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Allergens and Allergen Immunotherapy

Fourth Edition

edited by

Richard F. Lockey

Dennis K. Ledford

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Allergens and Allergen Immunotherapy

CLINICAL ALLERGY AND IMMUNOLOGY

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Allergens and Allergen Immunotherapy

Fourth Edition

edited by

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New York London

Informa Healthcare USA, Inc.
52 Vanderbilt Avenue
New York, NY 10017

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 1-4200-6197-6 (Hardcover)
International Standard Book Number-13: 978-1-4200-6197-0 (Hardcover)

Library of Congress Cataloging-in-Publication Data

Allergens and allergen immunotherapy / edited by Richard F. Lockey, Dennis K. Ledford. — 4th ed.
p. ; cm. — (Clinical allergy and immunology ; 21)
Includes bibliographical references and index.
ISBN-13: 978-1-4200-6197-0 (hb : alk. paper)
ISBN-10: 1-4200-6197-6 (hb : alk. paper) 1. Allergy—Immunotherapy. 2. Allergens—Therapeutic use. I. Lockey, Richard F. II. Ledford, Dennis K., 1950- III. Series.
[DNLM: 1. Hypersensitivity—therapy. 2. Allergens—immunology. 3. Allergens—therapeutic use. 4. Immunotherapy. W1 CL652 v.21 2008 / WD 300 A4305 2008]
RC588.I45A43 2008
616.97′06—dc22
2008011317

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To our friend and mentor, Samuel C. Bukantz, MD, Emeritus Professor of Medicine and Medical Microbiology and Immunology, Director Emeritus, Division of Allergy and Immunology, University of South Florida College of Medicine—a continual source of inspiration and guidance in our careers; and to Jewell Bukantz, his lovely wife and our dear friend.

Introduction

As I look over my library, one book is the most battle-scarred and well-worn: It is *Allergens and Allergen Immunotherapy*, the first edition published about 15 years ago. My partners and I read this book from cover to cover and used it to set up an allergy immunotherapy clinic that was as current as possible.

As an editor, I knew that I wanted to have the subsequent editions of this book in our series of books, *Current Allergy and Immunology*, because it was going to be a necessary read for everyone practicing legitimate allergy immunotherapy. In fact, not only have the subsequent editions of this book been in the series but the coauthor, Richard Lockey, is now my coeditor.

If anything defines the practice of allergy, it is allergen immunotherapy. As in all clinical disciplines, there are a variety of ways to practice a treatment program, but this series of books has tried to capture and accurately present what is the right (and wrong) way to practice this treatment program. In the several editions of the book, carefully considered discussions of what are the well-proven ways to perform immunotherapy protocols have been included. I personally have used this book for every aspect of directing our office's allergen injection therapy protocols.

The fourth edition brings us up-to-date on allergens, how they are manufactured, and the differences between Europe and the United States. But the novel focus of this edition is the focus on SCIT (subcutaneous immunotherapy) versus SLIT (sublingual immunotherapy). While SLIT is a potentially useful way to administer immunotherapy, the data are not yet complete. One has to be cautious about subscribing to treatments not yet investigated in the USA and therefore not having been proven effective or safe by our very stringent criteria. The discussions in *Allergens and Allergen Immunotherapy* provide considered opinions about the current state of SLIT and will guide the reader to understanding the issue of whether this immunotherapy method has a role in current allergy practice. I know that my partners and I will devour this edition as we have the past three editions. Speaking for the community of physicians who try to apply the latest knowledge to their use of immunotherapy, we welcome this valuable new edition and look forward to a long and pleasurable read of the core clinical feature of our specialty and where it is headed.

Michael A. Kaliner, MD

Preface

This fourth edition of *Allergens and Allergen Immunotherapy* has been especially created to highlight the most relevant information concerning allergens and allergen immunotherapy. We have seen the book grow from the first edition, published in 1991, with 13 chapters, to the third edition, published in 2004, with 41 chapters and over 800 pages. We have therefore elected to make the fourth edition more concise and practical for the reader by providing the most critical new updates in the field.

Since the last edition, the scientific information available on sublingual immunotherapy has increased substantially. Sublingual immunotherapy is used in many parts of the world, but not yet used in other parts of the world, pending additional investigative studies and approval by regulatory agencies. The knowledge and experience of physicians varies widely with respect to sublingual immunotherapy. Therefore, five chapters are devoted exclusively to this subject. Other areas of significant increase in scientific information include recombinant allergens, physicochemical characterization of allergens, and alternative forms of immunotherapy. The latter includes results of animal studies but is included as this knowledge will likely influence the future of allergen immunotherapy.

The chapters are still grouped into five parts.

Part I, Basics, details the history of the subject, definitions, immunologic responses, and knowledge about allergen nomenclature, so critical in a physician's formulation of an allergen vaccine.

Part II, Allergens, describes the inhalational, ingested, and injected allergens. The major and minor allergens and their cross-reactivity with other allergens are described. Biologic and immunologic characteristics are included.

Part III, Immunotherapy Techniques, describes the manufacture and standardization of allergens for injection and ingestion, instructions for their use, different routes of administration, and their labeling as allergen vaccines as recommended in 1998 by the World Health Organization.

Part IV, Other types of Immunotherapy, describes immunotherapy for food and latex allergy, alternative routes of immunotherapy administration, DNA vaccines, anti-IgE therapy, and novel approaches with inhalant allergens.

Part V, Prevention and Management of Adverse Effects, details how to avoid and treat adverse effects, including anaphylaxis. Instructions and consent forms are included for subcutaneous and sublingual immunotherapy.

All chapters have been updated and organized in a manner that will hopefully enhance this volume as a reference source for the use of allergens for immunotherapy.

Clemens Von Pirquet coined the word "allergy," hoping it would "facilitate new research workers to study the interesting phenomena in the field." With the advent of molecular biology, this has since been realized. While the understanding of the cellular and biochemical aspects of the "phenomena" has advanced with the use of molecular tools, the basic principles of allergen immunotherapy have not changed. This book is to prepare the clinician to know how, what, and why with regards to immunotherapy today and better understand and evaluate the many options of tomorrow. The editors thank Geeta Gehi for her essential contribution. Her attention to detail, gentle nudging, and skill enabled the completion of this edition.

Richard F. Lockey
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1 Allergen Immunotherapy in Historical Perspective

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IMMUNITAS

The term "immunitas" is derived from the Latin adjective "immunis" or its noun form "immunitas," which means exemption, freedom from cost, burden, tax, or obligation.

Original usage of term pertained to the inferior Roman class of plebeians, artisans, and foreign traders who—deprived of religious, civil, and political rights and advantages of the patrician gentes—were immune to taxation, compulsory military service, and civic obligations and functions. After 294 BC, with the transition of the monarchy to the Roman Republic, immunitas defined special privileges (e.g., exemptions from compulsory military service and taxation granted by the Roman Senate to sophists, philosophers, teachers, and public physicians). In later years, common use of the Anglicized descriptor immunity continued to have legal relevance. Into the Middle Ages, church property and clergy were granted immunity from civil taxes. In 1689, the English Bill of Rights formalized parliamentary immunity protecting members of the British Parliament from liability for statements made during debates on the floor. In France, a century later, a 1790 law prevented arrests of a member of the legislature during periods of legislative sessions without specific authorization of the accused member's chamber.

The first medically relevant usage of the term appears to be that of the Roman poet Lucan [Marcus Annaeus Lucanus (39–65 AD)] in "Pharsolia" on referring to the "immunes" of members of the North African Psylli tribe to snakebite. In the scientific literature with definitive medical usage, the term appeared in an 1879 issue of London's *St. George's Hospital Reports (IX:715)*: "In one of the five, instances . . . the apparent immunity must have lasted for at least two years, that being the interval between the two diphtheritic visitation." The following year the descriptor found a place in medical terminology with Pasteur's (Fig. 1) report of his seminal work on attenuation of the causal agent of fowl cholera, noting the "(induction) of a benign illness that immunizes (Fr. immunise) against a fatal illness" (1).

IMMUNITY THROUGH INTERVENTION

Anthropological records reveal that from the earliest times that humans sought to understand the factors that made for well-being, there were attempts to intervene to prevent deviations from health and well-being. Healers of antiquity, priest-doctors, secular sorcerers, medicine men, practitioners of folk medicine all played influential roles. In the ancient cradles of civilization—Mesopotamia, Babylonia, Assyria, Egypt—magic and mystic methods were created to ward off divine and cosmic-directed afflictions mediated through spirits and demons with tools of intervention such as incantations, rituals, sacrifices, amulets, and talismans. In the biblical era of the Old Testament, freedom from disease and affliction (which were believed to be divine punishment for sin) was sought through the power of prayer and left in the hands of rabbis who took on the dual role of healer. In sixth-century BC India, preventive practice became synonymous with following the enlightened morality teachings of Buddha [Gautama (566?–c. 480 BC)]. To herbs and dietary manipulations critical for maintaining health and disease promoting balances between internal Yang and Yin forces,

Figure 1 Louis Pasteur, ScD (1822–1895). Founding Director of the Institut Pasteur, Paris. *Source*: Courtesy of the National Library of Medicine.

ancient China added physical methods. To drain off Yang or Yin excesses, procedures employed insertion of needles (acupuncture) and heat-induced blistering (moxibustion at organ-related skin points along channels of vital flow). According to the tenets originating in classical Greece—with the writings of Hippocrates (460–370 BC)—and extended in Roman medicine by Claudius Galen (130–200 AD), it was the four internal humors (blood, phlegm, yellow bile, and black bile) that were determinants of health and disease. Their pathogenetic imbalances could be corrected by preventively draining off excesses of the humors through the interventions of bleeding, blistering (by cupping), sweating (by steam baths), purging, and inducing expectoration and emesis.

Regarding pestilence, the observation that survivors of an epidemic were spared from being stricken during return waves of the same illness was described by the ancient historians Thucydides (c. 460–400 BC) (2) (Fig. 2), who described the plague of Athens, and Procopicus of Byzantine (c. 490–562 AD), who wrote about the plague of Justinian that struck Mediterranean ports and coastal towns. First attempts to duplicate this natural phenomenon appeared in the eleventh century, when Chinese itinerant healers developed a method to prevent contracting potentially fatal smallpox. These healers were able to deliberately induce a milder transient pox illness through the medium of dried powder prepared from material recovered from a patient's healing skin pustules and blown into a recipient's nostrils. The practice disseminated along China-Persia-Turkey trade routes ultimately reached Europe and the American colonies following communications with England in 1714–1716 by Timoni, a Constantinople physician (3), and Pylorini, the Venetian counsel in Smyrna (Izmir) (4). Although effective in reducing susceptibility and incidence in epidemic attack, variolation presented difficulties; inoculations

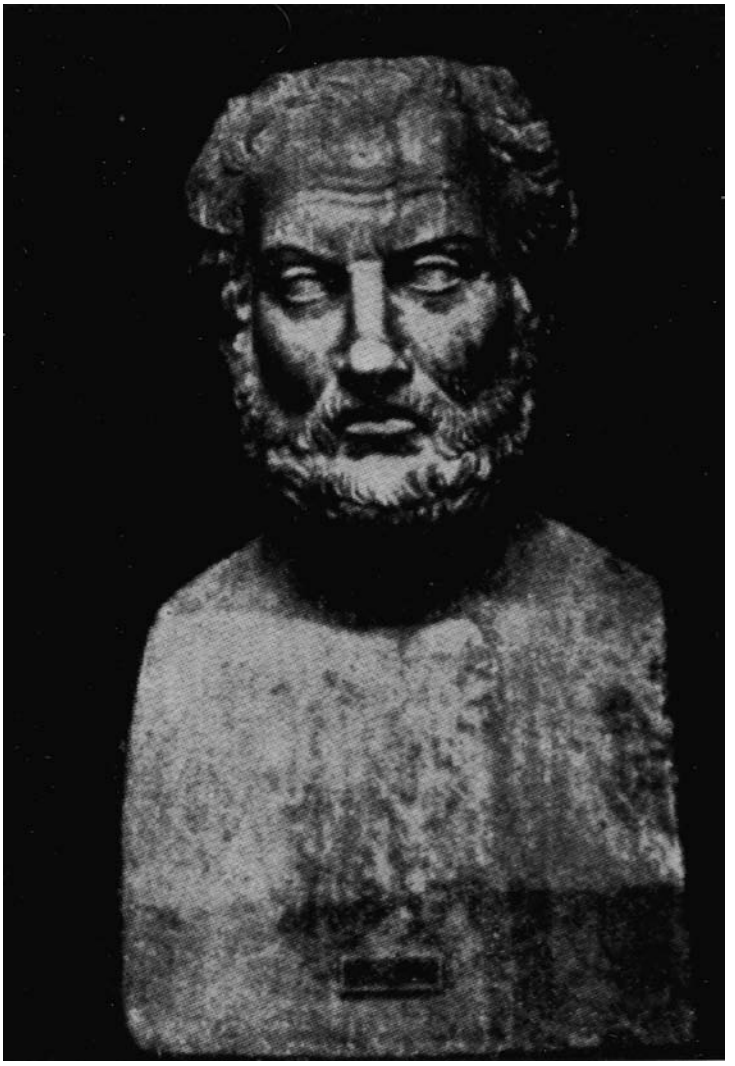

Figure 2 Thucydides (c. 460–400 BC). Greek historian. *Source*: From Gordon BL. Medicine Throughout Antiquity, 1949. Courtesy of F. A. Davis Company, Philadelphia.

sometimes resulted in severe, even fatal, primary illness and recipients could serve as sources of transmittable infection until all active lesions healed. A solution to the problem was found in the investigations of Jenner (Fig. 3), the English rural physician who in 1795 reported a new benign method to prevent smallpox by inducing a single pustule of a related, but different, skin disease, cowpox (vaccinia, from Latin word *vaccinus* meaning pertaining to a cow)—a lesion resembling smallpox only in appearance. From its name, the procedure became known as vaccination (5).

Jenner's carefully designed protocols carried out in 1796 stimulated experimental leads and raised a number of pertinent questions for future investigators: (*i*) Were disease-producing and protective (antigenic) qualities interdependent and equivalent? (Jenner had noted that some stored, presumably deteriorated, pox material did not evoke a vaccination lesion; however, he was unable to ascertain whether it still was capable of providing a protective effect.) (*ii*) Could two different agents share the ability to induce identical protective responses? (Jenner believed vaccination succeeded because smallpox and cowpox were different manifestations of the same disease.) (*iii*) Could the same agent induce both protection against disease and tissue injury? (Jenner's description of the appearance of a local inflammatory lesion after revaccination provided the earliest documentation of hypersensitivity phenomena as a function of the immune response.)

Figure 3 Edward Jenner, MD (1749–1823). Practicing physician in Cheltenham, rural England. *Source*: Courtesy of the National Library of Medicine.

Koch's and Pasteur's early endeavors to develop preventive vaccines were innovative giant steps in establishing immunization as an efficacious measure in disease prevention; they also served as models for later developments of allergen immunotherapy.

Pasteur's use of attenuated microorganisms as vaccines (*i*) in fowl cholera and sheep anthrax demonstrated that specific antigenic immunizing potential was not impaired by decreasing virulence of a bacterium (6). Later studies by Salmon and Smith (7) with heat-killed vaccines indicated that immunogenicity also did not require antigen viability.

Some unfortunate outcomes of early immunotherapeutic ventures temporarily hindered the future of immunotherapy with allergens. Koch was premature in introducing injectable preparations of glycerol extracts of tubercle bacilli cultures for the treatment of tuberculosis. His error revealed that violent systemic reactions could result from injection of antigens that acted as specific challenges in delayed hypersensitivity states (8). Pasteur's rabies vaccine met with enthusiastic success, but antigens of the rabbit spinal cords, used as culture medium for the aging rabies virus, also induced simultaneous production of antinervous tissue antibodies and adverse autoimmune neurological reactions (9).

Figure 4 Henry Sewall, MD, PhD (1855–1936). Professor and Chairman, Department of Physiology, University of Michigan. *Source*: From Webb GB, Powell D, Henry Sewall, Physiologist and Physician, 1946. Courtesy of Johns Hopkins, Johns Hopkins University Press, Baltimore.

> Practical approaches to immunization in the Western world might have had an earlier beginning had cognizance been taken of a centuries-old practice in Egypt. Dating back to antiquity, snake charmers in the temples—and later religious snake dancers among native Southwest American Indians—had found the key to protection from the danger of their craft. Beginning with self-inflicted bites from young snakes as sources of small amounts of venom, and progressing to repetition by large snakes led to tolerant outcomes of otherwise potentially fatal challenges. However, it was not until 1887 that Sewall's (Fig. 4) experimental inoculation of rattlesnake venom in an animal model introduced appreciation and development of antitoxins (10).

The discovery of diphtheria exotoxin (11) spurred the practice of inducing antitoxins in laboratory animals and their therapeutic use by passive immunization (12). The fact that the resultant antitoxins evolved into therapeutically effective agents was because of Ehrlich's (Fig. 5) studies on the chemical nature of antigen-antibody reactions and applications to biological standardization (13). Further, the methods by which antitoxins were obtained enabled early stages of development of allergen immunotherapy (14). Subsequently, development of severe life-threatening hypersensitivity reactions following injection of the antibodies in serum proteins of the actively immunized horse (15) created a virtually insurmountable obstacle in later attempts to initiate therapy of hay fever by passive immunization (14).

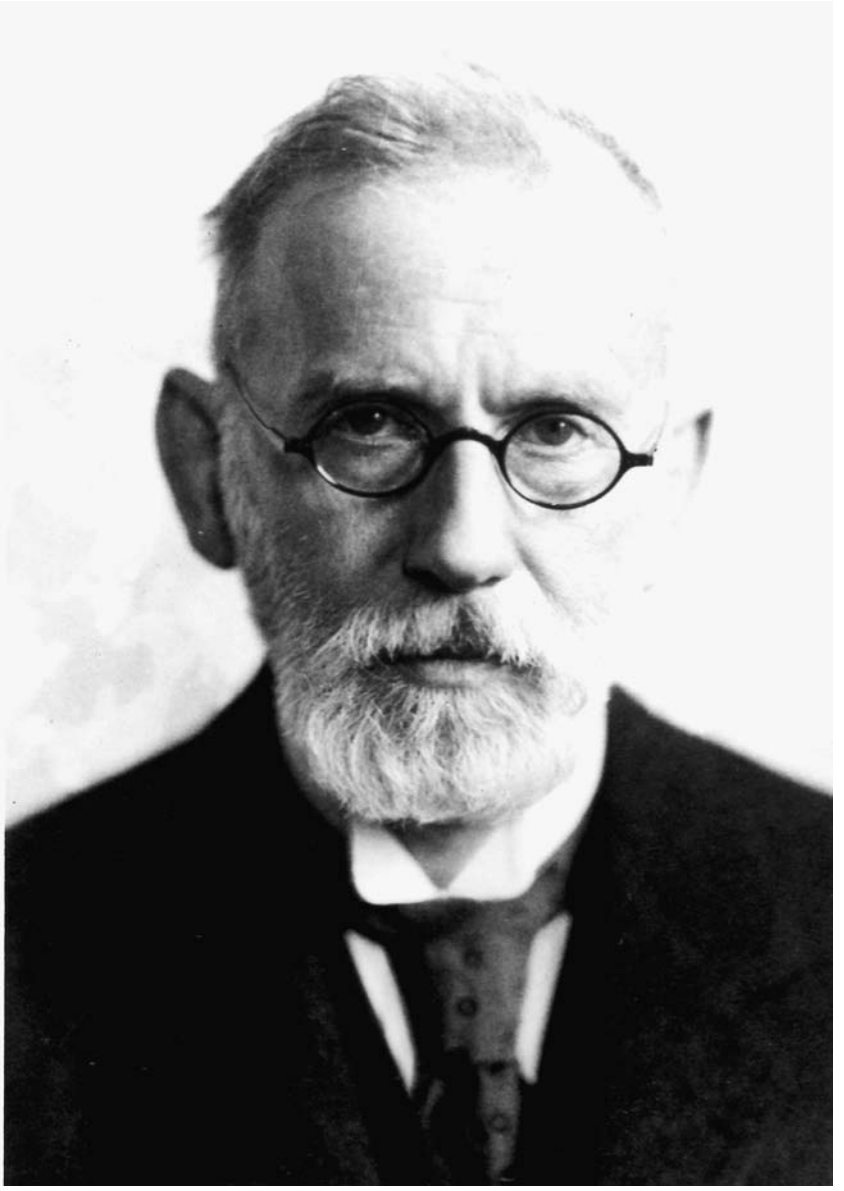

Figure 5 Paul Ehrlich, MD (1854–1915), Founding Director of the Institute for Experimental Therapy, Frankfurt. *Source*: Courtesy of the National Library of Medicine.

GENESIS OF ALLERGEN IMMUNOTHERAPY

Discoveries in immunity gave rise to another pioneering area of study within the newly established discipline, and the introduction of immunologically based therapies for infectious diseases soon followed. The impact of widening applications of immunotherapy was largely responsible, in the first half of the nineteenth century, for the evolution of allergy as a separate segment of medical practice. The forerunner of this relationship occurred in 1819, when Bostock, a London physician, precisely described his own personal experience and classical case history of hay fever (16). This landmark account of allergic disease was recorded only 23 years after Jenner's controlled demonstration of the ability of inoculation with cowpox to prevent smallpox (2).

Some 70-odd years after Bostock's report, Wyman identified pollen as the cause of autumnal catarrh in the United States (17). A year later, Blackley published confirmative descriptions on the basis of self-experimentation, which established that grass pollen was the cause of his seasonal catarrh, which was noninfective (18). He also made the first investigational reference to allergen immunotherapy when he repeatedly applied grass pollen to his abraded skin areas, but without resultant diminution of local cutaneous reactions or lessened susceptibility.

In 1900, Curtis reported that immunizing injections of watery extracts of certain pollens appeared to benefit patients with coryza and/or asthma caused by these pollens (19). Dunbar (Fig. 6) then attempted to apply the principle of passive immunization developed with diphtheria and tetanus antitoxin to the preventive treatment of human hay fever. He tried using "pollatin," a horse and rabbit antipollen antibody preparation. As a powder or ointment, it was developed for instillation in and absorption from the eyes, nose, and mouth and as pastille inhalational material for asthma (13). Subsequent attempts to immunize with grass pollen extract were abandoned because of severe systemic symptoms induced by excessive doses. Dunbar's associate, Prausnitz, had failed to diminish either the mucous membrane reactions or symptom manifestations of hay fever after "thousands" of ocular installations of pollen "toxin" (14). Dunbar then attempted immunization with pollen toxin-antitoxin (T-AT) neutralized mixtures—a technique that had been used with bacterial exotoxins (e.g., tetanus and diphtheria) (20).

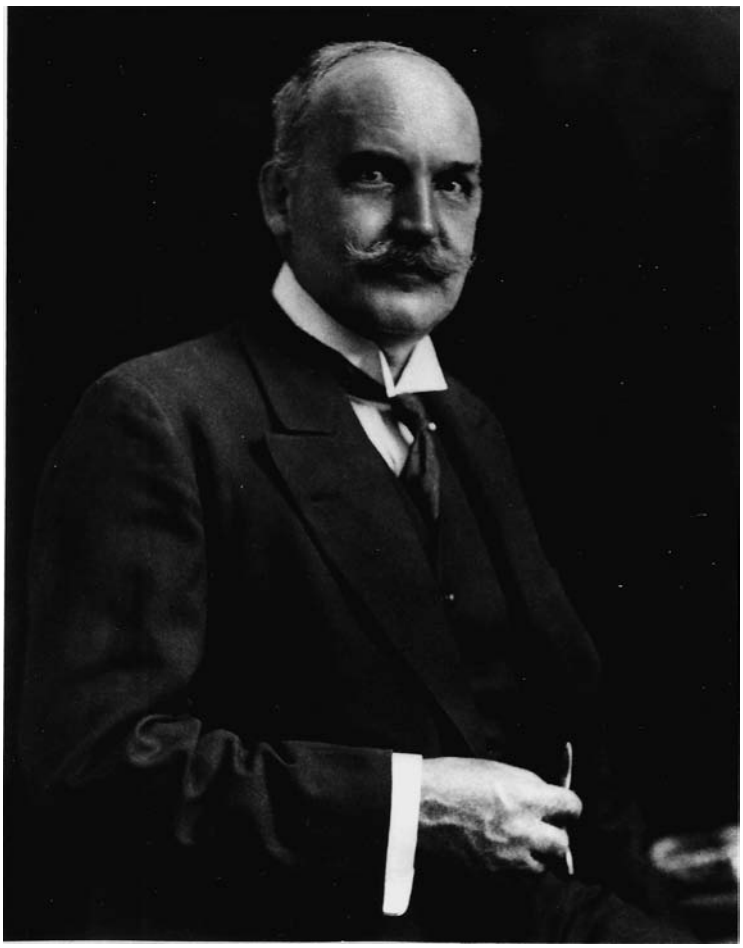

Figure 6 William Dunbar, MD (1863–1922). Director of the State Hygienic Institut, Hamburg. *Source*: Courtesy of the Hygienisches Institute, Hamburg, Germany.

While Dunbar's anecdotal reports of success could not be duplicated, the discovery of anaphylaxis formed a new concept of immunity and its relevance to immunotherapy. In 1902, Portier and Richet described anaphylactic shock and death in dogs under immunization with toxins from sea anemones (21). Four years later, these exciting and provocative animal experiments were followed by reports of sudden death in humans after the injection of horse serum antitoxins, and of exhaustive protocols with experimental animals that implicated anaphylactic shock as the likely mechanism (22). Smith made similar observations while standardizing antitoxins, which prompted Otto to refer to the findings as "the Theobald Smith Phenomenon" (23).

Wolff-Eisner applied the concept of hypersensitivity to a conceptual understanding of hay fever (24). Further, anaphylactically shocked guinea pigs were discovered to have suffered respiratory obstruction because of contraction and stenosis of bronchiolar smooth muscle that resulted in air trapping and distension of the lungs (25), similar to the characteristic pulmonary changes in human asthma. This finding led Meltzer to conclude that asthma was a manifestation of anaphylaxis (26). The role of the anaphylactic guinea pig as a suitable experimental model for the study of asthma was further enhanced by Otto's demonstration that animals that recovered from induced anaphylactic shock became temporarily refractory to a second shock-inducing dose (27). Additionally, Besredka (Fig. 7) and Steinhardt discovered that repeated injections of progressively larger, but tolerable, doses of antigen eventually protected sensitized guinea pigs from anaphylactic challenge (28). These results suggested that a similar injection technique might successfully desensitize the presumed human counterpart disorders of asthma and hay fever.

Investigational pursuit of active immunization for hay fever was soon begun in the laboratories of the Inoculation Department at St. Mary's Hospital in London, where Wright had provided the setting for interaction with visiting European masters of microbiology and

Figure 7 Alexandre Besredka, MD (1870–1940). Pasteur Institute, Paris. *Source*: Courtesy of the National Library of Medicine.

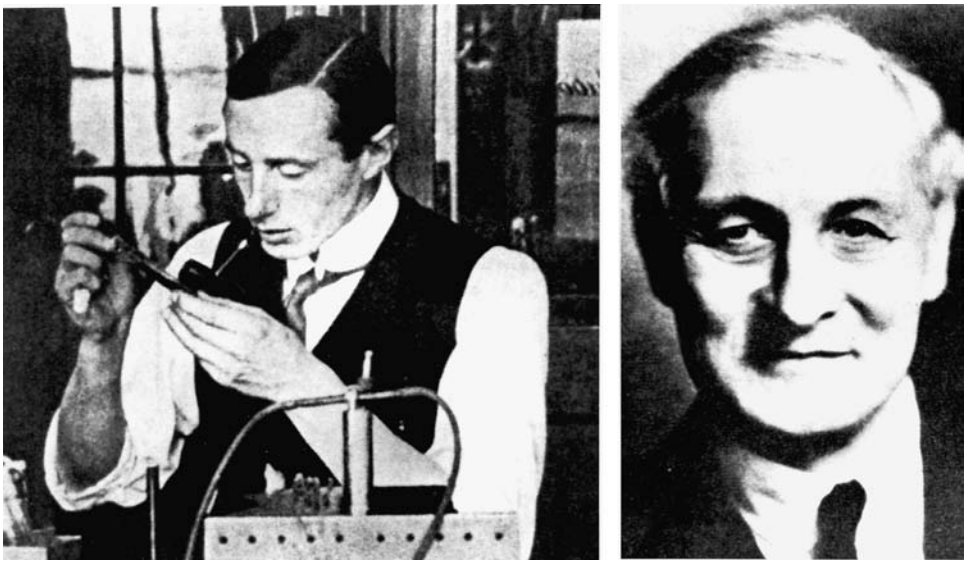

Figure 8 Leonard Noon, 1877–1913 (*left*) and John Freeman, 1877–1962, (*right*). Immunologists on staff, Inoculation Department, St. Mary's Hospital, London. *Source*: Courtesy of the College of Physicians of Philadelphia.

immunology, giving his students the opportunity to learn about the "new immunotherapy." Wright's enthusiasm was reflected in his frequent prediction that "the physician of the future may yet become an immunisator" (29).

Noon (Fig. 8), Wright's assistant, following Dunbar's concept, also believed that hay fever was caused by a pollen "toxin." To accomplish active immunization, he initiated clinical trials in 1910 with a series of subcutaneous injections of dosages of pollen extracts calculated on a pollen-derived weight basis (Noon unit), and thus introduced preseasonal immunotherapy. Noon's observations provided the following (still pertinent) guidelines: (*i*) a negative phase of decreased resistance develops after initiation of injection treatment; (*ii*) increased resistance to allergen challenge, measured by quantitative ophthalmic tests, is dose dependent; (*iii*) the optimal interval between injections is 1 to 2 weeks; (*iv*) sensitivity may increase if injections are excessive or too frequent; and (*v*) overdoses may induce systemic reactions (30). Noon's work was continued by his colleague, Freeman (Fig. 8), who in 1914 reported results of the first immunotherapeutic trial of 84 patients treated with grass pollen extracts during a three-year period. The protocols lacked adequate controls, but successful outcomes were recorded with acquired immunity lasting at least one year after treatment was discontinued (31). A cluster of related reports indicated that other clinical studies of immunization of hay fever patients by others had been underway, concurrently and independently (31–35).

Clinical and investigative aspects of this new modality were expanded with the beginnings of pioneering allergy clinics. The first in 1914 was started at the Massachusetts General Hospital by Joseph Goodale (Fig. 9), a rhinologist introduced to immunology at Robert Koch's Berlin Institute for Infectious Diseases. Francis Rackemann subsequently joined Goodale as clinic codirector. The next year, I. Chandler Walker (Fig. 10) initiated a clinic at Peter Bent Brigham Hospital, and in 1918, Robert Cooke (Fig. 11) at New York Hospital.

With the growing appreciation of pollens as allergens, the concept of pollen "toxin" faded and the objective of immunotherapy took on new meaning. Cooke, at a 1915 meeting at the New York Academy of Medicine, added his summary of favorable result—in a majority of 140 patients treated with pollen extracts (36)—to the series of 45 patients reported from Chicago by Koessler (34). Developments during the next 10 to 15 years were characterized by an eagerness to accept a continuing stream of favorable reports and adopt an arbitrary and relatively unquestioned technique of immunization therapy. A number of factors influenced the widespread use of this therapeutic method.

The scratch test introduced by Schloss in 1912 (37) was popularized by Walker (38) and by Cooke (36), who introduced the intracutaneous skin test technique in 1915. These new

Figure 9 Joseph L. Goodale (1868–1957), rhino-laryngologist and Associate Surgeon in Otolaryngology at Massachusetts General Hospital. In 1949, bringing hay fever patients into the hospital throat clinic for systematized study and treatment provided the nucleus for the first allergy clinic founded in the United States. *Source*: Courtesy of Robert L. Goodale, M.D.

Figure 10 I. Chandler Walker, MD (1883–1950). Founder of the first allergy clinic in the United States, at Peter Bent Brigham Hospital, Boston: Department of Medicine, Harvard Medical School. *Source*: Courtesy of Frederick E. Walker.

Figure 11 Robert A. Cooke, MD (1880–1960). Founding Director of the Institute of Allergy, Roosevelt Hospital, New York. *Source*: Courtesy of the National Library of Medicine.

diagnostic techniques obviated the need for the more limited ocular test site and permitted practical identification of a wide variety of allergenic substances that might be useful in treatment.

