome *14q* at position 13.1 [414]. This has also been observed in 33 patients with Nezelof syndrome [304]. A variety of mutations have been found in the PNP gene in patients with PNP deficiency [432]. Although ADA and PNP are both purine salvage pathway enzymes, PNP deficiency does not lead to as severe an ID as ADA deficiency. Patients have considerably reduced concentrations of serum and urinary uric acid. Numbers of T cells fall progressively, more than that of B cells (Table 22.3), just like the proliferating responses to mitogens and antigens, especially because PNP deficiency causes an intracellular accumulation of deoxy-GTP (guanosine triphosphate) inhibiting ribonucleotide-reductase and T- and B-lymphocyte proliferation, so combined T and B defects are critical. PNP-deficient patients are as profoundly lymphopenic as those with ADA deficiency, with absolute lymphocyte counts usually <500/mm^3. Ig levels and production of specific antibodies are all normal [19]. *Onset may be early,* as for SCID, but also delayed until the age of 3–5 years. The clinical pattern is dominated by recurrent bacterial, viral and fungal infections, with an abnormal susceptibility to opportunisic germs. Two-thirds

of patients suffer from neurological alterations, ranging from spastic symptoms and alterations, etc., to mental retardation and one-third from AIDs, the most common of which is AIHA. The consequence of severe infections, generalized vaccination, severe chickenpox, lymphosarcoma and GvHD caused by blood transfusions in the first decade of life is death [304, 397] unless BMT is successful [25, 58, 67, 83, 98]. However, poor neurodevelopmental progression may result [25] or may not [98]. Since the biochemical bases of PNP and ADA deficiencies are similar, it is hoped that genetic treatment will also be effective in children with this PID [76].

HLA Deficiency

HLA Class II Deficiency

This deficiency of HLA molecule expression occurs in the more severe forms of PID if they are class II: about 80 cases [140, 428] are known of this AR syndrome [140], heterogeneous for the numerous complementation groups the patients are divided into [76]. *HLA class II molecules are absent in all tissues* [400], to the extent that the cells of patients maintained in cultures for years preserve the negative phenotype [400]. There is a deficiency of class II gene transactivator (CIITA) codified by chromosome *15,* the expression of which plays an important role in T-cell activation: its absence makes class II gene expression impossible [473]. This function is shared with another protein mapped on chromosome *2,* RFX5, with a binding site in the promoter region of genes codifying class II chains [401]. Two additional class II-specific transcription factors are RFXAP and RFXANK [314]. These act on the class II promoter region and are essential and also nonreplaceable, to the extent that alternative routes cannot compensate for their absence [311]. Furthermore inactivation, or the deficiency of these factors, has a specific effect on the genes dictating HLA class II, the li chain and HLA-DM, because there is no indication that other regulating systems may be involved [401]. The absence of HLA class II is associated with a CD4 lymphopenia. HLA class I expression is normal in the patients tested and CD8 lymphocyte numbers are not reduced [428]. Interestingly, in a twin study, despite the deficiency, there were antibody responses and class II-dependent T cells; hence the authors envisage that this represented a HLA class II residual expression below the test sensitivity [540]. The clinical outline is dominated very early on, *before the age of 6 months* (range, 2 weeks to 12 months) [428], by severe and recurrent gastroenteric and pulmonary infections, with a severe and prolonged course, associated with malabsorption and failure to thrive [77]. Bacterial and viral infections, bronchopneumonia, hepatitis, cholangitis, viral meningoencephalitis and various autoimmune manifestations are common complications [140]. Even though an HLA class II deficiency is clinically less severe than SCID, the result is uniformly fatal during the first or second decade of life [162].

The most evident immune defect consists in the complete lack of reactivity to exogenous antigens, which in vivo reflects an anergy to SPTs, as well as the complete lack of HLA class II expression and absence of cellular and antibody responses to antigen stimulation [140], which are instead positive to mitogens (Table 22.8; Fig. 22.24) [508]. Laboratory investigations show a normal B lymphocyte number, but children may be agammaglobulinemic [140]. The thymus and other lymphoid organs are remarkably hypoplastic, with a severe CD4 lymphocyte depletion, while CD8 and B-cell levels are normal. The syndrome involving a deficiency of HLA antigens confirms an important HLA biological role in the complex system of T–B cooperation [77, 140]. Some studies suggest that there are more types of deficiency. When placed together in a culture, the B lymphocytes of these patients, previously transformed by EBV, the lymphocytes correct each other so as to allow HLA class II molecule expression. This has led to the identification of so-called complementary groups [251]. The specification that the gene is mapped on chromosome *19p13.3* can lead to an *earlier prenatal diagnosis* [166]. Long-term survival seems to depend primarily on HLA-identical and HLA-haploidentical BMT performed in the first 2 years of life, before the acquisition of chronic virus carriage and sequelae of infections [257, 140]. A child recently received a transplant [428] with a novel protocol [208]: the CD4 count increased up to 300 cells/μl [428]. A direct correction of the genetic defect is based on the transduction of cells from patients with lentiviral vectors encoding CIITA, RFXANK, RFX5, or RFXAP. The RFXANK vector restored class II expression in a T-cell line from one patient. The RFXAP vector corrected primary cells from a second patient [314].

HLA Class I Deficiency

The study of the common association of HLA class I molecule deficiency, already known as bare lymphocyte syndrome, has led to the identification of various patients with an isolated deficiency and of one patient with a deficiency associated with class II, the most severe [85]. The deficiency is caused by *TAP-1–TAP-2* mutation, accompanied by severe and chronic bacterial RRIs [115]. In two brothers with the AR HLA class I defect, the onset of RRIs took place between the ages of 4 and 7; the poor expression of NK cells was so severe that it led to the development of bronchiectasis [125]. The immunological structure is characterized by few CD8: the deficient expression of HLA class I molecules is diagnostic [115] (Tables 22.1, 22.3).

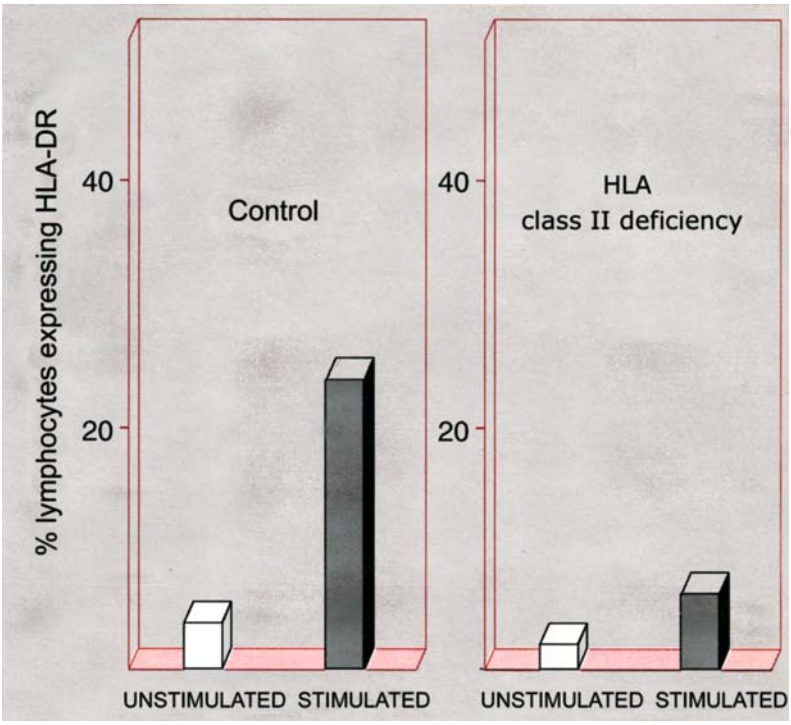


Fig. 22.24. Expression of DR antigen by B lymphocytes in a healthy control and in a 3-year-old boy with HLA class II deficiency, before and after in vitro stimulation with IFN-γ and PHA. Per cent of lymphocytes expressing HLA-DR

CD3γ, CD3δ, CD3ε, CD3ζ Deficiency

The CD3γ chain deficiency due to γ or ε gene mutations [19] determines a lack of CD8 and the absence of CD45RA [249]. In the first two cases described, one brother died at 31 months because of viral pneumonia after a clinical history indicating SCID with severe AIHA, while the other was asymptomatic at the age of 10 years, although with the same molecular defect [18]. The study of these brothers proved that, in spite of the absence of functioning γ chains and 50% of the expressive levels of the CD3/TcR complex, the lymphocytes were normal. According to the authors, other chains may act in the place of missing ones; however, the correlated scarcity of CD8 may have negatively interfered with the mechanisms discriminating between self and non-self, while γ chain deficiency could have modulated the onset of the deceased brother's severe autoimmune disease (AID) [18].

A CD3ε deficiency was found in a 4-year-old child with mild RRI symptoms and otitis media; the expression of the CD3/TcR complex was only 10%, but the stimulation with anti-CD3 induced a normal proliferating response. In fact, despite the ongoing mutation, a Northern blot analysis showed production of a low amount of transcribed RNA, corresponding to a small quantity of ε normal chains, even though their dimensions were smaller than normal ones [471, 496].

The ζ chain deficiency found in the two brothers is similar to the deficient expression of CD3/TcR [5]. In the younger brother, the thymus, markedly reduced, showed no Hassall bodies; the elder brother had similar chain mutations with no symptoms referable to an ID, therefore integrating a genetic heterogeneity. Two other brothers and both parents were healthy [5]. While *the ε chain deficiency produces modest clinical symptoms,* the other two are severe also from an immunological point of view: *in the γ chain deficiency resulting from the profound CD8 and CD45RA decrease* caused by altered thymic activity that leaves the CD45RO unaffected [249], and in those of *the ζ chains due to the severe thymic atrophy* [5] and thymocytes falling to 15% of normal levels, with limits at between 1% and 50% [249].

The CD3δ deficiency due to a heritable mutation of the CD3 gene that prevents the synthesis of the CD3 protein has been reported in 3 cases HZ for the CD3 mutation. Two cousins died at 2–3 months of age because of overwhelming infection. The thymus shadow is clearly visible on chest x-rays. The thymus becomes populated with developing thymocytes, with an arrest of differentiation *at the $CD4^-CD8^-$ stage of T-cell development*. A girl (3rd patient) survives after a BMT [111].

ZAP-70 Deficiency or Selective CD8 Deficiency

This rare deficiency transmitted as an AR trait is caused by mutations of the ZAP-70 gene, a non-src family protein tyrosine kinase (PTC) important in T-cell signaling (Tables 1.31–1.33). ZAP-70, known to be crucial for T cell activation, is a key player in TcR down-modulation and ζ degradation [136]. ZAP-70 has an essential role in

the positive and negative selection of maturing T cells in the thymus [342]. In several babies (most of Mennonite origin) with SCID [20, 92, 139, 179], the nonfunctional CD4 T cells (Table 22.3) were either normal or increased ($CD3^+$ $CD4^+$, 75%). CD8 absence in the thymus and in circulation ($CD3^+$ $CD8^+$, 0%–2%) [139, 179] suggests that the selective process is arrested during the transition from double-positive (DP) to mono-positive (MP) T cells [20, 140, 179]. Arrested thymocytes had terminated *RAG* gene expression and up-regulated TcR and bcl-2 expression, but failed to differentiate into mature CD4 or CD8 MP thymocytes, to be rescued from death by neglect or to sustain $IL_7R\alpha$ expression [294]. ZAP-70 deficiency results in an impairment of transendothelial migration that can be rescued by the transfection of ZAP-70 because cross-talk between the ZAP-70 signaling pathway and the chemokine receptor CXCR4 is required for T-cell migration [502]. Although the thymic architecture is normal with presence of Hassall bodies [179] and CD8 seem normal in the cortex, very few migrate to the medulla [20]. The near absence of $CD8^+$ cells and an increased CD4:CD8 ratio dominate [27]. The few CD8 coexpress $CD56^+$, the NK-cell marker; B cells appear normal and functional, $CD3^-$ $CD19^+$ is at a level of 20%–40% [139, 179], and serum Ig values are normal [27]. The same phenotype was found in the brothers [92]; other relatives were HET [20, 139]. *The absence of CD8 expression* was shown to correlate with a missense mutation in both Ig alleles of the CD8 α gene domain in a 25-year-old man and his sister, whereas high percentages of $CD4^-$ $CD8^-$ $TcR\alpha\beta^+$ T cells were found in the three siblings [114]. The proliferative responses in vitro to phorbol myristate acetate (PMA) and ionomycin, PKC activators (protein kinase C), were normal, unlike PHA (phytohemagglutinin), PWM, tetanic toxoid, anti-CD3, etc. [20, 139]. The positives operate below the TcR, while the negatives react directly with the CD3/TcR complex [92, 140, 179], confirming the ZAP-70 deficiency [20]. The CD4 are present despite the deficiency because Syk, the other member of the family, ensures a compensatory role in the infrathymic CD4 selection, although with a limited efficacy [179]. Seven months after BMT, a child was clinically well and immunologically recovered [27].

TAP-2 Deficiency

Studies in two siblings HZ for a stop mutation in the TAP-2 gene suggest that NK cells express still unknown inhibitory receptor(s) (the missing receptor, discussed in Chap. 1) capable of down-regulating the NK cell cytotoxicity on binding to surface ligand(s) expressed by T cell blasts. Functional analyses were consistent with the concept that this putative inhibitory receptor is expressed by virtually all TAP-2/NK cells, whereas it is present only in rare NK cells from healthy persons. Another prospect would be that TAP-2/NK cells are actually missing this still unidentified triggering receptor involved in NK cell-mediated killing of PHA blasts. Since cells derived from patients displaying defective expression of either of the TAP subunits are characterized by a strong reduction of mature HLA class I molecules at the cell surface, a *TAP deficiency is connected with HLA class I deficiency* [517].

NFAT Deficiency

As discussed in Chap. 1, NFAT (nuclear factor of the activated T cells) is a transcription factor that forms a powerful transcriptional activating complex and, by linking with specific DNA-regulating sites, plays a critical role in the synthesis of various T-cell ILs which, due to the deficiency or excessive migratory mobility of NFAT, although normal in number and in distribution, are incapable of activating and/or secreting the genes of IL_2, IL_4 and IFN-γ [86]. A 4-year-old girl with SCID presented during infancy with severe recurrent infections and failure to thrive; her mRNA was not produced for IL_{2-5} and IFN-γ due to poor T-cell proliferation, although these were normal in number and in distribution, to initiate the transcription of the relative genes, regulated by NFAT, with a binding site in the proximity in the 5′ region. This severe clinical picture is accompanied by evident hgG [19, 86].

NK-Cell Deficiency

NK-cell deficiency is found in SCID, CVID, reticular dysgenesis, Chédiak-Higashi syndrome, XLP, LAD in TAP-2 deficiency and in CFS (chronic fatigue syndrome), in particular CID such as SCID, suggesting an association between NK- and T-cell deficiencies [478]. There is one known case of an adolescent with an isolated numerical and functional deficiency of NK cells and of precursors, recurrent neutropenia, severe and recurrent EBV, CMV, *Herpes simplex* virus (HSV) infections and life-threatening chickenpox. Another child, diagnosed at the age of 2.5 years with a CD8 deficiency, suffers from severe viral and bacterial infections although he has antibodies to various viruses [49]. The growing list of human genetic defects that impair NK-cell function has been recently joined by NEMO-ID [356] which occurs in a group of patients with antibody deficiency combined with exquisite susceptibility to infection with nontuberculous mycobacteria. Infectious susceptibilities common to these disorders stress the important role for NK cells in host defense [59]. The *natural history of three boys with NEMO mutations* outside of the 10th exon has been described. Including these boys, there have been 22 families described as having NEMO-ID. The resulting estimated incidence of NEMO-ID is 1:250,000 live male births, making this disorder significantly less common [356].

Undifferentiated SCID

Human p56lck deficiency

p56lck deficiency is an AR SCID due to a defect of an src kinase critical for the generation of mature thymocytes in adult mice. p56lck is important in TcR signaling and phosphorylation of the ITAMs of the CD3/TcR complex proteins. Mutant mice lacking p56lck have pronounced thymic atrophy, a critical reduction in DP ($CD4^+CD8^+$) thymocytes, no detectable MP thymocytes, and only a few peripheral T cells. Both proliferation and development of a given defined cell subpopulation depend on meuse age. The absolute numbers and proliferation of DN and ISP (immature single positive) thymocytes only proliferate during fetal and early postnatal life up to 14 days after birth, whereas the proliferation is significantly decreased beyond that age, thus lck may have differential roles in the proliferation and maintenance of DN, ISP, and MP/DP thymocyte populations [151]. The first demonstration of a human SCID patient with an abnormal expression of p56lck is an SCID infant hospitalized at 2 months for dehydration, failure to thrive, and sepsis. The immune phenotype included hgG, selective CD4 lymphopenia, lack of CD28 expression on $CD8^+$ T cells and poor T cell blastogenic responses to various mitogens and IL_2. p56lck protein expression was only minimal with an unusual mRNA splicing pattern of the lck gene. The levels of p59fyn were normal and it is therefore possible that p59fyn played a role, albeit incomplete, in the development of his mature T cells. The child has since undergone an allogeneic BMT (at 32 months) from a matched unrelated donor (MUD) [185]. Unfortunately the boy died 2 months later due to CMV infection and GvHD (FD Goldman, pers. comm., 8 Nov. 2005).

Human whn deficiency

whn (winged-helix-nude) encodes for a transcription factor that is crucial for maturation of the thymus microenvironment [185]. nu/nu mice fail to develop a thymus and mature T cells due to a defect in the whn gene encoding a transcription factor necessary for terminal epithelial cell differentiation. A defective whn gene could lead to the disrupted early T cell development in the BM. T cell progenitors were associated with a lack of pTα gene expression and a failure to give rise to mature T cells in adoptive euthymic hosts. Wild-type HSCs rapidly matured into functional T cell progenitors in the marrow of euthymic or thymectomized but not nu/nu hosts. Therefore defects in BM prethymic T cell development can contribute to T cell deficiency in nu/nu mice [90]. In two sisters a severe SCID caused by mutation of the whn gene was associated with complete alopecia. HLA-identical BMT in one of the two girls resulted in a clear reconstitution of $CD4^+$ and $CD8^+$ CD45RA cells and a marked clinical improvement. These data indicate that the thymus is differentially required in the maintenance of the TcR repertoire complexity [380].

Predominantly T-Cell Defects

Primary CD4 T-Cell Deficiency

Known also as idiopathic lymphocytopenia, primary CD4 T-cell deficiency is revealed by a profound and persistent reduction in circulating CD4 and with a CMI deficiency. It is documented in patients suffering from infections caused by opportunistic germs such as cryptococcus-induced meningitis and oral candidosis, also including ten children and a number of adolescents, for whom the following minimum levels of CD4 per age have been established: <1,000 cells/mm^3 from 0 to 23 months and <300/mm^3 from 2 to 12 years, or a total lymphocyte count of <20% on two separate occasions without being HIV-infected [463]. A family has been reported involving two brothers aged 13 and 18 with T counts between 150 and 200/mm^3, recurrent respiratory, intestinal and cutaneous infections, and failure to thrive. The mother showed a low CD4:CD8 ratio [122], while the entire family showed normal levels of Ig and subclasses and HLA molecules [128]. Other symptoms included mental retardation, pansinusitis, bronchiectasis [168], but no infections caused by opportunistic germs such as those reported by the WHO Scientific Group [414].

Primary CD7 Deficiency

One case of primary CD7 deficiency is known of a child with SCID without genetic transmission of the deficiency. T-cell proliferative responses to mitogens were defective and IL_2R expression was deficient on his T lymphocytes, and B cells did not differentiate into antibody-secreting cells when provided with the help of normal T cells [245].

Primary CD45 Deficiency

The index patient for primary CD45 deficiency was the first child of consanguineous Kurdish parents. She presented aged 2 months with a rash, pyrexia, hepatosplenomegaly, lymphadenopathy, pneumonitis, pancytopenia, and disseminated CMV infection. Laboratory analysis showed absolute lymphopenia, low T cell numbers, with markedly low $CD4^+$ and low $CD8^+$ and normal B cell numbers. She responded well to anti-CMV treatment and at 8 months underwent a MUD BMT. T-cell engraftment was demonstrated 3 weeks after BMT. Despite continuous anti-CMV treatment, her

CMV reactivated, and she died 55 days after BMT [74]. A 6-bp deletion in the gene encoding CD45 resulted in the loss of glutamic acid 339 and tyrosine 340 in the first fibronectin type III module of the extracellular domain of CD45, identifying a region important for CD45 structural integrity and lack of surface CD45 expression. This was almost certainly responsible for the ID in this girl [494]. A second child presented at 2 months of age with severe CID, showing similar T-cell defects. Despite normal B-lymphocyte numbers, serum Ig levels decreased with age [272]. Introduction of a *functional CD45 minigene* was sufficient to overcome the main SCID-associated defects and represents a potential route to a gene therapy for human CD45-deficient SCID [516].

Multiple IL Defects

Two male infants born to consanguineous parents had SCID despite phenotypically normal blood lymphocytes. Their T cells were unable to produce IL_2, IFN-γ, IL_4 and TNF-α [154]. Another child with SCID had defective transcription of IL genes encoding IL_2–IL_5 [86]. DNA binding of activation protein 1 (AP-1), Oct, CREB, SP1, and NF-κ B was normal, but the binding of NFAT to its IL_2 promoter response element [154], or the ability of nuclear factors from the child's T lymphocytes to bind response elements present in the IL_2 regulatory region [86] was barely detectable [86, 154] both before and after T-cell stimulation [154]. These results indicate that the NFAT abnormality may underlie the multiple IL deficiency in these boys.

Nezelof Syndrome

Nezelof syndrome, also known as cellular ID with Ig, or combined with a predominant T-cell defect, or as a SCID variant, clinically *less severe compared to the previous ones,* is characterized by a form of AD, concentrations of IgE that may also be extremely elevated (Table 22.2), and normal or increased serum levels of other Ig classes [62]. The CMI study emphasized the mature T-cell reduction or absence, various expressions of immature cells, with cutaneous anergy to SPTs and a reduced or absent in vitro lymphocyte response to mitogens. From infancy, patients present recurrent or chronic pulmonary infections, pondostatural retardation, oral and/or cutaneous candidosis, chronic diarrhea, recurrent cutaneous and urinary tract infections, Gram-negative bacterial sepsis and a particularly severe form of chickenpox [394]. Differential diagnosis must include pediatric AIDS, also marked by proportionably increased Ig and a lack of antibody and T-cell function [79].

Fas (CD95) Deficiency

Inherited through AR modalities, CD95 deficiency has been observed in 8 children, two of whom were brothers, with mutations of the Fas gene, one HZ and 7 HET [166, 281], as well as in 9 unrelated children [468]. These mutations most often arise as a result of mutations in the gene encoding the lymphocyte apoptosis receptor Fas/APO-l/CD95. A novel mutation has been identified in the intracellular apoptosis signaling domain of Fas in 11 members of a family, with several members monitored for up to 25 years [228]. Thus, the deficiency is inherited in an autosomal dominant fashion but with a high degree of variability in clinical expression [228], but also in an AR fashion [510]. The clinical picture is dominated by imposing hepatosplenomegaly with an early onset, even neonatal, accompanied by T-cell hyperproliferation, chronic and persistent lymphadenopathy, and failure to thrive [468]. An extensive lymphocyte infiltration of lymph nodes, spleen and liver is observed, with T cells reaching 35,000/μl [$CD3^+$, $CD4^-$ $CD8^-$ (DN) equal to 35–60 cells/μl compared to 0–3 in controls], as in Omenn syndrome, also in the bloodstream, with possible oligoclonality of T cellularity. DN T cells expressed the α/β TcR [468]. Immune dysregulation is associated with G and A HgG (hypergammaglobulinemia, auto-antibodies and AIDs, especially of the hematological type, such as AIHA, and with a severe and recurrent thrombocytopenia [166, 281]. Autoimmune features are discussed in Chap. 18. An overlapping mechanism could belong to the etiopathogenesis of XLP and Omenn syndrome.

Other Well-Defined ID Syndromes

Wiskott-Aldrich Syndrome

WAS has a prevalence of approximately 4×10^6 live births [402]. It is transmitted as a recessive hereditary trait linked to the chromosome X, localized in a pericentrometric position on the short limb of chromosome X (*Xp11.22–p11.3*) [274]. It is therefore possible to identify the female carriers and to provide *prenatal diagnosis* [61].

The gene that codifies the WAS defective protein (WASp) has been isolated [458] and has 167 mutations distributed among all 12 exons of the entire gene, 110 of which are unique and 38 familiar, with two large deletions, one embracing exons 1–7 and one intron 8 [275, 444] (Fig. 22.25). Six novel mutations have been identified that involve nonsense mutations, or small deletions, all of which result in predicted truncation of WASp synthesis [57]. A new, recurrent mutation is V75M, due to a CpG island was found in a HZ girl, who showed microthrombocytopenia and infections to the same degree as her hemizygous father and brother. The amount of WAS protein was about 10% in platelets and 15% in mononucleated white cells [388].

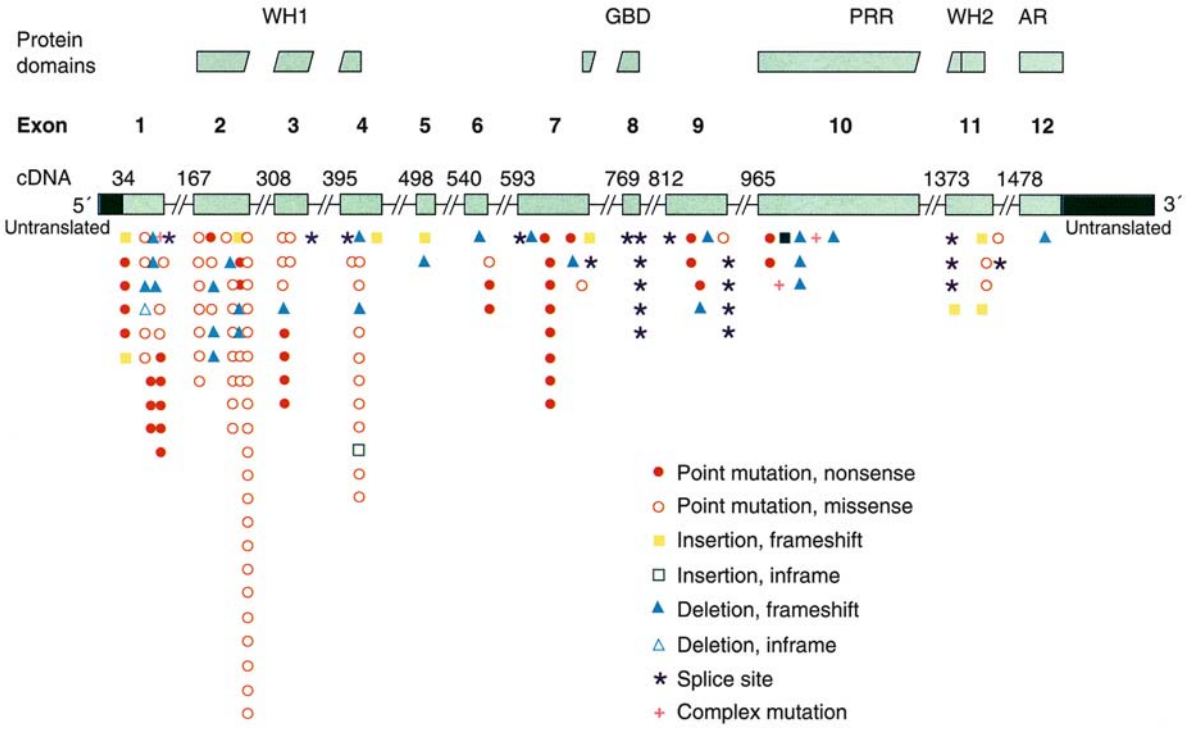


Fig. 22.25. Distribution of mutations across WAS exons and domains

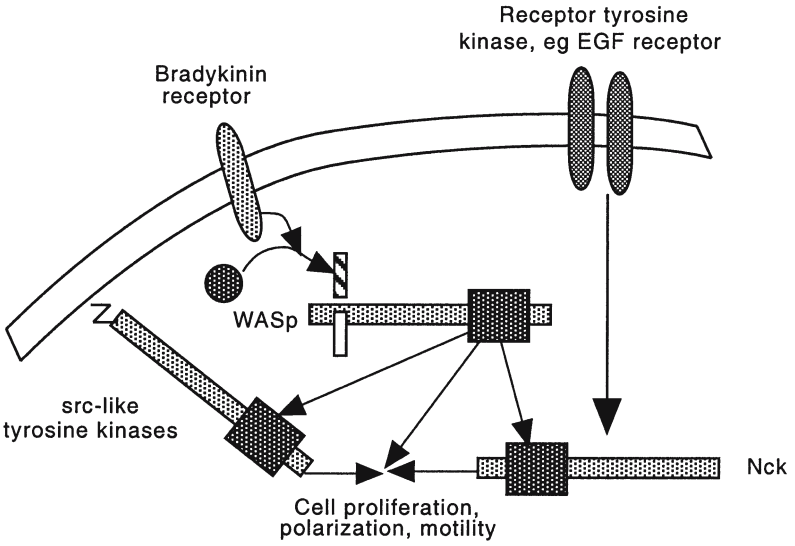


Fig. 22.26. WASp involved in cell signaling. Receptor signals (such as bradykinin receptor) cause exchange of GDP bound to Cdc42 (*dark shaded areas*) to GTP. Cdc42-GTP (*light shaded areas*) binds to WASp in a specific site (*white areas*), thus inducing cell-shape changes. WASp also binds its proline-rich region to SH3 domains in other signaling proteins such as Nck and Src-like tyrosine kinases. (Modified from [152])

Molecular biology has proven that WASp found only in blood cells binds the small GTPase CDC42H2 in the GTP but not in the GDP [23, 265, 490]. CDC42H2 plays a critical role in the assembly of actin filaments [490] and in T-cell polarization when they encounter a B-lymphocyte APC [481]. WASp activity is regulated by several proteins acting in concert to control WASp configuration. The WASp-interacting protein, when phosphorylated, releases WASp from its grip, allowing WASp to be activated by Rho-family GTPases [433]. Experimental data also indicate that Cdc42, WASp and actin might be involved in ensuring the T lymphocyte functional polyvalence, also explaining why microvilli and platelet defects are absent [152]. WASp and several related proteins (the WASp family) are all involved in the organization of the actin cytoskeleton. To carry out vital functions, cells have to rearrange their actin cytoskeletons [467].

The characteristics peculiar to WASp as a meeting point for the marking pathways is illustrated in Fig. 22.26 [152]. The WASp function is absent in 135 cases of WAS, in ten with attenuated WAS and in 23 with XLT [372]. In normal subjects, it is found in the cyto-

Table 22.12. Immune characteristics of WAS

1. Very elevated IgA and IgE concentrations
2. Decreased IgM concentrations
3. Normal total IgG concentrations
4. Quantitatively normal B lymphocyte, in progressive expansion
5. Progressively decreased T lymphocyte number and function (generally with preserved reciprocal rate) so that lymphopenia is almost never marked before age 6
6. No response to polysaccharide antigens, so patient serum is deprived of isohemagglutinins
7. Poor lymphocyte response to mixed lymphocyte culture and to mitogenic effects of antibodies to CD3

Data from [287, 413].

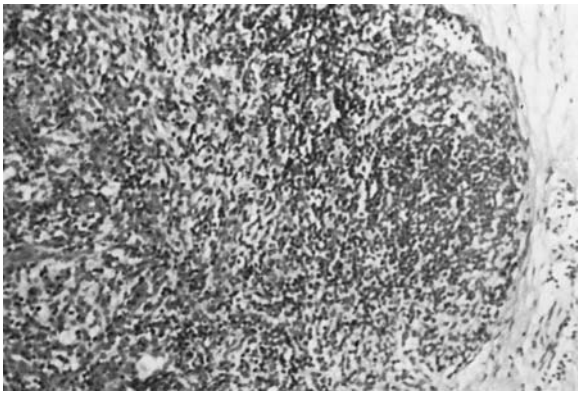


Fig. 22.27. A lymph node biopsy specimen from a 4-year-old boy with WAS. There is a moderate degree of depletion of lymphoid cells in both thymus-dependent and thymus-independent areas, with lack of follicular formation

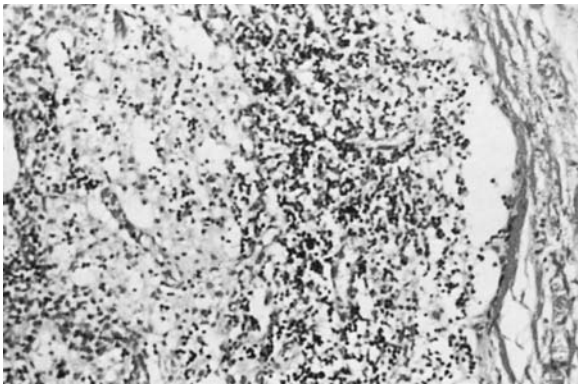


Fig. 22.28. A lymph node biopsy specimen from an 8-year-old boy with WAS, the older brother of the patient shown in Fig. 22.27. There is a greater degree of depletion of lymphoid cells in both thymus-dependent and thymus-independent areas, and no follicular formation

plasm but not in the nucleus of various cells such as platelets, T and B lymphocytes and monocytes [477]. The XLT gene is located on the same *locus* as the WAS and could therefore be a variant [444]; the main immunological anomalies are summarized in Table 22.12 [287, 413]. Children with WAS have *significantly elevated levels of IL_4 and IgE* (Table 22.2) and *decreased levels of IFN-γ* [217]. The pathogenetic mechanism unifying the symptom triad is not clear; the glycosylation defect has been proved, primarily concerning sialidation, therefore resulting in an instability on the membranes of platelets, neutrophils and lymphocytes expressing a glycoprotein sialopherin (CD43) [402], localized on chromosome 16, which makes it an improbable candidate, even though CD54 is indeed the binding agent of CD43 and could therefore play a role in T-cell maturation, differentiation and activation, thereby acquiring marking capacities that are independent of TcR/CD3 [19]. However, the TcR-mediated signaling defect is characteristic of WAS [259], in addition to the reduced expression of CD23 [458], which can explain immune and hematological defects.

In the lymph nodes, there is a shortage of lymphatic follicles and the thymus-dependent and -independent areas are depleted, moderately at the age of 4 years (Fig. 22.27) and to a greater extent at 8 years (Fig. 22.28). The predominant immunological outline is constituted by elevated IgA and IgE levels, low IgM and all IgG levels, as well as the absence of a response to polysaccharide antigens, which is why the children's serum lacks isohemagglutinin [278, 528]. In unweaned babies, the most striking finding is the CD4:CD8 ratio =5 [549], compared to 2.65 in normal children aged 0.63–3.06 (Tables 1.36–1.39).

WAS usually *starts at 13.7 months* (range, 1–58) [132] with hemorrhagic manifestations, petechiae and prolonged bleeding from the umbilical scar or the circumcision site, observed in newborn babies [402]. The clinical triad is characterized by cutaneous lesions that are practically *indistinguishable from rather severe AD* (70%), congenital thrombocytopenia (100%), a marked susceptibility to RRIs (91%) [132] (Figs. 22.29, 22.30) and gastroenteric symptoms such as hematemesis, melena and chronic diarrhea [132]. Other complications may include neutropenia (25%), arthritis (29%), skin vasculitis (22%), cerebral vasculitis (7%), inflammatory bowel disease (9%), and renal disease (3%) [132]. A reduced thrombopoiesis (level <50,000/ml), with microthrombocytes and an accelerated turnover in boys must allow for a suspected diagnosis [413]. It has recently been proven that the classic presentation is more common in children aged 6.8 months than in those aged 7.2 months (60% compared to 25%), unlike platelet counts [549]. However, only 27% of 154 unselected children with persistent thrombocytopenia, positive FH, small platelets and defects associated with T and/or B lines had the classic triad and 20% only thrombocytopenia before diagnosis [484].

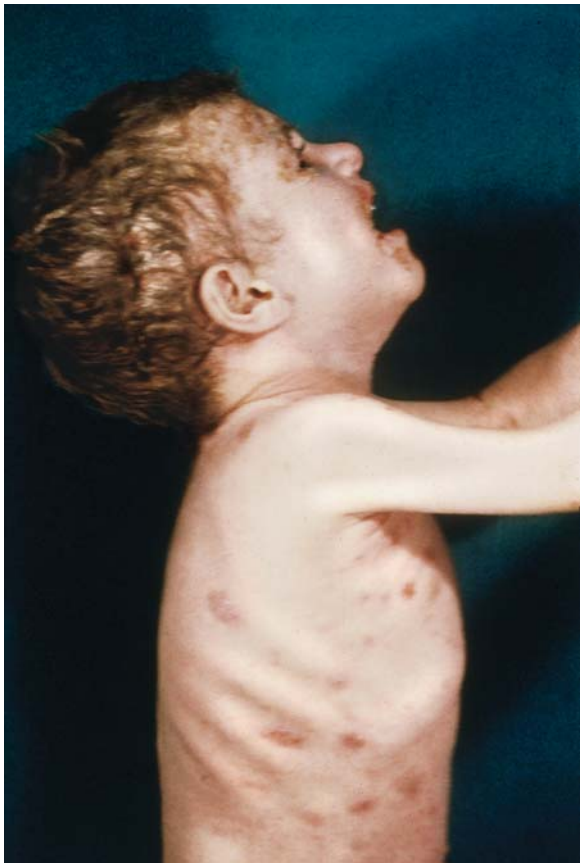


Fig. 22.29. Child with WAS (for details see text)

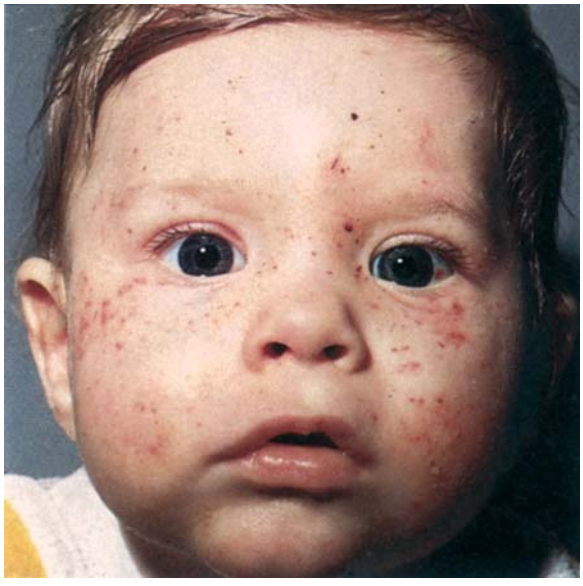


Fig. 22.30. Child with WAS, particularly of the face with eczematous dermatitis and some petechiae

Infections, appearing during the first months of life, are often marked by otitis media, pneumonia, meningitis and sepsis, caused by viruses (CMV and *Herpesvirus*) and by bacteria (*pneumococci* or other capsular polysaccharide). These are followed by more common infections caused by opportunistic germs, *Pneumocystis carinii* and mycetes such as *Candida albicans*. Differential diagnosis should also include a rare AR syndrome similar to WAS, also reported in female patients, characterized by AD, RRIs and thrombocytopenia with microthrombocytes [62]. When caring for these children one must monitor the platelet count, the immunological structure (Ig, lymphocyte and subpopulation counts) and the potential onset of autoimmunity and tumors [224]. AIHA may be found in 36% of children [132]. Prophylactic treatment for infections is done with IVIg; 500 mg/kg every 3 weeks) and sulfamethoxazole (25 mg/kg/2 days) after diagnosis [132]. Splenectomy may decrease the bleeding tendency [224], but early relapse of thrombocytopenia after splenectomy is predictive of a poor prognosis [132]. On average death occurs around the age of 11 (8 in untreated children), but can occur between 0.5 and 4.5 [549], with survival also >18. Death is caused by massive hemorrhages (23%), tumors (26%) and severe infections (44%) [484].

The second largest group of patients with ID given BMTs since 1968 are those with WAS, with *78.8% of children aged <5 years* [158]. Fourteen out of 18 patients underwent phenoidentical (n=1) or haploidentical (n=13) HSCTs; the other four died before HSCT could be undertaken [132]. Boys who had received a MUD HSCT transplant <5 years *had survival rates similar to those receiving HLA-identical sibling transplants,* but the success rate decreases dramatically at the age of 5–6 [158]. WAS-associated T-cell signaling defects can be improved upon retrovirally transduced HSCTs [258].

Recently, correcting the T-cell defects has been proposed. The potential for correction of the T-cell defects has recently been demonstrated by transduction with *an oncoretroviral vector encoding the WASp,* which resulted in correction of the deficient proliferative response to TcR stimulation characteristic of WAS [483].

Ataxia-Telangiectasia

ATA is a complex AR inherited syndrome, associated with neurological, immunological, endocrinological, hepatic and cutaneous abnormalities, characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, and increased susceptibility to RRIs [379] with an incidence estimated at 1:100,000–1,300,000 live births [61]. In Italy the frequency on the general population, is of 1.3×10^6, with an increase in HETs from 1.7% to 3.43% [94]. It is characterized by a genetic heterogeneity, which is reflected in the division into four main groups of complementation, to which one must add the Nijmegen and AT-Fresno variants, perhaps caused by

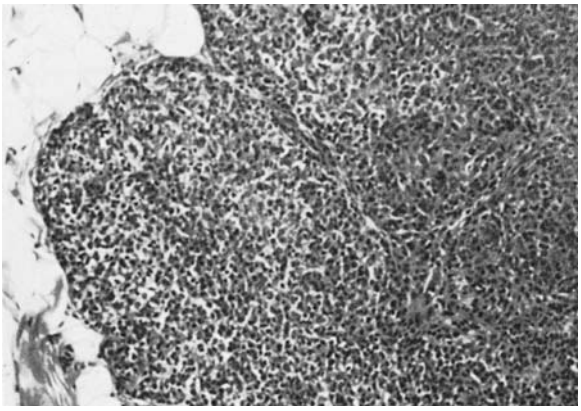


Fig. 22.31. Biopsy specimen of a thymus from a patient with ATA. Although some degree of cellularity is seen, there is no corticomedullary differentiation, and no Hassall corpuscles

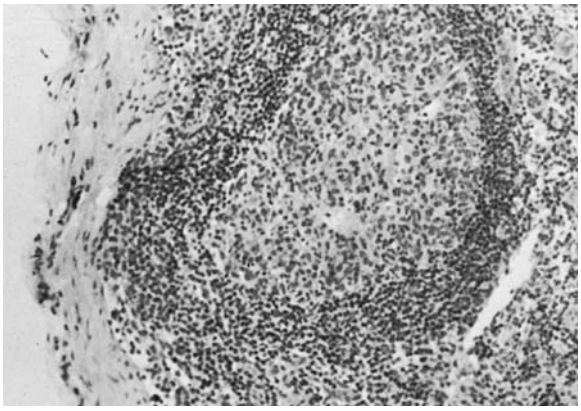


Fig. 22.32. A lymph node biopsy specimen from a 2-year-old child with ATA (for details see text)

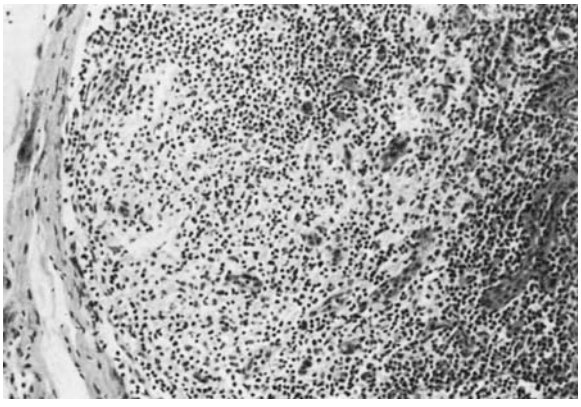


Fig. 22.33. A lymph node biopsy specimen from an 8-year-old child with ATA. In comparison with Fig. 22.32, the degree of depletion of lymphocytes is extensive in both thymus-dependent and thymus-independent areas

the same gene, also localized on the long arm of chromosome *11q22.23* [176].

The 12-kb gene, called ATM (AT mutated) because of its mutations by defective splicing in all patients with ATA, permits HET identification [435]. A DNA clone complementary to ATM shows considerable affinity to factors responsible for signals involved in regulating the cell cycle and codifying a protein similar to phosphatidylinositol-3-kinase (PI 3K) [435], involved in mitotic signal transduction, meiotic recombination, and cell cycle control. A result could be a recombination defect which interferes with B and T lymphocyte gene rearrangement, involving TcR and isotype switching, consequent to a damaged DNA triplication and therefore accounting for Ig deficiencies [176]. Cells from these patients progress too rapidly from the G_1 phase, in which they receive ionizing radiations, to the S phase, then continuing irradiation, to the G_2/M phase with further delay, evolving in apoptosis [279]. This hypothesis has received further credit after observing that the *p53* gene expression does not increase in human cells exposed to radiations [250]. The *p53* gene is part of the normal cell cycle and during the S phase provides time for the DNA physiological repair after exposure to radiation that may also be cosmic [279].

The thymic tissue is either absent or degenerated with a fetal appearance (Fig. 22.31): some follicles, also with B cells, are visible at the age of 2 (Fig. 22.32), at 8 there is complete cellular depletion (Fig. 22.33). Immune deficiencies are humoral and cellular (cutaneous anergy and depressed proliferative responses) [70]. The karyogram shows that the lymphocytes have common rupture points at the chromosomal level with *inversions and translocations involving precisely the TcR and Ig genes* [320]. Most chromosomal translocations involve the genes encoding TcR on chromosome 7 and the Ig H chains on chromosome 14: most breakpoints occur at the *loci* that encode Ig and TcR for antigen (regions *7q35*, *7p12*, *14q32*, *14q12*) [264], in areas typical for codification of molecules of immunological importance (Chap. 1). An important role is played by genes belonging to Ig gene superfamily (IgSF) (Table 1.4). Possibly the progressive ID of ATA, like its apparently unlinked manifestations, is at least in part linked to the accumulation of clonal anomalies affecting the TcR and the IgSF: this suggests the intervention of "illegitimate" recombinations damaging above all the T cells [162]. T-cell immunological deficiency is completed with lymphopenia, a decreased CD4:CD8 ratio due to the drop in cytotoxic CD8, and a rise of immature forms with TcRγδ [80].

Another consequence is the isotype deficiency: about 70% of patients present SIgAD; >50% are also affected by an IgG_2–IgG_4 deficiency with IgM becoming monoclonal, and 30% by serum IgG deficiency [364, 379, 528]. Anatomopathological studies explain the reason for the widespread and progressive cerebellar cortex degeneration, showing in the intermediate and deep layers rare

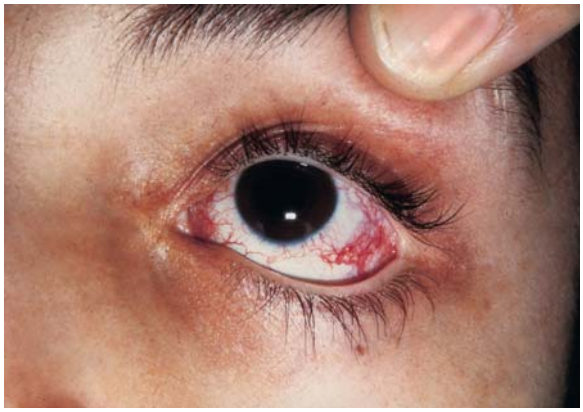


Fig. 22.34. Conjunctival telangiectasia in a girl with ATA

Purkinje cells (PCs) and degenerated granular cells. That the number of basket cells, so called because they form with the axons bunches of fibrils distributed so as to form a nest in which the nucleus of each PC settles, is almost normal, proving that PCs are probably normal at birth and degenerate only later [176].

Typical clinical manifestations are ataxia, telangiectasia of both auricular lobes and sclera, RRIs and an elevated incidence of neoplasia [489]. From a review of 331 patients [70], the percentages of symptoms are as follows: progressive ataxia (100%), typically cerebellar, becomes evident *when children start walking* or a little later, affecting intentional movement and becoming complicated by dysarthria (100%) and involuntary choreic movements (92%), causing the majority to be unable to walk by about the age of 10–12 [276]. At a later stage it is possible to observe nystagmus (67%), strabismus, oculomotor apraxia (88%), reduction or absence of reflexes (77%), and dyslalia, increasingly amplified and, in some patients, also mental retardation [176]. Telangiectasias develop between the ages of 1 and 6 on the bulbar conjunctiva (97%) (Fig. 22.34), on the flexor surfaces of the limbs and areas exposed to sun rays (17%). Height and weight are <10th percentile (64%) and the appearance is progeric (63%). Severe RRIs are common (70%), encouraged by antibody deficiencies. The pathogens involved can be bacterial or viral, often resulting in lung bronchiectasis, all starting after the onset of neurological manifestations [276, 489]. Associated neoplasia (6%) is usually lymphoreticular, less common than adenocarcinoma, with an eightfold increased trend for all kinds of tumors [224]. In cultures the fibroblasts of these patients are three times as sensitive, compared to controls, to ionizing radiations and to radiomimetic chemical substances, but not to UV rays, unlike what is observed in the cells of subjects affected by xeroderma pigmentosum [70]. In addition, the persistence of elevated serum α-1-fetoprotein (AFP) levels was observed in all patients. An interesting in vitro study has reported that by introducing a normal human chromosome 11 into cells, the chromosomal aberrations induced by X-rays were suppressed [261].

The rare AR *Nijmegen breakage syndrome*, so called because it was initially seen in two brothers of second-cousin parents living in that city, and at the moment observed in approximately 80 patients, has various characteristics of ATA but without ataxia, telangiectasia, or high concentrations of AFP. Clinical characteristics are singular: short stature and microcephaly with prenatal onset, bird-like profile, prominent midface, a long nose, low-set ears, cutaneous depigmentation with café-au-lait spots, an almost normal intelligence, and also RRIs and bronchiectasis. Humoral and cellular ID includes reduction of antibodies and lymphoproliferative responses [70, 224]. During an 8-year period of observation, the ID was found to be profound, highly variable, and with a tendency to progress over time in 40/50 children [193]. There is a high proclivity to expressing rearrangements of chromosomes 7 and 14 as in ATA [70, 224].

DiGeorge Syndrome

DGS is usually sporadic, with known cases of positive FH [224]. It is caused by a defective development of the 3rd and 4th branchial pouches which takes place before the 12th week of gestation, with consequent thymic hypoplasia or aplasia and parathyroid hypoplasia; the 5th and 6th pouches and branchial arches can also be affected [497]. The cause can be found in the neural crest cell incapacity to migrate and interact appropriately with endothermic cells of the brachial pouches and arches [295].

Deletions (often microdeletions) at the pericentrometric region of chromosome *22q11-pter* have been described in 80%–90% of cases [130]. A microdeletion *22q11.2* was recorded in 112 children aged 4–70 months, 54% of whom had developmental delays, mild hypotonia, as well as language and speech delays [181]. Another 80 children had deficits in the areas of attention, story and visuospatial memory, arithmetic performance relative to other areas of achievement, psychosocial functioning [541], and mental retardation in 73% of 44 children [11], thus indicating the need for early intervention beginning in infancy [181].

Overlapping alterations are present in the syndrome complex known as CATCH 22, which in turn includes the CHARGE association. Other cases of DGS can derive from microdeleted chromosome *10p* (fetal-alcoholic syndrome, retinoic embryopathy, maternal diabetes) [414]. This variable phenotype is reliably referred to microdeletion *22q11.2*; the greater it is the more complex is the associated phenotype [508]. Another difference depends on the variable spectrum of T-cell abnormalities in individuals with DGS who might have normal T-cell numbers and function, low T-cell numbers but fairly normal T-cell proliferative function [32] or no T cells

Table 22.13. Clinical manifestations of DiGeorge syndrome

External features	Malformations
Thymus	Aplasia
Parathyroids	Aplasia
Eyes	Hypertelorism, antimongoloid slant
Ears	Low-set, prominent with notched pinnae
Mouth	Micrognathia, absent/short labial philtrum, high arched palate
Heart	Interrupted aortic arch type B, common truncus arteriosus

Modified from [79].

Fig. 22.35. Infant with DGA (DiGeorge anomaly) with eye antimongoloid slant, micrognathia and low-set prominent ears

[305]. A second group is referred to as having partial DGS (DGSP) or transient forms (DGST), with mild symptoms. The designation "complete DiGeorge syndrome" (DGSC) is reserved for the third group of infants who have absence of thymic function in addition to other defects of the 3rd and 4th pharyngeal pouches, <1% of patients with DGS, although they can have high T-cell numbers that respond to mitogens [32, 305]. These patients have profound ID, with its associated clinical findings [308]. DGST includes cases with a spontaneous quantitative and qualitative T lymphocyte recovery [162]. The thymus can also be ectopic: in DGSC the T zones are depleted, the CD4/CD8 markedly reduced both in number and in function with SPT anergy, and B cells appear unaffected or increased [162]. In DGSP, the most common type, T-cell number and function are instead usually normal, as are the CD56/CD16 cells with a NK phenotype, or they may be moderately reduced [162]. The proliferative response to mitogens can be pathologically reduced [224] and the response to polysaccharide antigens may be absent [442]. From neonatal age, there are malformations of other structures that form during the first weeks of embryogenesis, presenting a *suggestive but not pathognomonic picture* (Table 22.13) [79].

Diagnosis is usually suspected within the first 2 days after birth, due to the presence of hypocalcemic tetany caused by hypoparathyroidism and cardiac malformation. The facial dysmorphism is also characterized by a small mouth with thin lips described as fish-like [79, 192] (Fig. 22.35). The two rare cardiopathies indicated in Table 22.13 depend on neural crest nonintegration, as mentioned, which accounts for >50% of the alterations alone [295]. Others can be observed affecting the right heart, such as Fallot tetralogy, pulmonary athresia with an interventricular septum defect, and pulmonary infundibular stenosis [508].

Babies surviving the neonatal period manifest from the very first months an increased susceptibility to infections, particularly those of the respiratory and digestive tract, viral and/or fungal, but also caused by *Pneumocystis carinii*, which can be fatal in DGSC [162]. Other findings include gastroesophageal reflux, speech delay, laryngomalacia, absent kidney, conductive or sensorineural deafness, 6th cranial nerve palsy, and hypothyroidism [535]. Treatment with high doses of vitamin D and diets enriched with Ca gluconate are needed immediately, also ensuring that calcemia remains at the lower limit of normal values so as to avoid SNC and renal damage. Subsequently the possible correction of cardiac malformations should be evaluated. ID may be severe, but can regress spontaneously with reconstitution of CMI and T functions; compensating hyperplasia of the residual parathyroid tissue can make it possible to discontinue Ca and vitamin D treatment [192]. *DGS natural history* is, however, complicated by mental retardation and the difficulties encountered in correcting cardiac malformations and in controlling hypoparathyroidism [224]. Because of variability in the ID severity, it is difficult to evaluate claimed benefits of BMT: in two cohorts of 8 [305] and 5 transplanted infants [306], the survivors were 3 out of 13 (23.1%). Recently, 5/6 and 7/12 infants underwent postnatal transplantation with cultured unrelated thymic tissue, with immunosuppression, with positive results [307].

Del22q11.2 syndrome, characterized by a 3-Mb deletion on chromosome *22q11.2* is the most frequent known chromosomal microdeletion syndrome, with an incidence of 1 in 4,000–5,000 livebirths. Patients show

cardiac abnormalities, T-cell deficits, cleft palate facial anomalies, and hypocalcaemia. At least 30 genes have been mapped to the deleted region. Recently, in 5/13 patients with del22q11.2 syndrome without *22q11* deletion mutations were found in T-box 1 that is a major genetic determinant of the del22q11.2 syndrome [545].

X-Linked Lymphoproliferative Syndrome

XLP is caused by a defect in the *SH2D1A* gene (Table 22.1), which binds to the cytoplasmic domains of CD150 SLAM (signaling lymphocyte activation molecule) and 2B4, and may regulate signals transmitted by these receptors in T and NK cells, respectively [345]. XLP has been reported in >270 males from >80 families [446, 460], and in an other 27 males [381], it is inherited with the X-linked model. It is set off in males aged 5–6 by an EBV infection that became manifest with a very polymorphous pattern, often with unusually severe or fatal infections mononucleosis caused by the immune system incapacity to respond to EBV, or evolving into a hgG with IgA and IgG deficiency and HIgMS, or medullar aplasia and/or a Burkitt type lymphoma [446]. The disease has been reported in 15 female subjects [381].

XLP polymorphism could be explained by the fact that the EBV receptor is expressed on differentiating B lymphocytes starting with the preceding isotypic conversion stage [500]. It has recently been verified that before EBV infection, males already suffer from dys- or pan-hgG, incapable of regulating the expression of Ig and/or containing B or T lymphoproliferation. Even after EBV infection, the immune system is unable to provide adequate Th2 responses, and therefore releases cytotoxic alloreactive CD8 and Th1-like T cell ILs, causing extensive damage to the entire parenchyma, exemplified by *fulminating hepatitis, cellular infiltrations and tissular necrosis.* The lymphoid tissues with an altered structure are also affected by necrosis, with a high incidence of mostly nonlocalized lymphomas [381]. The thymus is also affected by thymocyte rarification, with clinical outlines not unlike GvHD [446] (Fig. 22.36) [508], suggesting a possible connection to a Fas deficiency (CD95) or apoptosis syndrome. The SH2D1A gene was found altered in two families, thus indicating that XLP must be considered when more than one male patient with CVID is encountered in the same family, and SH2D1A must be analyzed *in all male patients with CVID* [330].

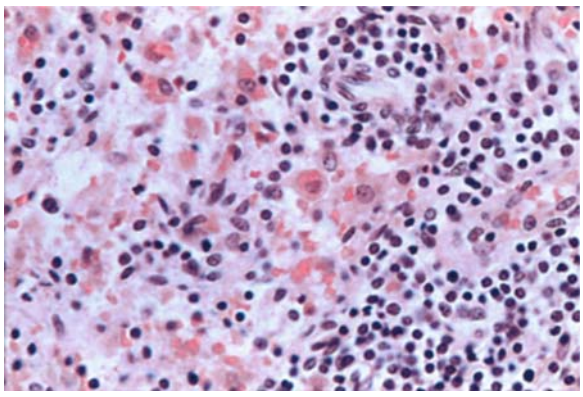


Fig. 22.36. Bone marrow biopsy specimen in a 3-year-old boy: numerous histiocytes in erythrophagocytosis

Hyper-IgE Syndrome

Recently a critical revisitation of this experiment in nature has allowed the identification of links between ID and allergy [62, 178, 196, 287]. The rare HIgES is associated with bacterial RRIs, chronic AD, coarse facial features and very elevated IgE levels [60, 250] (Table 22.2), up to 40,000 IU/ml [549]. Linkage to a region on chromosome *4q* has been demonstrated in several affected families; however, neither the fundamental host defect nor the defective gene has yet been identified [195]. FH is frequently positive for atopic disease, at times HIgES is combined with an unusual predisposition to *Staphylococcus aureus* infections [62, 178, 196, 287]. In Buckley's study, it was present in 36.4% of cases, of both sexes, indicating an autosomal dominant transmission with incomplete penetrance [62]. Onset occurs in the pediatric age in 90% of cases [64]. Clinical presentation is unusual: there are no complaints during the first months of life, toward the 3rd–4th month a *severe form of chronic AD* appears all over the body, which can be associated with other allergic manifestations, including asthma in 13.6% of cases [62]. Skin biopsy specimens reveal spongiosis and perivascular dermatitis and/or folliculitis with a predominance of eosinophils [91]. There is an excessive predisposition to cutaneous and respiratory tract infections (deep and superficial abscesses, otitis, pneumonia, sepsis) (Fig. 22.37), also encouraged by *neutrophil chemotactic deficiency* caused by defective cellular functions (Table 1.65), which, if present, is so pronounced that it becomes a characteristic, unlike AD where it is secondary [226]. The subcutaneous abscesses, described as cold, not covered by warm and reddened skin, are pathognomonic to HIgES but not essential to the diagnosis [144]. The abscess is filled with pus that always grows *Staphylococcus aureus*; in some cases mucocutaneous candidosis and chronic herpetic keratitis are associated [178].

Infections appear within the first 18 months [62]. The face shows coarse and dysmorphic features, midline facial defects such as a prominent nose and a high, arched palate, and disproportionate cheekbones and mandible; pondostatural growth notably retarded [64], pneumatocele (Fig. 22.38) and osteoporosis caused by reduced bone density with a tendency to fracture [287] complete the picture. Six consanguineous families have been reported with an AR form of HIgES, including 13

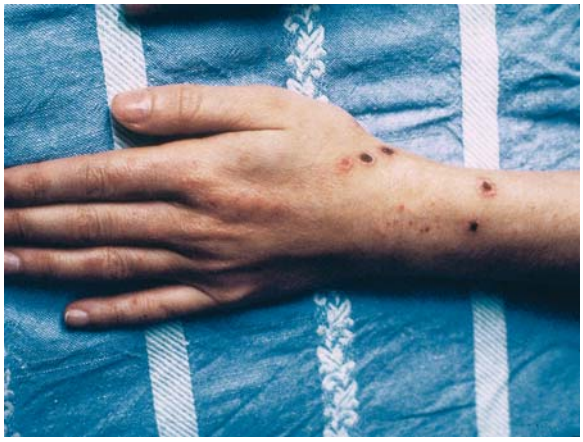


Fig. 22.37. Hyper-IgE syndrome (for details see text)

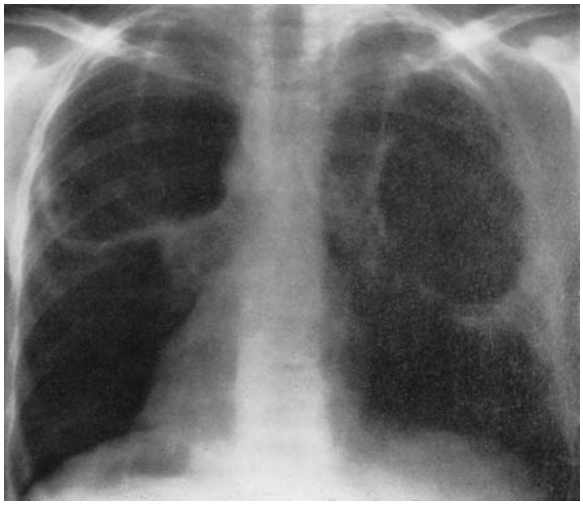


Fig. 22.38. Chest roentgenogram of a 12-year-old boy with hyper-IgE syndrome: evidence of giant pneumatoceles

affected children aged 15 months to 12.5 years, with AR-HIgES presenting with the classic immunological findings, including RRI, eczema, elevated serum IgE, hypereosinophilia, and severe recurrent fungal and viral infections [64]. Notably, patients with AR-HIgES did not have skeletal or dental abnormalities and did not develop pneumatoceles, as seen in autosomal dominant-HIgES [404].

Among the immunological characteristics (Table 22.14) [93, 287], cutaneous anergy to several antigens such as *Candida* and tetanic toxoid is characteristic, which is associated with the anomaly of proliferative responses by the T cells to antigens and mitogens, in contrast with the integrity of other functions tested in vitro [196]. T subpopulations appear to be normal [64]. However, the lymphocyte proliferation to anti-CD3/CD28 monoclonal antibodies can be impaired [226].

As noted, *IFN-γ deficiency* associated with a pathological Th2 prevalence has a fundamental impact on IgE

Table 22.14. Immunological abnormalities of HIgES

1.	Markedly elevated IgE levels (up to 40,000 IU/ml)
2.	Specific IgE directed against *Staphylococcus aureus*, *Candida albicans* and *Herpes simplex* antigens
3.	IgG-anti-IgE antibodies
4.	IgE-containing immune complexes
5.	Normal lymphocyte proliferation responses to PHA and PWM, but reduced to con-A
6.	DHST negativity
7.	Markedly variable chemotactic abnormalities of neutrophils
8.	Normal phagocytic and bactericidal activity
9.	Marked peripheral and local eosinophilia
10.	Reduced IFN-γ synthesis
11.	Selective CD8 deficiency
12.	Underexpression of chemokines ENA-78, MCP-3, and eotaxin

Data from [93, 287].
Con-A concanavalin A, *DHST* delayed hypersensitivity skin test, *PHA* phytohemagglutinin, *PWM* pokeweed mitogen.

hyper-production [178, 287]. In HIgES, some studies have confirmed IFN-γ deficiency compared to controls [120, 368], also due to an impaired response to IL_{12} [56], while others have not [62, 512]; however, compared to AD, normal levels of T producers of IL_4 are characteristic [120]. Considering the IFN-γ/IL_4+ correlation of AD, in HIgES no specific T-cell anomalies are noted, nor does the hyper-IgE explain this pediatric abnormal susceptibility to infections: high IgE levels are also seen in children with AD, who do not, however, have an unusual predisposition to abscess formation [226]. One typical characteristic is sIgE directed against microbial antigens: the anti-staphylococcal sIgE rise to 8.9% compared to normal levels of 0.2%–0.6%. Another constant finding is the increase in 100% of cases of eosinophil concentrations, which make up 6%–12% of leukocytes [64], reaching 30%–50% [178]. By expressing the CD40–CD154 duo, they stimulate the isotype B-cell switching to IgE. We studied children affected by severe AD, chronic FA-induced diarrhea and asthma. The allergens responsible were CM and Der p [73]. In case of HIgES caused by FA, atopic manifestations can clearly improve following an exclusion diet, reducing the frequency of infections and partially correcting the immune defect [420], revealing how FA can induce several immunological anomalies. *Diagnosis* is made on the basis of the data in Table 22.14; *differential diagnosis* with AD is schematized in Table 22.15 [287]. Treatment with cromolyn is extremely effective, anti-staphylococcal antibiotic treatment [64, 226] and if necessary antifungal therapy provide good results [144].

Table 22.15. Differential diagnosis between HIgES and AD

Features	HIgES	Atopic dermatitis
Age of onset	1–8 weeks	>2 months
Frequency	Very rare	Common
Coarse facies	Common	Rare
Dermatitis	Atypical eczema	Typical eczema
Growth	Often delayed	Normal
Osteoporosis	Present	Absent
Erythemas	Absent	Present
Abscesses	Typical	Absent
S. aureus infection	Deep-seated sepsis type	Superficial skin-limited
Other infections	Frequent	Rare
Respiratory allergy	Uncommon	Common
Kerato-conjunctivitis	Rare	Infrequent
IgE level	Extremely high	Normal to very high
Eosinophilia	Frequent	Frequent
Defect of chemotaxis	Frequent	Absent

Data from [61, 287].

Chédiak-Higashi Syndrome

The clinical features of this rare AR disease include oculocutaneous albinism and susceptibility to especially *S. aureus* and β-hemolytic *streptococcus* [549]. Approximately 85% of patients develop an accelerated phase of the disease, with deposition of lymphohistiocytes in the liver, spleen, lymph nodes and BM, resulting in hepatosplenomegaly, lymphadenopathy, BM infiltration hemophagocytosis, pancytopenia as well as fever, jaundice, prolonged bleeding, easy bruisability, neurological changes (nystagmus and neuropathy), mild mental retardation, and partial ocular and *cutaneous albinism* [285, 334, 526]. The cellular hallmarks of the disease include large lysosomal granules in leukocytes, giant melanosomes in melanocytes and affecting other cells of the body such as neural Schwann cells, renal tubular cells, gastric mucosa, pneumocytes, hepatocytes, Langerhans cells of the skin, and adrenal cells [229, 157]. The fundamental defect in this disorder was found to be caused by mutations in a gene mapped to chromosome *1q42–q43* [31] encoding a cytosolic protein on chromosome 1 named *lysosomal-trafficking* (LYST) *regulator*, encoding a 425-kD protein whose function remains unknown [285]. BMT is resolutive in these children [205].

Griscelli Disease

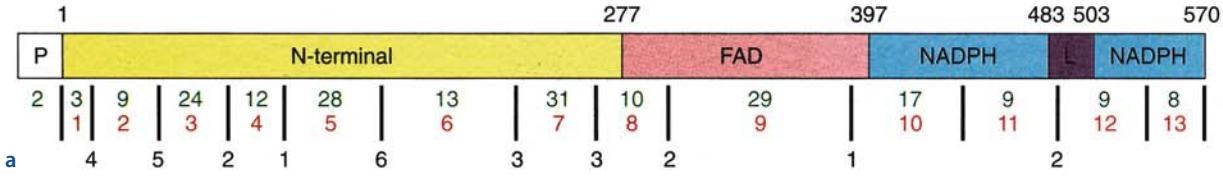
Griscelli disease, mapping to chromosome *15q21* [372], is an AR syndrome caused by mutations in the *MYO5A* (GS1), *RAB27A* (GS2), or *MLPH* (GS3) genes, all of which lead to a similar pigmentary dilution [50, 312]. The disease is also characterized by partial oculocutaneous albinism, predisposition to pyogenic infections and in most patients by abnormal regulation of the immune system, which results in a syndrome of macrophage hyperactivation, known as hemophagocytic lymphohistiocytosis [15]. Mutations in the GTP-binding protein RAB27A (GS2), which appears to be involved in an uncontrolled T lymphocyte and macrophage activation syndrome, leading to death in absence of BMT, occur in this syndrome [319]. A mutation was found in the *MYO5A* gene (GS1) associated primarily with neurological impairment [319]. Two identical twin boys aged 3 months were reported with persisting fever, mouth ulcers, hepatosplenomegaly, pancytopenia and failure to thrive [431], as was an 8-month-old infant [397]. Both infants had silvery-gray hair and pigment clumps on the hair shafts, and skin biopsy showed accumulation of melanocytes on melanosomes. Their parents were first cousins and a sibling with similar manifestations had already died, as did the twins. A genetic study revealed a 5-bp deletion in the *RAB27A* gene (510 del AAGCC in exon 5) [431]. In a 4-year-old child with hemophagocytic syndrome, ID, and secondary neurological disorders, typical melanosome accumulation was found in skin melanocytes and pigment clumps were observed in hair shafts. Two heterozygous mutant alleles of the *RAB27A* gene, a C-T transition (C352T) leading to Q118stop and a G-C transversion on the exon 5 splicing donor site (G467+1C) were found [50]. The finding of *gray strands of hair, gray eyebrows, and eyelids in childhood* should alert pediatricians to considering Griscelli syndrome since an early diagnosis is life- and health-saving [203].

Phagocyte Deficiency

The phagocyte system with the biochemical basis of CGD is analyzed within the framework of innate immunity.

Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) has an overall prevalence of 1:500,000 to 1:10^6, although this could be underrated (Table 22.4), considering that some subjects may have a very mild clinical phenotype that escapes diagnosis [501]. A US registry of birth rates found a prevalence of 1:200,000 to 1:250,000 live births for the period 1980–1989 [538]. The youngest patient was *27 days old* [337] and in 12 children with CGD the mean age at the onset of infections was *5 months,* with a median delay in diagnosis of 2.5 years [371]. Otherwise the



a

N-terminal domain

```
    1  MGNWAVNEGLSIFVILVWLGLNVFLFVWYYRVYDIPPKFFYTRKLLGSAL   50
                          C

   51  ALARAPAACLNFNCMLILLPVCRNLLSFLRGSSACCSTRVRRQLDRNLTF  100
       DS   LER                          @
        G

  101  HKMVAWMIALHSAIHTIAHLFNVEWCVNARVNNSDPYSVALSELGDRQNE  150

  151  SYLNFARKRIKNPEGGLYLAVTLLAGITGWITLCLILIITSSTKTIRRS  200
          T    R

  201  YFEVFWYTHHLFVIFFIGLAIHGAERIVRGQTAESLAVHNITVCEQKISE  250
                             L                S
                                              Y
                                              R

  251  WGKIKECPIPQFAGNPPMTWKWIVGPM                         277
```

FAD-binding domain

```
  278                                  FLYLCERLVRFWRSQQKVVITKV  300

  301  VTHPFKTIELQMKKKGFKMEVGQYIFVKCPKVSKLEWHPFTLTSAPEEDF  350
            K    -                        P    Y

  351  FSIHIRIVGDWTEGLFNACGCDKQEFQDAWKLPKIAVDGPFGTASED    397
            R    R    R                    A    -
```

NADPH-binding domain

```
  398                                                  VFS  400

  401  YEVVMLVGAGIGVTPFASILKSVWYKYCNNATNLKLKKIYFYWLCRDTHA  450
          R  E       H       P
             R       L

  451  FEWFADLLQLLESQMQERNNAGFLSYNIYLTGWDESQANHFAVHHDEEKD  500
                   C
  501  VITGLKQKTLYGRPNWDNEFKTIASQHPNTRIGVFLCGPEALAETLSKQS  550
              \/                          R
             HIWA

  551  ISNSESGPRGVHFIFNKENF                                570
                          K
                          ------
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Loop over NADPH-binding domain

```
  484  DESQANHFAVHHDEEKDVIT                                503
                          G
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b

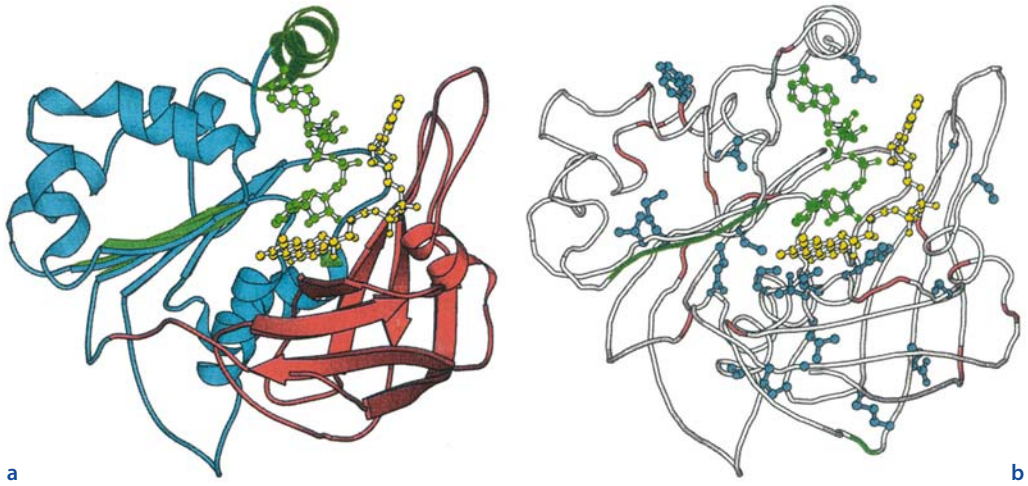

Fig. 22.40 a,b. Model of the three-dimensional structure of C-terminal domain of gp91^{phox}. **a** FAD is shown in *yellow*, NADPH is *green*, the FAD-binding domain in *red*, NADPH-binding domain in *blue* and the helix blocking the NADPH-binding site in *purple*. **b** Missense, nonsense and small in-frame deletions and insertions in the region shown in **a**, with the protein backbone as coil. Missense mutations are shown as *blue* side chains, nonsense mutations as *red* sections of backbone, deletions as *purple* sections and insertion/deletion combinations as a *green* section

median age at onset was 1.12 months, and the median age at diagnosis was 1.1 years [81].