Development of methods of extracting allergenic fractions from foods and airborne and environmental materials was extensively pursued by Wodehouse and Walker (Fig. 10) at the Peter Bent Brigham Hospital in Boston (39,40) and by Coca at a newly established Division of Immunology of New York Hospital (41). A variety of injectable materials became available for the treatment of allergic patients whose problems were not exclusively seasonal.

Botanists identified and collected pollens of regional indigenous trees, grasses, and weeds, and developed methods for aerobiological sampling to provide the information and technology essential for specific diagnosis (42–46).

Hospital and clinic sections devoted to diagnosis and treatment of allergic disorders (47) were established. Immunization procedures were extended and applied to the treatment of asthma. With favorable results recorded in the treatment of seasonal asthmatic manifestations by pollen immunization, similar benefit was sought for chronic asthma by injections of extracts of perennial allergens and bacterial vaccines (38,48,49).

Medications capable of relieving allergic and asthmatic manifestations were relatively unavailable. During those early years, only epinephrine and atropine were mentioned as primary therapeutic agents and iodide, acetyl salicylate, anesthetic ether, morphine, and cocaine and their derivatives (with cautious qualifications) as secondary medications (50). The

pharmacological action of ephedrine, with its limited value, was not defined until 1924 by Chen and Schmidt (51).

The strong leadership of Cooke and the dedication of Coca provided opportunities for training, experience, and structured courses on preparation and use of allergenic vaccines (52). From these endeavors, an increasing number of clinics were seeded in the U.S. cities (53).

Rapid dissemination and application of the newly developed methods for identification of specific agents of hypersensitivity and desensitization therapy for hay fever and asthma patients engendered a new set of problems and questions complicating logical approaches well into the 1940s (53). The era of grant-supported full-time institutional-based academic and research positions in allergy and clinical immunology was then still some three to four decades away. Meanwhile, awaiting definition through research-generated data, there developed wide variability in ideas, criteria for indications, usage of materials, and methods and design of injection treatment plans. Adding to the complexity, a role for airborne mold spores as allergens was introduced by Storm van Leeuwen in 1924 (54), after a searching comprehensive study of the seasonal pollen problem. Thommen (Fig. 12) formulated a set of postulates that offered rational guidelines for the assessment of specific tree, grass, and weed species in the etiology of hay fever and as a source of immunotherapeutic agents (55): (*i*) The pollen must contain an excitant of hay fever. (*ii*) The pollen must be anemophilous or wind borne, as regards its mode of pollination. (*iii*) The pollen must be produced in sufficiently large quantities. It is characteristic of wind-pollinated flowers in general that they produce pollen in far greater quantities than do flowers, which are insect pollinated. (*iv*) The pollen must be sufficiently buoyant to be carried considerable distances. (*v*) The plant producing the pollen must be widely and abundantly distributed.

Principles of preseasonal pollen desensitization were then applied to treatment of patients troubled the year round with vaccines of a variety of perennial allergens that had given positive skin reactions. Of these, house dust as an agent was described by Kern in 1921 (56) and its role became increasingly recognized as an important environmental allergen in respiratory disease. The high prevalence of positive skin tests to dust vaccines initiated widespread use of stock and autogenous house dust vaccines for injection treatment of perennial rhinitis and asthma. Although there often was insufficient evidence to define the

Figure 12 August A. Thommen, MD (1892–1943). Director of Allergy Clinic, New York University College of Medicine. *Source*: Courtesy of New York Public Library.

allergenic activity of house dust, a positive skin test alone—without differentiation of irritant properties of test materials—was frequently accepted as indication for its use. Some confusion in differentiating house dust—sensitive disease from nonallergic chronic respiratory disease led Boatner and Efron to develop a "purified" house dust vaccine with the objective of increasing the diagnostic significance of a positive skin test to house dust (57).

There was an obvious need to develop suitable guidelines for efficacious injection treatment methods with a minimum of untoward constitutional reactions. Progress depended on the availability of vaccines of uniform strength and stability, Cooke attempted to bypass the problems of variations in allergenic activity of different pollen batches (because of seasonal plant growth factors and/or inadequate storage of collected pollen) by using an assay of total nitrogen content in standardization, although he did note that total nitrogen and allergenic activity were not identical (58,59). Subsequently, with a collaborating chemist, Stull, he developed and championed a unit on the basis of measurement of the content of protein nitrogen as a more accurate representation of residual stable activity of allergenic fractions (59).

Early treatment programs were developed by trial and error, and efficacy varied accordingly. In general, skin test reactivity was used for determination of starting dosages, their increments, and frequency of administration, Perennial rhinitis, and asthma mandated uninterrupted treatment schedules, but the superiority of perennial versus preseasonal plans for treatment of hay fever could not be settled by impressions and anecdotal reports. Modifications of schedule were devised for applying the principle of desensitization within compressed time frames. Pollen extract injections were given in small daily doses when initiated after seasonal symptoms had already begun (60). An intensive schedule of daily injections was required if initiated within two weeks of the anticipated seasonal onset (61,62). Other modes and variations for pollen desensitization were described in 1921–1922 (63–67): (*i*) daily nasal and throat sprays with atomized vaccines (63), (*ii*) pollen-containing ointments applied to the nasal mucosa (64), (*iii*) oral administration (65), (*iv*) intracutaneous injections (66), and (*v*) a full-cycle return to Blackley's attempt 50 years earlier by contact at needle-puncture or skin-abraded sites (67).

THE EARLY DEVELOPMENTAL YEARS

In 1931 the (Western) Association for the Study of Allergy and the (Eastern) Society for the Study of Asthma and Allied Conditions established a Joint Committee of Survey and Standardization that achieved one objective by the mid-1930s: approval of medical school and hospital allergy clinics to meet guidelines for allergy training developed by the committee (68). However, the committee was unable to define standards for methods and materials. A lack of correlation between skin test results and allergic manifestations had been noted in too many patients. Also, the committee believed that proper standardization must await the isolation and purification of etiologically responsible components of allergen vaccine such as Heidelberger and Avery had accomplished by isolating and purifying the specific soluble substances (capular polysaccharide) of the pneumococcus (69).

In 1992, Cooke reported that cutaneous reactivity was not eliminated in patients receiving injection treatments for asthma or allergic conditions because of horse and rabbit danders and sera. This contrasted with desensitization that accomplished complete inactivation of antibody action in animal models of anaphylaxis. Cooke, perceiving that the differences were functions of different mechanisms, referred to the beneficial effects of allergen injections as because of hyposensitization rather than neutralization or desensitization (70). This concept was confirmed in 1926 by Levine and Coca (71) and Jadassohn (72), both of whom found clinical improvement and allergen activity to be independent of effect, if any, on skin-sensitizing (reaginic) antibody. Levine and Coca's study also demonstrated that a rapid (two- to fourfold) increase in serum reaginic antibody sometimes followed allergen injections. This finding helped to explain some paradoxical observations in treatment programs that had been designed to lessen specific hypersensitivities. For example, (*i*) severe constitutional reactions followed small increments or even repeated previously well-tolerated dosages, especially in early stages of injection schedules (73); (*ii*) local tolerance diminished even with reduced

vaccine dosages; and (*iii*) symptoms of the treated allergic disorder might increase rather than decrease.

Freeman, in 1930, introduced "rush desensitization" in which injections of pollen vaccines were given at 1.5- to 2-hour intervals over a daily 14-hour period, under close observation and in a hospital setting (74). Since the benefits to be derived were generally believed to be outweighed by the danger of severe reactions, rush desensitization found little receptivity in the United States.

In 1935, Cooke's group, relocated in a new Department of Allergy at New York's Roosevelt Hospital, presented evidence in favor of a protective serum factor induced by injection treatments (75). Further, the transferable nature of the factor was indicated by Loveless's report that blood transfusions from ragweed-sensitive donors treated with pollen vaccine injections conferred equivalent beneficial effects on untreated ragweed-sensitive recipients during the hay fever season (76). This finding provided the lead for extended investigation centered at the target tissue cell level.

The ability of posttreatment serum to inhibit reactions between serum-containing reaginic antibody and corresponding pollen allergen at passively sensitized cutaneous test sites by the technique of Prausnitz and Kustner (P-K test reaction) (76) was attributed to the effects of "blocking antibody" induced by injection treatment (77). Demonstration, in specifically treated patients, of coexistent, characteristically different—sensitizing and blocking—antibodies provided both the technique and stimulus for continuing study of hyposensitization phenomena. Additionally, relevant contributions by Cooke and associates included demonstrations of (*i*) production of the inhibiting factor (blocking antibody) by nonallergic individuals as a function of normal immune responsiveness (75), (*ii*) specificity of blocking antibody activity and its relationship to the pseudoglobulin serum factor (77), and (*iii*) decreases in serum reagin titers after long-term allergen immunotherapy (78).

Fortuitously, in 1955, the impetus to search for alternative explanations coincident with the emergence of the National Institute of Allergy and Infectious Diseases (NIAID), a body within the National Institutes of Health, spurred the establishment of the requisite resources to support relevant research endeavors. In an early project, Vannier and Campbell undertook pertinent immunochemical studies on the allergenic fraction of house dust (79). A lead project, on the basis of a large multicenter collaborative study, later focused on the characterization of other allergens, and a working group was organized under Campbell's chairmanship. Ragweed was the selected prototype for initial investigation by subcommittees for chemistry, animal testing, and clinical trials. The subsequent isolation of the major allergenic fraction of ragweed pollen, designated as antigen E, provided the first quantifiable reagent for standardization of skin test and treatment extracts (80).

BACTERIAL VACCINES

A belief that nasopharyngeal bacterial flora were involved in the pathogenesis of the common cold led to a study in London in which Allen developed a respiratory bacterial vaccine (81). The possibility that the immunizing effect of such an autogenous preparation might be of value in the treatment of respiratory illnesses other than the common cold led to its application to hay fever. The introduction, in 1912–1913, of bacterial vaccines for the management of seasonal rhinitis was integrated with an attempt to ameliorate nasopharyngeal and paranasal sinus infection as presumed factors in hay fever (82). Morrey reasoned that a nasal mucosa strengthened by bacterial vaccination would be resistant to the effects of whatever irritants were responsible for hay fever (83). Lowdermilk, in 1914, followed up both reports and utilized both Noon's pollen toxin and Allen's bacterial vaccine formulations in his introduction of immunotherapy (35).

Goodale's report of skin test reactions to bacterial preparations in vasomotor rhinitis (84) was followed by great interest in putative relationships between bacteria and asthma (85,86). Walker, in popularizing the scratch test, extended the technique to a number of bacterial species along with pollens, perennial inhalants, and foods, and introduced autogenous vaccines into the treatment of asthma (86,87). The groundwork for adopting the concept of bacterial allergy was already in place. It centered around demonstrations of (*i*) induced

sensitization to bacteria in guinea pig models of anaphylaxis (88), and (*ii*) skin test and systemic reactivity to bacterial products associated with active infection (e.g., tuberculin) (89).

Further clinical relevance was provided by Rackemann's classic study, which defined intrinsic asthma (90) as a subset in patients with infective asthma, eosinophilia, and family backgrounds of extrinsic allergic diseases—a disorder later characterized by Cooke as presumptively immunologically mediated (91). Subsequent studies of treatment programs demonstrated lack of specificity of positive scratch, intracutaneous, and subcutaneous test reactions to bacterial preparations (92), as well as lack of specific or enhanced efficacy of autogenous over stock bacterial vaccines (93). Although the concept of desensitization or hyposensitization mechanisms as responsible for beneficial effects in infective asthma was put aside, respiratory bacterial vaccines continued to occupy a prominent place in clinical practice. Cooke related respiratory tract infection—especially chronic sinusitis—to asthma, and exacerbations of asthmatic symptoms to incremental overdosages of bacterial vaccine. On the basis of his experiences, he was a strong proponent of immunotherapy with autogenous vaccines as adjuvants for prevention of recurrences after removal of focal infection, particularly from the paranasal sinuses and upper respiratory tract (94).

Respiratory bacterial vaccines became entrenched immunotherapeutic agents. The first report of controlled trials, however, did not appear until 1955 (95): within the next four years, publication of two additional studies followed (96,97). Each failed to find efficacy for bacterial vaccines in attempts to prevent or treat asthma that was demonstrably related to respiratory infection. Following these reports, subsequent critical observations, and the diminishing influence of the earlier investigators whose uncontrolled impressions had influenced the clinical scene, respiratory bacterial vaccines slowly fell out of favor.

CLINICAL TRIALS

A new initiative cut to the heart of the accepted role of allergen immunotherapy when Lowell—whose in-depth experience and analytical probing added credibility to his position—heralded the need for sound investigation to meet the requirements of statistical significance (98). A valid and unbiased evaluation of results of allergen immunotherapy, especially of pollenosis, was not available because controls for the many variables of periodic disease were found lacking in published trials. Sample sizes were too limited for tests of significance, and inconsistent seasonal, climatic, environmental, and biologically fluctuating factors had not been subjected to adequately controlled study.

"Controlled" studies presented during the preceding 10 years (99–101) were all found to be flawed. Reliance on historical features had not been replaced by placebo controls; double blinding of both subject and evaluator had not been followed; a single test group often consisted of pretreatment and newly entered patients; and comparable groups had not always been balanced for equivalent sensitivities (e.g., by skin test titrations). Lowell and Franklin then performed a double-blind trial of treatment of allergic rhinitis because of ragweed sensitivity. They reported that patients receiving injections of ragweed pollen vaccine had fewer symptoms and lower medication scores than a control group. The beneficial effect was specific for ragweed, and the effect diminished in varying degrees within 5 months after discontinuing treatments (102). The following year, Fontana et al. reported that any beneficial effect of hyposensitization therapy in ragweed hay fever in children was indistinguishable from differences likely to occur in untreated controls (103). Their study, however, looked only for the presence or disappearance of symptoms, rather than at comparable degrees of severity (104).

Immunotherapy gained credibility with the introduction of new evaluatory measurements [i.e., symptom index score and the in vitro measure of leukocyte histamine release (105)], especially in children (106).

ANTIGEN DEPOTS

During the late 1930s, allergen vaccines were modified in an effort to decrease the frequency of injections. Depot-like immunogenic materials were prepared to provide a slow, continuous release of allergen from injection sites. The first attempt used ground raw pollen suspended in

olive oil (107). Because particulate bacterial vaccines and modified toxoid proved to be effective immunogens, soluble pollen allergen vaccines next were converted to particulate suspensions by alum precipitation and alum adsorption (108,109). Other modifications included acetylation, heat, and formalin treatment (109), precipitation by tannic (110) and hydrochloric acids (111), and mixture with gelatin (112). Of these, only alum-adsorbed pollen extracts gained any popularity. Treatment of hay fever with an emulsified allergen vaccine was introduced by Naterman, who, in 1937, emulsified a pollen extract with lanolin and olive oil (113). Thirteen years later, he suspended grass and ragweed pollen tannates in peanut oil with aluminum monostearate (114). Malkiel and Feinberg, encouraged by evidence of slow absorption from new penicillin-in-oil depot formulations, prepared extracts of ragweed in sesame oil–aluminum monostearate. With these, however, they were unable to avoid constitutional reactions, while failing to reduce severity of symptoms (115). Furthermore, other investigators detected increased titers of neutralizing antibody in treated patients without clinical benefit, thus casting doubt on the clinical relevance of "blocking" antibody (116,117).

Clinical trials with repository therapy, initiated by Loveless in 1947 (118), gave highly favorable results as reported 10 years later (119). This stimulated the first major departure from conventional injection treatment schedules. Loveless, firmly believing that successful treatment was a function of induced "blocking" antibody, aimed her protocols at maintaining the highest possible humoral levels of blocking antibody. She was convinced that the threshold of conjunctival responses to graded local challenges was a valid measure of systemic sensitivity and that suppression of both depended on the generation of neutralizing factor. Although there were no data to equate desired results with those reported for influenza vaccine (120), she used the depot medium that Freund and McDermott had developed (121) as an immunogen adjuvant in experimental animal models. A large dose of pollen vaccine, calculated as the cumulative total that would be given in the course of a conventional preseason schedule, was emulsified in oil with an emulsion stabilizer, and administered as a single intramuscular injection (118,119). A number of anecdotal reports by Brown spoke of "thousands" of uniformly successful results of treatment with emulsified vaccines of pollen and other airborne allergens (122). However, adverse reactions consisting of late formation and persistence of nodules, sterile abscesses and granulomata, and a potential for induction of delayed hypersensitivity to injected antigens were found inherent in emulsion therapy. Furthermore, subsequent controlled studies failed to confirm significant therapeutic effectiveness (123–125). Finally, emulsion therapy was discontinued after a report that mineral oil and mineral oil adjuvants induced plasma cell myelomas in a certain strain of mice (126) and the U.S. Food and Drug Administration did not approve the repository emulsion for therapy.

ORAL ROUTE TO TOLERANCE AND DESENSITIZATION

Possibilities for inducing protection by feeding on causative agents date back to stories of poisons in antiquity. In the first century BC, Mithradates VI (131–63 BC) (Fig. 13), King of Pontus in Asia Minor, noted that ducks who fed on plants known to be poisonous to humans did not manifest any apparent ill effects. Applying this observation, he incorporated ducks' blood in an antidote he attempted to develop against poisons—an early concept of passive immunization. Further, in preparing himself for the ever-present possibility of a palace revolt, Mithradates sought to gain immunity from poisoning by swallowing small amounts of poisons—particularly toadstool toxins—in gradually increasing dosages (127). So successful was the outcome of his experiments that he later failed to achieve attempted suicide by ingesting large doses of the same poisons (128). For many subsequent centuries, the technique of gaining tolerance or active immunity through incremental dosage schedules continued to be known as mithradatising.

The renowned Greek physician who practiced in Rome, Claudius Galen (130–200 AD), had noted that snake venoms taken by mouth were devoid of the systemic toxic actions effected by snake bites (129). According to folklore, this knowledge allowed snake charmers of the classic Greco-Roman era to acquire protection against potentially fatal bites by drinking

Figure 13 Mithradates VI Eupator (c. 131–63 BC), King of Pontus in Asia Minor. *Source*: Courtesy of the Musee de Louvre, Paris.

from serpent-infested waters that contained traces of their venoms (130)—a less traumatic method than seeking protection through self-inflicted bites.

Moving to a more recent era and the beginning of the scientific study of immunity, in 1891 Ehrlich provided experimental evidence of orally achieved toxin tolerance in mice by feeding them the toxins ricin and abrin (131). Then germane to delayed hypersensitivity, in 1946 Chase demonstrated an inhibiting effect of prior feeding (132). The earliest recorded journal item of clinical relevance was noted in a description of plant-induced allergic contact dermatitis in 1829 (133). In his discussion, Dakin reported that chewing poison ivy leaves, both as a prevention and a cure, was recommended by some "good meaning, marvelous, mystical physicians," despite adverse side effects—eruption, swelling, redness, and intolerable itching around the verge of the anus. It was also a practice seen among native North Americans (Indians), who chewed and swallowed the juice of early shoots as a preventive against the development of poison ivy dermatitis during ensuing summer months (134). Apparently, this method had been found to be of some value since it was used in rural areas and by park

workers, and considered an example of effective homeopathic autotherapy (135). A novel modification reported partial immunity after drinking milk from cows deliberately fed poison ivy in grass mixtures (136).

The first move to explain the procedure that originated in folk medicine in terms of immune phenomena began with the approach of Strickler in 1918. Although unable to demonstrate circulating blood antibodies in patients affected by poison ivy and poison oak dermatitis, Strickler postulated the likely pathogenesis to be a form of "tissue immunity" to the plant toxins. Believing the mechanism to be similar to that of hay fever, he introduced an adaptation of desensitization for treatment and prevention of the plant-related contact dermatitis with extracts of the alcohol-soluble leaf fraction given by intramuscular injection (137). The following year, Schamberg introduced an oral approach to prophylactic desensitization utilizing incremental drop dosages of a tincture of *Rhus toxicodendron* (138). Strickler's follow-up report three years later indicated favorable acceptance of intramuscular injection, oral methods, and a combination of both (134). Although trials during subsequent years supported this early usage (139), there were differing reports varying from only short-term immunizing effects (140) to lack of either clinical benefit (141) or increased tolerance (142).

Despite divergence of opinion, the oral method of preventive therapy remained popular for 50-some years. Alcohol and acetone extracts in vegetable oils were prepared from a variety of plant source polyhydric phenols (e.g., the Rhus ivy-oak-sumac group, primula, geranium, tulip, and chrysanthemum). In 1940, Shellmire expanded the spectrum of plant sources of delayed hypersensitivity by identifying ether-soluble fractions of pollens responsible for producing allergic contact dermatitis through airborne exposure. These were distinct from water-soluble pollen albumins implicated in the immediate hypersensitivity phenomenon of hay fever. Through Shellmire's work, preparations of specific pollen oleoresins were then made available for oral desensitization (143).

Proponents in the 1940s and 1950s based their belief in the validity of desensitization methods for plant contact dermatitis on the concept of cell-associated "antibody" to chemical haptens in the pathogenesis of delayed cutaneous hypersensitivity. However, there were complicating problems in the nature of induced dermatitis at locally injected or previously involved distal sites, exacerbations of existing lesions, stomatitis, gastroenteritis, anal pruritus, and dermatitis from mucous membrane contact with oral preparations. Additionally, in the face of lack of convincing evidence of efficacy, the practice gradually faded from popular usage.

On a parallel track, similar thought was being given to treatment of another group of allergic disorders that Coca in 1923 characterized as atopic—hay fever, asthma, and eczema. The first case record of desensitization to an allergenic food came from England, in 1908, with Schoffield's report of successful reversal of severe egg-induced asthma, urticaria, and angioedema in a 13-year-old boy by the daily feedings of egg in homeopathic doses (144). Three years later, Finzio, in Italy, reported similar success with cow's milk in infants (145). Shortly thereafter, favorable results of trials of desensitization to foods in children were reported in the United States by Schloss—in a study that coincidentally established practicability of the scratch test in hypersensitivity (37)—and in work by Talbot (146). Because of possible anaphylactic reactions to only a minute amount of an allergenic food in an exquisitely sensitive individual, Pagniez and Vallery-Radot, in 1916, prefed patients with food digests consisting predominantly of peptones. Theoretically, these foods were reduced in allergenicity by the treatment process but retained immunogenic specificity (147,148). Acceptance of oral food desensitization plans declined with later negative experiences (149,150).

The first use of an orally administered pollen-related preparation appeared in the homeopathic literature of 1890 with the description of "ambrose," a tincture of fresh flower heads and young shoots, recommended for the treatment of hay fever (151). Impressed by an experience in which asthma caused by inhalation of ipecac was prevented with drop doses of syrup or tincture of ipecac, Curtis explored a like possibility in hay fever. In 1900—in conjunction with introduction of flower and pollen vaccines—he noted preliminary efficacious results with tincture and fluid extracts of ragweed flowers and pollen taken by mouth (152). Touart later reported varying responses in six patients given enteric-coated tablet triturates of grass and ragweed pollen (65). In 1927, Black demonstrated that large doses of orally administered ragweed extract effectively lowered nasal threshold responses to inhalational

challenges (153), but later reported a large series of patients with results less favorable than could be expected after injection treatments (154). Urbach attempted to bypass distressing gastrointestinal symptoms following ingestion of pollen vaccines by advocating oral administration of specific pollen digest peptones (propetan) (155). Since collection of pollen supplies was difficult, Urbach prepared peptone derivatives of blossoms of trees, grasses, and grass seeds for use as orally administered allergens (156). Passive transfer experiments by Bernstein and Feinberg calculated that more than a pound of raw pollen would be required orally to reach a circulating antigen concentration obtained by injection of maximally tolerated doses of pollen vaccine (157). Additionally convincing lack of efficacy confirmed by a later multicenter, collaborative, placebo-controlled study followed (158).

DRUGS AND BIOLOGICAL PRODUCTS

The purported effectiveness of oral desensitization to foods was soon applied to drug hypersensitivity, and a report of successful oral desensitization of a malaria patient with anaphylactic hypersensitivity to quinine appeared in the French literature (159). When the allergenic character of pharmaceutical and biological products derived from plant and animal sources became increasingly evident, attempts were made to desensitize reactive patients who otherwise would be deprived of essential specific therapy. An early problem was treatment of the horse-sensitive patient with horse antidiphtheria or antitetanus antiserum (160). The cautious injections of horse dander vaccine offered some measure of protection after long-term treatment (161). However, the potential for anaphylaxis resulting from the large volumes of therapeutic antisera required was too great. Even a minute dose could cause a fatal reaction (162), and early trials had failed to accomplish desensitization (163,164).

Success was achieved in use of dried and pulverized ipecacuanha plant root for treatment of ipecac-sensitive asthmatic pharmacists and physicians and of beef or pork insulin for desensitization injection of sensitive diabetics who required insulin replacement therapy (165,166).

Freeman's method of "rush inoculation" with pollen vaccines (74) was not generally accepted. However, the principle was effectively applied in treating drug hypersensitivities requiring prompt resumption of therapy, such as with insulin to control diabetes (167) and penicillin when required as the essential antibiotic to control a specific and severe infection (168). This procedure probably induced transient anaphylactic desensitization, as first demonstrated in the guinea pig (28), or by mechanism of hapten inhibition (169). Over 40 to 50 years, a number of publications affirmed effective desensitization to pharmaceutical products responsible for hypersensitivity reactions (170–172).

INSECT ANTIGENS

In classical Greece of the fourth century BC, the philosopher-biologist Aristotle, who had written extensively on the life history, types, and behavior patterns of bees, in his *Historia Animalia* noted their ability to sting large animals to death—even one as large as a horse. Yet it was recognized that beekeepers in the course of their work could be repeatedly or periodically stung without ill effect. No attempt was made to duplicate this observed natural phenomenon until the early years of the twentieth century, when the possibility of ameliorating insect hypersensitivity was provided by the description of favorable responses to injection treatments with extracts of gnats (173) and bees (174). Hyposensitization to other species was also explored using mosquito (175) and flea (176) extracts. Some failed attempts were not understood until the acquisition of knowledge that delayed (cell-mediated) hypersensitivity and biochemistry of inflammation were responsible mechanisms.

Whether hypersensitivity-induced states owed their reduction to the raising of blocking antibodies or to later defined mechanisms of regulatory control of IgE production, elements of cell-mediated immunity did not lend themselves to comparable diminishing effects sought in allergen immunotherapy for immediate hypersensitivity disorders.

Fine hairs and epithelial scales shed by swarming insects were also identified as airborne allergens responsible for conjunctivitis, rhinitis, and asthma, which could be managed by

hyposensitization (177,178). Benson reported extensive studies of Hymenoptera allergy and hyposensitization with whole-body vaccine. Efficacy of treatment was demonstrated for anaphylactic sensitivity to the venom of stings and for inhalant allergy to body parts and emanations incurred by exposed beekeepers (179). Hyposensitization therapy employed whole-body vaccines until Loveless—based on her discovery and definition of neutralizing (blocking) factor as therapeutically responsible for the efficacy of pollen hyposensitization in hay fever—sought the same objective for the Hymenoptera-anaphylactically sensitive patient. She then introduced several variations: (*i*) use of isolated contents of dissected venom sacs in conventional hyposensitization schedules, (*ii*) single repository immunization with venom emulsified in oil adjuvant, (*iii*) "rush" desensitization, and (*iv*) deliberate controlled stinging with captured wasps to ascertain establishment and maintenance of a protective state (180,181). Later studies confirmed the far greater efficacy of venom allergens (chap. 14)

NONSPECIFIC IMMUNOTHERAPY

Attempts were made to duplicate the benefits of specific hyposensitization by altering, initiating, or regulating immune system function through injections with a variety of nonspecific antigens (e.g., typhoid and mixed coliform vaccines, cow's milk, snake venom, soybean, and creation of a sterile fixation abscess with injection of turpentine) (182,183). It was thought that repeated injections of small doses of protein-digested peptones might evoke subclinical anaphylactic mechanisms with resultant desensitization to a multiplicity of allergens (184).

Another global approach employing the administration of autogenous blood visualized that injected (autohemato- and autoserotherapeutic) samples contained absorbed causative allergens in quantities too small to produce an attack, yet sufficiently minutely antigenic to induce tolerance (185).

Another indirect approach considered possible benefits that might be derived from attempted hyposensitization responses to antigens to which specific sensitization resulted from past infection but were concurrently inactive and unrelated to the etiology of asthma. Two such agents—tuberculin (186) and the highly reaginic and anaphylactic antibody-inducing extract of *Ascaris lumbricoides* (187)—were given to correspondingly positive skin test reactors according to conventional hyposensitization schedules.

If unable to accomplish specific hyposensitization, therapy attempted to neutralize the alleged mediator of allergic reactions (i.e., histamine). Histamine "desensitization" was first introduced in 1932 for treatment of cold urticaria in the expectation that daily incremental injections would achieve correspondingly increased degrees of tolerance to histamine and thereby diminish allergic symptoms (188). Enzymatic destruction of released histamine in urticaria and atopic dermatitis was then attempted with parenteral or oral administration of histaminase (189). An immune-mediated blocking of histamine was postulated through injections of a histamine-linked antigen [(histamine-azo-depreciated horse serum) hapamine] to induce antihistamine antibodies (190). While some of these modalities were initially encouraging, later studies failed to confirm their benefit. Favorable symptomatic improvements of empirical but nonspecific, treatment designed to modulate immune functions could not be determined without controlled clinical trials. The use of these agents fell by the wayside as new scientific knowledge of mechanisms of allergy were acquired (191).

CONCLUDING COMMENTS

In this review of the evolution of allergen immunotherapy (Table 1) as a method introduced into clinical medicine almost a century ago, two retrospective considerations are particularly noteworthy. The first relates to the several decades of trial and error, recorded observations, and the transition from loosely conducted trials to controlled clinical investigative protocols. Relevant knowledge of the value of allergen immunotherapy was not advanced much beyond appreciation that varied approaches helped some treated patients, some of the time, to variable degrees. Establishing a requisite informational base still looks to (*i*) epidemiological studies of a scope and design to provide in-depth understanding of the natural history of asthma and

Table 1 Pioneering Highlights Along the Pathway to the Development and Understanding of Allergen Immunotherapy

Time	Observation/finding	Credit
430 BC	First recorded perception of immunity; recovery from plague-endowed protection from repeated attack.	Thucydides
63 BC	Oral tolerance: method derived from repetitious ingestion of incremental, minute, subtoxic doses of plant poisons (127).	Mithradates VI
1712–1776	Variolation: ancient oriental method, introduction of induced active immunity (2,3).	Emanuel Timoni, Giacomo Pilorini
1798	Vaccination: immunity induced through biologically related inoculum (4).	Edward Jenner
1880–1884	Immune responses not dependent on pathogenicity (1) or viability (7) of inocula.	Louis Pasteur, Daniel Salmon, and Theobold Smith
1880	Conceptual method for exhausting susceptibility to hay fever by repetitious application of pollen to abraded skin (18).	Charles Blackley
1897	Immunizing method derived from inoculation series of minute sublethal doses of rattlesnake venom (10).	Henry Sewall
1890	Passive immunization with tetanus and diphtheria antitoxins; introduction of therapeutic antisera (12).	Shibasaburo Kitasato and Emil von Behring
1891–1907	Adverse outcomes: hypersensitivity disorders mediated by immunizing agents.	
	Severe nonantibody reactions to biological product of disease agent tuberculin (89); systemic cell-mediated delayed hypersensitivity.	Robert Koch
	Anaphylaxis; immediate hypersensitivity mechanism (21).	Paul Portier and Charles Richet
	Systemic foreign serum sickness (14) and local tissue reaction (Arthus phenomenon) (192); antigen-antibody complex mechanism.	Clemens von Pirquet and Béla Schick; Maurice Arthus
1897	Standardization of diphtheria antitoxin; introduction of concept of biological standardization with application to immunogens and antisera (13).	Paul Ehrlich
1903	Conceptual immunization for hay fever with grass pollen "toxin" (proteid isolate) and foreign species antisera (13).	William Dunbar
1907–1913	Protection against anaphylactic challenges: animal models. "Antianaphylaxis"; transient desensitization following recovery from anaphylactic shock because of temporary depletion of anaphylactic antibody (127).	Richard Otto
	Temporary protection (desensitization) induced by repeated subanaphylactic doses of antigen through neutralization or exhaustion of anaphylactic antibody (28).	Alexandre Besredka
	"Masked anaphylaxis," partial refractory state: antigen prevented from reaching shock tissue by excess of circulating anaphylactic antibody (193).	Richard Weil
1911–1914	First reported successful immunization against grass pollen "toxin" for hay fever (30,31).	Leonard Noon and John Freeman
1917–1919	"Injection treatments" for desensitization expanded to allergens beyond pollens (38).	I. Chandler Walker
1917	Development of techniques for extraction of allergens: availability of expanded testing and treatment reagents made available (39,40).	Roger Wodehouse
1919	Oral tolerance to plant oil-soluble fraction agent of contact dermatitis: derivitive modification of Native American preventive practice of chewing "poison ivy" shoots (134,137).	Jay Schamberg
1921	Differentiation between antibodies (Ab) involved in states of hypersensitiveness and desensitization: anaphylactic Ab, precipitin, and atopic reagin (194).	Arthur Coca and Ellen Grove
1922	"Desensitization" by procedure of Besredka in an anaphylactic animal model not attainable in human hypersensitiveness objective of hyposensitization" (70).	Robert Cooke
1922	Constitutional reactions from hyposensitization injection treatments: cause, nature, and prevention (73).	Robert Cooke

(*Continued*)

Table 1 Pioneering Highlights Along the Pathway to the Development and Understanding of Allergen Immunotherapy (*Continued*)

Time	Observation/finding	Credit
1922	Identification of house dust as a ubiquitous allergen: expanded scope of hyposensitization programs for the treatment of perennial rhinitis and asthma (195).	Robert Cooke
1926	Increase in serum reaginic antibodies following hyposensitization injection treatments explaining nature of reactions to injections of pollen vaccines (196).	Philip Levine and Arthur Coca
1932	Arbitrary incorporation of bacterial vaccines in hyposensitization treatments influenced by concept of immunological mechanism in infective asthma (91).	Robert Cooke
1933	Laboratory technique of assay of allergenic vaccines: protein nitrogen unit standardization for guide to hyposensitization schedule (197).	Arthur Stull and Robert Cooke
1935	Identification of blocking antibody as a product of hyposensitization treatment: its chemical and immunological differentiation and inhibiting action on atopic reagin + allergen, (75).	Robert Cooke and Arthur Stull
1937	Guideline for prevention of precipitin-mediated serum disease by desensitization: contraindication in coexisting presence of atopic reagins to foreign species antisera (198).	Louis Tuft
1940	Depot allergenic vaccines for delayed absorption: alum adsorption (109).	Arthur Stull, Robert Cooke, and William Sherman
1947–1957	Repository adjuvant therapy with single injection of water-in-oil emulsified vaccine (118,119).	Mary Loveless
1956	Desensitization to anaphylactic challenge of stinging insect venom (180).	Mary Loveless
1962	Densitization to anaphylactic drug hypersensitivity in penicillin model explained by hapten-inhibition mechanism.	Charles Parker and Herman Eisen
1967–1987	Identification and assay of immunoglobulin E as the reaginic antibody (199) and function of a cytokine, IL-4, in its synthesis (200); presenting new vistas for exploring applications of cellular and molecular immunological phenomena to allergen immunotherapy through regulatory control of IgE.	Kimishiga and Teruko Ishizaka; William Paul

allergic disease, and (*ii*) large-scale clinical trials from which to construct critical criteria for exact indications, and use of materials and methods by which immunotherapeutic regimens can be properly evaluated.

Second is awareness of the enormous impact and influence that allergen immunotherapy had on the launching, development, and continuation of allergy as a medical specialty. For 40 to 50 years following the original description of skin test and hyposensitization techniques, these modalities served as the mainstays of allergy when there was little else to offer in the way of adequate and feasible management. So firmly had arbitrary patterns of allergen immunotherapy been implanted in clinical practice that only recently was an internationally representative effort made to sort out bias and unproven impressions from verifiable fact, and an attempt made to reach consensus (191).

This review, then, leaves allergen immunotherapy with a major question: With the advent of newer, effective symptom-relieving pharmacological agents and new relevant knowledge on chemical mediators of inflammation, were the empirical aspects of allergen immunotherapy perpetuated beyond justification? At the same time, this consideration leaves the history of allergen immunotherapy in the midstream of new technologies in molecular biology, informational advances, and research opportunities. Current interests and activities in the design of modified antigens of enhanced efficacy, immunochemical characterization and standardization of allergen vaccines, and definition of responsible immune mechanisms and targeted responses ultimately may provide answers to questions pursued by a century of pioneering research in biomedical science—particularly immunochemistry and cellular immunology—and clinical investigation. Later chapters deal with many of these relevant advances.

SALIENT POINTS

Although "injection treatments" with pollen vaccines were introduced into clinical practice in the early 1900s, development of the method is rooted in the genesis and evolution of immune function dating back to antiquity. An appreciation of allergen immunotherapy viewed in this historical context follows.

Immunity, as a naturally occurring phenomenon, was recognized as early as the fifth century BC, with the observation that those who recovered from epidemic illness during the plague of Athens were not similarly stricken a second time (2).

By applications of the principles of nature, prototype methods introduced the phenomenon of induced immunity as a result of deliberate exposure to causative agents: (*i*) tolerance to plant poisons by ingestion of subtoxic doses (Mithradates VI, 63 BC) and (*ii*) protection from smallpox by contact with material recovered from disease lesions (variolation; eleventh-century Chinese healers).

Modification of variolation introduced methods for inducing immunity with reduced risk by inoculations of (*i*) biologically related agent of mild disease [vaccination (4)], (*ii*) nonpathogenic attenuated microorganisms (1), and (*iii*) killed bacteria (7). Although relatively harmless procedures, inocula demonstrated potential for producing inflammatory effects concurrent with immunity (later defined as sensitization mechanisms).

Demonstration of protection of an animal model from lethal snake venom by inoculation series of sublethal doses (10) provided the introductory approach to the development of methods for immunization against microbial toxins and identification of the antibody product, antitoxin, in blood serum (12).

Systemic shock reaction of anaphylaxis—discovered as an adverse effect of immunization (21)—provided animal models for the study of hypersensitivity as an aberrant immune phenomenon (22); particularly relevant was the challenged-sensitized guinea pig whose respiratory manifestations suggested a counterpart expression of human hay fever and asthma. Discovery of refractory state following recovery from shock—attributed to temporary depletion of anaphylactic antibody (23)—led to development of the method of "desensitization" by repeated injections of incremental tolerated doses of antigens (28).

In the erroneous belief that seasonal hay fever was caused by grass pollen toxin, serial injections of pollen solutions—designed to induce immunity by production of serum antitoxin—introduced the concept of allergen immunotherapy (30,31). This method was subsequently defined as an approach to reverse sensitization to pollen proteins and expanded in scope by employing vaccines derived from a variety of airborne seasonal and perennial allergens (39,40).

Serum factors associated with hypersensitivity and desensitization treatments were differentiated as skin-sensitizing antibody (ssa) and precipitating antibody (pa), respectively (194). Detection of concurrent induction of pa and increase in levels of ssa—identical with naturally occurring atopic disease reagins—following injections of allergen vaccines accounted for local and constitutional reactions associated with therapy (71).

Desensitization, as effected in animal anaphylactic models, when recognized as not attainable in allergen immunotherapy, aimed at the objective of inducing diminished (hypo) sensitization (70). Studies of antibody raised by allergen-hyposensitizing injections demonstrated its chemical properties and its "blocking" of reactions of skin-sensitizing (reaginic) antibodies with allergens to explain putative responsible immune mechanisms (75).

Demonstrated adjuvant effect of allergen vaccine incorporated in oil-in-water emulsion (76) had the inherent potential for inducing plasma cell neoplastic proliferation as a function of hyperimmunization (126), and was thus contraindicated in allergen immunotherapy.

Desensitization of anaphylactic drug reactivity (e.g., penicillin and insulin) was accomplished by a special rush protocol of immunotherapeutic injections designed to effect the mechanism of hapten inhibition (169).

ACKNOWLEDGMENTS

In the search and collection of original source material, we drew heavily upon the resources of the National Library of Medicine (NLM) and the archival and special collections of the NLM History of Medicine Division (HMD). For valued interactions and expert assistance graciously

extended by information specialists of the Library Reference Section and HMD staff, our many thanks and special appreciation. We also gratefully acknowledge and thank Patricia E. Richardson, NIAID editorial assistant, for dedicated technical skills and assistance in the assembly and organization of materials from which this chapter was constructed.

REFERENCES

1. Pasteur L. De l'attenuation du virus du cholra des poules. C R Acad Sci III 1880; 91:673.
2. Thucydides., Ch. 47. In: Smith CF, trans. The Peloponesian War—Book 2. Vol 2. Cambridge, MA: Harvard University Press, 1958:54.
3. Timoni E. A letter containing the method of inoculating the small pox; practiced with success at constantinople. Philos Trans R Soc London 1714; 339:72.
4. Pylarinum J. Nova et tuta Variolas per Transplantatonem Methodus, nuper inventa et in ufurn tracta. Philos Trans R Soc London 1716; 347:393.
5. Jenner E. An Inquiry into the Causes and Effects of the Variolae. London: Sampson Low, Soho, 1798.
6. Pasteur L, Chamberland C, Roux E. Compte Rendu Sommaire des experiences faites a' Pouilly-le-Fort, pres' Melun, sur la vaccination charboneusse. C R Acad Sci III 1881; 92:1378.
7. Salmon DE, Smith T. On a new method of producing immunity from contagious diseases. Proc Biol Soc Wash 1884/86; 3:29.
8. Koch R. Forsetzung der Muttheilungen "uber ein Hermittel gegen Tuberculose. Dtsch Med Wochenschr 1891; 9:101.
9. Pasteur L. Method pour prevenir la rage apres' morsure. C R Acad Sci III 1885; 101:765.
10. Sewall H. Experiments on the preventive inoculation of rattlesnake venom. J Physiol 1887; 8:205.
11. Roux PPE, Yersin AEJ. Contribution a' l'etude de la diphterie. Ann Inst Pasteur 1889; 2:629.
12. Behring EA von, Kitasato S. Ueber das zustandekommen der diphtherie-immunitat und der tetanus-immunitat bei thieren. Dtsch Med Wochenschr 1890; 16:1113.
13. Ehrlich P. Die Wertbestimmunung des Diphtherieheislserums. Klin Jb 1897; 6:299.
14. Dunbar WP. The present state of our knowledge of hay-fever. J Hyg 1902; 13:105.
15. Pirquet von Cesenatico C P, Schick B. Die Serumkrankheit. Vienna: F. Deutch, 1905.
16. Bostock J. Case of periodical affection of the eyes and chest. Med Chir Trans 1819; 10:161.
17. Wyman M. Autumnal Catarrh. Cambridge, MA: Hurd and Houghton, 1872.
18. Blackley CH. Hay Fever; Its Causes, Treatment, and Effective Prevention. London: Balliere, 1880.
19. Curtis HH. The immunizing cure of hay fever. Med News 1900; 77:16.
20. Park WH. Toxin-antitoxin immunization against diphtheria. J Am Med Assoc 1922; 79:1584.
21. Portier P, Richet C. De l'action anaphylactique de certains venins. CR Soc Biol 1902; 54:170.
22. Rosenau MJ, Anderson JF. A study of the cause of sudden death following the injection of horse serum. In: Hygienic Laboratory Bulletin 29. Washington, DC: Government Printing Office, 1906.
23. Otto R. Das Theobald Smithsche Phenomenon der Serum-Veberfindlichkeit. In: Gendenkschr. f.d. verstorb Generalstabsarzt, Vol 1. Berlin: von Leuthold, 1906:153.
24. Wolff-Eisner A. Das Heufieber. Munchen: J. F. Lehman, 1906.
25. Auer J, Lewis PA. The physiology of the immediate reaction of anaphylaxis in the guinea pig. J Exp Med 1910; 12:151.
26. Meltzer SJ. Bronchial asthma as a phenomenon of anaphylaxis. J Am Med Assoc 1910; 55:1021.
27. Otto R. Zur frage der serum-ueberempfindlichkeit. Munch Med Wochenschr 1907; 54:1664.
28. Besredka A, Steinhardt E. De l'anaphylaxie et de l'antianaphylaxie vis-a-vis due serum de cheval. Ann Inst Pasteur 1907; 21:117, 384.
29. Colebrook L. Almoth Wright. Provocative Doctor and Thinker. London: William Heinemann Medical Books Ltd., 1954:61.
30. Noon L. Prophylactic inoculation against hay fever. Lancet 1911; 1:1572.
31. Freeman J. Vaccination against hay fever; report of results during the last three years. Lancet 1914; 1:1178.
32. Clowes GHA. A preliminary communication on certain specific reactions exhibited in hay fever cases. Proc Soc Exp Biol Med 1913; 10:70.
33. Lowdermilk RC. Personal Communication to Duke WW. Cited in Duke WW. Allergy. Asthma, Hay Fever, Urticaria and Allied Manifestations of Reaction. St Louis: Mosby, 1925:222.
34. Koessler KK. The specific treatment of hayfever by active immunization. Ill Med J 1914; 24:120.
35. Lowdermilk RC. Hay-fever. J Am Med Assoc 1914; 63:141.
36. Cooke RA. The treatment of hay fever by active immunization. Laryngoscope 1915; 25:108.
37. Schloss OM. A case of allergy to common foods. Am J Dis Child 1912; 3:341.
38. Walker IC. Studies on the sensitization of patients with bronchial asthma (Study series III-XXXVI). J Med Res 1917:35–37.

39. Wodehouse RP. Immunochemical study and immunochemistry of protein series. J Immunol 1917; 11:VI. cat hair, 227; VII. horse dander, 237; VIII. dog hair, 243.
40. Wodehouse RP. IX. Immunochemical studies of the plant proteins: Wheat seed and other cereals. Am J Botany 1917; 4:417.
41. Coca AF. Studies in specific hypersensitiveness. XV. The preparation of fluid extracts and solutions for use in the diagnosis and treatment of the allergies, with notes on the collection of pollens. J Immunol 1922; 7:163.
42. Goodale JL. Preliminary notes on the anaphylactic skin reactions exacted in hay fever subjects by the pollen of various species of plants. Boston Med Surg J 1914; 171:695.
43. Goodale JL. Pollen therapy in hay fever. Boston Med Surg J 1915; 173:42.
44. Wodehouse RP. Hay Fever Plants. Waltham, MA: Chronica Botanica Co., 1945.
45. Durham OC. The contribution of air analysis to the study of allergy. J Lab Clin Med 1925; 13:967.
46. Unger L, Harris MC. Stepping Stones in Allergy. Minneapolis, MN: Craftsman Press, 1975:75.
47. Cohen SG. Firsts in allergy. N Engl Reg Allergy Proc 1983; 4:309; 1984; 5:48; 5:247.
48. Cooke RA. Protein sensitization in the human with special reference to bronchial asthma and hay fever. Med Clin North Am 1917; 1:721.
49. Cooke RA. Studies in specific hypersensitiveness. New etiologic factors in bronchial asthma. J Immunol 1922; 7:147.
50. Duke WW. Allergy, Asthma, Hay Fever, Urticaria and Allied Manifestations of Reaction. St. Louis: C. V. Mosby, 1925:237–241.
51. Chen KK, Schmidt CF. The action of ephedrine, the active principle of the Chinese drug MaHuang. J Pharmacol Exp Ther 1924; 24:192.
52. Cohen SG. Firsts in allergy: IV. The contributions of Arthur F. Coca, M. D. (18751959). N Engl Reg Allergy Proc 1985; 6:285.
53. Cohen SG. The American Academy of Allergy, An historical review. J Allergy Clin Immunol 1976; 64:322–466.
54. Storm van Leeuwen W. Bronchial asthma in relation to climate. Proc R Soc Med 1924; 17:19.
55. Thommen AA. Etiology of hay fever: Studies in hay fever. N Y State J Med 1930; 30:437.
56. Kern RA. Dust sensitization in bronchial asthma. Med Clin North Am 1921; 5:751.
57. Boatner CH, Efron BG. Studies with antigens. XII. Preparation and properties of concentrates of house dust allergen. J Invest Dermatol 1942; 5:7.
58. Cooke RA. Human sensitization. J Immunol 1916; 1:201.
59. Stull A, Cooke RA, Tenant J. The allergen content of protein extracts; its determination and deterioration. J Allergy 1933; 4:455.
60. Thommen AA. The specific treatment of hay fever. In: Coca AF, Walzer M, Thommen AA, eds. Asthma and Hay Fever in Theory and Practice. London: Balliere, Tindall, & Cox, 1931; 757–774.
61. Bernton HS. Plantain hay fever and asthma, J Am Med Assoc 1925; 84:944.
62. Kahn IS, Grothaus EM. Studies in pollen sensitivities. Med J Rec 1925; 121:664.
63. MacKenzie GM. Desensitization of hay fever patients by specific local application. J Am Med Assoc 1922; 78:787.
64. Caulfield AHW. Desensitization of hay fever patients by injection and local application. J Am Med Assoc 1922; 79:125.
65. Touart MD. Hay fever; desensitization by ingestion of pollen protein. N Y Med J 1922; 116:199.
66. Phillips EW. Relief of hay fever in intradermal injections of pollen extracts. J Am Med Assoc 1922; 79:125.
67. Le Noir P, Richet C Jr. Renard. Skin test for anaphylaxis. Bull Soc Med Hop 1921; 45:1283 (abstr); J Am Med Assoc 1921; 77:1770.
68. American Academy of Allergy, report of the Joint Committee on Standards. J Allergy 1935; 6:408.
69. Heidelberger M, Avery OT. The soluble specific substance of pneumococcus. J Exp Med 1924; 40:301.
70. Cooke RA. Studies in specific hypersensitiveness, IX. On the phenomenon of hyposensitization (the clinically lessened sensitiveness of allergy). J Immunol 1922; 7:219.
71. Levine P, Coca A. Studies in hypersensitiveness. J Immunol 1926; XX. A quantitative study of the interaction of atopic reagins and atopen. 11:411; XXII. On the nature of alleviating effect of the specific treatment of atopic conditions. 11:449.
72. Jadassohn W. Beitrage zun idosynkrasie problem. Klin Wochenschr 1926; 5(2):1957.
73. Cooke RA. Studies in specific hypersensitiveness. III. On constitutional reactions: The dangers of the diagnostic, cutaneous test and therapeutic injection of allergens. J Immunol 1922; 7:119.
74. Freeman J. Rush inoculation with special reference to hay fever treatment. Lancet 1930; 1:744.
75. Cooke RA, Barnard JH, Hebald S, Stull A. Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). J Exp Med 1935; 62:733.
76. Loveless MH. Application of immunologic principles to the management of hay fever, including a preliminary report on the use of Freund's adjuvant. Am J Med Sci 1947; 214:559.

77. Cooke RA, Loveless M, Stull A. Studies on immunity in a type of human allergy (hay fever): serologic response of non-sensitive individuals to pollen injections. J Exp Med 1937; 66:689.
78. Sherman WB, Stull A, Cooke RA. Serologic changes in hay fever cases treated over a period of years. J Allergy 1940; 11:225.
79. Vannier WE, Campbell DH. The isolation and purification of purified house dust allergen fraction. J Allergy 1959; 30:198.
80. King TP, Norman PS. Isolation studies of allergens from ragweed pollen. Biochemistry 1962; 1:709.
81. Allen RW. The common cold: Its pathology and treatment. Lancet 1908; 2(1):1589; (2):1689.
82. Farrington PM. Hay fever. Memphis Med J 1912; 32:381.
83. Morrey CB. Vaccination with mixed cultures from the nose in hay fever. J Am Med Assoc 1913; 61:1806.
84. Goodale JL. Preliminary notes on skin reactions excited by various bacterial proteins in certain vasomotor disturbances of the upper air passages. Boston Med Surg J 1916; 174:223.
85. Walker IC. Studies on the sensitization of patients with bronchial asthma to bacterial proteins as demonstrated by the skin reaction and the methods employed in the preparation of those proteins. J Med Res 1917; 35:487.
86. Walker IC. The treatment with bacterial vaccines of bronchial asthmatics who are not sensitive to proteins. J Med Res 1917; 37:51.
87. Walker JW, Adkinson J. Studies on staphylococcus pyogenes aureus, albus and citreus and on Micrococcus tetragenous and M. catarrhalis. J Med Res 1917; 35:373 (subsequent articles in this series appeared in 35:391, 36:293).
88. Kraus R, Doerr R. Uber bacterienanaphylaxie. Wien Klin Wochenschr 1908; 21:1008.
89. Koch R. Fortsetzung der muttheilungen uber ein Heilmittel gegen Tuberculose. Dtsch Med Wochenschr 1891; 9:101.
90. Rackemann FM. A clinical study of one hundred and fifty cases of bronchial asthma. Arch Intern Med 1918; 22:552.
91. Cooke RA. Infective asthma: indication of its allergic nature. Am J Med Sci 1932; 183, 309.
92. Walzer M. Asthma. In: Coca AF, Walzer M, Thomen AA, eds. Asthma and Hay Fever in Theory and Practice Springfield, IL: Charles C. Thomas, 1931:260–261.
93. Hooker SB, Anderson LM. Heterogeneity of streptococci isolated from sputum with active critique on serological classification of streptococci. J Immunol 1929; 16:291.
94. Cooke RA. Infective asthma with pharmacopeia. In: Cooke RA, ed. Allergy in Theory and Practice Philadelphia: W. B. Saunders Co., 1947:151–152.
95. Frankland AW, Hughes WH, Garrill RH. Autogenous bacterial vaccines in the treatment of asthma. Br Med J 1955; 2:941.
96. Johnstone DE. Study of the value of bacterial vaccines in the treatment of bronchial asthma associated with respiratory infections. Am J Dis Child 1957; 94:1.
97. Helander E. Bacterial vaccines in the treatment of bronchial asthma. Acta Allergy 1959; 13:47.
98. Lowell FC. American Academy of Allergy Presidential Address. J Allergy 1960; 31:185.
99. Brun E. Control examination of specificity of specific desensitization in asthma. Acta Allergol 1949; 2:122.
100. Frankland AW, Augustin R. Prophylaxis of summer hay-fever and asthma: Controlled trial comparing crude grass-pollen extracts with isolated main protein component. Lancet 1954; 1:1055.
101. Johnstone DE. Study of the role of antigen dosage in treatment of pollenosis and pollen- asthma. Am J Dis Child 1957; 94:1.
102. Lowell FC, Franklin W. A double blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. N Engl J Med 1965; 273:675.
103. Fontana VC, Holt LE Jr., Mainland D. Effectiveness of hyposensitization therapy in ragweed hay-fever in children. J Am Med Assoc 1967; 195:109.
104. Lowell FC, Franklin W, Fontana VJ, et al. Hyposensitization therapy in ragweed hay fever. J Am Med Assoc 1966; 195; 1071 (lett).
105. Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hay fever with ragweed antigen E: Comparisons with whole pollen extracts and placeboes. J Allergy 1968; 42:93.
106. Sadan N, Rhyne MB, Mellits ED et al. Immunotherapy of pollenosis in children. Investigation of the immunologic basis of clinical improvement. N Engl J Med 1969; 280:623.
107. Sutton C. Hay fever. Med Clin North Am 1923; 7:605.
108. Zoss AR, Koch CA, Hirose RS. Alum-ragweed precipitate: Preparation and clinical investigation; preliminary report. J Allergy 1937; 8:829.
109. Stull A, Cooke RA, Sherman WB et al. Experimental and clinical studies of fresh and modified pollen extracts. J Allergy 1940; 11:439.
110. Naterman H. The treatment of hay fever by injections of suspended pollen tannate. J Allergy 1941; 12:378.
111. Rockwell G. Preparation of a slowly absorbed pollen antigen. Ohio State Med J 1941; 37:651.

112. Spain W, Fuchs A, Strauss M. A slowly absorbed gelatin-pollen extract for the treatment of hay fever. J Allergy 1941; 12:365.
113. Naterman HL. The treatment of hay fever by injections of pollen extract emulsified in lanolin and olive oil. N Engl J Med 1937; 218:797.
114. Naterman HL. Pollen tannate suspended in peanut oil with aluminum monostearate in the treatment of hay fever. J Allergy 1950; 22:175.
115. Malkiel S, Feinberg SM. Effect of slowly absorbing antigen (ragweed) on neutralizing antibody titer. J Allergy 1950; 21:525.
116. Gelfand HH, Frank DE. Studies on the blocking antibody in serum of ragweed treated patients. II. Its relation to clinical results. J Allergy 1944; 15:332.
117. Alexander HL, Johnson MC, Bukantz SC. Studies on correlation of symptoms of ragweed hay fever and titer of thermostable antibody. J Allergy 1948; 19:1.
118. Loveless MH. Application of immunologic principles to the management of hay fever, including a preliminary report on the use of Freund's adjuvant. Am J Med Sci 1947; 214:559.
119. Loveless MH. Repository immunization in pollen allergy. J Immunol 1957; 79:68.
120. Henle W, Henle G. Effect of adjuvants of vaccination of human beings against influenza. Proc Soc Exp Biol Med 1945; 59:179.
121. Freund J, McDermott K. Sensitization to horse serum by means of adjuvants. Proc Soc Exp Biol Med 1942; 49:548.
122. Brown EA. II. The treatment of ragweed pollenosis with a single annual emulsified extract injection. Ann Allergy 1958; 16:28 through XI. Tree pollenosis effects of single annual injections of emulsified extracts in 560 multiply allergic patients. Ann Allergy 1960; 18:1200.
123. Feinberg SM, Rabinowitz HI, Pruzanski JJ et al. Repository antigen injections. J Allergy 1960; 31:421.
124. Sherman WB, Brown EB, Karol ES et al. Respository emulsion treatment of ragweed pollenosis. J Allergy 1962; 33:473.
125. Arbesman CE, Reisman RE. Hyposensitization therapy including repository: A double blind study. J Allergy 1964; 35:12.
126. Potter M, Boyce ER. Induction of plasma cell neoplasms in strain BALB/c mice with mineral oil and mineral oil adjuvants. Nature 1962; 193:1086.
127. Pliny. Pliny Natural History. Book 15, Vol 7. Jones WHS, trans. Cambridge, MA: Harvard University Press, 1956:139.
128. Appian. Chap. 16 Appian's Roman History. Book 12. White H, trans. Cambridge, MA: Harvard University Press, 1962:453.
129. Galen. De Temperamentis. Coxe JR. Writing of Hippocrates a) Id Galen (epitomized from the original Latin translation). Philadelphia: Lindsay and Blakiston, 1846:493.
130. Pliny. Cited by Urbach E, Gottlieb PM. Allergy. New York: Grune & Stratton, 1943:252.
131. Ehrlich P. Experimentelle intersuchungen uber immunitat. Dsch Med Wochenschr I. Uber ricin, 1891; 17:976; II. Uber abrin. 1891; 17:1218.
132. Chase MW. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc Soc Exp Biol Med 1946; 61:257.
133. Dakin R. Remarks on a cutaneous affliction produced by certain poisonous vegetables. Am J Med Sci 1829; 1:98.
134. Strickler A. The toxin treatment of dermatitis venenata. J Am Med Assoc 1921; 77:910.
135. Duncan CH. Autotherapy in ivy poisoning. J Am Med Assoc 1916; 104:901.
136. Diffenbach WW. Treatment of ivy poisoning. South Cal Pract 1917; 32:91.
137. Strickler A. The treatment of dermatitis venenata by vegetable' toxins. J Cutan Dis 1918; 36:327.
138. Schamberg JF. Desensitization against ivy poisoning. J Am Med Assoc 1919; 73:1213.
139. Blank JM, Coca AF. Study of the prophylactic action of an extract of poison ivy in the control of Rhus dermatitis. J Allergy 1936; 7:552.
140. Molitch M, Poliakoff S. Prevention of dermatitis venenata due to poison ivy in children. Arch Derm Syph 1936; 33:725.
141. Bachman LC. Prophylaxis of poison ivy: Use of an almond oil extract in children. J Pediatr 1938; 12:31.
142. Sompayrac LM. Negative results of rhus antigen treatment of experimental ivy poisoning. Am J Med Sci 1938; 195:361.
143. Shelmire B. Contact dermatitis from vegetation. Patch testing and treatment with plant oleoresins. South Med 1940; 38:337.
144. Schoffield AT. A case of egg poisoning. Lancet 1908; 1:716.
145. Finzio G (1911). Anaf. familiare per il latte di mucca. Tentativie di terapia antianaf. Pediatria 1911; 19:641.
146. Talbot FB. Asthma in children, III. Its treatment. Long Island Med J 1917; 11:245.
147. Pagniez P, Vallery-Radot P. Etude physiologique et therapeutique d'un cas d'urticaire geante. Anaphylaxie et anti-anaphylaxie alimentaires. Nouv Presse Med 1916; 24:529.

148. Luithlen F. Ueberempfindlichkeit und ernahrungstherapie. Wien Med Wschnsehr 1926; 76:907.
149. Rowe AH. Desensitization to foods with reference to propeptanes. J Allergy 1931; 3:68.
150. Rowe AH. Food Allergy. Its Manifestation and Control and the Elimination Diets, A Compendium. Springfield, IL: Charles C Thomas, 1972:71.
151. Wrightman HB, discussion of Iliff EH, Gay LN. Treatment with oral ragweed pollen. J Allergy 1941; 12:601.
152. Curtis HH. The immunizing cure of hay fever. Med News 1900; 77:16.
153. Black JH. The oral administration of pollen. J Lab Clin Med 1927; 12:1156.
154. Black JH. The oral administration of ragweed pollen. J Allergy 1939; 10:156.
155. Urbach E. Desensibilisiering pollen ullergischer individuen auforalem wege mittels art- spezitischer pollenpeptone. Klin Wochenschr 1931; 10:534.
156. Urbach E. Die biologiche behandlung des henfiebers. Munchen Med Wchnschr 1937; 84:488.
157. Bernstein TB, Feinberg SM. Oral ragweed pollen therapy Clinical results and experiments in gastrointestinal absorption. Arch Intern Med 1938; 62:297.
158. Feinberg SM, Foran FL, Lichtenstein ML. Oral pollen therapy in ragweed pollinosis. J Am Med Assoc 1940; 115:231.
159. Heran J, Saint-Girans F. Un cas d'anaphylaxie a la quinine chez un paludeen intolerance absolus et urticaria. Antianaphylaxie par voie gastrique. Paris Med 1917; 7:161.
160. Goodale JL. Anaphylactic reactions occurring in horse asthma after the administration of diphtheria antitoxin. Boston Med Surg J 1914; 170:837.
161. Feinberg SM. Allergy in Practice. Chicago: Year Book Publishers. 1946; 536.
162. Boughton TH. Anaphylactic deaths in asthmatics. J Am Med Assoc 1912; 73:1912.
163. Kerley CG. Accidents in foreign protein administration. Arch Pediatr 1917; 34:457.
164. Tuft L. Fatalities following injection of foreign serum; report of unusual case. Am J Med Sci 1928; 175:325.
165. Widal F, Abrami P, Joltrain E. Anaphylaxie a l'ipeca. Presse Med 1922; 32:341.
166. Jeanneret R. Desensitization in insulin urticaria. Rev Med Suisse Rom 1929; 49:99 (Abstr J Am Med Assoc 1929; 92:2197).
167. Corcoran AC. Note in rapid desensitization in a case of hypersensitiveness to insulin. Am J Med Sci 1938; 196:357.
168. Reisman RE. Rose NR, Witebsky E et al. Penicillin allergy and desensitization. J Allergy 1962; 33:178.
169. Parker CW, Shapiro J, Kern M, Eisen HN. Hypersensitivity to penicillenic acid derivatives in human beings with penicillin allergy. J Exp Med 1962; 115, 821.
170. O'Donovan WJ, Klorfajn I. Sensitivity to penicillin: Anaphylaxis and desensitization. Lancet 1946; 2:444.
171. Peck SM, Siegel S, Bergamini R. Successful desensitization in penicillin sensitivity. J Am Med Assoc 1947; 134:1546.
172. Crofton J. Desensitization to streptomycin and P. A. S. Br Med J 1953; 2:1014.
173. Clewes, cited by Freeman J. Toxic idiopathies; the relationship between hay and other pollen fevers, animal asthmas, food idiosyncracies, bronchial and spasmotic asthma, etc. Proc R Soc Med 1919–1920; 13:129.
174. Braun LIB. Notes on desensitization of a patient hypersensitive to bee stings. South Afr Med Rec 1925; 23:408.
175. Benson RL. Diagnosis and treatment of sensitization to mosquitoes. J Allergy 1936; 8:47.
176. Mclvor BC, Cherney LS. Studies in insect bite desensitization. Am J Trop Med 1941; 21:493.
177. Parlato SJ. A case of coryza and asthma due to sand flies, J Allergy 1929; 1:35.
178. Figley KD. Asthma due to May fly. Am J Med Sci 1929; 178:338.
179. Benson RL. Semenov H. Allergy in its relation to bee sting. J Allergy 1930; 1:105.
180. Loveless MH, Fackler WR. Wasp venom allergy and immunity. Ann Allergy 1956; 14:347.
181. Loveless MH. Immunization in wasp-sting allergy through venom-repositories and periodic insect stings. J Immunol 1962; 89:204.
182. Walzer M. Asthma. In: Coca AF, Walzer M, Thommen AA, eds. Asthma and Hay Fever in Theory and Practice. London: Balliere, Tindall & Cox, 1931; 297–304.
183. Feinberg SM. Allergy in Practice. Chicago: Year Book Publishers, 1946; 544–553.
184. Auld AG. Further remarks on the treatment of asthma by peptone. Br Med J 1918; 2:49.
185. Kahn MH, Emsheimer HW. Autogenous defibrinated blood in the treatment of bronchial asthma. Arch Intern Med 1916; 18:445.
186. Storm Van Leewuen W, Varekamp H. On the tuberculin treatment of bronchial asthma and hay fever. Lancet 1921; 2:1366.
187. Brunner M. In: Coca AF, Walzer M, Thommen AA, eds. Asthma and Hay Fever in Theory and Practice. London: Balliere, Tindall & Cox, 1931; 301–302.
188. Bray GW. A case of physical allergy: A localized and generalized allergic type of reaction to cold. J Allergy 1932; 3:367.