The deficiency appears in two forms (Table 22.1): *X-linked (X-CGD)* is caused by a mutation in the gene encoding the 91-kD (gp91^{phox}) [538], termed CYBB, a subunit of the cytochrome b558 component of the oxidase for which a database includes 304 patients from 261 families and 192 individual mutations [345, 412] (Figs. 22.39, 22.40). *The X-CGD* (56.3%–70.4% of cases) [335, 538], with an H-chain deficiency, is divided into four X91 subtypes (Table 22.16 [84, 97, 109, 112, 185, 501]), also identified on the basis of NBT results, depending on whether the X91 is absent (the most common form), reduced or present but inactive; subtype X91$^-$ is divided into two variants: in one of them the NBT is slightly positive in 80%–100% of cells (6% of patients), in the other in 5%–10% (3% of patients) [84, 109, 412, 501]. More precisely, the four subtypes are caused by mutations in the four gp91^{phox} regions, many of which depend on CYBB gene mutations, causing the X91^0 form, while 17 mutations depend on the NADPH-oxidase activity (X91$^-$ form) and eight others lead to a normal protein expression (X91$^+$ form), but with a total absence of oxidase due to incorrect binding [412].

AR-CGD is caused by a mutation in the genes encoding the remaining oxidases of 47 kD (p47^{phox}) (phox, phagocytic oxidase) [538] (NCF-1), p22^{phox} of 22 kD (CYBA), and p67^{phox} of 67 kD (NCF-2) [109, 501]. The AR-CGD forms (18.5%–22% of cases) [285, 458] are identified using the immunoblotting technique, depending on whether they affect the p22^{phox}, the p47^{phox} or the p67^{phox} [84, 97, 109, 501], with greater prevalence in an American study [97]. Patients with the X-CGD appear to have a more serious clinical phenotype than patients with the AR-CGD, based on the fact that they are diagnosed significantly earlier (mean, 3.01 years of age vs 7.81 years of age, respectively), have a significantly higher prevalence of infections and a higher mortality (21.2% vs 8.6%) [537]. Mutations in any of the 6 structural molecules (Table 22.16) lead to CGD. Mutation of Rac2 (see LAD), the predominant G protein in neutrophils, leads to defects in SO production, as well as in chemotaxis [416]. Activation of the NADPH oxidase requires complex rearrangements between the protein

←

Fig. 22.39. a Domain organization of gp91^{phox}. The *green numbers* indicate the number of families having mutations in the exons that are numbered in *red*. The number of families having intron mutations is shown in *black* below the exon-intron boundaries. Two families having mutations in the promoter (*P*) region are also indicated. **b** Mutations causing X-CGD. The sequences are arranged according to domains. The *underlining* in the N-terminal domain and the beginning of the FAD-binding domain indicates the hydrophobic residues that may be membrane spanning. Further *underlining* in the FAD-binding domain indicates residues that are supposed to be involved in FAD binding, and in the NADPH-binding domain for supposed NADPH-binding. The α-helices and β strands are indicated by *red* and *blue* sequences, respectively. The mutations given *below* the sequence lead to diminished protein expression and oxidase activity (X91 ~ CGD) (*indicated in italics*), normal protein expression and total lack of oxidase activity (X91 + CGD) (*indicated in bold*), or to unknown phenotypical expression (*indicated in normal print*)

Table 22.16. Structure, expression and distribution of CGD genes

Components	Component affected					
	gp91^{phox}	p22^{phox}	p47^{phox}	p67^{phox}	p21^{rac2}	p40^{phox}
Locus of genes	CYBB	CYBA	NCF-1	NCF-2		
Chromosomal location	*Xp21.1*	*16q24*	*7q11.23*	*1q25*	*22q12*	*22q13.1*
Gene/mRNA size	30 kb/4.7 kb	8.5 kb/0.8 kb	15.2 kb/1.4 kb	37 kb/2.4 kb	18 kb/1–5 kb	18 kb/1–2 kb
No. of exons	13	6	9	16	?	10
Tissue specificity	Myeloid; low levels in mesangial cells	mRNA ubiquitous, protein stable only in presence of gp91^{phox}	Myeloid	Myeloid	p21^{rac1} ubiquitous, p21^{rac2} restricted to myeloid cells	Myeloid
Inheritance	X	AR	AR	AR	AD	ND
No. of affected/incidence[a]	X91^0 50–63	A22^0 5–5	A47^0 33–23	A67^0 5–5	ND	ND
	X91$^-$ 6/3–4		(33)	(5)		
	X91$^+$ 3-?	A22$^+$ 1.5–?				

Chromosomal sites: see Table 22.1.
For details, see text.
The rates (%) consist of a first [109] and of a second number related to the European study [84]; US data regarding AR CGD are in parentheses [97].
Data from [84, 97, 109, 185, 501].
X X-linked, *AR* autosomal recessive, *AD* autosomal dominant inheritance, *ND* not done.
[a] The superscript symbols indicate the level of immunoreactive proteins: ^{0} undetected, $^-$ diminished, $^+$ normal protein levels.

subunits, which are in part mediated by noncovalent binding between src-homology 3 domains (SH3 domains) and proline-rich motifs [447].

CGD is a hereditary disease (Table 22.16) characterized by severe recurrent pyogenic infections. This marked susceptibility is caused by the phagocytes' incapacity to kill in particular the catalase-positive bacteria, because of a genetic defect of the NADPH-oxidase enzymatic system situated in the wall of the phagocytic vacuole. In CGD, phagocytosis occurs normally, but the NADPH-oxidase is unable to markedly produce anion superoxide ($O_2^{\bullet-}$), H_2O_2 and other O_2 free radicals, thereby permitting the survival of microorganisms within the cells, where they are protected from the antibodies and from most antibiotics [501]. Another consequence of the lack of O_2 radicals is the development with countless inflammatory episodes, which then result in typical granulomas [109]. The nitroblue tetrazolium (NBT) reduction test is based on the chemical characteristics: in fact, the phagocytes without $O_2^{\bullet-}$ are unable to reduce the yellow NBT of products activated by PHA aspecifically stimulated phagocyte O_2, or specifically with corpuscle particles such as preopsonized yeasts (Fig. 22.41). The result was 0% in 14 children [81]. At a molecular level, the genes that codify the two subunits of flavocytochrome b588, gp91^{phox} and p47^{phox} have been cloned: respectively the cytochrome, H (β) and L (α) chains situated on the phagosome vacuole membrane, and also the cytosolic factors p40^{phox}, p22^{phox} and p67^{phox}, deriving from the NADPH-oxidase activation, all proteins placed inside the cytoplasm and that belong to innate immunity. It has therefore been possible to identify molecular lesions at the CGD origin, with the exception of the p21^{rac1} [16, 412, 533].

The clinical pattern is severe in the X91^0 form and variable in the other two X91 forms; *onset occurs within the 1st year of life in 2/3 of cases, and in others within the 2nd year* [159], although it can appear also at the age of 16 [335]. Purulent recurrent infections, with a granulomatous evolution, predominantly affect the epithelial surfaces normally colonized by bacteria, such as cutaneous, subcutaneous, mucous membranes, the respiratory tract and the intestine: cutaneous and mucosal infections, and lymphadenitis lead to suppuration and fistulation (Fig. 22.42), pneumonia or lung abscesses (Fig. 22.43) are more frequently characterized by persistent fever and diarrhea [159] (Table 22.17) [109, 538]. Pneumonia was the most prevalent infection in 369 patients (79%) (mostly by *Aspergillus*), followed by suppurative adenitis (53%), subcutaneous abscess (42%) and liver abscess (27%); mostly by *Staphylococcus*, osteomyelitis (25%) mostly by *Serratia*, and sepsis (18%), and by *Salmonella* [538]. In a long-term trial, pneumonitis was the most prevalent infection (91%) followed by lymphadenitis (83%), aphthous stomatitis (58%), liver abscesses (25%) and chronic lung disease

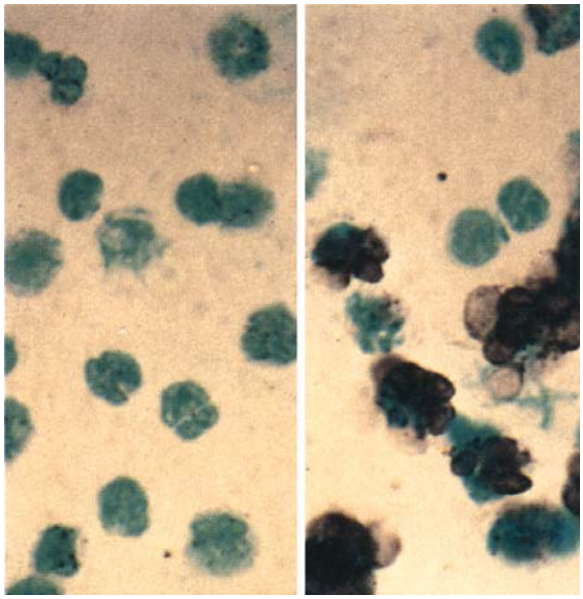

Fig. 22.41. NBT test. *Right*: in normal PMN and PBMC reactive superoxide generated by the respiratory burst reduces the soluble yellow NBT dye to the deep blue of formazan. *Left*: CDG patients cannot form superoxide, so the dye stays yellow

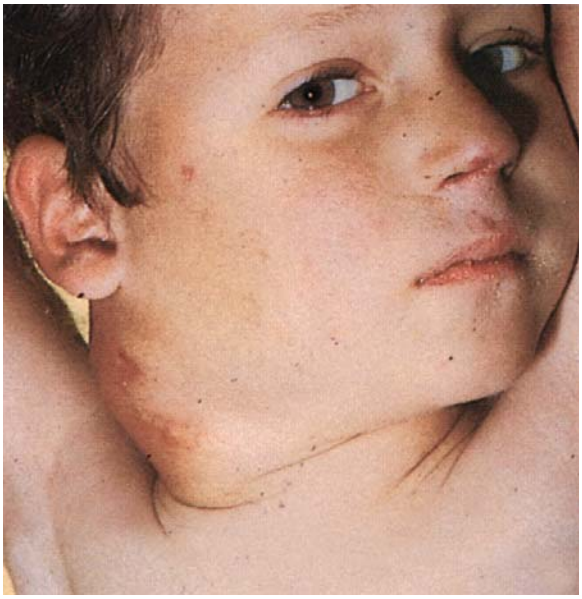

Fig. 22.42. Child with X-CGD. Abscess caused by *S. aureus*; those in both inguinal regions were surgically drained

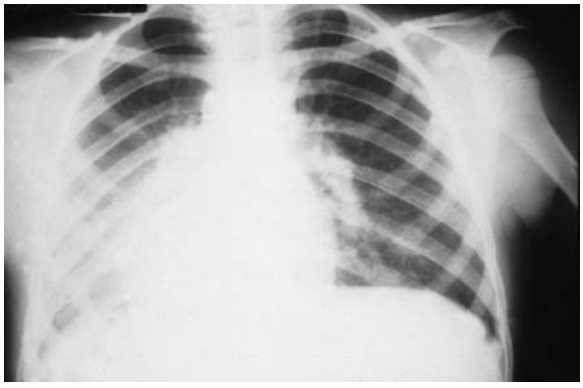

Fig. 22.43. Chest roentgenogram of a child with X-CGD (for details see text)

Table 22.17. Sites of infections in two cohorts with CGD (%)

Infections References	No. 550 [109]	No. 368 [538]
Pneumonia	75	79
Cutaneous infections	70	
Lymphadenitis	70	53
Hepatic/perihepatic abscess	35	27
Osteomyelitis	25	25
Septicemia/meningitis	17	13
Conjunctivitis	15	
Subcutaneous abscess		42
Perianal abscess	15	
Stomatitis	15	
Colitis enteritis		17
Urinary tract infections	10	
Enteric infections	10	
Gastric outlet obstruction	10	15
Urinary obstruction		10

(58%) [371]. Lymphadenitis, lung infections, enteral infections, and hepatic abscesses were the most frequent infections in a cohort of 48 children [335]. Staphylococcal liver abscesses are almost pathognomonic of CGD [447, 538].

Because the infections develop in areas drained by lymphatics, they tend to diffuse via the lymphohematogen route, thus causing *arthritis and osteomyelitis* and *abscess formation,* especially affecting *the bones, which are the most severe manifestation,* and *hepatitis with common upsurge of hepatosplenomegaly.* Lung infections are almost the rule: those initially segmented and parallel tend to gradually spread over the entire lobe [109, 538]. Histological examination shows widespread *granulomas in the entire lung parenchyma,* which are formed by mononucleates (Fig. 22.44a) with giant cells (Fig. 22.44b). The chronology of infection onset is summarized in Table 22.18 [335]: lymphadenitis is the earliest. Osteomyelitis is usually a worrying complication:

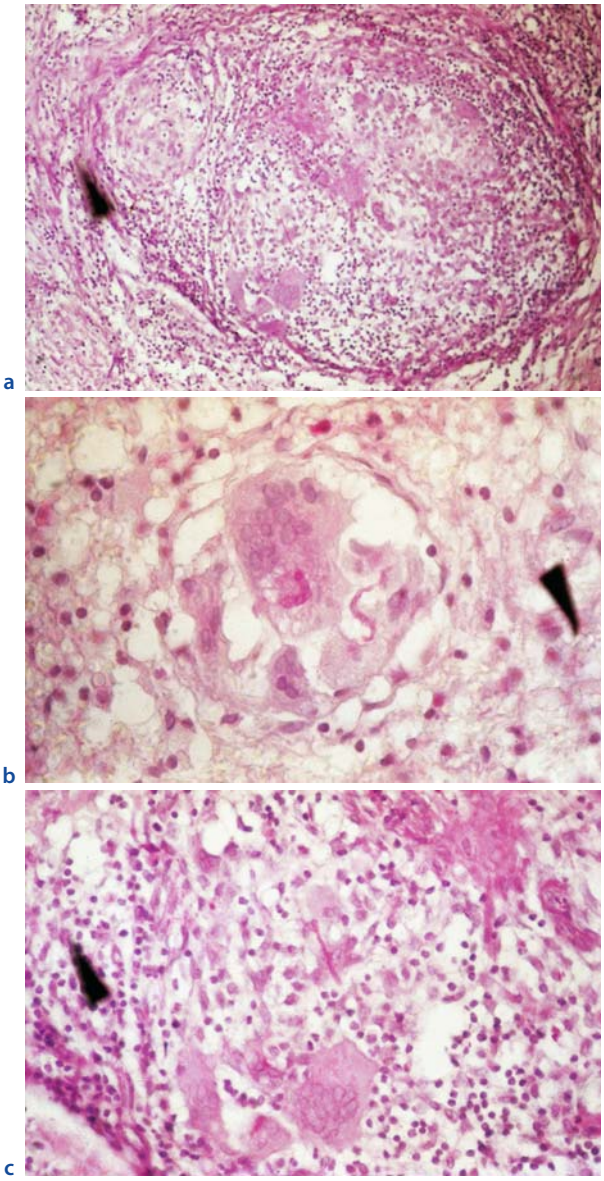

Fig. 22.44 a–c. Child with X-CGD. **a** Granuloma. **b** Giant cells. **c** Pulmonary aspergillosis

extensive bone destruction involves various segments, for example the vertebra, the metacarpus and the metatarsus, causing widespread damage, which is difficult to treat and is also irreversible [109, 501]. *Aspergillus*, pulmonary, bone (Fig. 22.44c) or encephalic infections constitute a severe therapeutic problem and are threatening events, with a mortality rate of 26%, but with specific treatment the prognosis is good as far as recovery is concerned [335]. The treatment includes prophylaxis with trimethoprim-sulfamethoxazole (TMP/SMX) (5 mg/day given in two divided doses), and IFN-γ (50 mg/m^2 subcutaneously thrice weekly) in all patients with CGD, regardless of genotype [81, 416]. Itraconazole therapy (5 and then 10 mg/kg/day)

Table 22.18. Onset age (mean + range) of infections in children with CGD

Infections	Mean (months)	Range (years)
Airway infection	55	0.1–14
Liver abscess	83	0.1–18
Gastroenteric infection	38	0.3–14
Lymphadenitis	16	ND

Data from [335].
ND not done.

has an excellent tolerance in all cases and was effective in 29 of 32 children (90.6%) [336].

Survival until the age of 21 and beyond is achieved by 20% of patients with CGD XL and 37% of those with CGD AR [339]. Because the prognosis is uncertain, as observed, the *only possibility for a definite resolution is with a BMT,* from family donors who are X-CGD or X-CGD-identical [393]. BMT was successful in 27 children out of 31 (87.1%) (see Table 22.30), including a 4-year-old boy with X-CGD who underwent successful HLA-identical peripheral blood SC transplantation during invasive pulmonary aspergillosis and osteomyelitis, which was unresponsive to antifungal treatment [48].

Leukocyte Adhesion Deficiency

LAD is due to mutations in the gene on chromosome 21 at position *q22.3* encoding CD18 (Table 22.1). It is divided into five types: *LAD type I to LAD type V* [24, 71, 138, 367]. The three subunits of the CD11/CD18 complex are involved in PID (LAD type I syndrome), AR, linked to the lack of $\alpha_M\beta_2$ equal to CD11b/CD18 (Table 1.46) surface expression on all leukocyte populations caused by 20 different mutations in the CD18 encoding gene, often severe in infancy [145, 202]. Children with a deficiency of these integrins have a defect above all in phagocyte action, suffer from severe infections from the neonatal period [202] due to absent β2-integrin activity, which impairs neutrophil ability to exit the circulation and travel to sites of infection. On the contrary, leukocyte movements are not prevented, indicating the normal involvement of CD54 and CD102 (Table 1.4). The clinical basis for defining this disease, described in over 200 cases [145], dates back to a study at the Soothill school in 1979 [215].

There are two forms of *LAD type I* [71]: if the deficiency is full blown (no detectable CD18), the clinical symptoms (Table 22.19) [71, 138] are dominated by severe and recurrent infections with a negative prognosis in the first years of life unless corrected by an allogenic BMT, the only resolutive treatment [492]. If instead it is a partial deficiency with residual CD18 expression, the

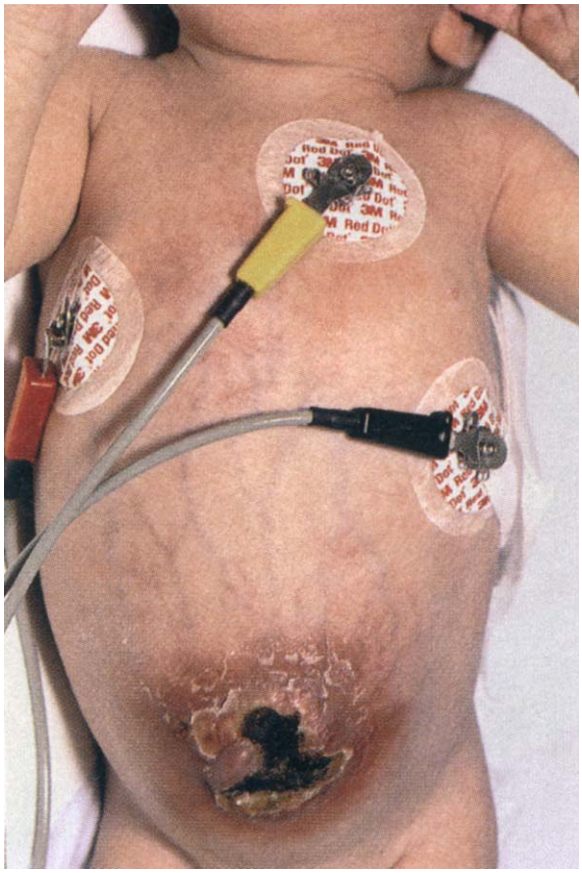

Fig. 22.45. Child with LAD, hepatosplenomegaly and omphalitis

Table 22.19. Prevalent clinical features of LAD

Almost total absence of leukocytes in lesional sites
Delayed umbilical cord severance[a] or infection
Delayed wound healing and/or infection of surgical wounds
Frequent, persistent leukocytosis (12–160 × 10^9/l)
Gingivitis or periodontitis
Infections involving skin and subcutaneous layers Cellulitis or abscess dependent on trauma or wounds Indolent subcutaneous abscess or cellulitis
Otitis media
Systemic infections Aseptic meningitis Necrotizing pharyngitis or tracheitis Omphalitis Perianal abscess Peritonitis Pneumonia Septicemia
Ulcerative stomatitis or pharyngitis

Modified from [71, 138].
[a] >2 weeks.

clinical outline is less severe and some patients, with appropriate treatment, can live to adult age [24].

LAD type II, AR (CD18 levels are 1%–10% of the normal levels), with a molecular base represented by an sLeX ligand (CD15s) is a defect common to CD62 E and P, which mediate neutrophil rolling. In the absence of a GDP-fucose transporter, the sLeX is not made. LAD type 2 results from mutations in this transporter that takes fucose into the Golgi apparatus for posttranslational fucosylation of newly synthesized proteins. This is the ligand for CD62E; without it, leukocytes cannot make initial attachment to vascular endothelium [7]. LAD has been described in two children aged 3 and 5 with mental retardation, from different families, but both with parents who were blood relatives [145]. It has a lower mortality rate [138]. Mice with a deficiency of both selectins show a LAD-like syndrome, providing a useful model for studying these syndromes [171].

LAD type III shows defective tethering and adhesion and bleeding diathesis. This is a new syndrome where in vitro leukocytes showed normal rolling along endothelial cell cultures but defective tethering and tight adhesion. Thus this is a defect in the capability of vascular integrins on circulating leukocytes to rearrange with their endothelial ligands at adhesive contacts and rapidly arrest on target vascular endothelium in response to endothelial-displayed chemoattractants. However, the expression levels of the major integrins on lymphocytes and neutrophils were largely conserved in the patient cells, ruling out a LAD-I syndrome. Patient leukocytes showed no LAD-II like fucosylation defect, since they expressed normal levels of the fucosylated marker CD15s, comprising the sLex carbohydrate selectin ligand [7].

Defects in both leukocyte and platelet functions that are biochemically and molecularly distinct from the adhesion disorders previously described suggest a mutation in an early myeloid pathway. The defect is associated with regulation of the GTPase activating protein Rap1, as demonstrated by the intact Rap1 expression and activation by phorbol esters, thus ruling out an LAD defect in Rap1 GTP loading [255].

LAD type IV manifests defective CD62E expression or tethering. A girl developed *Pseudomonas* omphalitis at *5 weeks of age,* recurrent ear and urinary tract infections, and had clinical evidence of impaired pus formation reminiscent of a LAD syndrome, but her neutrophils were functionally normal and expressed normal levels of CD18, CD62E, and sLex. However, the patient showed an absence of CD62E from the endothelium, although E-selectin mRNA was present. In contrast to patients with LAD 1, she had mild chronic neutropenia but appropriate leukocyte increases in response to infections or GM-CSF. A BM biopsy performed during a period of health showed normal cellularity for her age. Her FH is remarkable only for a previous sibling who had died at 32 weeks of gestation of a staphylococcal infection of the fetus, amniotic fluid, and

placenta. She also has two half-sisters who are completely well. The FH is negative for recurrent infections in either parent or more distant relatives [121].

LAD type V caused by Rac2 deficiency. *A 5-week-old* boy born to unrelated parents had delayed UC separation, perirectal abscesses, poor wound healing, and absent pus at sites of infection in the setting of neutrophilia, suggesting a neutrophil defect. His neutrophils exhibited decreased chemotaxis, polarization, azurophilic granule secretion, as well as significantly reduced stimulated superoxide production but had normal expression and up-regulation of CD11b. Rac2 constitutes more than 96% of the Rac in neutrophils [9]. A 1-year-old boy who had multiple recurrent, life-threatening infections characterized by leukocytosis and notable for the absence of pus in the inflamed tissues was reported. The presence and density of CD11b, CD11c, and CD18 were normal. The expression of CD62P and CD62L were also normal. A BMT was curative. The boy shared a phenotype that closely mimicked that of a mouse mutant deficient in the Rho GTPase, Rac2 [534]. The disease was shown to be attributable to an AD mutation in the Rho GTPase Rac2 at an amino acid needed for proper interaction with other intracellular proteins. Rac2 comprises >96% of the critically important G protein Rac in neutrophils. Each member of the family appears to control a distinct function of the actin cytoskeleton (chemotaxis and degranulation) and NADPH oxidase (superoxide production) function [9].

Deficiency of Multiple Leukocyte Integrins

A male child from the mother's first pregnancy was born at term from parents of Arab ethnic origin who were first cousins. He had a severe genetic disorder associated with functional defects in multiple leukocyte integrins, reflected in recurrent infections, profound leukocytosis and a bleeding diathesis. Platelet transfusions and antibiotic courses reduced the symptoms, which remained a significant clinical problem. At age 6 years, he died from disseminated fungal infection after a mismatched BMT. A younger brother presented with the same clinical and hematological phenotypes at birth and died at age 1 week from sepsis.

Glucose-6-Phosphate-Dehydrogenase Deficiency

G6PD converts G6P to 6-phosphogluconolactone, generating NADPH and a H^+ ion from $NADP^+$. NADPH oxidase catalyzes the monovalent reduction of O_2 to $O_2^{\bullet -}$, with the subsequent conversion to H_2O_2 by superoxide dismutase [285]. In the form of a partial deficiency, known as the cause of hemolytic anemia or favism, the enzyme's residual activity (20%–25%) permits normal bactericidal activity. The 400 G6PD variants have been classified by the level of residual enzyme activity and propensity for hemolysis and grouped into five classes: *class I,* severely deficient with chronic hemolytic anemia; *class II,* severely deficient with occasional hemolytic anemia (<10% residual activity); *class III,* moderately deficient (10%–60% residual activity); *class IV,* normal activity (60%–150%); *class V,* increased activity [310]. In a trial on 161 G6PD-deficient subjects originating from different parts of Italy, a greater molecular heterogeneity than described by others was observed, especially in Sardinia [310]. In a complete deficiency, sexually transmitted, whose gene is localized on the chromosome X at position *p28* and characterized by several mutations and their variants, with a consequent *deficiency of bactericidal activity,* the neutrophils are unable to kill *S. aureus*, *E. coli* and *Serratia*, and therefore there is an increased susceptibility to infections, rather like CGD [109]. The diagnostic work-up of children may reveal a child with recurrent infections who initially received the diagnosis of G6PD deficiency, subsequently shown to have the phenotype of X-linked CGD [3]. The disorder has a *higher incidence* in Mediterranean countries and Asia, in Japan (10.6%) than in Indonesia (4.3%), as ascertained with a novel screening kit [234], and is low in newborns in Tehran, Iran (2.1%) [1].

Myeloperoxidase Deficiency

Within the framework of oxygen-dependent killing defects, hereditary myeloperoxidase deficiency (MPO) is the most common neutrophil biochemical defect and plays an important role in the host defense mechanism against microbial diseases. The neutrophil disorder characterized by the lack of MPO activity is speculated to be associated with a decreased level of immunity. MPO is unusually accompanied by a specific pathology. AR transmitted, it appears far more common than previously suspected (1:2,000 for the partial deficiency to 1:4,000 for the total deficiency). It is a disorder that is prevalently recorded in entirely healthy patients and therefore, in most cases, a *random laboratory finding*. In addition to three already-known mutations, the genetic characterization of an Italian population showed the presence of six novel mutations: four missense mutations, a deletion of an adenine within exon 3 (*c.325delA*) and a mutation within the 3′ splice site of intron 11 (*c.2031-2A>C*). The *c.325delA* deletion causes a shift in the reading frame with the occurrence of a premature stop codon within the pro-peptide. The activation of a cryptic 3′ splice site located 109nt upstream of the authentic 3′ splice site causes a shift in the reading frame that may lead to the generation of an abnormal MPO precursor lacking the enzymatic activity [303]. In a Japanese patient with complete MPO deficiency, neutrophil function analysis revealed that MPO activity was

significantly diminished with slightly elevated superoxide production. Mutational analysis of the patient revealed a glycine to serine substitution (G501S) in the exon 9 region [356]. Because the granulocytes without MPO cannot kill *Candida*, some subjects, presumably carriers of a more extensive mutation and in association with other diseases, *present severe and recurrent Candida infections.* The MPO defect can be diagnosed via a cytochemical investigation or a quantitative count of enzyme levels [303].

Specific Granule Deficiency

Neutrophil-specific granule deficiency is a rare autosomal dominant disorder characterized by recurrent pyogenic infections, defective neutrophil chemotaxis and bactericidal activity, and lack of neutrophil secondary granule proteins [284]. The markedly decreased level of mRNA expression for the bactericidal/permeability-increasing (BPI) protein, the activation factor PU-1 and defensins in these patients suggests a role for CCAAT/enhancer binding protein (*C/EBPη*) gene in earlier phases of the myeloid differentiation program [187]. *C/EBPη* is a member of the leucine zipper family of transcription factors, expressed primarily in myeloid cells [284]. Recessive mutations in the *C/EBPη* gene were described in one patient; analyses of the *C/EBPη locus* indicated that the disorder could have resulted from HZ recessive inheritance of the mutant allele from an ancestor shared by both parents [187]. Loss of C/EBPη function is the primary genetic defect in this disease [455]. In a second individual lacking functional C/EBPη, analysis of peripheral blood leukocytes revealed aberrant expression of CD45, CD11b, CD14, CD15, and CD16 on the proband cells [455]. A male patient lacking neutrophil-specific granules died from complications of pneumonia at age 20 [284]. Neutrophil-specific granules contain important microbicidal components (Table 1.23). Among other deficiencies of oxygen-independent killing, this AR defect is characterized by severe recurrent bacterial deep-tissue skin infections without patients showing an increased susceptibility to a particular pathogen. They have defects in chemotaxis, disaggregation, and receptor up-regulation. Deficiencies of the oxidoreduction and microorganism-killing mechanisms have also been described. The markedly decreased level of mRNA expression for the bactericidal/permeability-increasing (BPI) protein, the activation factor PU-1 and defensins in these patients suggests a role for C/EBPη in earlier phases of the myeloid differentiation program[187]. The defect is identified through a blood test colored with a Wright reactive in which polymorphonucleates do not present the specific granules that normally contain lactoferrin. From a morphological point of view, the nuclei appear bilobated and the nuclear membrane may show intro- and extroversions. It is also possible to identify the membrane's lack of alkaline phosphatase [414]. Monocyte functional alterations in the second individual suggest that C/EBPη plays a critical role in monocyte/macrophage development of humans and implicates abnormalities in monocytes/macrophages and neutrophils in the onset and development of the disorder [455].

Neutropenia

Severe congenital neutropenia (SCN) and cyclic neutropenia are disorders of neutrophil production predisposing patients to recurrent bacterial infections. Recently, mutations of the gene encoding neutrophil elastase 2 (ELA2) have been indicated as the most common cause for SCN as well as the cause for autosomal dominant cyclic neutropenia [225]. Deficiency of ELA2 leads to regularly fluctuating levels of neutrophils [112]. Linkage analysis on 13 affected pedigrees have shown that cyclic neutropenia and sporadic cases of this disease are due to a mutation in the gene for ELA2, located at *19p13.3* [112]. This enzyme is synthesized in neutrophil precursors early in the process of primary granule formation [225]. A mutation in the *ELA2* gene was detected in one of three apparently autosomal dominant kindreds with familial SCN. No mutations were identified in the apparently AR families [12]. These results fit those showing that mutations were found in all five SCN families [112], but they suggest that not all cases of autosomal dominant SCN caused by mutations in *ELA2* [12]. However, the high frequency of HET mutations in the neutrophil elastase gene in sporadic SCN confirms a previous report [112]. Considering that four novel mutations and a low-frequency polymorphism were detected, nearly all cases of sporadic SCN may result from *de novo* HET mutations in *ELA2* [12]. In recurrent SCN, an absolute neutrophil count of <200 cells/mm^3 (or $<0.1\times10^9$/l) [12] oscillates with an approximate 21-day periodicity. Circulating neutrophils vary between almost normal numbers and zero [225]. In about 30% of patients with cyclic neutropenia, however, the cycles range from 14 to 36 days [42]. In 26 children referred during a 22-year period PIDs were as follows: cyclic neutropenia (30.7%), Shwachman-Diamond syndrome (26.9%), Kostmann syndrome (23%), and Chédiak-Higashi syndrome (19.2%). The mean absolute neutrophil count of children was 398.2±259.3 cells/mm (range, 74–1,152/mm) at the first visit. The children first experienced symptoms of infection suggesting neutropenia at a median age of 7.5 months (range 1 month to 10 years), also suffering from oral ulcer, otitis, pneumonia, diarrhea, cutaneous abscess, and oral candidiasis [398]. Fever, stomatitis, and periodontitis and skin infections occur during periods when the neutrophil count is low.

Cyclic Neutropenia

Cyclic neutropenia is an autosomal dominant disorder in which cyclic hematopoiesis causes intervals of neutropenia and susceptibility to opportunistic infection. In nine families whose children displayed typical blood patterns, pedigrees confirmed dominant inheritance without evidence of heterogeneity or decreased penetrance; three pedigrees suggested new mutations [369]. A wide spectrum of symptom severity, ranging from asymptomatic to life-threatening illness, was observed within the nine families. The phenotype changed with age. Children displayed typical neutrophil cycles with symptoms of mucosal ulceration, lymphadenopathy, and infections [369]. Patients are usually asymptomatic, but during the period of severe neutropenia, recurrent overwhelming infections, inflammation, and ulcers occur in about 10% of patients and can lead to significant chronic morbidity [298]. Severe neutropenia was shown by 21 children, moderate by 4, and mild by 1: 16 of these children had leukopenia, 7 anemia, 2 thrombocytopenia, and 1 monocytosis. During follow-up, respiratory infections developed in 24, oral manifestations in 20 children. The most common infections, in descending order of frequency, were otitis media, abscesses, pneumonia, oral ulcers, acute diarrhea, cutaneous infections, oral candidiasis, and periodontits. Sinusitis, cystitis, conjunctivitis, meningitis, and osteomyelitis were less frequently observed. Hepatomegaly was also detected in 10 children and splenomegaly in one; 3 children died of recurrent infections. Therefore, recurrent infections always deserve further evaluation for detecting such disorders [398]. Abdominal pain must be assessed aggressively because of the high frequency of *Clostridium* infections during the period of severe neutropenia [369]. During the course of SCN, BM shows lack of maturation of granulocyte precursors beyond myelocytes, and there is myeloid hyperplasia during the remainder of the cycle. Occasionally, there is a reduction in the severity of neutropenia and the accompanying infections over time [369]. *A complete clearing of symptoms and a significant increase in quality of life is* noteworthy in children [298]. However, while the disease is commonly described as benign, four children in three of the nine families died of *Clostridium* or *E. coli* colitis, documenting the need for urgent evaluation of abdominal pain [369]. Pediatric cyclic neutropenia is effectively treated with *rHuG-CSF* (recombinant human G-CSF), usually at doses of 1–5 µg/kg/day (median dose, 2.5 µg/kg/day) [449] or twice weekly, or once a month.

Severe Congenital Neutropenia (Kostmann Syndrome)

Typically, children are noted in early infancy to have persistent SCN with absolute neutrophil counts $<0.2\times10^9$/l lasting for months or years [12]. In children aged 4 days to 19 months, the initial and lowest median absolute neutrophil counts were 0.29×10^9/l and 0.06×10^9/l, respectively [289]. Usually, children suffer from long-term recurrent bacterial infections, and maturation arrest of myelopoiesis at the promyelocyte-myelocyte stage of BM development [12]. The disease begins during the 1st year of life, and its infectious complications include cellulitis, perirectal abscess, peritonitis, stomatitis, and meningitis, commonly as a result of infections with *S. aureus, E. coli* and *Pseudomonas aeruginosa* [42]. The numbers of circulating monocytes and eosinophils are often increased [42]. Missing the most important cells in the defense against bacterial infections, the neutrophil granulocytes, children suffer from episodes of severe, often *life-threatening bacterial infections* [42]. They spend many days in hospital, requiring IV antibiotic treatment. Recurrence of bacterial infections leads to irreversible tissue damage, for example in the lungs, requiring often disabling surgical interventions. A high incidence of significant bone mineral loss was seen in children with SCN [545]. The presence of qualitative and quantitative abnormalities of primitive myeloid progenitor cells expressing G-CSFR may play an important role in the impairment of granulopoiesis in these patients, thus nearly all patients have a response to pharmacological doses of rHuG-CSF: neutrophil counts rise, infection rates fall, and mortality is reduced [343]. Since the introduction of rHuG-CSF, most children enjoy a *normal life span and a greatly improved quality of life,* although they still have problems with infections, especially chronic gingivitis and periodontitis [82]. It is more likely that the bone loss was caused by the pathophysiological features of the underlying disease, but it is possible that *rHuG-CSF* accelerates bone mineral loss [545]. Prolonged administration of rHuG-CSF at a dose of 3 U/kg bw twice daily may be associated with increased bone resorption, mediated by osteoclast activation and leading to bone loss. In children, the resulting osteopenia can be successfully managed with antiresorptive bisphosphonate therapy with significant improvement in bone density [449]. A child maintained on long-term rHuG-CSF therapy developed acute myelogenous leukemia associated with a G-CSFR mutation. After having undergone successful allogeneic BMT, both *ELA-2* mutation and *G-CSFR* mutation became undetectable by PCR [237].

Shwachman Syndrome

Shwachman syndrome, a rare AR condition, characterized by pancreatic insufficiency, reduced mobility and neutrophil chemotaxis, cyclic neutropenia, thrombocytopenia, metaphyseal dysostosis, delayed growth and recurrent pyogenic infections, in two cases was associated with isolated GH deficiency [96, 268]. In addition to metaphyseal chondrodysplasia, neutropenia, and pan-

creatic exocrine insufficiency, the findings in children are noted as variable extremity shortening, cup deformation of the ribs, metaphyseal widening and hypoplasia of the iliac bones, and increased echogenicity of the pancreas with no change in size [43]. Recurrent infections begin during the 1st year of life and commonly involve the sinuses, lungs, bones, skin, and urinary tract [42]. Neutropenia, either cyclic or intermittent, occurs in all patients, and 10%–25% of patients also have pancytopenia [464]. Immune functions may be involved in this syndrome, including marked pan-hgG, especially of the IgA, normal/increased cellular immunity, but depressed humoral and NK cell immunity [268]. In 13 patients diagnosed in infancy, a significant growth improvement and a decreasing frequency of infections were observed over time, in addition to improvement or normalization of exocrine pancreatic function [96].

Leukocyte Mycobactericidal Defect

A continuous spectrum from systemic BCG infection to local recurrent nontuberculous mycobacterial infection covered by the clinical features of affected children has recently helped to identify several genetic defects in the monocyte-macrophage-Th1 T-cell pathway [509]. Different types of *mutations in four genes* (IFN-γR1, IFN-γR2, IL_{12}p40, IL_{12}Rβ1) *forming the IFN-γ/IL_{12} axis* [123] have revealed both allelic and nonallelic heterogeneity and result in different disorders whose common pathogenic pathway is impaired IFN-γ-mediated immunity [123, 403]. Several children have been reported who presented a new kind of hereditary ID with severe and/or recurrent infections caused by only one microorganism family, in opposition to other patients with classic PID. Five new syndromes may encompass these children with a genetic predisposition to infectious diseases. If the IFN-γ/IL_{12} axis is impaired, the host becomes highly susceptible to infection with organisms that replicate intracellularly (susceptibility to mycobacterial disease). STAT-1 (signal transducer and activator of transcription-1) deficiency predisposes to viral disease, NEMO and IRAK-4 (IL_1R-activating kinase-4) deficiencies predispose to infections caused by pyogenic bacteria [376].

IFN-γ Deficiency

This PID encompasses several defects: complete, partial, and *AR IFN-γR1 deficiency,* and complete, partial, and *AD IFN-γR2 deficiency* [122]. IFN-γ and the cellular responses induced by it are essential for controlling mycobacterial infections. Patients with AR mutations leading to complete loss of IFN-γR1 or IFN-γR2 expression have the most severe phenotypes, and they present *early in life* with disseminated severe infections, especially if they have received BCG vaccination, and have poor to absent granuloma formation [242]. *Salmonella* and certain viral infections [HSV, CMV, *parainfluenza,* and respiratory syncytial virus (RSV)] are also seen [126]. Most patients bearing an IFN-γR1 deficiency present gross mutations that truncate the protein and prevent its expression, giving rise to severe mycobacterial infections and, frequently, a fatal outcome [6]. Mortality in these children is high, and infections are severe and recurrent [242], as in an 8-year-old girl before receiving a BMT [405]. A point mutation may be fatal: an individual, probably HZ for the mutation, died from meningitis due to *Mycobacterium bovis* [6]. A HZ missense IFN-γR1 mutation was identified in two siblings who did not respond to low or intermediate concentrations, yet responded to high IFN-γ concentrations, probably for a reduced affinity of IFN-γR1 for its ligand [243]. Otherwise the mutation results in normal surface expression of IFN-γR1 that do not bind IFN-γ [244]. A dominant deletion in the *IFN-γR1* gene has been reported in a female patient HZ for a 4-bp deletion in exon 5 of *IFN-γR1* who developed postvaccinal disseminated BCG infection [417]. The AR form of partial IFN-γR1 deficiency was reported in 18 patients of 12 unrelated kindred with susceptibility to mycobacterial infection [403]. An 8-year-old girl with IFN-γR1 deficiency, also with recurrent mycobacterial infections and liver cirrhosis with portal hypertension, received *red cell-depleted BMT from her HLA-identical sister.* The transplantation course was uneventful and 4 years later the child remains in excellent clinical condition and free of mycobacterial infections [405].

A complete IFN-γR2 deficiency was found in a child due to a HZ dinucleotide deletion resulting in a premature stop codon in the protein extracellular domain. This gene defect emphasizes the critical role that IFN-γ plays in *host defense against mycobacteria* [126].

IL_{12} Deficiency

A girl with BCG and *Salmonella enteritidis* infection and a HZ recessive deletion in the p40 subunit of IL_{12} leading to a *complete IL_{12}p40 deficiency* has been reported. A large HZ deletion within the *IL_{12}p40* subunit gene was found, precluding *IL_{12}p70* (composed of p40 and p35 subunits) functional expression by activated DCs and phagocytes. The net result was a markedly impaired IFN-γ production by lymphocytes. However, addition of recombinant exogenous IL_{12}p70 in the assay was able to restore normal IFN-γ production in vitro [8]. The girl suffered from well-organized granulomas, possibly due to residual IL_{12}-independent IFN-γ production [8]. Another kindred [377] and two siblings and one unrelated patient [142] carried the same large deletion, also accompanied by disseminated infections. A 3-year-old female was repeatedly hospitalized since the age of 5 weeks for recurrent episodes of pneumococcal pneumonia with sepsis and other infections in the absence of

fever. She exhibited IL_{12} deficiency that was associated with an abnormality of the *$IL_{12}p40$* gene. Although present, IFN-γ was reduced [211].

A genetic lack of $IL_{12}R\beta1$ surface expression predisposes to severe infections by pathogenic mycobacteria or *Salmonella* and causes strongly decreased, but not completely abrogated IFN-γ production [408]. The deficiency may be complete as well as partial [291]. Several patients with these features have been reported [291]. Three unrelated individuals with severe, idiopathic mycobacterial and *Salmonella* infections were found to lack *$IL_{12}R\beta1$* chain expression. *$IL_{12}R\beta1$* sequence analysis revealed genetic mutations that resulted in premature stop codons in the extracellular domain [116]. A patient with severe infections as above and multiple adverse drug reactions had T cells unable to produce IFN-γ or proliferate in response to IL_{12}, despite the expression of wild-type *$IL_{12}R\beta1$* and *$IL_{12}R\beta2$* [186]. Defective $IL_{12}R$ signaling leads to low T-cell and NK-cell IFN-γ production [509]. $IL_{12}R\beta1$ and $IL_{23}R\beta1$ chains are associated in an AR deficiency with susceptibility to *Mycobacteria* and *Salmonella* infections [351].

The *STAT4* (2 forms, AD and AR [351]) *S721* mutant failed to restore IFN-γ production in STAT4-deficient $IL_{12}R\beta2$ transgenic cells [329]. STAT1, -3, and -5 activation by IL_{12} was lost, an impairment specific for IL_{12}; nor is activation of STAT4 alone sufficient for IL_{12}-induced IFN-γ production and proliferation [186]. Two unrelated infants HZ with respect to mutated STAT1 suffered from mycobacterial disease, but unlike patients with IFN-γR deficiency both died of viral disease [133]

Complement Deficiency

The complement is an integral part of the humoral defense system against infections and also for promoting inflammatory process (Figs. 1.63, 1.65). Complement deficiency was found in 6/176 Dutch patients (3.4%) over a 33-year period (0.1% × year) [156]. From the study of blood donors the prevalence may be of 0.03% in the general population [531]. Congenital deficiencies have been described for most of the proteins it is composed of (Tables 22.20 and 22.1F [167, 169, 453, 531], usually following the AR model. Properdin deficiency is the only complement deficiency that is X-linked [531]. HETs can be easily identified because their relevant component is present in the serum with a 50% concentration. The lack of one component at the HZ level serologically involves the blockage of enzyme release below and the absence of hemolytic activity, while that of controlling proteins causes its uncontrolled activation, consuming the factor that is the object of control and, in various ways, also of successive components [137]. Nonfunctional C1q variants have been observed, C1r and C1s deficiencies are often associated, probably because they are mapped on contiguous genes of chromosome 12 (C1q on 1) [167]. The B, C2 and C4 genes, situated on the short limb of chromosome 6, constitute along with others the HLA class III (Chap. 1). C6 and C7 are codified on chromosome *5p* and have a similar structure; C8 shows a different structure, because the molecule consists in three α, β and γ chains, united to form two subunits, α-γ and β dictated by different genes [495]. Alternative pathway deficiencies are extremely rare [328].

Complement deficiencies are accompanied by an *increased frequency of infectious pathologies* [155], although it is not rare to come across them in individuals who are apparently in good health, as in the case of C2 hereditary deficiency [422]. Also frequent are teens and young adults with autoimmune manifestations (Chap. 18). Classic pathway deficiencies are often associated with SLE-like diseases (systemic lupus erythematosus), ID of the early components of complement (C1-C3) are associated with risks of infections caused by encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, as well as by *meningococci* [363]. The incidence of SLE in patients with C1q, C4, or C2 deficiency is 90%, 75%, and 15%, respectively [378]. Partial C4 deficiency is also associated with SLE; 15% of patients with SLE exhibit C4A deficiency [531]. Several components are associated with development of membranoproliferative glomerulonephritis (Table 22.20 [167, 169, 453, 531]). In alternative pathway ID, the infections recognize pyogens as the most common etiological agents, while final common pathway ID (C5-C9) or properdin (P) have been associated with recurrent or invasive infections by *Neisseria (N) gonorrhoeae* or *N. meningitidis*, Gram-negative bacteria, and asplenia, agammaglobulinemia [167, 169, 363, 531]. It is estimated that the frequency of meningitis in subjects with HZ deficiency of the final C5-C9 pathway is 10%, 6,000-fold higher than in non-ID individuals [363, 488]. Some characteristics appear to associate the patients with complement deficiency and meningococcal disease: frequent recurrent episodes, an older age at the first onset, lower mortality compared to patients with a normal complement, and a prevalence of males [167].

C1 Deficiency

Over 50 patients with C1q, C1r and C1s deficiencies have been described, C1s deficiency only in two cases [239]. A selective and complete C1s deficiency in a 2-year-old girl with complex AIDs including SLE-like syndrome, Hashimoto's thyroiditis, and autoimmune hepatitis has been reported. Exon-specific amplification of genomic DNA by PCR followed by direct sequence analysis revealed a MZ nonsense mutation in the C1s gene exon XII at codon 534. Both parents were HET for this mutation [127]. A deficiency in one of these proteins is sufficient to block the classic pathway activation; deficiency results as a consequence of non-synthesis, which in the

Table 22.20. Inherited complement and complement-related protein deficiency

Deficient protein	Incidence		Reported prevalent clinical correlates	
	(%)	MW (K)	Infection	Other manifestations
Classic pathway				
C1q	1.5	450	Pyogenic	SLE-like; GN
C1r	0.5	85	Pyogenic	SLE-like
C1s	0.1	85	Pyogenic	SLE
C1r-C1s	0.5		Pyogenic	SLE-like
C1-Inh	56.7	105		Angioedema
C4	1.5	206	Pyogenic	SLE, GN, vasculitis
C2	8.7	102	Pyogenic	SLE, GN, JRA
Alternative pathway				
C3	1.8	190	Pyogenic	SLE, GN
Factor D	0.2	25	*Neisseria*	Recurrent infections
Factor H	0.8	150	Pyogenic	GN
Factor I	0.9	88	Pyogenic	Recurrent infections
Factor P	2.4	25	*Neisseria*	Fulminant infections
Common pathway				
C5	1.5	190	*Neisseria*	Meningococcemia
C6	5	128	*Neisseria*	Meningococcemia
C7	2.8	120	*Neisseria*	Meningococcemia
C8	3.7		*Neisseria*	Meningococcemia
C9	11.3	71	*Neisseria*	Meningococcemia

The incidence is based on the data from [167]; the inheritance is always AR, with the exception of C1-Inh deficiency (autosomal dominant) and Factor P deficiency (autosomal recessive or X-linked).
Data from [167, 169, 453, 531].
GN glomerulonephritis, *JRA* juvenile rheumatoid arthritis.

case of C1q amounts to 60% of cases, while in the remaining 40% the molecules are malfunctioning but cross-reacting with the native molecule [328]. C1r and C1s deficiencies are usually combined, due to the contiguity of the two genes; typically in these patients C1r is absent and C1s levels are reduced (20%–40%) [422]. Affected patients suffered from a SLE-like syndrome and sporadically from an extended predisposition to infections [155].

C1q Deficiency

Associated symptoms in C1q deficiency are SLE-like syndrome, rheumatic disease, and infection. Several children suffered from meningitis, recurrent septicemia, recurrent otitis media, pneumonia, and stomatitis; two died from meningitis septicemia [241].

C4 Deficiency

Unlike C2, HZ C4 deficiency is very rare and is caused by the non-expression of all 4 alleles (2 of C4A and 2 of C4B, 2 maternal and 2 paternal alleles), which can occur due to punctiform mutations, gene deletions, or other gene alterations that prevent gene transcription [28]. The two *4A* and *4B* genes are polymorphous, just like *C2*, *C3*, *C6*, *C4A* and *C4B* and the B factor (Bf); polymorphic variants of the other proteins are rare, with 12 different alleles for C4A and 23 for C4B identified at the moment. Moreover, two *loci* C4A and C4B null alleles *Q0* (quantity 0), do not codify for any phenotype, although often present in the general population [167]. In a 4-year-old Caucasian child who suffered from several bouts of pneumonia caused by respiratory viruses, eight episodes of acute otitis media, prolonged respiratory and urinary tract infections, molecular studies of the *C4* gene region revealed HZ deletion of HLA class III *CYP21A-TNXA-RP2-C4B*, generating total deficiency of

C4B and the flanking 5′ region up to C4A [230]. Moreover, in 7/13 cases the *C4A*Q0* alleles were related to a *C4A/CYP21P* gene deletion within the *HLA-B8 C2C BfS C4AQ0B1 DR3* haplotype. In 3/13 cases, the *C4B*Q0* allele was related to a *C4B/CYP21P* gene deletion within the *HLA-B18 C2C BfF1 C4A3BQ0 DR3* haplotype [212]. The *C4* null allele incidence is so elevated that 60% of the population expresses all *C4* genes, while 30% lacks 1–3 alleles [328]. The elevated number of *Q0* probably derives from the marked similitude of the two genes, which facilitates the unequal crossover, but this crossover in the HLA can modulate the expression of three *C4A* alleles and one *C4B* or vice versa; the *C4AQ0* allele spreading amplifies the risk of contracting SLE and juvenile RA (JRA).

C2 Deficiency

HZ C2 deficiency, *the most common in the Caucasian population,* has an incidence that varies between 1:10,000 and 1:28,000, whereas the HET carrier rate is 1.2% [456]. It is usually found in the *A25*, *B18*, *DR2*, *BFS*, *C2Q0*, *C4A*, *C4B2* haplotype context which, due to its considerable rarity, could assume a predictive value [238]. Two different types of C2 deficiency are known: in type I the synthesis is deficient due to the protein nontranslation, in type II there is a selective absence of secretion but not of synthesis, therefore C2 levels are 0.5%–2% of normal values [238]. HETs have a nonfunctioning gene, the complement profile is characterized by serum C2 concentrations equal to 50% of normal values; about 50% are asymptomatic, while the other half exhibit frequent infections and quite a few suffer from SLE and correlated syndromes [155]. HET C2 deficiency was associated with a 28-bp deletion in the *C2* gene (type I), mainly within the *HLA-A25 B18 C2Q0 BfS C4A4B2 DR2* haplotype [212]. In a certain number of cases, a C2 deficiency is accompanied by a partial Bf malfunctioning, genetically close to it. HZs can also have a deficient function of the alternative pathway [445]. Possibly this deficiency, like other quite common ones, may not always be reported in the literature: the total number of cases therefore underestimates the real prevalence, as is also found in children with *C7* deficiency [167]. *C2* deficiency must be suspected in all patients presenting pneumococcal infections after the age of 2 years [239].

Both *C2* and *C4* predispose to SLE, but this is not the expression of a particular genetic association caused by the same gene localization, because C1q, r, s, deficiencies, which also cause SLE, are situated as mentioned outside the HLA system [422].

C3 Deficiency

The molecular bases of this PID appear heterogeneous. The *C3* gene exists in different allelic forms, some of which have reduced functionality. One must remember the C3 important role in immune responses, also as far as APC and B cells are concerned, as well as the defensive role played in innate immunity along with C4. Since both pathways converge in the cleavage and activation of C3, there is no way that this defect can be corrected, and furthermore the opsonic power is greatly deficient, as is the C5 chemotaxis; therefore patients affected by HZ deficiency mostly present clinical symptoms totally similar to a congenital hgG with severe recurrent infections and at times the symptoms of CIC disease [239]. Although ID is severe, some patients apparently remain in good health and the syndrome may also in time become less severe, probably due to the higher number of immune experiences that allow a better effector function to antibody reactions mediated by the Fc receptor [167]. C3 protein was defective in noninfected Nigerian children with protein-energy malnutrition (PEM), but rose significantly in the presence of bacterial infection, thus sharing the values found in healthy controls [137].

C5 Deficiency

In its clinical expressions, C5 deficiency does not differ from the other deficiencies discussed here; the clinical consequences of absent C5a anaphylotoxin are unclear [328].

C6 Deficiency

One-fourth of all patients are asymptomatic. In Caucasians incidence is 1:60,000 [495]. Deficiencies associated with C6-C7 are rare but reflect the close genetic proximity of their pertinent genes; in C6-C8 deficiencies, 63% of patients lacking one component experience at least one severe episode of *Neisseria* infection and 5.5% one AID [239].

C7 Deficiency

Rare in Europe, C7 deficiency is the second most common complement deficiency in the Japanese (0.005%) [339]. In Italian children it has a prevalence of 10% and has been identified also in healthy siblings [531]. In Japan C7 deficiency is more associated with meningococcal meningitis than with *Neisseria* infections [339]. In a highly inbred Arab population, a C7 deficiency was associated with a mutation (*G1135C*) that is also prevalent among Israeli Jews of Moroccan ancestry [34].

C8 Deficiency

In the two forms of C8 deficiency (C8α + C8γ and C8β mapped on chromosome 1), the subunit not involved is present in the serum, also with reduced levels, and accompanied by altered functionality of the one involved. C8 deficiency has different characteristics due to the diverse associations of the β and α-γ chains; therefore Caucasians with this deficiency lack the β chain (10%), while colored patients lack the α and γ chains (90%) [495].

C9 Deficiencies

C9 deficiencies are poorly considered because they are often asymptomatic [339]; however, in Japan there is an incidence of 0.1% [339] and between 33% [155] and 25% of cases [339] present meningococcal meningitis. Congenital C9 deficiencies are very common among the Japanese (0.036%–0.1%) and represent 11.3% of all deficiencies (Table 22.20).

There have been >150 cases of congenital C5–C9 deficiencies reported, distributed unevenly between the various ethnic groups: C5 and C6 deficiencies are prevalent in colored patients and the C7 deficiencies appear to be more common in Caucasians [422]. An analysis of published studies shows that 14% of patients with sporadic meningococcal infections may have a C5–C9 deficiency [495]. Clinical patterns are overlapping.

C1 Inhibitor Deficiency

The most common complement deficiency is C1-INH deficiency (C1 inhibitor), responsible for hereditary angioedema that causes symptoms in HETs (Chap. 8).

Factor I Deficiency

At least 15 cases of factor I deficiency are known [328], with autosomal co-dominant transmission because parents show normal complement levels at 50% of factor I [239]. As in an H deficiency, serologically one has the alternative pathway activation, due to C3b non-catabolization that continuously forms C3 conversion with severe C3 deficiency, the levels of which do not exceed 15% of normal values [155]. In HZs, in addition to subsequent pyogenic infections, as in C3 deficiency [422], cutaneous rashes and urticaria caused by massive release of histamine and pro-inflammatory cellular products by anaphylotoxic fragments are reported [167].

Factor H Deficiency

A total deficiency of this 150-kD protein, inherited as an AR trait, has been described in a young patient with a hemolytic-uremic syndrome and in one case in Italy whose parents were first cousins [531]. Among 21 relatives of the proband studied, encompassing 3 generations, ten had low factor H levels, including her two children, indicating a HET factor H deficiency [157]. H deficiency results in uncontrolled breakdown of C3, and in depletion of Bf, P and C5 [157]. C3 and C9 components are decreased in varying degrees, while C3 and C5 are found in plasma in traces and only as activated molecules [167].

Factor D Deficiency

Inheritance of factor D deficiency is for the moment uncertain [488] (Table 22.20): a partial deficiency (6%–12% of normal concentrations) has been described in two MZ twins, and a total deficiency in one male. This deficiency, serologically characterized by the non-functionality of the alternative pathway, is clinically accompanied by an increased susceptibility to *Neisseria* infections [239], leading us once more to emphasize the alternative pathway significance as a substantial means of defense for a broad spectrum of damaging actions caused by bacterial infections.

Properdin Deficiency

Properdin deficiency is the only one inherited as a characteristic linked to the chromosome X and only affects males [190]. At the moment, >50 cases have been described. The deficiency can be materialized by a total P absence, with levels reduced to 10%, with normal levels, however, showing an altered functional activity [239]. Specific research has shown a remarkable reduction of C3A and B titers, which represent the C3-convertase proteins, with a consequent heightened consumption due to the alternative pathway spontaneous activation. Males are affected by septic episodes caused by *Neisseria*, sometime fulminating, with onset even occurring during the 1st year of life [488]. There is no evidence of increased susceptibility to CIC diseases or infections caused by other organisms [155], thus implying that a functioning alternative pathway is particularly important for a defense against infections [328].

Correctly identifying children with complement abnormalities is important and worthwhile if any of the following factors are present: ID (such as repeated or unusual infections with other organisms, FH, unusual course of the illness, etc.), repeated Neisserial infections, infection with an unusual serogroup, fulminant disease in males (P deficiency), coexisting angioedema, autoimmune, or connective tissue disorders [220].

Children with RRIs

The fact that a child during the initial period of life should experience a certain number of URTIs or LRTIs is within the norm: RRIs are mainly caused by immunological immaturity or inexperience, both transient. A typical symptom outline is difficult to define, and prevalence is also little known. In ID children, a basic pathology is present instead, which encourages the recurrence of infections.

Definition and Prevalence

Although a distinction between infection and associated disease is important, childhood infections might have a key role in stimulating the maturation of the immune system, and the microbial burden in early life has been invoked as a protective factor against wheezing and asthma (Chap. 4). Preschool-age children have an especially high frequency of VRIs, with most having three to eight infections per year and 10%–15% have ≥12 VRIs per year. The rate in children aged 0–4 has been about 1 in 200 children compared with about 1 in 500 for children aged 5–10, and about 1 in 1,000 for those aged 11–17 [413]. RRIs may be defined as >six episodes of URTI and/or >3 LRTIs in the previous year, or based on age and ≥8 episodes per year if aged <3 or ≥6 episodes per year if aged ≥3.

Predisposing Factors

Several risk factors can influence the onset and recurrence of infections [217]. It is clear that the younger the child is, the more he/she may fall ill: this is also related to serum Ig levels (Table 1.15). The dogma of primary and secondary responses also may not apply to infections, at least those caused by *Rotavirus,* which confers considerable protection only after various infectious events and in children aged 1 [511].

Environmental factors in the absence of a basic pathology are important. It is also obvious that the more crowded the environment the child lives in, the more probable that infection becomes. In addition to the number of siblings, other factors such as socioeconomic status, age (preschool children), contact with outside persons, especially babysitter, early social contacts, exposure to passive smoke, indoor and outdoor pollution may be found to be related to the hygiene hypothesis (Chap. 4). However, daycare attendance, which was considered to be an indicator of exposure to respiratory pathogens, and the presence of siblings, increased the risk of URTI in preschool children aged 4–5 [273], and in the 1st year of life for children with FHA [88]. Among children with FHA, the protective effect of day care attendance in early life against the development of atopy only begins by 2 years, and against wheezing this may not be observed until after 4 years [89]. The particular ease of smoking parents and/or relatives who fall ill with influenza has been known for some time, to the same extent that the children who live with them are affected by RRIs (Table 4.24). The impact on RRI incidence as it is related to children in kindergartens, has been reflected by a significantly increased morbidity observed in babies aged 3 months to 3 years who go to daycare, who show a number of more severe and longer lasting infections per year [523], with an incidence of 6.5% in children who stay at home compared to 13.1% of those in kindergartens [524] and an increase of 49% of OME persistence (Chap. 15). In children exposed to cigarette smoke, the risk increased 3-fold for LRTI [221] or by 3.5-fold, equal to ≤3 episodes of respiratory infections each year [26].

Studies on *environmental pollution* have identified the most damaging agents: conclusive data on fine particles in suspension and polluting derivatives is available, proving a significantly increased risk of infantile RRIs: Table 4.20 indicates that NO_2 reduces immune defenses against RTIs, provoking alterations of the epithelium and of the lymph node cells, with negative effects on mucociliary clearance and macrophages.

The *biological role played by* NO_2 in the *domestic pollution* derived from the home has been ascertained to be related to cooking and the smoke released by combustion [26]. Using wood for heating leads to SO_2 development, while radiators cause the air to dry, which in turn causes potentially infected particles to remain in suspension. Pollutants are increasingly responsible for indoor pollution (Chap. 4).

Although levels of *micro-pollution* are not easily ascertained, significant associations with acute RRIs and conditions such as polypnea and dyspnea have been reported, especially in <2 year-old children [507]. These children's capacity to evoke adequate responses is genetically controlled; however, it is commonly known that their parents or siblings have suffered similar illness as children. In subjects with physiological immaturity of the immune system, VRIs more easily cause infectious episodes, which are important factors to be considered only in the presence of recurrent or incompletely cleared conditions [165].

Predisposing factors related to a basic pathology derive from perinatal factors, more common in premature babies, which can lead to respiratory tract alterations and consequently to bronchopulmonary dysplasia; anatomical anomalies; cystic fibrosis, which can become recurrent pneumonia; adenoiditis causing otitis and OME; congenital ciliary dyskinesia; humoral deficiency and PID characterized by recurrent sinopulmonary infections [101] (Table 22.21) [79].

Viruses are the principal etiological agents, and over 159 different kinds have been isolated (see Chap. 15):

- *Pharynx: Rhinovirus, Coronavirus, Herpesvirus, Adenovirus, Coxsackie-virus, Influenza and Parainfluenza virus, EBV, CMV*

Table 22.21. Infections most frequently seen in patients with primary immunodeficiency

	Bacteria				Fungi			
	H. influenzae	*Pneumococcus*	*S. aureus*	*Campylobacter*	*P. carinii*	*Candida*	*A. fumigatus*	*Cryptococcus*
	Meninges and chest		Skin	Gut	Lungs			
Antibody deficiency	++	++	+	++	–	–	–	–
Combined T and B cell defects	++	++	+	+	++	++	++	+
Selective T cell defects	–	–	–	–	–	+	–	–

	Viruses					Protozoa		
	H. zoster	*CMV*	*H. simplex*	*Polio*	*ECHO virus*	*G. lamblia*	*Cryptosporidia systemic*	*T. gondii*
						Gut		
Antibody deficiency	+	–	–	+	++	++	+	–
Combined T and B cell defects	++	+	+	+	–	++	+	+
Selective T cell defects	++	++	–	–	–	–	–	–

++ Most frequent, + less frequent, – rare.
Data from [79].

- *Middle ear: RSV, Adenovirus, Influenza virus*
- *Larynx: RSV, Adenovirus, Influenza and Parainfluenza virus, Rhinovirus*

A novel member of the coronavirus family has been characterized, which is associated with cases of SARS (severe acute respiratory syndrome). Phylogenetic analyses and sequence comparisons showed that SARS-coronavirus is not closely related to any of the previously characterized coronaviruses [421].

As investigated by a 15-year study the overall prevalence is age-related, and different between children aged 0–4 (Fig. 22.46) [326] and those aged 5–19 (Fig. 22.47) [326]: *RSV and rhinovirus have a different impact on the first group* (58% vs 28%) *and the influenza viruses on the second group* (9%–48%). Incidence in young children was 1.75-fold higher than in those aged 5–19 [326]. RSV causes bronchiolitis in breast-fed babies, with a higher frequency the younger the child is (Tables 11.24, 11.25), and rhinitis in older siblings. Even an infectious agent neglected for some time, such as *Ureaplasma urealyticum*, causes a lung pathology in younger children while sparing those >3 years old [271].

The *bacteria* most commonly involved are: *Streptococcus pyogenes* (pharynx and larynx); *Haemophilus in-*

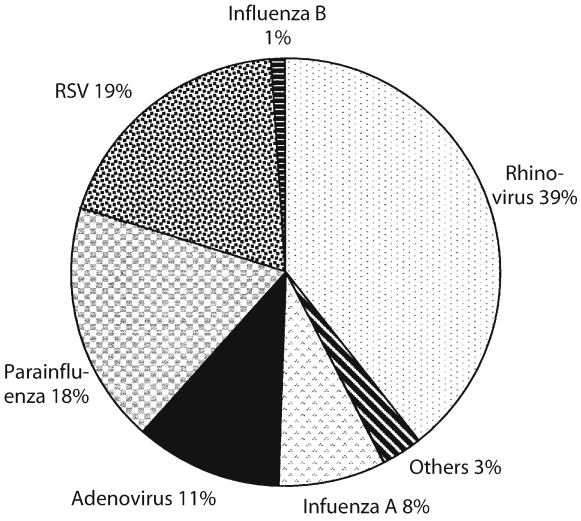

Fig. 22.46. Prevalence of respiratory viruses in children aged 0–4 years. Annual isolation and percentages. (Data from [326])

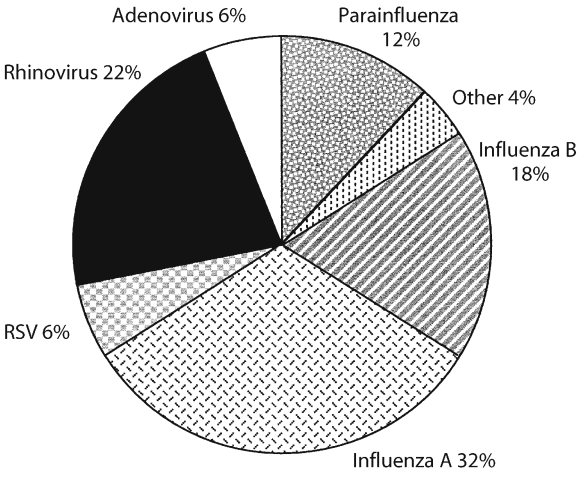

Fig. 22.47. Prevalence of respiratory viruses in children aged 5–19 years. Annual isolation and percentages. (Data from [326])

fluenzae (middle ear and larynx reaching the epiglottis); and *Streptococcus pneumoniae* (middle ear). There are often secondary bacterial infections such as complications caused by VRIs, which certainly contribute to recurrent infections and/or the onset of chronicity [508]. In Chap. 4 we reported several studies which concluded that early infections may protect from atopy development (hygiene hypothesis).

Immunodeficiency

We must distinguish PID outlines and pseudo-ID in children with RRIs.

Children with PID

Summarizing the aforementioned, severe and recurrent LRTI and sinusitis are the principal clinical manifestations in children affected by deficiencies prevalently involving *humoral immunity* (Table 22.1). These children fall ill *during the first weeks of their lives* and often contract infections caused by opportunistic agents, fungi, protozoa and viruses, and as months go by are also affected by malnutrition and failure to thrive. Episodes affecting the airways, particularly common in cellular and combined ID, tend to become longer or severe, especially if complicated by pneumonia [44]. Chronic disorders such as sinusitis and bronchiectasis (sinobronchial syndrome) are not rare; interstitial pneumonia is in most cases caused by *Pneumocystis carinii* and results in tachypnea and lung hyperinflation [409]. Infections supported by the *Herpes simplex*, EBV and CMV are also common: males with XLP exhibit a deficient response to EBV [446]. Severe and recurrent sinopulmonary, WAS, HIgES and ATA infections as well as severe RRIs in CGD, complement deficiencies and LAD 1 to V must also be borne in mind. Cases of recurrent pneumonia should be warning signals to rule in 4.8% pediatric cases of PIDs [392]. The second most important manifestation is *chronic diarrhea:* in some cases the infections are caused by rotavirus and enterovirus among which the ECHO: *Giardia lamblia, Salmonella* and *Campylobacter* can also cause chronic enteric infection; malabsorption resistant to treatment can be ascribed to *Cryptosporidium* [80].

Children with RRIs

RRIs are common in children. They reflect the immaturity of the immune system in its encounter with environmental antigens; this developmental delay during the first years of life fosters the development of RRIs. Thus, *RRIs are part of the growing-up process of any child* [47]. The consequences of RRIs can be of a profound and sometimes protracted alteration of the different immune defense mechanisms, which place the child in an undefended position, similar to the condition observed in children with PIDs, compromising the phagocytes, lymphocytes, NK cells, antibody production, and ILs at every occasion [419]. The responsible viruses for these infections have a development limited to surface mucous cells, spreading from cell to cell due to contiguity, while the viremic stage is absent or remains marginal. The incubation period is therefore brief, normally <3 days; consequently the immune response may not be capable of ensuring a protective function, or it only intervenes partially, clarifying the potentially unlimited number of infectious episodes [392]. From a pathogenetic point of view, the virus works by triggering the development of IgE and allergic sensitization and/or damaging the immune structures [322, 519]. In the first case, it is known that experimental infection in mice with RSV is capable of significantly increasing the absorption of ovalbumin (OVA) administered by aerosol, of IgG, IgE and anti-OVA sIgA (Fig. 4.26) and of increasing the synthesis of IgE and specific IgG to ragweed also administered by aerosol. The result is that the majority of infants become infected with RSV, although LRTIs develop in only about 20% [382]. Approximately 25%–50% of those subsequently experience recurrent acute asthma from VRI [530].

The mechanism by which VRIs induce atopic sensitization in experimental models is identified with antigen penetration and sIgE synthesis [322]. Studies show that viruses increase mucosal permeability, by modulating antigen uptake and altering antigen processing by the mucosa, which results in the IgE-suppressor T-cell depression, while IFN-ɣ-modulated histamine release further increases mucosal permeability [162]. It is probable that the immune deficiency is secondary to VRIs, because many viruses are capable of inducing transient

modifications of both humoral and CMI, therefore not only of antibody synthesis or phagocyte and neutrophil functions, but also of T lymphocytes and related ILs [457]. It is also possible to hypothesize that *persistent VRIs are capable of inducing Th2 activation* by antigens or super-antigens: Th2 T cells with IL_4 help induce virus-specific $CD8^+$ to produce IL_5, which recruits eosinophils in the respiratory tract, thus reducing IFN-ɣ secretion. Thus there may be an increased interaction of IgE mast cells in these subjects with immunoregulatory alterations, due to a marked lymphoproliferative response and the elaboration of other ILs, which consequently amplifies IgE production in the respiratory tract. The IgE response is thought to lead to a greater production of bronchoconstrictor mediators by effector cells; viral infections themselves may induce these cells to release histamine. It is also known that humoral deficiency, especially of IgA, very often opens the way to Gram-positive germs causing viral and bacterial infections, thereby completing the circle [409]. Interestingly, only 2/13 children have at least a significant production of antigen-specific salivary IgA against *Klebsiella pneumoniae* [418]. Even if the alterations are transient and aspecific in children with RRIs, their occurrence and persistence for a number of months, for a year and even longer, leads to believe that the immunosuppressive mechanisms set off by the first episode occasion more severe, profound and lasting consequences for the immune functions than those occurring in their normal peers [36, 273]. Having at least one physician-diagnosed LRTI in the 1st year of life was significantly associated with recurrent wheezing (OR, 2.0) and asthma (OR, 2.5) [89]. At the base of this exclusive predisposition in children for contracting RRIs, there is a NK cell reduction [419] and immune deficiencies related to the global lymphocyte population, the CD4 and the CD4:CD8 ratio, unbalanced toward CD4 T cells, prevalent in children expressing coughing compared to those with bronchial hyperreactivity (BHR) [374]. Other T-cell deficiencies in children with humoral anomalies include a considerable spontaneous production of IL_2 and IL_4, both generated by Th phenotypes, or alternatively by the Th0, which express both ILs [216]. It is similarly feasible that virus-specific CD8 deprived of cytolytic activity are converted into Th2-like T cells when IL_4 is present [216].