189. Laymon CW, Cumming H. Histaminase in the treatment of urticaria and atopic dermatitis. J Invest Dermatol 1939; 2:301.
190. Sheldon JM, Fell N, Johnson JH et al. A clinical study of histamine azoprotein in allergic disease: A preliminary report. J Allergy 1941; 13:18.
191. Thompson RA, Bousquet J, Cohen SG et al. Current status of allergen immunotherapy. Shortened version of World Health Organization/International Union of Immunological Societies Working Group Report. Lancet 1989; 1:259.
192. Arthus M. Injections rep&tees de serum de cheval chez le lapin. CR Soc Biol (Paris) 1903; 55:817.
193. Weil R. The nature of anaphylaxis and the relations between anaphylaxis and immunity. J Med Res 1913; 27:497.
194. Coca AF, Grove EF. Studies in hypersensitiveness XII. A study of the atopic reagins. J Immunol 1925; 10:445.
195. Cooke RA. Studies in specific hypersensitiveness; new etiologic factors in bronchial asthma. J Immunol 1922; 7:147.
196. Levine P, Coca AF. Studies in hypersensitiveness XXII. On the nature of the alleviating effect by the specific treatment of atopic conditions. J Immunol 1926; 11:449.
197. Stull A, Cooke RA, Tenant J. The allergen content of pollen extracts: Its determination and deterioration. J Allergy 1933; 4:455.
198. Tuft L. Clinical Allergy. Philadelphia: Saunders, 1937;739.
199. Ishizaka K, Ishizaka T. Identification of gamma-E antibodies as a carrier of reaginic antibody. J Immunol 1967; 99:1187.
200. Paul W, Ohara J. B-cell stimulatory factor-I/interleukin 4. Ann Rev Immunol 1987; 5:429.

2 Definition of An Allergen (Immunobiology)

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INTRODUCTION

A variety of terms are used to define the substance that stimulates an atopic reaction. Which words are used depends on the terms chosen to denote the sensitivity (1). In the context of a general immunological reaction, the triggering substance is called an antigen. In modern usage, an antigen is any substance that induces a state of sensitivity and/or resistance as a result of coming into contact with appropriate tissues of an animal body (2). The observed sequence—exposure to substance, latency period, manifestation of substance-specific sensitization upon reexposure to substance—is characteristic of immunological memory, and indicates that B cells and/or T cells are involved.

In 1923, Coca and Cooke coined the word "atopy" to describe a type of sensitized state (3); through the intervening years, the term subsequently has been defined as an adverse immune reaction involving immunoglobulin E (IgE). The term "allergen" has been used to define the substance that induces specific IgE antibodies; exposure can be by inhalation, ingestion, tactile contact, or by injection. Thus, allergens are defined in terms of the body's response to them. Interestingly, not all individuals have a demonstrable IgE response to "known" allergens. That there is a genetic basis for atopic predisposition has been recognized for nearly a century (4). Ultimately, the response to an allergen is complex and influenced by the interplay of multiple factors: characteristics of the host (including genetic susceptibility), the environment, and physical properties of the allergen itself (5). Although we choose to define an allergen as an antigen that will induce and interact specifically with IgE, the differences between allergens and antigens are blurred. The question therefore arises as to whether all antigens can be allergens under the proper conditions.

PROPERTIES OF AN ALLERGEN/ANTIGEN/IMMUNOGEN

An operationally defined antigen (*i*) shows immunogenicity (i.e., a capacity to establish a state of sensitivity and/or stimulate the formation of corresponding antibody) and (*ii*) reacts specifically with the responding tissue and/or those antibodies (2). The two properties are not always associated. If immunogenicity is observed, the molecule responsible is more broadly defined as an immunogen. Immunogenicity is not an inherent property of a molecule as is molecular weight. Haptens (low molecular weight compounds, such as drugs) are not immunogenic in and of themselves, but do possess/contain antigenic epitopes (6). In the proper context, such as may exist after a hapten has covalently bound to a larger protein, the hapten-protein complex taken up and processed by an antigen-presenting cell, and the hapten/antigenic epitope presented in the context of MHC (cell surface antigens of the major histocompatibility complex)—they produce and react specifically with the corresponding antibodies that were formed against hapten-protein complexes. A molecule acts as an antigen whenever an organism's immune system recognizes it; if that recognition results in identification as "foreign," the immune system responds to it. Thus, a molecule might function as an antigen in one organism but not in another. This chapter is primarily concerned with molecules recognized as antigens by the humoral system of humans.

Normally, antigen recognition by both T and B cells is required for elicitation of humoral immunity. B-cell recognition, and eventual specific antibody response, is directed toward a unique surface region of the antigen. B-cell epitopes are conformational and generally have a surface area of 500 to 1000 $Å^2$ (7,8). The antibody's antigen-binding region, composed jointly by

variable regions of the light and heavy chains, is called the "paratope" and forms a tightly fitting complementary surface with the antigen's B-cell epitope. The juxtapositioning of charges and hydrophobic mountains or valleys within epitope and paratope produces the free energy for the binding reaction. The precise fit of the two surfaces excludes most of the hydration water, tightening the complex (9). Because the antibody is directed toward a specific epitope, it will recognize another antigen if it carries the same or a very similar epitope. This is the basis for observed cross-reactivity between antigens (10,11). The surface of an antigen represents a quilt of putative epitopes (12). How many of those putative epitopes dominate the antibody response varies from case to case (8,11). The structure and position of dominating epitopes are being described for an increasing number of protein antigens (13). To date, the prediction of allergen cross-reactivity has mainly been based on protein homology (i.e., linear sequence data).

Next, consideration must be given to the function of antibodies in general and those specifically involved in interaction with allergens (14). The most important feature for an antibody is its ability to recognize an antigen and to form a complex with its target epitope. The function of an antibody to allergen is thought by many to arm an antibody receptor situated on effector cells, such as mast cells, and wait for the antigen/allergen to come and cross-link the receptors. It is the cross-linking reaction that an allergen, in general, must accomplish. For that purpose an allergen must carry at least two suitably separated epitopes, allowing the molecule to form a bridge. One epitope might be enough for the functioning of an antigen, but it is probably not for an allergen. For some purified allergens, the antibody response is predominantly toward three to four dominant epitopes (15). Both the IgG and IgE responses in the same sensitized individual recognize the same epitopes (16). From a receptor aggregation point of view, a favorable topography of epitopes would probably contribute to the potency of an allergen. One might argue that the necessary high affinity of antibody would limit the inherited libraries capable of producing such antibodies and thus restricting some antigens to become allergens. Although IgE affinities toward allergens are exceptionally high, nonallergic individuals are able to mount an equally high-affinity response of IgG to the same allergens acting in this case as antigens (15). Thus, high affinity by itself is not a necessary step toward atopy. In addition, in skin test negative clinically allergic people, lower-affinity antibodies can act in allergic reactions (17,18). Affinity is correlated to the ability to cross-link receptors (19). Thus, high affinity is correlated with the strength of atopic reactions, but achieving that affinity seems not to be the limiting factor in characterizing allergens among antigens.

The necessity of link formation of two separated epitopes might also induce a lower limit in molecular weight where crowding on small surfaces could limit a cross-linking activity. Studies of Amb a 5 reveal that at least three epitopes are present on that 2500 MW protein (16). How much smaller it can go without the necessity of dimerization or polymerization of the putative allergen is not known; it is likely, however, that the probability of finding an antigen with allergenic properties is lessened at the lower molecular weight.

For antigens to act as allergens, they must elicit T-cell-dependent responses and be able to form at least two, preferably three or four, spatially separated epitopes. This establishes some lower molecular weight limit and raises the question of whether the majority of T-cell-dependent antigens become allergens. They certainly have the ability, but whether they become an antigen depends on circumstances (20). A series of investigations of allergy-prone families found that although the tendency to be sensitive to allergens was inherited, the choice of allergens among antigens seemed to be totally random. Clinically, there was no correlation between the type of specific allergen sensitivity seen in the mother or father versus the children. Thus, all antigens encountered fulfilling the two criteria above may become allergens by a purely random process (21).

The sea of molecules acting as allergens is organized and named according to a schema proposed by World Health Organization/International Union of Immunologic Societies (WHO/IUIS). The molecules are labeled by the first three letters of the genus and the first letter of the species they are isolated from, and then by an Arabic numeral indicating the sequence of isolation. Der p 1 is the first isolate from *Dermatophagoides pteronyssinus*, a house-dust mite. The distinction between major and minor allergens is a functional classification; in current usage, a major antigen is one to which >50% of allergic patients react.

Antigens/allergens are generally proteins, glycoproteins, or lipoproteins of plant or animal origin. Many of the major allergens (including those from mites, animal danders,

pollens, insects, and foods) have been cloned and sequenced (22). For many of these, the three-dimensional structure is also now known; either because it has been directly visualized via crystallography or has been modeled, that is, the structure is inferred on the basis of sequence homology with other, solved, antigens. It does not appear that these molecule types can be divided into allergic/atopic or nonallergic/non-atopic on an a priori structural reason (23). Antigens are derived from proteins with a variety of biological functions, including proteases, pathogenesis-related proteins, seed storage proteins, ligand-binding proteins, lipid-transfer proteins, calcium-binding proteins, and other structural proteins; in toto, they have been identified as coming from more than 120 distinct protein families (24–26). However, the majority of the plant and animal allergens are clustered within just a few of these families (24). Classification of allergens into groups with structural similarity may help predict cross-reactivity or may provide other useful information (10). For example, biological function, such as the proteolytic enzyme allergens of dust mites, may directly influence the development of IgE responses (i.e., via direct cleavage of CD23 from the B-cell surface, thus inhibiting negative feedback regulation) (27). This same antigen might directly initiate inflammatory responses in the lung, such as those associated with asthma (28,29). An antigen's intrinsic structural or biological properties may also influence the extent to which it persists in the indoor and outdoor environments or retains its allergenicity while within the digestive tract (30).

In the future, structural biology and proteomics may continue to enable the identification of motifs, patterns, and structures of clinical and immunological significance.

ALLERGEN: ROUTE AND AMOUNT OF EXPOSURE

Exposure to allergen is typically necessary to develop an allergic IgE immune response. However, the presence of cross-reacting antibodies as well as autoantibodies may complicate the picture. The skin and mucosal surfaces, present in upper and lower respiratory tract, GI tract, genital tract, and mammary glands, are the body's barriers to encounters with allergens and other environmental factors. The presence of these barriers safeguards the internal milieu by keeping foreign items out. First, they act as physical barriers and thus prevent penetration of high molecular antigens; Schneeberger reported that the molecular weight cutoff above which nasal and alveolar membranes are impermeable is between 40,000 and 60,000 Da (31). Next, the skin and mucosal barriers are the first sites of contact with the innate and adaptive immune systems, both of which are involved in the defense of the body.

The innate immune system is the host defense mechanism that is encoded in the germline genes of the host (32). It involves barrier mechanisms such as the epithelial cells layers, secreted mucus layers, and epithelial cilia; soluble proteins and bioactive small molecules in biological fluids, that is, complement and defensin, released from cells (cytokines, chemokines and bioactive amines and enzymes); as well as cell surface receptors that identify by binding molecular patterns expressed on the surfaces of invading microbes and other foreign substances. Innate mucosal defense consists of many soluble and cellular elements, including complement, secretory leukocytes, protease inhibitors, surfactant protein, defensin, mucins, slatherin, lactoferrin, cystatins, lysozyme, mannose-binding lectin, thrombospondins, and collectin, as well as secretory agglutins.

The adaptive system exhibits specificity for its target antigens. It is based primarily on the antigen-specific receptors on the surfaces of the T and B lymphocytes (33). The antigen-specific receptors of the adaptive response are assembled by somatic rearrangement of germline gene elements to form both intact T-cell receptors (TCRs) and B-cell antigen-specific receptors (Ig). The adaptive mucosal immune system involves two main tissue systems: (*i*) the tonsils, Peyer's patches, and isolated lymphoid follicles and (*ii*) the diffuse mucosal immune system consisting of intra-epithelial lymphocytes and the lamina propria. IgA is the main mucosal antibody (34).

The organized mucosal tissues play an important role in the inductive stage of an immune response (35). The mucosal surfaces present in the upper and lower respiratory tract and in the GI tract are important routes of entry for development of allergic sensitization (36); they also represent key sites for initiation of non-IgE responses (37,38). Immune responses, whether healthy or aberrant, are induced via other routes of exposure as well. IgE sensitization has been found following injection of allergens, such as penicillin metabolite acting as a hapten, or enzymes delivered by stinging insects (14).

Once encountered, the amount/dose of exposure, duration, as well as other modulating pollutants are a few of the many environmental factors that influence the type of response to an antigen/allergen. Allergens appear to induce IgE production at relatively low doses (39). Marsh has estimated that the mean adult annual dosage of individual allergenic components is probably in the nanogram range (14). The ambient level of mite allergen that a normal individual is exposed to has been measured to fluctuate at around 100 pg/m^3. House dust with mite content >2 μg mite/g dust is associated with sensitization in children (expert consensus) (40). Clinical studies suggest that the duration of exposure needed for IgE sensitization varies; "days" for parasitic allergens, "months" for some constant/perennial allergen exposure, to "years" of exposure for seasonal allergens such as pollens are needed to develop IgE antibodies.

ALLERGIC SENSITIZATION

The innate and adaptive immune systems work together to protect the organism from foreign substances that may possess a diverse collection of pathogenic mechanisms. Though the innate and adaptive immune responses are different in their mechanisms of action, synergy between them is essential for a fully effective immune response (41,42). The innate system is the first line of host defense; it sets the stage for the development of an adaptive response to the antigen/allergen.

To explore the possible positioning of allergens within the antigen family, features of antigen in its function as an initiator of humoral response have to be considered. First, antigens (regardless of their allergenic properties) can be broadly divided based on whether or not they require T-cell help when eliciting a humoral response. The thymus-independent pathway allows direct activation of antigen-specific B-cell clones, thus eliminating the need for a T-cell epitope. Most bacterial sugar-based antigens belong to this class (43). Protein antigens, including allergens, are thymus dependent; this means that to act as antigen and trigger a humoral, antibody-based response, the molecule has to be able to first interact and activate antigen-specific T cells.

T cells are unable to recognize antigen in the absence of antigen presentation (with the exception of the superantigens) (44). The TCR is restricted to recognizing antigenic peptides displayed in the context of molecules of the major histocompatibility complex (MHC) (45). In humans, human leukocyte antigens (HLA) are encoded by genes of the MHC complex, located on chromosome 6. With the exception of some cell types, all cells are capable of presenting antigen and activating the adaptive response via MHC class I. Dendritic cells, macrophages, and B cells play a major role in the innate response, and also act as professional antigen-presenting cells (APCs). APCs phagocytize exogenous foreign substances, such as allergens, bacteria, parasites, or toxins in the tissues, and then migrate, via chemotactic signals, to T-cell-enriched draining lymph nodes (46). During migration, dendritic cells undergo a maturation process in which they lose phagocytic capacity and develop an increased ability to communicate with T cells. This maturation process is dependent on signaling through pattern recognition receptors, such as the members of the Toll-like receptor family, which occurs following binding of pathogen-associated molecules (41,47). Lysosome-associated enzymes digest phagocytized proteins into smaller peptides. These peptides are loaded into the antigen-binding clefts of MHC class II molecules for display (i.e., as "T-cell epitopes"); MHC class II molecules bind peptides that are 10 to 30 amino acids long with a core region of 13 amino acids containing primary and secondary anchor residues. On the APC surface the MHC-peptide antigen complexes are available for recognition by any naïve $CD4^+$ T cell passing through the lymph node. $CD4^+$ helper T lymphocytes are immune response mediators and play an important role in establishing and maximizing the capabilities of the adaptive immune response. Several different subtypes of $CD4^+$ T cell can be activated by professional APCs, with each type of T cell being specially equipped to deal with different foreign substance, whether it be allergenic, bacterial, viral, or a toxin (48). The type of T cell activated, and therefore the type of response generated, depends, in part, on the context in which the antigen was first encountered by the APC.

When a naïve T_H0 cell contacts an antigen and is stimulated through its antigen receptor, it begins to polarize along a lineage-determining developmental pathway (49,50). T_H1, T_H2, T_H17, and T regulatory (Treg) (Tr1) cells all develop from the same naïve T_H0 cell, under the

influence of genetic and environmental factors acting at the level of antigen presentation. The predominance of a given cytokine in the microenvironment of the responding T_H0 cell is an important modulatory factor in this process (51). Signals through contact molecules, as well as through cytokine receptors, elicit a complex series of molecular interactions that culminate in the binding of lineage-specific transcription factors to multiple regulatory elements in the promoters, and subsequent activation of a differentiation pathway (52). Following activation, naïve T_H0 cells differentiate toward T_H1 in the presence of IL-12, which upregulates IFN-γ via signal transducer and activator of transcription 4 (STAT-4), leading to IFN-γ-mediated activation and induction of the T_H1 lineage-determining transcription factor T-bet, subsequently leading to production of T_H1 cytokines, including IFN-γ, IL-2, and TNF-β. T_H2 cell differentiation occurs in response to IL-4, which activates STAT6, resulting in induction of the T_H2 lineage-determining transcription factor GATA-3, and leading to production of the T_H2 cytokines IL-4, IL-5, and IL-13 (53–55). The T_H17 subset develops in response to TGF-β plus IL-6; STAT-3 is activated and induces the T_H17 lineage-specific transcription factor RORγt, which is an orphan nuclear receptor, (56). Treg cells can also be generated from naïve T_H0 cells; TGF-β induces the transcription factor FOXP3, leading to production of the suppressive cytokines, TGF-β and IL-10 (57). The molecular mechanisms of these pathways involve cross talk on the level of the transcription factors: GATA-3 not only increases transactivation of the IL-4 locus promoter but also inhibits production of IFN-γ; T-bet interferes with T_H17 cells and directly blocks GATA-3 while binding to its own targets. For the T_H1, T_H2, and T_H17 lineages, the differentiation process includes a cascade of events that results in genetic imprinting—reorganization of the histone/chromatin structure such that the determined T-cell polarization is subsequently maintained (58–61). A sketch of the various T helper subsets and the cytokines thought to be key for induction and maintenance of their polarization is presented in Figure 1A.

As mentioned above, early IL-4 production favors T_H2 polarization, whereas IFN-γ and IL-12 in the absence of IL-4 promote T_H1 polarization. While the source of IL-4 produced in the beginning of the immune response is not fully understood (the naïve T_H0 cells themselves, mast cells, and/or basophils, NKT cells), both IL-12 and the IFNs responsible for T_H1 polarization are produced during innate immune responses (62). For example, many bacteria and viruses contain one or more components able to interact with the Toll-like receptors present on dendritic cells and NK cells; IL-12 and IFN are among the cytokines released as a consequence of that activation. It is likely that T_H2 priming can occur either as a default pathway in the absence of Toll-like receptor signaling (it prevents rejection of the developing fetus in utero) or through currently unidentified T_H2-activating receptors. Although T_H2 cells are characterized, defining the properties of allergen-specific T cells is difficult in human beings because of their low frequency within the T-cell repertoire.

Allergen-responsive B lymphocytes develop from bone marrow precursors before antigenic stimulation; they then migrate to and populate peripheral lymphoid tissues, where they complete their maturation and are available to interact with foreign antigen (63,64). Humoral responses are initiated by the recognition of antigens by B cells specific for each antigen (65). Antigen binds to the IgM and IgD receptors on naïve B cells and activates them; a clonal proliferation of antigen-specific cells results, and these cells continue to differentiate. Some of these become effector cells that actively secrete IgM antibody, others become memory B cells, and still others undergo heavy chain isotope class switch so that IgG, IgA, and IgE can be produced. Isotype switch is stimulated by $CD4^+$ helper T-cell signals, which include the various cytokines and CD40-CD40 ligand interaction. Affinity maturation can also occur at this point.

Primary sensitization may occur in predisposed naïve individuals on their initial encounter with the allergen. The cellular and molecular pathways that lead to sensitization are quite similar to those that lead to a future recognition reaction in sensitized people; however, the cellular participants are probably different. The cells recruited for sensitization response cannot come from the memory cell compartment but only from the naïve cell population. Furthermore, as heavy chain isotype switching and affinity maturation increase with repeated exposures to protein antigens/allergens, the absence of traces of high-affinity antibody favors cells that do not use the Ig as a receptor in antigen-presenting function. This may push the concentration limits for recognition higher than those that develop in sensitized individuals. For some purified allergens, the antibody response is predominantly toward three to four dominant epitopes (15). Both the IgG and IgE responses in the same sensitized individual recognize the same epitopes (16). The rough outline of this essential process leading to

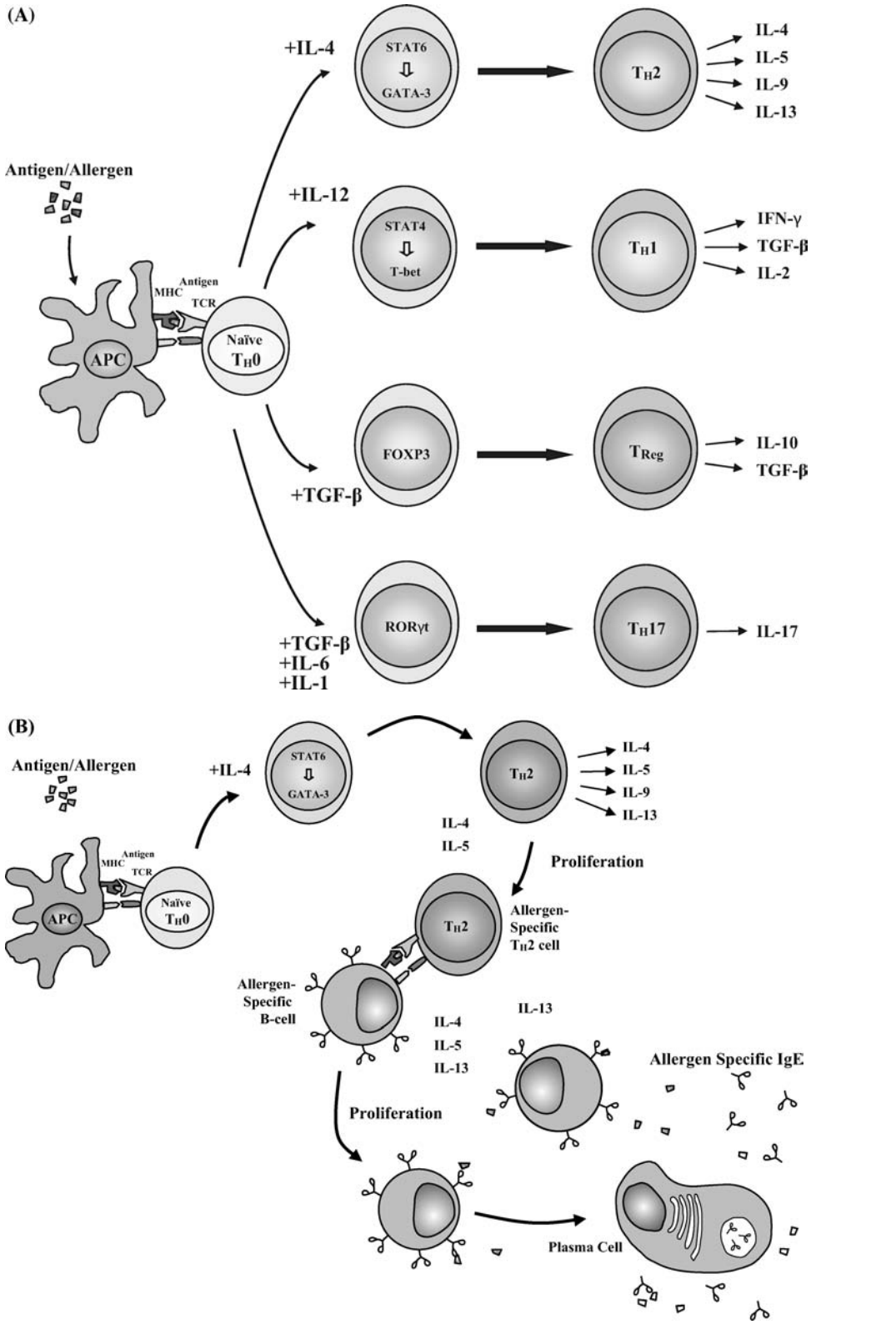

Figure 1 (**A**) T helper cells of various subsets are thought to develop from the same naïve T_H0 cell under the influence of both genetic and environmental factors acting at the level of antigen presentation. (**B**) Early exposure to and production of IL-4 results in polarization toward a T_H2 phenotype and is associated with production of allergen-specific IgE.

production of IgE antibody is sketched in Figure 1B. The process is similar for other subclasses of antibody production as well, except that different cytokines are involved.

In addition to the thymus-dependent and thymus-independent classes of antigens, a third antigen class exists: a "superantigen" is an antigen capable of triggering nonspecific activation of many T cells, leading to wide antibody response. These are proteins produced by many pathogens (including bacteria, mycoplasma, and viruses), which can bind to the variable region of the beta chain (V-beta) of the T-cell receptor and cross-link it to MHC class II molecules on the surface of APCs. Superantigens function as intact molecules; they are not processed and presented by APCs. The extent of T-cell stimulation is a function of the frequency of T cells bearing V-betas that can bind a specific superantigen. There has been some speculation about the superantigenic nature of some allergic responses (66).

Most people, both atopic and nonallergic, mount a vigorous response to antigens, utilizing all subclasses of immunoglobulins except IgE (67,68). The atopic people mount the same response, but in addition they have an IgE response (69). The major difference in immune antibody response to antigen and allergen is consequently quite narrowly localized. The additional production of high-affinity IgE is directed to the dominant epitopes of the antigen. The epitopes seem to be the same recognized by other antibody classes. Unusual patterns of response by other subclasses of antibodies has been frequently mentioned, especially that of appearance of enhanced IgG_4 response. This may appear in individual circumstances, but studies of large populations of immune-response profiles to allergens have not revealed any systematic differences.

There is intrinsically very little in the structure of antigens that determines whether they will become allergens or not. Allergens are created by the selective response to them as they are presented as normal antigens (70); consequently, whether a molecule is an "antigen" or an "allergen" probably ultimately rests in the circumstances under which the presentation takes place.

GENETIC FACTORS MODULATING THE IMMUNE RESPONSE TO ALLERGENS

The atopic immune response is a complex condition involving genetic as well as environmental factors (Fig. 2). The evidence for genetic factors being involved in the different phenotypes of atopy has consisted of their aggregation in families, increased prevalence in first-degree relatives, and increased concordance in monozygotic twins compared with dizygotic twins (71,72). Genetic investigations to determine where the genes are located have used many approaches including forward genetics, candidate genes, genome screens, fine mapping, and functional genomics using statistical linkage and association analysis (73). These

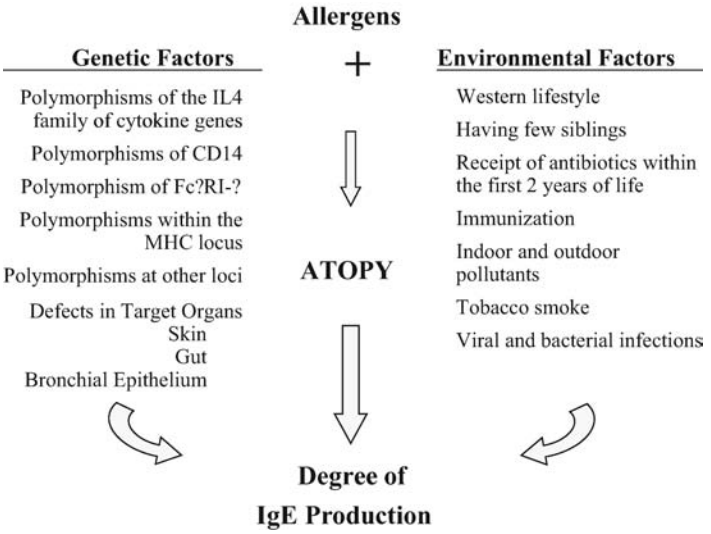

Figure 2 Examples of the environmental and genetic factors implicated in IgE response to allergen.

methods considered genetic heterogeneity, gene-gene interaction, and gene-environmental interaction. Atopy, defined as an adverse specific IgE immune reaction, has been studied using a variety of phenotypes including serum IgE levels, skin test reactivity, specific skin test reactivity, and specific serum IgE levels (74).

Jackola et al. demonstrated that all individuals usually respond with an immune response to a foreign allergen, but in atopy there is an increased production of a high-affinity IgE antibody while non-atopic individuals produce an equally high-affinity IgG_1 antibody (75). The clinical conditions resulting from an atopic mechanism may consist of asthma, some forms of urticaria, and allergic rhinitis. It is evident that the ultimate clinical picture of asthma will involve a variety of genetic factors that interact with each other and with environment exposures such as allergens, smoke, weight, infectious agents, diet, endotoxins, drugs, and diesel particles, all of which may change with time. There is no consensus regarding the mode of inheritance and/or the genes that are involved. Even less is known about the clinical course of these phenotypes (74). The main studies that have been used are the positional cloning or the genome screen and the candidate gene or association approaches (73).

Genome Wide Linkage Analysis for Asthma and Atopy and Related Phenotypes

The number of regions having linkage with atopy and related phenotypes such as asthma is at least 20. The replicated regions reported for the atopy and asthma phenotype have been on 1p, 2q, 4q, 5q, 6p, 12q, 13q, 14q, 19q, and 21q; for total serum IgE sites on 2q, 3q, 5q, 6p, 7q, and 12q; atopy on chromosomes 3q, 4q, 6,p, 11q, 17q, 20p; and blood eosinophil counts on 15q (73,76–78). Blumenthal et al. reported on a genome-wide search for an atopy gene in three ethnic groups from the Collaborative Study on the Genetics of Asthma (CSGA). They found linkage on chromosomes 11q and 20p (79). The same group investigated mite sensitivity and noted linkage for the combined group on chromosomes 19q and 20q (80). These studies indicate the complexity of these conditions.

Candidate Genes for Asthma and Atopy and Related Phenotypes

Genome-wide linkage analysis and/or biological factors are the basis used for the selection of candidate genes studied for association. A variety of genetic association investigations for atopy and asthma phenotypes have been performed in different populations, many times yielding some reproducible but also many nonreplicable results (73,76–78). The candidate genes for atopy and asthma include allelic variants of genes of known immunological significance; variants in over 100 genes have been described (73,78). Some of the most common candidate gene variants are mentioned in the following paragraphs.