CMI in these children consists above all in the transient T-cell numeric and functional depression, coinciding with a deficiency of ILs necessary for their activation, proliferation and differentiation. The virus toxic effect also acts directly on the T cells, inciting rough structural modifications including giant polynucleate cells, which jeopardize homing and recirculation capacities, to the point of immunosuppression, affecting the specific lymphocytes for that particular virus, thus favoring the attacking organism [552]. A condition of immunosuppression occurs also as a result of superantigen (SA) orchestration, which, stimulating a large number of lymphocytes to release ILs, deter the specific response addressed at them. Some SAs, especially the staphylococcal enterotoxins and the *Pseudomonas* endotoxins A (Table 1.29), seem to activate the cytolytic effector cells capable of destroying the host cells [323]. Viruses, on the other hand, can both induce and inhibit apoptosis (Table 1.19). The host is, however, ready to fight them and *can rely on well-organized defensive bases:* if the viruses recombine the DNA, the host T and B cells recombine the genes codifying the antigen receptors; if they mutate, and they can do so at each generation, B cells hypermutate their V exons by at least four times; if they invade different sites, B cells respond with their isotype machinery directing the more appropriate antibodies wherever requested [521]. The lack of NK cells, which are in the front line of defense against viral infections, and with which the altered production of IL_2 and factors activating the phagocytes are associated, appears significant, but it is unclear whether the deficiency is primary or secondary to viral infections [419]. The basic question with potential therapeutic consequences therefore remains unanswered, and is probably destined to remain so until more sophisticated tests are available to clarify this issue, although the NK-cell reduction in these children corroborates the first hypothesis.

Recent data emphasizes the important defensive activity of cytotoxic CD8 T cells and by different ILs, including IL_{10}, IL_{28} and IL_{29} active in antiviral defense: if the infected cells express on the surface the viral antigen processed in association with HLA class I molecules, cytotoxic CD8 take care of killing the cell. Normally the CD8 are activated by the virus itself or by soluble factors released by the infected cells and mediate the cellular lyses after recognizing HLA class I antigens on the same cell. However, if the APC is a macrophage, it can be parasitized by the virus, with consequently reduced chemotaxis and microbicidal activity and in particular the capacity to cooperate with T cells [322]. Considering that macrophages may have evolved specific mechanisms for directing T-cell development toward the Th1, since CMI can solve some infections, it is clear that their dysfunctions appear in the insufficient NK cell activation and inadequate Th1 development in response to infections. IFN-ɣ, produced not as a direct consequence of infection, but probably by IL_{12} and/or IL_{15}, stimulates the nearby cells to block the nucleic acid transcription and therefore the viral replication, preventing the infection of those cells to which virus spreads due to contiguity. Therefore the IFN-ɣ species-specific antiviral activity takes place at the very first stages of the infection, preceding the antibodies [322].

The response to germs' capsular polysaccharides, particularly deficient until the age of 2, although encounters with these germs are abundant during this period of time, still remains to be evaluated [508]. However, selected children, although normal from an immunological point of view, *may have a deficient antibody response* even aged 4–8 [195] with a percentage of

nonreactive children of 4%–19% [143, 430], rising to 13%–42% if with an IgA and/or IgG subclass deficiency [198, 216, 430]. While 50% of children have low anti-pneumococcus IgG_2, antibody responses are totally absent in 40% of dysimmunoglobulinemics with a virtual absence of IgA and IgG_2 [430], confirming previous data [198]. IgA and IgG_2-deficient children show a clinical pattern with elevated susceptibility to *S. pneumoniae* infections [430].

Von Waldeyer ring (NALT) is a significant constituent of immune response, and of resistance to NALT-dependent infections. Among the consequences of chronic adenotonsillitis (Table 15.2) the *statistically significant decrease in Ig levels, including sIgA*, must be evaluated eventually in relation to RRI complications (Chap. 15).

IgG Subclass Deficiencies and RRIs

IgG subclass deficiencies can be present in children with chronic and/or severe asthma, associated or not with SIgAD, in children affected or not by asthma with severe RRIs or with chronic nonallergic respiratory clinical symptoms, in children with SIgAD, ATA, WAS, CVID, SCID and in healthy subjects [80]. The anti-virus antibodies generally belong to the IgG_1 and IgG_3 isotypes while the IgG_3 protect from microbes with polysaccharide antigens, such as *S. pneumoniae* and group A and type b *H. influenzae* [153]. Table 22.7 shows normal values for IgG subclasses in subjects aged 0–15. Unlike total IgG concentrations, IgG_1 and IgG_3 reach normal levels within the 1st year, IgG_2 mature more slowly, ensuring an effective antibody response only after the 2nd year, and IgG_4 develop even more slowly. Various authors believe that the role played by IgG subclasses is unique and vital in defending from infections: IgG_2 deficiency is associated with an increased susceptibility to infections by bacteria expressing capsular polysaccharides, such as pneumococci, meningococci, *H. influenzae, Bordetella pertussis*, etc., as well as other factors capable of setting off an inflammation [113, 465]. IgG_4 deficiency instead seems associated with a marked predisposition for RRIs [333]. However, undetectable IgG_4 subclass levels are a common finding in normal individuals and an accurate detection of very low levels of IgG_4 is technically difficult to achieve [450, 465].

Babies aged ≥1 month have levels of circulating *lymphocytes secreting all four subclasses* in a higher number than adults; therefore the capacity to produce antibodies exists well before a full humoral response is developed.

Subnormal IgG subclass concentrations, especially of IgG_2, are also observed in healthy children who do not present an increased susceptibility to infections.

Low subclass levels do not necessarily indicate that the subject will experience immune disorders exclusively linked to these, nor do normal concentrations guarantee that the child will be spared complications. Subjects have been observed both with normal IgG_2 levels and with recurrent infections and with a subclass deficiency, which often do not form other types of antibodies [450].

IgG subclass determination does not indicate what the humoral level restricted to that molecule is: this is a *characteristic in children*, unlike adults, even though research was carried out using the same methods in the two studies [465].

Interesting hints come from studies involving children with RRIs:

Of children aged 2–10, with a confirmed diagnosis of susceptibility to infections, 67/567 (11.8%) had a *IgG subclass deficiency,* almost all concerning IgG_4, with several associations [283].

In other children, the *selective IgG_4 deficiency* was statistically significant compared to atopic controls without RRI, associated not so much with a well-defined state of ID as to a respiratory tract defense mechanism deficiency, in view of the fact that the relative prevalence of this subclass in secretions may indicate a role in the mucosal defense [333].

Other children with *typical symptoms of recurrent infections,* lymphadenopathies, failure to thrive and HgG exhibited low IgG_2 levels, confirming that normal levels of total IgG do not exclude a subclass deficiency [454].

In a cohort of young babies, IgG_4 deficiency was only present in 78/267 subjects (37%) [198]; however, the absence of a control group makes the results incomparable. An IgG subclass deficiency is therefore able to induce or worsen chronic respiratory symptoms in allergic and nonallergic children, especially if predisposed to developing these affections [113], or in subjects with SIgAD [327]. With time these deficiencies, and eventually also those associated with IgA, can normalize [29].

In conclusion, transient and persistent IgA and/or IgG_2 deficiencies have been reported in a small percentage of asymptomatic children [446], but even if IgA and IgG subclasses are not always required as such for a normal immune response, their deficiency may predispose to RRIs [160].

Atopy

As previously illustrated in Chap. 11, the close links between atopy and RRIs are known, and this is confirmed by the observation that asthmatic children have a higher incidence of RRIs than their nonasthmatic siblings. It has been known that RRIs during the early periods of life can play a role in the development of BHR and atopy: in the classic study by Frick et al [173], in 11 out of 13 allergic children sensitization was propitiated by RRIs. With continued observation, the authors noted the presence of high IgE levels, positive RAST and histamine released by leukocytes after infections [172]. In a cohort of 73 asthmatic children aged 0.8–3.1, the 21 affected by

RRIs had a higher incidence of FH positivity (p=0.015), increased IgE (p=0.021), as well as a combined IgA (p=0.038) and IgG (p=0.018) deficiency [296]. IgE hyperproduction could be the result, not only of the well-known association between IgG subclasses and IgE and their coregulation of IL_2 expression [296], but also of a virus-caused unbalanced CD4:CD8 ratio [374]. These results link atopy to RRIs, confirming that the state of chronic inflammation and BHR induced by allergic sensitization is an ideal substratum for the adhesion and chronic evolution of bacterial and/or viral infections.

Clinical Presentation

There are no specific clinical outlines for RRIs. On the contrary, symptoms are extremely varied, with, as previously mentioned, infections caused by bacteria and viruses. URTIs are common at age 4. During the last 12 months, 9.5% of the children experienced more than one bout of acute otitis media, 6.9% had more than one pharyngotonsillitis episode, 47.7% contracted >2 common colds, and 3.2% had rhinitis weekly or monthly [273]. There are children who, during the period of maximum exposure due to *biological immaturity and immunological inexperience,* suffer from one episode each month affecting different organ systems, as well as lymphadenopathies and failure to thrive. The capacity for inducing BHR in normal subjects and worsening the symptoms in those already ill are precisely caused by VRI, also facilitating greater penetration of inhaled viral allergens [530] (Table 11.10); RRIs in turn predispose to sinusitis. Lower IFN-ɣ levels produced by 18 of 53 children at 6 months of age were even greater if the comparison was made between children with RRIs and those with no or maximally one RRI during the follow-up period [382].

Rhinovirus-induced infections (Table 11.11) take the appearance of common rhinitis, but stimulate mastocytes to release histamine, contributing to BHR development and the perspective of delayed reactions.

Diagnosis and Differential Diagnosis

Children with PID

A differentiating feature is the respiratory infections in the ID child that may also result from opportunistic pathogens [80] (Table 22.21). Respiratory infections should be under control. A screening of humoral immunity revealed low Ig levels in 4.6%, low IgA levels in 2.3%, and SIgAD in 1.3% of children [270].

During the last few years, increasingly sophisticated diagnostic techniques have permitted *prenatal diagnosis* in many cases (Table 22.22) [61, 166, 389, 406, 414, 491]: in forms supported by RAG-1 and/or RAG-2 mutations a diagnosis even at the 10th–12th or at the 20th week of pregnancy is possible, so as to evaluate the immune phenotype in the fetal blood [491]. WCCs (white-cell counts) in CB and differential counts can be used to detect the lymphopenia that is commonly present in infants with SCID. However, subset analysis by flow cytometry is necessary to enumerate T, B and NK cells. Subsequently, SCID diagnosis will be suspected when overwhelming opportunistic infections occur [320]. Depending on whether one suspects a humoral, cellular or innate immune deficiency, we begin with the algorithm in Table 22.23 [107], positive if infants or children have ≥2 of these signs, then Tables 22.24–22.26 [101, 478, 522] on laboratory tests can be consulted. However, children with variable levels of antibody ID may end up with different diagnosis [269]. Children with HIgMS presented initially with a history of an increased susceptibility to infection including *Pneumocystis carinii* pneumonia [539] in 43% of children [290]. In PIDs affecting phagocytes, because of the relatively narrow spectrum of disease-specific infections (such as aspergillosis in CGD), careful attention to the microbiology laboratory early in the course of evaluation of a patient suspected of having a PID is crucial to orient the work-up in the appropriate direction [416]. In a male newborn referred to hospital at *27 days of age* for fever, hemodynamic failure and an inflammation syndrome caused by pulmonary infection, culture of tracheal, bronchoalveolar lavage samples and lung biopsy grew positive for *A. fumigatus*, enabling the diagnosis of CGD [337]. To investigate whether patients with undiagnosed ID could be identified in diverse inpatient hospital populations, a scoring algorithm and computer screening method was updated [107] on the basis of ICD-9 codes to survey the discharge diagnoses of all hospitalized patients over periods of time. Thus 17 ID patients were identified, eight of whom were children aged 2–10 (47%), two with neutropenia, two with IgG deficiency, one with LAD, one with DGS, etc. [108]. We also suggest including congenital phagocytic defects in the differential diagnosis of recurrent bacterial or fungal infections in a child [285]. A congenital complement deficiency should be suspected if levels of even one component are reduced [167]. Early diagnosis is essential for choosing the necessary treatment [44].

Differential Diagnosis

The differential diagnosis of PID will emphasize the different characteristics schematized in Table 22.27, to which one must add objective rarity, while FH and child gender become important [10]. In subjects suffering from Omenn syndrome, WAS, severe combined and cellular IDs, the screening of clinical symptoms may be useful at birth and during the first few months after birth (Table 22.28) [61, 80, 399]. The localization of infections is multiple, the ID child usually appears to be ill, and the peripheral lymph nodes and lymphatic

Table 22.22. Prenatal diagnosis in PID

PID	Suggested investigations
A. T lymphocytes	
X SCID	Lymphocyte subsets and function X-chromosome inactivation Nucleic acid sequence
AR SCID	Lymphocyte subsets and function
Nucleic acid sequence	
ADA deficiency	ADA in amnion cells
PID with hyper-IgM	Linkage, nucleic acid sequence
PNP deficiency	PNP in amnion cells
HLA class II deficiency	Absence of HLA class II molecules
ATA	Linkage, nucleic acid sequence
WAS	Fetal platelets, nucleic acid sequence
DiGeorge syndrome	FISH
B. B lymphocytes	
XLA	Mature B cells in fetal blood linkage Nucleic acid sequence X chromosome inactivation
CVID	Nucleic acid sequence
C. Phagocyte function	
LAD deficiency	CD11/CD18 by flow cytometry
CGD, XL	NBT, linkage studies
CGD, AR	NBT, nucleic acid sequence
Chédiak-Higashi syndrome	Giant lysosomal granules in fetal tissue

Data from [61, 166, 389, 406, 414, 491].
CGD chronic granulomatous disease, *FISH* fluorescence *in situ* hybridization, *LAD* leukocyte adhesion deficiency, *NBT* nitroblue tetrazolium, *SCID* severe combined immunodeficiency, *XLA* X linked agammaglobulinemia.

Table 22.23. Clinical algorithm for the screening of PID in infants and children

1. Family history of PID
2. Failure to thrive
3. Oral or cutaneous candidiasis after 1 year of age
4. One or more episodes of cellulitis, meningitis, osteomyelitis, or sepsis
5. Two or more episodes of pneumonia
6. Recurrent deep cutaneous or organ abscesses
7. Two or more episodes of sinusitis in 1 year
8. Two or more months on oral antibiotics with little effect
9. Need for IV antibiotics to clear infections
10. Eight or more episodes of otitis in 1 year

Modified from [107].

pharyngeal tissue are almost imperceptible [102, 196]. The seriously undernourished appearance should be noted, more often observed in children with SCID [162]. RRIs can be observed in other CMI forms [162]: deficiencies of CD3 γ and ε chains [18, 249, 471], ZAP-70 [92, 139] and HLA class II [77, 256]. Finally, children with AIDS will seem to be in severe general condition and this disease is a paradigmatic example of how HIV can overturn the T lymphocyte immune defense with regards to opportunistic infections [79]. In some cases of pediatric AIDS there is hgG that is indistinguishable from PID and that belongs to the differential diagnosis of severe recurrent infections during the first few months after birth [79]. As far as TIH is concerned, the confirmation of a normal presence of the B lymphocytes and low levels of intrinsically produced Ig is resolutive, compared to agammaglobulinemia [61].

Table 22.24. Laboratory tests in children with suspected humoral immunity deficiency

Screening tests
Quantitative IgA, IgG, IgM, IgE serum levels
Isohemagglutinin titers
Antibody responses to prior vaccine antigens (polio, diphtheria, tetanus, rubeola, measles, etc.), Schick test
Advanced tests
Number of circulating B cells (CD19 and/or CD20)
Secretory IgA levels (saliva, tears)
Antibody responses to new vaccine antigens (typhoid, pneumococcal, influenza, etc.)
Lateral pharyngeal X-ray to visualize adenoidal tissue
Specific tests
Advanced B-cell phenotyping
IgA and IgG subclass levels
IgA autoantibodies (selective IgA deficiency)
Lymph node biopsy
Surface markers
Suppressor-inducer cells (CD4/CD45RA)
Helper/inducer cells (CD4/CD29)
CD154 (CD40 ligand) on activated T cells HIgMS
Functional tests
LTT with B-cell mitogens (PWM)
Ig synthesis in vitro
Studies on T-cell function (CVID)
Suppressor cell function:
PWM-Ig synthesis in vitro
Con-A-induced suppression of autologous lymphocytes

Data from [101, 478, 522].
Con-A concanavalin A, *CVID* common variable immune deficiency, *LTT* lymphocyte transformation test, *PWM* pokeweed mitogen.

Table 22.25. Laboratory tests in children with suspected cellular immunity deficiency

Screening tests
Lymphocyte count and morphology
Thymic shadow on chest radiograph
Delayed skin test (tetanus toxoid, Candida, streptokinase, mumps, etc.)
Specific cytotoxicity assay (NK, ADCC, CTL, etc.)
Karyotype
HLA typing
Class I: all cells
Class II: B lymphocytes, monocytes, activated T cells
Advanced tests
Advanced T-cell phenotyping
Number of circulating T-cell subsets
Immature T-cell subsets
Biopsies (lymph nodes, liver, skin, thymus)
Enzyme assays (ADA, PNP)
Specific tests
Surface markers
Mature T cells (CD3)
Major T cell surface marker subsets (CD4, CD8, CD4:CD8 ratio, etc.)
Adhesion molecule typing (CD11a, CD18, selectin ligand)
Functional tests
LTT with mitogens (PHA, anti-CD3, PWM)
LTT with antigens (tetanus, Candida, PPD)
LTT and Ca release with PMA, ionophore, IL_2
Further surface markers (CD7, TcR), CD43, activation markers CD25, CD38
If signal transduction is deficient CD3-γ, CD3-ε, ZAP-70, etc.
Cytokine production (IL_2, IL_3, IL_4, IL_5, IFN-γ, IL_{12})
$IL_2R\ \gamma$ chain (X-linked SCID)
Chromosome fragility (ataxia-telangiectasia, Bloom's syndrome, etc.)

Data from [101, 478, 522].
ADA adenosine-deaminase, *ADCC* antibody dependent cell-mediated cytotoxicity, *CTL* cytotoxic T lymphocytes, *LTT* lymphocyte transformation test, *PHA* phytohemagglutinin, *PMA* phorbol myristate acetate, *PNP* purine nucleoside phosphorylase, *PPD* purified protein derivative.

Children with RRIs

An articulate case history often identifies the familiarity of RRIs, usually with an absent basic pathology and the frequent predisposing environmental factors (Table 22.21), among which passive cigarette smoking stands out. Maximum prevalence occurs during the first 2 years of life or during first contacts with school, the disease is limited in time, and there is usually a single location. In most cases the pseudo-immunodepressed child is clinically normal in all other respects [374]. In ten reported SARS-infected children from Hong Kong, fever, cough, and runny nose were common in the younger children, whereas teenagers presented with symptoms of malaise, myalgia, chill, and rigor [223] similar to those of adults [542]. Thus

Table 22.26. Laboratory tests in children with suspected innate immunity deficiency

Screening tests
PMNs absolute number and morphology, twice weekly over 4 weeks
CH_{50}
C_3
C_4
NBT dye test (now rarely used)
IgE levels
IgG and subclass levels
Cases with neutropenia anti-PMN autoantibodies
Cases with neutropenia without autoantibodies: bone marrow aspiration
Advanced tests
Leukocyte random mobility and chemotaxis
Phagocytosis assays
Bactericidal assays
Opsonic assays
Rebuck skin window
Complement activation assays (C3a, C4a, C5a, etc.)
Complement component assays
Specific tests
Leukocyte turnover
Chemiluminescence
Enzyme assays (MPO, G6PD)
Deformability, adherence and aggregation
Chemotactic factor assays
Complement alternative pathway activity
Functional assays
F actin, 47-kD and 88-kD proteins (regulate actin polymerization)
Defensins, cathepsin G (from specific granules)
Cases with neutropenia
Studies on PMN: hydrocortisone test, G-CSF R
Other studies
Rule out glycogenosis IB
Transcobalamin II
Function of exocrine pancreas
Cases without neutropenia
Killing test
If O_2 production is impaired with soluble and particulate stimuli:
CGD subtypes, G6PD
If O_2 production is impaired with a particulate stimulus only:
Adhesion molecules

Data from [101, 479, 522].
CGD chronic granulomatous disease, *CH_{50}* hemolytic complement 50%, *G-CSF* Granulocyte-colony stimulating factor, *R* recombinant, *G6PD* glucose-6-phosphate dehydrogenase, *MPO* myeloperoxidase, *NBT* nitroblue tetrazolium.

SARS seems to have a less aggressive clinical course in younger children [223].

When in doubt, a broad spectrum of laboratory tests are available: CBC, proteinemia and protidogram, serum Ig levels, or in secretions and IgG subclasses (Table 22.7), immunoelectrophoresis (homogeneous components, κ/λ), dosage of isohemagglutinin, five other natural antibodies, the sweat test [10], in strictly selected cases also a lymphocyte population and subpopulation count (Tables 1.34–1.39), and X-ray of paranasal sinuses. Analysis of the lymphocyte profile sometimes shows a number of deficiencies, statistically differentiated from those found in other children affected by an asthmatic pathology; however, none of the immunological deficiencies indicated (Table 22.29) are characteristic in pseudo-immunodepressed children.

Common diagnostic methods may not be capable of revealing a deficiency of IgG subclasses or of selective IgG_4: the chance that there may be abnormal IgG_2 or IgG_4 levels is not excluded by the normality of IgG serum concentrations [153]. Furthermore, the distribution of IgG into four subclasses makes it difficult to identify these deficiencies simply by measuring total serum IgG levels [113]; only for the past few years have there been highly specific reagents for measuring individual subclass levels and methods such as radial immunodiffusion (RID) [198, 317]. The AAAAI has recommended not relying on subclass levels [10], especially IgG_4 levels, which seem to be unmeasurable in 25% of the population [198]. RID, which has proven to be more sensitive than the ELISA used by the CDC in Atlanta, has shown that 25% of normal children have values below normal for at least one subclass [317]; a similar deficiency was present in 58% of children with RRIs [198]. A recent study measuring the IgG with both methods, has proved that the RID can show higher values of IgG_1 and IgG_2 in low serum levels of both Ig [387], data with an unquestionable negative effect in pediatrics.

In conclusion, at the moment our knowledge suggests that we should also carefully interpret low levels of one or more subclasses, because on the one hand this might indicate a transient or paraphysiological condition, on

Table 22.27. Pediatric clinical differential diagnosis between RRI and PID

Findings	RRI	PID
Family history	+	+ +
Environmental factors	+ +	±
Age <1 year	–	+ +
Sex	Indifferent	Male
Abnormal facial features	–	+
Fever	Short duration	Persistent
Antibiotic therapy	Facultative	Necessary
Recurrent respiratory infections	+ +	+++
Location	Only respiratory	Multiple
Airway localization	Upper	Lower
Pathogens	Common germs	Also opportunistic
Tonsil and lymph node	Normal	Hypoplastic
Course	Normal	Severe, prolonged
Immune abnormalities	Absent or slight and transient	Severe, persistent
Growth	Normal	Retarded
Malnutrition	–	+ +
Prognosis	Good	Short life span[a]

Data from references cited in the text.
PID primary immunodeficiencies, *RRI* recurrent respiratory infections.
[a] If not cured appropriately.

Table 22.28. PID screening in neonates, infants, and young children

Family history	Complement defects
Consanguinity	CMI defects
Early deaths in family	DiGeorge syndrome
Prominent symptoms in the neonatal period	SCID (all forms)
Hypocalcemic seizures	HIV infection and AIDS
Morbilliform rash in the very first days of life	3–6 months
Chronic candidiasis	Agammaglobulinemia
Delayed umbilical cord separation	Hypogammaglobulinemia
Autoimmune hemolytic anemia	Leukocyte adhesion defect
Systemic reactions to live virus or BCG vaccination	6–18 months
Lymphopenia (<1,000 mm^3)	Wiskott-Aldrich syndrome
Eosinophilia	Ataxia-telangiectasia
Cardiopathy	18 months and beyond
Absence or hypoplastic thymic shadow	Common variable immunodeficiency
Onset of symptoms	Hyper-IgE syndrome
Birth to 3 months	Secondary immunodeficiency
Neutrophil defects	

Data from [61, 80, 399].
BCG Bacillus Calmette-Guérin, *CMI* cell-mediated immunity, *SCID* severe combined immune deficiency.

Table 22.29. Differential diagnostic features of PID

Clinical features	Disorders
Autoimmune disease	Selective IgA deficiency, CVID, XLP
Blood	
Aplastic anemia	XLP
Hemolytic anemia	T- or B-cell ID
Neutropenia	HIgMS
Pernicious anemia	SIgAD
Thrombocytopenia	WAS, HIgMS, XLA
Gastroenterology	
Bloody stools	WAS
Diarrhea	SCID, HIgMS
Delayed umbilical cord separation	LAD
Hepatosplenomegaly	T- or B-cell ID, HIgMS, Omenn's syndrome, XLP
Malabsorption	CVID
Mouth ulcers	HIgMS
Thrush	HIgES, SCID
Growth	
Failure to thrive	SCID, T- or B-cell ID, Omenn's syndrome
Head	
Coarse features	HIgES
Oculocutaneous albinism	Chédiak-Higashi syndrome
Unusual facies	DiGeorge syndrome
Lymphadenopathy	Omenn's syndrome, XLP
Recurrent infections	T- or B-cell ID, Chédiak-Higashi syndrome, CGD, CVID, HIgES, HIgMS, LAD, SIgAD, WAS, XLP
Skin and integuments	
Abscesses, recurrent	HIgMS, CGD, LAD
Chronic dermatitis	HIgES, HIgMS
Dermatomyositis-like rash	XLA
Eczema + petechiae	WAS
Fine, hypopigmented hair	Cartilage hair hypoplasia
Lupus-like rash	Complement deficiencies
Maculopapular rash	SCID with GvHD
Oculocutaneous albinism	Chédiak-Higashi syndrome
Teleangiectasia	XLA
Skeletal	
Bony dysplasia	ADA deficiency, Shwachman syndrome

Data from references cited in the text.
CVID Common variable immunodeficiency, *GvHD* graft versus host disease, *LAD* leukocyte adhesion deficiency, *WAS* Wiskott-Aldrich syndrome, *XLA* X linked agammaglobulinemia, *XLP* X-linked lymphoproliferative syndrome.

the other a modest deficiency can result in clear hgG [451]. Subnormal IgG_2 levels can indeed be associated with various manifestations of immune dysfunctions; it is therefore advisable in this case to proceed with specific investigations, measuring the response to polysaccharide antigens and studying the lymphocyte activity in vitro [451]. In children with normal serum levels, who are instead lacking in antibody responses to polysaccharide antigens [143, 430], this is a conclusive investigation [470] (Fig. 22.47). A study of children aged >5, half atopic and half not, has confirmed this thesis, concluding that the answers were similar in both groups, therefore excluding a greater RRI predisposition in the atopic children [344]. Many patients with high IgE levels do not present atopic manifestations: it is thought that an increased concentration is related to a reduced inhibiting activity of the thymus in IgE synthesis [457].

Differential Diagnosis

Recurrent sinopulmonary infections must make one also consider cystic fibrosis and immotile cilia syndrome [457]. Children with malnutrition (Chap. 21) suffer from numerous IDs, prevalently concerning CMI; their vulnerability makes them succumb to severe bacterial ME infections and URTIs, often also risking death. Obese subjects may also be affected by RRIs due to a possible adipose tissue hypovascularization or to a defect in the granulocyte microbicidal activity [457].

Treatment

Antibody Deficiency

In the presence of antibody deficiency, antibiotic treatment is chosen as a preventive therapy in less severe cases, otherwise the *preferable therapy consists in IVIg* (Fig. 22.48). This treatment is restricted to a limited number of diseases, including some forms of ID, secondary or cytopenic ID, in which effectiveness has been proved in DBPC studies [135, 426, 439], like other positive forms of intervention described, while it appears to be of no use in uncomplicated THI [426, 439]. Two children aged 2.5 and 3 with HIgES and Kawasaki disease were administered 400 mg/die of IVIg for 5 days and one 2.5-year-old with HIgES received only one dose, with IgE levels falling from 4,000–14,000 to 600–5,000 UI/ml on the 28th day. Hence there was almost a normalization of IgE production with symptom relapse after 6 months; similar results using a single dose were also obtained in two children with HIgES and severe AD [254].

The following data represent a number of clinical and immunological parameters in children suffering from humoral PID and ATA, with IgG levels <100 mg/dl. Treatment with IVIg, also at a higher dosage, was very well tolerated by patients: all children presented a clear-cut reduction in all clinical parameters, with significant differences compared to IM therapy (Figs. 22.49, 22.50) [174]. IgG levels in all patients also rose considerably compared to previous treatment: the average levels in different determinations was >700 mg [174]. Finally, all children grew normally; the height achieved by each child is between the 3rd and 50th percentile, within the limits of theoretical values calculated on the height of their parents. Substituting therapy with IVIg has allowed patients to return to their normal activities, with a considerable *improvement in quality of life*. In all these years, we have never come across substantial unwelcome reactions or infectious complications [174], as also found by other authors [177, 461]. IVIg could also be effective for reducing the allergic symptoms discussed thus far: presuppositions are not lacking, such as the blocking of allergens and mastocyte FcR thanks to the modest quantities of IgG_4 present in the preparations [196]. In addition, the increased understanding of the IgG transplacental passage (Chap. 2) can absolve the function of timing their transfusion in the case of mothers with antibody ID, so that the fetal defenses can be complete and quantitatively adequate.

In SIgAD common IVIg preparations cannot be used, even if with a low content of IgA, nor enriched, both because of the extremely short IgA life-span, which would therefore suggest IgA administration every 2–3 days, and because the infused IgA do not reach the secretions [507]. Should IVIg be indicated for a deficiency associated with IgG_2, or should transfusions of blood derivatives become necessary, one must first investigate serum antibody anti-IgA levels (IgG and IgE) and, should these be positive, avoid infusions or administer them in a hospital under strict medical supervision, or use washed red blood cells [507]. The same precaution must be taken for subjects with ATA for whom IVIg, if is appropriately administered, also ensure beneficial effects on quality of life, while there are no known therapies for contrasting neurological symptoms. In patients with humoral deficiency, alongside IVIg, if appropriate, an antibiotic prophylaxis is suitable with monthly cycles, alternating amoxicillin, cephalosporin, co-trimoxazole, etc., bearing in mind family compliance. In CVID recurrent infections caused by *Giardia lamblia* should be treated using furazolidone (8 mg/kg/day) or methronidazole (15 mg/kg/day) for 10 days, if necessary to be repeated. CVID treatment in specialized centers involves recombinant IL_2, IL_{10} and cimetidine [456].

T-Cell PID

Some T-cell PIDs represent a severe clinical emergency, such as Omenn syndrome, in which hypovolemic shock and reticular dysgenesis are immanent in the battle for survival. Although precise figures are unavailable, thousands of patients worldwide with different forms of

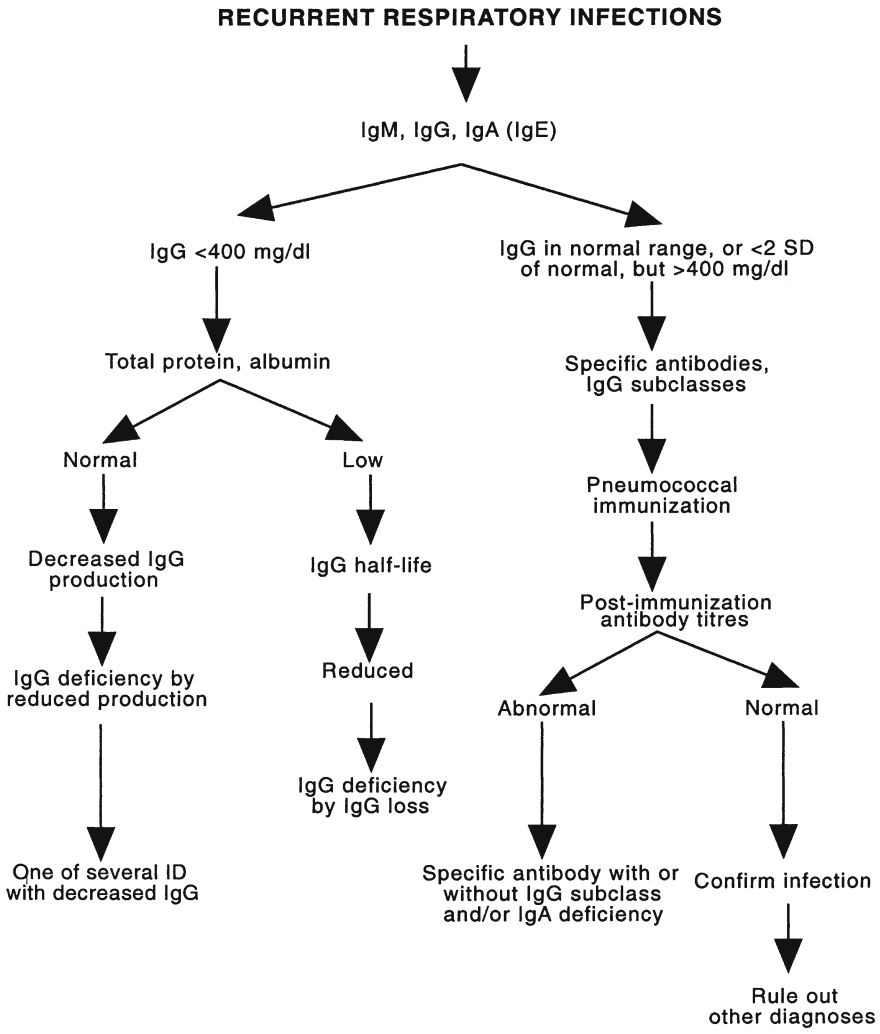

Fig. 22.48. Algorithm for the diagnosis of ID with antibody deficiency in children. The level of 400 mg/dl is arbitrary, but it is the mean value for babies aged 7–12 months (Table 1.15). Calculating IgG half-life after an IgG infusion is helpful to determine an IgG loss in children with diarrhea or nephrotic syndrome. For children with normal or borderline IgG levels, immunizing with pneumococcal vaccine and then measuring antibody titers along with specific pneumococcal antibody titers 4–6 weeks later is most practical. (Modified from [470])

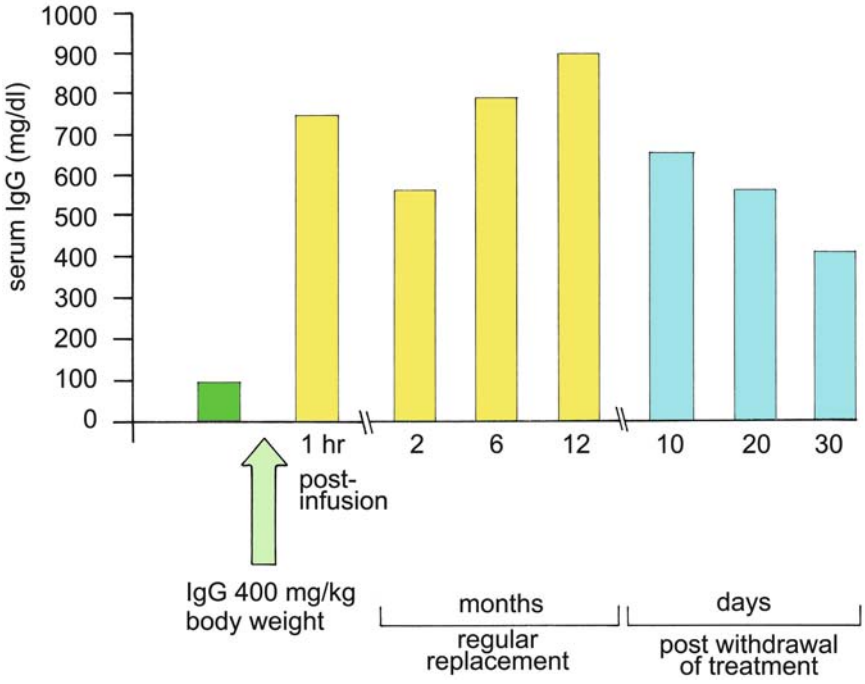

Fig. 22.49. IgG levels in a child with XLA following IVIg replacement therapy. The *first two bars* show IgG levels before and 1 h after receiving replacement therapy, then IgG levels 2, 6 and 12 months after start of therapy; the *last three bars* indicate IgG half-life

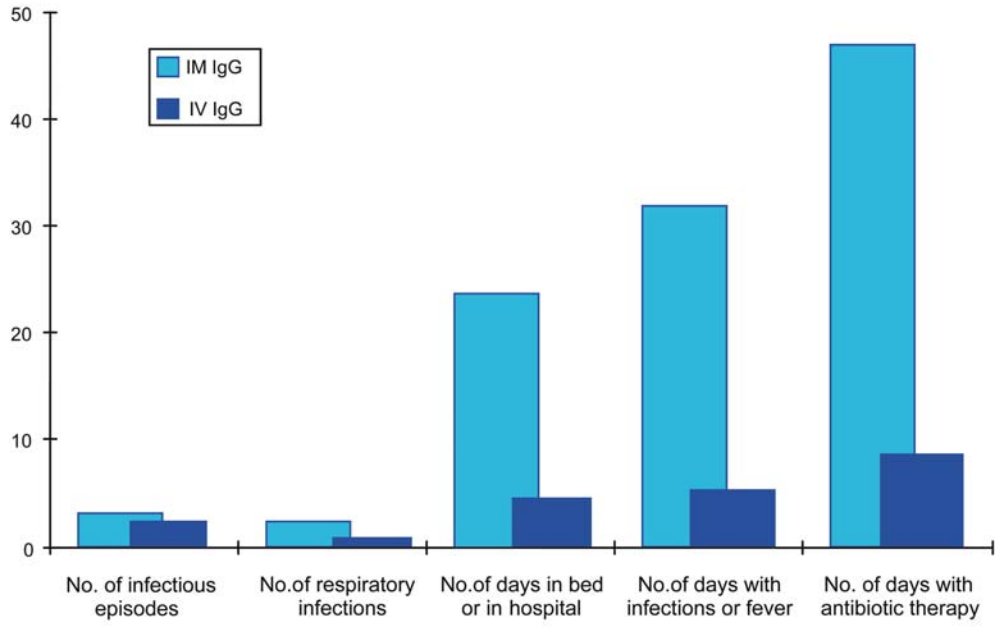

Fig. 22.50. Percent reduction of clinical manifestations (by year/child) in children with antibody deficiency disorders and treated with IM IgG compared to IV IgG-treated children. (Data from [174])

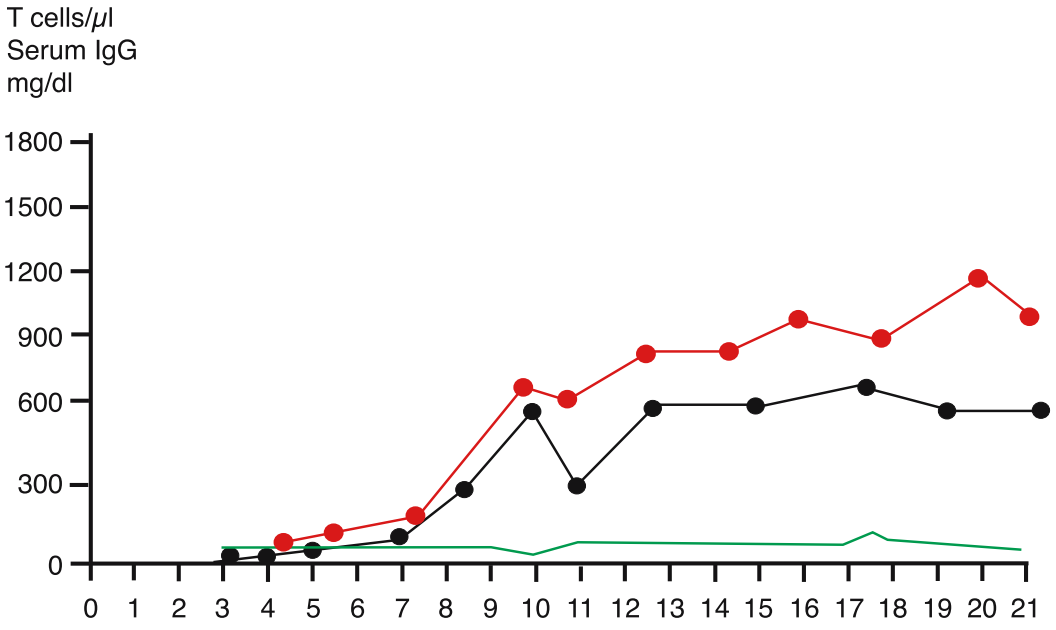

Fig. 22.51. Reconstituting the immune system following TCD haploidentical BMT in an infant with SCID. CD4 (*red line*), CD8 (*black line*), IgM levels (*green line*). (Data from [508])

genetically determined ID have been given BMT in attempts to correct their underlying ID [66], including a recent series [14]. Specific treatment for cellular PID consists in a BMT from a HLA-compatible donor [10]. The ideal SC donor is normally a sibling who shares identical HLA class I and class II *loci*. Without such a donor, these transplantations usually resulted in fatal GvHD. If death did not occur, event-free survival was severely affected by several factors [26]. Either HLA-identical marrow or T-cell-depleted (TCD) haploidentical parental marrow is the standard of care for SCID (Fig. 22.51). When histocompatible related donor BMT is unavailable, a BMT either with HLA-identical unfractionated or TCD haploidentical parental marrow is the standard of care for SCID [338]. All but one (95%) of 21 SCID infants who received TCD identical or haploiden-

tical BMT in the first 28 days of life are currently alive, with the period of survival ranging from 8 months to >19.2 years after transplantation. This compares favorably with a 74% survival rate of 96 infants receiving transplants at a median age of 190 days (range, 45–516 days) [338]. A girl with T^- B^- SCID received a full matched BMT from her sister *at age 2 weeks* [491]. A worldwide survey conducted by Buckley from 1994 through 1997, with subsequent additions of published cases from the literature, revealed that 239 of 302 (79%) patients with PID transplanted with HLA-identical marrow during a period of 34 years were alive [65]. There are >375 patients worldwide who have survived SCID as a result of successful transplantation of HLA-identical or haploidentical BM [67]. Most importantly, 34 of 35 infants (97%) undergoing transplantation *in the first 3.5 months of life* are currently alive [69, 338], compared with a cut-off at 6 months (97% vs 86%, children younger vs those aged >6 months) receiving BMT (OR 5.0 [14]). We stress that neonates developed higher lymphocyte responses to phytohemagglutinin and higher numbers of $CD3^+$ and $CD45RA^+$ T cells in the first 3 years of life than those receiving BMTs late. T-cell antigens peaked earlier and with higher values in the neonatal BMTs (181 days to 1 year) than in the late BMTs (1–3 years) [338]. Over the past 22 years 78% of all SCID patients (110/142) receiving BMT at Duke University Medical Center survive to varying ages up to ≥20 years after BMT. Only 16 had an HLA-identical donor. All others received rigorously TCD haploidentical BM from a parent, most often the mother. The soy lectin, SRBC rosetting technique was used (R. Buckley pers. comm. November 20th, 2004 and April 20th, 2005). An uncommon BMT to treat AR SCID was undertaken in *a 1-month-old girl*. The donor was her HLA-mismatched 6-year-old sister, who had previously received a BMT from her father [479]: presently, they are aged 5 and 13 and are affected by *Molloscum contagiosum* infection [547].

BMT, both HLA identical unfractionated and TCD HLA aploidentical [63]. A recent trial found that because only 10%–15% of affected children have a familial HLA-identical donor (RID), the alternative therapeutic options are BMT from a MUD or a haploidentical BMT or from HLA-mismatched related donors (MMRDs). Only 40% of these children may find a matched donor; therefore, the remaining PID-affected children are candidates for a TCD haploidentical BMT [277]. MUD HSCT is successful in young children [132], but the success rate decreases dramatically above the age of 5–6 years [158] (Table 22.30) [13, 15, 25, 27, 39, 46, 52, 55, 56, 58, 64, 67, 69, 80, 83, 85, 98, 111, 119, 131, 132, 163, 185, 188, 197, 205–208, 231, 233, 235, 237, 245, 252, 256, 257, 259, 260, 277, 280, 286, 288, 290, 305, 307, 334, 360, 366, 380, 405, 408, 428, 440, 448, 452, 456, 466, 474, 491, 496, 499, 503, 534]. Table 22.30 shows the treatment of choice for severe primary T-cell PIDs. Of 94 infants diagnosed as having SCID who received BMT between 1990 and 2004, 66 (70.2%) survived, 12 (92.3%) of 13 infants who received RID BMT, 33 (80.5%) of 41 who received MUD BMT, and 21 (52.5%) of 40 infants who received MMRD

Table 22.30. Bone marrow transplantation for PID disease

Deficiency	Survival rate	Time (years)	Reference(s)
T lymphocytes			
T^- B^+ SCID	18/27	3–13	[466]
	21/44	3	[474]
AR SCID	2/2	1.7	[260]
	17/20	P	[69]
Jak-3 deficiency	1/1	1.7	[260]
	4/4	2–23	[207]
	6/6	P	[69]
	9/10	4–18	[408]
γ-Chain deficiency	14/14	2–23	[207]
	34/43	P	[69]
SCID, unspecified	3/4	P	[277]
	4/4	2–23	[207]
	82/193	>0.5	[206][a]
T^-B^- SCID	6/12	3–13	[466]
	22/31	3	[474]
ADA deficiency	3/13	3	[474]
	11/13	P	[69]
Artemis deficiency	12/16	7	[360]
Reticular dysgenesis	1/1	2.5	[13]
	1/1	3	[474]
	1/1	P	[260]
	2/2	P	[119]
	3/5	P	[46]
T^-B^- SCID	1/1	3–13	[466]
	1/1	P	[491]
Omenn SCID	2/5	P	[67]
	3/3	P	[277]
	6/8	3	[474]
	6/9	4–11	[188]
IL_2 deficiency	1/1	P	[452]
PNP deficiency	1/1	1.3	[58]
	1/1	7	[58][b]
	1/1	1	[83]
	1/1	1.6	[98]
	1/1	P	[25]
	2/4	P	[67]

Table 22.30. (Continued)

Deficiency	Survival rate	Time (years)	Reference(s)
HLA class II deficiency	1/1	[a]	[85]
	1/1	[b]	[52]
	2/6	0.4	[428]
	2/9	1.7–3.3	[233]
	3/8	P	[163]
	13/33	2–10	[256, 257]
ZAP70	1/1	0.6	[27]
	1/1	2.5	[13]
	2/2	P	[67]
	3/8	3–13	[466]
	4/6	0.9–3.5	[56]
CD154 deficiency (HIgMS)	1/1	[c]	[499]
	1/1	[b]	[55]
	1/1	P	[197]
	1/1	P	[286]
	1/1	P	[208]
	1/2	P	[290]
	3/3	P	[67]
	4/7	P	[503]
	4/8	P	[252]
	5/11	P	[131]
	26/38	P	[180]
CD3δ	1/1	3	[111]
SCID with p56lck defect	1/1	[a]	[185]
SCID with whn defect	1/1	6	[380]
Combined ID (CID)	1/1	1.7	[260]
	1/1	P	[277]
	3/7	2.2–4	[231]
	11/27	P	[67]
Nezelof syndrome	1/1	1.7	[260]
Fas (CD95) deficiency	1/1	2	[39]
CD7 deficiency	1/1	P	[245]
WAS	1/1	1.5	[13]
	1/2	P	[67]
	3/3	1.5–2	[259]
	5/9	1.8–4.9	[231]
	11/18	P	[163]
	12/17	1.5–16.5	[366]
	14/118	P	[132]
	120/170[b]	5	[156]
XLP	4/7	3	[199]
DiGeorge syndrome	3/13	P	[305, 306]
	5/6	1–2.1	[309]
	7/12	1.2–8.5	[307]
Phagocyte system			
CGD	1/1	7	[288]
	1/1	P	[48]
	1/4	P	[334]
	2/2	P	[67]
	23/27	2	[448]
LAD type I	1/1	P	[235]
	1/1	P	[277]
	3/3	1.6–4.8	[280]
	5/8	P	[67]
	7/8	P	[163]
	10/13	1–12	[496]
LAD type II	2/2	2–4.6	[231]
LAD type V	1/1	0.3	[534]
Complete IFN-γR deficiency	1/1	4	[405]
	1/2	P	[67]
Chédiak-Higashi syndrome	1/1	3.1	[231]
	1/3	1.5	[334]
	2/2	P	[64]
	7/10	5.6[c]	[205]
Griscelli syndrome	1/1	P	[15]
	1/1	2	[440]
Severe congenital neutropenia	1/1	P	[237]

Several different BMT techniques have been adopted. Additional data from [65, 85, 246, 328].
[a] See details in the text for survival rate.
[b] Case reported to Broome et al [58].
[c] Median (1.5–13 years after BMT).
P, at the time of publication.

BMT survived. Compared with MMRD BMT, survival was significantly higher with RID or with MUD (45/54 = 83.3%) [201]. When an HLA-identical sibling as the donor is unavailable, a phenotypic HLA-matched unrelated BMT is needed, also used in CD154 deficiency [290, 499], with a clinical and immune outline normalization [246] and a variable effect on IgG subclasses

[153]. Possibly because of earlier diagnosis before untreatable opportunistic infections develop, the results have improved considerably during the last two decades [65]. BMTs have been successful when applied within the first *7–24 days of life* in 21 infants with SCID, 20 (95%) of those still alive range from 8 months to 19 years, not justifying *in utero* transplants. A completely normal T-cell function was obtained within 82–118 days [338] and in an other 83 patients was still present after 10–17 years [373]. Of the 58 children who received a mismatched parental BMT from 1980 to 1998, 43 (74%) remain alive with T-cell immune reconstitution, a median of 128.8 (range, 13–224.3) months after BMT [362]. Of 96 children out of 117 who received allogeneic BMT after the first 28 days of life, 71 (74%) are alive [69]. Three out of five children who received a HSCT are alive and well after 18–32 months [13]. Breast feeding appeared correlated to an earlier reconstitution when the donor was the mother [338]. *Intra-amniotic gene transfer* has been successfully carried out on a laboratory animal, registering in a dose-dependent manner the fetal gastroenteric and respiratory effects [222]. If confirmed in human beings, this method of treatment will certainly prove useful for prenatal correction of PID.

BMT/HSCT should be completed by conditioning regimens with busulfan and cyclophosphamide, less toxic than total lymphoid irradiation or a combination of nucleoside analogs and anti-lymphocyte antibody preparations [65]. For example, busulfan (16 mg/kg), melphalan (90 mg/m^2) and anti-thymocyte globulin (36 mg/kg) [83]. To enhance the engraftment rate in haploidentical BMT in PID, it was recently suggested to add donor peripheral SCs after mobilization with G-CSF (16 µg/kg for 5 days) and BM cells. With this procedure the cell load is increased, which allows intensification of the conditioning regimen for induction of faster engraftment [277].

In utero BMT suggested advantages include the sterile environment *in utero,* and immaturity of the fetal immune system enabling the prevention of clinical manifestations of the disease in the neonate, and the engraftment without the use of cytotoxic conditioning regimens: a child thus treated was well at age 11 months [164]. Two series of six and four patients [386, 504] and two additional case reports [182, 532] of *in utero* transplants have been published, yet failure of B-cell engraftment and function may result in long-term dependence on IVIg replacement [248]. Better results than those published for *in utero* BMT for SCID were implicit in 13 infants admitted and diagnosed *at a median age of 3 days* because of a FH of a previously affected infant. BMT was successful and all children are alive and well with follow-up to 11.5 years [248] or <30 days vs approximately 4 months [194]. However, it is suggested that in utero transplants may carry the risks associated with injecting the fetus and the inability to detect GvHD during gestation [65].

Umbilical CB transplantation (UCBT) was done in two children affected by a Zap-70 deficiency and an Omenn-like syndrome. Both are alive and well at 4.5 and 2.2 years after UCBT [148]. Unrelated UCBT in eight children with severe T-cell ID [260] and in three with WAS [259] resulted in consistent and stable T$^-$, B$^-$, and NK cell development [259, 260]. Faster availability of UCBTs is a meaningful advantage for patients requiring urgent transplantation: a median of 25 days more rapidly than did those receiving bone marrow [30]. In a large report [423], 40 patients with SCID, seven with WAS, and other unspecified PID received an unrelated UCBT. UCB was evaluated as a SC source for immune reconstitution in children with severe primary T-cell IDs such as SCID, reticular dysgenesis, thymic dysplasia, CID, DGS, and WAS when a matched sibling donor was unavailable, and has been used to date in more than 2,000 patients [194]. Three infants who rejected a TCD-mismatched parental BMT without prior cytoreduction engrafted after infusion of UCBT [69]. A 4.5-year-old girl with HLA class II deficiency had a successful related UCBT for graft failure following TCD nonidentical BMT [52]. A girl with reticular dysgenesis failed to engraft following her first transplant, but fully engrafted after a second unrelated UCBT. Five of six patients showed grade I GvHD, although one child experienced grade IV skin and gut GvHD. Immunological UCBT resulted in consistent and stable T-cell, B-cell, and NK-cell development [260]. Long-term event-free survival (≥27 months) with recovery of antigen-specific responses was reported following an unrelated UCBT in a child with Omenn's syndrome [38].

Gene therapy, which has revolutionized and could revolutionize even more PID treatment in the near future, is analyzed in Table 22.31 [76, 104]. The requisite for applying this form of genetic engineering treatment is that the responsible gene must be cloned; the most common techniques involve knock-out mice and inactivating a particle [104], or employing retroviral vectors (Table 22.32) [263], totally deprived of their genomic factors except for the normal copy of the gene to be inserted. This is indispensable for allowing the vector to reach the human nucleus, where it will integrate with the cellular genome [263]. In this case, PBLs are collected through leukapheresis and cultivated in vitro with retroviral particles containing a normal gene RNA copy: thus the healthy gene is introduced into the cell genome using a vector and the manipulated cells are reinfused, so as to restore normal immune functions. This system has been used to treat two children aged 2 months suffering from SCID [508] and from ADA deficiency by employing autologous PBLs [51], BM cells [54], and CB cells [262]. Three reports [4, 88, 204] of successful gene therapy in infants with X-linked SCID [88, 204] and in T$^-$ B$^-$ SCID [4] are a major step forward among repeated efforts to achieve better immune reconstitution in ADA-SCID with gene therapy than with BMT/SCT [161]. Immediately after the diagnosis had been made in two

Table 22.31. Gene therapy for PDI

T lymphocytes
SCID[a]
CD3γ deficiency
Omenn SCID[b]
ADA-SCID[a]
ZAP-70-SCID
JAK-3-SCID
X-linked hyper-IgM syndrome
IL_2 deficiency
HLA-SCID, including CIITA deficiency
PNP deficiency
B lymphocytes
XLA
Phagocyte system
LAD syndrome?
CGD $p47^{phox}$ and $gp91^{phox}$?

Data from [4, 76, 104].
CIITA class II transactivator, *CGD* chronic granulomatous disease, *XLA* X linked agammaglobulinemia; for other abbreviations see Table 22.1.
[a] Done with success, see text for details.
[b] *In utero* transplant of maternal stem cells.

Table 22.32. Gene delivery vectors

Viral vectors	Nonviral vectors
Retroviral	Liposomes
Adenoviral	DNA particles
Adeno-associated virus	Ligands
Lentivirus	

Data from [263].

children aged 8 and 11 months including a novel splice imitation in the common γc chain [183], haploidentical $CD34^+$ peripheral progenitor cells mobilized with GM-CSF were isolated to a purity of more than 99%. These cells were infused with no prior chemoablation and no prophylaxis against GvHD. Both children showed signs of T-cell reconstitution beginning 3 weeks after the $CD34^+$ infusion and were weaned from continuous cures. They are in excellent health, without GvHD, 34 and 68 months after transplantation. One child does not need replacement Ig. The other received a booster infusion of $CD34^+$ SCs from the original donor 1 year later to improve B-cell function and now receives Ig every 3 months. Both were followed for 10 months after gene transfer [88]. However, retroviral vectors have the capacity of wild-type, replication-competent retroviruses to cause leukemia in immunologically immature neonatal mice [263], and in humans (two children out of 11) [63, 88, 204]. Retroviruses can cause insertional oncogenesis, a long-known potential complication of retroviral gene transfer attempts, because gene integration occurs at random in the genome, thus deregulating the expression of cellular oncogenes [263]. This complication has been thought to be unlikely with such vectors, because they are capable of inserting only once into the cell's chromosomes and cannot repeatedly reproduce and integrate. Lentiviruses may be more effective than murine retroviruses for gene transfer into human hematopoietic SCs and T lymphocytes [263]. In ADA-SCID, *the safety and efficacy of HSC gene therapy* combined with nonmyeloablative conditioning for the treatment of SCID has allowed two children to live at home and clinically well, with normal growth and development [4]. On January 14, 2003, FDA placed on "clinical hold" all active gene therapy trials using retroviral vectors to insert genes into blood SCs after having learned that a second child treated in the French gene therapy trial developed a leukemia-like condition. Gene therapy is on hold despite enormous promise for certain SCID/CID variants.

Survival. In SCID, the European experience with unfractionated HLA-identical and TCD or non-TCD haploidentical or MUD BMTs in patients with SCID reported that between 1968 and 1999, a 3-year survival with sustained engraftment was significantly better after HLA-identical than after mismatched transplantation (77% vs 54%). Within the HLA-identical group, survival after BMT from genotypically or phenotypically identical related or MUDs was 81%, 72%, and 63%, respectively [14]. In non-SCID, 3-year survival after genotypically HLA-matched, phenotypically HLA-matched, MMRD, and MUD BMT was 71%, 42%, 42%, and 59%, respectively [14]. In a retrospective analysis of BMTs performed between 1977 and 1991 at 13 European centers in 149 children as young as 1 month with 11 different PIDs (excluding SCID), the overall survival among 53 recipients of HLA genetically identical BMT was 66%, 45.5% in 22 patients who received closely matched BMT, and 38% in 71 recipients of BMT with two or three mismatched HLA antigens. A significant improvement in survival has been achieved in most PIDs (overall survival, 81.5% vs 51.7%, primarily because of a decrease in the frequency of infectious complications [163]. In the similar analysis performed in 193 children with SCID at 18 European centers between 1982 and 1993, 116 out of 193 (60.1%) patients were alive with evidence of engraftment 6 months after BMT. However, 24 patients died >6 months post-BMT, mainly due to cGVHD and/or viral infection. Thus GvHD 6 months after BMT and B^-SCID vs B^+SCID were the main factors associated with a poor outcome [206]. The disease-free survival was significantly better for patients with B^+SCID (60.7%) than for those with B^-SCID (33.3%) [14]. In a

trial on children with PID receiving BM from HLA-non-identical related donors or from HLA-identical unrelated donors at 13 European centers between August 1990 and June 1993, 22 out of 28 children (76.6%) survived 22–58 months. BM was TCD by use of either erythrocyte rosetting or monoclonal antibodies to prevent GvHD [231]. Additional survival rates were reported previously (Table 22.30). In a series of consecutive UD BMTs 31/33 children with SCID and non-SCID PIDs who received a BMT with reduced-intensity conditioning (RIC) regimen between 1998 and 2001 survived after a 3.3-year follow-up, as well as 10/19 children who received a BMT with myeloablative conditioning (MAT) between 1994 and 1998 and survived after an 8.6-year follow-up. Therefore a RIC regimen results in improved survival and reduced BMT-related mortality compared with MAT in HR children undergoing an UD BMT [396].

In 170 transplanted patients with WAS, the 5-year probability of survival differed according to donor type: 87% with HLA-identical sibling donors, 52% with other related donors, and 71% with MUD. Significantly, boys who had received a MUD transplant before 5 years of age had survival rates similar to those receiving HLA-identical sibling transplants [158]. However, the time required to develop immune function after haploidentical SCTs is quite different from that after unfractionated HLA-identical BM. Lymphocytes with mature T-cell phenotypes and functions fail to rise significantly until 3–4 months after BMT; normal T-cell function is reached between 4 and 7 months [338]. B-cell function develops much more slowly, averaging 2–2.5 years for normalization; many do not have B-cell function, despite normal T-cell function.[338]. *Ex vivo* rigorous depletion of post-thymic T cells from donor marrow that cause GvHD is efficient and feasible, even in haploidentical settings [13], presumably because of more effective infection-control measures and better transplantation strategy [514]. For non-SCID, SCT can provide a cure, and grafts from unrelated donors are almost as beneficial as those from genetically HLA-identical relatives [45]. In most patients, deficient B-cell function persists after transplantation and requires lifelong IVIg therapy [69,207], which is necessary to prevent bacterial and common viral infections [69, 514]. Some patients also have persistent deficiencies of T-cell function after SCT [206, 373].

Children with RRIs

In children with RRIs, depending on the nature of the infection, the pediatrician will prescribe the most appropriate symptomatic and/or antibiotic therapy. In the presence of persistent inflammation, or during the winter, when the risk of close acute recurrent episodes is higher, anti-inflammatory preparations will be prescribed via aerosol, chromones, ketotifen, β_2-adrenergic and if necessary steroids for topical use, strictly depending on the need. We suggest monitoring measures, such as keeping a clinical diary, in which each acute episode should be briefly noted, continuing registration until clinical symptoms have not regressed for at least 15 days and returning to keep notes in the diary each time there is a cough and/or nasal and/or bronchial inflammation, completing this with PEF as well as some respiratory parameters right at the beginning and then every 6 months. It is obvious that if medical intervention is not resolutive a center specialized in infantile respiratory physiopathology should be contacted [47]. Children with SARS were treated with high-dose ribavirin, oral prednisolone, or IV methylprednisolone, with no short-term adverse effects [223].

Antibiotics must be used very carefully in these children because they can influence positively or negatively the innate, cellular or humoral immunity (Chap. 18), interaction with ILs and growth factors are not known, repeated use often causes phenomena involving allergy/intolerance [470], and most infection-prone children suffering from VRIs are given antibiotics unnecessarily.

In Italian children (54.6% of males) aged 6 months to 14 years (median, 4 years) with a history of RRIs, macrolide therapy of acute respiratory infections influenced the natural history of RRIs, probably because of their elective activity on atypical bacteria [508]. Considering the emergence of antibiotic-resistant bacterial stock, as for example *S. pneumoniae*, immunotherapy has been proposed as a means of preventing RRIs by providing children with small doses of inactive bacterial antigens liable to trigger specific and protective immune responses (Table 22.33) [36]. For example, OM-85 BV significantly reduces the URTI rate, particularly in a DBPC study in 232 children aged 3–8 with a history of acute URTIs [448], is active in preventing RRI episodes [36] with a meaningful reduction in the number of days of suffering acute URTIs [448]. Bacterial ribosomal and membrane proteoglycans of *S. pneumoniae*, which stimulate B cells with secretory responses, as well as memory cells, may be used for responding to future infections [36]. Ribosomal immunotherapy appears to be not only well tolerated, but also ideally targeted to induce mucosal responses [37]. Among the preparations reserved for specific use, a study of pidotimod in DBPC trials proved its effectiveness in a sample of 101 children with RRIs, also showing increased CD25, absent in placebo-treated children [72]. The use of immunostimulants should be limited to children with proven high susceptibility to acute URTI, or overexposed children attending daycare facilities, or attending kindergarten or elementary school [508]. However, according to a meta-analysis, immunostimulants are an effective treatment for the prevention of acute URTI in children [40]. Furthermore the *indiscriminate and purely empiric use of IVIg* must be discouraged in every child with RRI, whereas in a prospective, DBPC study of IVIg and co-trimoxazole, 106 of 130 children <8 years referred for recurrent bacterial RRIs became infection-free over a 4-month obser-

Table 22.33. Immunological therapeutic intervention for children with RRIs

Type	Procedure	Mode of action
Nonspecific	Leukocyte transfusion	Leukocyte
	Fresh frozen plasma	Antibody, complement
	Interleukins	IFN-γ
Specific	Fresh frozen plasma	Antibody
	IVIg	Antibody
	Specific immunity	Antibody (higher titers)
	Specific vaccines	Antibody
Combined	IBE	Pleiotropic effects

Modified from [36].
IBE immunoreactive bacterial extracts, *IVIG* intravenous immunoglobulins.

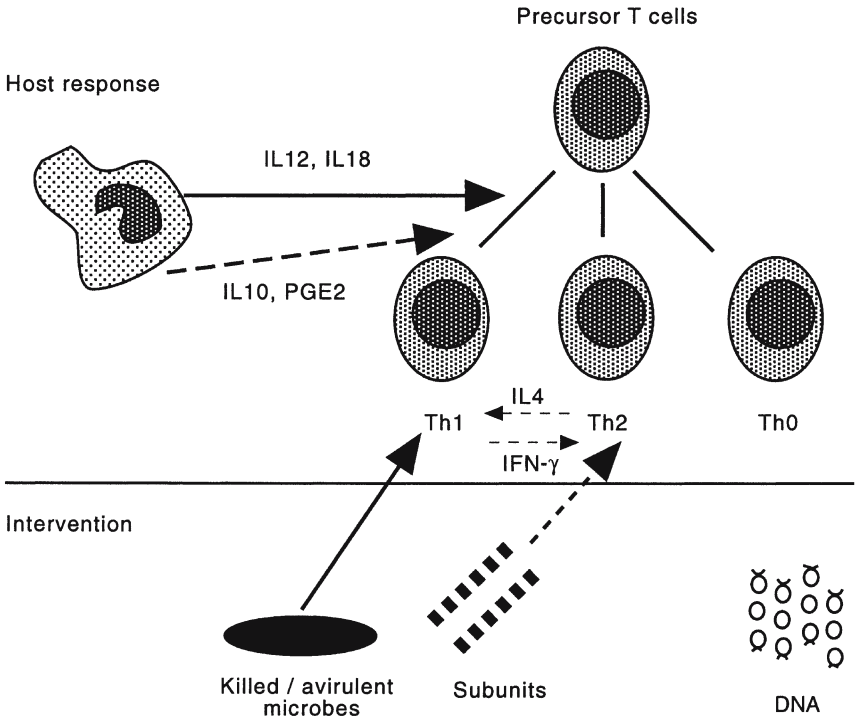

Fig. 22.52. Development of Th subpopulations during the natural immune response to intracellular pathogens. *Solid* and *dashed arrows* indicate positive and negative stimulation, respectively. (Modified from [340])

vation period [353], in addition to having an extremely unfavorable cost–benefit ratio [426]

Immunization

Children with RRIs and deficient antibody responses to germs expressing a capsular polysaccharide can be successfully vaccinated, but avoiding the administration of live virus vaccines and integrating this if necessary with an IgG replacement therapy [218]. Furthermore, in view of the availability of conjugated vaccines, it will be possible to induce antipneumococcus-IgG_2, providing an effective treatment for children with RRIs, especially if caused by pneumococci. Other kinds of vaccines have provided disappointing results: Fig. 22.52 [340] indicates the immune bases of a specific immunization and the possibility of specific interventions. In children with PID, one should bear in mind all the aforementioned facts.

Pediatricians, PID and RRIs

Until the past few years, there was a busy motion into the fundamental problems underlying a majority of these conditions. Many have now been mapped to specific chromosomal locations, and an impressive number

of the fundamental biological errors have been identified. The pediatrician is entrusted with a more difficult job, that of identifying as early as possible the possible existence of PID, remembering the suggestions for case history in Chap. 6, with the exception of clinical emergencies such as Omenn syndrome and reticular dysgenesis. This specific research becomes a necessity thanks to the new diagnostic and therapeutic advances that have been conceived over the past few years: the earlier one acts, on the one hand with a prenatal diagnosis and on the other with a BMT or SCT therapy, the greater the chance to increase life expectancy for these children, in addition to ensuring *better quality of life*. The discovery and cloning of the genes for these diseases have obvious implications for the potential of gene therapy. The rapidity of these advances suggests that there will soon be many more to come. One of the most common differential diagnoses will occur with a child affected by RRIs, for whom we believe the number of infections must be immediately clarified, although evaluated according to different numeric and epidemiologic factors, not associated with those which instead concern the severity and the site of the infection as well as the type of the pathogenic agent that characterize children with PID. However, antibiotics are banned by the supporters of the hygiene hypothesis (Chap. 24).

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Prevention of Allergic Disorders

Preventing Atopic March: A Priority

A heredity component in the pathogenesis of atopic disease has been shown in Chap. 4, and an increased prevalence of atopic disease in Chap. 5, so such diseases represent a very contemporary problem: even a cursory glance at the literature clearly demonstrates the growing prevalence of atopic disorders. In the past two decades, a number of factors have contributed to this increase: urbanization that has brought in pollen-carrying ornamental plants and has generated extensive pollution, excessive smoking that seems to have made inroads everywhere, increased exposure to allergens, and poor ventilation of modern homes, which are outfitted with everything that favors Der p proliferation. These figures alone demonstrate that atopic diseases must not be underestimated, regardless of their manifestations. This is not only because a mild clinical picture can quickly become complicated, but also because in cases that are serious from the outset, only an accurate and timely diagnosis – and a rapid therapeutic conclusion – can effectively resolve this contingency. This would compound the problems of an already difficult area of preventive medicine, although it may be pertinent to move attempts at intervention back into early pregnancy or even preconception. The ability to prevent atopic disease before its occurrence has been a strong desire of physicians dedicated to this pivotal field. In *On Medicine* (2, pp 17–18), Celsus said that *"food and drink... are not only the common aid to all disease but also to good health"*, and Cantani Sr that *"pure air, water and diet are the main and most powerful ways of staying healthy"* [53].

The focus of prevention of pediatric allergy and asthma has recently changed. In Chap. 13 we documented the resolutive effect of the desensitization to both food and inhalant allergens. This is a great opportunity in light of the marked increase in prevalence of the familial allergic diseases of children, including asthma, allergic rhinitis (AR), atopic dermatitis (AD), and food allergy (FA), as shown by our personal experience demonstrating that atopic disease afflicts 95% of infants and children with FHA (family history of atopy), as attested in the table in Chap. 5 illustrating this emergency and in our study on increased prevalence of pediatric asthma from 2001 to 2003.

Definition

Allergic diseases are multifactorial disorders, which are caused by genetic factors that predispose children to atopy and by numerous environmental factors that contribute to their phenotypic expression: given that modifying genotype is not possible, prevention thus focuses on modifying unfavorable environmental factors through appropriate manipulations recommended only for siblings of atopic subjects, therefore defined as genetically at high risk (HR) for allergy.

Methods

Allergic diseases can be prevented in three ways:

- *Primary prevention,* by acting on predisposing factors to prevent or at least delay sensitization before any IgE-mediated disease has occurred.
- *Secondary prevention,* based on the use of drugs as well as other measures, once IgE-mediated disease has developed, means prevention of further sensitization.
- *Tertiary or long-term prevention,* prevents complications and means attempting to reduce allergy expression once IgE-mediated disease has occurred.

Requirements

It has often been noted that prevention has two basic requirements: practicality and cost-effectiveness. On a day-to-day basis, however, the programs for preventing allergic disease may seem impractical and expensive to the families that need to implement them. As a result, they are not always easy to accept, as they often diverge from widespread habits and mentalities. For example, birth shortly before the pollen season is associated with a high prevalence of respiratory allergy during the next developmental stage [360] (Table 4.12), but it would be a difficult task to convince allergic parents to plan the birth of a child for less risky months. The hygiene hypothesis indicates that daycare center attendance by children at risk for atopy helps protect them from subsequent development of allergic diseases and infections. Nevertheless, we must bear in mind that the statistics on ETS (environmental tobacco smoke) [86], extended to

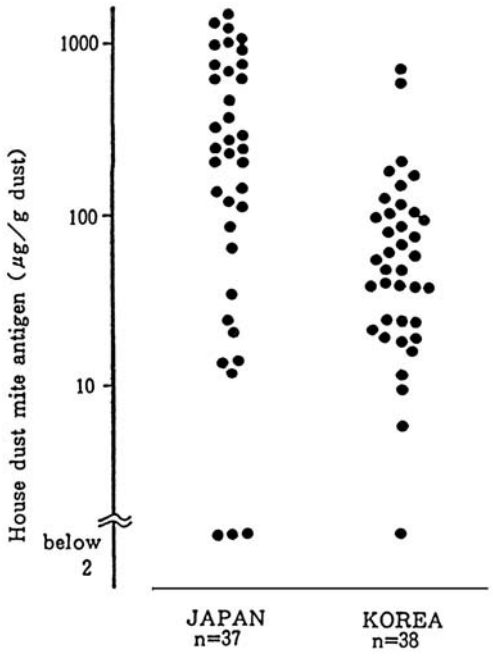

Fig. 24.1. Der p antigen content in dust samples collected from houses in Japan and Korea as measured by ELISA-inhibition method. In Korean houses with a traditional floor heating system and smooth plain flooring a lower Der p concentration was found

the risk of infections of the lower respiratory tract (LRTI) and of development of specific IgE (sIgE) (Table 4.25), involve a significant number of children – up to 100% – attending daycare settings. Fel d 1 and Can f 1 were detected in all daycare settings, evidently brought in by children and/or personnel in contact with cats or dogs, while Der p 1/Der f 1 were noted in 41% of cases [303]. Another observation involved the significant amount of dust that was collected, despite thorough cleaning of the rooms and furnishings [303] (Fig. 24.1) [22].

Exclusive breast-feeding for the first 6 months and delayed weaning over the 6th month of life is often at odds with real problems or with the widespread habit of early weaning. Even the suggestion that nursing mothers restrict the use of strong allergens in their diets – above all cow's milk (CM) – may be accepted reluctantly, due to the common opinion that "milk makes milk." Moreover, a great deal depends not only on the commitment of researchers in this area, but also on incisive campaigns to inform public opinion. Also important is the ability of pediatricians to explain to parents how necessary the proposed dietary–environmental measures are, a concept that cannot be appreciated by everyone. It is vital to emphasize to parents that a range of preventive measures, permitting timely protection against an excess of allergens, can help avoid or delay the appearance of allergic sensitization, or at least ensure that the disease will be less severe. Our studies in this field have been confirmed by the results on 67 children of atopic parents: *none of those exposed to a low allergen load* (<2 μg/g of dust) *became sensitized to airborne allergens* [396].

The prevention of the atopic march is not anecdotal [230] but a reality, as we will demonstrate. Our belief has been strengthened by the need to act positively in the early stages of life [189], by the significant preventive effects of breast-feeding in prospective birth to 7 [39, 123, 484], 11 [477], 15 [162], and 17 years of follow-up [360], also by the dramatic case of a 7-week-old baby for whom no preventive measures were taken despite a positive family history (FH), and who died after several changes in diet (Chap. 9). On this specific subject, we refer to Table 3.8, which summarizes the characteristics of newborns and nursing infants at a HR for atopy, and to Table 4.29, which lists the main genetic and environmental factors that are responsible for or contribute to the development of atopic diseases in HR infants and children.

Primary Prevention

The factors that can modulate the clinical expression of phenotype are as follows(Fig. 4.31):

1. Prenatal factors
2. Perinatal complications
3. Birth period
4. Diet during the first several months and weaning
5. Exposure to environmental factors
6. Immune interventions

Given that we have already discussed points 1–3 in Chap. 4 (summarized in Table 24.1) [23], we will examine the role that other factors play in primary prevention [45, 44, 46].

Role of Diet

Prenatal Role of Diet

Can children be born with allergy? We already answered this question in Chap. 3 by saying that this is a statistically rare possibility. The point we must emphasize, however, is that the fetus starts synthesizing IgE as of the 10th week of intrauterine life, and responds to food allergens that are ingested or inhaled by the mother during pregnancy (Chap. 4).

The theory of intrauterine sensitization has been studied by several authors. Anti-CM cord-blood IgE (CBIgE) was found in 3.3% of HR newborns, and CB lymphocyte stimulation test (LST) with ovalbumin and bovine serum albumin (BSA) was positive in a 2-year follow-up of 8/37 infants when it was repeated twice (Chap. 3). Nevertheless, it was clear that *sensitization does not occur during fetal life,* since CBIgE are the out-

Table 24.1. Environmental modulation of IgE mediated and non-IgE mediated reactivity

Effect of inhaled or ingested allergens
Feeding at-risk neonates/infants with formulas based on heterologous proteins entails an increase in IgE levels and/or of anaphylactic manifestations
Month of birth may be associated with the risk of IgE-mediated hypersensitivity in the subsequent years of life
Exposure to aeroallergens (pollens, house dust mites, molds, pets, etc.) increases the synthesis of specific IgE antibodies
Effect of infections
Viral infections (Epstein-Barr virus, respiratory syncytial virus, etc.) may be temporarily associated with priming of specific IgE antibodies
Effect of medications and other agents
Progesterone taken in pregnancy increased cord blood IgE antibodies
Passive smoking augments total IgE levels
Cortisone reduces the IgE low-affinity receptors on lymphocytes and monocytes of atopic children
Specific immunotherapy reduces specific IgE antibody levels
Hygiene hypothesis
Increased use of antibiotics in childhood, cleaner drinking water, improved sanitation, and widespread vaccination practices could be associated with an increased atopy prevalence

Modified from [23].

come of nonspecific synthesis rather than antigen stimulation. The lack of reactions in the 1st week of life and at initial exposure to CM [195] or hydrolyzed formulas (HF) [50] does not favor this hypothesis but at the same time, it does not exclude it [195].

Several studies have been conducted in different countries to establish whether a special diet during the final months of pregnancy can decrease allergy prevalence in children. Consequently, in atopic families that gave their consent, maternal diets were monitored during pregnancy, reducing the consumption of CM, eggs, fish and so on during the last trimester. Four hundred pregnant women were examined and they followed four different diets during the last trimester: total or partial elimination of the incriminated foods, free diet, or the daily consumption of an egg and 1 l of CM. This study [128] and others reported to date [254, 255, 486] have not revealed any statistically significant differences in either total IgE levels or in atopy development when comparing the offspring of mothers on a restricted diet and those of mothers who did not follow any diet [255]. The results at the 5-year follow-up [129] confirm that no benefits can be gained from this diet, as demonstrated by maternal avoidance of CM and egg during the third trimester of pregnancy and lactation yielding no reduction in AD and CM and egg sensitization in their HR German newborns [181]. We must note that the fetus is already immunologically active starting in the second trimester (Tables 2.4–2.6) and, therefore, the lack of success of the diets during pregnancy may depend on not starting any diet before the third trimester.

The anaphylactic reactions occurring in newborns or infants the first time they ingest CM (Fig. 9.14) imply that evidently it is not easy to protect the predisposed newborn after birth. These reactions may involve the transfer of maternal antigens to the antigen binding sites of anti-idiotype antibodies: if anti-polio anti-idiotype antibodies can be transferred from mother to child, other anti-idiotype antibodies may likewise replace antigens, mimicking their functional properties and thus being transferred from mother to fetus, acting like antigens in the prenatal period or subsequently.

Role of Postnatal Diet

Role of Foods in the Immediate Postnatal Period

In addition to the studies we have cited and those we discussed in Chap. 4 (repeated low doses of allergens can sensitize a predisposed individual), two infants presented anaphylactic shock the first time they were given CM proteins: but it was the second time, since they had received supplements while in maternity wards [45, 253, 265]. Likewise, HFs given to nine newborns at risk triggered anaphylactic reactions when the babies were weaned from the breast [50]. Other studies have shown that nurslings with adverse reactions to CM [196] or with positive skin prick tests (SPTs) to CM and sIgE to egg [256] *were fed CM during the first few days after birth*; moreover, total IgE titers at 5 days of life were significantly related to the amount of supplements *they were given immediately after birth* [371]. All the infants who developed CM allergy (CMA) during exclusive breast-feeding had been exposed to CM at maternity hospital [196]. Between 64 % and 100 % [196, 380, 403] of breast-fed infants who received CM supplements while in maternity wards developed CMA after 7 weeks (mean) [403]. Obviously, early exposure to CM (160 ml) was shown to be sufficient to increase the risk of atopic disease in the first 2 years of life [109]. Among 39 children who developed CMA, a supplement of only 40 ml of CM was adequate [195] (the "low doses," Chap. 3). It is unlikely that immune memory will be suppressed once activated. It is noteworthy that in a cohort of 6,209 newborn infants, significant risk factors of CMA development were full-term exposure to CM at the maternity hospital (87 %) (OR 3.5); long-term breast-feeding (OR

3.9 while breast-feeding supplemented with infrequent small amounts of CM) during the first 8 weeks (OR 5.7) induces the development of IgE-mediated CMA: 2.1% (OR 0.85) in those fed own mother's milk and 2.4% (OR 1.0) in those fed CM formula. More importantly, during the first 8 weeks of life, 54.5% of the infants were exposed to CM at home [358]. Exclusive breast-feeding for 3–4 months yielded statistically significant results [228, 250, 400]. In a trial on 3,903 children, those in the interventional subgroup had a significant protective effect on AD if compared with conventional CM formula (OR[adjusted], 0.64). [250]. Also in the PIAMA birth cohort study, a negative association with AD (OR, 0.6) was noted [228]. Finally, there was an association with an increased risk of AD in children with no parents with allergies (incidence rate ratio, [IRR], 1.29) but not for children with one (IRR, 1.11) or two (IRR, 0.88) parents with allergies [400]. To prevent hidden feeding of allergenic foods in maternity wards, a decision should be taken at a government level, as has been done in Scandinavia [23] and in a London hospital [280], where *supplements have no longer been permitted in maternity wards* for several decades. In contrast, giving supplements is a common practice in Denmark [285] and in Italy [90], and supplements are given to up to 73% of newborns [285] even the children who will be breast-fed receive a CM formula supplement during the night if they cry [90]. Consequently, it would be appropriate for public health agencies to closely supervise the quality of the messages targeting physicians, obstetrical and pediatric nurses and, indirectly, also mothers [141]. Above all, the utmost strictness is required in monitoring maternity wards and hospitals about CM supplements and the harmful use of bottles "by nurses with a pirate bottle ready behind the mother's back" [90]. In the US, since 1935 Ratner recommended to avoid giving CM to breast-fed infants during the neonatal period [340]. The negative effect of CM supplements is that they also shorten the duration of breast-feeding [28, 285], akin to maternal smoking [28, 263, 410], and terminate weaning in advance.

Role of Food in the Days and Month Following Birth

In terms of potential sensitization, the first few weeks of life seem to be particularly critical:

- The mechanisms responsible for *excluding and eliminating antigens* are physiologically immature, permitting protein macromolecules to pass across the intestinal barrier, which is more permeable, particularly if – as demonstrated in newborn rats – intestinal closure is delayed by early CM antigen feeding. This is paralleled by other data [211, 401].
- *A decisive role is played by a sIgA transient decrease* during the first 4 weeks; sIgA plays a protective role by blocking intestinally absorbed antigens, having the highest IgA antibodies concentrations in intestinal secretions [448].

Food allergens represent the most frequent cause of *sensitization early in life,* as they can pass across the intestinal barrier, thus priming IgE antibody generation. Based on this context it is clear that the immune protection imparted to newborns is fundamental:

– *From colostrum,* rich in immunoglobulins (Igs), sIgA and EGF (epidermal growth factor), IgE-suppressing factors [455], which contributes to maturing intestinal defense and to intestinal closure, and EFA (essential fatty acids) (Table 2.19). IgA high levels, of which the newborn – in the first day of life – absorbs about 4 g/l, equivalent to the daily production of a healthy adult, testify to the important immunological protection that comes from breast milk [292]. These IgA have λ chains rather than κ chains, as opposed to serum IgA, and as a result newborns have Igs available that are of vital importance for defense [292].

– *From breast milk* that, as shown in Table 2.12, is rich in crucial cells, important protective anti-inflammatory and immunological defense factors, above all sIgA, as a result of which IgA in breast-fed babies will increase 12%/year until the age of 7 years (p=0.002) [225]. It also contains EFA [377] (Table 2.14), with normal levels [377] that do not depend on gestational age [199] and are only partially dependent on maternal diet, generally containing C18:2ω6 and C22:6ω3, and nucleotides (Tables 2.16, 2.17), which build up the immune system [70], with positive effects on long-chain polyunsaturated fatty acids (LCPUFAs), unlike enriched formulas [471]. Lastly, it performs an immune-modulating action on infantile immune mechanisms, by the transfer of cell-mediated immunity (CMI) and cytokines [24] (Table 2.20). Dietary imbalances such as reduced proportions of regulatory PUFAs (ω3 PUFAs) may be a risk factor for the development of atopic disease [226].

Although breast milk is not as nutritious as adapted formulas, it is *the prototype of hypoallergenic food*, since it provides the *only proteins recognized as homologous by the infant* and they are automatically nonallergenic. Moreover, breast milk ensures an excellent growth standard: based on Bayley's indexes of mental and psychomotor development, breast-fed babies surpass bottle-fed peers in terms of intellectual [262], cognitive and behavioral development [248]. It is the only food that allows such *personal, intimate and emotionally important contact* between mother and child, also promoting breast milk production, impaired by maternal admission to the maternity ward and CM administration there [186].

The erythrocyte concentration of arachidonic acid (20:4ω6) remains the same in breast-fed infants, while it decreases in babies fed EFA-enriched formulas, even if not to the extent of causing undesired effects [269]. The option of feeding HR infants using formulas enriched with ω-6 and ω-3 in the same amounts existent in breast milk is important [276], or with those containing linoleic/α linolenic:10:1 acid (recommended dosage: ω6 0.5–0.7 g/kg/day and ω3 0.07–015 g/kg/day), in case there is a need to replace the missing amounts with an EFA preparation, selected from Table 7.21. Nucleotide addition to CM formulas would promote EFA conversion by enhancing the action of ω6 and, in the long term, making LCP supplements unnecessary.

Breast milk contains very high levels of sCD14 (endotoxin receptor, Table 1.2), which has been postulated to protect against the development of atopy, AD (atopic dermatitis), or both [213].

We stress that IgE-mediated sensitization is possible via breast milk (Table 9.1), due to small quantities of food allergens ingested by the mother that, passing into breast milk, cause atopic manifestations in her child. However, the quantity of β-lactoglobulin (βLG) contained in the CM received in the maternity ward (500–860 ml) is impressive [195, 371] compared to the ng or ng/ml of βLG present in breast milk (Appendix 9.6). To summarize, CM supplements can occasion sensitization in predisposed infants, while small amounts of CM allergen in breast milk can later trigger allergic reactions, labeled as *breast milk allergy* (BMA) or *intrauterine sensitization,* whereas they are caused by CM drunk by lactating mothers [253] (Table 9.1). This possibility must not be cited as a reason to discourage breast-feeding, which is a biological form of nutrition. Instead, it is the reason behind the advice to nursing mothers to avoid or reduce their ingestion of allergenic foods [192]. We feel that inadvertent exposures to CM [195] appear to be far more important than the very small amount of CM transmitted via breast milk, which may rather induce tolerization than allergic sensitization [37].

Late weaning is also important for atopy prevention. Several authors suggest that the protective effect of breast milk seems to be due largely to the delayed weaning of breast-fed children [44, 46, 55]. It is commonly believed that breast-feeding lowers the prevalence of allergic manifestations in children at risk for atopy, being the sole source of nourishment [52, 360], and protracted breast milk-feeding is combined with delayed weaning [134], also because solid food introduction shortens its duration [123]. Data on breast-feeding delayed for up to 9 months of life are controversial, with or without the addition of solids [10, 367]. Others suggest prolonging breast-feeding for up to 12 months if possible [269]. Moreover, the *thymus is significantly larger in volume at 8 months in infants exclusively breast-fed* until that age, and higher in those still being breast-fed at 10 months compared to infants weaned between 8 and 10 months [175]. It was instead emphasized that each month of breast-feeding increased the risk of developing AD in the first 7 years; however, half of the children studied received a CM formula in the very first days of life [19] Kajosaari and Saarinen [221] have demonstrated that introducing solids *in the first 4 months* of life exposes children to the *risk of chronic or recurrent AD*: 35% of 135 children at risk in whom solids were introduced in the 3rd month presented AD by the age of 1 year, with a rate of CM and egg allergy that was fivefold higher than the 14% found in babies exclusively breast-fed until the age of 6 months [221]. The risk of FA is 2.9-fold higher compared to children not weaned in the indicated period ($p<0.005$), increasing in proportion to the number of foods introduced [132, 134] (Table 4.30). While others disagree [19, 137], the confirmation of the benefits of delayed weaning is nevertheless meaningful [84, 466], and these benefits include the prevention of AD [243], FA [484, 486] and asthma [244, 245, 307, 309, 339, 360, 410]. A study on 2,612 infants followed up to age 2 confirmed that a delayed introduction of solids for 4 months of life may prevent AD and sensitization, while a delayed introduction beyond 6 months failed to provide any benefit for the AD prevention [490]. Only 3 studies prolonged the breast-feeding for 9 months [8, 185, 186] with a decrease of atopy.

These data should make us reflect on the disadvantages of certain additions to the diet – at times for no valid reason [485] – during the early days or months of life of nurslings at risk, also without the knowledge of motivated parents [172]. These considerations are reinforced by the observation that radical changes have occurred in the diet, not only because of the introduction of new compounds and the increased use of additives, so that even fresh products have no taste. Nothing is known about the possible effects these modifications can have on children [23]. The best protection is thus afforded by exclusive and extended breast-feeding in children enrolled at birth and followed up prospectively [192]: Table 24.2 [9, 10, 15, 17, 38, 39, 44, 46, 76, 78–81, 133, 162, 165, 166, 167, 170, 176, 183–186, 197, 218, 220, 221, 227, 240, 244, 261, 264, 272, 280, 289, 297, 331, 360, 361, 367, 374, 388, 433, 484, 486–488], or retrospectively: Table 24.3 [27, 156, 157, 161, 235, 238, 267, 470]. The prospective studies in Table 24.2 are further divided based on whether allergen avoidance was included or not in the study. Table 24.4 [44–46] lists dietary and environmental manipulations suggested for HR infants and children.

Table 24.2. Prevention of atopy based on breast-milk feeding on children followed-up since birth: results of prospective studies according to the publication year

Authors	Reference	Year	No. of cases		Feeding of S group	Duration of BM (w, m)	F-U (years)	Effect on atopic diseases	Results in the study group (1st no.) compared to controls (2nd no.) and significance; data in parentheses = OR
			S	C					
Halpern et al	[170]	1973	352	1,084	BM, SM	0.5–6 m	7	= Atopy	12.1/19.9 NS
Chandra	[76]	1979	37	37	BM	>5 m	2	↓ AD, Asthma	10.8/56.7, $p<0.001$; asthma 2.7/21.6 $p<0.01$
Saarinen et al	[361]	1979	54	105	BM	6 m	3	↓ AD	0/18 $p<0.05$; FA 4/24, $p<0.05$
Ziering et al	[340]	1979	25	25	BM	6 m	2	↓ AD	32/64, $p=0.0235$
Kaufman and Frick	[227]	1981	38	56	BM	6 w	2	↓ Asthma	5.3/17.9, $p<0.05$
Hide and Guyer	[183]	1981	204	62	BM	6 m	1	= AD	7.9/8.9; asthma 11.8/11.3, NS[e]
Gruskay	[162]	1982	48	201	BM	3 m	15	↓ AD, AR, Asthma	Only 1 case of AR at 15 years
Juto et al	[218]	1982	54	11	BM	>1 m	1	↓ Asthma	0.06±0.3/1.3±2.1, $p<0.001$
								↓ Atopy	1.4±1.8/3.2±4.5, $p<0.05$
Businco et al	[44]	1983	49	41	BM + SM	6 m	2	↓ Atopy	18/37, Fisher =0.0381
Fergusson et al	[133]	1983	202	908	BM	4 m	4	↑/= Asthma	8.5/6.6, NS
Kajosaari and Saarinen	[221]	1983	70	65	BM	6 m	1	↓ FA	7/37; AD 14/35 $p<0.001$–0.01
Pratt	[331]	1984	19	58	BM	>3 m	5	↓ AD	15.8/37.9, $p<0.05$
Hide and Guyer	[187]	1985	115	52	BM	6 m	4	= AD	14.8/17.3; asthma 10.4/11.5, NS
Moore et al[a]	[296]	1985	224	35	BM	3 m	1	↓ AD	13/20, $p<0.05$
Chandra et al	[79]	1986	35	20	BM	3 m	1	↓ AD	14/60, Fisher =0.0046
Vandenplas and Sacre	[433]	1986	47	228	BM	3 m	0.4	↓ Atopy	8.5/39.9, $p=0.0001$
Miskelly et al	[289]	1988	189	293	BM + SM	±3 m	1	↓ Asthma	21.7/42.7, $p<0.001$
Hattevig et al[b]	[176]	1989	65	50	BM + CH	±3 m	1.5	↓ AD	10.8/28, $p=0.033$
Chandra et al	[80]	1989	97	40	BM	±6 m	1.5	↓ AD	22/70, $p<0.001$
Chandra et al	[81]	1989	72	72	BM	±4 m	1.5	↓ AD	18/30; asthma 0/4.5; AR 1.5/7.5, $p<0.05$
Lucas et al	[261]	1990	38	37	BM	5 w	2	↓ AD	6/15 (3.6); asthma 8/11 (1.6); atopy 13/24 (3.6)
Chandra and Hamed[d]	[78]	1991	60	68	BM	4 m	1.5	↓ AD	20/35.8, $p=0.0484$
Arshad and Hide	[9]	1992	420	747	BM	3 m	1	↓ Asthma	6.7/12, $p<0.01$
Sigurs et al	[388]	1992	65	60	BM + CH	± 3 m	4	↓ AD	29.2/50, $p=0.0038$

Table 24.2. (Continued)

Authors	Reference	Year	No. of cases		Feeding of S group	Duration of BM (w, m)	F-U (years)	Effect on atopic diseases	Results in the study group (1st no.) compared to controls (2nd no.) and significance; data in parentheses = OR
			S	C					
Burr et al	[39]	1993	179	274	BM	±3 m	7	↓ Asthma	59/74, $p<0.001$; AD, AR, NS
Halken et al	[166]	1993	20	75	BM	6 m	1.5	↓ CMA	0/20, Fisher =0.0207
Kajosaari	[220]	1994	51	62	BM	6 m	5	↓ AR	20/37, $p=0.04$; asthma 8/15, NS
Høst et al	[197]	1995	88	75	BM + CH	6 m	5	↓ CMA/CMI	5.7/20, $p=0.0055$
Saarinen and Kajosaari	[360]	1995	48	102	BM	<1–>6 m	17	↓ Atopy, AD, Asthma	42/65, $p=0.02$; severe 8/54, $p<0.0001$
									AD, $p=0.03$; FA $p=0.02$; asthma $p=0.01$
Chandra	[77]	1997	60	68	BM	4 m	5	↓ AD, Asthma	AD 10/29.9, $p=0.0057$; asthma 6.6/23.9, $p=0.0079$
With environmental manipulations									
Matthew et al	[280]	1977	23	19	BM + SM	3 m	1	↓ AD	13/47.4, Fisher =0.0171
Businco et al	[46]	1987	179	65	BM + SM	6 m	3.6	↓ AD, Asthma	4.5/15.4, $p=0.0039$; asthma 7.2/20, $p=0.0073$
								↓ Atopy	14.5/38.5, $p=0.0001$
Savilahti et al	[367]	1987	142	31	BM	6–9 m	1	↑ Atopy	38/13, NS
Zeiger et al	[486]	1989	103	185	BM + CH	±6 m	2	↓ AD, FA	7.2/20.1, $p=0.005$
Arshad et al	[8]	1992	58	62	BM + SH	9 m	1	↓ AD, FA	4/12 (3.59); 3/7 (3.29)
								↓ Asthma	7/19 (4.13)
Halken et al	[165]	1992	105	85	BM + CH/WH	≥ 3 m	1.5	↓ Atopy	32/74 $p<0.01$; AD 14/31, $p<0.01$
								↓ Asthma	13/37, $p<0.01$; FA 6/17, $p<0.05$,
Zeiger et al	[487]	1992	103	185	BM + CH	±6 m	4	↓ Atopy, FA, AR	at 12 months, $p=0.05$–0.01
Bardare et al	[15]	1993	145	196	BM + SM	6m ?	1	↓ Atopy	13.3/28.9, $p=0.0044$
Bruno et al	[38]	1993	160	14	BM + SM	6 m	4.3	↓ Atopy	11/21, $p=0.001$
Hide et al	[185]	1994	58	62	BM + IS	9 m	2	↓ Atopy	29.3/58.1, $p<0.05$; AR 3.4/11.3, $p<0.1$
								↓ Asthma	(SPT+) 6.9/22.6, $p=0.0162$
Machado et al	[264]	1994	333	87	BM + SM	6 m	4	↓ Atopy	17/32, $p=0.0028$
Zeiger et al	[484]	1995	53	106	BM + CH	±6 m	7	↓ FA	10/22, $p=0.06^c$

Table 24.2. (Continued)

Authors	Reference	Year	No. of cases		Feeding of S group	Duration of BM (w, m)	F-U (years)	Effect on atopic diseases	Results in the study group (1st no.) compared to controls (2nd no.) and significance; data in parentheses = OR
			S	C					
Hide et al	[186]	1996	58	62	BM + SH	9 m	4	↓ Atopy	32.7/54.8 (2.73); AD 13.8/24.2 (3.4)
								↓ Asthma	(SPT+) 13.8/33.9, $p<0.02$
D'Agata et al	[103]	1996	30	15	BM	6 m	0.6	↓ AD, asthma	8% vs 53%
Marini et al	[272]	1996	117	47	BM	4–5 m	3	↓ AD, AR, wheezing	all 13.2% vs 42.1%, $p=0.0002$
Oddy et al	[219]	1999	1,087	978	BM	1–6 m	6	↓ Wheezing	Kaplan Meyer survival functions[f]
		1087	978					↓ Asthma	Kaplan Meyer survival functions[f]
Halken et al	[167]	2000	232	246	BM	6 m	1.5	↓ Wheezing	50/232 at 5, 12, and 18 months, $p=0.001$
Kramer et al	[240]	2001	8,547	7,895	BM	3–6 m	1	↓ AD	3.3% vs 6.3%; (0.54; CI, 0.31–0.95)
Kull et al	[244]	2002	3,013	773	BM	4 m	2	↓ Asthma	(0.7; CI, 0.5–0.8)
								↓ AD, AR	(0.8; CI, 0.7–1.0), (0.7; CI, 0.5–1.0)
Schoetzau et al	[374]	2002	865	1,121	BM	4 m	1	↓ AD	(0.47; CI, 0.30–0.74)
de Jong et al	[374]	2002	752	730	BM CH	3 m	5	↓ Atopy	25% vs 26.3%, RR 1.05
Becker et al	[17]	2004	246	230	BM CH	4 m	2	↓ Asthma	0.40 (0.20–0.82)
Total			19,626	18,369					

Hide and Guyer [184] have re-examined the 1981 group [183], Burr et al [39] the 1988 group [289], Chandra and Hamed [57] the 1989 group [81], concluded by Chandra at the 5th year [77], Kajosaari [220] the 1983 group [221], Zeiger et al[484] the 1989 and [486] and 1992 groups [487], Saarinen and Kajosaari [360] the 1979 group [221], Hide et al [186] the study of Arshad et al [10], Sigurs et al [388] the study of Hattevig et al [176]; the number shown represents the total number of children whatever the study (excluding from total the re-examined = 12.8% = 10.587). In the Oddy et al [307] and Kull et al [244] studies, we compared the babies breastfed for ≤4 or ≥4 months. de Jong et al [109] consider as controls BM-fed children who had received CM supplements (see text).

↓ Decrease, ↑ increase, = no difference, *w* weeks, *m* months, *S* study group, *C* controls, *F-U* median follow-up time (years), *FA* food allergy, *AD* atopic dermatitis, *atopy* atopic diseases, *BM* breast milk, *SM* soy milk, *CH* casein hydrolysate, *WH* whey hydrolysate, *SH* soy hydrolysate, *CMA* CM allergy, *CMI* CM intolerance, *NS* not significant, *ND* not done, *OR* odds ratio, *CI* 95% confidence intervals.

[a] At 3 months.

[b] At 8 months.

[c] At the follow-up at 7 years, the decrease was the same as noted in 1989.

[d] Extension up to 18 months.

[e] In this study there was a 71% rate of drop-outs among the breast-fed infants, and among the asthmatics were also included children with wheezy bronchitis. We have considered as reduced wheezing results concluding that the positive prevention regarded wheezy bronchitis (e.g., [167]). See the related discussion in Chap. 4.

[f] The Kaplan Meyer survival functions [307] indicate that the cumulative incidence of both asthma ($p=0.001$) and wheeze ($p<0.001$) was higher if other milk was introduced <4 months. We have included in the study group the children breast-fed >4 months and in the controls those weaned before that age.

Table 24.3. Prevention of atopy based on BM: results of retrospective studies according to the publication year

Authors	Reference	Year	No. of cases		Feeding of S group	Duration of BM (months) (w, m)	F-U (years)	Effect on atopic diseases (as above)	Results in the study group (1st no.) compared to controls (2nd no.) and significance; data in parentheses, OR
			S	C					
Grulee and Sanford	[161]	1936	1,8354	1,707	BM	9 m	0.75	↓ AD	0.2/3.5, p=0.0000
Koivikko	[235]	1974	73	486	BM	≥8 m	1	↓ Asthma	p<0.0005, = AD
Blair	[27]	1977	80	59	BM	>2 m	20	↓ Asthma	25/64, p<0.05
Kramer and Moroz	[239]	1981	59	102	BM	≥2 m	0.8	= AD	41.5/31.1, NS
Gordon et al	[157]	1982	112	85	BM	≥3 m	2	= AD; asthma	22/15 NS
Golding	[156]	1982	221	1,567	BM	≥3 m	5	↓ AD	1.8/8.8, p<0.001
								= Asthma	0.2/1.4, NS
Magnusson	[267]	1988	48	142	BM	>3 m	1.5	= Atopy	16.7/21.1, NS
Wjst et al	[470]	1992	484	2,352	BM	±2 m	1	= Asthma, AR	NS
Raisler et al	[339]	1999	2,869	4,223	BM	6 m	0.5	↓ Wheeze	(0.83; CI, 0.70–1.00 vs 1.0; CI, 0.83–1.19)
Total			22,354	10,723					
Final total			41,980	29,092=71,072					

The studies have been considered "with environmental prevention" when the pertinent controls were suggested along with the dietetic manipulations; we have taken into account the reviews of Burr [39], Zeiger [481], and Kramer [241] whose remarks we consider.

The mothers of children at genetic risk for atopy were greatly motivated in the above 58 prospective and retrospective studies, and have breast-fed their babies for 136 days (median 135), although breast-feeding was not always exclusive.

↓ Decrease, ↑ increase, = no difference, *w* weeks, *m* months, *S* study group, *C* controls, *F-U* median follow-up time (years), *FA* food allergy, *AD* atopic dermatitis, *atopy* atopic diseases, *BM* breast milk, *SM* soy milk, *CH* casein hydrolysate, *WH* whey hydrolysate, *SH* soy hydrolysate, *CMA* CM allergy, *CMI* CM intolerance, *NS* not significant, *ND* not done, *OR* odds ratio, *CI* 95% confidence intervals, *RR* relative risk.

Table 24.4. Dietary and environmental manipulations suggested for HR infants and children

Environmental
Absolutely no smoking in the house
Strict environmental controls for the elimination of house dust
No pets in the house
Avoidance of air pollution
Avoidance of lifestyle changes
Avoidance of pesticides and consumer products
Dietary
Avoid strict food limitations in pregnant women expecting HR offspring; such mothers must take supplemental calcium (up to 1,500 mg daily)
No CM formula in the maternity ward
Exclusive breast-feeding for the first 6 months of life
Do not introduce solid foods during dietary prevention
CM and dairy products gradually introduced after the 6th month and egg after the first birthday

See the section on the hygiene hypothesis.
Data from [44–46].

Role of Allergen Avoidance

Recent data underscore the importance of *environmental factors* in the sensitization of children to certain allergens that favor the development of asthma. In addition, the timing of the exposure, *in utero* or during the 1st year of life, is crucial. Therefore, primary prevention begins before birth, as we can see from Table 24.5 [44, 46, 60], and it is maximal when preparing the home for the newcomer. The need to ensure a healthy environment prior to the birth of a baby (Table 2.26) is demonstrated by a higher prevalence of severe forms of asthma and a higher number of asthma attacks observed per year in children living in areas with significant environmental pollution, as compared to those living in clean areas [6, 18].

In Chap. 4, we examined the effects of the *energy crisis:* this led to building homes with new features, but it also created a parallel increase in humidity levels above all trapped in rooms [23]. This has indubitably contributed greatly to an increased prevalence of pediatric asthma in Northern Europe and in other areas, and encouraged the development of environmental conditions favorable to Der p/f and mold growth [463]. Home construction year is associated with asthma at the age of 4 [245].

Preventive measures have become mandatory as a result of *high Der p 1 levels detected* in *amniotic fluid samples* at 16–17 weeks of gestation [188], in HR newborns [10] and infants [300, 396, 427] (Fig. 24.1). The measured levels, between 4.3–4.5 and 18.4 µg/g of dust (GM, geometric mean) [10, 396, 427], exceed the lowest limit of 2 µg/g and the highest of 10 µg/g [306, 327]. Sensitization can also occur at levels of <1 [201] or <2 µg of Der p/g [439], while in 25% of homes there were levels >2 µg of Der p/g [18]: this trend can be reversed only by adopting an active preventive strategy [274]. Exposure to 10 µg Der p/g leads to a fourfold increase in the risk of developing asthma in early childhood [397], but even to 1 µg may be associated with a higher incidence of AD; the presence of Der p 1 IgE antibody at 18 months of age was associated with a higher incidence of asthma [201] (Table 24.6) [230, 396, 479]. Environmental measures must be taken against house dust mites (HDM) [463] to reduce the level to <2 µg Der p/g *several months prior to the birth* of at-risk babies or before the family moves to the home, since Der p fecal matter may persist for several months despite strict allergen avoidance. Thus, allergen avoidance must be fulfilled before at-risk children present asthma.

In the *Isle of Wight study* [10, 185, 186] even a modest reduction in HDM allergen amounts in homes of infants at risk reduced the prevalence of sensitization to mites and overall manifestation of atopy during the 1st year of life and some benefits on allergic disease were recorded at age 4 years. At age 8, the results were confirmed with a fivefold reduction of HDM antigen in the preventive group compared to no reduction in the control group (1.28 vs 2.45 µg/g) [12]. Active measures to reduce prenatal and postnatal allergen exposure resulted in HR infants having fewer respiratory symptoms at 1 year of age [102]. Since infants usually spend 80% of their time at home, any home dampness must be considered of the utmost importance, paying attention both to wall stains and to moisture that condenses on glass windows, particularly in bedrooms, as this can represent an additional risk for asthma development [6, 463]. Another measure of primary prevention to be started several months before childbirth or moving elsewhere involves removing any *cats and dogs* in the house [22], without any halfway measures [474]. The dose that can sensitize infants ≥1 year with pets in the house is 3–300 ng/day or 1–100 µg/year, while ng of inhaled allergens/min can cause acute asthma and bronchial hyperreactivity (BHR) [118].

Passive smoke is a particularly harmful environmental factor that can play a leading role in the etiology of early childhood asthma, fostered by a significant rise by smoking parents of children at risk (31%) [165] (Fig. 4.23). A large body of epidemiological evidence emphasizes that *maternal smoking* can raise IgE levels in cord blood (CB) (Table 3.7), it is associated with childhood allergy [266, 267] and the onset of asthma and LRTIs in children <1 year, and lowers EGF [214] and LCP levels [2] in breast milk. Maternal smoking during pregnancy can compromise *the development of the respiratory system* [402] (Table 4.24) *and of the brain* [158], and have important implications for respiratory disease

Table 24.5. Prevention of atopic disease in high-risk children

Before birth
Stop maternal smoking during pregnancy
Free diet for pregnant women (from the allergological point of view)
Remove pets and have the house thoroughly cleaned
Prefer to live on a high storey (if feasible)
If feasible adopt an underfloor heating
Ensure adequate ventilation and humidity (anti-Der p measures)
Perinatally
Nontraumatic delivery
No hidden CM formula at the maternity ward
In infancy
Stop maternal smoking during breast-feeding
Encourage exclusive breast-feeding, prolonged if possible up to the 6th month of life
Consider an elimination diet of nursing mothers (CM, egg)
Clearly explain to parents, especially to grandparents, caregivers and baby-sitters the significance of such avoidance measures
Recommend a soy protein formula (SPF) or homemade meat-based formula (HMMBF) or Rezza's diet. as breast-milk back-up or replacement up to the 6th month of life (two pediatric guidelines prefer to avoid SPFs and suggest extensively HFs [5, 198]). We also use soy + pork collagen formulas
Consider supplemental calcium during restrictive diets
No solid food for the first 6 months; at that age vegetable broth with fresh lamb meat, rice flour, olive oil, and pear may be initiated
Delayed introduction of dairy products until 1 year of life, starting with parmesan at gradually increasing doses
CM- and egg-free pasta and cookies, and bread at 8 months (if the child is not allergic to cereals)
Delayed introduction of eggs up to 2 years, and fish, peanuts and nuts up to 3 years of age
Introduce new foods one at a time
Postpone feeding beef: BSE (Chap. 9) and antibiotics (Chap. 19)
Postpone foods potentially cross-reacting with pollens (Table 9.48)
Do not cook apples, spinach, citrus fruit in stainless steel pots, both new and used, or pressure cookers
Pediatrician or allergist suggestions or skin prick test results shall specify which potentially sensitizing foods could be introduced next
Environmental allergen avoidance
Wash baby with Marseille soap
Use underwear exclusively of white cotton to be washed in a washing machine with Marseille soap at a T >60 °C, wash blankets every 15 days
Absolute ETS cessation at home or in daycare center
Do not allow furred pets in the home
Reduce the early exposure to major allergens especially in the baby's bedroom, and limit any potentially sensitizing factor such as house dust, wool, molds, animal danders, cockroaches, etc.
Remove upholstered furniture and stuffed toys and replace with leather, plastic, vinyl, or wooden furniture and toys, removing plush toys, carpets, wall-to-wall carpeting, bedside rugs, wool and/or feather mattress and pillow
Encase in allergen-impermeable cover but water-permeable fabric, as discussed later, both mattress and pillow in the child's bedroom
Air the child's bedroom daily and vacuum it using a vacuum cleaner with an HEPA filter weekly, scrub bedsprings outside the room
Maintain at home a T of ≈ 20 °C and reduce indoor humidity up to <50 %
Consult pediatrician or allergist about any uncertainty and/or before taking any initiative

Data from [44, 46, 60].

in older children and adults [402]. In 545 HR infants, exposure to maternal smoking during pregnancy or the 1st year was *a risk factor for asthma at 2* [17] and at 4 years [245] and a 13.8 risk factor [12] for children on prevention [12, 17]. ETS is not only a triggering factor of respiratory allergy in babies at risk of atopy, but especially an additional genetic factor, since asthma can be more easily provoked if an atopic parent smokes (more if both parents smoke), and even in children of smoking parents who are not atopic [58]. Therefore, it must be strongly discouraged during pregnancy and following birth. CB cotinine levels were detectable in 61 %–70 % of

Table 24.6. Risk of sensitization to Der p

Threshold value of the risk of sensitization to Der p, of Der p-specific IgE and asthmatic symptoms: 2 µg Der p 1/g dust
Threshold value of the risk of developing acute symptoms: 10 µg Der p 1/g dust
Age of the first episode of wheezing inversely correlated to the greater exposure to Der p 1 allergen during infancy

Data from [230, 396, 479].

HR and low-risk babies [102]. When both cotinine and nicotine were measured in the hair of the newborns of smoking mothers, these values were *6- to 17.5-fold > those in children of non-smokers*; associated with cotinine and nicotine levels in the hair of children of *non-smoking mothers exposed to secondhand smoke during pregnancy* [122]. Moreover, trying to convince the parents of newborns to quit smoking is discouraging [476]: the very high cotinine levels of infants of smoking nursing mothers could not be differentiated from the control levels [86]. *Newborns born to smoking mothers already had a critical level in their CB* of >5 ng/ml; in 50% of newborns there was at least one smoker, and at the age of 1 year, in 51% of the infants the cotinine level was equal to 30 ng/mg of creatinine [18]. Another negative aspect is the reduced propensity for breast-feeding among smoking mothers: 43–51% vs 80% [263] at 6 weeks or 57% vs 70% [470], particularly when ≈11 cigarettes are smoked a day. In the case of a newborn at risk, a powerful appeal must be made to the parents' motivation. Here, encouraging data have come from a valid contribution made by widespread efforts of the Department of Health Services to convince and educate parents, which yielded positive results in 80% of parents (Chap. 4). The possible danger of nicotine contained in commonly consumed foods has been reduced significantly [180].

Causal relationships can also be established between *atmospheric pollutants* (Tables 4.13–4.16) and allergic manifestations possibly associated with felling of several trees in the city or district. Airborne substances can directly sensitize the organism, triggering allergic reactions. In some cases (particulate matters [PM]), they can also act as adjuvants, priming IgE response. Lastly, with an indirect mechanism they are not only able to modulate the host immune response but also act on the shock organ, inducing symptoms in sensitized but asymptomatic subjects [234]: daily life in our cities repeatedly demonstrates this.

Failure to observe these principles can cause inestimable damage to both the child and the entire family's health and well-being. To conclude, ETS, early contact with household dust, humidity, mold and domestic pets, and viral respiratory infections (VRIs) in the 1st year of life can quickly stimulate or not IgE synthesis, which can lead to atopic manifestation even many years later [24].

Immune Interventions

The flourishing investigations into the genetic basis of atopy have identified several candidate genes (Table 4.2), which may provide knowledge that could help in allergy prevention. A special immune intervention strategy, which still requires study, involves the prevention of initial Th2 sensitization to environmental allergens, to induce tolerance or better, immune deviation [189]. This would be achieved through three steps, to identify the allergens that cause atopic disease, the defense mechanism applied by the immune system to overcome the offending agent and the method for administering (vaccination) the allergen or an appropriate analog in a form that can stimulate the defense mechanism best able to induce the development of a protective immunological memory. This procedure would be performed in childhood, before the child is sensitized, so that his or her response to allergens can turn from hypersensitivity to tolerance [189]. Given that a cocktail of allergens is indicated for this purpose [189], we feel it would be difficult to specify the number of potential allergens, which include the pertussis virus, that can act as an adjuvant in promoting IgE. Thus, this procedure should also be scheduled far from whooping cough vaccinations. Other strategies that are still being investigated include CMA prevention, achieved in laboratory animals with a single IV injection of an IgG-βLG conjugate (Chap. 9), and genetically modified fruit to replace SIT via injection (Chap. 13). Dietary nucleic acids may play an important role in promoting a shift in Th1/Th2 balance toward Th1-dominant immunity, also suppressing serum ovalbumin-specific IgE and IgG_1 antibody levels as well as in vitro IL_4 and IL_{10} secretion, while enhancing both serum ovalbumin-specific $IgG_{2\alpha}$ antibody levels and in vitro IFN-ɣ [406] whose deficiency has been shown in children with CMA [328].

Dietary Prevention and Environmental Measures

For 70 years, countless studies have shown that based on breast-feeding and/or feeding with soy protein formulas (SPFs) or Rezza's diet and/or CM delayed introduction, with delayed weaning starting at 6 months, is the rationale underlying the potential benefit of early allergen avoidance to reduce or prevent the prevalence of allergic disease in neonates genetically at risk for atopy: breast milk delays the onset of asthma [245] and AD [243]. The PREVASC study has come to the point: infants in the intervention group were significantly more breast-fed ($p = 0.001$) [242] and/or significantly more received a hypoallergenic formula feeding than infants in the control group. The first intake of solid food was significantly more often postponed to the age of 6 months in the intervention group compared with the control group ($p = 0.0001$) [242, 376]. In 1936, Grulee and Sanford emphasized the protective effect of breast milk in a large population study, and reported that among breast-fed children the frequency of AD was 1,750% lower than in children fed CM [161]. Glaser and Johnstone [154] have demonstrated a significant correlation between feeding children SPF and a lower rate of AD. Since then, there have been both prospective (Table 24.2) and retrospective studies (Table 24.3). The data have been confirmed by a 17-year follow-up, showing statistical significance for respiratory allergy, FA and AD (Fig. 24.2) [360], and

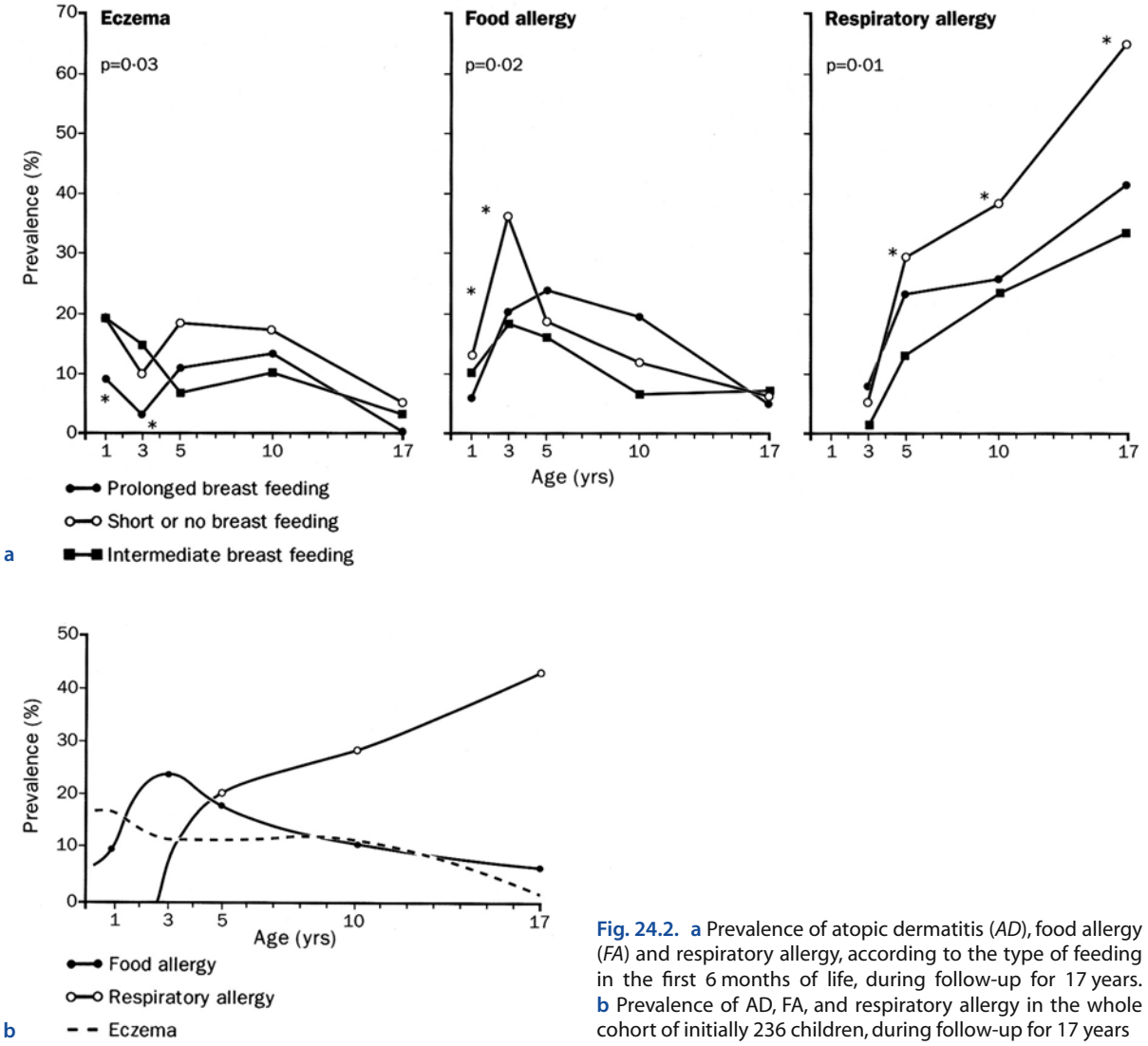

Fig. 24.2. **a** Prevalence of atopic dermatitis (*AD*), food allergy (*FA*) and respiratory allergy, according to the type of feeding in the first 6 months of life, during follow-up for 17 years. **b** Prevalence of AD, FA, and respiratory allergy in the whole cohort of initially 236 children, during follow-up for 17 years

by a 15-year follow-up, in which no breast-fed child developed FA and AD [162]. A 7-year follow-up of 545 children showed a protective effect on respiratory symptoms only if exclusive breast-milk feeding was continued for at least 15 weeks compared to bottle-fed children; solid feeding started before 15 weeks was associated with an increased risk of wheeze during childhood [466]. Table 24.4 shows that CM feeding should be limited, given that after a meal of CM *βLG persists for 3 days* [265]. Allergen avoidance should be enhanced and refined to prevent sensitization to aeroallergens [235] since dietary measures alone are insufficient [481, 484]. In a subsequent Australian study on the same children [307], breastfeeding for >4 months was a significant protective factor for wheezing LRTI, current asthma and atopy, following multivariate adjustment [309]. In a Swedish study in 4,089 babies with a 2-year follow-up, breast-feeding had a significant preventive impact on development of asthma, suspected AR, AD, and suspected allergic respiratory symptoms associated with exposure to pollen and/or pets during the first 2 years of life [244]. Exclusive breast-feeding for ≥4 months reduced the risk for AD (OR, 0.78; 95% CI, 0.63–0.96) [243] and for asthma (OR, 0.72; 95% CI, 0.53–0.97) [245] at the age of 4. In a US retrospective study [339], 7,092 babies were followed up for 6 months: compared with no breast-feeding, full breast-feeding infants had lower OR of coughing or wheezing, vomiting and diarrhea, and lower mean ratios of illness months and sick baby medical visits [339]. Breast-fed children had significantly reduced odds of being diagnosed with asthma or recurrent wheeze before 24 months of age compared with never-breast-fed children [87] A meta-analysis has noted more than a tripling in severe LRTIs resulting in hospitalizations for bottle-fed infants compared to infants exclusively breast-fed for 4 months [143].

The 61 studies on the effects of breast milk have involved over 71,000 children to date. The *protective value*

of breast milk (supplemented with other formulas in 31% of cases) in relation to allergic sensitization has been confirmed by 48/52 (92.3%) of the prospective studies and by 5/9 (55.5%) of the retrospective studies (p=0.0026; Fisher =0.0126). Inversely, 8/61 trials (13.1%) either failed to note any difference or they reported an increase in allergy among breast-fed children as opposed to those fed artificially (Tables 24.2, 24.3).

Important results regard breast milk's positive long-term health outcome on IgE levels in atopic children. In a birth cohort study of 1,246 children studied until the age of 11 years, the children who were breast-fed for >4 months had significantly lower serum IgE levels at both 6 and 11 years if their mothers had low serum IgE levels, whereas the opposite occurred in 16% of children with mothers who had higher serum IgE levels [477]. This result may be explained by individual variations in the levels of breast milk's immunological constituents: exclusively breast-fed children, especially those in the high IgE group, had a statistically lower mean IgE level at 1 and 2 years of age than babies fed adapted or HF formula [272].

Possible Causes of Controversial Results in Prevention Studies

Several controversies can arise from some shortcomings such as the parameters outlined in Table 5.1 or from differences in study design (cohort, case–control, cross-sectional), nonmasked evaluations, unconfirmed diagnoses, no documentation of adherence, short-term breast-feeding, and differential environmental control measures [483]. Kramer et al [237] closely analyzed 636 case histories to monitor whether diagnosis adhered to biological and methodological standards. They found that 34.3% of 470 infants were breast-fed (infant feeding), AD was severe in 17 cases out of 142 (11.9%) (atopic condition), on whom was based the relationship between AD severity and breast-feeding (statistical power). Methodology flaws can be found in other studies. Breast-feeding lasted <3 months [356], for example, in 47% of cases [147], or <6 weeks [170, 261, 382, 404], and solids were introduced early to the children, at 4 months in up to 75% of cases [133] (Table 4.30), with predictable effects [46].

Poor compliance was shown with the recommendation to follow exclusive breast-feeding for 6 months [166]. In Germany, breast-feeding is not widely practiced [18], and in the US it is practiced in 50% of cases, a figure dropping to <20% at 6 months [200]. A significant parameter is the number of dropouts, from 16% to 38% [436, 486] to 57% after 2 years, altering the ratio between breast-fed infants and control subjects [184], an evident contrast with the 11% figure after 7 years [40] and 36% after 17 [360]. As two studies [307, 410] point out, it is the age that CM was introduced rather than the duration of breast-feeding that was more strictly associated with asthma or atopy at 6 years [307]. A *program of allergen avoidance* was disregarded in many studies, or it was not followed adequately (Table 24.7) [46]. There were no such measures in a retrospective study that lasted >30 years, but babies were *breast-fed for ≥1 month* [404]; the criticism was impartial [359]. In a longitudinal study of approximately 1,000 New Zealand children to age 26, information regarding breast-feeding modalities and supplemental milk was assessed retrospectively when the children were 3 years old [382]. *Breastfeeding for ≥1 month* had a higher prevalence of allergen sensitization and asthma [382]. In 926 babies exclusively breast-feeding for <4 months in *13.5%* and >4 months in *16.6%* of cases, breast-feeding was associated with an increased risk of asthma at the age of 6 years, but only *for atopic children with asthmatic mothers* [478].

For example none of the mothers was requested to keep a diary of her child's illness and feeding history [307, 374, 375]: in two studies [382, 478], data were assessed by interviewers evaluating data collected by nurses during their first 2–3 years of life [382] or by physicians' reports or parental questionnaires at 18 months [478]. Similarly, in two Japanese studies breast-feeding was associated with an increased prevalence of allergy according to data gathered from students aged 12–15 and their parents [290] or from students aged 6–15 without their parents [408]. Kramer has shown that retrospective data collection of infant-feed-

Table 24.7. Possible causes of controversial results in studies on atopy prevention

1.	Selection criteria (atopic or nonatopic parents)
2.	Methods used to diagnose the atopic disease (parental diagnosis, general practitioners, pediatricians, allergists, dermatologists, questionnaires)
3.	Lack of supportive immunological data
4.	Lack of statistical analysis of data
5.	Social demographic characteristics
6.	Sex of babies
7.	Drop-out rates
8.	Small number of subjects (possible type 2 error)
9.	Exclusive nature and duration of breast-feeding
10.	Dietary restriction in mothers (cow's milk, egg)
11.	Age of solid food introduction
12.	Type of solid foods
13.	Maternal and child compliance
14.	Attendance at daycare facilities
15.	Allergen avoidance (smoking, dust, pollution)
16.	Duration of follow-up
17.	Prospective vs retrospective studies

Data from [46].

ing history is prone to recall bias, because mothers may forget details about duration and exclusivity of breast-feeding [237]. We know that 32% of fathers and 28% of mothers failed to recollect the feeding procedures [470]. In these studies, no information was given about breast-feeding modalities and supplemental milk, which could not be obtained retrospectively at age 2–3 [382, 478], 7 [404], 10 [470], 6–15 [408], or 12–15 years [290].

The exposure to CM formula in the maternity hospital has been highlighted [382].

In 6,535 children (65% of whom were breast-fed), breast-feeding had no benefit in preventing respiratory allergy, even if breast milk was administered for about 6 months [470]. However, the age at onset of asthma and AD was significantly delayed in breast-fed children and the risk of developing asthma and/or AR was statistically much lower [470]. Breast-feeding is not promoted because it supposedly offers only partial protection, its use is allegedly controversial, and cases have been reported of atopic disease occurring in exclusive breast-feeding [133, 367]. Although the overall beneficial effect of breast-feeding on a child's health *was never questioned,* it is only allegedly cited as ineffective in preventing or reducing atopic symptoms [126, 370].

The problem with the majority of these studies showing controversial findings is that of the confounding factors and that the infants cannot, for ethical reasons, be randomly assigned to breast-feeding or formula-feeding in a double-blind placebo-controlled study (DBPC) [31, 237]. In addition, the lingering discussion on the topic has gained a fresh revival by a reportedly increased risk of asthma and atopy in breast-fed New Zealanders [382] and Tucson, Arizona children who had an asthmatic mother [478]. Logically, these recent adverse reports could have negative public health implications on infant feeding practices [250]. To date, the 61 studies on breast-milk effectiveness – with or without other formulas – have involved over *1,000 children per year* in >70 years: 92% of 61 studies have demonstrated breast milk's advantages, while controversies are raised by 3/61 studies (4.9%), apart from 5/61 failing to show any differences (8.2%) (Tables 24.2, 24.3). This confirms that breast milk contains many immune factors that compensate for the undeveloped defence mechanisms of the gut of the newborn infant (Tables 2.15, 2.16). On the other hand, BMA is a frequently discussed question: The 21 exclusively breast-fed children with BMA were selected among approximately 50,000 children who attended our Division over 10 years. The incidence of BMA may thus range from as low as 0.042% to as low as 0.0042% per year [56] (See Table 9.1). Even if we extend the Kramer et al criticism [237], noting above all the short or very short duration of breast-feeding and the limited size of several cohorts and follow-ups, these studies have involved a large number of children, and convincingly demonstrate the value of breast-milk prophylaxis in neonates genetically at risk for allergy, in a statistically significant percentage of cases. It should not be surprising that infants at risk develop allergic disease (this follows the calculus of probability, Table 4.9) over the long term, above all since highly selected infants are involved.

Studies on the Prevention of Atopic Dermatitis and Food Allergy

In 26 studies, atopic dermatitis (AD) was prevented, four studies established that there was no difference, in seven studies FA/CMA was prevented, and 13 studies prevented atopy in general. Thus 43/52 prospective studies (82.6%) have demonstrated a positive effect of breast-feeding in preventing AD and FA, as well as a study on 4,089 children [243]. A meta-analysis identified 18 prospective studies (818 infants) that met the predefined inclusion criteria and concluded that exclusive breast-feeding during the first 3 months of life is associated with lower incidence rates of AD during childhood in children with a FHA (FH of atopy) (OR 0.84, CI 0.59–1.19). Thus, breast-feeding should be strongly recommended to mothers of infants with FHA as a means of preventing asthma and AD [145]. AD was prevented with asthma in several prospective studies [10, 46, 76, 81, 103, 165, 244, 272, 360, 361, 487]. CMA was specifically prevented in a study on 91 children enrolled at birth but with no control group. No child was sensitized to CM at 6 months and two at 12 months [181].

Studies on the Prevention of Respiratory Allergy

To date, 27 out of 52 prospective studies (51.9%) have noted a reduction in asthma and wheezing and/or AR, but were protected by 16/22 studies (72.7%) with environmental manipulations. The extension at age 8 [12] of the Isle of Wight study [10] showed that after adjusting for confounding variables, the prophylactic group was found to be at a significantly reduced risk for current wheeze, asthma, and atopy (OR: 0.11–0.26) [12]. The Kramer criteria have been applied to 12 studies on the association between breast-feeding and childhood asthma, establishing an OR of 0.70 for the protective effect of breast-feeding and a greater OR (0.52) in children with FHA [146]. In the 17-year study, the effect was positive for respiratory allergy (Fig. 24.2b). Regarding AR, another meta-analysis ascertained that exclusive breast-feeding during the first 3 months after birth is also protective according to six studies, either with positive FHA (OR, 0.87) or negative FHA (OR, 0.74) [288]. In the 20-year retrospective study [27], breast-feeding for >8 weeks ensured a long-term prognostic improvement in the asthmatic children. In the 15-year follow-up, no child developed respiratory allergy, only one child developed AR between 8 and 15 years [162].

In the Third National Health and Nutrition Examination Survey (NHANES III), ever breast-fed children had significantly reduced odds of being diagnosed with asthma and of having recurrent wheeze before 24 months of age [87]. We compared two contrasting studies, one with no effect of dietary manipulation on respiratory diseases [487] and another that instead demonstrated positive results on wheezing and recurrent coughing, with highly significant statistical differences [235]. The data show a significant reduction in asthma at age 4 [245] and at 6 years if exclusive breast-feeding is continued for ≥4 months after birth [245, 307]. The Kaplan-Meier survival functions showed that the cumulative incidence of both asthma and wheeze was significantly higher if other milk was introduced before 4 months [307]. Moreover, breast milk should be recommended to mothers of infants with FHA in first-degree relatives [12, 146]. The study on 16,442 infants [240] breast-fed for 3–6 months has established a solid scientific basis for future interventions to promote breast-feeding. Thus breast-feeding can beneficially impact health care by reducing the need to treat young children for asthma [87].

These observations may suggest that a longer duration of breast-feeding could extend its positive effect to respiratory allergy. Indeed, in a prospective birth cohort study of 2,602 Australian children, the risk of asthma increased if exclusive breast-feeding was stopped (other milk was introduced) before 4 months [308]. In several negative studies, *no dietary–environmental measures were followed* [290, 382, 404, 408, 470, 478], as also found in one study stressing the weight of maternal atopy as a risk factor for AD [356].

The cornerstone of the prevention of respiratory allergy is to discourage maternal smoking during pregnancy and afterwards [9, 10, 46, 49, 55, 58, 67]. Cigarette smoke causes respiratory allergy in an exponential fashion: children exposed to tobacco smoke *in utero* had an earlier onset of sensitization as compared with non-exposed children [205]. The importance of prophylactic measures was underscored in the prevention of asthma [9], in which the risk created by passive smoke must also be given due consideration. As early as the age of 3 months, it shows approximately a fivefold increase in the risk of allergy [10]. There are so many smoking parents as opposed to nonsmoking parents that they represent statistical differences throughout the entire 1st year of life [185].

In this context, important confirmation [10] comes from the fact that *environmental measures*, which have been proposed by many authors for years (Table 24.4), are highly effective in reducing the prevalence of allergic disease: 14% vs 40% depending on whether the group of children (all fed with breast milk and hydrolyzed SPF) applied these measures or not [10]. In a sample of infants divided into two groups in which preventive measures were or were not taken, the prevalence of atopic disease was higher in the group not taking preventive measures: 29.3% and 58.1%, respectively [185] or 20.0% and 46.8% [12]. In a multicenter, randomized study, children were subjected to dietary and environmental controls. At 1 year of age, the overall sensitization rate against the tested allergens (Der p and Der f) and food allergens (egg, CM) in the prophylactic group was 6.21% vs 10.67% in the control group [169]. In an unpublished, retrospective study, 289 children, 169 males and 120 females, aged 3.5–7.5 years, attending our department because they were affected with respiratory allergy, were breast-fed in 94.5% of cases (p=0.0225) for 136 days (mean 125 days, range 33–211). The control children were breast-fed in 69.6% of cases, for a mean of 98 days (range 10–110) (p=0.0001), although also in these children breast-feeding was not always exclusive. In our opinion, the results are positive when both the children and their families were followed up effectively: in 94% of cases, no pets were kept in the house and smoking was avoided in 67% (81% of mothers also during pregnancy) (p=0.0001) [38]. It must be noted that a favorable low allergic environment as occurs in Sweden significantly reduces sensitization rates in children [311].

Dietary Restrictions for Mothers Breast-Feeding Babies at Risk for Atopy

Dietary restrictions have long been implemented with evident success [362], also to minimize the risk of allergenic sensitization through breast milk. In short (Tables 24.4, 24.5):

- The nursing mother should *not freely consume CM and eggs,* or any food that contains them. We and others recommend lowering CM consumption to 150–200 ml/day and eggs to two a week [44, 46, 79, 145].
- If *the nursing infant presents disorders* attributable even to minimum amounts of these or other foods in breast milk, the mother should completely eliminate them from her diet [113].
- *Foods that contain any CM and/or egg* derivatives should be eliminated, as well as casein or sodium caseinate, etc. (Appendix 9.2).
- Meat consumption is permitted, avoiding beef if the nursing infant shows signs of allergy [135].
- *Peanuts, shellfish and mollusks* must be eliminated [362]. Peanuts eaten by a lactating mother provoked an allergic reaction in her 2-week-old infant [113].
- The mother should add *calcium orally* to her diet on a daily basis: Tables 9.23, 9.24.

CM, egg and fish elimination from the mother's diet for 3 months reduces the number of nursing infants who are sensitized in the first 3 months and of those with AD in the first 6 months [177]. At the 4-year follow-up, 19 of 65 (29.2%) children breast-fed by mothers on restriction diets presented AD, with 28 of 50 (56%) among those with no restrictions [388], but the incidence of AD was even lower, 14.2% vs 30.6%, respectively [54], or

10% at 52 months [38], and at 12 months in a study that negatively evaluated maternal diets [181], as opposed to 20% of other case studies [18]. However, one of two children sensitized to CM at 12 months belonged to the group whose mothers did not follow a restricted diet [181].

Allergy-prevention programs should take into account dietary restrictions, to be adopted only in selected cases with a genetically determined risk (Table 24.5). In a cohort of children whose mother did or did not avoid foods during lactation, at the 10-year check-up, the rates of atopic symptoms were the same between both groups [178]. However, a related meta-analysis concluded that an antigen-avoidance diet of mothers during lactation may substantially reduce the AD development in their high-risk child in early childhood [240]. Therefore, restrictive diets should not be recommended indiscriminately, because they can cause emotional and social stress for the family, as well as the danger of suboptimal nutrition for the nursing mother. More severe restrictions are not only unmotivated in many cases, but they also foster poor compliance, social isolation and malnutrition [230]. Poor compliance was more frequent among parents with a low level of education, young mothers, smoking mothers, and those who weaned their infant before the age of 2 months [375]. Doctors should be very precise in explaining all dietary rules to the father too [230], as well as to caregivers. In strictly controlled studies, in four out of five infants with a positive SPT for eggs, *the grandparents had given the infants eggs without the parents' knowledge* [172]. Some children presented sIgE for eggs and CM before these foods were allowed in the diet [176] and others who have never eaten eggs may experience anaphylactic shock at the first ingestion [296]. Infants were given CM, although it was not suggested [485]. Even mothers included in a preventive program can make certain dietary errors, mistakenly eating CM and/or fish [176]. Often, a careful discussion with the parents reveals that children also eat other foods absent in medical prescription, above all if he or she is cared for by several different people.

Results of Our Group's Preventive Studies

In an extensive study we conducted [46], at the median age of 3 years and 8 months (follow-up, 7 months to 8 years), we monitored 347 at-risk newborns, children of atopic parents identified by FH and/or CBIgE. They were part of a preventative program that included the dietary and environmental measures summarized in Table 24.4, including the dietary restrictions for nursing mothers as described above. Fifty-one children presented atopic symptoms during the follow-up: 26/179 (14.5%) fed BM and/or SPF and 25/65 (38.5%) fed CM (p=0.0000).

We conducted a study lasting ≈5 years on the prevention of allergic disease in newborns at risk for atopy, and they were followed up by us *at 1 month,* then *every 3 months up to 12 months,* and subsequently *once a year.* The purpose was to personally check the child's diet, note any atopic manifestations and child growth in weight and height [38]. At the age of 52 months, 19/174 children (11%) presented atopic symptoms. This rate dropped to 10% considering only the children of families who followed the preventative program. The incidence was 9% at 6 months and 4.5% at 36 months, decreasing gradually to 1% at 52 months, while the incidence at 24 and 52 months was much lower [38] (p=0.0001). The application of at least some of the recommendations we proposed helped limit symptom severity and the number of children with total IgE over 2 SDs dropped from 21% at birth to 11% at 3 years [38].

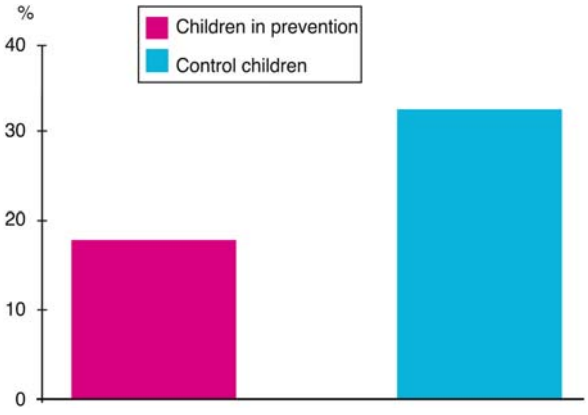

Fig. 24.3. Multicenter study. Prevalence rate of atopic disease in the study children and control children. p=0.0018

We undertook a multicenter study for the prevention of allergic disease in newborns at risk for atopy, based on dietary and environmental prophylaxis [49, 67]. At the last follow-up, 106 of 531 children (20%) had atopic symptoms: 11 (2%) AD; 69 (13%) asthma; five (1%) urticaria, 21 (4%) rhinitis. Of the 106 children, 73 (69%) were males and 34 (31%) females (p=0.0001); 81 (76%) had only one atopic family member and 25 (24%) had at least two (p=0.0001). During the follow-up, the prevalence of AD increased gradually starting at 6 months, peaking at 11% and then dropping to 2% at 4 years, while asthma increased progressively from 1% at 1 year to 13% at 4 years. Very significant statistical differences were found when comparing all types of diet, alone or combined with CM-based diets. The prevention program that was adopted led to a lower incidence of atopic disease in children followed as above, and the rate was significantly lower compared to that observed in the control children (p=0.0018) (Fig. 24.3); Fig. 24.4 shows the ratio of different types of nursing [264]. Figure 24.5 [49, 67, 264] summarizes the cases of atopic disease diagnosed in comparison with the data measured at 2 years, whereas the preventative effects in the 174 children we followed up [38] are summarized in Figs. 24.6 and 24.7. Interestingly, comparing the different rates of

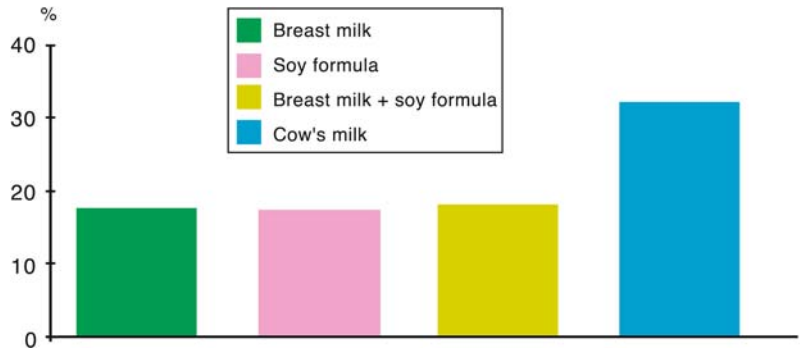

Fig. 24.4. Multicenter study. Relationship between prevalence rate of atopic disease and feeding received. Compared to cow's milk, breast milk p=0.0072, soy formula p=0.0115, breast milk and/or soy formula p=0.0134

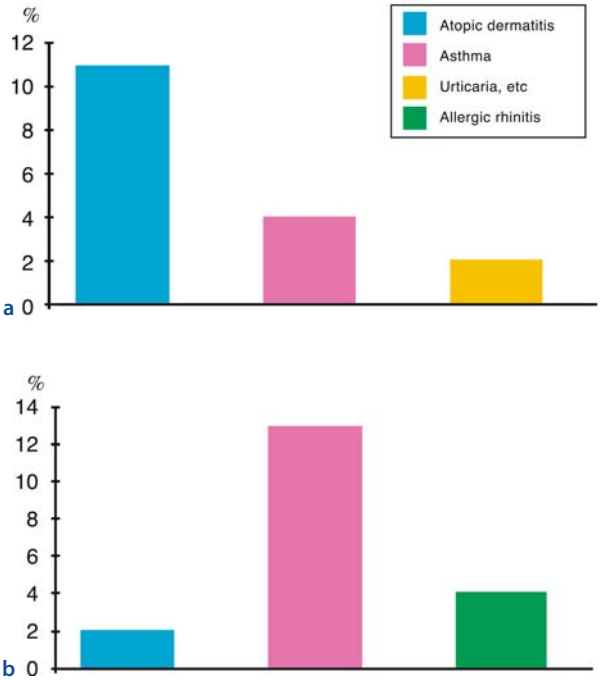

Fig. 24.5 a,b. Multicenter study. Prevalence of atopic disease in children, a at 2 years; b at 4 years. The different reciprocal rate of atopic dermatitis and asthma should be noted. The 3-fold increase of pediatric asthma in two years is evident. (Data from [49, 67, 264])

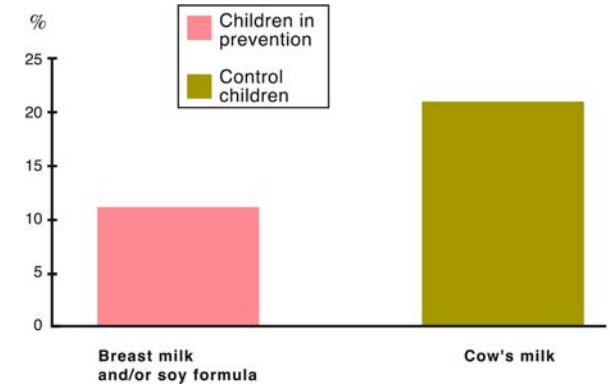

Fig. 24.6. Percentage of the 174 children with atopic disease compared with the control children according to the type of feeding, p=0.0001. (Data from [35])

Table 24.8. Possible causes of failure of allergy prevention programs

Prenatal sensitization
Allergens
Anti-idiotype antibodies?
Poor motivation of nonatopic parents
Lack of maternal compliance, namely, smoking and dietary restrictions
Superficial application of dietetic restrictions
Delayed diagnosis and identification of the offending food
Deliberate or accidental food mistakes
Hidden cow's milk or hydrolyzed bottle in maternity ward
Insufficient intake of important nutrients (such as, calcium)
Solid food introduction during the first 6 months of life
Grandmother effect, such as egg feeding
Cross-reacting allergens
Effect of infections

Data from [230].

Table 24.9. Potential risks of allergy prevention programs

Malnutrition of mother, fetus and infant
Inadequate nutrition
Overprotection of the infant/child
Family dysfunction
Economic consequence for the family
Family social isolation
Feelings of guilt if symptoms do occur despite the efforts
Possible misunderstandings with the clinician

Modified from [230].

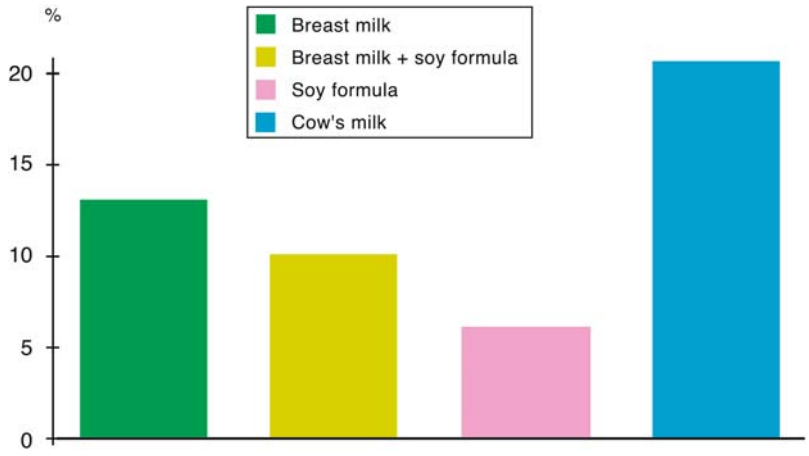

Fig. 24.7. Study of 174 children. Prevalence rate of atopic disease in the study children and control children according to the feeding received. (Modified from [35])

the two studies, in the 174 children followed up exclusively by our staff, the results are clearly lower (Figs. 5.3, 5.4) [38]. Tables 24.2 and 24.3 show that several data confirm our results and that soy allergy is uncommon in atopic children. Our prophylactic studies have regularly demonstrated that all symptoms were mild in children who developed atopic disease and, in general, corticosteroid use was not required. We attribute this to the clinical visits at regular intervals, more frequent in the 1st year of follow-up; moreover, mothers were free to contact us for any clinical manifestation of their babies, as suggested recently [375]. In conclusion, the plague of dropouts was virtually unknown to our studies.