Chromosome 2q Region

This site identified in genome screens to be linked with the atopy and asthma phenotype contains the interleukin 1 receptor antagonist (IL-1RN) and the cytotoxic T lymphocyte antigen-4 (CTLA4) genes. Single nucleotide polymorphisms (SNPs) in CTLA4 are associated with asthma, serum IgE, asthma severity, airway responsiveness, and asthma (73,81–86). P-selectin has been reported as an atopy susceptibility locus (87).

Chromosome 5q and 16q Regions

Activation of the interleukin 4 receptor (IL-4RA) stimulates the production of total serum IgE. Interleukin 4 (IL-4) is located on chromosome 5q31 and IL-4RA on chromosome 16p12, both of which are found on genomic regions linked to the asthma phenotype. Many studies demonstrate that functional SNPs in the promoters of IL-4 and IL-4RA are associated with the atopy and asthma phenotypes (73,76,78). An article by Basehore et al. suggests that the 3017G/T variant or the haplotypes it identifies influence IL-4's ability to modulate total serum IgE levels (73,77,88). They suggest that inconsistencies with previously reported IL-4 associations might be due to population differences in allele frequencies, linkage disequilibrium with this SNP or haplotype, or both. Vercelli further discusses the discrepancies reported regarding CD14 polymorphisms, atopy, and endotoxin switch (89). Other genes on chromosome 5, including interleukin 13 (IL-13), monocyte differentiation antigen CD14 (CD14), serine protease inhibitor kazal type 5 (SPINK5), and leukotriene C4 synthase (LTC4S) have been

studied. SNPs in the promoter and coding regions of these genes are associated with immune response and asthma phenotypes (73,76–78,90).

Chromosome 6p21 Region
Human leukocyte antigen DRB1 (HLA-DRB1), tumor necrotic factor alpha (TNF-α), and lymphotoxin alpha (LTA) are associated with asthma phenotypes (73). HLA class II molecules are involved in controlling the immune response to allergens (73). Early association studies demonstrate that several purified allergens, such as ragweed Amb a 5 and 6, olive Ole e 1, and *Lolium perenne* 1, 2, and 3, are associated with HLA. Functional SNPs and/or haplotypes in HLA-DRB1 are associated with allergic specific IgE responses. Moffatt et al. noted a relationship of atopy, respiratory function, and HLA-DR in Aboriginal Australians (91).

Chromosome 11q13 Region
The gene for the beta chain of the high-affinity receptor for IgE on chromosome 11q13, a genome region linked to atopy, has been reported. SNPs and/or haplotypes in FCERIB are associated with atopy, asthma, and their associated phenotypes (73,78).

Chromosome 12q Region
SNPs and haplotypes for the STAT-6 and nitric oxide synthase 1 (NOS1) are described to be associated with asthma and atopy (73,76,78).

Chromosome 17q Region
The predominant eosinophil chemoattractant on chromosome 17q is the gene for eotaxin (SCYA11), which is involved in allergic inflammation (73,76,78).

These are a few of the many atopy and asthma susceptibility genes suggested in the literature. Even though the loci and variants are identified, their function needs to be established. This is done through functional studies that involve SNPs in coding sequences of genes (promoter, enhancers, and chromatin structure). Over 55 genes with functional information have been reported; a selected few of these are noted in Table 1. Polymorphisms that may have function effects include IL-10, CD14, CCL5, Filaggrin, IL-17, IL-13, and the cysteinyl leukotriene receptors (73,78,92).

Asthma Susceptibility Loci Identified by the Positional Cloning Approach

Five potential asthma susceptibly genes or complexes have been identified using a positional approach (93). These are ADAM33, DPP10, PHF11 and SETDB2, GPRA, and SPINK5 (94–98). The replication studies of ADAM33 and asthma have resulted in conflicting results (99–102). To date, there is no definite replication of the association between GPRA, PHF11 and DPP10, and asthma and/or atopy phenotypes by groups independent from those publishing the original reports (73,76,78,93).

Gene-Environmental Interactions

The influence of environmental factors, including pets, endotoxin, viruses, smoke, and pharmacological agents, on the expression of genes and the ultimate clinical phenotype is being investigated and will be further summarized in a following section "Environmental Factors Modulating the Immune Response to Allergens." Investigation of gene-environmental interactions will be important in understanding the genetic basis of asthma and atopy. Even though there is genetic predisposition, environmental factors probably modulate the effect, causing either tolerance or susceptibility (73,74).

Summary

The current understanding of the immune system suggests that the upregulation of IgE synthesis in atopy is due to the induction of IgE isotype utilization at the DNA level in B cells. The start of IgE synthesis appears to involve a number of signals followed by direct T- and B-cell interaction. They require prior engagement of the TCR with antigenic fragments (peptides)

Table 1 Candidate Genes of Atopy and Allergy

Type	Examples
Cytokines influencing atopic phenotype	
Eosinophil growth-, activation-, and apoptosis-inhibiting factors	IL-5, IL-3, GM-CSF, CCL11, CCL5
Mast-cell growth factors	IL-3, IL-9, IL-10, SCF, TGF-β
Histamine-releasing factors	CCL2 (MCP-1), CCL7 (MCP-3), CCL5
IgE isotype switch factors	IL-4, IL-13
Inhibition of IgE isotype switch	IFN-γ, IL-12, IL-18, IL-23
Lipoxygenase pathway metabolism	5-LO, 5-LO-activating peptide, leukotriene C4 synthase
Proinflammatory cytokines	IL-1α, IL-1β, TNF-α, IL-6
Anti-inflammatory cytokines	TNF-β, IL-10, IL-1Rα
Receptors	
Antigen receptors	T-cell receptors (α/β, γ/δ), B-cell receptor (IG, κ/λ light chains)
IgE receptor	FcεRI β chain, FcεRII (CD23)
Cytokine gene receptors	IFN-γR β chain IL-1R, IL-4R, TNF receptors, common γ-chain
Adhesion molecules	VLA-4, VCAM-1, ICAM-1, LFA-1
Corticosteroid receptor	Grl-hsp90
Neurogenic receptors	β2-Adrenergic, cholinergic receptors
Nuclear transcription factors	GATA-3, T-bet, NF-κB, IkB, NFAT, STAT-1/2, STAT-4, STAT-6
Other molecules of importance	
MHC and antigen processing	HLA class I and II molecules, TAP-1 and TAP-2, LMP
Cell signaling	CTLA-4, CD28, JAK1
Barriers and other defense	SPINK5, Clara cell protein 16, endothelin 2

Abbreviations: CCL, chemokine ligand; GM-CSF, granulocyte-macrophage colony–stimulating factor; HLA, human leukocyte antigen; hsp, heat-shock protein; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LFA, lymphocyte function-associated antigen; LMP, large multicatalytic proteosome; LO, lipoxygenase; MHC, major histocompatibility complex; SCF, stem cell factor; TAP, transporter associated with antigen processing; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

that are recognized on MHC class II molecules on antigen-presentation cells. INF-α appears to be a major downregulator of IgE synthesis. There are at least two major genetic controls of atopy. One, which is non-epitope specific, is noted using the phenotypes of total serum IgE levels and skin test reactivity in general. The genes may reside on a variety of different loci and chromosomes, that is, IL-4 on chromosome 5q, IgE receptor on chromosome 11q, and IFN-γ on chromosome 12q. Another is epitope specific and appears to be associated with the HLA system. Therefore, there appears to be several levels involved in selectivity: (*i*) epitope-specific level that is related to the HLA system, (*ii*) purified allergen level (molecular selection) that is only partially HLA associated and dependent on size, and (*iii*) complex or natural allergen level involving many epitopes selective for organisms. There are probably too many surface epitopes, and too many HLA polymorphic types to demonstrate any specific HLA association.

In view of the complexity of atopy, it is not completely clear how many genes and environmental factors are involved. The genome screens suggest that there may be many genes with modulatory effects. It is likely that many of the genes that influence immunity will prove to be polymorphic. As a result, almost any immune response gene may be found to have an effect on any immune-mediated disease. To determine the clinical relevance of these polymorphisms, they should be tested in case-control studies involving patients with different manifestations and severity of the disease. The samples should be obtained from representative populations with environmental factors being defined. A good understanding of these relationships is needed for the proper management of atopy and its related conditions.

ENVIRONMENTAL FACTORS MODULATING THE IMMUNE RESPONSE TO ALLERGENS

A substantial increase in the prevalence of atopic diseases has occurred in the western world over the last few decades (103). These increased numbers cannot be explained simply on the basis of heightened awareness (leading to increased diagnosis of existent disease). Nor would

we expect to detect change/drift in the genetic composition of the affected populations during this time period. Since expression of the atopic phenotype occurs as a result of complex interplay between genetic and environmental factors, the environment must be of major importance in the development and increased prevalence of atopy. Western living conditions, allergens, air pollution such as smoke and diesel fumes, and microbial exposure all may impact the immune system, and together influence an individual's risk for being atopic (104,105). The hygiene hypothesis postulates that reduced early childhood exposure to farm animals, microbes, and endotoxin or other microbial products, due to a combination of smaller family size, improved living standards, higher personal hygiene, changes in infant diet, and early/increased use of antibiotics, might result in increased risk for developing atopic disease (106). It was based on the observations, many of which have not been consistently confirmed, that there may be an inverse relationship between some infections and atopic parameters and that children who grow up in a farming environment may have less asthma (atopic sensitization, asthma, "hay fever") than children of the same age and living in the same communities, but not growing up on a farm (107–110).

A possible explanation for the protective effects of exposure to bacteria or their products during the period when sensitization occurs in early life is via increased stimulation to IFN-γ production; the lack of microbial stimulation of sufficient intensity may, paradoxically, influence the maturation of the immune system, causing a predominance of T_H2 cell subtype in genetically susceptible individuals (111–114). Early exposure to allergens from domestic pets, such as dogs and cats, is related to atopic sensitization (115,116). Like farm animals, domestic animals can be a source of endotoxin and may thus bias the overall immune response away from an atopic or T_H2 response. It is also postulated that only those infections that stimulate a strong cell-mediated immune response and long living memory immunity may play a positive role resulting in a shift toward the T_H1 type response and prevention of asthma and atopy (117).

The results seen in some studies may be a result of selection and environmental bias. There are many problems in accepting the hygiene hypothesis. These include the finding that asthma and atopy are more prevalent in the core city compared with the suburbs and the observations that autoimmune processes, which are thought to involve T_H1, are increasing. Other environmental factors such as diesel fumes, occupational inhalants, and allergen exposure have been noted to affect the immune response to allergens and the resulting clinical picture. It appears that environmental factors may enhance either sensitization or normalization (118,119).

ALLERGIC ATOPIC REACTIONS AND INFLAMMATION (INCLUDING PATHOLOGY)

The resulting clinical allergic reactions may vary from having symptoms of sneezing, nasal discharge, and nasal congestion associated with allergic rhinitis; coughing, wheezing, and shortness of breath with evidence of reversible airway obstruction; as well as certain forms of urticaria and angioedema, and anaphylaxis. Inflammation is an important feature of these conditions as summarized above; it is a dynamic process that consists of cytological and histological reactions that occur in tissues in response to injury or abnormal stimulation caused by a physical, chemical, or biological agent.

Once the individual begins to develop sensitization to an allergen, inflammation is initiated. Upon reexposure to the allergen, the immune system is further activated, resulting in more inflammation. This ultimately determines the clinical picture of allergy/atopy. The allergic reaction results from the involvement and interactions of a variety of cell types, ranging from monocytes and macrophages to T cells involved in the development of the specific immune reaction, as well as the granulocytic cells of the myeloid series (i.e., mast cells, eosinophils, neutrophils, and platelets). The interactions of all these cells are of importance in the inflammatory response. One of the steps following reexposure to the allergen involves the interaction with its specific IgE, attached by way of FcεRI and FcεRII to cells containing mediating substances. Important mediators are thought to include histamine, cytokines, and leukotrienes. Mediators released by some cells regulate the functions of others. The acute symptoms of allergies, such as sneezing, wheezing, and

urticaria, may be due to the release of mediators from the mast cells, such as histamine, whereas the chronic symptoms such as bronchial hyperreactivity may be explained on the basis of eosinophil-mediated tissue damage. T cells are of the T_H2 type and produce IL-4 and IL-5, which potentiate the terminal differentiation and activation of eosinophils. Basic proteins, together with the platelet-activating factor and leukotrienes secreted by eosinophils, probably also contribute to these chronic symptoms. Cell-adhesion molecules are also important in inflammation. A series of cell-adhesion molecules mediate interaction between vascular endothelium and leukocyte cell surfaces. The three major families of adhesion molecules, which contribute to this process are integrins, selectins, and immunoglobulin-like receptors. Other mediators of the inflammatory response that may be important are the complement system and heat-shock proteins. Therefore, as a result of the interaction of the allergen in a sensitized individual, a variety of cells and humoral components are activated, which results in inflammation and determines the clinical picture. The end result for exposure to an allergen is transient and/or chronic inflammation. The molecular and tissue changes found are common to all inflammatory processes. The difference between atopic allergy and all other inflammatory processes lies in causation. In the case of atopic allergy, it is linked to aberrant humoral response to foreign molecules whether these responses are IgE, IgG, or direct cellular reactions, as in the case of some late-phase reactions (120,121).

The nature of the immune reaction to an allergen and the resulting clinical picture is dependent on many steps influenced by host and environmental factors such as properties of the allergen, route of exposure, as well as genetic controls.

SALIENT POINTS

- Allergens/antigens have two properties: (*i*) immunogenicity (i.e., the capacity to stimulate the formation of the corresponding antibody and/or a state of sensitivity) and (*ii*) the ability to react specifically with those antibodies and/or the responding tissue. The two properties are not always associated.
- Allergens are antigens that induce the production of an IgE-specific antibody that will interact with the inducing antigen.
- From the chemical standpoint, there seems to be little to differentiate allergens from other antigens.
- There appear to be four restrictions for a molecule to become an allergen: (*i*) it must possess a surface to which the antibody can form a complementary surface, (*ii*) it must have an amino acid sequence in its backbone able to bind the MHC II alleles of the responding individual, (*iii*) the free energy of interaction of the allergen with the antibody should be adequate to ensure binding at low concentrations, and (*iv*) it must form at least two epitopes able to act as a bridge.
- The nature of the immune reaction to an allergen is dependent on many steps influenced by host and environmental factors.
- Genetic factors include multiple genes regulating non-epitope-specific factors, such as those on chromosome 5q, as well as those that are allergen epitope-specific, including genes in the MHC on chromosome 6.
- The duration, route, and amount of exposure, as well as other modulating pollutants, are a few of the environmental factors that influence the type of response seen to an allergen.
- Atopy clinically defined is an inflammatory condition resulting from an allergen producing an adverse immune reaction.

ACKNOWLEDGMENTS

This work was supported in part by NIH grants 5U01HL49609 and M01-RR00400 from the National Center for Research Resources, National Institutes of Health.

DEFINITIONS

Antigens are substances that have immunogenicity, leading to production of antibodies with which the antigens will react.
Allergens are a subclass of antigens that stimulate the production of and combine with the IgE subclass of antibodies.
Haptens are substances that are not immunogenic in/of themselves (cannot stimulate humoral response without the help of carrier substances) but can combine specifically with antibody once it is formed.
Immunogens are substances that stimulate specific immune response such as the production of an antibody.
B-cell epitopes are specific surface areas on antigen toward which the specificity of a single antibody is directed.
T-cell epitope is a proteolytic antigenic fragment (approximately 13 amino acids long) displayed by MHC; MHC-restricted recognition is required for activation of antigen-specific T cells.
T helper cell 2 (T_H2) profile is a specific pattern of effector molecules, of which IL-4 and IL-5 are dominant derived from activated T cells.
T helper cell 1 (T_H1) profile is a specific pattern of effector molecules, where INFγ is dominant, derived from activated T cells.

REFERENCES

1. Blumenthal MN. Historical perspectives. In: Bjorksten B, Blumenthal MN, eds. Genetics of Allergy and Asthma: Methods for Investigative Studies. New York: Marcel Dekker, 1996:1–8.
2. Pirquet CV. Allergy. In: Gell PGH, Coombs RRA, eds. Clinical Aspects of Immunology. Philadelphia: FA Davis Company, 1963:1457.
3. Coca AF, Cooke RA. On the classification of the phenomena of hypersensitiveness. J Immunol 1923; 8:163–182.
4. Cooke RA, vander Veer A. Human sensitization. J Immunol 1916; 1:201–205.
5. Church MK, McGill JI. I am atopic, but why don't I develop allergy? The association between atopy and clinical expression of allergic disease. Clin Exp Allergy Rev 2005; 5:12–15.
6. Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. J Exp Med 1935; 61:643–656.
7. Wilson IA, Stanfield RL. Antibody-antigen interactions: new structures and new conformational changes. Curr Opinion Struct Biol 1994; 4:857–867.
8. López-Torrejón G, Díaz-Perales A, Rodríguez J, et al. An experiment and modeling approach to locate IgE epitopes of plant profiling allergens. J Allergy Clin Immunol 2007; 119:1481–1488.
9. Davies DR, Padlan EA, Sheriff S. Antibody-antigen complexes. Ann Rev Biochem 1990; 59:439–473.
10. Aalberse RC. Assessment of allergen cross-reactivity. Clin Mol Allergy 2007; 5:2.
11. Egger M, Mutschlechner S, Wopfner N, et al. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy 2006; 61:461–476.
12. Jemmerson R. Epitope mapping by proteolysis of antigen-antibody complexes. Protein footprinting. Methods Mol Biol 1996; 66:97–108.
13. Greenbaum JA, Andersen PH, Blythe M, et al. Towards a consensus on datasets and evaluation metrics for developing B-cell epitope prediction tools. J Mol Recognition 2007; 20:75–82.
14. Marsh D. Genetics of allergy. In: Sela M, ed. The Antigens. New York: Academic Press, 1975:271–359.
15. Pierson-Mullany LK, Jackola DR, Blumenthal MN, et al. Characterization of polyclonal allergen-specific IgE responses by affinity distributions. Mol Immunol 2000; 37:613–620.
16. Kim KE, Rosenberg A, Roberts S, et al. The affinity of allergen specific IgE and the competition between IgE and IgG for the allergen in Amb a V sensitive individuals. Mol Immunol 1996; 33:873–880.
17. Pierson-Mullany LK, Jackola DR, Blumenthal MN, et al. Evidence of an affinity threshold for IgE-allergen binding in the percutaneous skin test reaction. Clin Exp Allergy 2002; 32:107–116.
18. Jackola D, Liebeler C, Blumenthal MN, et al. Allergen skin test reaction patterns in children. Int Arch Allergy Immunol 2003; 132:364–372.
19. Mita H, Yasueda H, Akiyama K. Affinity of IgE antibody to antigen influences allergen-induced histamine release. Clin Exp Allergy 2000; 30:1582–1589.
20. Bredehorst R, David K. What establishes a protein as an allergen? J Chromatogr B 2001; 756:33–40.
21. Jackola DR, Liebeler CL, Blumenthal MN, et al. Random outcomes of allergen-specific responses in atopic families. Clin Exp Allergy 2004; 34:540–547.
22. Chapman MD, Pomés A, Breiteneder H, et al. Nomenclature and structural biology of allergens. J Allergy Clin Immunol 2007; 119:414–420.

23. Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol 2000; 106:228–238.
24. Breiteneder H, Ebner C. Atopic allergens of plant foods. Curr Opin Allergy Clin Immunol 2001; 1:261–267.
25. Breiteneder H, Mills C. Molecular properties of food allergens. J Allergy Clin Immunol 2005; 115:14–23.
26. Breiteneder H, Mills C. Nonspecific lipid-transfer proteins in plant foods and pollens: an important allergen class. Curr Opin Allergy Clin Immunol 2005; 5:275–279.
27. Hewitt CR, Brown AP, Hart BJ, et al. A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. J Exp Med 1995; 182: 1537–1544.
28. King C, Brennan S, Thompson PJ, et al. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. J Immunol 1998; 161:3645–3651.
29. Peake HL, Currie AJ, Stewart GA, et al. Nitric oxide production by alveolar macrophages in response to house dust mite fecal pellets and the mite allergens, Der p 1 and Der p 2. J Allergy Clin Immunol 2003; 112:531–537.
30. Pomés A. Intrinsic properties of allergens and environmental exposure as determinants of allergenicity. Allergy 2002; 57:673–679.
31. Schneeberger EE. The permeability of the alveolar-capillary membrane to ultrastructural protein tracers. Ann N Y Acad Sci 1974; 221:238–243.
32. Janeway CA, Medzhitov R. Innate immune recognition. Ann Rev Immunol 2002; 20:197–216.
33. Pancer Z, Cooper MD. The evolution of adaptive immunity. Ann Rev Immunol 2006; 24:497–518.
34. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. J Path 2006; 208:270–282.
35. Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. Ann Rev Immunol 2002; 20:495–549.
36. Tada T, Ishizaka K. Distribution of gamma E forming cells in lymphoid tissues of the human and monkey. J Immunol 1970; 104:377–387.
37. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. Cell 2004; 118:229–241.
38. Mazmanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122:107–118.
39. Erwin EA, Platts-Mills TAE. Allergens. Immunol Allergy Clin N Am 2005; 25:1–14.
40. Kuehr J, Frischer T, Meinert R, et al. Mite allergen exposure is a risk for the incidence of specific sensitization. J Allergy Clin Immunol 1994; 94:44–52.
41. Kabelitz D, Medzhitov R. Innate immunity—cross-talk with adaptive immunity through pattern recognition receptors and cytokines. Curr Opin Immunol 2007; 19:1–3.
42. Lee HK, Iwasaki A. Innate control of adaptive immunity: dendritic cells and beyond. Semin Immunol 2007; 19:48–55.
43. Rijkers GT, Sanders LA, Zegers BJ. Anti-capsular polysaccharide antibody deficiency states. Immunodeficiency 1993; 5:1–21.
44. Guermonprez P, Valladeau J, Zitvogel L, et al. Antigen presentation and T cell stimulation by dendritic cells. Ann Rev Immunol 2002; 20:621–667.
45. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. Ann Rev Immunol 2006; 24:419–466.
46. Underhill DM, Ozinsky A. Phagocytosis of microbes: complexity in action. Ann Rev Immunol 2002; 20:825–852.
47. Gay NJ, Gangloff M, Weber AN. Toll-like receptors as molecular switches. Nat Rev Immunol 2006; 6:693–698.
48. Kemper C, Atkinson JP. T-cell regulation: with complements from innate immunity. Nat Rev Immunol 2007; 7:9–18.
49. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol 2002; 2:933–944.
50. Weaver CT, Hatton RD, Mangan PR, et al. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Ann Rev Immunol 2007; 25:821–852.
51. Woodfolk JA. T-cell responses to allergens. J Allergy Clin Immunol 2007; 119:280–294.
52. Rothenberg EV. Cell lineage regulators in B and T cell development. Nat Immunol 2007; 8:441–444.
53. Fields PE, Kim ST, Flavell RA. Cutting edge: changes in histone acetylation at the IL-4 and IFN-γ loci accompany Th1/Th2 differentiation. J Immunol 2002; 169:647–650.
54. Soutto M, Zhou W, Aune TM. Cutting edge: distal regulatory elements are required to achieve selective expression of IFN-γ in Th1/Tc1 effector cells. J Immunol 2002; 169:6664–6667.
55. Hwang ES, Szabo JS, Schwartzberg PL, et al. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science 2005; 307:430–433.
56. Stockinger B, Veldhoen M. Differentiation and function of Th17 cells. Curr Opin Immunol 2007; 19:281–286.
57. Bacchetta R, Gambineri E, Roncarolo M-G. Role of regulatory T cells and FOXP3 in human diseases. J Allergy Clin Immunol 2007; 120:227–235.
58. Ansel KM, Djuretic I, Tanasa B, et al. Regulation of Th2 differentiation and IL4 locus accessibility. Ann Rev Immunol 2006; 24:607–656.

59. Spilianakis CG, Flavell RA. Epigenetic regulation of Ifng expression. Nat Immunol 2007; 8:681–683.
60. Chang S, Aune TM. Dynamic changes in histone-methylation 'marks' across the locus encoding interferon-γ during the differentiation of T helper type 2 cells. Nat Immunol 2007; 8:723–731.
61. Krangel MS. T cell development: better living through chromatin. Nat Immunol 2007; 8:687–694.
62. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 2004; 113:395–400.
63. Gould HJ, Sutton BJ, Beavil AJ, et al. The biology of IGE and the basis of allergic disease. Ann Rev Immunol 2003; 21:579–628.
64. Cobaleda C, Schebesta A, Delogu A, et al. Pax5: the guardian of B cell identity and function. Nat Immunol 2007; 8:463–470.
65. Carrasco YR, Batista FD. B cell recognition of membrane-bound antigen: an exquisite way of sensing ligands. Curr Opin Immunol 2006; 18:286–291.
66. Gould HJ, Takhar P, Harries HE, et al. The allergic march from Staphylococcus aureus superantigens to immunoglobulin E. Chem Immunol Allergy 2007; 93:106–136.
67. Akdis M, Verhagen J, Taylor A, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 2004; 199:1567–1575.
68. Akdis M. Healthy immune response to allergens: T regulatory cells and more. Curr Opin Immunol 2006; 18:738–744.
69. Jackola DR, Pierson-Mullany LK, Liebeler CL, et al. Variable binding affinities for allergen suggest a 'selective competition' among immunoglobulins in atopic and non-atopic humans. Mol Immunol 2002; 39:367–377.
70. Akdis CA. Allergy and hypersensitivity; mechanisms of allergic disease. Curr Opin Immunol 2006; 18:718–726.
71. Blumenthal MN. Overview of genetic factors in the allergic response. In: Liggett SB, Meyers DA, eds. Genetics of Asthma: Lung Biology in Health and Diseases. New York: Marcel Dekker, 1996:479–494.
72. Le Souëf PN. Using twin studies to determine genetic and environmental components of allergy and asthma. Clin Exp Allergy 2006; 36:1353–1354.
73. Blumenthal MN. The role of genetics in the development of asthma and atopy. Curr Opin Allergy Immunol 2005; 5:141–145.
74. Blumenthal JB, Blumenthal MN. Genetics of asthma. Med Clin North Am 2002; 86:937–950.
75. Jackola DR, Liebeler CL, Lin C-Y, et al. Evidence that negative feedback between antibody concentration and affinity regulates humoral response consolidation to a non-infectious antigen in infants. Mol Immunol 2005; 42:19–30.
76. Hoffjan S, Ober C. Present status on the genetic studies of asthma. Curr Opin Immunol 2002; 14:709–717.
77. Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic diseases: a comprehensive review of the literature. Respir Res 2003; 4:14.
78. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. Genes Immun 2006; 7:95–100.
79. Blumenthal MN, Langefeld CD, Beaty TH, et al. A genome-wide search for allergic response (atopy) genes in three ethnic groups: Collaborative Study on the Genetics of Asthma. Hum Genet 2004; 114:157–164.
80. Blumenthal MN, Ober C, Beaty TH, et al. Genome scan for loci linked to mite sensitivity: the Collaborative Study on the Genetics of Asthma (CSGA). Genes Immun 2004; 5:226–231.
81. Laitinen T, Polvi A, Rydman P, et al. Characterization of a common susceptibility locus for asthma-related traits. Science 2004; 304:300–304.
82. Gohlke H, Illig T, Bahnweg M, et al. Association of the interleukin-1 receptor antagonist gene with asthma. Am J Respir Crit Care Med 2004; 169:1217–1223.
83. Pessi T, Karjalainen J, Hulkkonen J, et al. A common IL-1 complex haplotype is associated with an increased risk of atopy. J Med Genet 2003; 40:e66.
84. Joki-Erkkila VP, Karjalainen J, Hulkkonen J, et al. Allergic rhinitis and polymorphisms of the interleukin 1 gene complex. Ann Allergy Asthma Immunol 2003; 9:275–279.
85. van Oosterhout AJM, Deurloo DT, Groot PC. Cytotoxic T lymphocyte antigen 4 polymorphisms and allergic asthma. Clin Exp Allergy 2004; 34:4–8.
86. Yang KD, Liu C-A, Chang J-C, et al. Polymorphism of the immune-braking gene CTLA-4 (+49) involved in gender discrepancy of serum total IgE levels and allergic diseases. Clin Exp Allergy 2004; 34:32–37.
87. Bourgain C, Hoffjan S, Nicolae R, et al. Novel case-control test in a founder population identifies P-selectin as an atopy-susceptibility locus. Am J Hum Genet 2003; 73:612–626.
88. Basehore MJ, Howard TD, Lange LA, et al. A comprehensive evaluation of IL4 variants in ethnically diverse populations: association of total serum IgE levels and asthma in white subjects. J Allergy Clin Immunol 2004; 114:80–87.
89. Vercelli D. Learning from discrepancies: CD14 polymorphisms, atopy and the endotoxin switch. Clin Exp Allergy 2003; 33:153–155.

90. Kabesch M, Carr D, Weiland SK, et al. The association between polymorphisms in serine protease inhibitor, kazal type 5 and asthma phenotypes in a large German population sample. Clin Exp Allergy 2004; 34:340–345.
91. Moffatt MF, Faux JA, Lester S, et al. Atopy, respiratory function and HLA-DR in Aboriginal Australians. Hum Mol Genet 2003; 12:625–630.
92. Hoffjan S, Stemmler S. On the role of the epidermal differentiation complex in ichthyosis vulgaris, atopic dermatitis and psoriasis. Br J Dermatol 2007; 157:441–449.
93. Cookson W, Moffatt M. Making sense of asthma genes. N Engl J Med 2004; 351:1794–1796.
94. Van Eerdewegh P, Little RD, Dupuis J, et al. Association of the ADAM-33 gene with asthma and bronchial hyper-responsiveness. Nature 2002; 418:426–430.
95. Allen M, Heinzmann A, Noguchi E, et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. Nat Genet 2003; 35:258–263.
96. Zhang Y, Leaves NI, Anderson GG, et al. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. Nat Genet 2003; 34:181–186.
97. Jang N, Stewart G, Jones G. Polymorphisms within the PHF11 gene at chromosome 13q14 are associated with childhood atopic dermatitis. Genes Immun 2005; 6:262–264.
98. Laitinen T, Polvi A, Rydman P, et al. Characterization of a common susceptibility locus for asthma-related traits. Science 2004; 304:300–304.
99. Howard TD, Postma DS, Jongepier H, et al. Association of disintegrin and metalloprotease 33 (ADAM 33) gene with asthma in ethnically diverse populations. J Allergy Clin Immunol 2003; 112:717–722.
100. Werner M, Herbon N, Gohlke H, et al. Asthma is associated with single-nucleotide polymorphism in ADAM 33. Clin Exp Allergy 2004; 34:26–31.
101. Lind DL, Choudhry S, Ung N, et al. ADAM 33 is not associated with asthma in Puerto Rican or Mexican population. Am J Respir Crit Care Med 2003; 168:1312–1316.
102. Raby BA, Silverman EK, Kwiatkowski DJ, et al. ADAM33 polymorphisms and phenotype associations in childhood asthma. J Allergy Clin Immunol 2004; 133:1017–1078.
103. Blumenthal MN. Epidemiology and genetics of asthma and allergy. In: Kaplan AP, ed. Allergy. Philadelphia: W.B. Saunders, 1997:407–420.
104. Leonardi S, Miraglia del Giudice M, La Rosa M, et al. Atopic disease, immune system, and the environment. Allergy Asthma Proc 2007; 28:410–417.
105. Kalinski P, Moser M. Consensual immunity: success-driven development of T-helper-1 and T-helper-2 responses. Nat Rev Immunol 2005; 5:251–260.
106. Strachan DP. Hay fever, hygiene, and household size. Br Med J 1989; 299:1259–1260.
107. Stern DA, Riedler J, Nowak D, et al. Exposure to a farming environment has allergen-specific protective effects on TH2-dependent isotype switching in response to common inhalants. J Clin Allergy Immunol 2007; 119:351–358.
108. Bach J-F. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 2002; 347:911–920.
109. Celedón JC, Milton DK, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. J Allergy Clin Immunol 2007; 120:144–149.
110. Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. J Allergy Clin Immunol 2006; 117:969–977.
111. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy 2000; 55:688–697.
112. Yabuhara A, Macaubas C, Prescott SL, et al. TH2-polarized immunological memory to inhalant allergens in atopics is established during infancy and early childhood. Clin Exp Allergy 1997; 27:1261–1269.
113. Vercelli D. Mechanisms of the hygiene hypothesis—molecular and otherwise. Curr Opin Immunol 2006; 18:733–737.
114. Romagnani S. Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. J Allergy Clin Immunol 2007; 119:1511–1513.
115. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA 2002; 288:963–972.
116. Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 2003; 3:169–176.
117. Chen W, Khurana-Hershey GK. Signal transducer and activator of transcription signals in allergic disease. J Allergy Clin Immunol 2007; 119:529–541.
118. Liu AH, Murphy JR. Hygiene hypothesis: fact or fiction? J Allergy Clin Immunol 2003; 111:471–478.
119. Liu AH, Leung DY. Renaissance of the hygiene hypothesis. J Allergy Clin Immunol 2006; 117:1063–1066.
120. Lympany PA, Lee T. Inflammation. In: Bjorksten B, Blumenthal MN, eds. Genetics of Allergy and Asthma: Methods for Investigative Studies. New York: Marcel Dekker, 1996:241–280.
121. Barnes P. Inflammation. In: Weiss EB, Stein M, eds. Bronchial Asthma: Mechanisms and Therapeutics. Boston: Little, Brown and Co., 1993:80–94.