Table 24.5 summarizes the main recommendations for preventing atopic disease in HR children. Similarly, Table 24.8 [230] summarizes the causes that can undermine the outcome of preventative programs, Table 24.9 [230] the possible negative aspects. We must emphasize that inadvertent contact with traces of food or the inhalation of fumes or dust (Table 7.22), through the skin and/or airways, can reach the nursling's immune system [230].

Analysis of Formulas Substituting Breast Milk in Atopic Prevention Programs

Both the Nutritional Committees from the American Academy of Pediatrics (AAP) [5] and jointly the European Society for Pediatric Allergology and Clinical Immunology and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition [198] have published recommendations to prevent and treat FA. The recommendations of these guidelines may have some differences in FA prevention [483].

Soy Protein Formulas

SPFs, as we were the first to demonstrate [61], are the safest and most valid food to use in at-risk newborns, as a substitute or supplement to breast milk if the latter is not available or is insufficient. SPFs are less allergenic than CM and permit regular growth even if used from birth and for a long time [61]. In showing its nutritional [61] (Fig. 9.22) and preventive [38, 44, 46, 61] effectiveness, we do not point to any alterations in the immune response, nor to the increase in morbidity reported by other studies [48]. Therefore, based on previous discussion and data in the literature (Tables 24.2, 24.3) and our 20-year personal experience with more than 3,000 newborns followed-up prospectively for many years [38, 44, 46, 49, 60, 61, 62], we feel that SPFs should be considered a first choice when substituting breast milk in programs for preventing atopy in at-risk newborns [61]. As already noted, the 1953 study [154] yielded highly positive results, but it has been criticized not only because it was retrospective, but also because there was already a high rate of atopy in the control subjects. An extremely low prevalence of SPF allergy has been found by DBPC food challenge (DBPCFC) in children with FA [61] and in infants of atopic parents fed SPFs from birth or very early in life [38, 46, 61]. (See Tables 9.26 and 9.27 including several young children.) Table 24.10 [15, 26, 36, 49, 77, 78, 80, 81, 162, 170, 215, 231, 264, 289] summarizes the results of studies that have specifically compared SPFs with CM in atopy prevention. The study by Bardare et al [15] confirms the *indispensable need for compliance* among subjects at risk and emphasizes the important, lower prevalence of atopic disease with exclusive breastfeeding and a specific diet by nursing mothers [15]. Moreover, in a study usually indicated as negative [36], the rate of atopy in children on an SPF diet was 10.6%, while in controls not on this diet it was 13.3%. Based on this table, we can see that only nonrandomized studies (except [215]) have very statistically significant differences. The prevalence of 74% is notable because it is parent-reported [231].

On the contrary, other authors do not approve of SPF use in preventing atopy and have expressed their criticisms (see [61]), which were overturned by the recent AAP statement (Chap. 9). It may be that in the negative studies, many highly motivated families did not follow the recommendation to avoid CM when nursing HR

Table 24.10. Prevention of atopic disease in at-risk infants: results of 14 studies comparing SPF(s) with CM feeding

Authors	Reference	Year	No. of infants		F-U (years)	R	Results	Diagosis	Significance (*p*)
			Fed SPF	Fed CM					
Johnstone and Dutton	[215]	1966	115	120	3	Yes	AD, S 5%; CM 2%	M	NS
							AR, S 7%; CM 17%		<0.001
							BA, S 6%; CM 23%		<0.05
Brown et al	[36]	1969	85	196	2	Yes	S 10.6%; CM 13.3%	M	NS
Halpern et al	[170]	1973	632	1,084	7	No	S 0.5%; CM 1.8%	M	0.02[d]
Kjellman and Johansson	[231]	1979	23	25	3	Yes	S 74%; CM 60%	PR	NS
Gruskay	[162]	1982	79	201	15	No	3 y, S 20%; CM 25%	M	NS
							15 y, S 53%; CM 53%		NS
Miskelly et al[a]	[289]	1988	228	233	1	Yes	AD, S 41%; CM 34%	ND	NS
							BA, S 33%; CM 33%		NS
Chandra et al	[80]	1989	41	40	1	Yes	AD, S 63%; CM 70%	M	NS
Chandra et al	[81]	1989	68	67	1	Yes	S 37%; CM 36%	SPT	NS
Chandra et al[b]	[78]	1991	68	67	1.5	Yes	S 27%; CM 17%	SPT	NS
Businco et al	[49]	1991	193	156	2	No	S 5%; CM 13%	M	0.0069[d]
Bardare et al	[15]	1993	158	218	1	No	S 13.3%; CM 28.9%	OFC	0.0003[d]
Burr et al	[26]	1993	215	231	7	Yes	AD S 17%; CM 15%	C	NS
							BA, S 13%; CM 32%	PEFR	NS
Machado et al[c]	[264]	1994	139	139	4	No	S 10%; CM 32%	M	0.0134[d]
Chandra[e]	[77]	1997	68	67	5	Yes	AD, S 28%; CM 30%	DBPCFC	NS
							BA, S 21%; CM 24%		

The authors have not always disclosed the precise numbers of the two groups, therefore some overlapping data are possible.
F-U follow-up, *R* randomization, *SPF* soy protein formula, *CM* cow's milk, *BA* bronchial asthma, *AD* atopic dermatitis, *AR* allergic rhinitis, *S* significant, *NS* not significant, *M* medical, *C* clinical, *PR* parental reporting, *SPT* skin prick tests, *OFC* open food challenge, *DBPCFC* double-blind, placebo-controlled food challenge, *PEFR* peak expiratory flow rate.

[a] In the trial by Miskelly et al [35], 189 of the 482 children were breast-fed.
[b] Extension up to 18 months and 5 years of the Chandra et al original follow-up [79].
[c] Extension up to 4 years of the Businco et al [49] and Cantani et al [67] original follow-ups.
[d] The comparison with the infants not subjected to preventive measures is even more significant, $p=0.0001$.
[e] Extension to 5 years of the previous studies.

children, as in the study by Zeiger et al [485]. A Cochrane Database review based on the analysis of five papers suggests that SPFs should not be recommended for the prevention of allergy or food intolerance in infants at high risk for allergy [312]. A new concern has arisen about the potential hormonal effects from exposure of SPF-fed babies to levels of phytoestrogens or isoflavones [56]. Setchell et al [384] studied 24 male infants aged 1–4 months, seven of whom were SPF-fed. The results of the study stress that the total isoflavone exposure for a SPF-fed 4-month-old infant is 6–9 mg/kg b/w per day. Total plasma levels of isoflavones and genistein in SPF-fed infants range from 2.0 to 6.6 and 1.5 to 4.4 µmol/l, respectively, and were 200-fold greater than plasma levels in infants fed CM formula or human breast milk. They also reported that phytoestrogens circulate in such infants at concentrations 13,000–22,000 times higher than plasma estradiol levels found in early life [384]. However, the isoflavones are commonly present in all SP foods: following ingestion they are absorbed and metabolized by the gut, processed by the liver, and after undergoing enterohepatic recycling are excreted in urine [97, 384].

Largely as a result of research in animal models, concerns have been voiced regarding isoflavones in infant SPFs in relation to nutritional adequacy [385], sexual development and neurobehavioral development [405], immune function and thymus dysfunction [480], but available evidence from infant populations indicates that dietary isoflavones in infant SPFs do not adversely affect human growth, development, or reproduction [56, 284]. Infants fed CM formulas excrete significant amounts of isoflavones in urine, including the bacterially derived metabolite equol, thus confirming the presence of isoflavones in CM [97]. It was suggested that breast milk is a useful source of phytoestrogens (perhaps another advantage of breast-feeding) [391] but recent data [385] do not support this contention.

See Chap. 9 for SAM22 in Bet v 1-allergic patients and for Rezza's diet in FA children.

Hydrolysate Formulas

Studies on HFs nutritional adequacy [481] are lacking, and there are no data from long-term studies apart from three reports [77, 437, 482]. Based on immunological and physiological considerations, we advise against their prophylactic use in the diets of at-risk newborns for atopy [47, 50, 64, 66].

Through breast milk, the GALT (gut-associated lymphoid tissue) of immunodeficient newborns receives sIgA, which plays a decisive role not only in inducing tolerance but also in the defense mechanism for excluding antigens. Moreover, the dietary stimulus and the absorption of various nutrients regulate the production of hormones, some of which – along with high EGF levels in breast milk – appear to be crucial for gut closure. Exposure to allergens when the barrier is immature can predispose the newborn to a deficiency in immunological and other defense mechanisms [13] and in atopic sensitization when allergen concentrations are high [189], promoting the development of Th2-primed IgE [177] within the 1st year. In the first few months, there is active recognition of allergens, and the immune system in maturation reacts with T-cell responses that are initially low-profile and heterogeneous, including allergen-specific Th1- and Th2-like clones that compete reciprocally. After numerous restimulations, one of these Th phenotypes becomes dominant in the response, thus constituting a reserve of CD45 that will then direct the immune response against the individual allergen [189]. At this stage, the genetic predisposition for atopy is also established: the difference between normal and atopic subjects lies in the slow maturation of CD8 affecting the immune deviation and in the absence or reduction of IFN-γ [189]. If allergens step in, if even small peptides (13–17 amino acids) can constitute an epitope associated with class II molecules and thus stimulate specific T-cell clones that can trigger IgE synthesis (Chap. 1), from a biological standpoint it is neither proven nor acceptable to define formulas as hypoallergenic simply because they contain few peptides with MW >6,000 D (Table 9.30). Experimental studies show that HFs cannot induce tolerance, nor can they stimulate defense mechanisms against antigens. Gut closure is delayed among newborns fed HFs in the first 3 days after birth, as compared to newborns not receiving HFs (Fig. 24.8) [211].

To produce HFs that are nonallergenic for the immature GALT and are thus not cross-reactive, intended for atopy prevention in newborns genetically at risk, we must identify enzymes that can fully split the polypeptide chain even of unmasked epitopes of the native HFs. Given that it is technically impossible to destroy all epitopes present on CM proteins using the methods currently adopted to prepare HFs [21], it is easy to understand that these epitopes still have allergenic potency and can be recognized by cell-bound IgE of sensitized children and can trigger even severe allergic reactions [139] due to cross-reactivity. Obviously, the degree of hydrolysis with which CM proteins are processed will significantly influence the presence of residual immunoreactive epitopes: in fact, extensive HF (EHF) have fewer epitopes, while partial HF (PHF) contain a large number. Consequently, the latter should *never be used for prevention in a child who is at risk*, as already specified for CMA therapy. The casein hydrolysate formulas (CHF) produced with a high level of hydrolysis have fewer epitopes that can bind IgE [142], although in many cases they have provoked reactions [139] (Table 9.31). Likewise, if a whey HF (WHF) contains significant quantities of casein, and a CHF contains significant quantities of whey protein that have both escaped enzymatic hydrolysis [260, 345], they can trigger severe reactions in children with IgE-mediated allergy [355] (Tables 9.31, 9.32). We have demonstrated the *direct sensitizing effect of the hydrolysates through breast milk* [43]: 39 nursing mothers of HR infants ingested 400 ml/day of

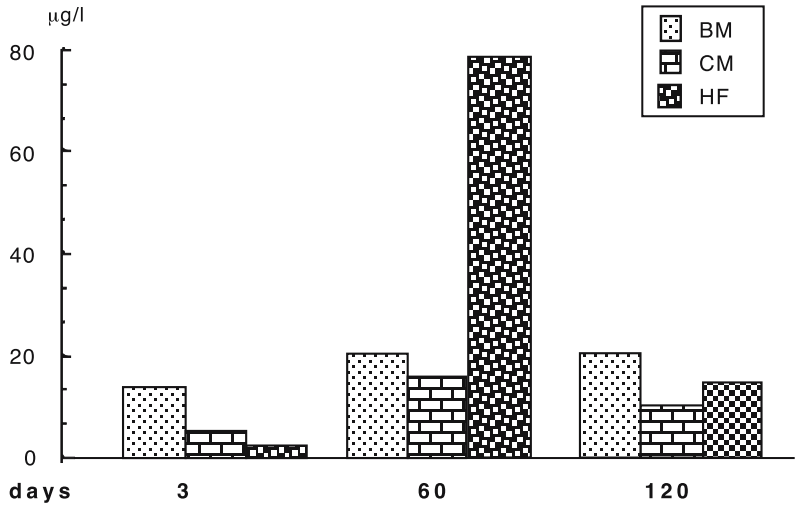

Fig. 24.8. α-Lactalbumin levels in children fed breast milk (*BM*), cow's milk (*CM*) and hydrolyzed formulas (*HF*). (Modified from [211])

a formula of partially WHF (PWHF) for all 6 months of nursing and 39 mothers with the same basic characteristics took 400 ml/day of CM. At the ages of 6 and 12 months, the prevalence of nurslings with total IgE and IgE >2 SDs compared to normal values for this age was significantly higher in children whose mothers had consumed the formula declared as hypoallergenic (p=0.02) [43]. Therefore, all protein epitopes, native or manipulated, are potentially immunogenic, but they can only be defined as such in sensitized individuals. All HFs contain peptides with an MW ranging from 0.5% to 18% greater than 6,000 D (Table 9.30), and variable amounts of residual βLG with allergenic activity (Appendix 9.7). We know that conformational epitopes can be processed through digestion or chemical methods, but if the primary amino acid sequence with at least one epitope persists in peptide fragments, the latter can interact with surface Igs: if two epitopes are intact, they establish a cross-link or they bridge to B-cell receptors, obviously activating them and triggering their clonal proliferation.

The fragments can also contain sequential epitopes that are taken up by APCs and recognized by allergen-specific T lymphocytes [448]; the presence of IgM vs βLG predigested with pepsin and trypsin confirms the persistence of epitopes despite treatment [210]. Naturally, memory lymphocytes can give rise to cross-reactions with these or even noncorrelated epitopes. To get an idea of how difficult it is to eliminate all the epitopes of CM proteins, suffice it to say that in given circumstances, nurslings can become sensitized to these proteins through breast milk. The allergenic molecules in question resist cooking (denaturation). Once ingested by the mother, the proteins are first hydrolyzed in the maternal intestine by gastrointestinal (GI) enzymes and undergo further denaturating and assimilative processes while passing through biological membranes, going to the blood and to the mammary gland (enteromammary axis) (Fig. 2.18). Via breast milk they reach the nursling's intestine, where the peptides undergo digestion again (second hydrolysis), are further absorbed and enter APCs where the hundredth transformation occurs. Despite such treatment, however, CM proteins are still immunogenic and allergenic [1]. If a minute amount of βLG remains in breast milk that can trigger anaphylaxis [253, 265], despite sIgA being there, which contributes to eliminating allergens, it would indeed be singular, on the basis of the peculiarity of the atopic condition – which by definition responds to minute allergen quantities – that a large amount of undegraded proteins contained in HFs can fail to be strongly sensitizing in a genetically predisposed newborn [21]. Even if part of the immunogenicity is destroyed during the various manipulations to which an HF is subjected, epitopes can persist or be unmasked [1] and their immunogenicity *can be just as strong as or even stronger than that of the native protein*. This means that they can thus stimulate the child's immunocompetent cells, induce IgE synthesis and trigger *immediate allergic manifestations* in the HR or sensitized child.

There are EHFs containing very significant quantities of βLG that can thus provoke this effect: we must remember that the products of protein splitting maintain their allergenicity, and that only their antigenicity is reduced [202]. To calculate the amount of βLG contained in a PWHF marketed for allergy prevention, as compared to casein EHF, refer to the data in Appendix 9.7 showing the βLG rate per liter of reconstituted formula compared to CM [21, 43]. βLG allergenicity is highest *in the window period,* as the neonatal intestinal barrier is immature and allows allergenic macromolecules to move into circulation [369]. In fact, βLG is not degraded, resisting proteolysis by pepsin [369], and as a result it goes into the duodenum undigested, where the degradation by pancreatic enzymes may be too slow to prevent absorption of peptide macromolecules and intact proteins into the circulation. This occurs because the *immature intestine is more permeable to macromolecules than in adults* [366, 448], but also because the characteristics of the infantile stomach do not favor

Table 24.11. Nonfatal anaphylactic reactions to a partially hydrolyzed whey protein formula (PHWF)

No.	Age (months)	Sex	Atopic disease	Food	Place
1	3	M	Atopic dermatitis	PHWF	Home
2	5	M	Atopic dermatitis	PHWF	Home
3	6	M	Atopic dermatitis	PHWF	Home
4	4	F	Atopic dermatitis	PHWF	Home
5	5	F	Atopic dermatitis	PHWF	Home
6	5	F	Unknown	PHWF	Home
7	3	M	Unknown	PHWF	Home
				PHWF	Hospital
8	9	F	Atopic dermatitis	PHWF	Hospital
9	1	F	Atopy prevention	PHWF	Home
10	4.5	M	Atopy prevention	PHWF	Home
11	3	M	Atopy prevention	PHWF	Home
12	4	F	Atopy prevention	PHWF	Home
13	5	F	Atopy prevention	PHWF	Home

Data from [50] (+ four cases communicated by L. Businco and + four further cases).

pepsin activity. Pepsin is secreted in very low amounts in nurslings, given the optimal pH of about 2 for most whey proteins, while stomach pH up to the age of 2 months is about 3–4, rising to 6 after meals. Moreover, the production of acid is delayed [311]. The persistence of high levels of whey protein epitopes can bind to preformed IgE or induce an immune response that may represent the process underlying CMA [369]. Experimental data have shown that even by incubating pepsin at pH 4, the residual antigenicity/allergenicity of α-lactalbumin (ALA) and BSA remained high compared to a rate of peptides, with an MW >5–14 kD eliminated by ultrafiltration at 3 kD, a procedure recommended for all HFs intended for children [369].

The data reconfirm the allergenicity of βLG, ALA and casein: therefore, these HFs are at least as sensitizing as CM, if not more so. A casein EHF that, according to the authors, contained only peptides <1,200 kD (Table 9.30), given to newborns for the prevention of diabetes, induced humoral and cellular responses [420]. Nutrition with these HFs also leads to an increase in ICAM-1 (CD54) and CD62L adhesion molecules, which can generate an immune response against bovine proteins, also because of the important costimulatory role of CD54 in activating T lymphocytes [317].

From a clinical standpoint, Chandra et al [77, 78, 81] noted a reduced prevalence of atopic manifestations in nurslings with genetic HR who were fed GS (Good Start) for preventive purposes: SPTs were positive to CM proteins in 4/5 symptomatic nurslings fed GS, as opposed to 2/25 of those given SPF [81] (Fisher, 0.0026), suggesting that sensitization to CM proteins in GS-fed babies is 72% more frequent than in SPF-fed babies. The next study [78] did not specify the number of children who completed the study, nor the allergy diagnosed in symptomatic children – omissions that, to a certain extent, weaken the data. The most recent study [77] reports that there were no statistically significant differences either in the cases of AD or asthma between children fed GS or SPF. In other entrants given HFs, 3.3% presented repeated diarrhea at 4–5 months and 26%–34% developed a respiratory allergy, compared to 15% in breast-fed babies [166]. In a group of 130 healthy newborns, three different types of nursing were established for the first 3 days of life, after which all babies were breast-fed. When the infants were examined at age 4 months, there were two cases of CMA only in CHF-fed babies [219]. In another cohort, the number of children was too small at the 18-month check-up to avoid type II errors [311]: the only significant difference involved the cumulative incidence of atopic disease for the CHF vs CM, while the open food challenge (OFC) for eggs yielded no differences. It must be noted that the highest rate of dropouts was observed in the group fed a PWHF (11.8%) and that only with this formula, there were two reactions to the DBPCFC (4.1%); CMA incidence was 2% [311]. In addition to the five children reported in Table 9.28 [47], we report 14 other cases of HR nurslings presenting nonfatal anaphylaxis at weaning (two instances in one baby) (Table 24.11) [50]. They all received small doses of PWHF at birth, demonstrating the *immunogenicity of HFs prescribed to young infants for prevention* (Table 24.12) [50]. From Tables 9.31 and 9.32, we see that other young infants experienced anaphylaxis or apparent life-threatening reactions when receiving these formulas: $CD45RO^+$ cells proliferate rapidly following sub-

Table 24.12. Details of the immunogenicity of a partially hydrolyzed whey protein formula (PHWF) administered to two at-risk babies

Feeding and effects					
Case 1	PHWF	Breast milk[a]	PHWF (10 ml)	Soy milk	PHWF (1 ml)
	3 days	3 months	Anaphylaxis	3 months	Lip edema urticaria
Case 2	PHWF	Breast milk *	PHWF (10 ml)	Soy milk	PHWF (1 ml)
	3 days	4 months	Anaphylaxis	3 months	Lip edema urticaria
Investigations					
Case 1	Prick test	RAST			
Cow's milk	+++	6.31			
PHWF	++++	18			
Soy milk	–	0			
Case 2					
Cow's milk	+++	14			
PHWF	++++	16			
Soy milk	–	0			

Data from [50].
[a] Cow's milk elimination and derivatives during breast-feeding.

sequent contact with the antigen. It has also been calculated that 80 mg of raw material identifiable as casein (ICRM) in 100 g of HF with the lowest percentages (0.08% Pepti Junior and 0.05% Nutrilon Pepti) is equivalent to what a 2-month-old infant ingests in about 10 days. Nevertheless, there are so-called hypoallergenic (HA) formulas with *ICRM that are 10-fold higher* [327]. These figures clearly indicate that HFs can be immunogenic, triggering an IgE-mediated response in predisposed subjects. Another possibility, even if not directly transferable from animals to humans, is that artificial nursing can alter the function of the intestinal barrier in immature newborns [401], as demonstrated in Fig. 24.8, opening the door to systemic invasion by pathogenic bacteria [401].

Table 24.13 [10, 77, 78, 80, 81, 112, 165–167, 173, 271, 272, 310, 311, 358, 432, 434, 437, 444, 465, 482, 486, 487] outlines the preventative studies based on HFs: Only one study on 3,473 children found an incidence of 1.5% for HF and 2.4% for CM [358]. It should be noted that in various studies, there is a 43%–74% prevalence of CMA in the control group [77, 78, 165, 173, 432, 435, 487], which is not matched in other studies focusing on prevention, apart from the results of Van Asperen et al [423] (42%) in HR children who were not subjected to preventive measures; this is assumed to be based on imprecise inclusion criteria [21]. For example, Halken et al [165] followed 105 HR infants from birth to 18 months, and dietary measures and avoidance measures were strictly followed in 85% of cases. Consequently, the reported prevalence of atopy is unexplainable (32% in the study group and 74% in the control children), while the prevalence of 15% in the 20 children exclusively breast-fed from birth is very low [166]; their IgE levels were significantly lower compared to the other groups [166]. Breast-fed HR babies recruited at birth had a lower development of atopy and of CM IgE levels than those HF-fed [310]. In other HR children *who were not subjected to any preventive measures*, CMA prevalence was 20%, while in HR children undergoing prevention it was 3.6%–5.6% [166], rates decidedly lower than those of 32%–74% [165]. FH positivity was 39% in 92 children nourished with HFs and 35% in the control children, with a high prevalence of atopy at 2 years of age: 24.3% and 62%, respectively [271]. In HR children divided into four groups based on FH and CBIgE, the latter accounted for only 20.5% [465]. Moreover, it was not clear how they were distributed, so that the benefit gained by these dietary manipulations seems to be limited to non-HR children [66].

It is also true that HFs have provoked CMA in at least 25%–40% of cases [77, 78, 112, 165, 310, 311, 432, 452, 482, 487]. In our studies, breast milk was ineffective in 11% (Fig. 24.6)–18% (Fig. 24.3) of cases. Breast milk has never provoked the reactions documented in Tables 9.31, 9.32.

There are few studies documenting the nutritional adequacy of CM-protein HF in HR children fed exclusively with these formulas for an extended period (Tables 24.13, 24.14). In addition, these studies have been meta-analyzed [378], with the conclusion that there were several methodological flaws in the studies, similar to those concerning the protective effect of breast-feeding [237]. Only five studies on PHF [78, 81, 272, 311, 435]

Table 24.13. Prevention of atopic disease: results of studies carried out with hydrolysate formulas (HF)

Authors	Reference	Year	F-U (years)	No. of cases		Type of formulas	Results (%)		Diagnosis	Atopic disease significance	
				HF	CM		HF	CM			
Vandenplas et al[a]	[434]	1988	0.3	30	15	W partial	0	40	OFC	↓ CMA, atopy	ND
Chandra et al	[80]	1989	1.5	43	40	C high	21	70	CI	↓ AD	$p<0.005$
Chandra et al[b]	[81]	1989	0.5	68	67	W partial	7	36	SPT, sIgE	= AD	(0.20–0.04; 0.94)
Zeiger et al[b]	[486]	1989	2	103	185	C high	16	27	SPT, sIgE	↓ FA	
Chandra/Hamed[b]	[78]	1991	1.5	68	67	W partial	26	43	SPT, sIgE	↓ AD	
Arshad et al[c]	[10]	1992	1	58	62	SC high	13	40	CI, SPT	↓ FA, asthma, atopy	
Halken et al[c]	[165]	1992	1.5	105	54	C/W high	32	74	CI	↓ FA, asthma, atopy	
Mallet/Henocq	[271]	1992	4	87	78	C high	16	31	CI	↓ AD	NS
Vandenplas	[444]	1992	1	32	35	W partial	6	40	SPT, sIgE	= CMA	(0.05–0.01; 1.01)
										AD	(0.39–0.09; 1.71)
										Atopy	(0.24–0.08; 0.76)
Vandenplas	[432]	1992	3	28	30	W partial	25	57	CI	↓ AD	Fisher =0.0141
										= Asthma	
Zeiger et al[c]	[487]	1992	4	103	185	C high	35	60		SPT, sIgE, OFC	↓ FA, Atopy (at 12 months)
Halken et al	[166]	1993	1.5	158	75	C/W high	4	20	OFC	↓ CMA	ND
Willems et al[d]	[465]	1993	1	30	92	W partial	7	36	CI	↓ Atopy	$p=0.0021$
De Seta et al	[112]	1994	2	23	39	W partial	35	45	CI	= Atopy	(0.62–0.21;1.80)
										Asthma	(0.56–0.17;1.83)
Vandenplas[e]	[437]	1995	5	28	30	W partial	29	60	SPT, sIgE	=	
Zeiger[c]	[482]	1995	7	103	185	C high	35	60	SPT, sIgE	As at 12 months	
Marini et al	[272]	1996	3	49	46	W partial	18	42	STP, sIgE	= Atopy	(0.82–0.18; 3.74)
										AD	(0.44 –0.10; 1.88)
Odelram et al	[310]	1996	1	32	39	W high	40	47	SPT, sIgE	=	

Table 24.13. (Continued)

Authors	Reference	Year	F-U (years)	No. of cases		Type of formulas	Results (%)		Diagnosis	Atopic disease significance	
				HF	CM		HF	CM			
Oldæus et al[f]	[311]	1997	1.5	106	49	C/W partial	33	13	DBPCFC	↓ Atopy	(1.65–0.71; 3.88)
										↓ AD	(1.44–0.57; 3.63)
										= CMA	(0.19–0.02;1.66
										FA	(0.50–0.04; 5.72)
Chandra[b]	[77]	1997	5	68	67	W partial	32	60	DBPCFC	↓ AD, asthma	$p<0.05$
Halken et al	[167]	2000	1.5	246		C/W high-part			OFC/DBPCFC =		
Saarinen et al	[358]	2002	1	1,715	1,758	W high	1.5	2.4	OFC, SPT, IgE	↓ CMA	(0.38–1.00; 0.61)
Han et al	[173]	2003	0.5	32	23	W partial	20	59	CI	↓ AD	$p<0.05$
Von Berg et al[g]	[444]	2003	1	797	286	C, W partial/high	15.6	11.4	CI	↓ FA	(0.54–0.90; 0.30–1.5)
Total				2,627	2,619						

This table includes some groups of children present in Table 24.2, but the authors have not always indicated the content of the two groups; therefore some overlapping data may be noted. *F-U* follow-up period, *HF* hydrolysate formulas, *CM* cow's milk, *CMA* cow's milk allergy, *CI* clinic, *OFC* oral food challenge, *SPT* skin prick tests, *sIgE* specific IgE antibodies, *FA* food allergy, *AD* atopic dermatitis, *C* casein, *SP* soy + pig collagen, *W* whey, high-partial highly or partially hydrolysate formulas, *NS* not significant, *ND* not done.

[a] Fifteen of 30 babies provided with HF received CM formula since the 31st day.

[b] Follow-up at 6, 18 months, and 5 years.

[c] The number of babies who received only HF is not known; the numbers are the same after 2 and 4 years.

[d] The distribution of 25 children (with no FHA) with high IgE levels among four groups is not known.

[e] The cumulative prevalence includes diarrhea and colic as single manifestations of CMA, as previously discussed.

[f] Prevalence at 9–12 months of C is 20%, of W partial is 13%. OFC with CM or egg failed to show statistical differences. There is a significant difference in the cumulative incidence of atopic disease at 9 and 18 months for C vs CM.

[g] In the study of von Berg et al we have reported the mean of the 3 HFs used; AD was not prevented.

were appropriate for comparison [378], among which a study that yielded significant OR results [311]. A recent study [167] fed 79 infants a CHF, 82 an EWHF, and 83 a PWHF, but significant results were only in 232 breast-fed babies (Table 24.2).

The evaluation of growth thus represents a tangible problem: feeding healthy infants a WHF or an adapted formula, from birth to 3 months of age, seems to provide adequate nutrition that is essentially indistinguishable between the two groups, by evaluating weight and length gain and various laboratory parameters, except for cranial circumference [436]. The Fe-binding capacity was higher in the first group and ferritinemia is lacking, which is a more sensitive indicator of any iron deficiency. With regard to an evident reduction in nitrogen retention (p=0.009), the authors cite a study in adults [392], in whom nitrogen use was equally lacking. Nevertheless, a PHF diet ensured greater retention even compared to solid foods [392]. Therefore, we cannot conclude that weight gain was similar in both groups [436].

As compared to breast-fed newborns, healthy newborns fed for a few days with a PWHF demonstrated significant abnormalities in their amino acid profile [347], which dropped on average at the age of 34 days. Threonine values were double, while those for tyrosine, proline and phenylalanine were significantly lower, as were total proteins [348]. In newborns fed as above, the increase in urea nitrogen and the altered distribution of amino acids was again confirmed, in contrast with breast-fed newborns [152]. We should not be surprised, as CM-protein HFs are made exclusively from casein or whey proteins, thus leading to an imbalance in amino acid serum composition. Therefore, to prevent this, adapted formulas should contain an adequate proportion of whey proteins and casein, which should be 60% and 40%, respectively.

Several formulas made with PWHF (Table 9.30) are marketed as preventive against atopic manifestations in general and FA in particular, based on studies that have supposedly demonstrated that a specific tolerance can be induced by early HF feeding (Table 24.14) [49]. The producing industries claim that these formulas may contain undegraded CM proteins or peptides with a high MW of up to >6,000 D, since they are products recommended *for CMA prevention and not for treatment*. What we do not understand is which clinical and experimental data differentiate the doses of allergens triggering symptoms in sensitized babies from those sensitizing not yet allergic babies [21, 43]. The label "HA" can create a false sense of security: this is an ambiguous term that may be erroneously employed [365], but the problem has not been discussed in a proper forum [93]. Instead, in the US the GS producers (Beba HA and Nidina HA in Europe) have been *banned from printing the acronym HA on the label or package*, with the obligation to indicate clearly that if CMA is suspected, GS must be used solely under medical supervision [380]. Re-examining the studies showing the *negative effects of these formulas, regardless of whether they are highly or partially hydrolyzed* (Tables 9.28–9.30), we can see that numerous studies have strongly recommended that their non-immunogenicity be ascertained before using them for preventative purposes or before prescribing them to children with CMA [93, 363]. Cochrane Database Syst Rev. (CD003664) evaluated HFs for prevention of allergy and FA in infants. Based on the analysis of 11 studies, it was concluded that there is *no evidence to support feeding with an HF for the prevention of allergy* in preference to exclusive breast-feeding. However, in HR infants who are unable to be completely breast-fed, there is evidence that prolonged feeding with an HF compared to a CM formula reduces infant and childhood allergy and infant CMA. Incremental costs of formula and the effect on compliance should be measured.

Table 24.14. Properties of hydrolysate formulas

Possible cross-reactivity with CM proteins (more frequent with whey-protein hydrolysate formulas)
Variable levels of unaltered proteins (casein in whey-protein hydrolysate formulas and vice-versa)
Possible allergenicity (partially hydrolysate or whey-protein hydrolysate formulas)
Lower antigenicity than that of CM protein
Nutritional adequacy not known in long-term studies
Unpleasant taste (except partially hydrolysate formulas)
Cost 80% more than a CM formula (except partly hydrolysates)

Data from [63].

The data provided so far are too limited with respect to the need to know definitively whether HFs are safe and nutritionally adequate. To our knowledge, the fair request to clarify – at least to the prescribing doctor – that the HF composition or the hydrolysis method used (which varies from one formula to another) has yet to be met [365]. The opinion has correctly been expressed that, if necessary, HFs could be used to reduce an existing sensitization, not to prevent it [50]. This is confirmed by data demonstrating that several PHF and EHF HA formulas have peptide bands with a high MW [344] (Fig. 9.24) and, if made of whey proteins, they have variable casein contents [345], making them unsuitable for prevention, particularly in sensitized subjects. The indiscriminate labeling of HFs as HA, merely on the basis of the degree of CM hydrolysis, without considering the allergenicity of the residual components and, therefore, their safety in children with CMA, poses the risk of adverse reactions in these children, by the gut detection of residual native or processed components of these HAs [114]. In conclusion, the option of using an HF in HR children should be evaluated on a case-by-case basis, after in vivo tests have been done [54, 363, 380], with data in Table 9.30 at hand. Lastly, the HF unpalatability should be considered, as this has led 50% of expectant

mothers to refuse HFs [128], as well as their cost: for both of these parameters, SPFs offer substantial benefits (Tables 9.20, 9.21). Both the soy + pork collagen HF [368] and Rezza's diet may be available alternatives.

It is evident *per se* that marketing many HA formulas intended for all newborns could pose a further threat to breast-feeding [123]. Mothers should not be led to believe, by expanding on [253] BMA cases, that HF is safer and thus preferable to breast milk from this standpoint. *Subtle propaganda recommending HFs while waiting for commencement of lactation* as a breast-milk supplement or substitute – a practice leading to the *immediate loss of colostrum, the principal immunological defense of the immunodeficient newborn* [292] – could foster the belief that breast-feeding is inferior to artificial milk: if this has occurred [93], the measures that were once invoked are still current today [54]. Giving a newborn any CM formula whatsoever – in 86% of cases in both Milan and Rome – means depriving the child of the immunological factors contained in human colostrum and of anti-infective, anti-inflammatory and immunomodulating factors in breast milk. These foremost effects, associated with allergen avoidance, strengthen breast milk's advantages.

In specialized journals and magazines aimed at the general public, there are frequent advertising inserts that emphasize the advantages of this or that HF: a producer of two baby formulas spent £3 million in England in just 1 year to advertise them [4], while the British Department of Health in turn spent only 1/60 of that amount to promote breast-feeding [4]. The policy of distributing milk formulas for children should not precede upcoming progress in the knowledge concerning children's diets [93]. We can only hope that the agreement reached by European Union (EU) governments, banning advertising that can further jeopardize breast-feeding, will be a long-lived one. In 2001, the prevalence of the initiation of breast-feeding and breast-feeding to 6 months of age in the US reached the highest levels recorded to date, 69.5% and 32.5%, respectively. Comparing rates in 2001 and 1996, increases in the initiation of breast-feeding and continued breast-feeding to 6 months of age were observed across all sociodemographic groups [357]. A recent systematic review shows that community-based trials of breast-feeding promotion and extra support from professionals with special skills in early infant feeding are effective in prolonging breast-feeding [389].

Secondary Prevention

When an atopic disease is manifested, all preventative measures that can avoid relapses must be taken. The cornerstones of this type of prevention can be summarized as follows:

- *Avoiding allergen contact*
- *Preventing allergen entry* or mast cell degranulation
- *Modifying the state of sensitization* using available aids

The usefulness of secondary prevention is demonstrated by the fact that effective measures, intended to identify and eliminate allergens, can represent a valid alternative to drug therapy in children and they are thus virtually ignored [321, 451]. Among strictly pediatric studies [395, 417, 451, 452], only one devotes any attention to avoidance measures [417]. We emphasize that these measures are instead pivotal for prevention.

Avoiding Allergen Contacts

If a child must avoid contact with allergens to which he or she has become sensitized, an elimination diet or environmental measures can be adopted, or even both in some cases. As part of secondary prevention, *establishing effective measures against HDMs has significantly reduced the severity of cutaneous symptoms in children with AD* [409] *and of BHR in asthmatic children* [121]. Above all, it is remarkable that, for the first time, a significant reduction has been noted in AD compared to placebo-treated controls and to Der p 1 concentration [409]. Likewise, Swedish children in the ETAC study, showed the lowest rate of sensitization to Der p and to Fel d 1 compared to other countries [311].

For the atopic child, secondary prevention involves:

- Airborne allergens
- Environmental pollutants
- Food allergens

Airborne Allergens

Effective avoidance is primarily based on physical rather than chemical measures, requires a full regimen in the bedroom(s) and is only relevant to children who are specifically allergic [324].

Dust Mites

The first step is to ascertain the presence of HDMs. To do this, there is an easy-to-use semiquantitative test that is inexpensive and simple to interpret (Acarex). The levels of guanine exhibited by a given sample make it possible to measure the mite infestation level [82, 168, 335]. A simple kit is commercially available.

Other tests measure the number of live HDMs per m^2 of collected samples:

- The mobility test (MT), whereby a rug is covered with a layer of adhesive tape, held in place with a heavy plate and left *in situ* for 24 h at T 21 °C and a humidity of 75%.
- The heat escape method (HEM), in which a flat heating panel is placed on the underside of the rug. It is then heated gradually until the internal layers reach a temperature (T) of 60–70 °C. HDMs tend to move away from the

source of heat, shifting toward the colder upper areas, where they are captured by the adhesive tape.

Depending on the limits of applicability, the following data are obtained [20]:

- MT does not reveal the absolute total, but demonstrates HDM presence in the child's bed, pillows and blankets, with a ratio of 2:1 compared to the mattress, while in the parents' bed the number diminished with virtually the opposite ratio.
- HEM determines the HDM number and is seemingly more sensitive, but it is not applicable to essentially smooth surfaces (carpets, clothing, sweaters, warm-up suits, etc.). It is 6- to 13-fold more sensitive than MT.

These methods measure the live HDMs: *conventional methods reveal 0.3 %–0.6 %* of the above values [20].

The measures applied in the home of a child allergic to Der p 1 will be effective only if they lead to an 80 %–98 % allergenic load reduction [174, 241, 399, 409]. Children benefit from this more quickly than adults [399], suggesting that *histological changes typical of asthma become less reversible* as exposure is prolonged [399]. Environmental manipulations are effective if they are started promptly and completely: surrogates can lead to an improvement in symptoms, but not in pulmonary function testing (PFT) [275]. At the same time, targeted help can come from parents active in adopting an avoidance strategy to reduce allergens [427, 439].

Preventive Measures

The Child's Bedroom

Briefly, the child's bedroom should be sunny, well-ventilated and dry, uncluttered by excess furniture and any items that can act as Der p 1 niches [399, 417], because babies spend a larger proportion of their lives in their bedroom than do adults [398]. If a crib is necessary, this and portable crib mattresses should be encased in mite-proof material [101].

Beds: if possible, there should be *only one bed* in the room, above and below which the child should not be allowed to play, jump, do somersaults, have pillow fights, etc. [306]: Der p also colonizes the floor under the bed [479].

The *mattress* should not be made of latex, wool, horsehair or feathers, but of artificial fibers and polyester. It must be encased with mite-proof covers (with pores ≤2 mm) to permit steam dispersion from transpiration while preventing HDM passage. The slipcover should also be made of dust-proof synthetic fibers. It must be washable and have a *self-sticking closing* for its junctions [456], and it must be covered with another washable slipcover [91]. Commercial mite-proof covers for mattress, pillow, and quilt are effective and inexpensive [101, 321], while cheaper vinyl covers should be avoided as they are totally impermeable [174]. If a second bed in the room is unavoidable, it must be prepared

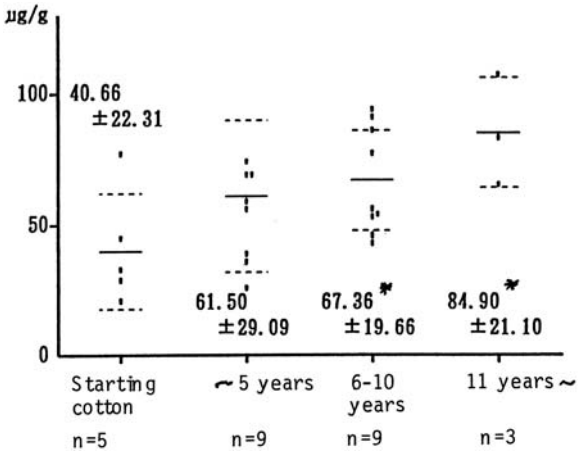

Fig. 24.9. Der p antigen content (μg/g) of traditional beddings by years of use in asthmatic children as measured by ELISA-inhibition method

in the same way [174]. We advise against *zippers* (dust goes through the teeth); if they cannot be replaced, the entire length of the zipper should be covered with adhesive tape. By adopting these measures, after 3 months Der p 1 concentrations in the mattress dust *were reduced to only 1 %* compared to uncased ones [313], and after 6 months the average percentage was significantly lower compared to the base figure [456]. In addition, polyester fibers have proven excellent for reducing not only Der p 1 levels in children, *but also BHR* [121]. According to some, the mattress should be changed every 5 years on the average [91] (Fig. 24.9), while others claim that buying new ones does not offer any benefits, because they quickly accumulate allergens [209] with levels correlated with those of any carpets in the bedroom [100]. The likeliest explanation is Der p 1 and Der f 1 contamination through the clothes of people who handled them, but also Fel d 1 and Can f 1 [107]. Vacuuming the mattress for infants before instalment will remove part of the dust. After installment, regular vacuuming is advisable [107]. Pillows must be treated as above, while sheets, pillowcases, blankets, bedspreads, quilts and so on must be made of synthetic material [241]. One year after the application of HDM impermeable covers on the parents and children's bed the concentration of HDM allergens, measured at the living room floor, the mattress of the parents and the mattress of the baby, was statistically significant decreased in the intervention group, but not in the control group [242, 376].

Furniture should be reduced to a minimum, simple with a regular shape and a smooth surface. Furniture should be wooden, metal, vinyl, and leather, and solid wood is preferable to particle board and laminated wood, as long as the wood is finished with natural paint and not *formaldehyde-based glues.* The objective is to ensure items that do not provide sites for mites to grow [323]. Furniture should be raised from the floor to make the room easier to clean. Avoid upholstered chairs, arm-

chairs and sofas; otherwise, the upholstery must be made of synthetic material. If wardrobes are a must in the room, parents are advised to empty them completely of regularly used clothes, bringing the contents elsewhere and using adhesive tape to seal them. Their function must be minimal, they can become containers to be opened twice a year at the seasonal wardrobe change, nothing more [91]. Books, booklets, magazines, records and so on must be banned, as they can collect HDMs, unless they are kept in dustproof cabinets or boxes, keeping out a book or periodical to read at night, if so desired [417].

If possible, *clothing* should be kept out of the room and stored in boxes or bags made of heavy plastic and prepared as indicated above, which are to be wiped often with a damp cloth. Shoes should be kept elsewhere in tightly closed plastic bags [174]. Likewise, wool blankets and clothing can be kept in sealed wardrobes in the summer, avoiding mothballs or limiting their use, or airing the items in the sun periodically, as with regularly used items.

Curtains, which must be made of synthetic material, must be small and lightweight, and they must be washed frequently [174].

Walls should be as bare as possible and painted. Thus, they should not be covered with unwashable wallpaper, and should not have paintings, prints, posters and other hanging items [417]. Beware of *casein contained in milk paints,* plasters, and wallpaper, since it is not known how commonly organic proteins are used in indoor construction materials [268].

Floors should preferably be smooth and washable (marble, ceramic, tiled, wood, vinyl and linoleum), paying attention to corners and cracks; concrete tends to trap moisture. Make sure that parquets are not glued using *casein or formaldehyde adhesives* [241]. For cleaning, use furniture polish that traps dust, making sure not to raise any dust in the room. Carpets, bedside rugs, wall-to-wall carpeting, animal feather, mats and so on are banned, as they are the most effective dust-collectors and receptacles: if they cannot be removed, for example, wall-to-wall carpeting, they must be covered completely with a polyvinyl sheet fastened at the edges with adhesive tape [306]. Note that synthetic carpeting and rugs have far less Der p than wool ones. Moreover, due to static electricity they tend to trap Der p, preventing its release in the air [333]. A danger for small children is crawling in close contact to house dust probably containing casein [268]. In one study a whey protein *(βLG) was detected in house dust* [469].

Children's *toys*, including dolls, puppets and so on, must be made of washable material and must never be wool, plush or padded. Even if they are new, they become filled with Der p 1 within 4 months [305]. After use, these items must be placed in a closed box. This also hold's for children's favorite bedtime items.

The room must be cleaned thoroughly every day: everything must be wiped carefully with a damp or oily cloth, otherwise room cleaning procedures will promote the circulation of mite-rich dust, increasing its levels in the air [323]. *A high-filtration vacuum cleaner* should be provided with a *HEPA microfilter* (high-efficiency particulate air filter) [306]. While it is true that there are not always significant differences with the use of filters [409], normal filters have no effect on HDMs attached to carpet fibers [306]. Other measures for the child's bedroom and other bedrooms used by children are summarized in Table 24.15 [174, 319, 414, 479]. Cleaning of the bed items must include the seams, where Der p tends to accumulate the most [174].

Other cleaning methods for carpets and similar items include exposing them to direct sunlight for several hours, which is effective for inactivating the allergens [414]. Otherwise, they can be wet-cleaned in the summer using a commercial detergent and applying an acaricide after about 1 month [108]. Radical measures include a T of –20 to –25 °C [108, 319] and the use of a steam cleaner to reach the T of 150 °C [92], but carpets in basements are almost impossible to keep dry [323].

Several strictly necessary measures are recapitulated in Table 24.16 [174, 283, 431, 479]: they are justified because HDMs multiply when T exceeds 25 °C and relative humidity is >75% (for humans, the optimum level is between 25% and 50%), and HDM can survive up to T 60 °C [464]. Therefore, blankets, bedspreads, quilts and so on must be washed at T >60 °C, every 2 weeks on average [283]. T <55 °C does not totally eliminate HDMs, reducing Der p 1 by 95% and Der p 2 by 91% [454], nor is this improved by adding detergents or other chemicals [283]. Instead, dry-cleaning the blankets is effective in 70% of cases [454]. T 60 °C is effective in 50% of cases only if maintained for a full hour. To achieve 100% results also for Der p 2, this would theoretically require T 130 °C for 30 min [319]. *Clothing* must be washed frequently: this is an unsuspected source of high levels of Der p, particularly if the clothes are made of wool, which shows an accumulation ten times higher than cotton [20] and polyester.

Table 24.17 [409, 479] shows a timetable for the measures described here. Two measures are urgent: encasing the mattress, which is truly effective [101, 121, 409] and can reduce the allergenic load by 98% [409], and using mite killers [101, 409].

Environmental Measures

- *To reduce humidity* in the kitchen, bathroom or other rooms, windows may be opened to change the air even during the winter, for 30 min twice a day. Closed windows increase humidity. For the same reason, laundry should not be hung inside the home to dry [426].
- If *the heating system* uses wall radiators, they must always be cleaned with a damp cloth or a high-filtration vacuum cleaner. Radiators collect far more dust that one would imagine and if they are electric this can also pose

Table 24.15. Prevention of atopic disease in high-risk children: rooms in the house

A. In the child's bedroom
Air the bed, blanket, and pillows daily
Scrub mattress and pillows and beat them with a rug beater; if feasible, expose all to sunlight
Clean mattress and pillow covers with a damp cloth and wash in the washing machine every 3–4 months
Change both sheets and pillow slip at least weekly
HEPA air cleaner should be used at least weekly to remove airborne dust particles from mattress and pillow
Air the rooms in the morning when Der p 1 levels are lower
When the room is vacuumed, the child should avoid being in the room while vacuuming is occurring until at least 20 min after it has been completed
The room should remain closed the whole time the child is away
Keep the doors and windows of the bedroom closed as much as possible when not using the room
B. In the other rooms
Do in the same manner:
In the parents' bedroom and the other bedrooms, even if the child does not stay or sleep there daily
In all other rooms where the child frequently goes
In the home of relatives
In holiday houses
If possible in hotel beds the mattress covering should be used
Prepare the other rooms where the child goes, although less frequently, such as, where the child watches television, in the same manner, removing the armchair upholstery, heavy curtains and carpets
Children should stay away from normally damper and not sunny rooms
Remove nonsucculent green plants and domestic pets; leaving them in the garden, courtyard or balcony is not a useful approach
Avoid aspecific irritants, including kerosene, cosmetics, formaldehyde, gas oil, insecticides, turpentine, paints, chemicals and strong odors
An aquarium may increase the humidity level
Prefer synthetic fibers for curtains, blankets, bedcovers and the like; mites burrow in the weft of closely woven cotton or wool fabric and hold on tight; thus they are unlikely to be completely eliminated

Data from [174, 319, 414, 479].

Table 24.16. Additional home avoidance measures

Maintain environmental T ≤20 °C
Uniform heating throughout the home, avoiding T differences
Maintain indoor relative humidity not >50 %
Wash all bedding, including mattress cover, blankets, curtains, etc. at T >60 °C
Dry cleaning of wool clothes, plush, stuffed toys, etc., at T >60 °C, then wash in cold water

Data from [174, 283, 431, 479].

Table 24.17. Suggested chronology for home avoidance measures

A. Primary measures (within 1 month)
Bedroom
Encase mattress and pillow with plastic impermeable covers
Increase T for washing bedding (Table 24.15)
Remove quilted bedcovers and wool blankets or encase as mattress
Remove stuffed toys, clutter and eliminate or encase upholstered furniture
Replace curtains with washable blinds
Remove carpets and wall-to-wall carpeting
Alternatively expose carpets to sunlight
Other rooms
Use HEPA filters
B. Medium- and long-term measures
Bedroom
Replace carpets and wall-to-wall carpeting with smooth floor or vinyl covering
Alternatively spray carpets with acaricides
Change bedrooms to second floor (if feasible)
Other rooms
Remove carpets and upholstered furniture that is not encased
Replace curtains with washable blinds
Reduce humidity by increasing ventilation with fresh air, or using either a dehumidifier or air conditioning
In general
Prefer older buildings with less insulation and better ventilation

Data from [409, 479].
HEPA high-efficiency particulate (or particle arresting) air.

a risk [206]. Hot air rising from radiators promotes airborne allergen dispersion [174]. Some feel that baseboard radiation panels are best [241] (Fig. 24.1).

- *Air conditioning* is helpful for minimizing excessive heat and humidity, which stimulate HDM proliferation. The following rules must be followed: if there is a vent in the room, we recommend covering it with a filter, which can be made of various layers of thick fabric or nylon, to be changed frequently. If there are cracks or holes around the vent, they should be filled with putty. The filters of the heating and/or air-conditioning ducts must be cleaned thoroughly every 2–4 weeks [174]. The effectiveness of this is demonstrated by the significant reduction in HDM number in rooms treated according to these standards [257]. Make sure that pollen does not filter into the models [174].
- An automatic system for *air cleaning* without opening the windows should be installed. In 20 asthmatic children sensitized and exposed to pets in the home, application of air cleaners in living rooms and bedrooms was accompanied by a significant improvement in BHR and a decrease in peak flow amplitude [425].
- If *humidifiers* are used – generally contraindicated [462], particularly in the child's bedroom [206] – verify that the relative humidity is always ≤40%–50%, and clean the tanks regularly.
- *A dehumidifier is* helpful in damp houses or in periods of excessive humidity, and can reduce the allergenic load by >50% [51]. These effects were not confirmed using a portable device [99] and thus their effectiveness is unsure.
- *Acaricides* are effective in reducing environmental HDMs, as long as they are repeatedly applied on carpets and soft furniture [101, 475], as demonstrated by clinical improvement of patients [10, 69, 102, 185, 186, 298, 334, 372, 475]; in fact, the symptoms reappeared in not dust-free rooms, demonstrating that removal of allergens improves clinical manifestations [298]. Antigen avoidance procedures associated with acaricide use reduced the mean concentration of Der p 1 from 25.9 µg/g of dust at birth to 6.0 µg/g at age 9 months [334] and resulted in the development of atopic symptoms at age 1 year in only 13.8% of infants, as opposed to 40.3% of the control subjects [10]. Other authors have failed to note the same effectiveness [121, 399, 456]. It was observed that poor effects on mattresses but significant on living-room upholstery depend on the difference in the thickness of Der p layers and/or the lack of associated cleaning: to be effective, an acaricide must kill not only Der p but also its eggs, denature fecal residue [298] and achieve a tenfold reduction in the allergen quantity per area unit [479]. Several studies [160, 409, 415, 450] tested the validity of Allersearch DMS, composed of a mixture of benzyl alcohol and tannic acid; it is nontoxic and has a dual action, killing HDMs and reducing residual antigenicity. In asthmatic children, a significant reduction was observed in SPT positivity to Der p in their homes [450]; others do not agree [334]. Colloff et al [91] have published a list of producers and/or suppliers of these products: however, many household products may have poor or nonexistent effects on allergens in the air or in dust samples and should be tested objectively before they are commercialized [83]. In addition, a major decrease in allergen was not recorded compared with that seen in the control population [324].

Measures in the Home

The main risk factors for the home are summarized in Table 24.18 [241, 426, 439, 462, 464]: humidity is generally ≥7 g/kg in homes with several floors as opposed to single-story homes, where a higher HDM number thrive well ($p<0.001$) [462]. These measures should best be applied in selecting a new home or before moving, ensuring that the home is insulated from the ground and the apartment is on an upper floor.

Several studies have demonstrated that early intervention may modulate the natural course of atopic disease. We recommend adopting all the above measures – at least the essential ones – several months before the baby is born or the family moves to the home. An antenatal intervention to reduce HDM allergen levels in children's beds had a limited effect since even with expert control measures in place, HDM allergen levels remained high by international standards [286]. In an extension at 18 months, HDM avoidance intervention was ineffective but was associated with a lower use of medications [287]. However, a substantial reduction in Der p 1 levels in beds and in airborne dust in a humid region with naturally high mite allergen levels can be achieved and sustained in children with use of occlusive covers and a rigourous washing routine [438]. HDM sensitization can be reduced by providing school age children with HDM impermeable mattress covers and advice on environmental measures to reduce exposure to HDM allergen. Significantly, three out of 117 (2.56%) children in the prevention group and nine out of 96 (9.38%) controls developed mite sensitization [11]. A pediatric DBPC trial reported no effect of the inter-

Table 24.18. Main home-related risk factors

Solid brick outer walls [426]
Lower storeys [242, 462]
Tight, poorly ventilated homes [242]
Absolute indoor humidity >7 g/kg [464]
Condensation on the inside of double-glazed windows [464]
Concrete slab basement [464]
Wall-to-wall carpets and wooden floor, associated with older floor coverings [426, 439]
Underfloor heating [241]

vention in children aged 2 [236], but at an earlier age the clinical benefits can be barely detectable [37]. In the homes of children born to allergic mothers, use of mite allergen-impermeable mattress covers reduced mite allergen exposure [428]. Active avoidance measures used *during pregnancy and in the 1st year of life* in homes of infants at risk of atopy including dust samples from the parent's bed, the infant's mattress, several floors, and Der p 1 levels demonstrated that recovered Der p 1 from the mother's mattress was reduced by 97.25%, with the effect persisting for 6–12 months [101]. Total Der p 1 from the bedroom floor in the active group was reduced by 53.7%; Der p 1 levels in the crib mattress and nursery floor in the active group were extremely low. The total amount of allergen recovered at age 1 year was 29-fold higher in the control group than in the active group [101]. Another study compared the effect of allergen-impermeable encasings on the mattresses, pillows and bedcovers over 1 year with matching placebo encasings. The use of antiallergic mattress covers resulted in significant reductions in Der p 1 concentrations in carpet-free bedrooms; however, there was no consistent variation in the clinical manifestations of the patients [349]. Others have demonstrated [429] that mite-impermeable mattress encasings have a significant but modest effect on dust and HDM levels of mattresses with low initial HDM levels, compared to placebo. A recently published meta-analysis of mite-preventive measures in mite-sensitive asthmatics suggested that current chemical and physical methods aimed at reducing exposure to allergens from HDMs might be ineffective in the tertiary prophylaxis of asthmatic patients. The authors judged quite ineffective the methods for mite eradication since these methods did not adequately reduce levels of mite antigens [159], but the analysis was contested [98] because there was a diversity of results between different types of treatment to prevent asthma. The use of mite allergen-impermeable mattress encasings was the cornerstone of a pediatric study associated with other mite and food allergen-preventive measures, which substantially lowered the incidence of sensitizations in the 1st year of life [169].

Special Preventive Measures

Holidays in areas patently infested with HDMs are not prudent: spending a vacation of ≥14 days in these places is considered a significant risk [462]. In cities as well, some places are dustier than others, such as theatres, cinemas, etc., Residence in the mountains can cause a range of immunological effects (Table 24.19) [29, 64, 82, 88, 322, 364, 390, 398, 421]: for example, a great difference in mite allergen content in dust of mattress in samples taken from high-altitude areas as opposed to those collected at sea level [85]. If the environment is strictly dust mite-free, an extended mountain stay can lead to appreciable improvement [422]. Nevertheless, parents and doctors should note that these improvements are limited to staying at high altitude, similar to what is achieved in a hospital setting – real or simulated (Chap. 7), but PFT deteriorates and asthma symptoms worsen in children following the return to sea level [88]. Considering the effects as a whole, these stays are sometimes justified by the need to ensure that the airways get a rest. Recently, a critical review was made of the role of certain allergens at high altitudes: even at 2,300 m, sensitization to environmental allergens is possible, including Fel d 1 [398]. Moreover, at 2,600–5,800 m, as many as 112 to 280 Der p/g of dust were noted, independently of T and relative humidity [364]. The study in Los Alamos, New Mexico, a high-altitude desert area and an environment in which there are very few HDMs, shows that in children born and raised there, nearly 17% were diagnosed as having asthma at some time with 14% taking asthma medication [398]. Consequently, although the prevalence of HDM sensitization is reduced in such areas, the prevalence of asthma continues to be a serious problem at altitudes considered free of airborne allergens. At home, other measures require banning smoking and avoiding irritants such as insecticides, mothballs, deodorants in general and so on.

Table 24.19. Results of high-altitude climate therapy

Favorable data
The number and concentration of mites vary inversely with altitude [364]
A t 1,400 m (4,267 ft), SPTs to Der p decrease remarkably (90%) [82]
Serum IgE levels decrease up to 40% and Der p-specific IgE up to 50% after a 9-month stay at 1,400 m [64]
PD_{20} increases after 80 days of allergen avoidance at 1,756 m (5,352 ft) [322]
Reduced histamine release by Der p-specific basophils at 1,756 m [322]
Reduced markers of eosinophil activation at 1,756 m [29]
Reduced peripheral blood CD4 (CD25) T lymphocyte activation (increased CD45RA/CD45RO ratio) and eosinophilia at 1,570 m (4,875 ft) [390]
Unfavorable data
High populations of mites are prevalent at 2,655–5,820 m (8,087–17,727 ft) [364]
At 2,800–3,000 m (8,534–9,144 ft), asthmatic children ranging in age from 8 months to 19 years are most frequently sensitized to Der p and Der f [421]
At 2,360 m (7,189 ft), a sensitization to Fel d 1 is likely [398]
The positive effects decrease invariably after renewed allergen exposure at sea level [88]
Cost/benefit ratio of the change of residence and move to mountain sites

Comment. Comparing the levels of HDMs in the homes of 3- to 15-month-old atopic and nonatopic children, only 50%–56% of parents of atopic children had made any changes to the flooring or beds, and 34%–46% to bedroom carpeting. Among these children, 56%–60% had mattresses and blankets that were not new – as opposed to 61%–68% of nonatopic subjects, although in 80% of these cases the mattresses were encased with an occlusive plastic sheet cover, with a difference compared to non-coverage (2.2 vs 3.1 µg/g) (GM) [427]. However, anti-Der p measures are applied so attentively in Sweden that in the ETAC study sensitization to these allergens was 0% (Fig. 5.22).

Pollens

With pollens, primary prevention is technically impossible, given the pollen ubiquity. It would be best to move to the mountains or out to the open sea for the entire duration of the pollination period, but this would entail a heavy financial investment, as short stays would lead to immediate (and more severe) relapses upon return to the city. Ideally, one should live permanently in a beach town where the wind blowing in from the sea brings pollen-free air, or at an altitude of >1,000 m above sea level if one is monosensitive to *Parietaria*, which does not thrive at these altitudes.

Often, due to the geographic and latitude characteristics that differ from one region to the next, there are pollination periods and types of pollen in the air that vary substantially between areas. To help pollinosis sufferers, sampling points have been set up extensively across the world and they are equipped with counters that take daily or weekly measurements of pollen (and mold) concentrations. As a result, the *aerobiological calendar* of an area of residence and/or study and/or work can be consulted for prevention [104]. Almost everywhere, there are measurement stations that deliver this information weekly via appropriate media (national television, Internet, daily newspapers, medical press, etc.), whereas doctors can obtain more detailed information at the headquarters of local aerobiology associations or from local monitoring centers [104]. Currently, there are short-term previsions of pollen concentrations based on the work done to monitor bioclimatic parameters (T, relative humidity, barometric pressure, wind direction, etc.). A computerized mathematical model correlates the databank of pollen counts for recent years with weather forecast data. A personalized study has also been proposed, with daily monitoring of the pollen count using the IPC (Individual Pollen Collector) or similar, which can furnish data on individual pollen exposure [297].

Preventive Measures

Preventive measures should be adopted essentially during the pollination period that, as should be remembered, is favored by hot, dry climates [104, 413, 479].

- Find out the pollination period of the plants to which the subject is allergic, taking steps when symptoms are most accentuated (for grasses pollination peaks between April and September: from 100 to 500 pollen grains/m^3 of air). We can summarize the other risk periods as follows: when it is windy and/or sunny, and in places such as plains, valleys, the countryside and outdoors in general. Cold and/or rainy days are the most reassuring, as is one's own home if improvements have been instituted.
- As much as possible, avoid spending time outdoors when pollination is peaking. For example, do not go camping or picnicking. Children with grass pollinosis who roll around in the grass can develop contact urticaria (Chap. 8).
- If possible, spend the hotter hours of the day – when pollen air concentration is highest – at home or at work, keeping windows closed. If possible, postpone airing the rooms until the evening.
- If you need to travel, try to leave on cloudy or rainy and windless days. As to rain, heavy and extended rain is ideal, as it can purify the pollen-rich air, while pollen on the ground percolates into the soil.
- Do not ride bicycles, scooters or motorcycles.
- Use air conditioners to reduce pollen concentration in the filtered air. If installing air conditioning in your car is planned, be sure in advance that it is compatible with the child's symptoms [252].
- Do not go in the country or to parks while grass is being mowed or has been mowed recently.
- If possible, put the child in the coolest room on the side least exposed to wind, so he or she can stay closed indoors even in the summer, weather permitting.
- If this is unfeasible, close the windows between noon and sunset, when pollen density peaks.
- Pollen enters through open doors and windows and is found in high concentrations in furniture, beds and carpets [395].
- Avoid eating honey, as it can contain pollen allergens (Chap. 9).
- After the child has been playing outside, have him or her take a shower and then change clothes in a room other than the bedroom.
- Dry laundry indoors, because pollen can adhere to fabrics put outside to dry.
- Avoid staying in rooms or places with an aquarium: pollinosis sufferers show allergic symptoms more often than nonallergic subjects (Chap. 4).
- Grass-sensitive children often have SPTs positive to plant foods. Therefore, when they eat these foods during the pollination period they may have symptoms of oral allergy syndrome (OAS) (Chap. 9).

Specific Measures

- Wear anti-allergy masks in risk situations.
- Scrupulously follow the prescriptions of your pediatrician.
- Before taking the child on a trip or on vacation, check the pollen calendar and pay attention to pollen density bulletins [104].
- Prophylaxis for car asthma [252]: in addition to the measures to be taken during the pollination season, do not allow pets or birds to be transported and ban smoking.

Molds

Some of the reasons that have stirred great interest in fungal allergens (Fig. 1.72) are summarized in Tables 24.20 [105, 330], 24.21 [105, 330], 24.22 [105, 330], 24.23 [330, 479], 24.24 [42, 330, 426, 479] and 24.25 [295], since prevention of mold allergy has gradually become more and more important [105]. Fungi are found both indoors and outside homes (Tables 24.21, 24.22), from which they should be removed by effective prophylaxis. High concentrations are found on school floors, both uncovered and wall-to-wall carpeted [119]. Fungi develop best at T 18–36 °C and a relative humidity of 75%–95%, and are active in spring and autumn, often with the highest concentrations between late summer and early fall [42, 94]. *Alternaria* and *Cladosporium* sensitivity was present in 1.5% and 0.5%, respectively, of children in prevention studies [17]. The *preventive measures* are summed up in Table 24.23 and more comprehensive recommendations for environmental mold avoidance in Table 24.24.

Prahl [330] has confirmed that avoidance measures are significantly effective, underscoring that the main measures to be adopted include:

- Immediate repair of defective insulation in homes or rooms
- Continuous cleaning of air conditioning systems
- Increased airing of rooms where mold growth is noticed (30 min at least twice a day)
- Increased bathroom ventilation
- Uniform heating everywhere
- Increased cleaning and hygiene procedures
- No indoor laundry drying
- Fewer indoor houseplants [330]

Mold spores can contaminate common foods, forming aflatoxins that can cause symptoms of intoxication (Chap. 10) and induce *intestinal and respiratory* symptoms and *skin lesions*. Therefore, an elimination diet is indicated as it can significantly improve symptoms, inducing their relapse (Table 24.25). It should be noted that *Penicillium glaucum* is used to produce gorgonzola or blue cheese, while other varieties are used for camembert, roquefort, etc. Among other possible factors, we must consider that CM pasteurization eliminates possibly 50% of aflatoxins and the UHT method gets rid of 25%.

Table 24.20. Difficulties in prevention studies of mold allergy

1.	Spores are ubiquitous and can be found either indoors or outdoors or both
2.	Fungal growth depends on several substrates
3.	Existence of hundreds of different species
4.	A single mold produces many allergenic substances
5.	Local microclimate, especially humidity, influences mycelia, spore and enzyme growth
6.	No growth of some species in culture media
7.	Saprophyte fungi have the ability to become pathogenic
8.	Skin tests are often weakly positive
9.	Poor correlation between skin tests, RAST and bronchial provocation tests
10.	The more studied molds are the fungi imperfecti, *Alternaria*, *Aspergillus* and *Cladosporium*
11.	Different methods of preparation of mold extracts from one supplier to another
12.	Humans inhale mold spores, unlike the other aeroallergens, whereas the extracts are based on mold mycelia and metabolic products
13.	Fungi are quite different from other aeroallergens, since inhaled particles consist of entire living cells

Data from [105, 330].

Table 24.21. Indoor ecological niches most frequently preferred by molds

Rooms with insufficient ventilation: bathroom, kitchen, woodroom, storerooms	
Other areas where molds collect	
a.	Wall cracks communicating with outside
b.	Window frames where condensation is prominent
c.	Humid floors and walls
d.	Wallpaper on cold walls
e.	Wool, cotton, kapok clothes
f.	Damp fabric or leather (shoes, boots)
g.	Dead leaves of houseplants
h.	Inappropriately stored foods, such as, in refrigerator even at 5 °C
i.	Air conditioning and humidification systems

Data from [105, 330].

Table 24.22. Outdoor ecological niches most frequently preferred by molds

1. Ground
2. Organic material in decomposition (dead leaves, garbage piles, barns, rotten fruit, rotting wood, etc.)
3. Particular work environment
a. Food industry (cheese factory, pork products)
b. Drug industry
c. Large storehouses: depository, docks, silos
d. Paper mill
e. Tree nursery and greenhouse
f. Stable
4. Places remaining closed for a long time
a. Cellar
b. Attic
c. Garage
d. Holiday dwellings

Data from [105, 330].

Table 24.23. Prevention of mold allergy

1. Especially in critical periods do not go to contaminated places, and avoid the following activities:
Going for a walk in the woods, especially with fog or after rain, in recently cut meadows, in harvested fields
Play or work for prolonged amounts of time in basements, cellars, attics, etc.
Mow lawn or make hay
Take dead leaves in the garden or elsewhere
Stay in the vicinity of barn, granary, stables, dunghills, containers with sand, hay, fertilizers
2. Allergic children and teenagers should wear a mask and maximize ventilation when working in areas with high mold growth
3. Decrease sources of mold in house by avoiding overwatering and keeping indoor watered plants or those with dead leaves
4. Air previously closed rooms where children go
5. Prune or cut trees giving shade to the dwelling
6. Reduce general humidity in house (Table 24.17) and water drainage
7. Wash daily air filters on air conditioners, which usually retain spores; these reproduce inside the filter, multiply and cross the filters, and sporulate freely in the environment; wash dehumidifiers with soap and water and nebulizer tubing
8. Supervise that there are no deteriorated or moldy foods in home, pantry, refrigerator, etc., discarding them whenever mold spots appear
9. Remove wallpaper as soon as possible
10. Exposure is reduced by closing windows in the house and car windows
11. Clean humid areas in the kitchen and bathroom, using:
Bleach diluted 1:4–6 parts water or vinegar also diluted
Benzalkonium chloride 1:10,000, or diluted alcohol, or trioxymethylene, which may be left to evaporate in container in closed places, such as, holiday dwellings, basements, cellars, attics or rooms remaining closed for a long time, to discourage fungal growth; the places should be aired by adequate ventilation, avoiding that mold-allergic children enter the spot while the vapor is not wholly dissolved
We do not suggest using formaldehyde regularly; it is a potent skin and airway irritant even in small amounts, above all by being highly hydrosoluble (Chaps. 4 and 8).

Data from [330, 479].

Table 24.24. Plan of a mold-free environment and its maintenance

In house
Buy a hygrometer
Maintain, if feasible, indoor humidity <30%, but never >50%
Ensure adequate ventilation
Increase the ventilation with fresh air where moisture is noted
Use air conditioning during the warmer days and months and at other times of high humidity levels
Use a dehumidifier in the basement or other areas of dampness
The air filters on air conditioners should be washed with soap and water to remove mold and airborne debris, and sprayed with commercially available fungicides as soon as a mold odor is smelled, whereas the dehumidifier should de regularly emptied of the air inside
When the dehumidifier is utilized also in winter:
Avoid excessive humidity
Change the water frequently to discourage mold growth
Clean the inside occasionally with bleach solution as above
No indoor clothes drying (not on indoor clotheshorse), cooking equipment should also be vented to the outside
Household plants should not be watered too much: a common alert of moisture are the circles of a whitish color at the base of the pots, formed by the excessive water used
In the kitchen
Verify the hood efficiency and use a kitchen fan to eliminate the steam formed when cooking
Periodically clean the work surfaces, sink and food containers with vinegar or comparable agents
Bring in firewood for immediate use only
Refrigerator
Molds often proliferate around and under the rubber seal of the door, trays and sinks
Empty the defroster basin regularly
To prevent food deterioration by molds, it is best to put food in impermeable containers or wrap with plastic sheet, aluminium foil, etc.; contaminated foods should be discarded, even if no mold spores are seen the characteristic mold odor is smelled
Clean refrigerator more times a year
Empty and clean the garbage can carefully
Reduce collections of standing water and water leakage
In the bathroom
No bath mat or the like
Apply a window fan to eliminate the steam formed while bathing or showering with hot water
Clean and dry shower curtains, furniture drawers, cabinet inside, and wash-basin, bathtub, shower tubing, etc. using bleach or vinegar as above
Change shower curtains when mold growth is noted
Caulk any possible crack (useful also as anti-cockroach measure)
Bedroom(s)
Eliminate or reduce dust
Remove wall-to-wall or felt carpeting, carpets, bedside rugs, wallpaper if feasible or spray with available fungicides
Mattress and pillows: act as for Der p (bedding is an ideal mold niche)
Condensation on window pane is cause of humidity and favors fungal growth
Hangings, books, upholstered furniture, stuffed toys and the like should be removed
Put shoes and boots back in the closet after an adequate drying
Wood room, store rooms, etc.
Do not allow mold-allergic child in these rooms
Low-wattage bulb lamps may prevent fungal growth
Basements, cellar, garage etc.
Mold-allergic children should not live in basements
Install a dehumidifier
Eliminate water infiltrations or leakage
Miscellaneous
Replace wall-to-wall carpeting with plastic materials
Maintain rooms dust-free, eliminate unnecessary objects that may become mold niches
Solid brick outer walls can be sprayed with fungicide agents
Outdoor activity
Besides what we have mentioned above, greenhouse, antique and embalmer shops, sleeping-bag, sauna, holiday
Dwelling, auto air-conditioners, and hotel rooms are areas where mold reigns

Data from [42, 330, 426, 479].

Table 24.25. Foods to be eliminated in the yeast-sensitive patient

Foods containing yeast as an additive ingredient in preparations
All preparations containing mushrooms and/or yeast extracts
Canned foods not consumed quickly
Breads
Cake and cake mixes
Cookies
Crackers
Foods or substances containing yeast or yeast-like substances because of their manufacture or preparation
Buttermilk
Citric acid, almost always a yeast derivative
Citrus fruit juices not home-squeezed, whether frozen or canned
Dried fruits of all types
Fermented beverages of all types, whether bottled or canned
Fermented cheeses of all types
Malted products of all types
Monosodium glutamate, a potential yeast derivative
Sour cream
Sour milk
Soy sauces
Truffles
Yoghurt
Foods or substances containing vinegar of all types
Apple, pear, grape and distilled, used as such or in the following foods:
Baby cereals
Catsup
Chili
Condiments
French dressings
Green olives
Horseradish
Mayonnaise
Mince pie
Peppers
Pickled beets
Pickles
Salad dressing
Sauerkraut
Tomato sauce
Flour enriched with vitamins from yeast
Hamburger buns
Hot dog buns
Milk, fortified with vitamins
Meat, fish or fowl, fried in cracker crumbs
Pastries
Pretzels
Meat rollé, homemade or canned
Salt-rising bread
Yeast-containing medications
Vitamin B_{12} and all vitamin B_{12}-containing medications
Moreover
All foods stored in the refrigerator for several days

Data from [295].

Pets

Preventive Measures

We are convinced that if the pet is already part of the family, and thus of the child's emotional world (Fig. 4.17), complete removal of the animal is possible only in exceptional cases, as demonstrated by the fact that 36% of asthmatic children complained that they were not allowed to play with pets [416]. If anyone considers these precautions to be excessive in any way, we recall that prevention is always better than taking steps too late: a cat in the house of a newborn or nursling means sensitization by the age of 1 year [9], associated with high IgE titers [311], above all due to the spread of Fel d 1 (Fig. 24.10). An epidemiological study has shown that cats were less frequently kept in families with parental asthma, AR, or pet or pollen allergy (3.5%–5.8%) than in families without FHA (10.8%–11.8%). Dogs were less common in families with (3.3%) than in families without (5.9%) parental AD. Cat ownership decreased from birth to 2 years of age, especially in families with FHA [3].

Although the association between pet ownership in childhood and subsequent allergic disease is controversial [273], the hygiene hypothesis even reversing previous knowledge [33, 41, 182, 314, 325, 343, 353, 489], the main preventive recommendations are as follows [91, 106]:

- Remove the pet from the house or keep it confined away from the child, taking the necessary precautions, given the psychological impact this can have on the child [395].

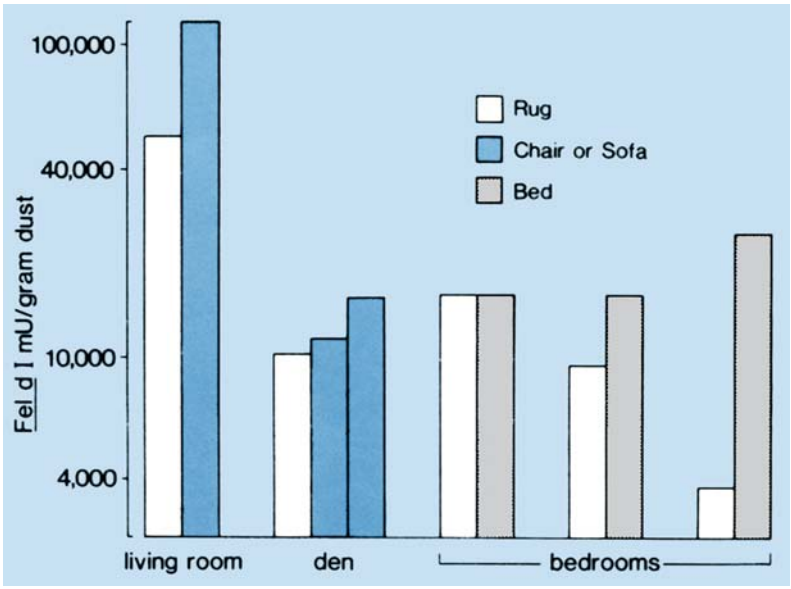

Fig. 24.10. Levels of Fel d 1 from various sites in different rooms of a house. Levels in the living room were five to ten times higher than those in the cat den

• Clean the house thoroughly after the pet has been removed: pet allergens can persist at length in furniture, upholstery, carpets and litters.
• If a cat is involved and the above measures are difficult to carry out, it is fundamental to:
– Bathe the cat as thoroughly and as often as possible: regularly bathing a cat on a weekly basis reduces Fel d 1 levels from 30–90 ng/m^3 to ≤7 ng/m^3 or less, a substantial difference that helps keep BHR away [106]. Other authors feel this is ineffective, on a par with other treatments [233]: because Fel d 1 is in the saliva, obviously a cat spreads it on its coat when it licks itself clean. Therefore, bathing it with cotton balls soaked with distilled or deionized water helps removing the allergens.
– Brushing cats and dogs helps in removing HDMs, which use skin waste as food, abundant in all furry animals.
– Dust or clean home walls thoroughly, allergens concentrate there in 97% of cases, unlike Der p 1 [474].
– Eliminate everything the animal seems to prefer – mattresses, pillows and so on – clearing rugs and upholstered furniture from the room where the pet spends most of its time, also removing a pet's favorite furniture, fixtures and clothing from other rooms as well.
– Air out the home: Fel d 1 levels drop with a ventilation of 0.5 ach, although this does not affect Can f 1 concentrations [301].
– Use a vacuum cleaner only if necessary: as we noted, filters, microfilters and special HEPA filters are not impermeable to allergens [119, 302]. This could be due to the continuous outside allergen supply and not to draperies and walls included in the daily cleaning routine [302].
– Treat rugs, wall-to-wall carpeting and upholstery with tannic acid spray, which is effective against Fel d 1 and Can f 1 [302]. This treatment lasts an average of 1–2 weeks [302, 413, 475] (Figs. 24.11, 24.12 [302]) and can be increased to intervals of <1 week, but other results suggest that only radical measures are effective [475]. It should be noted that while tannic acid is not toxic if used as indicated here, it stains fabrics and carpets [395].
• Do not let the child visit houses with cats, even if the pet is absent or if contact is improbable. If visiting the home is necessary, a pretreatment may be advisable using cromolyn and bronchodilators, or by having the child *wear a filter mask* (Chap. 7).
• In particular cases when epidermal feathers can be left on the pet-owners' clothing, avoid direct contact between the allergic child and these persons [124].
• After visiting houses or other places where cats or dogs are present, at home undress in the room furthest from the child's bedroom, keeping the door closed and the windows open, energetically brushing one's own clothing. For children who attend daycare settings, this can be avoided by changing clothes in special *exchange areas* [304].
• There must not be any animal products in the child's room such as furs, quilts, sleeping bags, skins or pelts, etc. [479].

If these measures do not yield the desired effect, it is clear that this is due to indirect contact [302] such as contamination by visitors and even by inattentive family members [124]: for example, at schools and daycare settings, high levels of Fel d 1 and Can f 1 have been detected, brought in on clothing of classmates, teachers and personnel who own pets. They were found above all on desks, but also on the floor [300, 302, 304], occurring 11-fold more frequently on wall-to-wall carpeting than on uncarpeted ones [304]. RAST inhibition recorded the levels of these allergens, particularly Fel d 1, which can sensitize children and induce perennial symptoms in already asthmatic children [119].

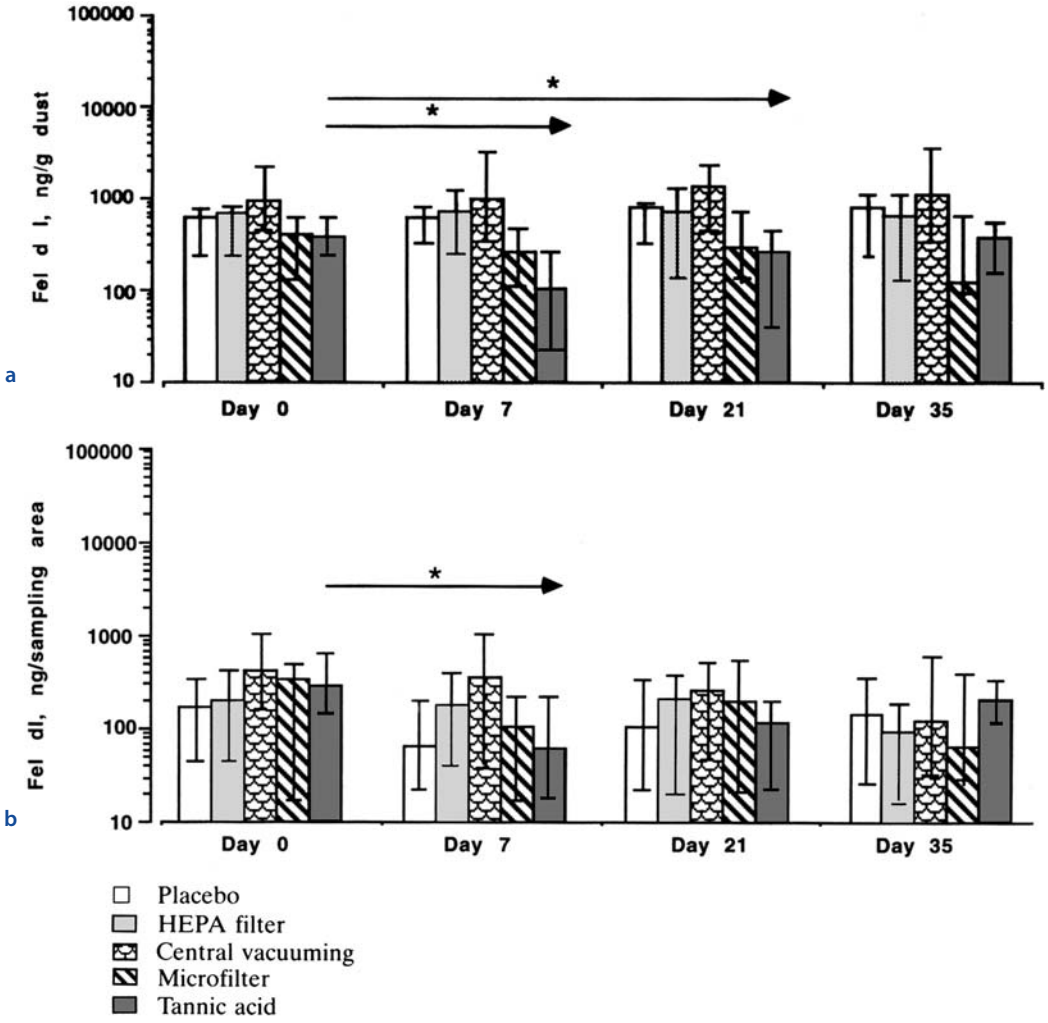

Fig. 24.11 a,b. Cleaning effects on Fel d 1. Influence of different cleaning techniques on **a** concentration of dust and **b** total amount/sampling area of Fel d in dust from living rooms. The *arrow* indicates significant decrease compared to placebo

To summarize, we emphasize that [479]:

- Animal hair has a high allergenic power.
- Current exposure to pets in children in prevention brings about a 43.1 risk factor for cat and a 34.5 for dog [12]. Among children with asthma 3.8% were sensitized to cat and 0.6% to dog [17]. All subjects with respiratory allergy who live in contact with pets can easily become sensitized: 30% of children with asthma/AR have SPTs positive for dog and cat dander [301].
- Being allergic to one species generally means being allergic to all species.
- Even animals not shedding hair can trigger allergy, since the allergens are in the fur as well as the dander.
- Ambient dust, which can easily be conveyed by pets, increases exposure of young patients.
- The more frequent and intense contacts with the animal, the faster sensitization occurs.
- Finding Fel d 1 in many public places (Chap. 4) multiplies the preventative measures.
- Fel d 1 are universally present in UK homes. Levels that have been associated with an increased risk of allergic sensitization were found even in homes without pets [8].
- Given the growing social importance of daycare facilities, extremely low levels of allergenicity can be achieved with a frequent scheduled cleaning [115].

The results of various studies are divergent: 20 weeks following the pet departure, 70% of houses had allergen levels in carpeting equivalent to those of houses without cats [475], or 100% after about 3 years [473], or 75% after 2–6 years [450]. However, it takes >5 years to normalize levels in mattresses [424].

Hymenoptera

Prevention is based above all on measures to avoid places and circumstances that attract these insects.

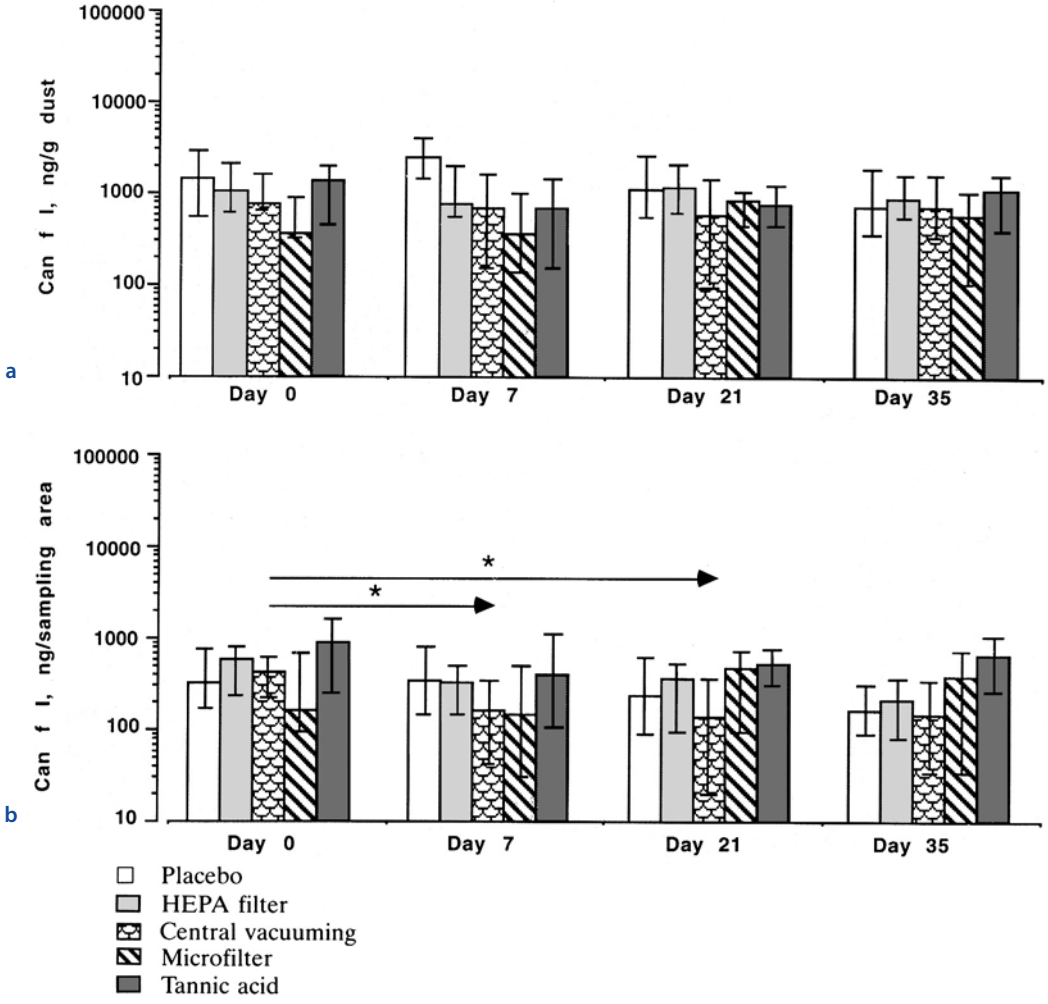

Fig. 24.12 a,b. Cleaning effects on Can f 1. Influence of different cleaning techniques on **a** concentration of dust and **b** total amount/sampling area of Can f in dust from living rooms. The *arrow* indicates significant decrease compared to placebo

Preventive Measures [318, 443]

- If you notice an insect near you, *do not make any sudden movements or try to swat it away*. The insect generally does not sting unless it is stimulated. If you are not wearing a cap, slowly cover your head with your arms or clothing.
- In case of bee stings, try to remove the stinger quickly to prevent anaphylaxis in susceptible subjects. The type of instrument used is not important and *speed is imperative* [443]: wasting time to find the most suitable tool allows the venom to spread [443] (Chap. 17).
- If you are stung near a beehive, move away from it as quickly as possible, because *an alarm pheromone is secreted at the base of the stinger* and, perceived by other worker bees, it helps them locate the victim and trigger their aggressiveness [443].
- When travelling by car, check the inside of the car to be sure there are no insects and keep the windows closed.
- On bicycles or motorcycles, wear gloves, coveralls and a safety helmet.
- When outdoors, particularly during outings, when camping and picnicking, etc., *cover yourself completely,* wearing socks, a long-sleeved shirt, closed shoes, and possibly with gloves and a hat or cap. Do not wear loose clothes, as insects can get trapped in them.
- Avoid wearing *clothes with floral motifs or bright colors,* favoring neutral tones like white, green, khaki, etc.
- Do not wear *perfume and scents* in the broadest sense of the term: scented hair gel, hair lotions and aftershave, shampoo or hairspray, cosmetics, cologne, heavily scented suntan lotion, etc.
- Do not stay *in the sun if you are wet or sweaty* or have rubbed tanning oil on your body.

- When playing or taking walks in the country, do not move rocks, tree trunks or branches lying on the ground with your hands or feet: they often hold wasp nests.
- Likewise, do not go near open flowers, very ripe fruit and/or dry trunks that have fallen from trees, bushes, stacks of wood, bundles of branches.
- In these situations, absolutely *avoid walking barefoot* (many insects are on the ground).
- When eating outdoors, *keep everything closed or covered*. Avoid bringing sweets or ice cream, opening containers with sweetened beverages, leaving food or waste out: they always attract bees and wasps.
- Stay away from fruit stands, as well as mangers or other places where animals are fed.
- During outdoor activities, especially in HR areas or places where trash has collected, consider wearing protective masks, boots and gloves in addition to full-length clothing.
- Hymenoptera are also attracted by perspiration and by the CO_2 produced by hyperventilation during athletic activities.
- Always keep bedroom windows closed or mount insect screens on them.
- Keep trashcans closed.
- If there are nests or beehives next to your home or office, call specialized personnel to remove them.
- Always wear *an ID bracelet* or tag, as recommended; purchase a *cellular phone* if possible.
- Always have an emergency kit with you containing self-injectable epinephrine drugs (that have not expired) and instructions on *emergency treatment*.
- Consult your pediatrician even after performing all the above measures.
- If the history of children with multiple stings of the same insect shows an essentially favorable prognosis, it is suggested to submit only children with severe reactions to SIT [318].

With regard to other insects (Tables 17.11–17.13), attention must be paid above all to *mosquitoes*: the main preventive measure is to avoid overwatering the plants on terraces and balconies and in the yard. Water should not puddle, as this promotes breeding. Similar measures are recommended for the *tiger mosquito*, which lays its eggs not only in stagnant water but also in gutters, manholes and puddles in general. The *fire ant* is found in mounds composed of fresh soil that may be at least several inches high and may extend 30–60 cm in diameter. These ants are very aggressive, particularly if their mounds are disturbed. With regard to available defense systems, children ≤2–3 years should not sleep in rooms where mosquito repellents have been used, given that repeated exposure can turn into sensitization: an *insect screen is safer*. Mosquitoes are also attracted by certain types of perspiration and have receptors for CO_2, through which they identify their prey even 15 m away. It seems that these subjects get bitten more frequently, as do women who use cosmetics containing stearic acid.

Birds

The only prevention is removing them. However, antigens can persist in the home even >18 months, making it necessary that children change bedrooms or, temporarily, even their home, depending on the clinical condition [95].

Horses

Horse allergy can also extend to mules, donkeys and similar animals. Removing the animal responsible for the allergy is imperative. When sensitization is underway this is of clinical importance.

Rodents

Mice and rats have numerous cross-reactive allergens (Table 1.73). Likewise, rabbits, small mice, hamsters and guinea pigs must be removed [413].

Animal fur and/or feathers reviewed so far decompose into a very fine powder that, if inhaled, can trigger asthmatic manifestations in sensitive children. In addition, the dust they generate remains for long periods, thereby prolonging contact.

Cockroaches

Allergen levels are generally low in cold regions with dry winters, prevailing instead in homes with poor ventilation and increased humidity with a 0.6% sensitivity in children in prevention [17, 301]. Cockroach allergen was associated with increased risk for wheezing [258]. Other preventive measures are part of normal cleaning routines [479].

- Keep the *kitchen scrupulously clean* and avoid leaving out leftover food and dirty dishes.
- Keep *food carefully preserved* and protected, or in well-sealed plastic, glass or metal containers.
- Use *specific waste containers.*
- *Caulk holes and cracks* in the walls near faucets, water pipes, bathroom fixtures, etc.
- *Change the seals* on leaky faucets and get rid of humidity on the pipes.
- *Improve ventilation* and remove damp areas.
- Every day, sweep the kitchen and dining room, or any other rooms where food is eaten.
- Use insecticides *monthly.*

After cockroach extermination, it takes 6 months for cockroach allergen levels to decrease by 70%–90%, thus leaving substantial levels of residual allergen [479].

Environmental Pollutants and Irritants

Various factors act as irritants to the air passages and can provoke asthma or aggravate it in subjects with BHR.

1. ETS

A *total ban on smoking* in the house of asthmatic children – and wherever they are – is a vital environmental measure. This measure, which is fundamental not only for prophylaxis but also for therapy, absolutely requires the collaboration of parents, the whole family and any smokers who visit the house of any allergic child. In fact, friends and relatives rarely refrain from smoking when they come to visit, nor are they asked not to smoke, even when the parents are motivated and attentive not to expose their children to ETS. Above all during cold weather, when the windows are kept shut for extended periods of time and asthmatic children spend nearly all day in the house, this creates a highly negative environment for their airways. Anyone who cannot forgo smoking should leave the child's home, closing the door. Many of the misleading methods that are commonly adopted, such as smoking fewer cigarettes, opening the windows or turning on a fan, as well as smoking in another room, out on the balcony or outside the front door, are completely useless and equally pathogenic as far as exposure is concerned. As has clearly been demonstrated, the child's cotinine levels do not change with any of these actions as compared to controls adopting none at all [468]. Maternal smoke during pregnancy has effects *in utero* and postpartum, predisposing the child for developing asthma; see Table 4.25 on pediatric morbidity associated with household smoking. Risk factors for persistent sensitization compared with those for transient early sensitization were maternal smoking during pregnancy, which significantly increased the risk for persistent sensitization (OR, 2.27; 95% CI, 1.14–4.52), also a significant risk factor for asthma at school age (OR, 2.46; 95% CI, 1.28–4.73) [205]. NHANES III stresses that breast-feeding might protect children against ETS-related asthma and recurrent wheeze by promoting postnatal Th2/Th1 switching [87]. However, children were less likely to have been breast-fed if their mothers smoked while pregnant (40.9%) or they lived in households with one or more smokers (36.8%) [87].

Preventive Measures. The asthmatic child must live in ETS-free rooms and/or very far from those visited regularly by smokers: in all restaurants and public buildings, there must be non-smoking areas. Likewise, smoking must be forbidden in cars [252], public transport, trains, ships, tourist buses, airplanes, etc. Smoking a single cigarette produces about 150 g of PM_{10} (particulate matters <10 µm). In a room of 100 m^3, PM_{10} reaches 1,500 when the PM_{10} level to block traffic is 40 µg/m^3/24 h [208]. Prevention becomes even more meaningful for older children and teenagers, and it is not comforting to note that younger generations are taking up smoking (Fig. 4.25). Paradoxically, however, they are also most exposed to ETS. As a specific preventive measure, proposals have been made to tax tobacco companies at twice the revenues generated by sales to young people under the age of 18 [153]. The younger generation is the one that, in fact, is targeted by tobacco companies to recruit new smokers, and the industry has even been accused of increasing cigarette nicotine content to bolster its ability to create a dependency [351]: once a neophyte becomes a slave to tobacco, each one is expected to generate a daily revenue income for at least 20 years [153]. Given the advertising effects, bolstered by widespread failure to remove this advertising from the areas immediately around schools, viewed by these companies as a highly profitable long-term investment [164], any company that prefers to avoid gearing advertising messages to minors and that modifies its marketing and distribution system to reduce tobacco consumption in the pediatric range most at risk would be exempt from this hypothetical tax [153]. An important study indicates that smoking prevalence in New Hampshire declined among middle school students during 2000–2001 and among high school students during 1995–2001 [291].

As we have repeatedly insisted, the actions of pediatricians and teachers are vitally important: it is very helpful for them to explain how important it is for children's health to ban all smoking at home. At times, they will have to use different methods to repeat the imperative measures for preventing infantile asthma, but the time that pediatricians devote to informing the family about the damage caused by ETS will indubitably be rewarded by the therapeutic success that is achieved [444]. In various countries, the number of smoking physicians is as high as 50%–56%: in England, following incisive campaigns it dropped from a 65% rate in 1950 to the current 5% figure [140]. Moreover, parents of at-risk infants and of asthmatic children, as well as older children and teenagers, must be informed about the pathogenic role of smoking. Despite the PREVASC intervention program focussed on avoidance of prenatal and postnatal passive smoking in children at high risk of developing asthma, no difference was found in the intervention compared with the control group concerning the exposure to tobacco smoke except maternal postnatal smoking [242, 376]. Therefore, family smoking of any kind should be strongly discouraged, helping them quit [140]. The action must be extended to urge government agencies *to reinforce nonsmoking measures and ensure that they are enforced* and *to introduce information on the relationship between smoking and disease in university pediatrics courses* [140]. Especially now, since Florentine researches have created the GMO tobacco, with accelerated flowering and bean multiplication (Press report, Feb. 27, 2007).

2. Environmental Pollutants

Environmental pollutants in the air act as adjuvants, irritating the airways, with negative effects on health.

Given the magnitude of the observed effects, a study on 1,759 children has shown that current levels of air pollution have chronic effects on lung development in children from the age of 10 to 18 years. Similar associations were significant in nonasthmatic and nonsmoking children [144]. Prevention is an indispensable measure because exposure at 19–36 months is a significantly higher risk factor (OR, 4.1) than exposure in general [394].

Preventive Measures. *Inside* the home of the asthmatic child, volatile substances with a strong and pungent odor (perfume, toiletry products, scented cosmetics, talcum powder, insecticide spray, cooking fumes, household detergents, cleaning products, room fresheners, fresh dyes and paints, etc.) [230] are to be avoided above all, in addition to other ascertained risk factors such as a humidifier in the child's room and electric heaters [206].

Outside it is more difficult to get away from exhaust fumes, atmospheric dust, industrial smog, SO_2, O_2, NO_2 (Table 2.26) and electrosmog.

- With regard to O_2, farseeing administrators advise parents against taking small children outside during hours that are most at risk based on sample measurements, generally in the afternoon. Therefore, it seems more responsible and sensible to plan morning activities.
- Pediatricians should inform parents of the risks connected with O_2 and, if necessary, recommend the use of special masks [234], suggesting that parents carry their children in their arms if taller strollers are not available, while awaiting more specific measures. The return of older children should be planned so that they avoid benzene in peak traffic hours.

3. Pesticides and Toxins

Infants and children are far more exposed to these products than adults, considering that, by weight, they drink more water, eat more food and breathe more air than adults do. For example, in their first 6 months, nurslings drink three- to fourfold more water kg, children aged 1–6 eat up to twice as much food kg than the average adult, and a resting infant inhales twice as much air as an adult. Considering that infants will put everything in their mouth and play on the floor, this increases their ingestion and/or inhalation of toxins found in dust or on the ground, as well as pesticide and pollutant fumes. The average daily dose (ADD) is drawn from studies on test animals and does not consider pesticide interactions once they are introduced into the body. Furthermore, the calculations are calibrated on adult weight and not on infant and child weight, who thus face greater exposure to pesticides and toxic products (Chap. 4). This is aggravated in France and Italy because they rank high in pesticide consumption (Fig. 10.5). A 1996 study by the Italian Health Ministry estimated the incidence of pesticide residues in fruit and vegetable products, and while it is true that the irregular samples amounted to 2.1%, only 64.5% of the samples were totally residue-free Table 24.26 lists the *foods potentially containing dioxin.*

Table 24.26. Foods potentially containing dioxin

Foods	%
Fish	34
Pork meat	21
Dairy products	20
Eggs	9
Beef, cow's milk, poultry	14[a]

According to WHO, the tolerable dioxin threshold is 1–4 pg/kg/die; the allowed exposure per day is 2 pg/kg.
Source: Istituto Superiore di Sanità, Rome.
[a] Shared with water, air and soil.

Additional Measures. We repeatedly recommend rinsing and rubbing fruit and vegetables under cold or lukewarm running water, if possible with the addition of salt or $NaHCO_2$, then peeling fruit and removing the outer leaves of vegetables. Although most phytomedicines penetrate inside fruit and vegetables, the Environmental Protection Agency of California deems these measures useful. We have warned that a great deal of fruit, including pears, due to prolonged storage far from sunny places and the use of *gas still defined as natural,* can lose their natural characteristics. We already noted that the common use of adding citrus peel to beverages must be given up. Foods treated with ionizing radiation to destroy or deactivate microorganisms may raise problems of nutritional values: the European Union limits this radiation, for example, to potatoes, onions and garlic.

4. Smoke from Gas Heaters, Gas and Wood Stoves and Fireplaces

This type of smoke is listed as a 63.8 risk factor [12]: while it does not fall into the pathogenesis of LRTIs, there is a substantial association with asthma [379]. A meta-analysis ascertained a mean 86% PFT reduction in 40,179 children aged 5–15 exposed to gas from cooking and heating [234].

5. Other Measures

For other predisposing factors, we have cited in several chapters the necessary preventive recommendations (exercise, emotional factors, GER, nocturnal asthma, cough-variant asthma, etc.). Appropriate recommendations for VRIs and sinusitis can be found in the respective chapters.

Table 24.27. Prevention of allergic reactions to drugs

Before prescribing medications
Consider whether
It is definitely necessary
There were no prior adverse reactions to the drug
The child has an increased risk of allergic reactions
Evaluate the option of prophylaxis
During treatment
Method of drug administration
Parent- or child-appropriate information of potential reactions
Oral administration as the safest route
Prescription of concurrent antiallergic drugs
Avoidance of intermittent or multiple therapy
Strict child observation during injection therapy
Facility in place to handle emergency
Potential desensitization
After treatment
Early prediction of the onset of allergic reactions

Data from [203].

6. Drugs and Additives

Among drugs provoking or exacerbating bronchospasm, usually with very severe and difficult-to-control reactions, the most often cited are the drugs listed in Table 19.24. Pediatricians must know whether the drug possibly prescribed can trigger undesired reactions, without waiting for the onset of symptoms following drug intake [203]. A great deal of caution should be exercised in administering antibiotics, antipyretic and anti-inflammatory drugs to asthmatic children [203]. Exposure to antibiotics *in utero* is a potentially important risk factor in the development of asthma and AD in a dose-related manner [282]. Moreover, kanamycin use during infancy promotes a shift in the Th1/Th2 balance towards a Th2-dominant immunity [406]. The indiscriminate use of antibiotics for veterinary and human purposes is the main cause of the appearance of antibiotic resistance in organisms. Adequate probiotic intervention after antibiotic treatment may prevent the Th2-shifted immunity induced by neonatal antibiotic use [406]. However, other evidence finds antibiotic use not associated with a greater prevalence of allergy and asthma [74, 204]. Table 24.27 [203] lists suggestions for prescribing drugs correctly and responding to general prevention criteria.

The type of foods eaten has changed drastically with the introduction of new industrial products with numerous additives, which are detected [203]:

- In all foods unless otherwise specifically indicated: foremost among these are monosodium glutamate, sodium metabisulfite and sodium benzoate.
- In alcoholic beverages including wine and beer, which can contain various excipients, yeasts and other grape components, and metabisulfites, used to stop the wine fermentation process.
- In drugs: again metabisulfites, which can be added to certain bronchodilators, aerosol or nebulizing preparations, and as dyes in oral preparations.
- A US study recorded that 72.5% of 102 oral preparations failed to specify the excipients they contained (natural and synthetic dyes, preservatives, flavorings, sweeteners); sodium benzoate and parabens were introduced, respectively, in 41.2% and 26.5% of cases (Chap. 10). Thus even fresh food items differ in many respects from those available only a few decades ago.

7. Contact Allergens (Tables 8.7, 8.8)

Parents of children allergic to latex must be given a list of all items potentially made of latex (Tables 8.10–8.12), so that they avoid their use. Doctors should be informed of this risk prior to any operation. Parents of sensitized children should be given a list of fruits cross-reacting with natural rubber (Table 8.14). Latex is also considered a hidden allergen: atopic subjects experience episodes resembling anaphylaxis *by eating food prepared and/or served by people wearing latex gloves.* It is impossible to record all possible functions of rubber gloves, some of which are difficult to imagine. For example, they are worn to make metal mesh or to join sheets of wood, which are contained in certain items [57]. Other preventive recommendations include wearing an ID bracelet at all times, always carrying an Epi-pen autoinjector and notifying family members, friends and teachers that epinephrine is required immediately in case of anaphylaxis, and carefully examining items and/or purchases to be sure that they are latex-free [57]. Another material to be avoided is nickel, found in several metal items and common foods. In addition to these items, low-karat gold, silver, white gold and more may contain Ni. For cooking purposes, it is best to use aluminium or teflon-coated pots and pans. For an elimination diet, follow Table 8.5.

Dietary Factors

Unmasking food allergens concealed in the most unobtrusive packages is difficult but vitally important: we are not referring solely to hidden sources of CM (Table 9.34) and numerous other foods (Appendix 9.10), but also the possibility that allergen is hidden in restaurant food, sandwiches, pizza and so on, or in prepackaged preparations whose ingredients fail to list the offending food, triggering severe reactions (Tables 20.5, 20.6), also with the consent of current laws [171]. Therefore, *pay attention when reading package labels*, with the help of Ap-

pendix 9.10: the wording depends on the professionalism of the production industry because by law, they are often considered natural flavorings, vegetable oils or the like [467]. We therefore suggest that labeling be improved with *plain-English terminology and allergen warnings* and parents be meticulously educated about deciphering labels. We recall six cases of fatal anaphylaxis and seven near-fatal cases in 13 children and teenagers aged 2–17 years who had various allergic diseases. Sampson et al reported (Chap. 20) that *no parent was aware* that allergens triggering fatal or nonfatal anaphylactic shock are found in common foods such as sweets, cookies, candy, cereals, sandwiches, and hamburgers. Moreover, precautions are not always successful: although five patients allergic to peanuts carefully avoided eating them, they nevertheless presented acute anaphylaxis (fatal in two cases) because foods served at restaurants contained hidden peanuts, or because the appearance deceived the customer and even the waiter. Severe reactions have also occurred in children with CMA because of the unspecified presence of *CM allergens in foods the producers guarantied were CM-free* [141, 150].

The EU has recently banned the usual jargon of the labels, thus no more casein, ovalbumin, but CM and egg proteins. Moreover, even the unknown ingredients contained in mg doses should be listed, and we hope also the food additives allowed in infant foods.

Four babies aged 4–13 months have been reported with peanut allergy provoked by *peanut oil* contained in the CM formula fed to them and in 11 of 45 (24.5%) of the formulas examined [293]. The authors have ascertained that peanut oil constituted 67%–80% of the total lipids present in two formulas, and thus ingestion amounted to 7 g/day [293]. In England, three of seven of the leading producers of formulas for nursing infants use this oil as a vegetable oil, and the same occurs in vitamin preparations [138]. We have ascertained that in CM formulas made for healthy nurslings, part of the animal fats are replaced with vegetable oils (peanut, sunflower, corn, soy, MCT oils, etc.). Therefore, it would be appropriate for producers to specify if the formulas and the foods they market for allergic children contain them. Peanut oil is an ingredient of various creams and oils used for children as well as of other products for topical application, most often for patients with AD [111]. Recent data of a birth-cohort study showed a significant independent relation of peanut allergy with the use of skin preparations containing peanut oil (OR, 6.8) [247]. The common use of prescribed and over-the-counter preparations containing refined peanut oil, applied to the skin of peanut-sensitized infants may be an important cause of cutaneous sensitization [247]. Heated refined oil would not cause allergic reactions [247]; however, excessive heating may enhance the allergenicity of the residual protein [270]. Peanut oil is also found in certain vitamin D preparations: a cohort of entrants aged 4–35 months who took daily drops containing this oil had positive SPTs to peanuts [111]: OR was high if the product was administered both in the neonatal period (5.47) and later (4.82) [111]. It has been demonstrated that if peanuts are cold-pressed, the oil derived can contain up to 3.3 μg/ml of protein, which can trigger anaphylactic reactions in sensitized subjects [187], who, as mentioned above, can respond to SPTs at a dilution of $1:10^7$. Therefore it is healthier to use olive oil. Unfortunately, it can get blended with hazelnut oil, which shares cross-reactions with walnuts (Table 9.9). Another important chapter covers non-food contaminants such as additives, bacteria, mycetes and excipients potentially added to foods, which can even provoke anaphylactic reactions (Chap. 20). Peanut allergen (Ara h 1) was detected in chocolate-containing food products [442].

A 10-month-old boy with severe AD was found to react to soy oil and was treated with an HF and an amino acid formula (AAF), which were ineffective. The AAF contained a soy lipidic emulsion (in addition to βLG; Appendix 9.4) and was replaced with another AAF; complete recovery was observed 6 weeks later [294]. *Soy lecithin* was positive in an oral challenge test (OCT) in a peanut-sensitive 4-year-old boy; the first case of *egg lecithin* also positive in an OCT occurred in a 15-month old girl [315]. A single reaction to soy lecithin diffused worldwide is a rarity.

Given that onset of atopic disease is usually in early childhood (Table 5.5), often acquiring severe traits, we can understand the importance of scrupulously following dietary and environmental measures. As we have emphasized, establishing avoidance measures should not have the effect of an exaggerated protection of the allergic child, nor should the family feel to be in a constant state of siege, with all the well-known repercussions. The request is that the various parties involved establish *the legal obligation to specify every ingredient in a way that is also understandable to any non-expert* (including the ingredients that are undeclared because they are natural foods). Ambiguous terms are listed in Appendix 9.2. We have noted that HF composition should be listed on the package. Parents cannot always rely on the completeness and accuracy of the ingredient declarations [411]. Accurate interpretation by parents of FA children of the food labels ranged from just 7% for CM to 22% for soy, 54% for peanut, 88% for wheat, and 93% for egg [216]. It is an indispensable intervention – also on a legislative level – to remove CM, eggs and so on from the list of natural foods, which consequently are *not mentioned on food packages.* This provision should be extended to all the examples listed in Appendix 9.10. However, in the greater part of Europe, an ingredient does not even have to appear on a label if it constitutes <25% of the product. As a second line of defense, restaurants, pizzerias, ice cream shops, bakeries and other foodservice operators could be requested to display a list of ingredients at their facilities, and/or to set up a toll-free number to be consulted in case of need

(difficult terminology, possible cross-reactions, etc.). When labels were studied for accuracy by comparison of raw ingredients with finished product labels, 25% of 85 establishments, including bakeries and candy and ice cream manufacturing facilities were found to have omitted raw ingredients, including peanuts and tree nuts, from the final labels [136]. *Pediatric allergy sufferers appear to be a subpopulation of children not always escorted by an adult able to use Epi-Pen.*

Recently, the US has recognized that food recalls can play a role in preventing or reducing the number of allergic reactions that may occur after a product containing an undeclared allergen has been introduced on the market. Three principal factors contributed to the presence of undeclared allergens in the recalled products: ingredient-statement omissions and errors (51% of all recalled products), manufacturing equipment cross-contact (40%), and errors by ingredient suppliers or manufacturing firm employees (5%). Significantly, consumers were the party most often responsible for identifying that an undeclared allergen was present in a product (56% of recalled products) [441]. Unfortunately, these numbers are still likely to be a significant underestimation of the true problem because there are many allergens that are less common but are important for children allergic to those foods [472].

Mad Cow Disease. Mad cow disease (MCD) or bovine spongiform encephalopathy or BSE, or acquired human prion disease, a variant Creutzfeldt-Jakob Disease (vCJD), the human equivalent of BSE, was first reported in 1996; the youngest patient developed symptoms at 16 years of age. Among children there were two fatal cases of definite vCJD and one case of probable vCJD; all reported in 1999. One girl was 12 years old at onset, the youngest ever case of vCJD [440]. Although the nature of the responsible agent of BSE/CJD is uncertain, it is characterized by the pathognomic *accumulation, within the central nervous system of the infected individual, of a normal protein from the host organism,* the PrP (prion protein) (CD230). Differences are noted between the PrP isolated from normal individuals (PrP-c) and PrP isolated from infected individuals (PrP-res) [117]. Apart from neurosurgical procedures, human exposure to prions usually occurs peripherally, especially by ingestion or inadvertent inoculation [68]. Postexposure prophylaxis against transmissible BSE is a necessary step. The absence of an immune response to prions may be related to the fact that these prions lack in nucleic acids. To investigate this, healthy mice were inoculated unmethylated deoxycytidyl-deoxyguanosin dinucleotide (CpG) oligodeoxynucleotides (ODNs). This postexposure prophylaxis resulted in longer survival, and even longer survival after repeated injections [386]. CpG ODNs have been shown to activate both innate and acquired immune responses via a signaling pathway involving toll-like receptor 9 (TLR 9) [419]. CpG-A ODNs induce plasmacytoid dendritic cells (DCs) to secrete very high levels of IFN-α, which secondarily induce purified monocytes to secrete high levels of the Th1-promoting chemokine IFN-γ-inducible protein-10 (IP10), thus creating a Th1-like environment [26]. Development of improved postexposure prophylaxis is urgently needed, given the unknown prevalence of prion exposure associated with the BSE outbreak [68].

Transgenic Foods. In 1998, GMOs gained a world role when Dr. Pusztai was fired from his post when his research discovered that rats fed genetically engineered potatoes experienced jejunal lesions [127]; thus he defined the potatoes, without formality, as *Frankenstein food*. But criticizing reports of research, as the Royal Society did with the Pusztai data, before they were reviewed and properly published, will only intensify *public skepticism about GMOs* [194]. Perhaps the speculation that the lectin GNA (*Galanthus nivalis* agglutinin) caused jejunal crypt hyperplasia is not wholly justified [299]. Possibly the use of transgenic plants expressing GNA to protect food plants highlights that terminal mannose moieties interacting with GNA are synthesized by human glycosylation pathways in white cells [131].

We already examined GMOs in Chap. 1 (Tables 1.78, 1.79 and Fig. 1.81), important above all for the identification and labeling of these GMOs. Currently, about 100 million acres are planted with genetically modified crops, with 72% in the US, 17% in Argentina and 10% in Canada. Considering these data, we can see that they are plants that have been genetically modified to yield products with a longer shelf life but that they lose their natural flavor. Nevertheless, can allergic subjects run the risk of anaphylaxis due to the introduction of new allergens even in very common foods? Virtually nothing is known about the possible influence of such dietary changes on childhood allergy [23]. There are different methods for manipulating food allergenicity, but one thing is to select some with a low allergenic content – for example, different types of apples contain different quantities of allergens that can cause OAS in people allergic to birch – or to reduce the allergenic content by altering the ratios of the individual elements in the food, a procedure that has been achieved with the perfection of hypoallergenic rice, as we discussed in Chap. 9. It is quite another manner to increase allergenicity, such as the introduction of allergens of different provenances by genetic manipulation, thus risking anaphylactic reactions as detailed in Chap. 4. The standardization of the methods used to control potential allergens is required, but this control program for determining the allergenicity of modified foods should be established on an international level. Figure 1.81 illustrates all the necessary steps: both FAO and WHO indubitably monitor the diversity between normal foods and GMOs [130]. On January 20, 2001, the Royal Society of Canada issued a document stating that 53 new procedures should be

fulfilled before new permission to cultivate GMOs could be granted. The use of substantial equivalence as a decision threshold by regulatory agencies is, in the Society's view, scientifically unjustifiable when used to exempt new products from full scientific scrutiny [354]. The BSACI warns that genetic manipulations may cause an inadvertent alteration in allergenicity because a change in an amino acid sequence within the new food might increase the potential of the food to cause sensitization and some neoallergens may evade detection. New allergenic B-cell epitopes and new sensitizing T-cell epitopes may stem from such manipulations; however, the unfolding caused by GMF processing would expose linear T-cell epitopes (Fig. 1.18) to the immune system that would not normally be seen [246]. Similarly, many conventional crosses involve different plant species and/or wild plants that we do not usually consume and thus the genes are new to our organism [418]. Table 20.4 lists 110 foods that can provoke anaphylaxis or anaphylactoid reactions, and several hidden allergens are listed in Tables 20.5 and 20.6. Normally children allergic to a given food avoid its ingestion, but are *unprepared to recognize hidden allergens,* nor are they assisted by food labels. GMO soy-lecithin is so widespread that it escapes any control. Immunoblot results have shown a 25-kD protein of GMO soybean reacting with IgE of some patients, a problem of the hour because 60% of processed foodstuffs contain soy produce [246]. In addition, GMO food labeling would be generic and thus of little value to children with specific FA [412]. So as not to undermine consumer confidence, the label should not only indicate if the food has or has not been genetically modified, but also how much transgenic product it contains. Currently, on a par with fish derived from monosexualization or from doubling maternal DNA [63], the regulations do not specify these characteristics on the labels that accompany products, a failure we have often emphasized in other fields as well. The fact that labels do not include GMO contents equal to 0.9% (EU) poses difficult measurement problems: even *minute doses of allergens* (μg of food) *are enough for primary sensitization* (see Tables 1.78, 1.79). Worried consumers expect that a collaboration between the EU and the US gives them the protection they need [155]. Nobody has evaluated whether intrauterine and infant exposure to GMOs with antibiotic resistance markers can have profound permanent and irreversible consequences even in adult life [63]. Figure 10.6 shows GMO production in European countries.

Hygiene Hypothesis

Our personal everyday experience in the Pediatric Allergy and Immunology Division, where we have worked since its foundation, allows us to conclude that the *hygiene hypothesis may have a very limited effect:* a) >95% of the children cared for have an evident FHA; b) in Table 1.74 we see that 98% of allergens act independently of the hypothesis; c) in taking the child's history, our experience has shown that only 10% of atopic families may have a pet at home; d) ETS is everywhere (Table 4.23) and resistant to any means (see "New Frontiers"). We will also discuss the possible inverse relationship between Th1 and Th2 disorders [217, 387].

A longitudinal study of subjects from age 9 years to age 26 years has found that asthma in early childhood continues with adult asthma. The earlier the age at onset, the greater the risk of relapse (OR, 0.89) per year of increase in the age at onset [381].What is presently known as the hygiene hypothesis is the decline in the infectious environment and in the pattern of microbial exposure during childhood, which consequently is a critical factor underlying the increasing severity and prevalence in allergic diseases in industrialized Western countries [278]. One theory under investigation suggests that increased use of antibiotics in childhood, cleaner drinking water, improved sanitation, and widespread vaccination practices have led to a lower cumulative exposure to microbial pathogens [458]. Thus conditions that are found in industrialized countries could be associated with increased atopy prevalence [259]. Epidemiological studies have shown that atopic diseases are more prevalent in Western industrialized countries [352]. Since it is difficult to change *the water supply and house sanitation,* in theory we should suggest discontinuing necessary antibiotics and no longer immunize children even against life-threatening infection, but *in practice we do the contrary.* In normal children there is a balance between Th1 and Th2 lymphocytes, but abating the protective exposure to infection the Th1 phenotype may be underexpressed and the Th2 phenotype overexpressed [277]. This imbalance forms the basis of the hygiene hypothesis for atopy, so several avoidance measures, for example, those followed by the second part of studies in Table 24.2 should be reversed. According to this hypothesis, immune responses to viruses and perhaps other organisms that generate Th1-like ILs, such as IL_{12} and IFN-γ, down-regulate Th2 responses that predominate in the neonatal immune system. Early exposure to respiratory viral infections through contact with older siblings or by attending daycare centers [14, 207], by the activity of Th1-like ILs, would help T-cell immune responses to mature into a Th1-like phenotype, less likely to favor allergen sensitization, thus *with a protective effect* [204]. Thus, childhood infections might have a key role in stimulating the maturation of the immune system away from the Th2 profile that predominates at birth toward a predominantly Th1-type phenotype [376].

According to data discussed in Chap. 4, the hygiene hypothesis reverses some avoidance measures listed in Table 24.4:

1. One effect of *infection exposure early in life* is to stimulate a shift away from a predominantly Th2 response, but the acquisition of commensal flora might affect up-regulation of Th1 reactivity and thereby affect

down-regulation of Th2 allergy-promoting responses. This immune switching is critical during early postnatal life, when exposure to high levels of antigen first occurs, because the response that evolves is likely to determine life-long reactivity to that antigen. However, early infection also plays an important role in maturation across the immune system, including down-regulation of both Th1 and Th2 responses [25, 259].

2. Along this line is *the suggestion of eating dirt, or moving to a farm:* at best they are theoretical rather than practical clinical recommendations to prevent asthma [458]. Epidemiological reports suggest that there has been a decrease in the frequency of allergy and asthma among children of farmers in Western industrialized countries than in children from nonfarming families, suggesting *a protective effect of the farming environment* against the development of atopic disease [34, 125, 193, 346, 446]. Children exposed to stables and/or farm milk during their 1st year showed substantial protection against the development of asthma, AR, and allergic sensitization [346]. Alterations in gut microbial flora might explain the inverse relationship between exposure to farm animals [25, 32]. Among 300 New Zealand farm and nonfarm children, there was an inverse association between AR, wheeze, and SPT positivity and dairy farming during the 1st year of life, but no such association with contact to specific farm animals. A reason may be found in the temperate climate of New Zealand, which allows animals on large farm holdings to stay outdoors throughout the year [461], as in Crete where animals are kept outdoors all year [16]. In a study on childhood allergy conducted in an urban area and in rural communities of Crete, atopy was twice as common among urban children compared to rural. However, among rural children any difference disappeared between intensity of animal contact and expression of atopy [16].

3. Several results have demonstrated that *cat and dog exposure in early infancy* is associated with later sensitization, but it is intriguing to find some reporting a *protective effect* [33, 41, 182, 314, 325, 343, 353, 489], in children without a maternal history of asthma [72], or with asthma unrelated to pet ownership [249], or in low-risk children without FH of asthma [343]. High-dose cat allergen exposure might induce tolerance to cat allergens and might therefore explain why animals in the house can decrease the risk of allergic diseases [489]. In a recent paper [12] on the *primary prevention of atopy and asthma* the children in prophylactic group had pets at home in 45.2%–51.6% of cases and the children in the control group hat pets in 34.5%–43.1% of cases (NS). The success of the primary prevention was dependent on the pet presence, as advocated by the hypothesis. In farmer's children, current contact with dogs was associated with a reduced risk of AR, asthma, wheezing, and sensitization to cat allergen and prass pollen. The inverse association between exclusive exposure to cats only during the first year of life was limited to wheezing, atopic asthma and grass pollen sensitization. Although higher levels of endotoxin were found in mattress dust of farm children, no significant differences were observed with respect to Fel d1 levels [453]. In 225 cat-sensitized children, we assessed the following atopic manifestations: asthma (38 = 45%), AR (15 = 18%), oculorhinitis (12 = 14%), AD (3 = 4%), FA (1 = 1%), urticaria (2 = 2%) and (14 = 16%) multiple sensitizations, with a high prevalence of respiratory allergy (77%). *Atopy* was present in 42.2% of children and *increased up to 91.7% in children with positive SPTs only for cat epithelium* [62]. A child we have treated reacted with wheezing when in the vicinity of a cat-owner peer. A further meta-analysis concluded that pet exposure increased the risk of wheezing in older children [7]. Some studies were cross-sectional and retrospective, others prospective [62, 249, 314, 343]; moreover, selection bias could not be excluded in two studies reporting that cat ownership was significantly related to a reduced risk of sensitization only in children with positive FH [33, 353]. Three prospective birth cohort studies have demonstrated that early exposure to cats [102] or to cat allergen [447] was associated with an increased risk of manifesting allergy to cat in the 1st year of life [102] and during the first 3 years of life [447] and in contrast, that exposure to > one dog or cat in the 1st year of life may reduce the risk of allergic disease *at age 6–7 years* [314]. Surprisingly, PTF results were better in boys but *not in girls* [314]. Moreover, keeping a single pet provided no protection compared with keeping no pets [459]. In asthmatic children *aged 6–8 cat exposure reached a 27.5% level* and in nonasthmatic a 33.3% level [232]. Thus, keeping a cat in the home is consistent with higher exposure to strong allergic sensitization and can therefore contribute to the *higher risk of atopy in later life and of wheezing episodes* [1]. Additional findings specify a dose-response relation between exposure to cats and specific sensitization within 1 year of age, thus cat ownership was significantly associated with sensitization to cats [102]. Overall, the presence of a cat in the household during the first 3 months of life was associated with an increased risk of sensitization to cats (OR, 2.1) and an interaction between early high-dose exposure to birch pollen and cats in the household was suggested for sensitization to cats [229]. Children exposed to a high concentration of cat allergen made a modified Th2 response characterized by IgG_4 antibody to cat proteins not associated with an IgE response [325], whereas a potential protective effect of high cat allergen levels is caused by increased proportions of $CCR5^+$ $CD8^+$ Th2 cells predominating *in children without FHA* [30]. On the contrary, high cat-induced IL_9 levels were associated with asthma in 12-year-old children [212]. At this stage, we would not advise people who wish to prevent allergic disease in their children to rid their homes of pets. We would also not recommend pet ownership as prophylaxis against asthma [273], especially to families where FHA is prevalent [12, 62]. An inverse association be-

Table 24.28. Evaluation of utility and carrying out procedures of allergen avoidance measures suggested to 96 asthmatic children aged 2–12 years (mean 6.9 years) + control children

	Control group		Study group	
	Yes	No	Yes	No
Pet elimination	27	69	84	12
Indoor plant elimination	48	48	77	19
Measures for humidity reduction	34	62	83	11
Smoke reduction (parents)	52	54	84	12

Study group: skin prick test positive to Der p (66 M, 30 F, p=0.0002). Control group: nonatopic children (56 M, 40 F, age 1–13 years, mean 7.6 years). Statistics: study vs control children in all rows, p=0.0001, smoking, p=0.0302. Unpublished personal data.

tween pet keeping and AD was found in a recent study [344]. Moderate exposure to cat allergen can result in the maximum prevalence of cat sensitization in children who have never lived in a household with a cat [182, 447, 451]. In high-risk families with at least one child or first-degree family member, pets are usually kept outside [242, 376]. Deliberate avoidance of pets might be an explanation for the protective "pet effect" observed in several studies [453]. Current cat possession represents a significant risk for sensitization to cats, *if cats are allowed indoors* [353], and in young children [62]. Contrary to what is normally seen, we show that the parents who wheezed in the first 6 years of life now with asthmatic children successfully organized efficient avoidance measures, especially related to pet elimination (Table 24.28). Cat allergen exposure was significantly lower on parental mattresses in families with allergic mothers [429], since allergic families live in homes with fewer triggers such as pets, no smoking and no carpets than the nonallergic families [37].

4. *Child daycare attendance during the 1st year of life* is an environmental factor possibly protecting against the development of allergies and asthma in childhood [228]. In children with FHA, the protective effect of daycare attendance in early life against the development of atopy begins by 2 years of age, and this protective effect in early life against wheezing may not be observed until after 4 years of age [73]. The development of asthma is less common among children with more exposure to other children at home or in daycare during the first 6 months of life than among children with little or no such exposure [14]. On the contrary, daycare attendance increases the risk of URTI (upper respiratory tract infections) and LRTI in the 1st year of life for children with FHA, especially specific environmental exposures within daycare (presence of pets or having a rug or carpet in the sleeping area) [71]. However, among children with maternal history of asthma, daycare in early life had no protective effect on asthma or recurrent wheezing at the age of 6 years but was instead associated with an increased risk of wheezing in the first 6 years of life [75]. Daycare attendance was a significant risk factor for AD in children born to asthmatic mothers (OR, 2.9) [228]. Concerning the effect of infections, differences have been shown in the microflora from the feces of allergic compared with nonallergic infants [191]. In two prospective studies, allergic infants were GI tract-colonized with more *Clostridia* and *Staphylococcus aureus*, whereas nonallergic infants had more enterococci, bifidobacteria, and bacteroides. If the microbial flora drives the maturation of the immune system, differences in the neonatal gut microflora such as changes in its composition might play a role in the development of atopy, suggesting a crucial role of the balance of indigenous intestinal bacteria in the development of and protection from allergy [25, 222].

5. A large multinational project involving children resident in Germany, Austria, and Switzerland [35, 346] has found that children exposed to stables and/or farm milk during their first year showed *substantial protection* against the development of asthma, AR, and allergic sensitization [346]. In a cross-sectional study involving 812 children aged 6–13 from farming and nonfarming households in rural areas of central Europe, the investigators found a relation between higher levels of *endotoxin* in the mattress dust and a protective effect against the development of AR, allergic asthma, and allergic sensitization in these children [35]. However, three of the four ILs that were down-regulated in the highly exposed children (IL_{10}, IL_{12}, IFN-ɣ) *are among those best known to suppress allergic responses mediated by Th2 T cells* (Table 1.5). In particular, IL_{10} down-regulates the immune responses mediated by both type 1 and type 2 helper T cells [458]. Endotoxin levels in the homes of children suffering from AR were lower than in families without sufferers [461]. However, endotoxin has been shown to be a *double-edged sword*. Longitudinal prospective birth cohort studies suggest that endotoxin exposure in infancy is associated with less AD, as possible evidence of an effect on the allergic march of early childhood [151]. Still, in this and two other birth cohort studies, high endotoxin exposure has a negative effect since it is associated with wheezing in the 1st year of life among infants with a familial predisposition to asthma and allergies [316]

and with an increased risk of physician-diagnosed respiratory infections and cough with respiratory infection, bronchitis, or both during the first 6 months of life, also protecting from AD [149]. Thus can endotoxin not only *prevent the development of atopy and atopic disease* but also down-regulate pre-existing atopic activity [117]? Only one prospective Austrian study involving 11 SPT-positive farm children followed for 3 years suggests that endotoxin might downregulate atopic sensitization [193]. Blood cells from farmer's children expressed significantly higher amounts of CD14 and TLR2 than cells from nonfarmer's children, thus suggesting that the innate immune system responds to the environmental microbial burden and modulates the development of allergic disease [230]. In 609 children of European farmers' and nonfarmer's parents, farmer's children had higher endotoxin concentrations in their homes [120] as in a previous study [346] but TLR genetic variation was a major determinant of their susceptibility to asthma and allergies. However, children of the high-endotoxin group benefited from a genetic variation in TLR4 inversely associated with sIgE to common aeroallergens [120]. High exposure to HDE was associated with an increased risk of wheezing over the period of observation, but this risk rapidly decreased over time [179], and of wheezing episodes, and was independent of the effects of HDE exposure [258]. On the contrary, households with detectable allergen levels but low endotoxin levels may provide a predisposing environment for pet allergen sensitization [149]. The modern metropolitan homes of allergen-sensitized infants had significantly lower levels of HDE compared with homes of nonsensitized infants and compared with farm and rural homes, but levels can still be significant, as well as with farm and rural homes [148]. HDE exposure in 9–24-month-old infants may protect against allergen sensitization by tilting the immune balance in favor of IFN-ɣ producing CD4 T cells (Th1 immunity) [148]; therefore it might shift the developing immune system to a predominantly Th1-type responsiveness postneonatally, thus protecting against asthma and allergies [279]. Whether there is an atopy-reducing benefit to the child *in utero* when the mother is in such an environment remains to be determined.

6. An unexpected result of the emphasis placed on the hygiene hypothesis might be *a prolonged fetal pattern of immune response into the first years of life* [279] when the Th1/Th2 profile is already being shaped [338]. Thus, the first encounter with common aeroallergens commonly occurs in an environment where antigen exposure in a germ-free environment favors the development of Th2 responses [406], which might explain why the newborn shows Th2-skewed reactivity [337]. The response that evolves is likely to determine life-long reactivity to that antigen, programmed into long-term immune memory. Therefore, strategies for primary prevention should be based on reestablishing conditions for the rapid development of Th1 functions during early infancy. This is a necessary step for fetal adaptation to the outside world and for the establishment of a balanced Th1/Th2 response [279].

7. Although endotoxin exposure has been shown to play a bidirectional role in conferring long-lasting protection against asthma as well as increasing the lifelong risk of the asthma onset [110], *muramic acid,* a constituent of peptidoglycan, is present in Gram– and Gram+ bacteria in the environment and may serve as an additional marker of microbial exposure. In a cohort of 553 farm and nonfarm school children from several European countries, children with higher mattress dust muramic acid concentrations had *a significantly lower prevalence of wheezing.* The prevalence of sensitization and respiratory symptoms was not different when comparing the two groups, data that could not be extrapolated to urban children. Independent of the endotoxin concentration, muramic acid can be considered as an independent marker for microbial exposure. Increasing mattress dust muramic acid concentrations are therefore associated with a lower incidence of wheezing, regardless of farming status and endotoxin exposure, and possibly of atopic sensitization among rural school children [430].

Too many paradoxical findings make it difficult to accept the hygiene hypothesis, and it does not explain the increase in asthma incidence in the last 40 years of the twentieth century [326]. Not only is there no credible immune mechanism to explain the hypothesis, but diverse influences on hygiene have been associated with a higher prevalence of atopy [326]. Will the hygiene measures abate the growing worldwide prevalence of allergic disease, as assessed in Chap. 5? A new hypothesis has been proposed in some recently published papers. These suggest that high-dose allergen exposure is associated with a lower risk for specific sensitization compared to moderate exposure to such allergen [170, 325]; similar results were found for cat, horse and pollen allergen [170]. Forerunners of the hygiene hypothesis envisaged that large doses of CM should be popularized, to feed nurslings not at risk of atopy (Chap. 4), and again the only result was that atopy prevalence grew in geometric progression. *Does high-dose pollen exposure induce tolerance* [182]? Thus, is it good hygiene to inhale pollens in early life? Among 592 children with severe asthma seen by us between January 2001 and June 2003, several pollen-sensitized children inhaled so much pollen that they had to be urgently admitted to the ED. Or, does eating dirt protect against mite sensitization [458]?

Changes in family size over the last 30 years do not appear to explain much of the reported increase in asthma or AR prevalence [460]. Increases in asthma mortality in New Zealand reflected a high prevalence of severe asthma in a country where approximately 50% of the homes have cats [276]. Progressive increases in lifestyle changes provide no convincing explanation for the steady increase in asthma hospitalization rate in both

black and white children aged 0–18 years [96]. Moreover, childhood asthma prevalence has increased in several large cities of Africa encompassing the informal settlements of Cape Town, where conditions could not be described as hygienic [457].

The number of siblings is discussed in Chap. 4.

In conclusion, there are prominent challenges to the Th1/Th2 paradigms, which, as currently formulated, might be an oversimplification, as shown by a cohort of 20,043 individuals [387]. The findings in 72 Estonian 4- to 6-year-old children do not even support the hypothesis of an immune deviation with down- and up-regulated Th1 and Th2 responses leading to atopic disease [217]. In a trial on 18,156 subjects, an FHA or FN of asthma was associated with *a higher risk of developing asthma* and a lower chance of remission (HR, 0.79) throughout life. No matter what one's FHA was, *early, acute respiratory infections* were associated with an increased lifelong risk of asthma onset (pooled HR, 3.19) [110]. So the hypothesis is restricted to nonasthmatic children with negative FHA. We feel it is encouraging that the parents of a group of asthmatic children have accepted to carry out successful avoidance measures, with a 52.8 % reduction in the number of smoking parents within the span of 1 year (Table 24.27). Application of the measures suggested in Tables 24.4, 24.16 and 24.17 is sufficient for keeping the levels of Der p constantly low [399]. Table 24.29 [213, 350] summarizes the pros and cons of the hygiene hypothesis. Table 24.30 lists the associations between endotoxin exposure and outcomes in children [35, 147, 251, 346].

Aid to Prevention

An aid to primary prevention comes from *Lactobacillus acidophilus*, possibly advantageous to maturation of the infant's immune system, and effective in preventing atopic disease in HR children [328, 407]. The gut microflora, still largely unexplored, might be a source of natural immunomodulators and *probiotics* [223], which may act by improving the intestinal ecosystem [407]. The use of probiotics in newborns to prevent atopy and atopic disease reduced the prevalence of AD; however, SPT positivity and total and specific IgE levels remained unchanged [223]. Thus the probiotic affected the manifestation of atopic disease but not atopy itself. Others, though, report that oral *lactobacillus* supplementation for mothers of exclusively breast-fed infants in the first 6 months of life offers a safe and effective mode of promoting the immunoprotective potential of breast-feeding and reduced threefold the prevalence of AD by age 2 in children receiving supplementation [342]. *Lactobacillus raises IFN-γ production* of PBMCs in infants with CMA and in infants with IgE-associated AD and may thus provide helpful Th1 immunomodulatory signals [328]. Dietary supplementation with *ω-3 fatty acids* might have a beneficial effect on the prevalence of wheeze during the first 18 months of life [274].

Table 24.29. Factors for and against the hygiene hypothesis

A. Pro
Early infections are inversely associated with atopy
Populations with high prevalence of parasitic infestation have low prevalence of AR and asthma
Exposure to cats, dogs, farm animals decreases the allergy risk
Gastrointestinal flora with high *lactobacillus* counts in children with low prevalence of atopy
Children raised in a farmhouse have fewer allergies than nonfarmers' children in the same area
Early exposure to group daycare is inversely associated with atopy
CD14 gene polymorphism may explain the variable prevalence of atopy
B. Con
Parasitic infestation associated with higher risk of urticaria and atopic eczema
Several microorganisms (*B. pertussis*, *respiratory syncytial virus*) increase IgE production
Active tuberculosis does not decrease Th2 reactivity
Pertussis vaccination can protect children from atopy by preventing pertussis infection
High prevalence of allergy in poor areas of developing countries or inner-city slums'
Air pollution is associated with higher prevalence of respiratory atopy
The striking increase in IgE-mediated diseases (allergy, asthma, AD) that has occurred over the last few decades
Reduced levels of sCD14 in the fetal and neonatal gastrointestinal tract is associated with the development of atopy, AD, or both
Fetuses and neonates show Th2-skewed reactivity

Data from [213, 350].

Table 24.30. Associations between endotoxin exposure and outcomes in children

1. Farm children were exposed to more endotoxins (as measured in house dust, barn dust, and mattress dust) [35, 147]
2. Greater endotoxin exposure was associated with less allergen sensitization, hay fever symptoms, and atopic asthma, in a dose-dependent manner [35]
3. Farm children's blood expressed higher amounts of CD14 and Toll-like receptor 2 [251]
4. Early life exposures to farm barns and unpasteurized milk had strong associations with low asthma and allergy prevalences [346]
5. High levels of endotoxin exposure were associated with an increased prevalence of nonatopic wheeze [35]

Preventing Allergen Entry or Mast Cell Degranulation

Prevention must also deal with infections: VRIs can cooperate with irritants, such as smoke, favoring allergenic invasion as well as increased IgE, as seen in Chap. 4, and thus atopic sensitization [479]. In Chaps. 7, 9 and 11, we illustrated pathogenic mechanisms and preventive properties of cetirizine, levocetirizine, cromolyn, ketotifen and nedocromil sodium. Asthma (including exercise-induced asthma, AIA) and FA are effectively prevented by these prophylactic – not curative – drugs.

Modifying the State of Sensitization Using Available Aids

Once the sensitization condition has set in, if a respiratory allergy is involved it can be modified or cured by *SIT* and if FA is involved by food desensitization, especially in a world in which a large proportion of the population is taking tablets or inhalers every day [324].

In the ETAC study, 795 nurslings with AD for at least 1 month and with an atopic parent were given cetirizine for 18 months, on a DB basis, at a dosage of 0.25 mg/kg twice a day (or placebo), followed by an equivalent observation period [450]: this prevented the development of asthma in 50% of children sensitized to pollen or Der p (Figs. 24.13–24.15). Thus, this study comes to the fore as an urgent priority, given the data illustrated in Tables 5.5, 5.8 and 5.15.

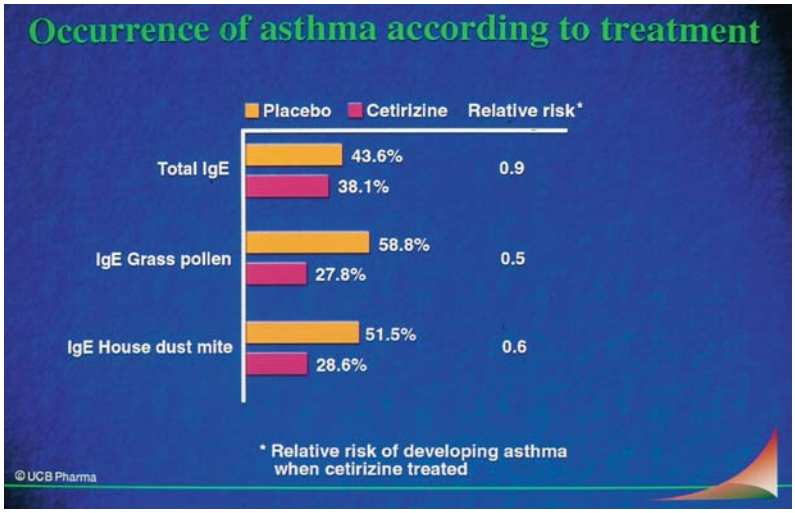

Fig. 24.13. ETAC study group. Occurrence of asthma according to treatment. Children receiving cetirizine who began the study with raised levels of total IgE and specific IgE to grass pollens and/or Der p had a reduced relative risk of developing asthma compared to children who began the study with raised levels of these IgE who received placebo

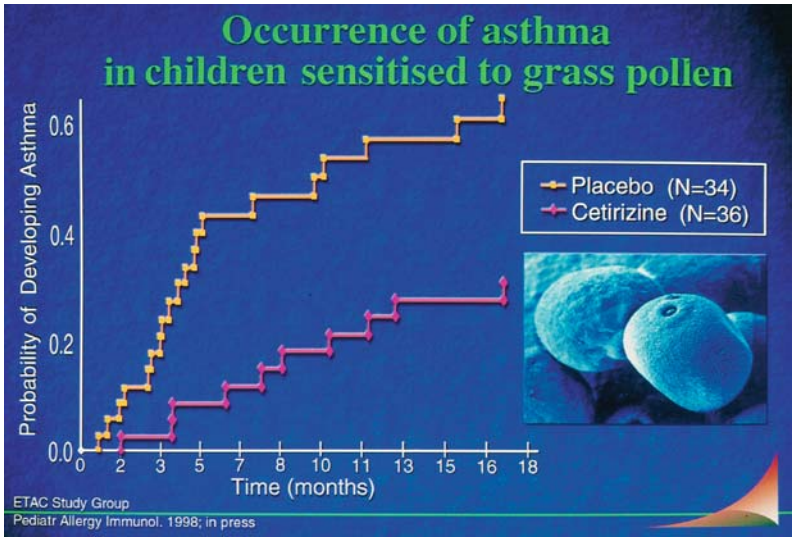

Fig. 24.14. ETAC study group. Occurrence of asthma in children sensitized to grass pollens. Cetirizine prevents asthma from developing in one out of two children who was sensitized to grass pollen and who had AD and a parent or sibling with a history of atopic disease

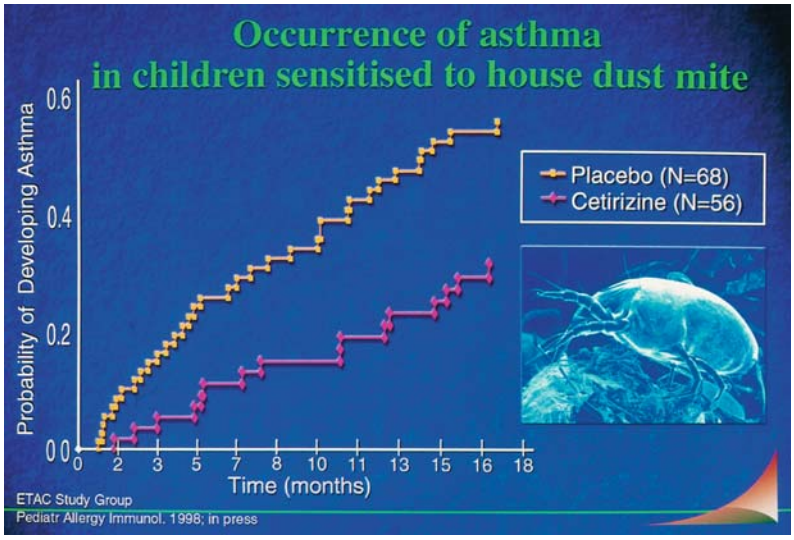

Fig. 24.15. ETAC study group. Occurrence of asthma in children sensitized to Der p. Cetirizine prevents asthma from developing in one out of two children who was sensitized to Der p and who had AD and a parent or sibling with a history of atopic disease

Tertiary Prevention

Tertiary prevention is the stage in which pediatricians treat children and adolescents to stop the atopic march. This goal is best accomplished by avoiding recurrence of symptoms, by using acceptable interventions, minimizing adverse effects, and providing cost-effective outcomes [483]. Parents often turn to their child's pediatrician or allergy specialist to find out if this or that measure they are about to adopt can have a boomerang effect. The advice sought most frequently by parents of allergic children involves the following aspects.