3 Allergen Nomenclature

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HISTORICAL INTRODUCTION

As with most biochemical disciplines, the history of allergen nomenclature dates back to the time when allergens were fractionated using a variety of "classical" biochemical separation techniques, and the active (most allergenic) fraction was usually named according to the whim of the investigator. Early attempts were made to purify pollen and house-dust allergens, using phenol extraction, salt precipitation, and electrophoretic techniques in the 1940–1950s. In the 1960s, ion exchange and gel filtration media were introduced and ragweed "antigen E" was the first allergen to be purified. This allergen was named by King and Norman because it was one of five precipitin lines (labeled A–E) that reacted rabbit polyclonal antibodies to ragweed in Ouchterlony immunodiffusion tests. Following purification, precipitin line E, or antigen E, was shown to be a potent allergen (1). Later, Marsh, working in Cambridge, England, isolated an important allergen from rye grass pollen (*Lolium perenne*) and used the name "Rye 1" to indicate that this was the first allergen purified from this species (2,3). In the 1970s, many allergens were purified from ragweed, rye grass, insect venoms, and other sources. The field was led by the laboratory of the late Dr. David Marsh, who had moved to the Johns Hopkins University, Baltimore, Maryland. At Hopkins, ragweed allergens, Ra3, Ra4, Ra5, and Ra6, and rye grass allergens, Rye 2 and Rye 3, were isolated and used for immunological and genetic studies of hay fever (4–6). At the same time, Ohman et al. identified the major cat allergen (Cat-I) (7) and Elsayed purified allergen M from codfish (8,9).

The state of the art in the early 1970s was reviewed in a seminal book chapter by Marsh in *The Antigens* (ed. Michael Sela), which described the molecular properties of allergens, the factors that influenced allergenicity, the immune response to allergens, and immunogenetic studies of IgE responses to purified pollen allergens (10). This chapter provided the first clear definition of a "major" allergen, which Marsh defined as a highly purified allergen that induced immediate skin test responses in >90% of allergic individuals in contrast to a "minor" allergen, to which <20% of patients gave skin test responses. Today, a major allergen is generally regarded as one to which >50% of allergic patients react (11).

With the introduction of crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) for allergen identification by Lowenstein and colleagues in Scandinavia, there was a tremendous proliferation of the number of antigenic proteins and CIE/CRIE peaks identified as allergens. Typically, 10 to 50 peaks could be detected in a given allergen based on reactivity with rabbit polyclonal antibodies or IgE antibodies (6,11–13). These peaks were given a plethora of names such as Dp5, Dp42, Ag 12, etc. Inevitably, the same allergens were referred to by different names in different laboratories, e.g., mite Antigen P_1 was also known as Dp42 or Ag12. It was clear that a unified nomenclature was urgently needed.

Three Men in a Boat

The origins of the systematic allergen nomenclature can be traced to meeting among Drs. David Marsh (at that time, Johns Hopkins University, Baltimore, U.S.), Henning Lowenstein (at that time, University of Copenhagen, Denmark), and Thomas Platts-Mills (at that time, Clinical Research Centre, Harrow, U.K.) on a boatride on Lake Boedensee, Konstanz, Germany, during the 13th Symposium of the Collegium Internationale Allergologicum in July 1980 (14). The idea was simply to develop a systematic nomenclature based on the Linnean system, with numerals to indicate different allergens. It was decided to adopt a system whereby the allergen was described based on the first three letters of the genus and the first

letter of the species (in italics) and then by a Roman numeral to indicate the allergen in the chronological order of purification. Thus, ragweed antigen E became *Ambrosia artemisiifolia* allergen I or *Amb a* I and Rye 1 became *L. perenne* allergen I or *Lol p I*.

An allergen nomenclature subcommittee was formed under the auspices of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) and criteria for including allergens in the systematic nomenclature were established. These included strict criteria for biochemical purity as well as criteria for determining the allergenic activity of the purified protein. A committee chaired by Marsh and including Lowenstein, Platts-Mills, Drs. Te Piao King (Rockerfeller University, New York, U.S.), and Larry Goodfriend (McGill University, Canada) prepared a list of allergens that fulfilled the inclusion criteria and established a process for investigators to submit names of newly identified allergens. The original list, published in the Bulletin of the WHO in 1986, included 27 highly purified allergens from grass, weed and tree pollens, and house-dust mites (15).

The systematic allergen nomenclature was quickly adopted by allergy researchers and proved to be a great success. It was logical, easily understood, and readily assimilated by allergists and other clinicians who were not directly involved with the nitty-gritty of allergen immunochemistry. The nomenclature, *Der p* I, *Fel d* I, *Lol p* I, *Amb a* I, was used at scientific meetings and in the literature, and expanded rapidly to include newly isolated allergens.

THE REVISED ALLERGEN NOMENCLATURE

Allergens

The widespread use of molecular cloning techniques to identify allergens in the late 1980s and 1990s led to an exponential increase in the number of allergens described. Many allergen nucleotide sequences were generated from cDNA cloning or PCR-based sequencing, and it soon became apparent that the use of Roman numerals was unwieldy (e.g., *Lol p* I through *Lol p* XI) (16,17). The use of italics to denote a purified protein was inconsistent with nomenclature used in bacterial genetics and the HLA system, where italicized names denote a gene product and regular typeface indicates expressed proteins. In 1994, the allergen nomenclature was revised so that the allergen phenotype was shown in regular type and Arabic numerals were adopted. Thus, *Amb a* I, *Lol p* I, and *Der p* I in the original 1986 nomenclature are now referred to as Amb a 1, Lol p 1, and Der p 1 in the current nomenclature, which has been published in several scientific journals (18–20).

Inclusion Criteria

A key part of the systematic WHO/IUIS nomenclature is that the allergen should satisfy biochemical criteria, which define the molecular structure of the protein, and immunological criteria, which define its importance as an allergen. Originally, the biochemical criteria were based on establishing protein purity (e.g., by SDS-PAGE, IEF, or HPLC and physicochemical properties including MW, pI, and N-terminal amino acid sequence) (20). Nowadays, the full nucleotide or amino acid sequence is generally required. An outline of the inclusion criteria is shown in Table 1. An important aspect of these criteria is that they should provide a "handle" whereby other investigators can identify the same allergen and make comparative studies. Originally, this was achieved by purifying the protein, developing monospecific or monoclonal antibodies to it, and providing either the allergen or antibodies to other researchers for verification. Nucleotide and amino acid sequencing unambiguously identifies the allergen and enables sequence variation between cDNA clones of the same allergen to be defined (21–24). Allergen preparations, sequences, and antibodies submitted for inclusion in the systematic nomenclature are expected to be made available to other investigators for research studies.

A second set of inclusion criteria involves demonstrating that the purified allergen has allergenic activity, both in vitro and in vivo. Researchers use a variety of techniques for measuring IgE antibodies in vitro, including radioallergosorbent (RAST)-based techniques, immunoblotting, radioimmunoassays using labeled allergens, enzyme immunoassay (ELISA), and fluorescent enzymeimmunoassay (FEIA). It is important to screen a large number of sera from an unselected allergic population to establish the prevalence of IgE reactivity. Ideally,

Table 1 Allergens: Criteria for Inclusion in the WHO/IUIS Nomenclature

The molecular and structural properties should be clearly and unambiguously defined, including
Purification of the allergen protein to homogeneity.
Determination of molecular weight, pI, and carbohydrate composition.
Determination of nucleotide and/or amino acid sequence.
Production of monospecific or monoclonal antibodies to the allergen.
The importance of the allergen in causing IgE responses should be defined by
Comparing the prevalence of serum IgE antibodies in large population(s) of allergic patients. Ideally, at least 50 or more patients should be tested.
Demonstrating biological activity, e.g., by skin testing or histamine release assay.
Investigating whether depletion of the allergen from an allergic extract (e.g., by immunoabsorption) reduces IgE-binding activity.
Demonstrating, where possible, that recombinant allergens have comparable IgE antibody-binding activity to the natural allergen.

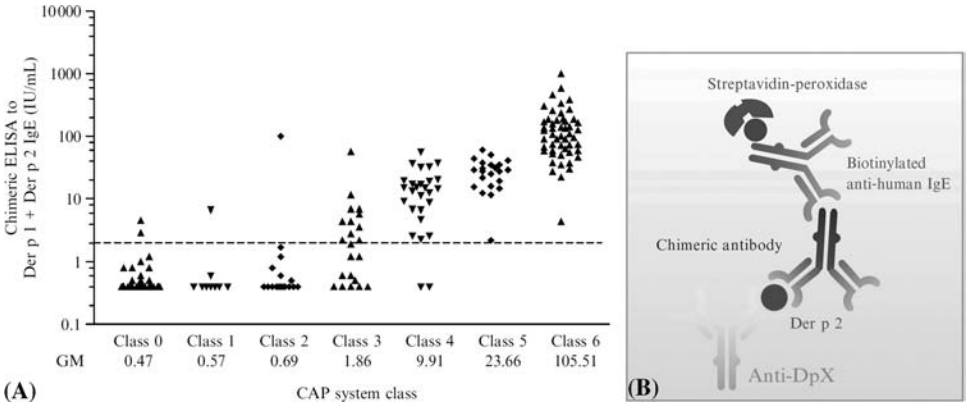

Figure 1 Chimeric ELISA for measuring allergen-specific IgE. (**A**) Schematic graphic of the ELISA. Microtiter plates are coated with monoclonal antibody followed by the relevant allergen and incubated with patient's serum. IgE antibodies that bind to the allergen complex are detected using biotinylated anti-IgE and streptavidin peroxidase. A chimeric IgE anti-Der p 2 is used to generate a control curve, and IgE values for patient's serum are interpolated from this curve. (**B**) Correlation between the chimeric ELISA for IgE antibody to Der p 1 and Der p 2 and FEIA (ImmunoCAP) for measuring IgE to house dust mite. There was an excellent quantitative correlation between the results for 212 sera from patients with asthma, wheezing, and/or rhinitis ($r = 0.86$, $p < 0.001$). *Source*: From Ref. 25.

50 or more sera should be screened, although allergens can be included in the nomenclature if the prevalence of IgE reactivity is >5% and if they elicit IgE responses in as few as five patients (Table 1) (20).

Several new methods for measuring IgE ab to specific allergens recently have been developed. "Chimeric" ELISA systems allow large numbers of sera to be screened for allergen-specific IgE ab by using a capture monoclonal antibody (mAb) to bind allergen. Serum IgE antibodies bind to the allergen complex and are detected with biotinylated anti-IgE (Fig. 1). The assay is quantified using a chimeric mouse anti-Der p 2 and human IgE epsilon antibody and provides results in ng/mL of allergen-specific IgE. Chimeric ELISA results for IgE ab to Der p 1, Der p 2, and Fel d 1 correlate with IgE measurements obtained by FEIA (25). A streptavidin-CAP assay using biotinylated allergens enables IgE antibodies to specific allergens to be routinely measured by FEIA (26). As with other diagnostic tests, chimeric ELISA and FEIA use separate tests to measure each IgE response, and these procedures use relatively large amounts of serum. Static or suspension microarray systems also have been developed that enable IgE antibodies to multiple allergens to be measured simultaneously. Microarrays provide a profile of IgE responses to specific allergens. One commercial test uses a static allergen array on allergen-coated glass slides to measure IgE antibodies in four sera to ~75 purified allergens at the same time. Results obtained with the microarray correlate with

FEIA using allergen extracts and the microarray uses only 30 μL serum (27–29). The sensitivity of the microarray is comparable to FEIA. Similarly, fluorescent multiplex suspension array technology has been developed in which allergens are covalently coupled to polystyrene microspheres containing different ratios of fluorescent dyes. Each microsphere bead can be distinguished by laser flow cytometry and forms a solid phase to which IgE antibodies bind and can be detected using biotinylated anti-IgE and streptavidin phycoerythrin. The fluorescent microarray currently measures total IgE and specific IgE to 10 purified allergens simultaneously using 20 μL serum (30). Array technologies are especially suited to large population surveys or birth cohorts for monitoring IgE responses to multiple allergens and for pediatric studies where serum is often in short supply.

Demonstrating that the allergen has biological activity in vivo is important, especially since many allergens are now produced as recombinant molecules before the natural allergen is purified (if ever). Several mite, cockroach, and fungal allergens (e.g., *Aspergillus, Alternaria, Cladosporium*) have been defined solely using recombinant proteins, and it is unlikely that much effort will be directed to isolating their natural counterparts. Ideally, the biological activity of recombinant proteins should be confirmed in vivo by quantitative skin testing or in vitro by histamine release assays. Skin testing studies were carried out using a number of recombinant allergens, including Bet v 1, Asp f 1, Bla g 4, Bla g 5, Der p 2, Der p 5, and Blo t 5 (22,31,32). These allergens showed potent biological activity and gave positive skin tests at the picogram level.

Resolving Ambiguities in Nomenclature

Early on it was recognized that because the system had Linnaean roots, some unrelated allergens would have the same name: *Candida* allergens could be confused with dog allergen (*Canis domesticus*); there are multiple related species of *Vespula* (Vespid) allergens; and *Periplaneta americana* (American cockroach) allergen needs to be distinguished from *Persea americana* (avocado)! These ambiguities have been overcome by adding a further letter to either the genus or species name. Examples thus become Cand a 1 (*C. albicans* allergen 1); Ves v 1 or Ves vi 1, to indicate *V. vulgaris* or *V. vidua* allergens, respectively; Per a 1 and Pers a 1 for the cockroach or avocado allergens. Dog allergen is referred to as Can f 1, from *Canis familiaris*. Many allergens have biochemical names that describe their biological function and which precede the allergen nomenclature. Examples include egg allergens (ovomucoid and ovalbumin); insect allergens (phospholipases and hyaluronidases); and tropomyosins from shrimp, mite, and cockroach. Sequence homology searches have assigned allergens to particular protein families and have provided important clues to their biological function. To some extent, allergens segregate among protein families that are according to whether they are indoor allergens, outdoor allergens, plant and animal food allergens, or injected allergens:

1. Indoor allergens (mite, animal allergens, cockroach, and molds): proteolytic enzymes (serine and cysteine proteases), lipocalins (ligand-binding proteins), tropomyosins, albumins, calcium-binding proteins, protease inhibitors (22,33)
2. Outdoor allergens (grass, tree and weed pollens, and mold spores): plant pathogenesis-related (PR-10) proteins, pectate lyases, β-expansins, calcium-binding proteins (polcalcins), defensin-like proteins, trypsin inhibitors (21,23,34,35)
3. Plant and animal food allergens (fruits, vegetables, nuts, milk, eggs, shellfish, and fish): lipid-transfer proteins, profilins, seed storage proteins, lactoglobulins, caseins, tropomyosins, parvalbumins (36–38)
4. Injected allergens (insect venoms and some therapeutic proteins): phospholipases, hyaluronidases, pathogenesis-related proteins, asparaginase (39,40)

Allergens belonging to these protein families are likely to have biological functions that are important to the host. Proteolytic enzymes are involved in digestion, tropomyosins and parvalbumins in muscle contraction, and profilins in actin polymerization in plants. The mouse lipocalin allergen, Mus m 1, is produced in the liver of male mice, secreted in large amounts in the urine and serves to mark the territories of male mice (41). The cockroach lipocalin allergen, Bla g 4, is produced in accessory glands of the male reproductive system and has an as yet unknown reproductive function (42,43) (Fig. 2). Crystallographic studies

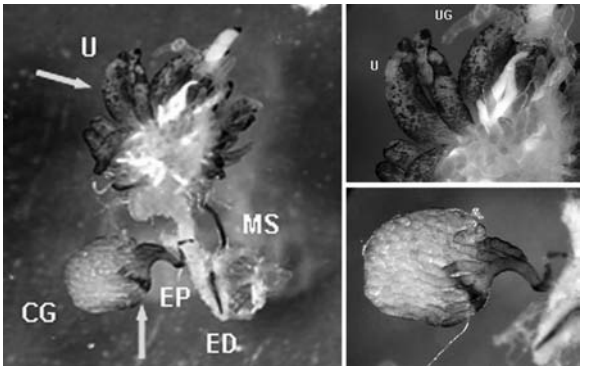

Figure 2 (*See color insert.*) Localization of German cockroach allergen Bla g 4 to the male reproductive tissues [conglobate glands (CG) and utricles (U)] by in situ hybridization (*left panel*). Right panel shows higher magnification. Bla g 4 is only found in male accessory reproductive glands and is transferred to the female during copulation. *Source*: From Ref. 42.

showed that Bet v 1, a plant pathogenesis–related (PR-10) protein, contained a hydrophobic pocket that could bind brassinosteroids and functions as a plant steroid carrier. The PR-10 proteins are important in plant defense, growth, and development (44).

In the allergy literature, it is preferable to use the systematic allergen nomenclature. However, in other contexts, such as comparisons of biochemical activities or protein structure, it may be appropriate or more useful to use the biochemical names. A selected list of the allergen nomenclature and biochemical names of inhalant, food, and venom allergens is shown in Table 2. There are now over 50 three-dimensional allergen structures in the Protein Database (PDB) and allergens are found in ~150 protein families in the Pfam protein family database (www.sanger.ac.uk/Software/Pfam). Breiteneder has argued that this is a relatively small number, given that almost 9000 protein families reside in Pfam (23,45). However, the 150 allergen protein families that have been identified still represent a huge degree of diversity at both the structural and biological level. Such diversity precludes any common structural feature, e.g., amino acid sequence motif or protein structure, which makes an allergen an allergen (21,24).

Isoallergens, Isoforms, and Variants

Originally, isoallergens were broadly defined as multiple molecular forms of the same allergen, sharing extensive antigenic (IgE) cross-reactivity. The revised nomenclature defines an isoallergen as an allergen from a single species, sharing similar molecular size, identical biological function, and $\geq$67% amino acid sequence identity (8). Some allergens, which were previously "grand fathered" into the nomenclature as separate entities, share extensive sequence homology and some antigenic cross-reactivity, but are named independently and are not considered to be isoallergens. Examples include Lol p 2 and Lol p 3 (65% homology) and Amb a 1 and Amb a 2 (65% homology). The word "Group" is now being used more often to describe structurally related allergens from different species within the same genus, or from closely related genera. In these cases, the levels of amino acid sequence identity can range from as little as 40% to ~90%. Similarities in tertiary structure and biological function are also taken into account when describing allergen Groups. Examples include the Group 2 mite allergens (Der p 2, Der f 2 and Lep d 2, Gly d 2 and Tyr p 2) showing 40% to 88% homology, and the Group 5 ragweed allergens (Amb a 5, Amb t 5, and Amb p 5) showing ~45% homology. The *Dermatophagoides* Group 2 allergen structures have been determined by X-ray crystallography and nuclear magnetic resonance spectroscopy (NMR). The structures of the Group 2 allergens from other species were modeled on the *Dermatophagoides* structures (Fig. 3). This enabled the structural basis for antigenic relationships between members of the Group to be defined (20–22).

The term "variant" or "isoform" is used to indicate allergen sequences that show a limited number of amino acid substitutions (i.e., polymorphic variants of the same allergen). Typically, variants may be identified by sequencing several cDNA clones of a given allergen. Variants have been reported for Der p 1, Der p 2, Amb a 1, Cry j 1 and, for the most prolific Bet v 1, for which 42 sequences have been deposited in the GenBank database. Isoallergens and variants are denoted by the addition of four numeral suffixes to the allergen name. The first two numerals distinguish between isoallergens and the last two between variants. Thus, for

Table 2 Molecular Properties of Common Allergens

Source	Allergen	MW (kDa)	Homology/function
Inhalants			
Indoor			
House-dust mite (*Demlatophagoides pteronyssinus*)	Der p 1	25	Cysteine protease[b]
	Der p 2	14	Lipid binding protein
	Der p 3	30	Serine protease
	Der p 5	14	Unknown
Cat (*Felis domesticus*)	Fel d 1	36	Secretoglobin[b]
Dog (*Canis familiariss*)	Can f 1	25	Cysteine protease inhibitor?[b]
Mouse (*Mus muscularis*)	Mus m 1	21	Lipocalin (territory marking protein)
Rat (*Rattus norvegicus*)	Rat n 1	21	Pheromone-binding lipocalin[b]
Cockroach (*Blattella germanica*)	Bla g 2	36	Inactive aspartic protease
Outdoor			
Pollens—grassses			
Rye (*Lolium perenne*)	Lol p 1	28	Unknown
Timothy (*Phleum pratense*)	Phl p 5	32	Unknown
Bermuda (*Cynodon dactylon*)	Cyn d 1	32	Unknown
Weeds			
Ragweed (*Artemisia artemisiifolia*)	Amb a 1	38	Pectate lyase[b]
	Aruba 5	5	Neurophysins[b]
Trees			
Birch (*Betula verrucosa*)	Bet v 1	17	Pathogenesis-related protein[b]
Foods			
Milk	β-Lactolobulin	36	Retinol-binding protein[a,b]
Egg	Ovomucoid	29	Trypsin inhibitor
Codfish (*Gadus callarias*)	Gad c 1	12	Ca-binding protein (muscle parvalbumin)
Peanut (*Arachis hypogea*)	Ara h l	63	Vicilin (seed-storage protein)[b]
Venoms			
Bee (*Apis mellifera*)	Api m 1	19.5	Phospholipase A_2[b]
Wasp (*Polistes annularis*)	Pol a 5	23	Mammalian testis proteins
Homet (*Vespa crabro*)	Ves c 5	23	Mammalian testis proteins
Fire ant (*Solenopsis invicta*)	Sol i 2	13	Unknown
Fungi			
Aspergillus fumigatus	Asp f 1	18	Cytotoxin (mitogillin)
Alternaria altenata	Alt a 1	29	Unknown
Latex			
Hevea brasiliensis	Hev b 1	58	Elongation factor
	Hev b 5	16	Unknown—homologous to kiwi fruit protein of unknown function

[a]Most allergens have a single polypeptide chain; dimers are indicated.
[b]Allergens of known three-dimensional structure are also indicated.

ragweed Amb a 1, which occurs as four isoallergens, showing 12% to 24% differences in amino acid sequence, the nomenclature is as follows:

Allergen: Amb a 1
Isoallergens: Amb a 1.01; Amb a 1.02; Amb a 1.03; Amb a 1.04

Three variants of each isoallergen occur, showing >97% sequence homology

Isoforms: Amb a 1.0101, Amb a 1.0102, Amb a 1.0103
Amb a 1.0201, Amb a 1.0202, Amb a 1.0203, etc.

The Group 1 allergens from tree pollen have an unusually high number of isoallergens and variants. The 42 Bet v 1 sequences are derived from 31 isoallergens, which show from 73% to 98% sequence homology and are named from Bet v 1.0101 through Bet v 1.3101. The Group 1 allergen from hornbeam (*Carpinus betulus*), Car b 1, has three isoallergens that show 74% to

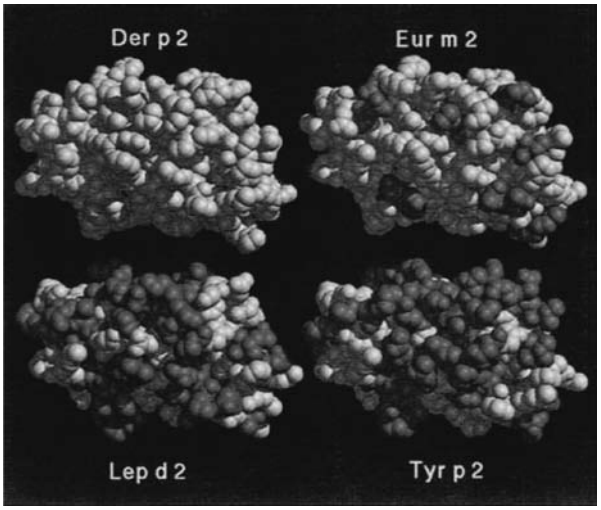

Figure 3 (*See color insert.*) Space-filling models of Group 2 allergens from house-dust mite. Amino acid substitutions are shown in gray scale. The space-filling model of Der p 2 (*Dermatophagoides pteronyssinus*) was generated from nuclear magnetic resonance spectroscopy studies and has subsequently been confirmed by X-ray crystallography (22). Eur m 2 (*Euroglyphus maynei*) shows 85% sequence identity with Der p 2 and seven of the substituted amino acids are shown in gray on the surface structure. There is extensive cross-reactivity between Der p 2 and Eur m 2. In contrast, Lep d 2 and Tyr p 2 show only 40% amino acid identity with the other Group 2 allergens. They show many substitutions on the antigenic surface of the molecules and show limited antigenic cross-reactivity for mAb and human IgE. *Source*: From Refs. 51, 52.

88% homology (Car b 1.01, 1.02, and 1.03) and the nomenclature committee's most recent records show 15 sequences of Car b 1. Ten variants of hazel pollen allergen, Cor a 1, have also been recorded. The reasons why the Group 1 tree pollen allergens have so many variants are unclear. Latex provides another example of distinctions in nomenclature. Hevein is an important latex allergen, designated Hev b 6, which occurs as a 20-kDa precursor with two fragments all derived from the same transcript. These moieties are all variants of Hev b 6 and are distinguished as Hev b 6.01 (prohevein, 20-kDa precursor), Hev b 6.02 (5-kDa hevein), and Hev b 6.03 (a 14-kDa C-terminal fragment). Studies have also uncovered a prodigious number of isoforms among mite Group 1 and Group 2 allergens. High fidelity PCR sequencing of environmental isolates of dust mites revealed 23 isoforms of Der p 1 and 13 isoforms of Der p 2) (24). Because isoforms differ in only a few amino acid substitutions, analysis of immunoreactivity to isoforms can be useful in defining antibody-binding sites and T-cell epitopes on allergens (46).

NOMENCLATURE FOR ALLERGEN GENES AND RECOMBINANT OR SYNTHETIC PEPTIDES

In the revised nomenclature, italicized letters are reserved to designate allergens genes. Two genomic allergen sequences have been determined from animal dander allergens: cat allergens, Fel d 1; and mouse urinary allergen, Mus m 1. Fel d 1 has two separate genes encoding chain 1 and chain 2 of the molecule, which are designed *Fel d 1A* and *Fel d 1B*, respectively (24). Genomic sequences of Bet v 1, Cor a 1, and apple "allergen," Mal d 1, have also been determined (47,48). Mal d 1 is an example of an incomplete or nonsensitizing allergen, i.e., an allergen that can interact with IgE antibodies but is unable to induce the production of IgE. Thus, symptoms of oral allergy syndrome in birch pollen allergic patients who eat apple are due to IgE cross-reactivity between Bet v 1 (the primary sensitizer) and Mal d 1 (with which the IgE anti-Bet v 1 interacts).

When recombinant allergens were first introduced, researchers often used the term "native allergen" to distinguish the natural protein from the recombinant allergen. However, because "native" has implications for protein structure (i.e., native conformation), it was decided that the term "natural allergen" should be used to indicate any allergen purified from natural source material. Natural allergens may be denoted by the prefix (n) to distinguish them from recombinant allergens, which are indicated by the prefix (r) before the allergen name (e.g., nBet v 1 and rBet v 1). There is no distinction between recombinant allergens produced in bacterial, yeast, or mammalian expression systems. Synthetic peptides are indicated by the prefix (s), with the particular peptide residues indicated in parentheses after the allergen name.

Thus, a synthetic peptide encompassing residues 100 to 120 of Bet v 1.0101 would be indicated: sBet v 1.0101 (100–120). At this point, the nomenclature, while technically sound, begins to become cumbersome and rather long-winded for most purposes. There are also additional refinements to the nomenclature that cover substitutions of different amino acid residues within synthetic peptides. This aspect of the nomenclature (which is based on that used for synthetic peptides of immunoglobulin sequences) is detailed in the revised nomenclature document to which aficionados are referred for full details (20).

THE IUIS SUBCOMMITTEE ON ALLERGEN NOMENCLATURE

Allergens to be considered for inclusion in the nomenclature are reviewed by the IUIS Subcommittee, which is currently chaired by Dr. Heimo Breiteneder (Medical University of Vienna, Austria) and has 19 members from all over the world (Table 3). The committee meets annually at an international allergy/immunology meeting and discusses new proposals it has received during the year, together with any proposed changes or additions to the nomenclature. There is also a committee-at-large, which is open to any scientist with an interest in allergens, to whom decisions made by the subcommittee are circulated. The procedure for submitting candidate names for allergens to the subcommittee is straightforward. Having purified the allergen and shown that it reacts with IgE ab, investigators should download the "new allergen name" form from the nomenclature committee web site (www.allergen.org) and send the completed form to the subcommittee prior to publishing articles describing the allergen. The subcommittee will provisionally accept the author's suggested allergen name, or assign the allergen a name, provided that the inclusion criteria are satisfied. The name will later be confirmed at a full meeting of the subcommittee. Occasionally, the subcommittee has to resolve differences between investigators who may be using different names for the same allergen, or disputes concerning the chronological order of allergen identification. These issues can normally be resolved by objective evaluation of each case.

Table 3 The World Health Organization and International Union of Immunological Societies Sub-Committee on Allergen Nomenclature, 2006–2008[a]

Name	Institution	Country
Heimo Breiteneder, PhD, Chairman	Medical University of Vienna	Vienna, Austria
Stefan Vieths, PhD, Secretary	Paul Ehrlich Institute	Langen, Germany
Wayne R Thomas, PhD, Past Chair	Western Australia Institute for Child Health	Perth, Australia
Naveen Arora, PhD	Institute of Genomics and Integrative Biology	Delhi, India
L. Karla Arruda, MD, PhD	School of Medicine of Ribeirão Preto, University of São Paulo	Ribeirão Preto, Brazil
Martin D Chapman, PhD	Indoor Biotechnologies, Inc.	Charlottesville, VA, USA
Fatima Ferreira, PhD	University of Salzburg	Salzburg, Austria
Richard Goodman, PhD	University of Nebraska	Lincoln, NE, USA
Donald Hoffman, PhD	East Carolina University	Greenville, NC, USA
Viswanath P Kurup, PhD	Medical College of Wisconsin	Milwaukee, WI, USA
Jørgen N Larsen, PhD	ALK-Abelló	Hørsholm, Denmark
Jonas Lidholm, PhD	Phadia AB	Uppsala, Sweden
Kåre Meno, PhD	ALK-Abelló	Hørsholm, Denmark
Andreas Nandy, PhD	Allergopharma Joachim Ganzer KG	Reinbek, Germany
Thomas AE Platts-Mills, MD, PhD	University of Virginia	Charlottesville, VA, USA
Christian Radauer, PhD	Medical University of Vienna	Vienna, Austria
Marianne van Hage MD, PhD	Karolinska Institute	Stockholm, Sweden
Ronald van Ree, PhD	Academic Medical Centre	Amsterdam, The Netherlands

[a]Past chairs of the committee: David G Marsh, PhD (1980–1989); Te Piao King, PhD (1990–1994); Henning Løwenstein, PhD (1994–1997); Wayne R. Thomas, PhD (1997–2006). Henning Løwenstein, PhD, DMSc (Hørsholm, Denmark) is an Emeritus member of the committee.