- *Vaccinations:* misunderstanding of the hygiene hypothesis in the lay press should be reversed because the substantial progress and reduction in morbidity and mortality achieved through the introduction of vaccinations should be continued for the benefit of decreasing morbidity from infectious illness. There are no contraindications for allergic children, for example, for the measles vaccine (Appendix 9.11), which should also be given to all atopic children [320]. Likewise, SIT is not contraindicated for coincident immunizations with both viral and bacterial vaccines: the only precaution is that they should not be given on the same day.
- The *choice of the most suitable sport:* we have commented that the asthmatic child feels limited in his or her athletic activities. Instead, even with severe asthma these children can participate in a number of sports: anaphylaxis and EIA can be prevented in most cases. SIT-treated children can also do sports, with the exception of the day they are given their dose of vaccine, due to the greater possible absorption of the allergenic extract during exercise.
- The *choice of vacations:* often (and rightly) parents ask about the most suitable destination. In general, there are no particular limitations: allergic children will benefit from the seaside or the mountains, as long as they spend as much time as possible outdoors. All winter skiing holidays are fine, and they also offer the additional benefit of less pollution, low humidity, and few specific and aspecific asthma-inducing factors. As to the mountains during the summer, children with pollen-induced asthma must avoid staying there in the months of July and August, when grasses are in the middle of their pollination period to varying extents, depending on altitude. With regard to HDMs, whose number seems to increase parallel to height (Table 24.19), the city child allergic to Der p should go to an aseptic house [85], and not to vacation homes that are shut down for the entire off-season period and are often covered with wall-to-wall carpeting. What yields the best results is common sense in the choice and furnishings of the accommodations, which easily helps avoid problems. Based on common experience, most hotels at mountain resorts open up for the season and there is a risk of being assigned a room that has long been closed and, at most, was opened and aired just a few hours in advance. With regard to the beach, parents should bear in mind that, as noted in Chap. 7, children with AD benefit enormously from the beachside sun and that asthmatic children can enjoy the water just like other children, engaging in swimming as we advised. Contrary to popular belief, there is no need to head to the beach at daybreak.
- *Military service:* the atopic boy who has suffered from inhalant-induced asthma, particularly if sensitive to Der p, should be evaluated carefully before being considered fit. The often dusty barracks, long marches often in unfavorable weather and, for subjects with FA, uncontrolled meals are all factors that can cause allergic manifestations, even in young men whose disease has been under control for years. Thanks to SPTs, RAST, PFT, and food and bronchial provocation tests, today it is possible to single out allergic subjects much better than in the past.
- *Laboratory tests or surgery:* it is important that the attending pediatrician be put in touch with the radiologist or surgeons to alert them that the child is atopic. In particular, hereditary angioedema requires special

Table 24.31. Advantages and disadvantages of preventive strategies

Primary prevention	Secondary prevention
Advantages	
Permits prenatal and postnatal prevention	Greater predictive capacity of atopic disease
Optimizes early parental cooperation	More convincing to some parents
Potential prevention of early sensitization/atopy	Prospective follow-up for atopy encouraged
Bridges atopy's window period and critical period	Pediatricians are more informed and motivated
Disadvantages	
Low sensitivity of predictive markers	Potential jeopardy of missing the critical period of prevention
Involves several families not at risk of atopy	Atopic disease may have developed in the meantime
Effective preventive efforts scarcely known	Usual medical care may not encourage prospective follow-up
Prevention costs and effectiveness are undetermined	Prevention costs and effectiveness are undetermined

Modifed from [482].

attention, even for simple dental extractions. We must emphasize that it is not a good idea to discuss hospitalization, surgery and related topics in front of the child: here again, the parent's loving preparation is required, if necessary with the pediatrician's help, particularly if he or she is the child's friend.

- *Travel* requires thorough preparation, and a number of precautions must be taken in addition to the ones indicated so far, depending on the means of transport, travel plans, destination and its microclimate. Pollen sufferers must obtain information on the plants and flowers prevalent in the region or country they are visiting. In Europe there is a special connection module with EAN (European Aeroallergen Network) for this purpose, to permit the exchange of information on aeropollen concentrations in various countries. By car, bus or train, potential allergens other than the ones indicated here include industrial irritants. The windows and/or air vents must be closed tightly: pollen trapped in the closed space of the car (5,000/m^3) or compartment can reach risk levels [252], above all on tree-lined streets and in the country. If you set off on a long car trip, turn on the ventilation/air conditioning 10 min before you leave, to clear the system of these inhalants. If you are travelling by air, check the air quality in the cabin and choose a seat as far as possible from the smoking area. However, if the child has suffered recently from sinusitis, OME and/or nasal blockage, the trip should be postponed. Subjects allergic to Der p must bring a mite-proof mattress cover and pillowcase with them, even if staying with friends or relatives. With FA, the danger lies in foreign foodservice and thus it is imperative to verify that the selected dish does not contain specific allergens. If necessary, bring an auto-injector of epinephrine. If the child is being treated with SIT, we have noted that in England it is difficult to find a doctor willing to give a booster shot. Therefore, when vacationing, it is best to take advantage of the free period. Before leaving, go to your pediatrician, who can draw up a letter for any colleague who may be required to intervene, detailing the diagnosis and current therapy. As far as drugs are concerned, bring a sufficient quantity in the original packaging, keeping a copy of the prescription at hand. If nebulization or peak-flow measurements are required when travelling abroad find out the prevalent voltage in the destination country. If necessary, obtain an adapter if the device does not have one, given that battery-operated models are expensive. As an alternative, there are portable ones that can be plugged into the car cigarette-lighter (only for this use).

Table 24.32. Bill of Rights of atopic disease prevention

Right to be breast-fed, not formula-fed
Right to live in dust-mite-free homes
Right to be free from pets
Right to be free from passive tobacco smoking
Right to breathe clean, unpolluted air
Right to be screened and advised for allergy prevention by the Ministry of Health

Modified from [482].

Table 24.31 [482] lists the advantages and disadvantages of primary and secondary prevention, Table 24.32 [482] outlines the charter of rights for the prevention of allergic disease, both in children at high risk of atopy.

New Frontiers

One of the crucial dilemmas of the allergy world is the endlessly repeated concept of the sensitization window during early infancy. This concept stems from a vast array of epidemiological studies showing that postnatal exposure to high levels of allergens maximizes the risk for subsequent expression of allergic reactivity to those

allergens in adult life. We are now aware that the fetus immune system (FIS) can be immunocompetent from the 18–20th week of intrauterine life, and that from the 22nd week the FIS is able to initiate responses to IgE antibodies and to a wide spectrum of food and inhalant antigens of maternal origin, thus suggesting an advancement of preventive measures. Discovering Der p 1 in the amniotic fluid and the fetal circulation is direct evidence of transamniotic and transplacental allergen exposure [188]. However, at birth the balance is shifted toward a predominance of Th2 T cells and related cytokines. Regardless of when allergen-specific memory first develops, poorly defined factors appear to be operating early to predispose allergen challenge in the immune system during infancy, so that the early formative period may progress toward expression of long-term Th2-skewed allergen-specific immune memory. Development of clinical allergic disease occurs within months of birth in many infants, but the key question is the timing of first exposure to individual allergens, which is likely to be variable before and after birth, generally occurring when the neonatal immune system is immature and more vulnerable to foreign influences [336]. After the report that nucleic acids are contained in breast milk in a much higher quantity than CM, and that these nucleic acids are capable of inhibiting the Th2-mediated responses, the key etiological factor in atopic disease may not be the acquisition of Th2 hegemony, but the efficiency of immune deviation mechanisms [190]; in the breast milk-fed infant the nucleic acids and their relative components may redirect the FIS responses toward the Th1 phenotype [406].

However, the fetus should be protected. Since the incidence of prenatal sensitization in selected infants (0.49%) (Table 3.2) is minimal, there is no advantage to advising expectant mothers to follow a restrictive diet during the last trimester of pregnancy. Maternal smoking in pregnancy may provoke hypoplasia of fetal lungs and reduces the number of alveoli and/or alter passive respiratory mechanics [402]. Pregnant mothers should refrain from corticosteroid treatments since their effects might be transmitted to the fetus, impairing its skeletal growth and development.

The atopic march increases progressively in infants and children, while it is difficult to compare recent epidemiological studies with data published over the past few years. It can be stated that the prevalence is at least 20%–25% in childhood, and that *atopy now rises to second place among the main pediatric pathologies.* Atopy interferes in the pediatric arena at all ages, albeit at varying levels of severity, and also *differs sharply from the adult forms* in etiopathogenesis, prevention, treatment and prognosis. Most cases of pediatric allergy (90% of cases start during this age) have a transitory course and they often go into remission as the child grows. A touchy point is the constant increase in expenses related to special food, drugs, absence from school, time off work and so on, and this underscores the importance of prophylaxis: prevention is always possible, and at a lower cost. Likewise, in the allergy field there have been many positive factors: the ongoing explosion of discoveries and significant research, on both a scientific and clinical level, is undeniable, and this means great progress. So far, these disease have not been endowed with growing importance – as they rightfully should be – in university courses and postgraduate courses, and as a result, greater information in the daily practice of doctors and pediatricians is not guaranteed. We believe that by dedicating ourselves to the young patient with scientific precision, clinical experience, perseverance and love, we can achieve a great deal in the field of diagnosis, treatment and prevention.

The strategy for preventing allergic disorders generally means intruding in the life of the entire family, often confronting deeply rooted habits or mentalities. The dietary-environmental strategy central to our prevention program, as shown in Table 24.4, is sometimes met with perplexity, not only because of the notable psychological and economic burden that these preventive programs entail [44, 46, 172, 176, 470, 485], but also because of the *little attention paid by consensus.* Instead, the active cooperation shown by several families and the encouraging results achieved only serve to *confirm their appropriateness in children at risk of atopy*.

Perhaps sharing a limited view of the unquestionable results that have been achieved, some people question the benefits of these programs, often demanding and complex. This may depend on the annoyance in adhering to a strict protocol and as a result many families with babies at HR of atopy find participation too demanding [310, 481], so that avoiding allergens may simply involve postponement rather than absolute prevention of allergy development [9]. Instead, we deem that dietary-environmental measures are proposed to these families to modify the natural history of allergic disease, reducing an established risk of genetic disorders, and that the possibility of success is tied to the equal involvement of both parents and family members who live with the child or take care of the child outside the home. On our part, the commitment must involve establishing extended supervision through adolescence, to verify if the preventive effect of allergic manifestations continues over the years and becomes extended to respiratory allergy, as recently documented (Fig. 24.2). Returning to the fatal and nonfatal cases reported in Chap. 20, we must theorize that the pediatricians had no knowledge of the preventive measures in children at risk, which have long been known [44, 393], and that, moreover, none of them was present to save dying children.

Jean-Jacques Rousseau once said, "*L'homme naît bon, c'est la société qui le corrompt*" (human beings are born good, it is society that corrupts them). As a pediatrician, and above all as an immunologist-allergist, I agree with the celebrated philosopher that neonates potentially enjoy good health and that the environment corrupts them. Today's world is so rich in pollution and in less

natural foods, which can predispose children to allergy development, that, if we consider the countless portrayals of the Infant Jesus at Mary's breast, we feel we must repeat that *breast-feeding is a measure of incalculable importance for preventing atopic disease.*

Colarizi observed that "no one stimulates mothers to breast-feed" [90]. He saw nurses entering the neonatal ward with a bottle disguised behind their back, because the babies should not be underweight [90]. EU countries have finally agreed to safeguard breast-feeding and in several countries legislation was passed banning both propaganda and distribution of CM formula during the first few months of life. While it is true that any initiative of this kind must be supported, the fact that these messages are being circulated by platforms of international consensus is also an acknowledgment of the active work done along these lines by pediatricians, as we have stressed in all chapters of this book. Oddly enough, the EU has also given all pediatricians a book praising the virtues of CM but not the immunological properties of breat milk, and yet the WHO and UNICEF recommendations should prevent these forms of advertising as well. In several hospitals in Great Britain, hidden forms of advertising have appeared [332] and in several US states, in Europe and in Italy, free samples of formula or coupons for free formula are mailed directly to the homes of nursing and even expectant mothers, and these mailings are repeated when the infant is 1 month old. The campaign is completed by incisive promotions directly targeting the public [200]. The effect is even more serious, considering the significant percentage of young and inexperienced mothers who are logically unable to distinguish advantages from disadvantages, immunological or nonimmunological [281]. It is easy to foresee that the next step will be the arrival in the hospital of false experts who will directly provide the means "to pluck the nipple from the boneless gums" of the newborn. Nonetheless, the work to inform and convince women to breast-feed usually achieves excellent results if it targets expectant mothers, while the results are poor if the message is instead conveyed by pediatricians after the baby is born [357]. This is proof of an initial temporal integration of specialized teams.

We appreciate to the highest degree today's legislation in force in Europe, which protects the working nursing mother: recent data (the ETAC study) show that 66% of European babies are exclusively breast-fed for 1 month (Fig. 24.16) and 42% for 3 months (Fig. 24.17). Even if it is desirable to extend this protection fully, we should point out that several countries in the van of progress in other social fields neglect this aspect of protecting children, to the point that a social-epidemiological trial has revealed that in one of these countries, only 8% of 2-month-old infants and 2% of 6-month-olds are breast-fed [281]. The results of a wealth of studies emphasizing the manifold advantages of breast-feeding [345, 360] should be viewed as a proof that exclusive breast-feeding in the first months of life, the most crucial period in the natural history of asthma [163, 381], should be seen as a true intervention by public health, since it may reduce the morbidity and prevalence of infantile asthma and allied atopic diseases (Tables 24.2, 24.3), with unquestionable benefits for the quality of life.

"An ounce of prevention is worth a pound of cure." It may also be true that a public television advertising campaign to promote breast-feeding and provide strong educational messaging for breast-feeding to expectant mothers was ultimately "watered down" by private interests (personal communication from Organic Consumers Association). We know that a 10- to 20-week fetus is able to advance sensitization to the first trimester of intrauterine life. If we cannot change the atopic inheritance after birth, can we start early and effective preventive measures before birth? In 1922, new frontiers were carved on the portal of our clinic: *in puero homo*, which means "in the infant is the seed of the future man." This ancient philosophy was revisited by the polyspecialistic

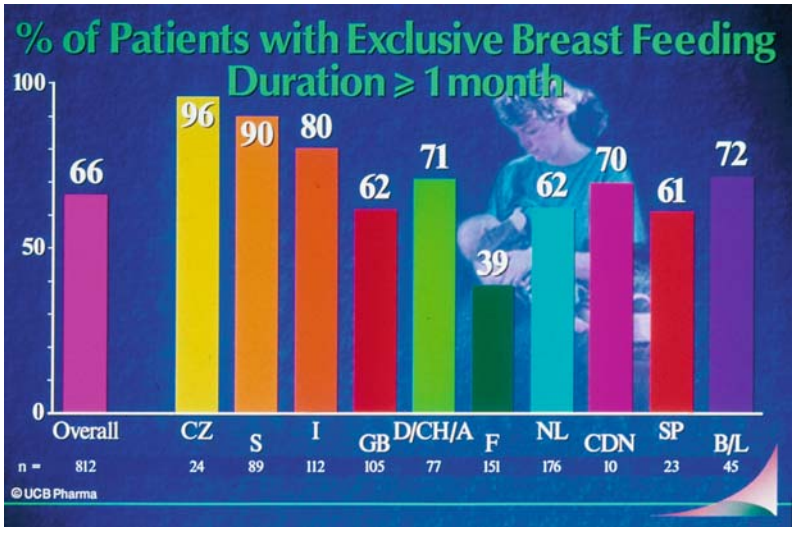

Fig. 24.16. ETAC study group. A mean of 66% of children received exclusive breast-feeding for 1 month or more. The highest frequency was in the Czech Republic (96%), the lowest in France (39%)

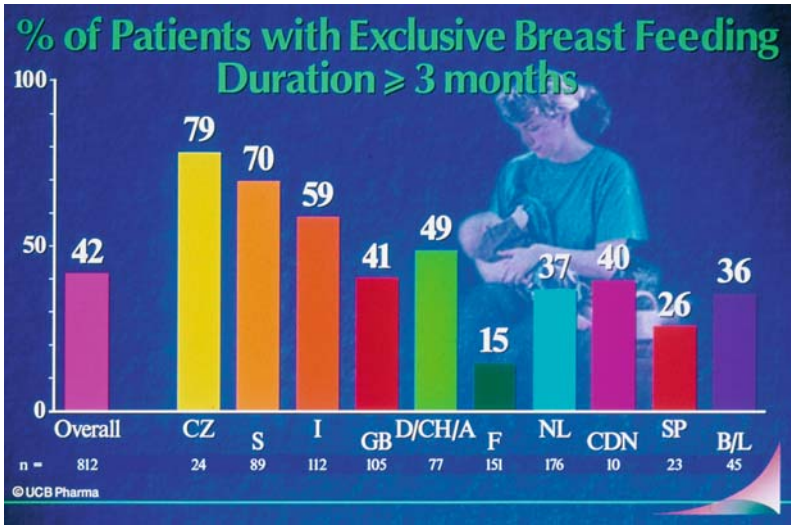

Fig. 24.17. ETAC study group. A mean of 66% of children received an exclusive breast-feeding for 3 months or more. The highest frequency was in the Czech Republic (79%), the lowest in France (15%)

pediatrics of Professor Colarizi. Thus, our goal is not only to reduce morbidity and mortality, but mainly to ensure the best quality of life for both infants and adults, by preventing, diagnosing and curing childhood atopic diseases to avoid their persistence in adults, as Ratner recommended as early as 1951 [341]. If we are unable to modify the genotype by appropriate systems of molecular biology, can we occasion, before birth, successful preventive and defense mechanisms, early and effectively, overall recognized and applied? Such a strategy is given significant impetus by finding specific responses in a domain that can be interpreted as a no-man's land. Mother Nature teaches that in the neonatal immature intestine the first line of defense is made up of a group of specialized factors [393]. It may be that *the first line of defense should be moved forward.* In Vom Kriege (*About the War*) (1832–1834), von Clausewitz (1780–1831) taught that the arrow shot by the bow can be more efficient than a huge gun, and that a company of trained soldiers works better than a haphazard concourse. Therefore, the frontiers of prevention should be moved forward and entrusted to a specialized team of gynecologists, neonatologists, pediatricians and allergists-immunologists. They act one at a time, one doctor for one mother, and should prime a further commitment: atopy prevention. The progress along the frontiers of prevention has been and will continue to be made from individual and collective contributions of these and other disciplines. We hope that the new, bonding relationship will integrate the separate disciplines of gynecology, neonatology, pediatrics and allergy-immunology into a unified intervention directed at moving forward in the prevention of allergic disease, which will ultimately benefit future generations.

Future challenges therefore appear to be centered on the effort to coordinate, through new bonds, the divisions of gynecology, neonatology, pediatrics, allergy and immunology, different as yet, to form a unified partnership, aimed at atopy prophylaxis that will eventually reduce the great demands of atopic disorders in physical, psychological and economic terms. The bonds unifying these disciplines vastly outweigh the ones differentiating them. Much remains to be learned about the mechanisms by which breast-feeding protects against atopy. We are a long way from seeing the complete picture, but the pieces are starting to fit together, and the image is gradually coming into focus. Evidence is already accumulating and we can see what it will look like when it is all put together. It may well be that *the picture is becoming clearer.*

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Appendices

Appendix 1.1. Nomenclature for factors of the HLA system

HLA Class I alleles

HLA-A alleles	HLA specificity
*A*010101*	A1
*A*010102*	A1
*A*0102*	A1
*A*0201*	A2
*A*0103*	–
*A*0104 N*	Null
*A*0105 N*	
*A*0106*	–
*A*0107*	A1
*A*0108*	A1
*A*0109*	–
*A*020101*	–
*A*020102*	–
*A*020103*	–
*A*020104*	–
*A*020105*	–
*A*020106*	–
*A*020107*	–
*A*020108*	–
*A*020109*	–
*A*0202*	A2
*A*0203*	A203
*A*0204*	A2
*A*0205*	A2
*A*0206*	A2
*A*0207*	A2
*A*0208*	A2
*A*0209*	A2
*A*0210*	A210
*A*0211*	A2
*A*0212*	A2
*A*0213*	A2
*A*0214*	A2
*A*0215 N*	A "Null"
*A*0216*	A2
*A*021701*	A2
*A*021702*	A2
*A*0218*	A2
*A*0219*	–
*A*022001*	A2
*A*022002*	A2
*A*0221*	A2
*A*0222*	A2
*A*0223*	–
*A*0224*	A2
*A*0225*	A2
*A*0226*	–
*A*0227*	–
*A*0228*	–
*A*0229*	A2
*A*0230*	–
*A*0231*	–
*A*0232 N*	–
*A*0233*	–
*A*0234*	–
*A*0235*	–
*A*0236*	–
*A*0237*	–
*A*0238*	–
*A*0239*	–
*A*0240*	–
*A*0241*	–
*A*0242*	–
*A*0243 N*	–
*A*0244*	–
*A*0245*	–
*A*0246*	–
*A*0247*	–
*A*0248*	–
*A*0249*	–
*A*0250*	–
*A*0251*	–
*A*0252*	–
*A*0253N*	–
*A*0254*	–
*A*0255*	–
*A*0256*	–
*A*0257*	–
*A*0258*	–
*A*0259*	–
*A*0260*	–
*A*0262*	–
*A*03010101*	A3
*A*03010202N*	A3
*A*030103*	A3
*A*03011*	
*A*0302*	A2
*A*0303 N*	Null
*A*0304*	A3
*A*0305*	A3
*A*0306*	–
*A*0307*	–
*A*0308*	–
*A*0309*	–
*A*0310*	–
*A*110101*	A11
*A*110102*	A11
*A*1102*	A11
*A*1103*	A11
*A*1104*	A11
*A*1105*	A11
*A*1106*	–
*A*1108*	–
*A*1109*	–
*A*1110*	–
*A*1111*	–
*A*1112*	–
*A*1113*	–
*A*1114*	–
*A*2301*	A23(9)
*A*2302*	A23 (100)
*A*2303*	–
*A*2304*	–
*A*2305*	–
*A*2306*	–
*A*2307N*	Null
*A*2308N*	Null
*A*2309*	–
*A*2401*	–
*A*2402101*	
*A*2402102L*	
*A*240202*	
*A*240203*	
*A*240204*	
*A*240301*	

Appendix 1.1. (Continued)

HLA Class I alleles					
HLA-A alleles	**HLA specificity**	*A*2603*	A26(10)	*A*3104*	A31(19)
*A*240302*		*A*2604*	A26(10)	*A*3105*	A31(19)
*A*2404*	A24(9)	*A*2605*	A26(10)	*A*3106*	–
*A*2405*	A24(9)	*A*2606*	A26(10)	*A*3107*	–
*A*2406*	A24(9)	*A*2607*	A26(10)	*A*3108*	–
*A*2407*	A24(9)	*A*2608*	A26(10)	*A*3109*	
*A*2408*	A24(9)	*A*2609*	–	*A*3201*	A32(19)
*A*2409N*	A "Null"	*A*2610*	A10	*A*3202*	A30(19)
*A*2410*	A9	*A*2611N*	Null	*A*3203*	–
*A*2411N*	A "Null"	*A*2612*	–	*A*3204*	–
*A*2413*	A24(9)	*A*2613*	–	*A*3205*	–
*A*2414*	A24(9)	*A*2614*	–	*A*3206*	–
*A*2415*	–	*A*2615*	–	*A*3207*	–
*A*2416*	–	*A*2616*	–	*A*3301*	A33(19)
*A*2417*	–	*A*2617*	–	*A*3302*	
*A*2418*	–	*A*2618*	–	*A*3303*	A33(19)
*A*2419*	–	*A*29010101*	A29(19)	*A*3304*	–
*A*2420*	–	*A*29010102N*		*A*3205*	–
*A*2421*	A9var	*A*290201*		*A*3206*	–
*A*2422*	A9 A24(100)	*A*290202*		*A*3207*	–
*A*2423*	A24(9)	*A*2903*	A29(19)	*A*3301*	A33(19)
*A*2424*	–	*A*2904*	–	*A*3303*	A33(19)
*A*2425*	–	*A*2905*	–	*A*3304*	–
*A*2426*	–	*A*2906*	–	*A*3305*	–
*A*2427*	A24(9)	*A*2907*		*A*3306*	–
*A*2428*	–	*A*2908N*		*A*3307*	–
*A*2429*	–	*A*2909*		*A*3401*	A34(10)
*A*2430*	–	*A*3001*	A30(19)	*A*3402*	A34(10)
*A*2431*	–	*A*3002*	A30(19)	*A*3403*	–
*A*2432*	–	*A*3003*	A30(19)	*A*3404*	–
*A*2433*	–	*A*3004*	A30(19)	*A*3405*	–
*A*2434*	–	*A*3006*	–	*A*3601*	A36
*A*2435*	–	*A*3007*	–	*A*3602*	
*A*2436N*	Null	*A*3008*	–	*A*3603*	
*A*2437*		*A*3009*	–	*A*3604*	
*A*2438*		*A*3010*	–	*A*4301*	A43
*A*2501*	A25(10)	*A*3011*		*A*6601*	A66(10)
*A*2502*	A10	*A*3012*		*A*6602*	A66(10)
*A*2503*	–	*A*310102*	A31(19)	*A*6603*	A10
*A*2504*		*A*31011*		*A*6604*	–
*A*2601*	A26(10)	*A*3102*	–	*A*680101*	A68(28)
*A*2602*	A26(10)	*A*3103*	–	*A*680102*	A68(28)

Appendix 1.1. (Continued)

HLA Class I alleles					
HLA-A alleles	**HLA specificity**	*B*0704*	B7	*B*0816*	
*A*6802*		*B*0705*	B7	*B*1301*	B13
*A*680301*	A28	*B*0706*	B7	*B*1302*	B13
*A*680302*		*B*0707*	B7	*B*1303*	–
*A*6804*	–	*B*0708*	–	*B*1304*	–
*A*6805*	–	*B*0709*	B7	*B*1306*	
*A*6806*	–	*B*0710*	–	*B*1307N*	
*A*6807*	–	*B*0711*	B7	*B*1308*	
*A*6808*	A68(28)	*B*0712*	–	*B*1309*	
*A*6809*	–	*B*0713*	–	*B*1310*	
*A*6810*		*B*0715*	B7	*B*1311*	
*A*6811 N*	Null	*B*0716*	B7 B7short	*B*1401*	B64(14)
*A*6812*	A28 A28 short	*B*0717*	–	*B*1402*	B65(14)
*A*6813*	–	*B*0718*	–	*B*1403*	–
*A*6814*	–	*B*0719*	–	*B*1404*	–
*A*6815*	–	*B*0720*	B7short	*B*1405*	–
*A*6816*	A68(28)	*B*0721*	–	*B*140601*	B14
*A*6817*	–	*B*0722*	–	*B*140602*	B14
*A*6818N*	Null	*B*0723*	–	*B*15010101*	B62(15)
*A*6819*	–	*B*0724*	B7 B7weak	*B*15010102N*	B62(15)
*A*6820*		*B*0725*	–	*B*150102*	
*A*6821*		*B*0726*	–	*B*150103*	
*A*6822*		*B*0727*	–	*B*150104*	
*A*6823*		*B*0728*	–	*B*1502*	B62(15)
*A*6824*		*B*0729*	–	*B*1503*	B72(70)
*A*6901*	A69(28)	*B*0730*	–	*B*1504*	B62(15)
*A*7401*	A74(19)	*B*0731*	–	*B*1505*	B62(15)
*A*7402*	A74(19)	*B*0801*	B8	*B*1506*	B62(15)
*A*7403*	A19	*B*0802*	B8	*B*1507*	B62(15)
*A*7404*	–	*B*0803*	B8	*B*1508*	B62(15)
*A*7405*	–	*B*0804*	–	*B*1509*	B70
*A*7406*		*B*0805*	–	*B*1510*	B7(70)
*A*7407*		*B*0806*	–	*B*151101*	B15
*A*7408*		*B*0807*		*B*151102*	B15
*A*7409*		*B*0808N*		*B*1512*	B76(15)
*A*8001*	A80	*B*0809*		*B*1513*	B77(15)
HLA-B alleles	**HLA specificity**	*B*0810*		*B*1514*	B76(15)
*B*0701*		*B*0811*		*B*1515*	B62(15)
*B*070201*	B7	*B*0812*		*B*1516*	B63(15)
*B*070202*	B7	*B*0813*		*B*15170101*	B63(15)
*B*070203*	B7	*B*0814*		*B*15170102*	B63(15)
*B*0703*	B703	*B*0815*		*B*1518*	B71(70)

Appendix 1.1. (Continued)

HLA Class I alleles					
HLA-B alleles	**HLA specificity**	*B*1561*	–	*B*270505*	B27
*B*1519*	B76(15)	*B*1562*	–	*B*270506*	B27
*B*1520*	B62(15)	*B*1563*	–	*B*27051*	B27
*B*1521*	B75(15)	*B*1564*	–	*B*2706*	B27
*B*1522*	B35	*B*1565*		*B*2707*	B27
*B*1523*	–	*B*1566*		*B*2708*	B2708
*B*1524*	B62(15)	*B*1567*		*B*2709*	B27
*B*1525*	B62(15)	*B*1568*		*B*2710*	B27
*B*1526N*	Null	*B*1569*		*B*2711*	B27
*B*1527*	B62(15)	*B*1570*		*B*2712*	B27
*B*1528*	B15	*B*1571*		*B*2713*	B27
*B*1529*	B15	*B*1572*		*B*2714*	–
*B*1530*	B75(15)	*B*1573*		*B*2715*	–
*B*1531*	B75(15)	*B*1574*		*B*2715*	–
*B*1532*	B62(15)	*B*1575*		*B*2716*	–
*B*1533*	B15	*B*1576*		*B*2717*	B27
*B*1534*	B15	*B*180101*	B18	*B*2718*	–
*B*1535*	B15	*B*180102*	B18	*B*2719*	B27
*B*1536*	–	*B*1802*	B18	*B*2720*	B27
*B*1537*	–	*B*1803*	B18	*B*2721*	–
*B*1538*	–	*B*1804*	–	*B*2722*	B27
*B*1539*	–	*B*1805*	B18	*B*2723*	–
*B*1540*	–	*B*1806*	B18	*B*2724*	–
*B*1542*	–	*B*1807*	–	*B*2725*	–
*B*1543*	–	*B*1808*	–	*B*350101*	B35
*B*1544*	–	*B*1809*	B18	*B*350102*	B35
*B*1545*	B62(15)	*B*1810*	–	*B*3502*	B35
*B*1546*	B72(70)	*B*1811*	–	*B*3503*	B35
*B*1547*	–	*B*1812*	–	*B*3504*	B35
*B*1548*	B62(15)	*B*1813*	–	*B*3505*	B35
*B*1549*	–	*B*1814*	–	*B*3506*	B35
*B*1550*	–	*B*1815*	–	*B*3507*	B35
*B*1551*	B70	*B*1816*	–	*B*3508*	B35
*B*1552*	–	*B*1817 N*	Null	*B*350901*	B35
*B*1553*	–	*B*1818*	–	*B*350902*	–
*B*1554*	–	*B*2701*	B27	*B*3510*	–
*B*1555*	B15 B15Bw6(12)	*B*2702*	B27	*B*3511*	B35
*B*1556*	–	*B*2703*	B27	*B*3512*	B35
*B*1557*	–	*B*2704*	B27	*B*3513*	B35
*B*1558*	B62(15)	*B*270502*	B27	*B*3514*	B35
*B*1559*	B35 B35(13)	*B*270503*	B27	*B*3515*	B35
*B*1560*	–	*B*270504*	B27	*B*3516*	–

Appendix 1.1. (Continued)

HLA Class I alleles					
HLA-B alleles	**HLA specificity**	*B*3806*	–	*B*4005*	B4005
*B*3517*	B35	*B*3807*	–	*B*40060101*	B61(40)
*B*3518*	B35	*B*3808*	–	*B*40060102*	
*B*3519*	B35	*B*3809*	–	*B*4007*	–
*B*3520*	B35	*B*390101*	B3901	*B*4008*	–
*B*3521*	–	*B*390103*	B3901	*B*4009*	B61(40)
*B*3522*	–	*B*390104*	B3901	*B*4010*	B60(40)
*B*3523*	–	*B*39012*		*B*4011*	B40
*B*3524*	–	*B*390201*	B3902	*B*4012*	–
*B*3525*	–	*B*390202*	B3902	*B*4013*	–
*B*3526*	–	*B*3903*	B39(16)	*B*4014*	–
*B*3527*	B35	*B*3904*	B39(16)	*B*4015*	–
*B*3528*	–	*B*3905*	B16	*B*4016*	–
*B*3529*	B35	*B*390601*	B39(16)	*B*4017*	–
*B*3530*	B35	*B*390602*	B39(16)	*B*4018*	B61(40)
*B*3531*	–	*B*3907*	–	*B*4018*	–
*B*3532*	–	*B*3908*	B39(16)	*B*4020*	B61(40)
*B*3533*	–	*B*3909*	B39(16)	*B*4021*	–
*B*3534*	–	*B*3910*	B39(16)	*B*4022N*	Null
*B*3535*	–	*B*3911*	–	*B*4023*	–
*B*3536*	–	*B*3912*	B39(16)	*B*4024*	–
*B*3537*	–	*B*3913*	B39(16)	*B*4025*	–
*B*3538*	–	*B*3914*	–	*B*4026*	B21
*B*3539*	–	*B*3915*	–	*B*4027*	B61(40)
*B*3540 N*	Null	*B*3916*	–	*B*4028*	–
*B*3541*	–	*B*3917*	–	*B*4029*	B61(40)
*B*3542*	–	*B*3918*	–	*B*4030*	–
*B*3543*		*B*3919*	–	*B*4031*	B60(40)
*B*3544*		*B*3920*	–	*B*4032*	–
*B*3545*		*B*3921*	–	*B*4033*	–
*B*3701*	B37	*B*3922*	–	*B*4034*	B60(40)
*B*3702*	–	*B*3923*	B39(16)	*B*4035*	–
*B*3702 N*	Null	*B*3924*	B39(16)	*B*4036*	–
*B*3703*	–	*B*3925 N*	Null	*B*4037*	–
*B*3704*	–	*B*3926*	–	*B*4038*	–
*B*3705*		*B*3927*	–	*B*4039*	–
*B*3801*	B38(16)	*B*400101*	–	*B*4040*	–
*B*380201*		*B*400102*	–	*B*4042*	–
*B*380202*		*B*400103*	–	*B*4043*	–
*B*3803*	B16	*B*4002*	B61(40)	*B*4044*	–
*B*3804*	–	*B*4003*	B61(40)	*B*4045*	–
*B*3805*	B38(16)	*B*4004*	B61(40)	*B*4101*	B41

Appendix 1.1. (Continued)

HLA Class I alleles

HLA-B alleles	HLA specificity				
*B*4102*	B41	*B*4429*	–	*B*5104*	B51(5)
*B*4103*	B41	*B*4430*	–	*B*5105*	B51(5)
*B*4104*	–	*B*4431*	–	*B*5106*	B5
*B*4105*	–	*B*4432*	–	*B*5107*	B51(5)
*B*4106*	–	*B*4433*	–	*B*5108*	B51(5)
*B*4201*	B42	*B*4434*	–	*B*5109*	B51(5)
*B*4202*	B42	*B*4435*	–	*B*5110*	–
*B*4203*	B42	*B*4501*	B45(12)	*B*5111 N*	Null
*B*4204*	B42	*B*4502*	–	*B*5112*	–
*B*4205*	B42	*B*4503*	–	*B*5113*	–
*B*44020101*	B44(12)	*B*4504*	–	*B*5114*	–
*B*44020102*	B44(12)	*B*4505*	–	*B*5115*	–
*B*440202*	–	*B*4506*	–	*B*5116*	B52(5)
*B*440203*	B44(12)	*B*4601*	B46	*B*5117*	B51(5)
*B*440301*	B44(12)	*B*4602*	–	*B*5118*	B51(5)
*B*440302*	B44(12)	*B*47010101*	B47	*B*5119*	–
*B*4404*	B44(12)	*B*47010102*	B47	*B*5120*	–
*B*4405*	B44(12)	*B*4702*	–	*B*5121*	–
*B*4406*	B44(12)	*B*4703*	–	*B*5122*	–
*B*4407*	B44(12)	*B*4704*	–	*B*5123*	–
*B*4408*	B44(12)	*B*4801*	B48	*B*5124*	B51(5)
*B*4409*	B12	*B*4802*	B48	*B*5126*	–
*B*4410*	–	*B*4803*	–	*B*5127 N*	–
*B*4411*	–	*B*4804*	–	*B*5128*	–
*B*4412*	B44(12)	*B*4805*	B48	*B*5129*	–
*B*4413*	B44(12)	*B*4806*	–	*B*5130*	–
*B*4414*	–	*B*4807*	B48	*B*5131*	–
*B*4415*	B12	*B*4901*	B49(21)	*B*5132*	–
*B*4416*	B47	*B*4902*	–	*B*5133*	–
*B*4417*	–	*B*4903*	–	*B*5134*	–
*B*4418*	–	*B*5001*	B50(21)	*B*520101*	–
*B*4419N*	Null	*B*5002*		*B*520102*	–
*B*4420*	–	*B*5003*		*B*520103*	–
*B*4421*	–	*B*5004*	–	*B*520104*	–
*B*4422*	–	*B*510101*	–	*B*5202*	–
*B*4423N*	Null	*B*510102*	–	*B*5203*	–
*B*4424*	–	*B*510103*	–	*B*5204*	–
*B*4425*	–	*B*510104*	–	*B*5205*	–
*B*4426*	–	*B*510105*	–	*B*5301*	B53
*B*4427*	–	*B*510201*	–	*B*5302*	–
*B*4428*	–	*B*510202*	–	*B*5303*	–
		*B*5103*	B5103	*B*5304*	–

Appendix 1.1. (Continued)

HLA Class I alleles					
HLA-B alleles	**HLA specificity**	*B*5802*	B58(17)	*Cw*0207*	
*B*5305*	–	*B*5803*		*Cw*0301*	
*B*5306*	–	*B*5804*	–	*Cw*030201*	Cw3
*B*5307*	–	*B*5805*	–	*Cw*030202*	Cw3
*B*5308*	–	*B*5806*	–	*Cw*030301*	Cw9(w3)
*B*5309*	–	*B*5807*	–	*Cw*030302*	Cw9(w3)
*B*5401*		*B*5901*	B59	*Cw*030303*	Cw9(w3)
*B*5402*		*B*6701O1*	B67	*Cw*030401*	Cw10(w3)
*B*5501*		*B*670102*	B67	*Cw*030402*	Cw10(w3)
*B*5502*		*B*6702*	–	*Cw*0305*	–
*B*5503*	–	*B*7301*	–	*Cw*0306*	–
*B*5504*	B55(22)	*B*7801*		*Cw*0307*	Cw3
*B*5505*	B22	*B*780201*		*Cw*0308*	–
*B*5507*	B54(22)	*B*780202*		*Cw*0309*	–
*B*5508*	–	*B*7803*	–	*Cw*0310*	Cw3
*B*5509*	–	*B*7804*	–	*Cw*0311*	–
*B*5510*	B55(22)	*B*7805*	–	*Cw*0312*	–
*B*5511*	–	*B*7901*		*Cw*0313*	–
*B*5512*	–	*B*8101*	B81	*Cw*0314*	–
*B*5601*	B56(22)	*B*8201*	B81	*Cw*0315*	–
*B*5602*	B56(22)	*B*8202*	B81	*Cw*0316*	–
*B*5603*	B22	*B*8301*	–	*Cw*04010101*	Cw4
*B*5604*	B56(22)	**HLA-C alleles**	**HLA specificity**	*Cw*04010102*	Cw4
*B*5605*	B56(22)	*Cw*0101*		*Cw*040102*	Cw4
*B*5606*	B78	*Cw*0102*	Cw1	*Cw*0402*	–
*B*5607*	B56(22)	*Cw*0103*	Cw1	*Cw*0403*	–
*B*5608*		*Cw*0104*	–	*Cw*0404*	–
*B*5609*		*Cw*0105*	–	*Cw*0405*	–
*B*5610*		*Cw*0106*	–	*Cw*0406*	–
*B*5611*		*Cw*0107*	–	*Cw*0407*	–
*B*570101*	B57(17)	*Cw*0108*	–	*Cw*0408*	–
*B*570102*	–	*Cw*0109*	–	*Cw*0409N*	Null
*B*5702*	B57(17)	*Cw*0201*	–	*Cw*0410*	
*B*570301*	B57(17)	*Cw*020201*	–	*Cw*0411*	
*B*570302*	B57(17)	*Cw*020202*	Cw2	*Cw*0501*	Cw5
*B*5704*	B57(17)	*Cw*020203*	Cw2	*Cw*0502*	Cw5
*B*5705*	–	*Cw*020204*	Cw2	*Cw*0503*	–
*B*5706*	–	*Cw*020205*	Cw2	*Cw*0504*	–
*B*5707*	–	*Cw*0203*	Cw2	*Cw*0505*	–
*B*5708*	–	*Cw*0204*	Cw2	*Cw*0506*	–
*B*5709*		*Cw*0205*	Cw2	*Cw*0601*	
*B*5801*	B58(17)	*Cw*0206*		*Cw*0602*	Cw6

Appendix 1.1. (Continued)

HLA Class I alleles

HLA-C alleles	HLA specificity
*Cw*0603*	Cw6
*Cw*0604*	Cw6
*Cw*0605*	Cw6
*Cw*0606*	–
*Cw*0607*	–
*Cw*0608*	–
*Cw*0609*	–
*Cw*070101*	Cw7
*Cw*070102*	Cw7
*Cw*07020101*	Cw7
*Cw*07020102*	Cw7
*Cw*0703*	Cw7
*Cw*070401*	Cw7
*Cw*070402*	Cw7
*Cw*0705*	–
*Cw*0706*	Cw7
*Cw*0707*	–
*Cw*0708*	–
*Cw*0709*	–
*Cw*0710*	–
*Cw*0711*	–
*Cw*0712*	–
*Cw*0713*	–
*Cw*0714*	Cw7
*Cw*0715*	Cw7
*Cw*0716*	Cw7
*Cw*0717*	
*Cw*0718*	
*Cw*0719*	
*Cw*080101*	Cw8
*Cw*080102*	Cw8
*Cw*0802*	Cw8
*Cw*0803*	Cw8
*Cw*0804*	Cw8
*Cw*0805*	Cw8
*Cw*0806*	Cw8
*Cw*0807*	–
*Cw*0808*	–
*Cw*0809*	–
*Cw*1101*	
*Cw*1201*	
*Cw*120201*	–
*Cw*120202*	–
*Cw*120203*	–
*Cw*120301*	–
*Cw*120302*	–
*Cw*120401*	–
*Cw*120402*	–
*Cw*1205*	–
*Cw*1206*	–
*Cw*1207*	–
*Cw*1208*	–
*Cw*1209*	
*Cw*1301*	
*Cw*1401*	
*Cw*140201*	–
*Cw*140202*	–
*Cw*1403*	–
*Cw*1404*	–
*Cw*1405*	–
*Cw*1501*	
*Cw*150201*	–
*Cw*150202*	–
*Cw*1503*	–
*Cw*1504*	–
*Cw*150501*	–
*Cw*150502*	–
*Cw*1506*	–
*Cw*1507*	–
*Cw*1508*	–
*Cw*1509*	–
*Cw*1510*	–
*Cw*1511*	–
*Cw*1601*	–
*Cw*1602*	–
*Cw*1603*	–
*Cw*160401*	–
*Cw*16042*	
*Cw*1605*	
*Cw*1606*	
*Cw*1701*	–
*Cw*1702*	–
*Cw*1703*	–
*Cw*1801*	–
*Cw*1802*	–

HLA-E alleles	HLA specificity
*E*0101*	–
*E*0102*	–
*E*010301*	–
*E*010302*	–
*E*010303*	–
*E*0104*	–

HLA-F alleles	HLA specificity
*F*0101*	–

HLA-G alleles	HLA specificity
*G*010101*	–
*G*010102*	–
*G*010103*	–
*G*010104*	–
*G*010105*	–
*G*010106*	–
*G*010107*	–
*G*010108*	–
*G*0102*	–
*G*0103*	–
*G*010401*	–
*G*010402*	–
*G*010403*	–
*G*0105N*	–
*G*0106*	–

Appendix 1.1. (Continued)

HLA class II alleles

DRA/DRB alleles	HLA-DR serological specificities	HLA-D associated (T-cell-defined) specificities
*DRA1*0101*		
*DRA1*010201*		
*DRA1*010202*		
*DRB1*010101*	DR1	Dw1
*DRB1*010102*	–	–
*DRB1*010201*	DR1	Dw20
*DRB1*010202*	DR1	Dw20
*DRB1*0103*	DR103	Dw″BON″
*DRB1*0104*	DR1	–
*DRB1*0105*	–	–
*DRB1*0106*	–	–
*DRB1*0107*	–	–
*DRB1*0108*	–	–
*DRB1*0108*		
*DRB1*0109*		
*DRB1*0110*		
*DRB1*030101*	DR17(3)	Dw3
*DRB1*030102*	DR17(3)	Dw3
*DRB1*030201*	DR17(3)	Dw″RSH″
*DRB1*030202*	DR17(3)	Dw″RSH″
*DRB1*0303*	DR18(3)	–
*DRB1*0304*	DR17(3)	–
*DRB1*030501*	DR17(3)	–
*DRB1*030502*	–	–
*DRB1*0306*	DR3	–
*DRB1*0307*	DR3	–
*DRB1*0308*	–	–
*DRB1*0309*	–	–
*DRB1*0310*	DR17(3)	–
*DRB1*0311*	DR17(3)	–
*DRB1*0312*	DR3	–
*DRB1*0313*	–	–
*DRB1*0314*	DR3	–
*DRB1*0315*	–	–
*DRB1*0316*	–	–
*DRB1*0317*	–	–
*DRB1*0318*	–	–
*DRB1*0319*	–	–
*DRB1*0320*	–	–
*DRB1*0321*	–	–
*DRB1*0322*	–	–
*DRB1*0323*	–	–
*DRB1*0324*	–	–
*DRB1*0325*	–	–
*DRB1*040101*	DR4	Dw4
*DRB1*040102*	DR4	Dw4
*DRB1*0402*	DR4	Dw10
*DRB1*030301*	–	–
*DRB1*030302*	–	–
*DRB1*0404*	DR4	Dw14
*DRB1*040501*	DR4	Dw15
*DRB1*040502*	DR4	Dw15
*DRB1*040503*	DR4	–
*DRB1*040504*	–	–
*DRB1*0406*	DR4	Dw″KT2″
*DRB1*040701*	DR4	Dw13
*DRB1*040702*	DR4	–
*DRB1*040703*	–	–
*DRB1*0408*	DR4	Dw14
*DRB1*0409*	DR4	–
*DRB1*0410*	DR4	–
*DRB1*0411*	DR4	–
*DRB1*0412*	DR4	–
*DRB1*0413*	DR4	–
*DRB1*0414*	DR4	–
*DRB1*0415*	DR4	–
*DRB1*0416*	DR4	–
*DRB1*0417*	DR4	–
*DRB1*0418*	–	–
*DRB1*0419*	DR4	–
*DRB1*0420*	DR4	–
*DRB1*0421*	DR4	–
*DRB1*0422*	DR4	–
*DRB1*0423*	DR4	–
*DRB1*0424*	DR4	–
*DRB1*0425*	DR4	–
*DRB1*0426*	DR4	–
*DRB1*0427*	–	–
*DRB1*0428*	DR4	–
*DRB1*0429*	DR4	–
*DRB1*0430*	–	–

Appendix 1.1. (Continued)

HLA class II alleles					
DRA/DRB alleles	**HLA-DR serological specificities**	**HLA-D associated (T-cell-defined) specificities**	*DRB1*0811*	DR8	–
*DRB1*0431*	DR4	–	*DRB1*0812*	DR8	–
*DRB1*0432*	DR4	–	*DRB1*0813*	–	–
*DRB1*0433*	–	–	*DRB1*0814*	DR8	–
*DRB1*0434*	–	–	*DRB1*0815*	–	–
*DRB1*0435*	–	–	*DRB1*0816*	DR8	–
*DRB1*0436*	–	–	*DRB1*0817*	DR8	–
*DRB1*0437*	–	–	*DRB1*0818*	–	–
*DRB1*0438*	–	–	*DRB1*0819*	–	–
*DRB1*0439*	–	–	*DRB1*0820*	–	–
*DRB1*0440*	–	–	*DRB1*0821*	–	–
*DRB1*0441*	–	–	*DRB1*0822*	–	–
*DRB1*0442*	DR4	–	*DRB1*0823*	–	–
*DRB1*0443*	–	–	*DRB1*0824*	–	–
*DRB1*0444*	–	–	*DRB1*090102*	DR9	Dw23
*DRB1*070101*	DR7	Dw17	*DRB1*090111*	–	–
*DRB1*070102*	DR7	–	*DRB1*0902*	–	–
*DRB1*0702*	–	–	*DRB1*100101*	DR10	–
*DRB1*0703*	DR7	–	*DRB1*100102*	DR10	–
*DRB1*0704*	DR7	–	*DRB1*110101*	DR11(5)	Dw5
*DRB1*0705*	–	–	*DRB1*110102*	DR11(5)	Dw5
*DRB1*0706*	DR7	–	*DRB1*110103*	DR11(5)	Dw5
*DRB1*0707*	–	–	*DRB1*110104*	DR11(5)	–
*DRB1*080101*	DR8	Dw8.1	*DRB1*1102*	DR11(5)	Dw"JVM"
*DRB1*080102*	–	–	*DRB1*1103*	DR11(5)	
*DRB1*080201*	DR8	Dw8.2	*DRB1*110401*	DR11(5)	
*DRB1*080202*	DR8	Dw8.2	*DRB1*110402*	DR11(5)	
*DRB1*080203*	DR8		*DRB1*1105*	DR11(5)	–
*DRB1*080302*	DR8	Dw8.3	*DRB1*110601*	DR11(5)	–
*DRB1*08031*	–	0	*DRB1*110602*	–	–
*DRB1*080401*	DR8	–	*DRB1*1107*	–	–
*DRB1*080402*	DR8	–	*DRB1*110801*	DR11(5)	
*DRB1*080403*	DR8	–	*DRB1*110802*	DR11(5)	
*DRB1*080404*	DR8	–	*DRB1*1109*	DR11(5)	–
*DRB1*0805*	DR8	–	*DRB1*1110*	DR11(5)	–
*DRB1*0806*	DR8	–	*DRB1*1111*	DR11(5)	–
*DRB1*0807*	DR8	–	*DRB1*111201*	–	–
*DRB1*0808*	DR8	–	*DRB1*111202*	DR11(5)	–
*DRB1*0809*	DR8	–	*DRB1*1113*	DR11(5)	–
*DRB1*0810*	DR8	–	*DRB1*1114*	DR11(5)	–
			*DRB1*1115*	–	–
			*DRB1*1116*	DR11(5)	–

Appendix 1.1. (Continued)

HLA class II alleles		
DRA/DRB alleles	**HLA-DR serological specificities**	**HLA-D associated (T-cell-defined) specificities**
*DRB1*1117*	–	–
*DRB1*1118*	–	–
*DRB1*1119*	DR11(5)	–
*DRB1*1120*	DR11(5)	–
*DRB1*1121*	DR11(5)	–
*DRB1*1122*	–	–
*DRB1*1123*	DR11(5)	–
*DRB1*1124*	–	–
*DRB1*1125*	DR11(5)	–
*DRB1*1126*	DR11(5)	–
*DRB1*112701*	DR11(5)	–
*DRB1*112702*	DR11(5)	–
*DRB1*1128*	–	–
*DRB1*1129*	DR11(5)	–
*DRB1*1130*	–	–
*DRB1*1131*	–	–
*DRB1*1132*	–	–
*DRB1*1133*	–	–
*DRB1*1134*	–	–
*DRB1*1135*	–	–
*DRB1*1136*	–	–
*DRB1*1137*	–	–
*DRB1*1138*	–	–
*DRB1*1139*	–	–
*DRB1*1140*	–	–
*DRB1*1141*	–	–
*DRB1*1142*	–	–
*DRB1*1143*	–	–
*DRB1*120101*	DR12(5)	–
*DRB1*120102*	DR12(5)	–
*DRB1*120201*	DR12(5)	–
*DRB1*120202*	DR12(5)	–
*DRB1*120302*	DR12(5)	–
*DRB1*12031*	–	–
*DRB1*1204*	DR5	–
*DRB1*1205*	DR12(5)	–
*DRB1*1206*	DR12(5)	–
*DRB1*1207*	–	–
*DRB1*1208*	DR12(5)	–
*DRB1*130101*	DR13(6)	Dw18, DRw6a
*DRB1*130102*	DR13(6)	–
*DRB1*130103*	DR13(6)	–
*DRB1*130201*	DR13(6)	Dw19
*DRB1*130202*	DR13(6)	–
*DRB1*130301*	DR13(6)	Dw"HAG"
*DRB1*130302*	DR13(6)	Dw"HAG"
*DRB1*1304*	DR13(6)	–
*DRB1*1305*	DR13(6)	Dw 6"PEV"
*DRB1*1306*	DR13(6)	–
*DRB1*130701*	DR13(6)	–
*DRB1*130702*	DR13(6)	–
*DRB1*1308*	DR13(6)	–
*DRB1*1309*	–	–
*DRB1*1310*	DR13(6)	–
*DRB1*1311*	DR13(6)	–
*DRB1*1312*	–	–
*DRB1*1313*	–	–
*DRB1*131401*	DR13(6)	
*DRB1*131402*	DR13(6)	
*DRB1*1315*	–	–
*DRB1*1316*	DR13(6)	–
*DRB1*1317*	DR13(6)	–
*DRB1*1318*	DR13(6)	–
*DRB1*1319*	DR13(6)	–
*DRB1*1320*	DR13(6)	–
*DRB1*1321*	–	–
*DRB1*1322*	DR13(6)	–
*DRB1*1323*	–	–
*DRB1*1324*	–	–
*DRB1*1325*	–	–
*DRB1*1326*	–	–
*DRB1*1327*	DR13(6)	–
*DRB1*1328*	–	–
*DRB1*1329*	DR6	–
*DRB1*1330*	–	–
*DRB1*1331*	–	–
*DRB1*1332*	–	–
*DRB1*1333*	–	–
*DRB1*1334*	–	–
*DRB1*1335*	–	–
*DRB1*1336*	DR13(6)	–

Appendix 1.1. (Continued)

HLA class II alleles					
DRA/DRB alleles	**HLA-DR serological specificities**	**HLA-D associated (T-cell-defined) specificities**	*DRB1*1418*	DR6	–
*DRB1*1337*	–	–	*DRB1*1419*	DR14(6)	–
*DRB1*1338*	–	–	*DRB1*1420*	DR14(6)	–
*DRB1*1339*	–	–	*DRB1*1421*	DR14(6)	–
*DRB1*1340*	–	–	*DRB1*1422*	DR14(6)	–
*DRB1*1341*	–	–	*DRB1*1423*	–	–
*DRB1*1342*	DR13(6)	–	*DRB1*1424*	–	–
*DRB1*1343*	–	–	*DRB1*1425*	–	–
*DRB1*1344*	–	–	*DRB1*1426*	DR14(6)	–
*DRB1*1345*	–	–	*DRB1*1427*	DR14(6)	–
*DRB1*1346*	–	–	*DRB1*1428*	–	–
*DRB1*1347*	–	–	*DRB1*1429*	DR14(6)	–
*DRB1*1348*	–	–	*DRB1*1430*	–	–
*DRB1*1349*	–	–	*DRB1*1431*	–	–
*DRB1*1350*	–	–	*DRB1*1432*	–	–
*DRB1*1351*	–	–	*DRB1*1433*	–	–
*DRB1*1352*	–	–	*DRB1*1434*	–	–
*DRB1*1353*	–	–	*DRB1*1435*	–	–
*DRB1*1354*	–	–	*DRB1*1436*	–	–
*DRB1*1355*	–	–	*DRB1*1437*	–	–
*DRB1*140101*	DR14(6)	Dw9	*DRB1*1438*	–	–
*DRB1*140102*			*DRB1*1439*	–	–
*DRB1*1402*	DR14(6)	Dw16	*DRB1*1440*	–	–
*DRB1*1403*	DR1403	–	*DRB1*1441*	–	–
*DRB1*1404*	DR1404	–	*DRB1*1442*	–	–
*DRB1*140501*	DR14(6)	–	*DRB1*1443*	–	–
*DRB1*140502*	–	–	*DRB1*1444*	–	–
*DRB1*1406*	DR14(6)	–	*DRB1*1445*	–	–
*DRB1*140701*	DR14(6)	–	*DRB1*150101*	DR15(2)	–
*DRB1*140702*	DR14(6)	–	*DRB1*150102*	DR15(2)	–
*DRB1*1408*	DR14(6)	–	*DRB1*150103*	–	–
*DRB1*1409*	–	–	*DRB1*150104*	–	–
*DRB1*1410*	DR14(6)	–	*DRB1*150201*	DR15(2)	–
*DRB1*1411*	DR14(6)	–	*DRB1*150202*	DR15(2)	–
*DRB1*1412*	DR14(6)	–	*DRB1*150203*	DR15(2)	–
*DRB1*1413*	DR14(6)	–	*DRB1*1503*	DR15(2)	–
*DRB1*1414*	DR14(6)	–	*DRB1*1504*	DR15(2)	–
*DRB1*1415*	DR8	–	*DRB1*1505*	DR15(2)	–
*DRB1*1416*	DR6	–	*DRB1*1506*	DR15(2)	–
*DRB1*1417*	DR6	–	*DRB1*1507*	DR15(2)	–
			*DRB1*1508*	DR2	–
			*DRB1*1509*	–	–

Appendix 1.1. (Continued)

HLA class II alleles

DRA/DRB alleles	HLA-DR serological specificities	HLA-D associated (T-cell-defined) specificities
*DRB1*1510*	–	–
*DRB1*1511*	–	–
*DRB1*1512*	–	–
*DRB1*1513*	–	–
*DRB1*160101*	DR16(2)	Dw21
*DRB1*160102*	DR16(2)	Dw21
*DRB1*160201*	DR16(2)	Dw22
*DRB1*160202*	DR16(2)	Dw22
*DRB1*1603*	DR2	–
*DRB1*1604*	DR16(2)	–
*DRB1*1605*	DR16(2)	–
*DRB1*1607*	–	–
*DRB1*1608*	–	–
*DRB2*0101*		
*DRB3*010101*	DR52	Dw24
*DRB3*01010201*	DR52	–
*DRB3*01010202*	–	–
*DRB3*01012*	–	–
*DRB3*0101202*	DR52	–
*DRB3*010103*	DR52	–
*DRB3*010104*	DR52	–
*DRB3*0102*	DR52	–
*DRB3*0103*	–	–
*DRB3*0104*	–	–
*DRB3*0105*	–	–
*DRB3*0106*	DR52	–
*DRB3*0107*	DR52	–
*DRB3*0108*	–	–
*DRB3*0109*	–	–
*DRB3*0110*	–	–
*DRB3*0201*	DR52	Dw25
*DRB3*020201*	DR52	Dw25
*DRB3*020202*	DR52	–
*DRB3*020203*	DR52	–
*DRB3*020204*	DR52	–
*DRB3*0203*	DR52	–
*DRB3*0204*	–	–
*DRB3*0205*	–	–
*DRB3*0206*	–	–
*DRB3*0207*	DR52	–
*DRB3*0208*	DR52	–
*DRB3*0209*	DR52	–
*DRB3*0210*	DR52	–
*DRB3*0211*	DR52	–
*DRB3*0212*	–	–
*DRB3*0213*	–	–
*DRB3*0214*	–	–
*DRB3*0215*	–	–
*DRB3*0216*	–	–
*DRB3*0217*	–	–
*DRB3*030101*	DR52	Dw26
*DRB3*030102*	DR52	Dw26
*DRB3*0302*	DR52	–
*DRB3*0303*	DR52	–
*DRB4*010101*	–	–
*DRB4*010101102N*	DR53	–
*DRB4*0102*	DR53	–
*DRB4*01030101*	DR53	–
*DRB4*01030102N*	Null	–
*DRB4*010302*	–	–
*DRB4*010303*	–	–
*DRB4*010304*	–	–
*DRB4*0104*	–	–
*DRB4*0105*	DR53	–
*DRB4*0106*	–	–
*DRB4*0201N*	Null	–
*DRB4*0301N*	Null	–
*DRB5*010101*	DR51	Dw2
*DRB5*010102*	DR51	Dw12
*DRB5*0102*	DR51	Dw12
*DRB5*0103*	–	–
*DRB5*0104*	–	–
*DRB5*0105*	–	–
*DRB5*0106*	–	–
*DRB5*0107*	DR51	–
*DRB5*0108 N*	Null	–
*DRB5*0109*	–	–
*DRB5*0110 N*	Null	–
*DRB5*0111*	–	–
*DRB5*0112*	–	–
*DRB5*0201*	–	–

Appendix 1.1. (Continued)

HLA class II alleles

DRA/DRB alleles	HLA-DR serological specificities	HLA-D associated (T-cell-defined) specificities
*DRB5*0202*	DR51	Dw22
*DRB5*0203*	DR51	–
*DRB5*0204*	–	–
*DRB5*0205*	–	–
*DRB6*0101*	–	–
*DRB6*0201*	–	–
*DRB6*0202*	–	–
*DRB7*010101*	–	–
*DRB7*010102*	–	–
*DRB8*0101*	–	–
*DRB9*0101*	–	–
DQA/DQB/DOA alleles	**HLA-DQ serological specificities**	**HLA-D associated (T-cell-defined) specificities**
*DQA1*010101*	–	Dw1
*DQA1*010102*	–	–
*DQA1*010201*	–	Dw2, w21, w8
*DQA1*010202*	–	Dw21
*DQA1*0103*	–	Dw18, w12, w8
*DQA1*010401*	–	Dw9
*DQA1*010402*	–	Dw9
*DQA1*0105*	–	
*DQA1*0106*	–	
*DQA1*0201*	–	Dw7, w11
*DQA1*030101*	–	Dw4, w10, w13
*DQA1*03012*	–	–
*DQA1*0302*	–	Dw23
*DQA1*0303*	–	–
*DQA1*040101*	–	Dw8, Dw"RSH"
*DQA1*040102*	–	–
*DQA1*0402*	–	–
*DQA1*050101*	–	Dw3, w5, w22
*DQA1*050102*	–	Dw5
*DQA1*05013*	–	–
*DQA1*0502*	–	–
*DQAI*0503*	–	Dw16
*DQAI*0504*	–	–
*DQAI*0605*	–	Dw5, Dw22
*DQAI*060101*	–	Dw8
*DQAI*060102*	–	–
*DQB1*0201*	DQ2	Dw3
*DQB1*0202*	DQ2	Dw7
*DQB1*0203*	DQ2	–
*DQB1*030101*	DQ7(3)	Dw4, w5, w8, w13
*DQB1*030102*	DQ7 (3)	–
*DQB1*0302*	DQ8(3)	Dw4, w10, w13
*DQB1*030302*	DQ9(3)	Dw23, w11
*DQB1*030303*	DQ9 (3)	–
*DQB1*0304*	DQ7(3)	–
*DQB1*030501*	DQ8(3)	–
*DQB1*030502*	–	–
*DQB1*0306*	DQ3	–
*DQB1*0307*	–	–
*DQB1*0308*	–	–
*DQB1*0309*	–	–
*DQB1*0310*	DQ8(3)	–
*DQB1*0311*	–	–
*DQB1*0312*	–	–
*DQB1*0313*	–	–
*DQB1*0401*	DQ4	Dw15
*DQB1*0402*	DQ4	Dw8, Dw"RSH"
*DQB1*050101*	DQ5(1)	Dw1
*DQB1*050102*	DQ5(1)	–
*DQB1*050201*	DQ5(1)	Dw21
*DQB1*050202*	–	–
*DQB1*050301*	DQ5(1)	–
*DQB1*050302*	DQ5(1)	–
*DQB1*0504*	DQ5(1)	–
*DQB1*060101*	DQ6(1)	Dw12, w8
*DQB1*060102*	DQ6(1)	Dw12, w8
*DQB1*060103*	DQ6(1)	–
*DQB1*0602*	DQ6(1)	Dw2
*DQB1*0603*	DQ6(1)	Dw18, Dw"FS"
*DQB1*060401*	DQ6(1)	Dw19
*DQB1*060402*	DQ6(1)	–
*DQB1*060501*	DQ6(1)	Dw19
*DQB1*060502*	DQ6(1)	Dw19
*DQB1*0606*	–	–
*DQB1*0607*	–	–
*DQB1*0608*	DQ6(1)	–
*DQB1*0609*	DQ6(1)	–

Appendix 1.1. (Continued)

HLA class II alleles				
DQA/DQB/DOA alleles	**HLA-DQ serological specificities**	**HLA-D associated (T-cell-defined) specificities**	*DPB1*020104*	DPw2
			*DPB1*020105*	DPw2
			*DPB1*020106*	DPw2
*DQB1*0610*	–	–	*DPB1*02011*	–
*DQB1*061101*	DQ1	–	*DPB1*0202*	DPw2
*DQB1*061102*	DQ1	–	*DPB1*030101*	DPw3
*DQB1*0612*	DQ1	–	*DPB1*030102*	DPw3
*DQB1*0613*	–	–	*DPB1*0401*	DPw4
*DQB1*0614*	DQ6(1)	–	*DPB1*0402*	DPw4
*DQB1*0615*	–	–	*DPB1*0501*	DPw5
*DQB1*0616*	–	–	*DPB1*0601*	DPw6
*DQB1*0617*	–	–	*DPB1*0801*	–
*DQB1*0618*	–	–	*DPB1*0901*	–
*DQB1*0619*	–	–	*DPB1*1001*	–
*DQB1*0620*	–	–	*DPB1*1101*	–
DPA1/DPB1 alleles	**Associated HLA-DP specificities**		*DPB1*11011*	–
*DPAI*010301*	DPw4		*DPB1*11012*	–
*DPAI*010302*	–		*DPB1*1301*	–
*DPAI*0103*	–		*DPB1*1401*	–
*DPA1*0104*	–		*DPB1*1501*	–
*DPA1*0105*	–		*DPB1*1601*	–
*DPA1*0106*	–		*DPB1*1701*	–
*DPA1*0107*	–		*DPB1*1801*	–
*DPA1*0108*	–		*DPB1*1901*	–
*DPAI*020101*	–		*DPB1*200101*	–
*DPAI*020102*	–		*DPB1*200102*	–
*DPAI*020103*	–		*DPB1*2101*	–
*DPAI*020104*	–		*DPB1*2201*	–
*DPAI*020105*	–		*DPB1*2301*	–
*DPAI*020106*	–		*DPB1*2401*	–
*DPA1*020201*	–		*DPB1*2501*	–
*DPA1*020202*	–		*DPB1*260101*	–
*DPA1*020203*	–		*DPB1*260102*	–
*DPA1*0202*	–		*DPB1*2701*	–
*DPAI*0203*			*DPB1*2801*	–
*DPA1*0301*	–		*DPB1*2901*	–
*DPA1*0302*	–		*DPB1*3001*	–
*DPA1*0401*	–		*DPB1*3101*	–
*DPB1*010101*	DPw1		*DPB1*3201*	–
*DPB1*010102*	DPw1		*DPB1*3301*	–
*DPB1*020102*	DPw2		*DPB1*3401*	–
*DPB1*020103*	DPw2		*DPB1*3501*	–

Appendix 1.1. (Continued)

HLA class II alleles

DPA1/DPB1 alleles	Associated HLA-DP specificities
*DPB1*3601*	–
*DPB1*3701*	–
*DPB1*3801*	–
*DPB1*3901*	–
*DPB1*4001*	–
*DPB1*4101*	–
*DPB1*4201*	–
*DPB1*4301*	–
*DPB1*4401*	–
*DPB1*4501*	–
*DPB1*4601*	–
*DPB1*4701*	–
*DPB1*4801*	–
*DPB1*4901*	–
*DPB1*5001*	–
*DPB1*5101*	–
*DPB1*5201*	–
*DPB1*5301*	–
*DPB1*5401*	–
*DPB1*5501*	–
*DPB1*5601*	–
*DPB1*5701*	–
*DPB1*5801*	–
*DPB1*5901*	–
*DPB1*6001*	–
*DPB1*6101 N*	Null
*DPB1*6201*	–
*DPB1*6301*	–
*DPB1*6401 N*	Null
*DPB1*6501*	–
*DPB1*6601*	–
*DPB1*6701*	–
*DPB1*6801*	–
*DPB1*6901*	–
*DPB1*7001*	–
*DPB1*7101*	–
*DPB1*7201*	–
*DPB1*7301*	–
*DPB1*7401*	–
*DPB1*7501*	–
*DPB1*7601*	–
*DPB1*7701*	–
*DPB1*7801*	–
*DPB1*7901*	–
*DPB1*8001*	–
*DPB1*8101*	–
*DPB1*8201*	–
*DPB1*8301*	–
*DPB1*8401*	–
*DPB1*8501*	–
*DPB1*8601*	–
*DPB1*8701*	–
*DPB1*8801*	–
*DPB1*8901*	–
*DPB1*9001*	–
*DPB1*9101*	–
*DPB1*9201*	–
*DPB1*9301*	–
*DPB1*9401*	–
*DPB1*9501*	–
*DPB1*9601*	–
DOA–DOB/DMA/DMB alleles	
*DOA*01011*	–
*DOA*0101201*	–
*DOA*0101202*	–
*DOA*0101203*	–
*DOA*01013*	–
*DOA*0101401*	–
*DOA*0101402*	–
*DOA*01015*	–
*DOB*0103*	–
*DOB*01012*	–
*DOB*01021*	–
*DOB*01022*	–
*DOB*010110*	–
*DOB*0101101*	–
*DOB*0104101*	–
*DOB*0104102*	–

DMA/TAP/MICA	HLA alleles
*DMA*0101*	
*DMA*0102*	
*DMA*0103*	
*DMA*0104*	
*DMB*0101*	
*DMB*0102*	
*DMB*0103*	
*DMB*0104*	
*DMB*0105*	
*DMB*0106*	
*MICA*001*	
*MICA*00201*	
*MICA*00202*	
*MICA*004*	
*MICA*005*	
*MICA*006*	
*MICA*00701*	
*MICA*00702*	
*MICA*00801*	
*MICA*00802*	
*MICA*00803*	
*MICA*00901*	
*MICA*00902*	
*MICA*010*	
*MICA*011*	
*MICA*01201*	
*MICA*01202*	
*MICA*013*	
*MICA*014*	
*MICA*015*	
*MICA*016*	
*MICA*017*	
*MICA*018*	
*MICA*019*	
*MICA*020*	
*MICA*021*	
*MICA*022*	
*MICA*023*	
*MICA*024*	

Appendix 1.1. (Continued)

HLA class II alleles		
*MICA*025*	*MICA*037*	*MICA*049*
*MICA*026*	*MICA*038*	*TAP1*0101*
*MICA*027*	*MICA*039*	*TAP1*0102 N*
*MICA*028*	*MICA*040*	*TAP1*0301*
*MICA*029*	*MICA*041*	*TAP1*0401*
*MICA*030*	*MICA*042*	*TAP1*02011*
*MICA*031*	*MICA*043*	*TAP1*02012*
*MICA*032*	*MICA*044*	*TAP2*0101*
*MICA*033*	*MICA*045*	*TAP2*0102*
*MICA*034*	*MICA*046*	*TAP2*0103*
*MICA*035*	*MICA*047*	*TAP2*0201*
*MICA*036*	*MICA*048*	

Some not sufficiently defined specificities are indicated with a "w" (workshop).
In several studies previous definitions may be found, which are replaced by the new factor, for example, B27=B*2708, DR1=DR103, DR2=DR15, DR16, DR3=DR17, DR18, DR5=DR11, DR12, DR6=DR13, DR 14, DR1403, DR 1404.
Data from WHO Nomenclature Committee for Factors of the HLA System (January 2004) [640].

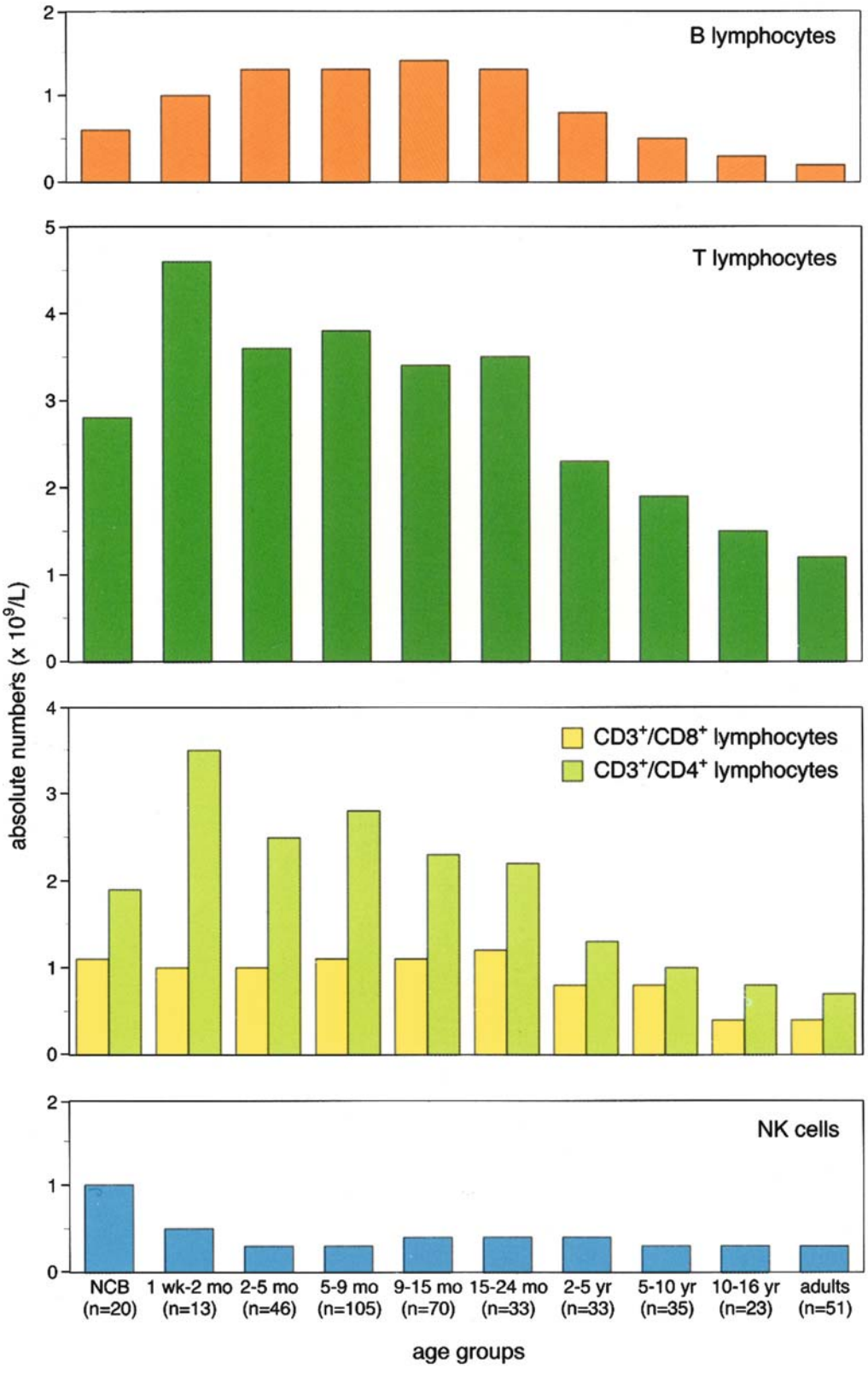
Appendix 1.2. Receptors and surface molecules expressed by eosinophils, basophils and mast cells

	CD	Eosinophils	Basophils	Mast cells
Cytokine receptor				
Il-1RI	121a	–	–	–
IL-1RII	121b	–	+	–
IL-2Rα	25	±	+	–
IL-2Rβ	122	–	–	–
IL-3R	123	+	+	–
IL-4R	124	+	+	±
IL-5R	125	+	+	
IL-6Rα	126	–	–	–
IL-7R	127	–	–	–
IL-8R	128	+	+	
IL-10R	NA		–	–
M-CSFR	115	–	–	–
GM-CSFR	116	+	±	–
G-CSFR	NA	–	–	–
IFN-α/βR	118		+	
IFN-γR	119	–	+	
TGF-βR	105	–	–	–
TNF-RI	120a	–	–	–
TNF-RII	120b	–	–	–
NGF-R (homologous)	40	–	±	–
SCF-R	117	–	±	+

Appendix 1.2. (Continued)

	CD	Eosinophils	Basophils	Mast cells
Surface antigens associated with complement				
CR1	35	+	+	–
CR2/C3dR	21	–	–	–
CR3/C3biR	11b/18	+	+	–
CR4	11c/18	+	+	–
C5aR	88	+	+	–
MCP	46	+	+	+
DAF	55	+	+	+
MACIF	59	+	+	+
Integrins				
β1, Common	29	+	+	+
β1, VLA-2α	49b	–	–	–
β1, VLA-4α	49d	+	+	+
β1, VLA-6α	49f	+	–	–
β2, Common	18	+	+	–
β2, LFA-1α	11a	+	+	–
β2, C3biR	11b	+	+	–
β3, Common	61	–	–	+
β3, IIbIIIa	41	–	–	
β3, VNRα	51	–	–	+
Receptors				
	54	+	+	
	102	–	+	+
	50	–	+	+
	103	–	–	–
	106	–	–	–
	31	+	+	–
	62	–	–	–
	11a/18	+	+	–
	58	+	+	+
	43	+	+	+
	15	+	–	–
	44	+	+	+

Data from [606].
NA not assigned CD; blank boxes correspond to not tested.