Table 4 Online Databases for Allergen Nomenclature and Structural Biology

Database	Locator
WHO/IUIS Allergen Nomenclature Sub-Committee	**www.allergen.org**[a]
Structural Database of Allergenic Proteins (SDAP)	http://fermi.utmb.edu/SDAP
Food Allergy Research and Resource Program (Farrp)	www.allergenonline.com
Protall	www.ifr.bbsrc.ac.uk/protall
ALLERbase	www.dadamo.com/allerbase
Allergome	www.allergome.org
Central Science Laboratory (York, UK)	http://www.csl.gov.uk/allergen/
AllFam	http://www.meduniwien.ac.at/allergens/allfam/

[a]Official website of the WHO/IUIS Sub-committee on allergen nomenclature.

Allergen Databases

The official web site for the WHO/IUIS Subcommittee on Allergen Nomenclature is www.allergen.org. This site lists all allergens and isoforms that are recognized by the Subcommittee and is updated on a regular basis. Over the past five years, several other allergen databases have been generated by academic institutions, research organizations, and industry sponsored groups (Table 4). These sites differ in their focus and emphasis, but are useful sources of information about allergens. The Structural Database of Allergenic Proteins (SDAP) was developed at the Sealy Center for Structural Biology, University of Texas Medical Branch, and provides detailed structural data on allergens in the WHO/IUIS nomenclature, including sequence information, PDB-files and programs to analyze IgE epitopes. Amino acid and nucleotide sequence information is also compiled in the SWISS-PROT and NCBI databases.

The Farrp and Protall databases have a focus on food allergens and provide sequence similarity searches (Farrp) and clinical data (skin tests and provocation tests) on food allergens (Protall). The Allergome database provides regular updates on allergens from publications in the scientific literature. Recently, a new database, AllFam, was developed that merges the Allergome allergens database with data on protein families from the Pfam database. Allfam contains all allergens with known sequences that can be assigned to at least one Pfam family. The database is maintained by Drs. Breiteneder and Radauer at the University of Vienna and can be accessed at: http://www.meduniwien.ac.at/allergens/allfam/.

CONCLUDING REMARKS

The three men in a boat did a remarkably good job! The use of the systematic allergen nomenclature has been extremely successful, has significantly enhanced research in the area, and continues to be revised and updated. The use of the generic terms "major" and "minor" allergen continues to evoke discussion. Relatively few allergens fulfill the criteria originally used by Marsh to define a major allergen (i.e., an allergen that causes IgE responses in $\geq$90% of allergic patients, such as Bet v 1, Fel d 1, Der p 2, Lol p 1) (10). However, there are a large number of allergens that cause sensitization in >50% of patients and Lowenstein et al. used this figure of 50% to define major allergens in the early 1980s (6). Scientists like to describe their allergen as "major" because it is effective in promoting their research and carries some weight in securing research funding. The question continues to be "What defines a major allergen"? Demonstrating a high prevalence of IgE-mediated sensitization and that the protein has allergenic activity is a minimal requirement, given the increasing sensitivity of assays to detect IgE antibodies. The contribution of an allergen to the total potency of the vaccine should be considered (e.g., by absorption studies), as well as the amount of IgE antibody directed against the allergen, compared with other allergens purified or cloned from the same source. Other criteria include whether the allergen induces strong T-cell responses and, for indoor allergens, whether it is a suitable marker of exposure in house-dust and air samples. The author, together with Dr. Rob Aalberse (University of Amsterdam), has developed eight criteria for "Allergens that make a difference."

It is clear from many studies that some allergens play a pre-eminent role in causing immune responses in atopic individuals; are better marker proteins for immunological, clinical, and epidemiological studies; and are usually considered to be high-profile targets for

Table 5 Eight Criteria for Defining Allergens That Make a Difference

1. A sensitization rate of >80% (>2 ng allergen specific IgE/mL) in a large panel of allergic patients
2. A significant proportion of total IgE (>10%) can be allergen specific
3. Absorption of the allergen from the source material significantly reduces the potency of the extract
4. Absorption of serum with purified allergen significantly reduces specific IgE to the allergen extract
5. The allergen accounts for a significant proportion of the extractable protein in the source material
6. The allergen can be used as a marker for environmental exposure assessment
7. Both antibody and cellular responses to the allergen can be measured in a high proportion of allergic patients
8. The allergen has been shown to be effective as part of an allergy vaccine

allergy diagnostics and therapeutics. Table 5 lists the eight criteria for defining the properties of these "allergens that make a difference." Examples of allergens that we consider to fulfill most of these criteria are as follows:

Mite	Group 1 and Group 2 (*Dermatophagoides* sp) allergens
Animal	Fel d 1, Mus m 1, Rat n 1
Tree pollen	Bet v 1 (and structurally homologous allergens); Ole e 1
Grass pollen	Phl p 1, Phl p 5
Weed pollen	Amb a 1
Peanut	Ara h 1, Ara h 2
Shellfish	Pen a 1 and other tropomyosins from shellfish
Insect allergens	Api m 1 (and homologous insect venom allergens)

Some of these recombinant allergens have already been shown to be effective as vaccines in clinical trials (Phl p 1 and Phl p 5), and most of the other allergens listed are being used as targets for vaccine development (4,22,49,50).

For most purposes, allergists need only be familiar with the nomenclature for allergens, rather than isoallergens, isoforms, peptides, etc. As measurements of allergens in diagnostics and vaccines, and in environmental exposure assessments become a routine part of the care of allergic patients, allergists will need to understand more about the structure and functions of allergens and how to distinguish them. Having a systematic nomenclature is an important part of this process. The systematic nomenclature is a proven success and is versatile enough to evolve with advances in molecular biology and proteomics that will occur over the next decade.

SALIENT POINTS

- A systematic nomenclature for all allergens that cause disease in humans has been formulated by a subcommittee of the WHO and the IUIS.
- Allergens are described using the first three letters of the genus, followed by a single letter for the species and an Arabic numeral to indicate the chronological order of allergen purification (e.g., *Dermatophagoides pteronyssinus* allergen 1 = Der p 1).
- To be included in the systematic nomenclature, allergens have to satisfy criteria of biochemical purity and criteria to establish their allergenic importance. It is important that the molecular structure of an allergen is defined without ambiguity and that allergenic activity is demonstrated in a large, unselected population of allergic patients.
- Modifications of the nomenclature are used to identify isoallergens, isoforms, allergen genes, recombinant allergens, and synthetic peptides. For example, Bet v 1.10 is an isoallergen of Bet v 1, and Bet v 1.0101 is an isoform or variant of the Bet v 1.10 isoallergen. Allergen genes are denoted by italics; e.g., *Fel d* 1A and *Fel d* 1B are the genes encoding chain 1 and chain 2 of Fel d 1, respectively.

This chapter has reviewed the systematic IUIS allergen nomenclature as revised in 1994. Other views expressed in the chapter are personal opinions and do not necessarily reflect the views of the WHO/IUIS Subcommittee on Allergen Nomenclature. The author is grateful to

Dr. Anna Pomés for assistance in preparing this chapter, and to Drs. Chad Gore and Coby Schal for permission to reproduce Figure 2.

REFERENCES

1. King TP, Norman PS. Isolation of allergens from ragweed pollen. Biochemistry 1962; 1:709–720.
2. Johnson P, Marsh DG. 'Isoallergens' from rye grass pollen. Nature 1965; 206(987):935–937.
3. Johnson P, Marsh DG. Allergens from common rye grass pollen (Lolium perenne). II. The allergenic determinants and carbohydrate moiety. Immunochemistry 1966; 3(2):101–110.
4. Marsh DG, Bias WB, Santilli J Jr., et al. Ragweed allergen Ra5: a new tool in understanding the genetics and immunochemistry of immune response in man. Immunochemistry 1975; 12(6–7):539–543.
5. Platts-Mills TA, Chapman MD, Marsh DG. Human immunoglobulin E and immunoglobulin G antibody responses to the "minor" ragweed allergen Ra3: correlation with skin tests and comparison with other allergens. J Allergy Clin Immunol 1981; 67(2):129–134.
6. Lowenstein H, King TP, Goodfriend L, et al. Antigens of Ambrosia elatior (short ragweed) pollen. II. Immunochemical identification of known antigens by quantitative immunoelectrophoresis. J Immunol 1981; 127(2):637–642.
7. Ohman JL Jr., Lowell FC, Bloch KJ. Allergens of mammalian origin. III. Properties of a major feline allergen. J Immunol 1974; 113(6):1668–1677.
8. Elsayed SM, Aas K. Characterization of a major allergen (cod.) chemical composition and immunological properties. Int Arch Allergy Appl Immunol 1970; 38(5):536–548.
9. Elsayed S, Bennich H. The primary structure of allergen M from cod. Scand J Immunol 1975; 4(2):203–208.
10. Marsh DG. Allergens and the genetics of allergy. In: Sela M III, ed. The Antigens. New York: Academic Press, 1975:271–350.
11. Lowenstein H. Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. Prog Allergy 1978; 25:1–62.
12. Lind P, Korsgaard J, Lowenstein H. Detection and quantitation of Dermatophagoides antigens in house dust by immunochemical techniques. Allergy 1979; 34(5):319–326.
13. Lowenstein H. Timothy pollen allergens. Allergy 1980; 35(3):188–191.
14. DeWeck A, Ring J. Collegium Internationale Allergologicum. History and Aims of a Special International Community Devoted to Allergy Research, 1954–1996. Munich, MMV Medezin Verlag 1996:1–120.
15. Marsh DG, Goodfriend L, King TP, et al. Allergen nomenclature. Bull World Health Organ 1986; 64 (5):767–774.
16. Scheiner O, Kraft D. Basic and practical aspects of recombinant allergens. Allergy 1995; 50(5):384–391.
17. Thomas WR. Mite allergens groups I-VII. A catalogue of enzymes. Clin Exp Allergy 1993; 23(5): 350–353.
18. King TP, Hoffman D, Lowenstein H, et al. Allergen nomenclature. WHO/IUIS Allergen Nomenclature Subcommittee. Int Arch Allergy Immunol 1994; 105(3):224–233.
19. King TP, Hoffman D, Lowenstein H, et al. Allergen nomenclature. Allergy 1995; 50(9):765–774.
20. King TP, Hoffman D, Lowenstein H, et al. Allergen Nomenclature. Bull World Health Org 1994; 72:797–800.
21. Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol 2000; 106(2):228–238.
22. Chapman MD, Smith AM, Vailes LD, et al. Recombinant allergens for diagnosis and therapy of allergic disease. J Allergy Clin Immunol 2000; 106(3):409–418.
23. Radauer C, Breiteneder H. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. J Allergy Clin Immunol 2006; 117(1):141–147.
24. Chapman MD, Pomes A, Breiteneder H, et al. Nomenclature and structural biology of allergens. J Allergy Clin Immunol 2007; 119(2):414–420.
25. Trombone AP, Tobias KR, Ferriani VP, et al. Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis. Clin Exp Allergy 2002; 32(9):1323–1328.
26. Erwin EA, Custis NJ, Satinover SM, et al. Quantitative measurement of IgE antibodies to purified allergens using streptavidin linked to a high-capacity solid phase. J Allergy Clin Immunol 2005; 115 (5):1029–1035.
27. Harwanegg C, Laffer S, Hiller R, et al. Microarrayed recombinant allergens for diagnosis of allergy. Clin Exp Allergy 2003; 33(1):7–13.
28. Wohrl S, Vigl K, Zehetmayer S, et al. The performance of a component-based allergen-microarray in clinical practice. Allergy 2006; 61(5):633–639.
29. Hiller R, Laffer S, Harwanegg C, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J 2002; 16(3):414–416.
30. King EM, Vailes LD, Tsay A, et al. Simultaneous detection of total and allergen-specific IgE using purified allergens in a fluorescent multiplex array. J Allergy Clin Immunol 2007; 120(5):1126–1131.

31. Schmid-Grendelmeier P, Crameri R. Recombinant allergens for skin testing. Int Arch Allergy Immunol 2001; 125(2):96–111.
32. Pauli G, Purohit A, Oster JP, et al. Comparison of genetically engineered hypoallergenic rBet v 1 derivatives with rBet v 1 wild-type by skin prick and intradermal testing: results obtained in a French population. Clin Exp Allergy 2000; 30(8):1076–1084.
33. Arruda LK, Vailes LD, Ferriani VP, et al. Cockroach allergens and asthma. J Allergy Clin Immunol 2001; 107(3):419–428.
34. Radauer C, Willerroider M, Fuchs H, et al. Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. Clin Exp Allergy 2006; 36(7): 920–929.
35. Gadermaier G, Dedic A, Obermeyer G, et al. Biology of weed pollen allergens. Curr Allergy Asthma Rep 2004; 4(5):391–400.
36. Breiteneder H, Radauer C. A classification of plant food allergens. J Allergy Clin Immunol 2004; 113(5): 821–830.
37. Reese G, Schicktanz S, Lauer I, et al. Structural, immunological and functional properties of natural recombinant Pen a 1, the major allergen of Brown Shrimp, Penaeus aztecus. Clin Exp Allergy 2006; 36(4):517–524.
38. Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. Ann N Y Acad Sci 2002; 964:47–68.
39. Henriksen A, King TP, Mirza O, et al. Major venom allergen of yellow jackets, Ves v 5: structural characterization of a pathogenesis-related protein superfamily. Proteins 2001; 45(4):438–448.
40. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. Int Arch Allergy Immunol 2000; 123(2):99–106.
41. Hurst JL, Payne CE, Nevison CM, et al. Individual recognition in mice mediated by major urinary proteins. Nature 2001; 414(6864):631–634.
42. Fan Y, Gore JC, Redding KO, et al. Tissue localization and regulation by juvenile hormone of human allergen Bla g 4 from the German cockroach, *Blattella germanica* (L.). Insect Mol Biol 2005; 14(1):45–53.
43. Gore JC, Schal C. Cockroach allergen biology and mitigation in the indoor environment. Annu Rev Entomol 2007; 52:439–463.
44. Markovic-Housley Z, Degano M, Lamba D, et al. Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. J Mol Biol 2003; 325(1):123–133.
45. Jenkins JA, Griffiths-Jones S, Shewry PR, et al. Structural relatedness of plant food allergens with specific reference to cross-reactive allergens: an in silico analysis. J Allergy Clin Immunol 2005; 115(1): 163–170.
46. Piboonpocanun S, Malainual N, Jirapongsananuruk O, et al. Genetic polymorphisms of major house dust mite allergens. Clin Exp Allergy 2006; 36(4):510–516.
47. Schenk MF, Gilissen LJ, Esselink GD, et al. Seven different genes encode a diverse mixture of isoforms of Bet v 1, the major birch pollen allergen. BMC Genomics 2006; 7:168.
48. Gao ZS, van de Weg WE, Schaart JG, et al. Genomic cloning and linkage mapping of the Mal d 1 (PR-10) gene family in apple (*Malus domestica*). Theor Appl Genet 2005; 111(1):171–183.
49. Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005; 116(3):608–613.
50. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol 2006; 6(10):761–771.
51. Smith AM, Benjamin DC, Hozic N, et al. The molecular basis of antigenic cross-reactivity between the group 2 mite allergens. J Allergy Clin Immunol 2001; 107(6):977–984.
52. Gafvelin G, Johansson E, Lundin A, et al. Cross-reactivity studies of a new group 2 allergen from the dust mite *Glycyphagus domesticus*, Gly d 2, and group 2 allergens from Dermatophagoides pteronyssinus, Lepidoglyphus destructor, and Tyrophagus putrescentiae with recombinant allergens. J Allergy Clin Immunol 2001; 107(3):511–518.

4 Immunologic Responses to Subcutaneous Allergen Immunotherapy

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INTRODUCTION

Immunotherapy is highly effective in appropriately selected patients with allergic disease. Whereas conventional vaccination strategies are employed to initiate and then boost Immunologic memory, allergen immunotherapy aims to subdue established immune responses mediated by IgE and allergen-specific memory T cells through controlled exposure to the offending allergen. Since subcutaneous injection [subcutaneous allergen immunotherapy (SCIT)] of sufficient native allergen to invoke immunoregulatory mechanisms can trigger unwanted IgE-mediated reactions, the amounts of allergen contained in injections are increased incrementally from low levels until a safe but sufficient maintenance dose can be achieved. Patient selection is important and the risk/benefit ratio must be assessed on an individual basis. The underlying mechanisms are important since they provide insight into the mechanisms of allergic disease and induction of clinical tolerance. In addition, allergen injection immunotherapy is allergen-specific. This enables one to observe the effects of specific modulation of the immune response in a patient in whom the provoking factor(s) (common aeroallergen or venom) are known. The effects of the allergen exposure may be observed either during experimental provocation in a clinical laboratory or during natural environmental conditions. Similarly, the influence of immunotherapy on clinical, Immunologic, and pathologic changes may be observed under controlled conditions. This is in contrast to other Immunologic diseases where the antigen is unknown and no antigen-specific treatment is available. Sublingual allergen immunotherapy (SLIT) is emerging as a viable clinical alternative to the traditional subcutaneous route, although there are very few published, well-controlled mechanistic studies (Chap. 2). This chapter will therefore focus entirely on mechanisms of injection immunotherapy, reflecting a collective research effort spanning several decades.

SCIT results in a rapid inhibition of allergen-induced late responses, with a slower and proportionately smaller decline in early responses. Biopsies taken from skin and nasal mucosa reveal reductions in inflammatory cell numbers, including mast cells, basophils, and eosinophils. Around six to eight weeks after starting weekly immunotherapy updosing injections, increases in allergen-specific IgG, particularly of IgG4 isotype, are observed. These antibodies block IgE effector mechanisms, including basophil histamine release and IgE-facilitated antigen presentation to T cells. Induction of allergen-specific IgA is also observed, and these antibodies can induce monocytic cells to produce interleukin-10, an immunoregulatory cytokine. These humoral responses likely reflect modulation of allergen-specific T-cell responses. Immunotherapy modifies peripheral and mucosal Th2 responses to allergen in favor of Th1 cytokine and IL-10 production (Fig. 1). The latter may be a key early event and IL-10-producing T cells are detectable within a few weeks of the first injection. IL-10 inhibits mast cell, eosinophil, and T-cell responses, as well as acting on B cells to favor IgG4 production. These IL-10 producing T cells may be so-called T_R1-type inducible regulatory T cells. The mechanism leading to development of these cells is yet to be elucidated, though similar populations can be experimentally induced by tolerogenic dendritic cells.

While current treatment regimes are effective, refinement of immunotherapy, both in terms of efficacy and safety profile, remains an important goal. In addition, the possibility of developing immunoregulatory vaccines for nonallergic immune diseases ensures considerable ongoing interest in underlying mechanisms. Novel approaches being clinically tested include the combination of allergens with immunomodulatory adjuvants to potentiate responses. These include bacterially derived modified lipid compounds or CpG-rich immunostimulatory

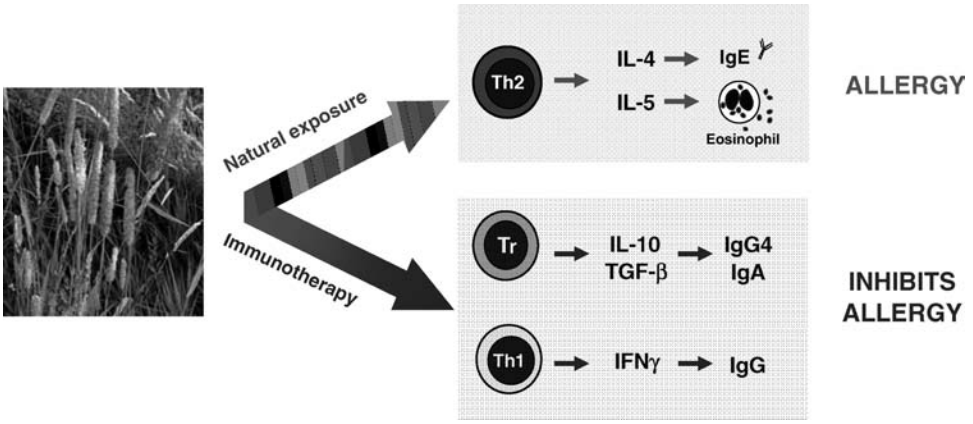

Figure 1 The effects of immunotherapy on T-cell responses: immunotherapy readdresses the balance between Th2/Th1 responses, in favor of Th1 responses. An increase in IL-10 and TGF-β-producing T cells, possibly regulatory T cells, has also been reported. IL-10 directly inhibits activation of inflammatory leukocytes including T cells, mast cells, and eosinophils. Additionally, IL-10 favors IgG4 production, which can block IgE-dependent pathways of effector cell activation.

oligodeoxynucleotides, which act through toll-like receptor-4 and receptor-9, respectively. Alternative strategies include the use of allergen-derived peptides or modified recombinant allergen vaccines. These aim to maintain the beneficial effects of vaccines while minimizing the immediate IgE-dependent complications, which currently require SCIT to be conducted cautiously and under specialist supervision.

THE ALLERGIC RESPONSE

Early and Late Responses

Experimental allergen exposure in the nose, eyes, or bronchi leads to development of mast cell–dependent sneeze, itch, watery discharge, and bronchospasm, maximal at 15 to 30 minutes and resolving within 1 to 3 hours. This "early response" is triggered by activation of mast cells through cross-linking of allergen-specific IgE molecules pre-bound to high-affinity IgE receptors (FcεRI). The sequela of this activation is the release of numerous preformed and newly synthesized mediators, including histamine, tryptase, TAME-esterase, bradykinin, leukotrienes (including LTC_4, LTD_4, and LTE_4), prostaglandins [including PGF_{2a} and PGD_2 (specific for mast cells)], and platelet-activating factor. These mediators collectively induce vasodilatation, increased vascular permeability, mucosal edema, increased mucus production from submucosal glands and goblet cells within the respiratory epithelium, and smooth muscle contraction in the lower respiratory tract.

In a proportion of individuals, the early response resolves to be followed by a late response. Airflow obstruction is usually the predominant symptom in both upper and lower airways. Skin challenge testing provokes similar cutaneous early and late responses with wheal and flare followed by late-onset localized edema and tenderness. Late responses are maximal at 6 to 12 hours and resolve within 24 hours. The late response is characterized by recruitment of eosinophils, basophils, activated T cells, and dendritic cells to the site of allergen exposure. These activated leukocytes are a rich source of potentially pathogenic mediators such as Th2 cytokines (IL-4, IL-5, IL-9, and IL-13), which modulate inflammatory cell function and upregulate adhesion molecule expression and B-cell IgE synthesis. The late response is also at least partially dependent on IgE-mediated mechanisms since anti-IgE therapy (omalizumab) is associated with inhibition of cutaneous allergen-induced late responses in addition to early responses (1). The immunopathologic changes in the mucosa during the late response are largely representative of those seen during "natural" chronic allergen exposure (e.g., during the pollen season).

Effects of SCIT

SCIT inhibits the late responses in the skin (2), nose (3), and lung (4), though it has not been established that this effect is predictive of clinical efficacy. In comparison, the effect on the early response appears to be relatively modest (and variable). For example, certain investigators describe only temporary inhibition of the early response in the skin (5) and no inhibition in the lung (4). Within a group of house-dust mite–sensitive children, suppression of the early skin response appears to be predictive of prolonged suppression following discontinuation of treatment, though this requires confirmation in a prospective study (6). The evolution of early and late response inhibition has been examined during a conventional grass pollen immunotherapy regime (7) (Fig. 2). Remarkably, late responses to intradermal challenge testing are reduced two weeks into treatment, by which time patients had received less than 1% of the total cumulative allergen dose administered weekly during the two-month updosing phase. It is established that immunotherapy with low doses of allergens is ineffective. Therefore, this finding suggests that late-response suppression alone is insufficient to account for the clinical efficacy of immunotherapy. In this same study, the decline in the sizes of early responses is proportionally much less but evolves slowly over a time frame more in keeping with clinical protection from symptoms.

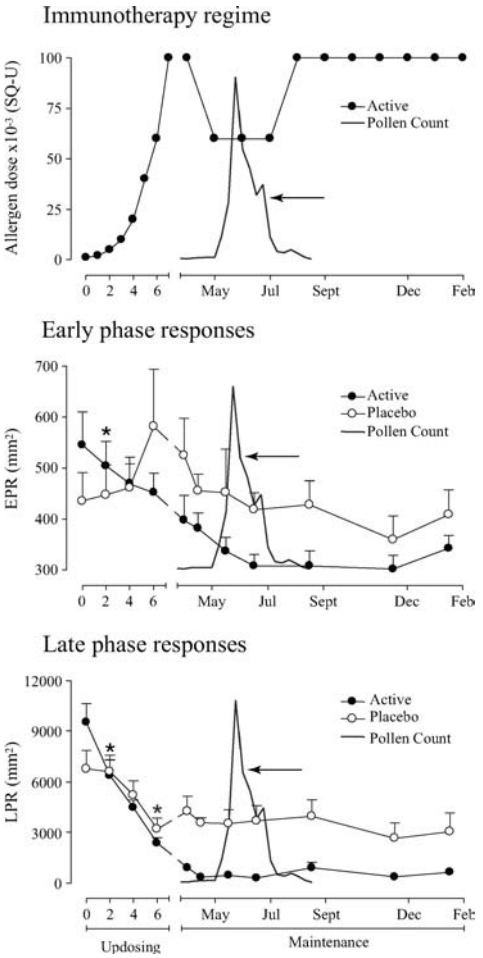

Figure 2 Time course analysis of clinical measurements during the first year of grass pollen immunotherapy with the dose of grass pollen allergen administered at each visit represented in the top panel. Early and late skin responses were assessed at 15 minutes and 24 hours following intradermal challenge with grass pollen allergen. Data are expressed as mean ± standard error. The solid line (see arrows) represents pollen counts in London, United Kingdom. $^*p < 0.05$ versus pre-immunotherapy value. *Source*: From Ref. 7.

EFFECTOR CELL RECRUITMENT

Mast Cells and Basophils

Nasal scrapings from children receiving house-dust mite immunotherapy reveal reductions in metachromatic cells, which are likely to have been mast cells (8). Seasonal allergic rhinitis induced by grass pollen exposure is also associated with migration of tryptase-positive mast cells into the nasal mucosa but did not appear to be inhibited by immunotherapy (9). Reexamination of mast cell numbers using the c-kit/stem cell factor receptor transmembrane tyrosine kinase as a marker subsequently revealed a marked recruitment of c-kit+ cells during the pollen season, which is suppressed by immunotherapy (10) (Fig. 3). In the same study, nasal mucosal expression of mRNA encoding IL-9, a growth factor for mast cells, is also lower in immunotherapy-treated patients. Basophils were examined using the 2D7 monoclonal antibody in the nasal mucosa of grass pollen immunotherapy patients during natural exposure (9). No effect of treatment is seen on basophil numbers in the lamina propria. However, infiltration into the epithelium could be demonstrated in approximately 35% of placebo-treated rhinitis but only 5% of immunotherapy patients. Thus, data exist to suggest that immunotherapy could act to reduce the seasonal recruitment of both mast cells and basophils into the nasal mucosa.

Eosinophils

SCIT is also associated with reduced eosinophil recruitment into tissue after allergen challenge. For example, reductions in the cutaneous late response to grass pollen allergen provocation are accompanied by a trend for lower eosinophil numbers in skin biopsies (11). Ragweed immunotherapy is also associated with lower eosinophil numbers in nasal lavage fluid collected during ragweed-induced nasal late responses (12). In the nasal biopsy model, mucosal eosinophils were also examined after grass pollen immunotherapy. Treatment is associated with inhibition of eosinophil recruitment both during the allergen-induced late response (13) and natural seasonal exposure (14). In contrast, the available data suggest that mucosal neutrophil numbers are not affected by immunotherapy. The effect of immunotherapy on lower airway eosinophilia was also examined in subjects with birch pollen–induced seasonal asthma (15). Bronchoalveolar lavage was examined for eosinophils and eosinophil cationic protein (ECP) during the birch pollen season. Treated subjects develop less lung

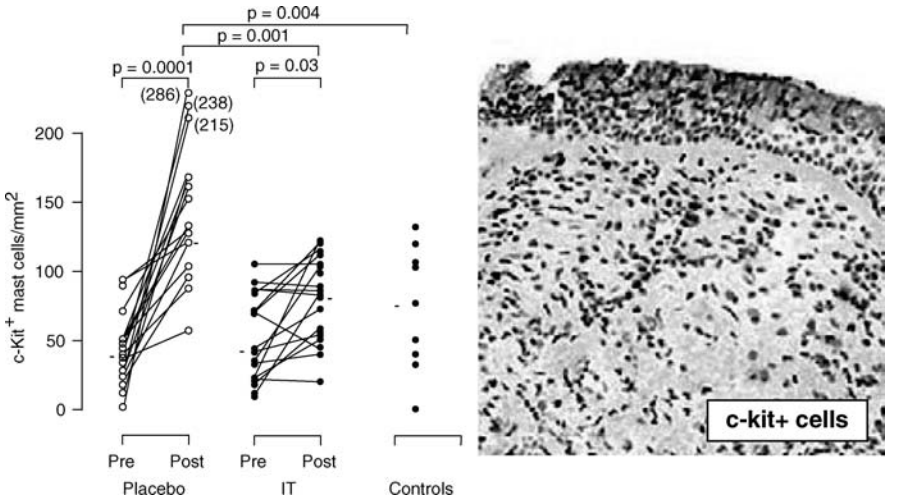

Figure 3 Effects of natural seasonal grass pollen exposure and immunotherapy on numbers of c-kit+ mast cells in the nasal mucosa. Nasal biopsies were collected outside the pollen season before immunotherapy (pre) and during the peak of the pollen after immunotherapy or placebo treatment (post). As a further control, biopsies were also collected from a cohort of nonatopic subjects during the peak of the pollen season (controls). The right panel shows an example of nasal biopsy section immunostained with a monoclonal antibody specific for c-kit. *Source*: Adapted from Ref. 10.

symptoms on exposure with lower measurable bronchial hyperactivity, eosinophil counts, and ECP concentrations.

ANTIBODY RESPONSES

Isotype Changes

Conventional pollen immunotherapy appears to have little effect on allergen-specific serum IgE concentrations (16), although increases in IgE during the pollen season are blunted (17). Following treatment with birch pollen extract, serum immunoblotting analysis suggests that a proportion of individuals developed new IgE responses to components of the vaccine (18). Nevertheless, the clinical effect of these new sensitizations is unknown, and the concentrations of IgE produced are relatively low. The functional significance of the humoral response to immunotherapy was first addressed in the 1930s by Cooke, who was able to demonstrate that transfer of sera from immunotherapy patients inhibited allergic responses. Platts-Mills and colleagues identified that antibodies from nasal lavage of immunotherapy patients inhibit histamine release from basophils in vitro (19). Allergen-specific IgG1, IgG4, and IgGA isotypes are all increased by immunotherapy with inhalant allergens (16,20). Of these, IgG4 shows the greatest proportionate increase. IgG4 antibodies are not proinflammatory in humans leading to the proposal that they act as "blocking antibodies" by competing for binding sites on allergens with surface-bound IgE (i.e., bound to FcεRI high-affinity receptors). IgG4 produced in response to immunotherapy blocks allergen-induced IgE-dependent histamine release by basophils in vitro (21). The properties of IgA antibodies induced by immunotherapy have also been examined (22). Cross-linking of polymeric IgA2 is a potent inducer of IL-10 expression by monocytes. IL-10 favors B-cell production of IgG4 and is an important immunoregulatory cytokine (discussed below).

Effects on Antigen Presentation

In addition to blocking allergen binding by cell surface FcεRI-bound IgE, IgG4 antibodies also inhibit formation of IgE-allergen complexes in solution. Formation of these multivalent complexes is required for IgE binding to low-affinity FcεRII (CD23) IgE receptors expressed by antigen-presenting cells (APC), including B cells, dendritic cells, and monocytes. This process appears to allow these cells to bind and process low concentrations of allergen for presentation to T cells by human leukocyte antigen class II molecules. van Neerven and colleagues demonstrated that sera from patients allergic to birch pollen facilitates antigen binding to B cells and ensuing activation of birch-specific T cells (23). This process is inhibited by sera from birch and grass pollen immunotherapy patients and the inhibitory activity co-purified with the IgG fraction (24–26). In contrast, IgA fractions do not demonstrate significant blocking activity in cellular assays (22). When the development of grass pollen allergen–specific IgG4 and corresponding blocking activity were studied over a detailed time course, responses took six to eight weeks to achieve significant levels during a conventional updosing regime (7).