Appendix 1.3. Graphic visualization of data reported in Table 1.34
Histogram showing the median absolute values of the main lymphocyte subpopulation in neonates, children and adults, with the pertinent age limits. *NCB* cord blood, *yr* years, *mo* months, *wk* weeks.
Courtesy of Dr. Comans-Bitter [93].

Appendix 1.4. Interactions of hormones with lymphocytes

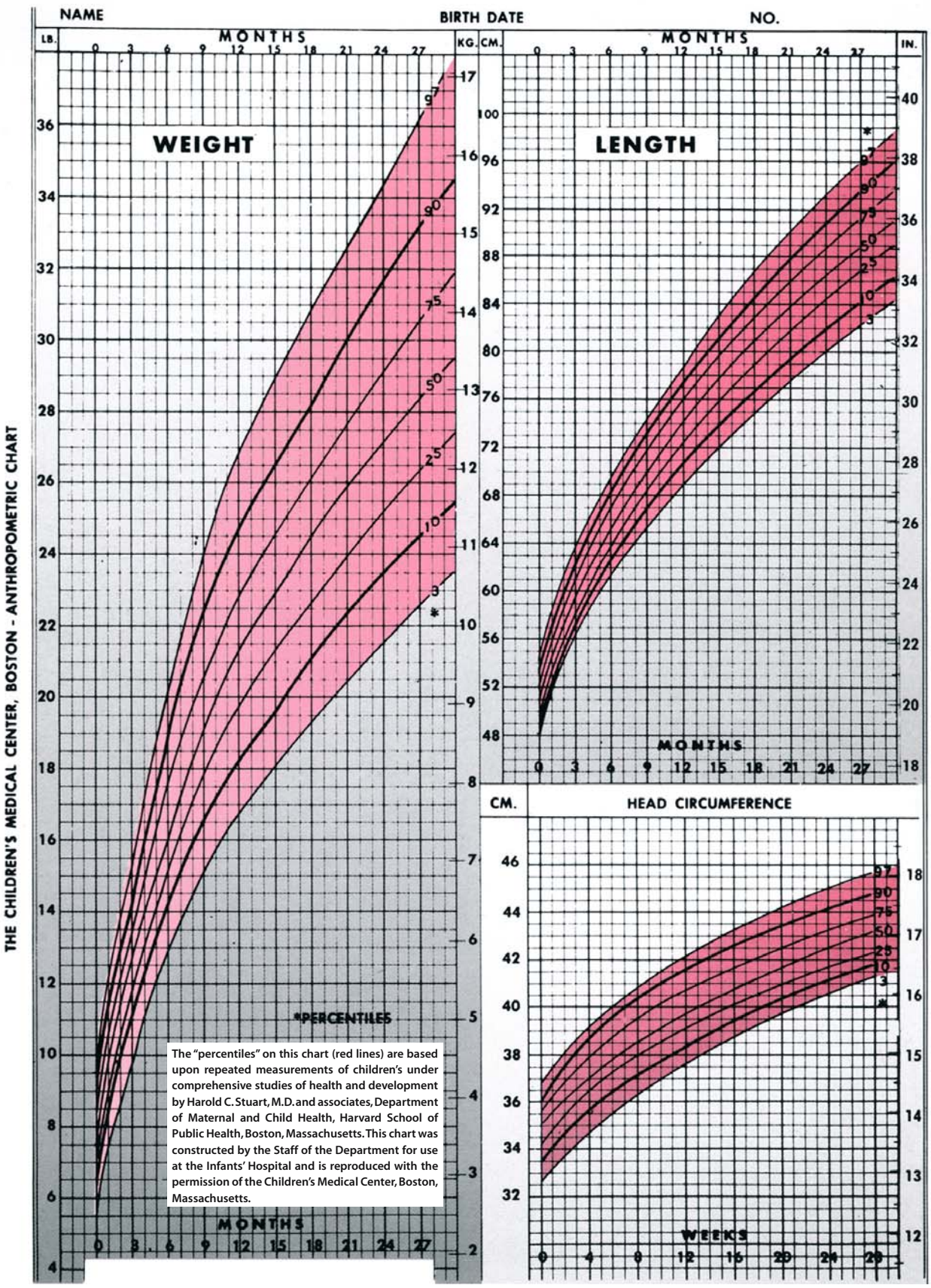
Hormone	Production	Targets	Principal effects
Glucocorticoids	Adrenal cortex	B and T cells	↓ Antibody production, NK activity and cytokine production
ACTH	Pituitary-activated lymphocytes	B and T cells	Glucocorticoid induction, variable effects On: antibody production, cytokine production and proliferation
Enkephalins	Pituitary	B and T cells	Low dose: T cell activation
			High dose: suppression
β-Endorphin	Pituitary	B and T cells	Variable effects: usually suppresses cell activation and antibody synthesis
Melatonin	Pituitary	T cells, thymocytes	↑ Antibody synthesis, cell proliferation
Catecholamines	Adrenal medulla	B and T cells	↑ T cell mobilization, accelerated immune responses sympathetic nerves
Thyroxine	Thyroid	Lymphocytes	↑ T cell activation, plaque-forming cells
Prolactin	Pituitary	B and T cells, macrophages	↑ Macrophage activation ↑ IL2 production
GH	Pituitary	Mononuclear cells	Macrophage activation ↑ Antibody synthesis
Vasopressin and oxytocin		Pituitary	T cells ↑ Proliferation

Modified from [337].

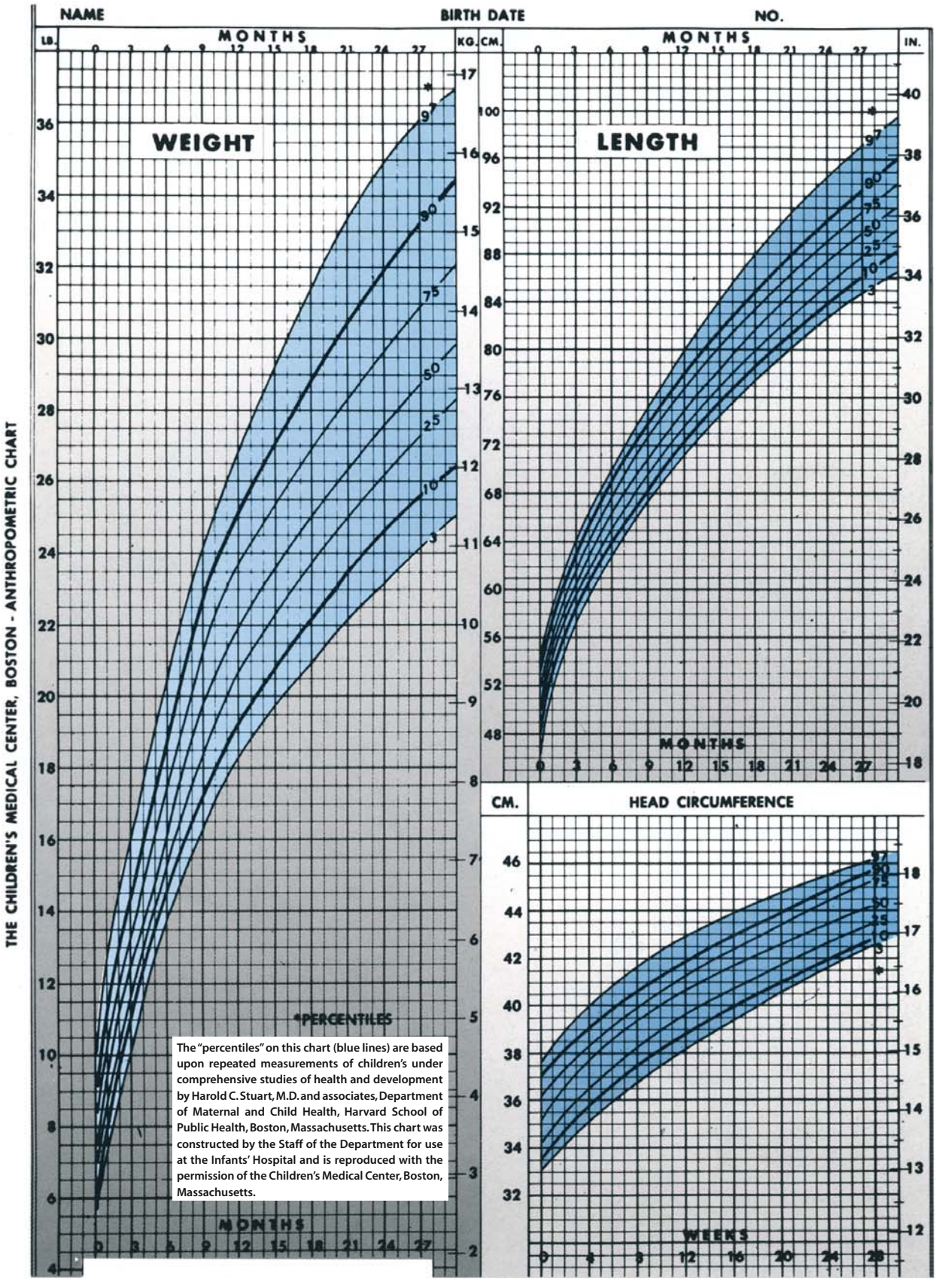
Appendix 4.1. Threshold levels per cubic meter of the main environmental pollutants

	CO	NO_2	O_3	PM_{10}[a]	TSP[a]	SO_2[a]
Alert	15 mg	200 µg	180 µg	50 µg	90 µg	125 µg
Warning	30 mg	400 µg	360 µg	100 µg	180 µg	150 µg

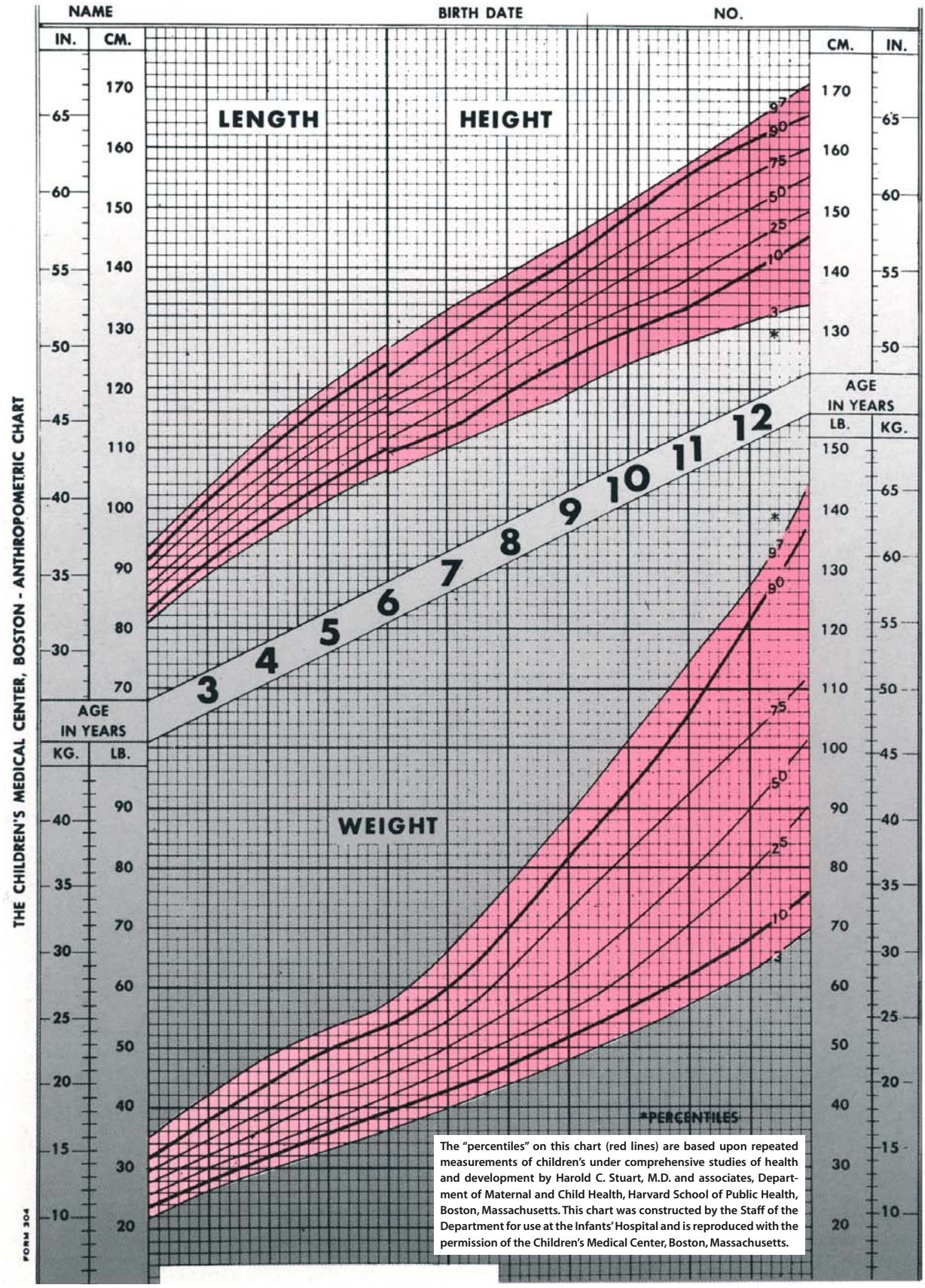
Note that the WHO "alert" value indicates when pollution levels are such that sensitive populations (children and people with respiratory diseases) will begin to feel detrimental health effects. "Unhealthy for Sensitive People": 150–250 µg/m^3.
The WHO "warning" value indicates when pollution has reached a level that everyone will be affected by the pollutant. The USEPA AQI gives different levels only for PM10. "Unhealthy for all People" PM10 levels: 250–350 µg/m^3.
In Europe all cities are "allowed" to register PM_{10} values >50 µg/m^3 for only 35 days/year.
Data from WHO and USEPA AQI (http://www.epa.gov/oar/oaqps/psiaqi.html); WHO Guidelines for Air Quality, WHO, Geneva.
TSP Total suspended particulate.
[a] Daily mean, all others hourly mean.



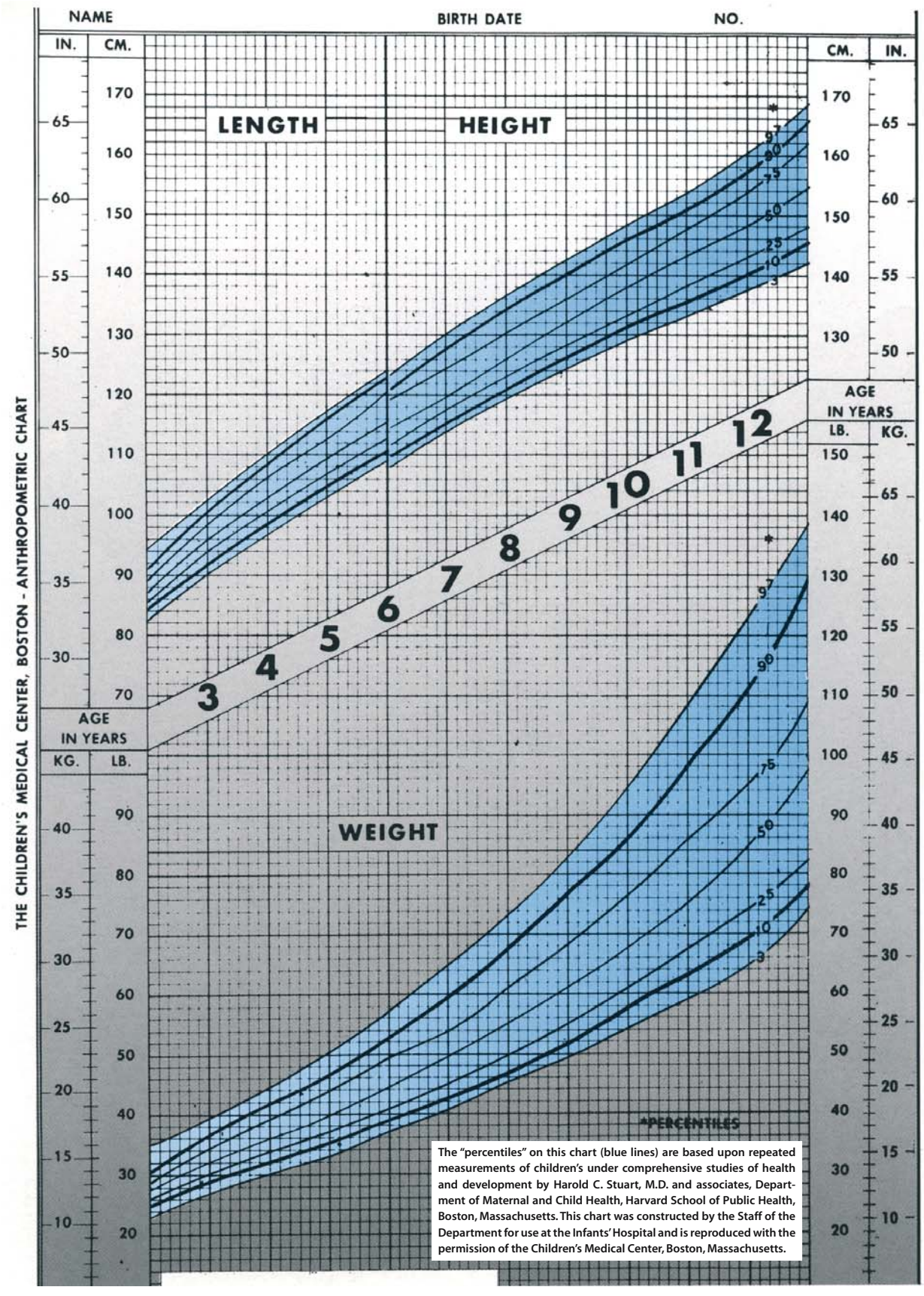
Appendix 6.1. Percentiles for length and weight for age, and for HC. Girls, birth to 2 years



Appendix 6.2. Percentiles for length and weight for age, and for HC. Boys, birth to 2 years



Appendix 6.3. Percentiles for stature and weight for age, girls, 3–12 years



Appendix 6.4. Percentiles for stature and weight for age, boys, 3–12 years

Appendix 6.5. Examination of nasal eosinophils and clinical evaluation

Instruct the child to blow his or her nose directly on a piece of wax paper or on a glass microscope slide, or apply and spread the mucus from the paper to the slide with a cotton-tipped applicator. Otherwise, place a cotton-tipped applicator on the mucosa of the anterior nasal cavity and leave it in place for 2–3 min, then remove, spread the adherent mucus on a glass microscope slide and allow the smear to air dry.

Staining of the nasal smear (the same staining can also be used for conjunctival mucus):

- With the Hansel stain if eosinophils are the only cells of interest
- With the Wright-Giemsa stain if metachromatic cells are also being studied

Examination of the smear. Examine with a microscope under low power (×100) to determine the adequacy of the specimen and individuate the areas of interest. Then examine under a high-power lens (×1,000) and oil immersion. Count the number of eosinophils (pink cytoplasmic granules with blue, bilobed nuclei) and the total number of polymorphonuclear leukocytes (PMNs), which have pale pink cytoplasm and blue, multilobed nuclei. Nasal epithelial cells have abundant pale blue cytoplasm and blue, unlobulated nuclei.

The interpretation of nasal cytograms is best done by the same person.

The percentage of eosinophils is calculated after having examined 1,000 cells, since only 100 cells do not ensure reproducible results. The absolute number of peripheral eosinophils/mm^3 is expressed by the ratio of eosinophils to the total number of peripheral PMNs.

The total number obtained (mean count of the two slides) is evaluated as follows:

No. of eosinophils

- <5%: not significant eosinophilia
- 5%–10%: low eosinophilia
- 10%–50%: increased eosinophilia
- >50%: high eosinophilia

Note that if there are 10% or more eosinophils, allergic rhinitis is suggested (see also Appendix 12.2)

From [14, 59].

Appendix 6.6. Equations for the predictive determination of mean PEF values in children aged 4–16 years, employing Vitalograph Peak Flow Monitor and Wright and Mini Wright Peakflow Meters [181]

Age and length (m) instrument	Sex	C	β1	β2	σ
4–7 years 1.00–1.40 m					
Mini Wright	M	−67.17			
			82.06	27.80	29.9
	F	−90.84			
Vitalograph	M	−55.29			
			59.51	29.15	28.8
	F	−77.47			
7–16 years 1.20–1.80 m					
Mini Wright	M	−46.72			
			97.20	17.38	48.5
	F	−68.96			
Wright	M	−13.84			
			94.72	13.18	43.4
	F	−23.81			
Vitalograph	M	−112.31			
			124.59	17.18	56.5
	F	−136.72			

Equation: $PEFR = C + \beta1 \times (length)^2 + \beta2 \times age$.
See Figs. 6.26–6.31 with ready-made graphs.
The calculations can be made more easily and rapidly with a pocket ruler [181].

Appendix 6.7. Suggestion for an informed consent form

I ..... parent or legal guardian of.....
have been fully and clearly informed by Doctor .....
about mechanisms, administration schedule, purposes and potential side effects of
in vivo diagnostic test .....or[a]
Specific immunotherapy for .....
I agree that my son/my daughter is subjected to this test/treatment[a], am aware that the treating physician will monitor and minimize adverse effects of the treatment. I also confirm that I am aware that I need to follow precautionary measures both before and after the injection/the test, in particular to wait with my son/my daughter in the medical facility for at least 30 minutes following
in vivo diagnostic test .....or[a]
Specific immunotherapy for .....……
dispensing the specialist of every responsibility, in the event I should leave without respecting the necessary observation period and the above-mentioned precautionary measures.
Date .....……
Signature .....……

From [38].
[a] Omit what may be unnecessary.

Appendix 7.1. Clinical evaluation of atopic dermatitis lesions

Severity criteria	Lesion assessment
Mild	Features: erythema, pigmentary changes, mild scaling without significant excoriations (apart from scratching) or lichenifications
	Distribution: relatively little spreading
	Course: episodic, inconstant
Moderate	Intermediate characteristics between mild and severe
Severe	Features: papule, vesicles, crusts, marked erythema, scaling with significant excoriations and/or lichenifications
	Distribution: widespread with face, trunk and limb involvement
	Course: chronic with persistent symptoms independently of local therapy

The SCORAD index (Chap. 6) gives a scoring evaluation.

Appendix 7.2. Levels of free fatty acids in breast milk of European and African mothers

EFA	Mean of 14 studies done in Europe (*n*=329)	EFA	Mean of 10 studies done in Africa (*n*=259)
ω-6 PUFA		ω-6 PUFA	
C18:2ω-6	11.0 (6.9–16.4)	C18:2ω-6	12.7 (5.7–17.2)
C18:3ω-6	0.54 (0.16–0.9)	C18:3ω-6	0.2 (0.1–0.3)
C20:2ω-6	0.35 (0.2–0.5)	C20:2ω-6	0.41 (0.3–0.83)
C20:3ω-6	0.32 (0.2–0.7)	C20:3ω-6	0.4 (0.2–0.5)
C20:4ω-6	0.55 (0.2–1.2)	C20:4ω-6	0.66 (0.3–1.0)
C22:4ω-6	0.08 (0.0–0.1)	C22:4ω-6	0.08 (0.0–0.1)
C22:5ω-6	0.15 (0.1–0.3)	C22:5ω-6	0.15 (0.1–0.3)
ω-3 PUFA		ω-3 PUFA	
C18:3ω-3	0.87 (0.7–1.4)	C18:3ω-3	0.86 (0.1–<2.0)
C20:3ω-3	0.06	C20:3ω-3	0.14
C20:5ω-3	0.23 (0.04–0.6)	C20:5ω-3	0.22 (0.1–0.48)
C22:5ω-3	0.2 (0.1–0.52)	C22:5ω-3	0.19 (0.1–0.39)
C22:6ω-3	0.29 (0.1–0.59)	C22:6ω-3	0.38 (0.1–0.9)
Modified from [134]		Modified from [317]	

Appendix 7.3. Recommended dietary allowances: ω6 and ω3 free fatty acids

	Age (years)	EFA (g)	
		ω6	ω3
Infants	0.5–1	4	0.5
Children	1–3	4	0.7
	4–6	4	1
	7–10	4	1
Males	11–14	5	1
	15–17	6	1.5
Females	11–14	4	1
	15–17	5	1

Source: Società Italiana di Nutrizione Umana.

Appendix 8.1. Waxes, gums, spices and flavoring agents that can cause contact urticaria

Waxes and gums
Acacia, beeswax, benzoin, carnauba, guar, karaza, spermaceti, tragacanth
Spices and flavoring agents
Oil of almond, anise, bergamot, camomile, capsicum, caraway, cinnamon, cloves, coconut, ginger, laurel, mace, menthol, minoneme, mustard, nutmeg, palm, peppermint, sesame

Data from [184].

Appendix 8.2. Use of fruit extracts cross-reacting with latex (Table 8.14)

Chymopapain and Papain (from papaya)
Most diffuse use: to tenderize foods (e.g., meat), to clarify drinks (e.g., beer)
Foods
Beer
Cakes
Coca cola
Crackers
Drinks with fruits
Fruit juices and salad
Meat (tenderizes)
Yogurts with fruits
Non-foods and other uses
Cosmetics
Toothpaste
Chemonucleolysis (Chap. 20)
Cleaners for dental prosthesis and soft contact lenses
Drugs: anti-inflammatory, laxatives, gastrointestinal drugs, etc.
Bromelin (pineapple)
Foods
Beer
Cheese
Meat
Other uses
Anti-inflammatory drugs
Diet pills
Ficin (fig)
Foods
Beer
Cheese
Meat
Other uses
Laxative and deworming drugs
Leather and textile industry
Oil of avocado
Cosmetics

Modified from [36].

Appendix 9.1. Dietary products that must be eliminated in children allergic to cow's milk and/or egg proteins

CM and powdered CM
Soups
Cream soups, all soups made with CM/egg, or CM/egg products
Cheese, cottage cheese
Cream of rice cereal
Wheat macaroni and pasta
Breads and cereals
Any prepared with CM or CM products, including biscuits, candy bars, crackers, hot breads, pancakes, rolls, waffles
All cereals precooked and prepared with added CM solids
High-protein cereals
Meat and meat substitutes
Breaded or creamed meat, fish, poultry
Delicatessen meats
Eggs and eggs cooked with CM or CM products
Egg substitutes such as egg beaters
Luncheon meats made with CM
Margarines
Poultry with stuffings
Sausage products such as frankfurters, bologna
Potato and vegetables
Any potato or potato substitute prepared with CM or CM products, including mashed potatoes and macaroni and cheese
Any vegetable prepared or creamed with CM, such as creamed spinach
Salad dressings and mayonnaise containing CM or CM products
Desserts
Any dessert prepared with ingredients that are not allowed
Chocolate (milk chocolate, check plain chocolate)
Commercial cakes, cookies, pies, puddings
Commercial ice cream, sherbet, yogurt
Nondairy whipped topping
Prepared mixes
Fruits and fruit juices

Appendix 9.1. (Continued)

Beverages	
Cocoa, egg nog, malts, milkshakes	
Miscellaneous	
Au gratin dishes	
CM chocolate	
Cream sauce	
Curd, whey	
Special diet preparations	
Egg and powdered egg	
Soups	
Cream soups, all soups made with CM/egg, or CM/egg products	
Macaroni and pasta made with egg	
Mock turtle and egg noodle soup, any egg-cleared stock soup, including consommé, bouillon, etc.	
Breads and cereals	
Commercial doughnuts, French toast, muffins, pancakes, waffles	
Prepared mixes for muffins, pancakes, waffles	
No cereals	
Meat and meat substitutes	
Any serving made with egg as a binding agent, such as casseroles, croquettes, hamburger, meat loaf, sausage, etc.	
Breaded foods in which egg is used as breading	
Canned or stuffed meat	
Cheese fondue, soufflé, cheese puffs	
Meat and fish sauces containing egg butter	
Potato and vegetables	
Any vegetable prepared or combined with egg sauces, including corn custard, hollandaise sauces, spinach omelet	
Duchesse potatoes, croquettes, egg noodles, potato cakes	
Commercial salad dressings and mayonnaise	
Desserts	
Any dessert prepared with ingredients that are not allowed	
Commercial cakes, cookies, frosting, ice creams, icing, pastries, pies, puddings, sherbets and fruit sherbets, yogurts	
Custard	
Meringue, marshmallow, nougat	
Pie crust brushed with egg	
Fruits and fruit juices	
Fruit served with custard sauce	
Fruit whips	
Beverages	
Coffee cleared with egg white	
Commercial candies made without egg may be brushed with egg white	
Ovomaltine and Ovomalt	
Miscellaneous	
All prepared mixes, frozen dinners, etc., unless label clearly indicates egg absence	
Baking powder containing egg white or albumin	
Divinity fudge	
Flour-coated frozen foods	
Thousand Island dressing	
Tartar sauce	

Warning: all labels on foods must be read for products containing milk, egg, and/or soy products: the composition of any food product may be changed without notice.
Lamb meat or products containing it and goat milk may induce cross-reactions in CM-allergic children while chicken or chicken-containing products can induce cross-reactions in egg-allergic children (see text).
The collaboration of M.S. Campostrini in compiling this appendix is acknowledged.
Data from [27, 516].

Appendix 9.2. Dietary products and ingredients with potential hidden forms of cow's milk and/or egg proteins

Ingredients or labels that indicate CM proteins:
Seasonings/flavorings with raw sugar, brown sugar coloring, caramel flavoring/color, natural flavorings, Simplesse [418]
Ingredients that may contain CM proteins:
Artificial butter flavor, butter fat, butter solids, buttermilk, buttermilk also dried or powdered, casein, casein hydrolysate, caseinates (ammonium, calcium, magnesium, potassium, sodium, etc.), cheese, cottage cheese, cream, curds, delactosed whey, demineralized whey, dried or powdered milk, half and half, heavy cream, hydrolysates (casein, milk protein, protein, whey), milk (condensed, dried, evaporated, homogenized, instant nonfat dry milk powder, lactose, lactose-free, low-fat, low-protein milk, low-sodium milk), malted products, margarine, nondairy whipped topping, non-fat butter spread, nougat, protein, rennet casein, skim, sour cream, sour cream solids, sour milk solids, sweet acidophilus, whey, whey protein concentrates, whey protein hydrolysate, yogurt, etc. [8]
Ingredients that indicate masked CM proteins
Brown sugar flavoring, natural flavoring, chocolate, caramel flavoring, high-protein flour, margarine
Potential hidden sources of egg proteins
Bagels
Breads
Coffee
Fillings
Flour-coated frozen foods
Fruit sherbet
Ice creams
Mayonnaise
Meringue
Pastas
Pastries
Pretzels
Root beer
Salads
Sausage
Soups
Egg servings (baked, creamed, deviled, emulsified, fried, frozen, hard- or soft-boiled, poached, scrambled egg, egg salad, egg sandwiches, egg sauces, meringues, omelet, soufflé)
Ingredients or labels that indicate egg proteins:
Albumin, egg (white, yolk, dried, powdered, solids), egg substitutes, eggnog, globulin, livetin (seralbumin, α_2-γ lycoprotein, serum gamma-globulin), ovalbumin, ovomucin, ovomucoid, ovovitellin, Simplesse, yogurts

Products with hidden forms of CM or CM proteins are listed in Table 9.34.
Data from [27, 516].
The collaboration of M. S. Campostrini in compiling this appendix is acknowledged.

Appendix 9.3. Composition of some soy protein formulas

Brand name	Protein	Carbo-hydrates	Fat	PUFA	Minerals	Electrolytes					
						Na	K	Cl	Ca	P	Fe
Alsoy	1.9	7.4	3.3	0.70	0.35	9.7	20.1	13.5	710	413	12.2
Frisosoy	2.0	7.1	2.5	0.6	0.29	5.7	28.9	15.9	460	270	7.0
Gerber Soy	2.0	6.8	3.6	0.70	0.4	13.9	20.0	16.6	640	500	12.2
Humana-sl	1.9	7.7	3.5	0.58							
Isomil	1.8	6.9	3.69	0.67	0.27	12.9	18.7	11.8	710	510	12.2
Milupa som 1[a]	2.0	7.6	3.5	0.58	0.4	27	78	46	560	360	8
Milupa som 2[a]	2.0	7.7	3.6	0.7	0.46	33	100	57	930	570	17
Nursoy	1.8	6.9	3.6	0.65	0.3	8.7	17.9	10.5	600	420	12
Nutrilon Soya 1[a,b]	1.8	6.7	3.6	0.5	0.3	18.0	65.0	40.0	540	270	8.0
Nutrilon Soya 2[a,b]	2.2	7.7	3.6	0.8	0.46	23.0	100	57.0	930	570	13
Prosobee	2.0	6.8	3.6	0.67	0.4	10.4	21.0	15.2	710	560	12.2
Soyalac	2.1	6.8	3.7	1.9	0.4	13.0	0.4	13.0	635	370	13

[a] The electrolytes Na, K, Cl are in mEq/l, Ca, P, FE in mg/l (normal dilution), all other nutrients in g/dl (normal dilution).
[b] Nutrilon soya 1 is for infants aged 0–4 to 6 months, Nutrilon soya 2 for infant older than 4–6 months.

Appendix 9.4. Composition of some whey protein hydrolysate formulas

Brand name	Protein	Carbo-hydrates	Fat	PUFA	Minerals	Electrolytes					
						Na	K	Cl	Ca	P	Fe
Alfaré	2.3	7.0	3.3	0.40	0.42	17.0	20.6	19.2	540	340	7.8
Aptamil[a]	1.5	7.2	3.6	0.43	0.30	7.8	21.2	11.2	580	350	8.0
Good Start	1.6	7.4	3.4	0.80	0.28	7.0	16.5	11.3	430	340	10.0
Nan 1	1.3	7.8	3.4	0.46	0.23	6.9	16.9	12.3	430	210	8.1
Nan 2	2.2	7.9	2.9	0.44	0.50	14.0	26.9	21.3	810	660	11.9
Nativà HA	1.8	6.9	3.6	0.60	0.26	7.4	17.4	13.0	410	210	8.1
Nidina HA	1.6	7.4	3.4								
Nutrilon Pepti 1[b,c]	1.6	6.9	3.6	0.45	0.27	19.0	71.0	45.0	520	260	5.0
Nutrilon Pepti 2[b,c]	1.8	7.2	3.6	0.48	0.41	27.0	85.0	51.0	880	510	12.0

MW molecular weight, *kD* kiloDalton.
[a] Casein-whey hydrolysate.
[b] The electrolytes Na, K, Cl are in mEq/l, Ca, P, FE in mg/l (normal dilution), all other nutrients in g/dl (normal dilution).
[c] Nutrilon pepti 1 is for infants aged 0–4 to 6 months, Nutrilon pepti 2 for infants older than 4–6 months.

Appendix 9.5. Nutrient distribution of some hydrolysate formulas

Brand name	Protein g/l	Fat g/l	MCT (%)	Carbohydrate			Energy kcal/l	Osmolarity mosm/l
				g/l	Type	(%)		
Pregestimil	18.8	27.2	+	91	Glucose polymer		660	245
Nutramigen	19	26	+	91	Glucose polymer		660	245
Alfaré	25	36	(50)	77.5	Maltodextrin	(90)	729	200
					Lactose	(1)		
Alimentum	18.6	37.5	(50)	69	Tapioca starch	(33)		
					Sucrose	(67)	676	
Aptamil HA	15	36	–	72	Lactose	(100)	670	324
Nidina HA	17	36	–	69	Lactose	(100)	670	282
Nutrilon	20	38	–	71	Lactose	(38)	700	260
Nutrilon Pepti	16	36	–	68	Lactose	(38)	650	275
					Corn syrup	(50)		
					Maltose	(12)		
Pepti Plus					Corn syrup	(50)		
					Maltose	(12)		
Profylac	15	33	–	70	Maltodextrin	(50)	660	
					Starch	(30)		
					Sucrose	(20)		
Pregomin	<5	2.0	3.6	8.6	Maltodextrin	(80)	750	202
Soy + pork					Starch	(20)		

MCT medium chain triglycerides.

Appendix 9.6. Levels of β-lactoglobulin in cow's milk, breast milk, and some hydrolysate formulas assayed by ELISA and/or RAST inhibition

Food Brand name	β-Lactoglobulin concentration ready-to-use			Ratio CM/ CM/hydrolysate %	RAST inhibition relative potency
	Dry weight µg/g (M±SD)	Product µg/l (M±SD)	Residual %		
CM	117,000	4,000,000	100		100
Beba HA	200				0.06
LHA	200±24	31.200±3.744	0.78	130	
Good Start	83±14	12.438±1.990	0.31	320	
Alfa Ré	0.12±0.016	14.5±1.8	0.00036	280,000	
Profylac	0.066±0.01	8.9±1.3	0.00022	450,000	<0.01
Pregomin	0.065±0.002	9.7±0.29	0.00024	410,000	
Pepti Junior	0.0061±0.0008	0.91±0.12	0.000023	4,400,000	
Alimentum	0.03				<0.01
Nutramigen	0.0056±0.0005	0.84±0.07	0.000021	4,800,000	0.05

A subsequent study shows different values (µg/g): Peptide-Tutteli (EWHF), Nutramigen, Nutri-Junior, Neocate, and Beba HA [235] have values of 0.011, 0.014, 0.031, 0.0016, 84, respectively. The Nutramigen value is greater than 0.4-fold higher in comparison with the above figures.
Data from [235, 317, 318, 368].

Appendix 9.7. β-Lactoglobulin levels in breast milk (ng/ml)

Axelsson et al [17]	5–800
Høst et al [226]	0.9–150 (mean, 4.2)
Jakobsson et al [238]	5–33
Machtinger and Moss [314]	0.1 pg/ml to 6.4 ng/ml
Sorva et al [451]	Basal, 0.0–3.5 (mean, 0.01) 1 h, 0.01–7.84 (mean, 0.12)[a]
	2 h, 0.01–2.34 (mean, 0.07)[a]

[a] After drinking 400 ml of CM.

Appendix 9.8. Nutrient distribution of some special formulas based on amino acids

Brand name	Protein g/dl	Fat g/dl	AA g/dl	Carbohydrate			Energy kcal/l	Osmolarity mosm/l
				g/dl	Type	%		
Neocate	1.95	3.5	2.3	8.1	Glucose		71	342
Neocate Advance	2.5	4.0	3.0	14.6	Glucose	(9)	71	298
Nutri 2000	2.9	3.4	1.7	15.0	Maltodextrin	(99)	100	520
Pepti-Junior	1.8	3.6	–	6.9	Maltodextrin	(90)		
					Glucose		(1)	
					Others	(8)	67	190
Pregomin AS	2.0	3.5	–	8.6	Glucose		73	300

Minerals and trace elements are added to all formulas; α-linolenic acid is added to Neocate and Pepti-Junior.

Appendix 9.9. Calcium-rich foods of the most common cow's milk substitutes for allergic children

Foods	Mg/dl of edible part
Beet	80
Horse	12
Lamb	10
Lean pork	9
Lettuce	50
Pear	13
Potato	9
Rabbit	17
Rice	12
Soy milk	**mg/dl of reconstituted product**
Alsoy	60
Humana SL	49
Isomil	70
Multisoy	51
Neo Soyal 1	71
Neo Soyal 2	90
Nutrilon Soya	54
Soyalac	63

Appendix 9.10. Number of egg-allergic children regularly vaccinated with measles vaccine or MMR and of so-called anaphylactic reactions provoked by MMR vaccination

Authors	Year	No. of vaccinated children	Adverse reactions No. of cases
Aickin et al	1994	410	4[a]
Andrews et al	1998	35	
Baxter	1996	199	1[b]
Beck et al	1991	28	
Bruno et al	1993	125	
Fasano et al	1992	140	
Freigang et al	1994	500	
Greenberg and Birx	1988	15	
Hermann et al	1983	8	2[a]
James et al	1995	54	
Juntunen-Backman et al	1987	122	2[c]
Kamin	1963	11	
Kamin	1965	11	
Keible et al	1995	59	
Kemp et al	1990	35	
Lavi et al	1990	111	3[d]
Panayiotopoulou et al	1993	63[e]	
Puvvada et al	1993	8	
Tounian et al	1993	17	
Trotter et al	1994	12	2[a]
Total		1,963	13

[a] The reported reactions have not caused modifications of vital parameters, nor have they required the administration of adrenaline, antihistamines or other medications, and the children completed the normal vaccination.
[b] One child developed anaphylaxis after a subsequent intradermal test.
[c] Two children developed mild generalized urticaria 24 h after MMR vaccination.
[d] The children presented with urticaria, which spontaneously remitted, and were subsequently vaccinated according to the desensitization protocol of Herman.
[e] The children were regularly vaccinated following the above protocol, without reactions.

Appendix 9.11. Number of immediate or anaphylactic-type reactions to measles or MMR vaccination in children not allergic to egg

Authors	Year	Immediate reactions No. of cases	Anaphylactic reactions No. of cases
Aukrust et al	1980	6	0/6
Fasano et al	1992	2	0/2
Juntunen-Backman	1987	4	0/4
Kalet et al	1992	5	0/5
Kelso et al	1993	1	1/1[a]
McEwen	1983	15	0/15
Pollock and Morris	1983	9	0/9
Thurston	1987	2	0/2
Van Asperen et al	1981	3	0/3
Patja et al	2001	3	2/3[b]
Total		50	3/50

[a] Kelso et al [265] demonstrated by the immunoblotting method the presence of gelatin-specific IgE in an egg-tolerant 17-year-old female who developed anaphylaxis after MMR vaccination.
[b] Two of the three children had sIgE to gelatin.
Data from [94, 243, 378].

Appendix 9.12. Potential hidden sources of wheat, peanuts, corn, nuts, soy, sesame, coconuts and dates, anise, beef, and shellfish

A. Products and ingredients potentially containing wheat
All products made with wheat flour (cake, cookies, ice cream cones, pastries, pie)
Any breaded or floured food or prepared with wheat flour
Bran
Biscuits, doughnuts, dumplings, French toast, muffins, pancakes
Bouillon cubes (some types)
Bread or bread crumbs made with wheat flour, bread and cracker stuffing
Bulgur
Cake flour
Canned meat
Cereal extracts
Cereals containing wheat
Commercial candies containing wheat products
Commercial gravy, gravy made with wheat flour
Commercial salad dressings thickened with wheat flour
Couscous
Cracked wheat flour
Cracker meal, crumbs
Enriched flour, farina flour, gluten flour
Frosting
Kamut
Hydrolyzed vegetable protein
Hyperproteinic floor
Meats containing filler such as meat loaf, bologna, luncheon meats, wieners
Natural flavoring
Noodles, macaroni, spaghetti
Packaged puddings
Pastry flour
Pepper powder (some types)
Phosphated flour
Potatoes or rice prepared with wheat flour (example: escalloped potatoes)
Prepared chocolates and mixes
Pretzels
Rye bread or corn bread made with wheat flour
Sauces of any type prepared with wheat
Seitab
Semolina of all types
Soups containing alphabets, dumplings, noodles, spaghetti
Soy sauce
Strained fruits with added cereals
Vegetable gum, starch
Wheat crackers

Appendix 9.12. (Continued)

Ingredients and labels that indicate masked presence of wheat protein

- All-purpose flour, bread crumbs, cake flour, cereal extract, cracker meal, farina, gelatinized starch, gluten, graham flour, high-gluten flour, high-protein flour, hydrolyzed vegetable protein, mixed grains flour, modified food starch, pastry flour, self-rising flour, semolina flour, triticate, vital starch, wheat bran, wheat germ bran, wheat gluten, wheat meal, wheat starch, whole wheat flour

Ingredients and labels indicating potential presence of wheat protein

- Gelatinized starch, modified food starch, modified starch, natural flavoring, soy sauce, starch, vegetable gum, vegetable starch

B. Products and ingredients potentially containing peanuts

African, Chinese, Thai and other ethnic dishes

Baked goods (pastries, cookies, etc.)

Bakery products

Breakfast cereals (some types)

Candy, cream-filled pastries

Canned fish

Chili

Chocolate (candy, candy bars)

Egg rolls

Ice creams

Margarine (check the ingredients)

Marzipan

Nougat

Pastries (they are often minced and therefore unrecognizable)

Sauces for meats

Sausages

Ingredients and labels that indicate masked presence of peanut protein

- Hydrolyzed plant protein, hydrolyzed vegetable protein
- Cold-pressed peanut oil, ground nuts, mixed nuts, Nu-Nuts (artificial nuts), peanut butter (check the ingredients), peanut flour

NB, Peanuts are employed as peanut oil:

- In CM formulas for babies
- In vitamin oil preparations

C. Products and ingredients potentially containing corn

Foods potentially containing corn starch

- Mixed vegetable creams and/or flours

Any food cooked or fried with corn oil

Baby foods

Bacon

Baking powder biscuits, baking mixes, corn fritters, pancakes

Batters for frying

Breaded or fried foods

Cakes

Candies

Canned or frozen fruits or juices "with sugar added"

Canned peas, frozen vegetables, pork and beans

Cereals for breakfast

Cheese, cheese spreads

Chewing gum

Chili

Chocolate milk

CM, in paper cartons

Coated rice

Coffee, instant

Cold cuts, ham, sausage, wieners, processed meats

Confectioner's sugar, powdered sugar

Cookies

Corn flakes, corn cereals, presweetened cereals

Eggnog

English muffins, tamales, tortillas

Fish sticks, prepared and processed fish

French dressing

Frosting

Fruit juices, fruit pies

Fruits, canned and frozen

Frying fats

Gelatin capsules, gelatin desserts

Glucose and fructose products

Grape juice

Gravies

Ice creams

Instant coffee, instant teas

Jam and jellies

Leavening agents and yeasts

Margarines and shortenings

Peanut butter, butter for frying

Appendix 9.12. (Continued)

- Polenta
- Popcorn
- Potatoes or rice fried in corn oil
- Puddings and custards
- Salad dressings
- Sandwich spreads
- Sauces for fish, meats, sundaes, etc.
- Sausages
- Soy milk
- Soy sauce
- Vegetable soups, commercial soups
- Chemical substances
- Caramel color
- Citric acid
- Dextrin
- Dextrose
- Fructose
- Glucose
- Maltodextrins
- Mannitol
- Monosodium glutamate
- Ingredients and labels that indicate masked presence of corn protein
  - Baking powder, blue corn, corn alcohol, corn bran, corn flour (blue, white, or yellow), corn germ, corn meal (blue, white, or yellow), corn pastas, corn products, corn syrup, corn syrup solids, corn sweetener, cornstarch, dent corn, hominy grits, maize, popcorn, puffed corn, sweet corn, whole dried corn, whole hominy
- Ingredients and labels that indicate peanut protein
  - Food starch, modified food starch[a], vegetable gum, vegetable starch
- Ingredients and labels indicating masked presence of corn protein
  - Corn sugars, corn flour (all types), hydrolyzed vegetable protein, margarine, natural and artificial emulsifiers and stabilizers, thickeners, vegetable broth, vegetable oil

D. Products and ingredients potentially containing nuts (all types), almonds, cashews[b]

- Almond paste
- Artificial nuts
- Baked goods (pastries, cookies, etc.)
- Bakery products
- Brazil nuts
- Cashews
- Chestnuts
- Chocolate with hazelnuts, nut chocolate (chocolate with chopped toasted nuts)
- Commercial sauces for pasta or meats
- Commercial vegetable courses
- Filberts/hazelnuts
- Hickory nuts
- Ice creams
- Macadamia nuts
- Marzipan
- Nougat
- Nut butters (i.e., cashew butter)
- Nut oil
- Nut paste (i.e., almond paste)
- Pecans
- Pine nuts (pinion nuts)
- Pistachios
- Pollack (surimi)
- Sugared almonds
- Torrone paste
- Torrone, chocolate torrone
- Walnuts

E. Products and ingredients potentially containing soybean[c]

- African, Chinese, Thai and other ethnic dishes
- All products made with soy flour
- Baked goods
- Biscuits and biscuit mixes containing soy oil
- Bouillon cubes (some types)
- Breads containing soy oil
- Canned foods
- Canned meat
- Caramel candies
- Cereals containing soy oil, soy flour
- Chocolate
- Commercial ice creams
- Corned beef
- Crackers
- Dietetic food
- Dough mixes
- Filler foods, especially meat-based
- Fish canned in soy oil

Appendix 9.12. (Continued)

Hamburger with soy protein
Hard candies, nut candies
Lecithin (added to biscuits, candies, chocolate and substitutes, ice creams, margarine, spaghetti, etc.)
Luncheon meats
Margarine or shortenings
Meat substitutes
Milk and coffee substitutes
Milkshakes that are not homemade
Minced meat not homemade
Products fried or cooked with soy oil or soy margarine
Salad dressing containing soy oil
Sausages or cold cuts with added soy protein
Soups containing soy or soy products
Soybean oil
Spaghetti prepared with soy flour
Steak sauce
Tea cakes
Toasted soybeans
Tuna canned in soy oil
Vegeburgers made with textured vegetable protein (TVP)
Vegetables served with a soy sauce or dressed with soy oil
Ingredients and labels that indicate masked presence of soy protein
Hydrolyzed vegetable protein, miso, protein fillers, soy flour, soy grits, soy nuts, soy oil, soy protein, soy protein concentrate, soy protein isolate, soy sauce, soy sprouts, soybean (curd, granules), TVP, tofu
Ingredients and labels indicating potential presence of soy protein
Emulsifiers, flavorings, gum arabic, hydrolyzed plant protein, hydrolyzed soy protein, hydrolyzed vegetable protein, natural flavoring, vegetable broth, vegetable gum, vegetable starch
F. Products and ingredients potentially containing sesame
Bread crumbs
Candies
Fillings
Hamburger
Nut and almond substitutes
Restaurant and commercially prepared salads
Vegetable hamburger
G. Products and ingredients potentially containing coconuts or dates
Biscuits
Commercial ice creams
Cookies, pies and puddings that are not homemade
Pastries
H. Products and ingredients potentially containing anise
Candies
Commercial pastries
Cookies, pies and puddings that are not homemade
Liquors
I. Products and ingredients potentially containing beef
Biscuits (containing beef "gelatin")
Bouillon cubes (even if "from poultry")
Canned beef (containing beef "gelatin")
Cheese curd (from calf)
Chopped meat
Commercial Ca preparations (containing beef "gelatin")
Sausages
J. Products and ingredients potentially containing shellfish[d]
There is no particular name masquerading shellfish: see also Table 9.9
The main problem is the use of alternative names, often foreign names:
Calamari for squid
Crevette for shrimp
Escargot for snails
Langouste, langoustine, scampi for lobster
Mollusks for shellfish

Data from [25, 27, 207, 276, 301, 390, 478, 516, 523].

[a] Can also be derived from other ingredients, including potatoes, tapioca, and wheat. Corn may also be used as an adhesive for postage stamps and envelopes, etc. The same problem also concerns fish-allergic children. Corn is used in many medicines and vitamins. Paper containers may contain corn, and the inner surface of plastic food wrappings and waxed paper cartons may be coated with cornstarch. Read every label, some foods may be eaten if checked with the manufacturer and found to be starch-free, best of all is home preparation of all foods.

[b] Nu-Nuts are peanuts first deflavored and then reflavored with a nut like pecan or walnut.

[c] Soybean flour may be added to bakery products to keep them moist; most soy-allergic children may safely eat products containing soy lecithin and/or soy oil. In Chap. 24 a child reacting to soy lecithin is reported.

[d] Beef "gelatin" is made from beef skin and bone; for all commercial products, check whether they effectively contain the offending food.

Appendix 9.13. Clinical and laboratory data to be checked during a more or less prolonged elimination diet

Medical examination of child
Body mass index (BMI), Mid-arm circumference (MAC), Mid-arm muscle circumference (MAMC), plicometry
Control of both the diet and diary
Control of food nutritional value
Height (Appendices 6.1–6.4)
Weight (Appendices 6.1–6.4)
Calcium serum levels
Ferritin serum levels
Iron serum levels
Lipid fractions
Nitrogen balance
Plasma aminoacidemia
Protidemia with electrophoresis
Serum and urine electrolytes
Transferrine serum level
Whole blood count
Zinc serum levels

Data from [516].

Appendix 10.1. Foods and non-foods potentially containing gelatin or vegetable gums

Gelatin	**Arabic gum, acacia gum E 414**
Gelatin of all types	Candy
Brawn	Commercial puddings
Canned beef	Diabetic foods
Chewing gum	Ice creams
Desserts	Iced confectionery
Fruit juices	Sherbet
Fruit yogurt	**Carob seeds, locust gum E410**
Gummy candies	Canned meat
Instant cakes	Chocolate, cocoa and coffee surrogate
Instant pudding	Flour
Instant whipped cream	Fruit juices and preserve
Licorice	Iced confectionery
Pastry	Jam
Pudding	Mustard
Whipped cream	Yogurt
Yogurt	**Nonfoods**
Nonfoods	Cigarettes
Measles vaccine	Cosmetics
	Toothpaste

Appendix 10.1. (Continued)

Guar gum E412	Cosmetics
Canned, commercial soups	Toothpaste
Cheese	**Adragant gum E413**
Dietetic food preparations	Cod-liver oil
Ice creams	Fresh, soft cheese
Salad dressing	Hamburger
Yogurt	Margarine
Non-foods	Mayonnaise
Pharmacological compounds	Soft drinks
Creams	**Non-foods**
Emulsions	Toothpaste
Gummy pills	Vitamin soluble preparations
Lotions	
Suspensions	
Toothpaste	

Gelatin has a widespread use as a stabilizer pharmaceutically and in foods.
Data from [91, 106].

Appendix 11.1. Respiratory rate per minute at different ages in healthy children when awake or asleep

Age	Awake			Asleep		
	Mean	Median	SD	Mean	Median	SD
<2 months	48.0	47	9.1	39.8	39	8.7
2–6 months	44.1	42	9.9	33.4	32	7.0
6–<12 months	39.1	38	8.5	29.6	28	7.0
12–<18 months	34.5	34	5.8	27.2	26	5.6
18–<24 months	32.0	32	4.8	25.3	24	4.6
24–<30 months	30.0	30	6.2	23.1	23	4.6
30–36 months	27.1	28	4.1	21.5	21	3.7
	Mean	Range		Mean	Range	
4–6 years	26	19–30		18	14–23	
6–8years	23	15–30		17	13–23	

Data from [544] (0–3 years) and [302] (4–8 years).

Appendix 11.2. Biological diagnosis of cough in children

Airway obstruction

1. Allergic rhinitis
2. Diphtheria
3. Foreign bodies
4. Maxillary sinusitis
5. Nasal secretions
6. Tonsillitis

Inspiratory stridor

1. Innervation anomalies and malformations
   - Congenital laryngeal malformations
   - Epiglottis or arytenoid cartilage flaccidity
   - Innervation anomalies associated or not with soft palate anomalies
   - Vocal cord dysfunctions
   - Vocal cord traumatic dislocation
2. Epiglottitis
3. Hypertrophied tonsils
4. Laryngeal stenosis
5. Laryngitis
6. Laryngospasm
7. Malformations
8. Acute tracheal stridor
   a. Foreign bodies
   b. Infections
9. Chronic tracheal stridor
   a. Vascular ring
   b. Tracheomalacia
   c. Tumors

Expiratory stridor – dyspnea

1. Bronchiolitis
2. Asthma

Pulmonary dyspnea

1. Congenital cysts
2. Diaphragmatic paralysis
3. Lobar emphysema
4. Pleural disease
5. Pulmonary disease
   a. Bronchopulmonary dysplasia
   b. Pulmonary fibrosis
6. Pulmonary malformations
7. Pickwick syndrome

Cardiogenic dyspnea

Breathing difficulties on expiration and/or inspiration

Dyspnea attributable to metabolic causes

1 Acidosis
   a. Poisoning especially by salicylate-containing drugs
   b. Ketonemia
   c. Uremia

2. Alkalosis
   a. Forced diuresis hypochloremic alkalosis
   b. Hyperaldosteronism
      Primary
      Secondary
      Bartter's syndrome
   c. Hyperventilation syndrome respiratory alkalosis
      Poisoning
      Fever
      Heart failure
   d. Hypokalemia syndrome
   e. Psychogenic hyperventilation
   f. Repeated gastric lavage
   g. Uncontrollable vomiting

Dyspnea due to cerebral cause

1. Biot's breathing
2. Cheyne-Stokes respiration

Dyspnea associated with hypoventilation

1. Birth injury
2. Congenital myotonia
3. Respiratory muscle pareses

Rare causes

1. Alveolar-capillary block syndrome
2. Bland-White-Garland syndrome
3. Endocardial fibroelastosis
4. Generalized arterial calcification
5. Hamann-Rich syndrome
6. Histiocytosis X
7. Idiopathic pulmonary hemosiderosis
8. Kofferath syndrome
9. Kugel-Stoloff syndrome
10. Macleod's syndrome
11. Mounier-Kuhn syndrome
12. Ondine's syndrome
13. Pompe's disease
14. Pulmonary alveolar proteinosis
15. Wilson-Mikity syndrome

Data from [186, 469, 479].

Appendix 12.1. Main causes of chronic nasal blockage in infancy and childhood

Congenital factors	Vasomotor rhinitis
Choanal posterior stenosis	**Allergy**
Congenital choanal atresia	Seasonal allergic rhinitis
Dermoid	Perennial allergic rhinitis
Encephalocele	Recurrent airway symptoms
Glioma	**Drug-induced**
Malformations	Alcohol
Teratoma	Aspirin
Tornwaldt's cyst	β-Adrenergic drugs
Mechanical factors	Cocaine
Adenotonsillar hypertrophy	Guanethidine
Direct or penetrating injury	Methyldopa
Foreign body	Nasal decongestants
Meningocele	Phenothiazine
Nasal polyps	Reserpine
Septal deviation	**Miscellaneous**
Tumors of the nasopharynx	Acromegaly
Infections and Inflammations	Cystic fibrosis
Adenoiditis	Hypothyroidism
Atrophic rhinitis	Pregnancy
Bacterial and viral infection	Sarcoidosis
Chronic rhinitis	Wegener's granulomatosis
Chronic sinusitis	**Iatrogenic causes**
Congenital ciliary dyskinesia	Rhinitis medicamentosa
Immunodeficiency	Stenosis of the nasopharynx
Infectious rhinitis	

Modified from [107].

Appendix 122. Examination of nasal and conjunctival secretions and their interpretation

In interpreting nasal cytograms, the following disorders are suggested in children:
Presence of 10% or more eosinophils:
1. Allergic rhinitis
2. Eosinophilic nonallergic rhinitis (ENR)
3. Nonallergic rhinitis with eosinophils (NARES)
4. Rhinitis associated with non-IgE-mediated asthma
5. Acetylsalicylic acid hypersensitivity
Presence of an increased number of metachromatic cells:
1. Conditions listed above for increased eosinophils
2. Nonallergic basophilic rhinitis
3. Primary nasal mastocytosis
If neutrophils are increased:
1. If associated with intracellular bacteria → infectious rhinopharyngitis or sinusitis
2. If associated with ciliocytophoria (epithelial cells with clumping of chromatin material) → viral upper respiratory infection
In interpreting conjunctival cytograms, the following disorders are suggested in children
Presence of eosinophils, metachromatic cells:
1. Allergic conjunctivitis
2. Vernal conjunctivitis
3. Giant papillary conjunctivitis
4. Keratoconjunctivitis
Presence of neutrophils → infectious or irritant conjunctivitis
Presence of lymphocytes → viral conjunctivitis

Prior to collecting nasal specimens, the child should clear the nose of excess secretions; in infants a rubber bulb might be used to aspirate excess mucus. Then, using a disposable plastic curette, under direct illumination and visualization of the nasal cavity, sample the medial or inferior portion of the anterior turbinate posteriorly, avoiding the anterior bulb area. Gently press the probe cupped tip on the mucosal surface and move outwards 2–3 mm. We suggest repeating the motion twice and withdrawing the probe without touching the nasal vestibule to prevent contamination. Staining is described in Appendix 6.5.

Appendix 131. Schematic dosage schedule for specific immunotherapy for inhalant allergy[a]

A. Start with vial no.1 (1:10,000) with the following doses:		
1st injection	0.10 ml	
2nd injection	0.20 ml	
3rd injection	0.40 ml	At 7-day intervals
4th injection	0.60 ml	
5th injection	0.80 ml	
→ Discontinue vial no. 1 even if there is residual vaccine and		
BContinue with vial no.2 (1:1000) with the following doses:		
1st injection	0.10 ml	
2nd injection	0.20 ml	
3rd injection	0.40 ml	At 7-day intervals
4th injection	0.60 ml	
5th injection	0.80 ml	
→ Discontinue vial no. 2 even if there is residual vaccine and		
C. Continue with vial no.3 (1:100) with the following doses:		
1st injection	0.05 ml	
2nd injection	0.10 ml	
3rd injection	0.15 ml	At 7-day intervals
4th injection	0.20 ml	
Continue with vial no. 3 with the dose of 0.20 ml at 2-week intervals		
→ This maintenance dose, if well tolerated, after a 1-year period will gradually be increased up to 0.50–0.60 ml and the administrations will be first continued at 3-week intervals and then at 4-week intervals.		

[a] SIT for Der p can be started in any season, whereas SIT for grasses should be started in fall (and not during summer) (in Europe) to reach the maximal tolerated dose before the start of the pollinating season. In this period, it is suggested to reduce slightly the maintenance dose in highly sensitive children.

Source: Division of Pediatric Allergy and Immunology, University of Rome "la Sapienza."

Appendix 13.2. Schematic dosage schedule for rush SIT and ultra-SIT for venom

	Rush SIT (in children)		Ultra-rush (in children and adults)	
Week no.	Day no.	Venom dose (in μg)	Minutes	Venom dose (in μg)
1	1	0.01; 0.1; 1; 3[a]	0	0.1
2	8	5	30	1
3	15	10	60	10
4	22	20	90	20
5	29	40	120	30
6	36	70	150	40[b]
7	43	100		

[a] Cumulative dose: 4.11 μg. From [184].
[b] Cumulative dose: 101.1 μg. From [27, 160] for adults and from G. Patriarca (pers. comm.).

Appendix 13.3. Schematic dosage schedule for modified ultra-rush venom immunotherapy

	Modified ultra-rush venom immunotherapy[a]	
Day	Time (h)	Insect venom (μg)
1	0	0.01
	0.5	0.10
	1.0	1.00
	1.5	10.00
	2.0	20.00
	2.5	40.00
	3.0	80.00
2	0	100.00

[a] All children were discharged from the hospital on the second day, with 8 children already discharged 4 hours after the first maintenance dose of 100 μg insect (Dr. Steiß, pers. comm., Dec 19, 2005).

Appendix 13.4. Oral desensitization to cow's milk, egg and fish

Protocol for oral desensitization with cow's milk.
Starting dilution: 10 drops of milk in 10 ml of water

Days	*Daily dose*
1–3	1 drop
4–6	4 drops
7–9	6 drops
10–12	10 drops
13–15	12 drops
16–18	18 drops
Pure milk	
19–21	1 drop
22–24	2 drops
25–27	3 drops
28–30	4 drops
31–33	6 drops
34–36	8 drops
37–40	10 drops
41–44	16 drops
45–48	24 drops
49–52	32 drops
53–56	40 drops
57–60	40 drops × 2
61–64	40 drops × 3
65–68	40 drops × 4
69–72	50 drops × 4
73–76	60 drops × 4
77–80	4.5 ml × 3
81–84	6 ml × 3

Appendix 13.4. (Continued)

85–88	6 ml × 4
89–92	7 ml × 4
93–96	12 ml × 4
97–100	15 ml × 4
101–104	15 ml × 5
105–108	20 ml × 3
Days	*Daily dose*
109–112	30 ml × 3
113–116	40 ml × 3
117–120	50 ml × 2
121–124	65 ml × 2
125–128	80 ml × 2
129–132	100 ml
133–136	120 ml
Maintenance dose: 120 ml of milk (about one glass) at least two or three times a week[a]	

Oral specific desensitization in patients allergic to egg

Starting dilution: 10 drops of shaken egg (albumen and yolk) in 100 ml of water	
Days	*Daily dose*
Days 1–3	4 drops
Days 4–7	4 drops × 2
Days 8–11	4 drops × 3
Days 12–14	8 drops × 3
Days 15–17	16 drops × 3
Days 18–20	36 drops × 3
Pure shaken egg	
Days 21–23	1 drop
Days 24–26	2 drops
Days 27–29	3 drops
Days 30–32	4 drops
Days 33–35	6 drops
Days 36–38	12 drops
Days 39–41	10 drops × 2
Days 42–44	10 drops × 3
Days 45–47	15 drops × 3
Days 48–50	20 drops × 3
Days 51–53	25 drops × 3
Days 54–56	35 drops × 3
Days 57–59	50 drops × 3
Days 60–62	5 ml × 2
Days 63–65	5 ml × 3
Days 66–68	5 ml × 4
Days 69–71	10 ml × 3
Days 72–74	10 ml × 4
Days 75–77	15 ml × 3
Days 78–81	15 ml × 4
Days 82–85	15 ml × 5
Days 86–90	30 ml × 3
Maintenance dose: one egg two or three times a week	
A homogenous dilution was obtained by shaking one egg for 3 min	

Oral specific desensitization in patients allergic to fish

Starting dilution[b]**:**	
Days	*Daily dose*
Days 1–3	4 drops
Days 4–6	8 drops
Days 7–9	12 drops
Days 10–12	24 drops
Days 13–15	32 drops
Days 16–18	48 drops
Days 19–21	72 drops
Days 22–24	108 drops
Pure fish extract	
Days 25–27	15 drops
Days 28–30	30 drops
Days 31–33	45 drops
Days 34–36	60 drops
Days 37–39	5 ml
Days 40–42	10 ml
Days 43–45	15 ml

Appendix 13.4. (Continued)

Cooked fish (boiled cod)		*Days*	*Daily dose*
Days	*Daily dose*	Days 85–87	32 g
Days 46–48	1 g	Days 88–90	40 g
Days 49–51	2 g	Days 91–93	48 g
Days 52–54	3 g	Days 94–96	56 g
Days 55–57	4 g	Days 97–99	64 g
Days 58–60	5 g	Days 100–102	72 g
Days 61–63	6 g	Days 103–105	95 g
Days 64–66	8 g	Days 106–108	110 g
Days 67–69	10 g	Days 109–111	130 g
Days 70–72	12 g	Days 112–114	150 g
Days 73–75	15 g	Days 115–117	175 g
Days 76–78	18 g	Days 118–120	200 g
Days 79–81	22 g	**Maintenance dose:**	
Days 82–84	27 g	200 g of boiled fish almost once a week	

All patients undergo an oral desensitizing treatment; the patients who are allergic to more than one food undergo one desensitizing protocol at a time. The oral desensitizing treatment is given 55 times, according to standardized protocols: at first a diluted food is administered and then is administered the pure food at increasing doses. The starting dilutions used for the desensitization protocols were lower than those used for the DBPCFC. Sometimes, at the beginning of the treatment, sodium chromoglycate (SCG) (250 or 500 mg, according to the patient's age) should be administered 20 min before food ingestion; if no reactions occur, this pretreatment can be dropped out in a few days. After completing the treatment, all patients should be advised to continue eating the allergenic food approximately twice a week, so as not to lose the state of tolerance.
Data from [187] and G. Patriarca, pers. comm.

[a] A recent protocol for oral desensitization in CM-allergic children [138] dilutes CM 1:25 and administers increasing diluted doses until day 42, when undiluted doses may be started under the cover of cetirizine. Overall, 15 of 21 children (71.4%) achieved the daily intake of 200 ml during a 6-month period; 3/21 children (14.3%) tolerated 40–80 ml/day of undiluted CM; 3/21 children (14.3%) failed the desensitization because they presented allergic symptoms after ingesting minimal amounts of diluted CM.

[b] 1.5% eel, 1.5% cod, 1.5% sardine, 1.5% anchovy.

Appendix 13.5. Oral rush desensitization to cow's milk

Day No.	CM dilution (ml)	CM (3.5% fat) (ml)
1	1:100	1
	1:100	2
	1:100	4
	1:100	8
2	1:100	16
	1:100	32
	1:10	1
	1:10	2
	1:10	4
	1:10	8
3	1:10	16
	1:10	32
	Fresh CM	1
	Fresh CM	2
	Fresh CM	4
	Fresh CM	8
4	Fresh CM	16
	Fresh CM	32
	Fresh CM	64
	Fresh CM	100
	Fresh CM	200
5	Fresh CM	200

Continue with daily intakes of 200 ml of fresh CM.
See also the notes for Appendix 13.4.
Data from [20].

Appendix 13.6. SLIT desensitization to latex

Day	Concentration	Dose administered	Total dose
1	From 10^{-18} to 10^{-10}	1 drop of each solution	28×10^{-10} mg of NRL
2	From 10^{-9} to 10^{-1}	1 drop of each solution	2.8 mg of NRL
3	Pure solution (500 mg/ml)	1, 2, 3, 4, 10 drops	500 mg of NRL
4	Pure solution (500 mg/ml)	1 ml	500 mg of NRL

Data from [186].
NRL Natural rubber latex.

Appendix 13.7. Children (<16 years) allergic to latex who successfully underwent SLIT

Children	Sex	Age (mean)
4	2 M/ 2 F	10 (9–11 years)

Data from [186].

Appendix 14.1. Examination of conjunctival secretions and their interpretation

When interpreting conjunctival cytograms, the following disorders are suggested in children
Eosinophils, metachromatic cells
1. Allergic conjunctivitis
2. Atopic keratoconjunctivitis
3. Vernal keratoconjunctivitis
4. Giant papillary conjunctivitis
Neutrophils
1. Infectious conjunctivitis
2. Irritant conjunctivitis
Neutrophils and eosinophils
Allergic conjunctivitis
Lymphocytes
Allergic conjunctivitis
Seromucous secretions
Allergic disease
Mucopurulent secretions
Infectious disease

To obtain conjunctival specimens, invert the inferior lid, have the child look up, and gently scrape the palpebral conjunctiva 1–2 mm with the same type of probe used for nasal specimens. A topical ophthalmic anesthetic can be used for sensitive children. Stain and examine with low-power microscope as shown in Appendix 6.5. Normal epithelium consists of several epithelial cells such as columnar cells both ciliated and nonciliated, and goblet and basal cells. These cells stain light blue, and contain no eosinophils or metachromatic cells (mast cells and basophils). A few neutrophils and bacteria can commonly be observed. Depending on the cells seen, a presumptive diagnosis can be made.
Modified from [91].

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Subject Index

Note: Page numbers in **boldface** show where the topic is treated exhaustively, those in *italics* indicate that there is a figure. If the page number is repeated, there is an illustration on the same page.

Basic concepts are indexed only where they are first mentioned. Common abbreviations are listed as such (e.g., HIV, IgE), less common abbreviations (e.g., PaO_2, SLE) are spelled out. The reader is asked to excuse any repetitions.

A

C

D

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Note: For sake of brevity the index lists corticosteroids, interleukins, values, etc. which in the text are also referred to as glucocorticoids, cytokines, levels, etc.; other such terms include cromolyn/disodium chromoglycate, Der p/house dust mite, AIDS/HIV infection, and bronchospasm/wheezing. The names of single medications in widespread use (antihistamines, anti-HIV medications, β2-adrenergics, corticosteroids, etc) are not included, and the reader is referred to the Tables with doses indicated under the single speciality. In infants and children refer to the whole pediatric population. The innumerable drugs cited in Chapter 19 are not included.

Abbreviations:
BHR = Bronchial hyperreactivity
FPT = Food provocation test
MIP = Macrophage inflammatory protein,
NAP-1 and 2 = Neutrophil activating factor-1 and 2
PID = Primary immune deficiency
SDS-PAGE = Sodium dodecylsulfate-polyacrylamide gel electrophoresis
sIgE = specific IgE
TAP-1 and -2 = Transporter associated with antigen presentation 1 and 2

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