A common objection to the blocking antibody model is the weak correlation between allergen-specific IgG concentrations and the clinical response to immunotherapy (27–29). However, data from our group show that two years following discontinuation of grass pollen immunotherapy, grass pollen allergen–specific IgG4 levels decline by approximately 80% (unpublished observations), though functional blocking activity in vitro persists during this period, as does clinical remission. It is possible that a population of long-lived high-avidity memory IgG+ B cells selectively persists following immunotherapy withdrawal, perhaps because of ongoing low-dose natural antigen exposure.

T-CELL RESPONSES

Considerable attention has focused on characterizing T-cell responses to SCIT. These studies have led to two major mechanistic hypotheses: first, that deviation of Th2 responses occurs in favor of a Th0/Th1 phenotype (assumed to be less pathogenic), and second, that allergen-specific T-cell

responses are suppressed by newly induced regulatory T cells or through inhibition of antigen presentation by IgG.

IMMUNE DEVIATION

A body of evidence suggests that immune deviation of T-helper responses occurs in successful immunotherapy, though no consistent pattern of change has emerged that is common to all studies. Many in vitro studies of peripheral blood T cells from immunotherapy patients have identified reductions in proliferative responses to allergen, usually in conjunction with a shift in the patterns of cytokine production from a Th2/0 toward Th1/0 profile (30–33). Others have not succeeded in reproducing these findings, reporting no changes in proliferative responses or cytokine production following SCIT (34–37). Potential reasons for these discrepancies include variations in laboratory methodology and lack of standardisation of allergen extracts used for SCIT. Another possible explanation is that inhibition of *peripheral* T-cell proliferation and Th2 cytokine production is not required for immunotherapy, and these responses poorly reflect the critical immune interactions and responses in lymphoid and mucosal tissues.

The effects of immunotherapy on the cytokine profile of T cells recruited into tissue have been investigated (35,36). Nasal mucosal biopsies performed during the allergen-induced late response of grass pollen immunotherapy patients reveal increases in IFN-γ mRNA–expressing cells (13). The relevance of this finding is supported by the inverse correlation between numbers of IFN-γ mRNA+ cells and clinical symptoms. A potential inducer of IFN-γ expression by mucosal T cells is IL-12 (38), and there is evidence that this mechanism may operate in grass pollen immunotherapy. Skin biopsies collected during the cutaneous late response were examined for IL-12 mRNA by in situ hybridization (39). In immunotherapy but not placebo-treated patients, there is concomitant late-response suppression and IL-12 mRNA expression. The latter correlates directly with IFN-γ, and the principal sources of IL-12 mRNA are the CD68+ macrophages. Similar studies demonstrate that immunotherapy significantly inhibits seasonal increases in IL-5- and IL-9 mRNA–expressing cells in the nasal mucosa (10,14). These studies underline the probable relevance of studying "end organ" immune responses rather than in the peripheral blood, particularly for diseases induced by inhalant allergens such as grass pollen.

Regulatory T Cells and IL-10

Studies also suggest that SCIT induces regulatory T cells that are able to modify allergen-specific T-helper cell responses. One subset of regulatory cells, first identified as CD4+CD25+ cells by Sakaguchi (40), appear to arise predominantly in the thymus and express the transcription factor Foxp3 (41). The inhibitory properties of human CD4+CD25+ cells in vitro and in vivo are described in numerous studies (42) and depend, at least in part, on cell-cell contact. Functional roles for membrane cytotoxic T-lymphocyte-associated protein 4 (43), surface-bound transforming growth factor beta (TGF-β) (44), and the glucocorticoid-induced tumor necrosis factor receptor (GITR) (45) have been proposed. These cells appear to regulate allergen-specific T-cell responses in healthy nonatopic individuals. In contrast, in subjects with allergic rhinitis, purified CD4+CD25+ T cells fail to suppress activation of CD4+CD25− T cells by grass pollen allergen (46). An additional population of "inducible" regulatory cells, often referred to as T_R1 cells, has also been described. Inducible regulatory T cells are generated in vitro through stimulation (47–49) and are classically described as not expressing Foxp3 (50), suggesting that these cells represent a subset distinct from the naturally occurring CD4+CD25+ regulatory T cells. Inducible regulatory T cells can produce cytokines such as IL-10 and TGF-β, both of which have important immunomodulatory properties.

Expression of IL-10 by T cells after SCIT is a consistent finding of several studies (31,37,51). IL-10 is expressed by both Th1 and Th2 cells, B cells, monocytes, dendritic cells, mast cells, and eosinophils and is associated with protection from immune-mediated pathology in diverse murine disease models, including pulmonary allergy (52). IL-10 acts on B cells to induce production of IgG4 in the presence of IL-4 (53). In addition, it has numerous potential antiallergic properties, including inhibition of mast cell activation by IgE (54), Th2 cytokine production, including IL-5 (37), and induction of T-cell hyporesponsiveness (55) through IL-10 receptor–dependent blockade of CD28 phosphorylation (56). The latter is an essential costimulatory pathway for T cells during antigen-induced activation by APC.

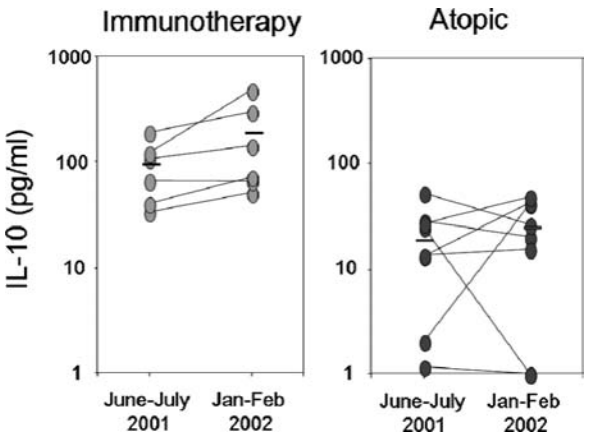

Figure 4 Effect of grass pollen immunotherapy on IL-10 production by peripheral blood T cells. Peripheral blood mononuclear cells were isolated from patients who had received at least 18 months of conventional grass pollen immunotherapy (*light grey circles*) and untreated hay fever subjects (*dark grey circles*) during and after the 2001 grass pollen season in London. Peripheral blood mononuclear cells were stimulated for six days with *Phleum pretense* (timothy grass) extract and culture supernatants tested for interleukin-10 concentrations by ELISA. *Source*: Adapted from Ref. 37.

IL-10 production by peripheral blood T cells is described following immunotherapy with bee venom (31), house-dust mite (20), and grass pollen (37) (Fig. 4). IL-10 mRNA expression is also evident in nasal biopsies of grass pollen immunotherapy patients, and expression appears to be additionally dependent on concurrent antigen exposure, that is, during the pollen season (26). The mucosal IL-10 response does not represent a restoration of the "normal" immune response as expression is not seen in pollen-exposed nonatopic control subjects. Surprisingly, only 15% of IL-10 mRNA signals colocalized to CD3+ T cells, with a further 35% being accounted for by CD68+ tissue macrophages (26). However, numbers of cells expressing IL-10 mRNA need not necessarily correlate with secretion of bioactive IL-10 protein, and it is quite possible that the contribution of T cells to IL-10 production is underestimated by this methodology. In peripheral blood studies following bee venom immunotherapy, monocytes, B cells, and T cells are all cell sources of IL-10 (51). The endogenous IL-10 produced in these cultures suppresses CD4+ T-cell responses. Subsequently, T-cell phenotypic subsets were analyzed for IL-10 production by flow cytometry. Using this approach, IL-10 production is associated with CD4+CD25+ T cells following SCIT with bee venom (50), grass pollen (37), or house-dust mite (20). In the case of house-dust mite immunotherapy, these CD4+CD25+ T cells produce both IL-10 and TGF-β and suppress immune responses to allergen in vitro. An important issue is how this phenotype of IL-10-producing T cells fits within the current model of "naturally occurring" CD4+CD25+ and "inducible" T_R1 regulatory cell subsets. One difficulty is that CD25 is not exclusively a marker of naturally occurring regulatory T cells, being expressed also by activated T cells. Indeed, in vitro stimulation is a common feature of many studies using peripheral blood T cells. However, Jutel and colleagues did separate CD4+CD25+ and CD4+CD25− T cells from house-dust mite immunotherapy subjects before in vitro stimulation and IL-10 production compartmentalized to the former subset (20).

How Do Immunotherapy Vaccines Induce IL-10 Responses?

Identifying the mechanism by which immunotherapy induces IL-10-producing T cells could be important in the design of new vaccines. Murine models provide some insights. Nevertheless, important differences exist between SCIT administered to patients to modulate mature Th2 responses and animal models where the emphasis is on tolerizing regimes given before sensitization. Tolerizing animals by oral or intranasal exposure to ovalbumin prior to intraperitoneal sensitization induces IL-10-producing regulatory T cells (57,58). In the intranasal tolerance model, induction of these T_R1-like cells is dependent on pulmonary lymph node dendritic cells expressing IL-10 and the inducible costimulatory molecule ligand (ICOSL) ligand. Indeed, adoptive transfer of these dendritic cells or T_R1 cells alone is sufficient to confer tolerance on the recipients (57,59). Human peripheral blood plasmacytoid dendritic

cells stimulated with a toll-like receptor 9 agonist express ICOSL ligand and induce differentiation of T_R1 cells from naïve T cells in vitro (60). Furthermore, cross-linking of the high-affinity IgE receptor (FcεRI) on plasmacytoid dendritic cells by allergen also induces IL-10 expression (61). Although these mechanisms are not directly demonstrated in immunotherapy, they do illustrate how dendritic cell subsets could provide the necessary tools to induce T-cell IL-10 responses following allergen vaccination.

NOVEL IMMUNOTHERAPY STRATEGIES

Immunotherapy performed with modern vaccines is a relatively safe form of treatment in trained hands. However, administering native allergen to IgE-sensitized individuals can trigger both local and systemic reactions. Immunotherapy regimes therefore tend to be cautious, involving numerous injections of gradually increasing allergen doses performed under specialist supervision. The aim for novel immunotherapy strategies should be to reduce the potential for IgE-mediated side effects while exposing patients to fewer injections and making fewer demands on resources.

Circumventing IgE-Mediated Side Effects

The first category of novel therapies is based on the hypothesis that immunotherapy works primarily through stimulating T cells at high antigen doses and that this directly leads to regulatory T-cell induction. This class of therapies is also based on the assumption that IgE-dependent mechanisms mediate immunotherapy side effects but are not necessary for vaccine efficacy. Strategies tested include genetically modified allergen proteins with reduced IgE binding but intact T-cell epitopes. For example, recombinant fragments of major birch pollen allergen Bet v1 have been generated with minimal allergenicity in cutaneous and nasal challenge models (62,63). These recombinant proteins successfully induce Bet v1–specific IgG1 and IgG4 responses that block basophil histamine release triggered by exposure to wild-type Bet v1 (64). Another approach is the use of allergen-derived peptides that stimulate T cells but that cannot cross-link IgE. Overlapping peptides representing the cat allergen Fel d1 have been examined in small clinical studies with promising results (65). Finally, a randomized double-blind placebo-controlled trial examined the value of pretreatment with a humanized anti-IgE monoclonal antibody (omalizumab) on side effects in a rapid updosing ("rush") protocol of ragweed immunotherapy (66). Such accelerated immunotherapy regimes balance increased convenience and compliance with a higher risk of systemic reactions. Omalizumab resulted in a fivefold reduction in the risk of anaphylaxis during the one-day rapid updosing period.

Adjuvants

The second category is based around the use of adjuvants to potentiate the Immunologic effects of vaccination with whole allergen proteins. Ideally, such adjuvants should not only potentiate Th2 to Th1 immune deviation, but should also favor induction of regulatory T-cell responses. The type-B immunostimulatory phosphorothioate oligodeoxynucleotide 5′-TGACTGTAACGTTCGAGATGA (ODN-1018) was tested in ragweed-stimulated peripheral blood mononuclear cells (PBMC) responses. It promotes Th1 cytokine and IL-12 responses at the expense of Th2 cytokine production. This activity could be further enhanced by conjugation of ODN-1018 with the major ragweed allergen, Amb a1. (67). A functionally similar type-B phosphorothioate oligodeoxynucleotide (ODN-2006) activates human plasmacytoid dendritic cells through the toll-like receptor-9 and induces regulatory T cells (60,68). A randomized double-blind placebo-controlled phase 2 trial examined the effects of six weekly injections of the ODN-1018–Amb a1 conjugate on ragweed-induced allergic rhinitis (69). Treatment is associated with improvement in peak season visual analogue, nasal, and quality of life symptom scores. Another clinically tested adjuvant is monophosphoryl lipid A, a derivative of bacterial lipopolysaccharide. Monophosphoryl lipid A acts through toll-like receptor-4 to induce IL-12 production and promote Th1 responses to allergen by human PBMC (70). In a randomized double-blind placebo-controlled study, a vaccine containing monophosphoryl lipid A and tyrosine-absorbed glutaraldehyde-modified extracts of grass pollen (Pollinex Quattro®) reduces hay fever symptoms and increases allergen-specific IgG when administered as four preseasonal injections (71).

In addition to guiding development of novel treatment approaches, knowledge of immunotherapy mechanisms is also likely to enable the development of effective biomarkers in order to predict patients who are likely to respond to immunotherapy and to predict relapse following discontinuation. Measurement of the biological activity of so-called "blocking" antibodies holds some promise (72). The sublingual route is also emerging as an effective and safe alternative (73). Meanwhile, the subcutaneous route using standardized natural allergens remains the gold standard against which to test putative biomarkers and novel immunomodulatory approaches.

SALIENT POINTS

1. Allergen injection immunotherapy is effective in selected patients with IgE-mediated disease and sensitivity to one or limited numbers of allergens.
2. Allergic disorders in humans are characterized by expression of IL-4 and IL-5.
3. SCIT inhibits allergen-induced early and late responses in nose, skin, and lung.
4. SCIT inhibits recruitment of mast cells, basophils, and eosinophils to the nose and lung.
5. SCIT increases allergen-specific IgA and IgG, especially IgG4. IgG antibodies inhibit some of the effects of IgE in vitro, but the clinical importance remains to be established.
6. SCIT induces immune deviation in favor of Th1 responses as well as T-cell IL-10 production in the nasal mucosa and peripheral blood. IL-10 has numerous antiallergic properties and promotes IgG4 production by B cells. Dendritic cells are the most likely candidates for inducing IL-10 responses.
7. Novel approaches include non-IgE-binding recombinant allergens, allergen-derived peptides, and combining conventional vaccines with anti-IgE (omalizumab). Bacterial-derived oligonucleotides and lipids acting through toll-like receptors to promote Th1 and regulatory T-cell responses also hold promise.

REFERENCES

1. Ong YE, Menzies-Gow A, Barkans J, et al. Anti-IgE (omalizumab) inhibits late-phase reactions and inflammatory cells after repeat skin allergen challenge. J Allergy Clin Immunol 2005; 116(3):558–564.
2. Pienkowski MM, Norman PS, Lichtenstein LM. Suppression of late-phase skin reactions by immunotherapy with ragweed extract. J Allergy Clin Immunol 1985; 76(5):729–734.
3. Iliopoulos O, Proud D, Adkinson NF Jr., et al. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. J Allergy Clin Immunol 1991; 87(4):855–866.
4. Warner JO, Price JF, Soothill JF, et al. Controlled trial of hyposensitisation to Dermatophagoides pteronyssinus in children with asthma. Lancet 1978; 2(8096):912–915.
5. Walker SM, Varney VA, Gaga M, et al. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. Allergy 1995; 50(5):405–413.
6. Des RA, Paradis L, Knani J, et al. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. V. Duration of the efficacy of immunotherapy after its cessation. Allergy 1996; 51(6):430–433.
7. Francis JN, James LK, Paraskevopoulos G, et al. Grass Pollen Immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. J Allergy Clin Immunol 2008; 121(5):1120–1125.
8. Otsuka H, Mezawa A, Ohnishi M, et al. Changes in nasal metachromatic cells during allergen immunotherapy. Clin Exp Allergy 1991; 21(1):115–119.
9. Wilson DR, Irani AM, Walker SM, et al. Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium. Clin Exp Allergy 2001; 31(11):1705–1713.
10. Nouri-Aria KT, Pilette C, Jacobson MR, et al. IL-9 and c-Kit+ mast cells in allergic rhinitis during seasonal allergen exposure: effect of immunotherapy. J Allergy Clin Immunol 2005; 116(1):73–79.
11. Varney VA, Hamid QA, Gaga M, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. J Clin Invest 1993; 92(2):644–651.

12. Furin MJ, Norman PS, Creticos PS, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. J Allergy Clin Immunol 1991; 88(1):27–32.
13. Durham SR, Ying S, Varney VA, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. J Allergy Clin Immunol 1996; 97(6):1356–1365.
14. Wilson DR, Nouri-Aria KT, Walker SM, et al. Grass pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. J Allergy Clin Immunol 2001; 107(6):971–976.
15. Rak S, Lowhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. J Allergy Clin Immunol 1988; 82(3 pt 1):470–480.
16. Gehlhar K, Schlaak M, Becker W, et al. Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen-specific IgG4 to IgG1 correlates with clinical outcome. Clin Exp Allergy 1999; 29(4):497–506.
17. Lichtenstein LM, Ishizaka K, Norman PS, et al. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. J Clin Invest 1973; 52(2):472–482.
18. Moverare R, Elfman L, Vesterinen E, et al. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. Allergy 2002; 57(5):423–430.
19. Platts-Mills TA, von Maur RK, Ishizaka K, et al. IgA and IgG anti-ragweed antibodies in nasal secretions. Quantitative measurements of antibodies and correlation with inhibition of histamine release. J Clin Invest 1976; 57(4):1041–1050.
20. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003; 33(5): 1205–1214.
21. Garcia BE, Sanz ML, Gato JJ, et al. IgG4 blocking effect on the release of antigen-specific histamine. J Investig Allergol Clin Immunol 1993; 3(1):26–33.
22. Pilette C, Nouri-Aria KT, Jacobson MR, et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. J Immunol 2007; 178(7):4658–4666.
23. van Neerven RJ, Wikborg T, Lund G, et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. J Immunol 1999; 163(5):2944–2952.
24. Wachholz PA, Soni NK, Till SJ, et al. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. J Allergy Clin Immunol 2003; 112(5):915–922.
25. van Neerven RJ, Arvidsson M, Ipsen H, et al. A double-blind, placebo-controlled birch allergy vaccination study: inhibition of CD23-mediated serum-immunoglobulin E-facilitated allergen presentation. Clin Exp Allergy 2004; 34(3):420–428.
26. Nouri-Aria KT, Wachholz PA, Francis JN, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 2004; 172(5):3252–3259.
27. Djurup R, Malling HJ. High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens. Clin Allergy 1987; 17(5):459–468.
28. Ewan PW, Deighton J, Wilson AB, et al. Venom-specific IgG antibodies in bee and wasp allergy: lack of correlation with protection from stings. Clin Exp Allergy 1993; 23(8):647–660.
29. Bodtger U, Ejrnaes AM, Hummelshoj L, et al. Is immunotherapy-induced birch-pollen-specific IgG4 a marker for decreased allergen-specific sensitivity? Int Arch Allergy Immunol 136(4):340–346.
30. Jutel M, Pichler WJ, Skrbic D, et al. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. J Immunol 1995; 154(8):4187–4194.
31. Bellinghausen I, Metz G, Enk AH, et al. Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. Eur J Immunol 1997; 27(5):1131–1139.
32. Ebner C, Siemann U, Bohle B, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. Clin Exp Allergy 1997; 27(9):1007–1015.
33. Benjaponpitak S, Oro A, Maguire P, et al. The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. J Allergy Clin Immunol 1999; 103(3 pt 1):468–475.
34. Till S, Walker S, Dickason R, et al. IL-5 production by allergen-stimulated T cells following grass pollen immunotherapy for seasonal allergic rhinitis. Clin Exp Immunol 1997; 110(1):114–121.
35. Klimek L, Dormann D, Jarman ER, et al. Short-term preseasonal birch pollen allergoid immunotherapy influences symptoms, specific nasal provocation and cytokine levels in nasal secretions, but not peripheral T-cell responses, in patients with allergic rhinitis. Clin Exp Allergy 1999; 29(10):1326–1335.

36. Wachholz PA, Nouri-Aria KT, Wilson DR, et al. Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1:Th2 cytokine ratios. Immunology 2002; 105(1):56–62.
37. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. J Allergy Clin Immunol 2003; 111(6):1255–1261.
38. Varga EM, Wachholz P, Nouri-Aria KT, et al. T cells from human allergen-induced late asthmatic responses express IL-12 receptor beta 2 subunit mRNA and respond to IL-12 in vitro. J Immunol 2000; 165(5):2877–2885.
39. Hamid QA, Schotman E, Jacobson MR, et al. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. J Allergy Clin Immunol 1997; 99(2):254–260.
40. Sakaguchi S, Sakaguchi N, Asano M, et al. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995; 155(3):1151–1164.
41. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299(5609):1057–1061.
42. Sakaguchi S, Sakaguchi N. Regulatory T cells in immunologic self-tolerance and autoimmune disease. Int Rev Immunol 2005; 24:211–226.
43. Takahashi T, Tagami T, Yamazaki S, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000; 192(2):303–310.
44. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 2001; 194(5):629–644.
45. Ling EM, Smith T, Nguyen XD, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet 2004; 363 (9409):608–615.
46. McHugh RS, Whitters MJ, Piccirillo CA, et al. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity 2002; 16(2):311–323.
47. Levings MK, Sangregorio R, Roncarolo MG. Human cd25(+)cd4(+) t regulatory cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. J Exp Med 2001; 193(11):1295–1302.
48. Sundstedt A, O'Neill EJ, Nicolson KS, et al. Role for IL-10 in suppression mediated by peptide-induced regulatory T cells in vivo. J Immunol 2003; 170(3):1240–1248.
49. Barrat FJ, Cua DJ, Boonstra A, et al. In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. J Exp Med 2002; 195(5):603–616.
50. Vieira PL, Christensen JR, Minaee S, et al. IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. J Immunol 2004; 172(10):5986–5993.
51. Akdis CA, Blesken T, Akdis M, et al. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998; 102(1):98–106.
52. Tournoy KG, Kips JC, Pauwels RA. Endogenous interleukin-10 suppresses allergen-induced airway inflammation and nonspecific airway responsiveness. Clin Exp Allergy 2000; 30(6):775–783.
53. Jeannin P, Lecoanet S, Delneste Y, et al. IgE versus IgG4 production can be differentially regulated by IL-10. J Immunol 1998; 160(7):3555–3561.
54. Royer B, Varadaradjalou S, Saas P, et al. Inhibition of IgE-induced activation of human mast cells by IL-10. Clin Exp Allergy 2001; 31(5):694–704.
55. Groux H, Bigler M, de Vries JE, et al. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. J Exp Med 1996; 184(1):19–29.
56. Akdis CA, Joss A, Akdis M, et al. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. FASEB J 2000; 14(12):1666–1668.
57. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol 2001; 2(8):725–731.
58. Zhang X, Izikson L, Liu L, et al. Activation of CD25(+)CD4(+) regulatory T cells by oral antigen administration. J Immunol 2001; 167(8):4245–4253.
59. Akbari O, Freeman GJ, Meyer EH, et al. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat Med 2002; 8(9): 1024–1032.
60. Ito T, Yang M, Wang YH, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J Exp Med 2007; 204(1):105–115.

61. Novak N, Allam JP, Hagemann T, et al. Characterization of FcepsilonRI-bearing CD123 blood dendritic cell antigen-2 plasmacytoid dendritic cells in atopic dermatitis. J Allergy Clin Immunol 2004; 114(2):364–370.
62. Nopp A, Hallden G, Lundahl J, et al. Comparison of inflammatory responses to genetically engineered hypoallergenic derivatives of the major birch pollen allergen bet v 1 and to recombinant bet v 1 wild type in skin chamber fluids collected from birch pollen-allergic patients. J Allergy Clin Immunol 2000; 106(1 pt 1):101–109.
63. Hage-Hamsten M, Johansson E, Roquet A, et al. Nasal challenges with recombinant derivatives of the major birch pollen allergen Bet v 1 induce fewer symptoms and lower mediator release than rBet v 1 wild-type in patients with allergic rhinitis. Clin Exp Allergy 2002; 32(10):1448–1453.
64. Niederberger V, Horak F, Vrtala S, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. Proc Natl Acad Sci U S A 2004; 101(suppl 2):14677–14682.
65. Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. Lancet 2002; 360 (9326):47–53.
66. Casale TB, Busse WW, Kline JN, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2006; 117(1): 134–140.
67. Marshall JD, Abtahi S, Eiden JJ, et al. Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes T(H)1 cytokine expression while downregulating T(H)2 cytokine expression in PBMCs from human patients with ragweed allergy. J Allergy Clin Immunol 2001; 108(2):191–197.
68. Moseman EA, Liang X, Dawson AJ, et al. Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of CD4+CD25+ regulatory T cells. J Immunol 2004; 173(7):4433–4442.
69. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006; 355(14):1445–1455.
70. Puggioni F, Durham SR, Francis JN. Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favor of Th1 responses. Allergy 2005; 60(5):678–684.
71. Drachenberg KJ, Wheeler AW, Stuebner P, et al. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. Allergy 2001; 56(6):498–505.
72. Shamji MH, Wilcock LK, Wachholz PA, et al. The IgE-facilitated allergen binding (FAB) assay: validation of a novel flow-cytometric based method for the detection of inhibitory antibody responses. J Immunol Methods 2006; 317(1–2):71–79.
73. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005; 60(1):4–12.

5 Immunologic Responses to Sublingual Allergen Immunotherapy

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INTRODUCTION

The physiopathology of the allergic immune responses is complex and influenced by several factors, including genetic susceptibility, route of exposure, allergen dose, and in some cases the structural characteristics of the allergen (1). The allergic immune response requires sensitization and development of specific immune response toward the allergen. During sensitization to allergen, priming of allergen-specific CD4+ T helper 2 (Th2) cells results in the production of Th2 cytokines [such as interleukin-4 (IL-4) and IL-13] that are responsible for class switching to the ε heavy chain for IgE production by B cells, mucus production and activation of endothelial cells for Th2 cell, and eosinophil migration to tissues (2,3). IgE sensitizes mast cells and basophils by binding to the high-affinity receptor for IgE (FcεRI) expressed on their surface. Upon cross-linking of the IgE-FcεRI complexes by allergen, mast cells and basophils degranulate, releasing vasoactive amines (principally histamine), lipid mediators (prostaglandins and cysteinyl leukotrienes), cytokines and chemokines, all of which characterize the immediate phase of the allergic reaction (2,3).

After the sensitization phase, clinical allergic inflammation and reactions to allergen challenge are observed in the target organ leading to the development of allergic rhinoconjunctivitis, eczema, asthma, and systemic anaphylaxis. T cells constitute a large population of cellular infiltrate in allergic inflammation, and a dysregulated immune response appears to be an important pathogenetic factor. Cardinal events during allergic inflammation can be classified as activation, organ-selective homing, survival and reactivation, and effector functions of immune system cells (4,5). T cells are activated by aeroallergens, food antigens, autoantigens, and bacterial superantigens in allergic inflammation. They are under the influence of skin, lung, or nose-related chemokine network and show organ-selective homing. A prolonged survival of the inflammatory cells in the tissues and consequent reactivation is observed in the subepithelial tissues. Finally, T cells display effector functions, which result in elevated IgE levels, eosinophil survival, and mucus hyperproduction. They also interact with bronchial epithelial cells, smooth muscle cells, and keratinocytes causing their activation and apoptosis (4,6). Peripheral T-cell tolerance to allergens can overcome all of the above pathological events in allergic inflammation because they all require T-cell activation.

Allergen-specific immunotherapy (SIT) is the only treatment that leads to prolonged tolerance against allergens due to restoration of normal immunity. Although very limited studies have been performed, immunological studies on mechanisms of sublingual immunotherapy (SLIT) might show similar immune regulation patterns compared with subcutaneous immunotherapy (SCIT). In addition, animal studies of mucosal tolerance indicate generation of T regulatory (Treg) cells and their cytokines by application of vaccines via sublingual, oral, and other mucosal routes. The induction of a tolerant state in peripheral T cells represents an essential step in SCIT and peptide immunotherapy (PIT) (7). Peripheral T-cell tolerance is characterized mainly by suppressed proliferative and cytokine responses against the major allergens and its T-cell recognition sites. It is initiated by autocrine action of IL-10 and/or transforming growth factor β (TGF-β), which are increasingly produced by the antigen-specific Treg cells. Tolerized T cells can be reactivated to produce either distinct Th1 or Th2 cytokine patterns, thus directing allergen-SIT toward successful or unsuccessful treatment. Treg cells directly or indirectly influence effector cells of allergic inflammation, such as mast cells, basophils, and eosinophils. In addition, there is accumulating evidence that they may suppress IgE production and induce IgG4 and IgA production against allergens. By the

application of the recent knowledge in mechanisms of allergen-SIT, more rational and safer approaches are awaiting for the future of prevention and possibly cure of allergic diseases.

NONINJECTION IMMUNOTHERAPY

Concerns about the safety of subcutaneous administration of allergens have inspired the search for effective noninjection routes of administration for allergen-SIT. These routes include oral immunotherapy (OIT), local bronchial immunotherapy (LBIT), local nasal immunotherapy (LNIT), and SLIT.

First reports on the oral administration of allergen (immediate swallowing of allergen) date back as far as 1927 (8). OIT has been abandoned in clinical practice due to a lack of statistically significant improvement of symptoms (only three studies have shown clinical benefit of OIT (9), high costs, and frequent side effects). However, given the large number of animal studies that show oral tolerance, it still remains a fruitful approach.

LBIT has been performed in only two double-blind placebo-controlled studies, both on house dust mite–allergic patients (10,11). Only one of these studies reported clinical improvement and patients suffered from many unwanted effects. Unproven efficacy and high risk levels have essentially stopped research in this area.

More studies have been performed using LNIT during the last few decades. Aqueous allergen extracts were proven highly effective, but frequently induce rhinitis. Dry powder extracts did not cause this problem and showed minimal side effects. Despite this and clinical success, the use of LNIT is in decline. The requirement of highly skilled staff to administer the allergens correctly (without the powder entering the deep airways) and indications that LNIT might not maintain its clinical benefit after it is discontinued favor SCIT over LNIT.

Many studies have been performed with sublingual administration of allergen extracts, either with or without subsequent swallowing of the extract, beginning in the mid-1980s. SLIT has been successfully used to treat allergic rhinitis and asthma, although efficacy varies greatly among studies (12,13). Because of potentially severe, albeit infrequent, side effects associated with SCIT, mucosal routes of administration are being investigated (14). While its safety and efficacy are now largely documented, much remains to be investigated on the immunological mechanisms underlying efficacy of SLIT. A meta-analysis of the double-blind, placebo-controlled trials performed in the past decade show that SLIT is clinically efficacious although, at present, the treatment benefit is approximately half that achieved with SCIT (15). SLIT seems to be effective in the amelioration of clinical symptoms, drug consumption, and bronchial hyperreactivity (16–18), although definitive evidence from large randomized controlled trials is lacking.

A longitudinal, double-blind, placebo-controlled, parallel-group study that included 51 centers from 8 countries, aimed to confirm the efficacy of a rapidly dissolving grass allergen tablet in patients with seasonal rhinoconjunctivitis. Subjects were randomized (1:1) to receive a grass allergen tablet or placebo once daily. A total of 634 subjects with a history of grass pollen–induced rhinoconjunctivitis for at least two years and confirmed IgE sensitivity (positive skin prick test and serum-specific IgE) were included in the study. Subjects commenced treatment at least 16 weeks before the grass pollen season, and treatment was continued throughout the season. The primary efficacy analysis showed a reduction of 30% in rhinoconjunctivitis symptoms and 38% rhinoconjunctivitis medication scores compared with placebo. Side effects mainly comprised mild itching and swelling in the mouth that was, in general, tolerated and led to treatment withdrawal in less than 4% of participants. There were no serious local side effects or severe systemic adverse events. In this multicentral study, SLIT grass allergen tablets were effective in grass pollen–induced rhinoconjunctivitis and represents a safe alternative to SCIT (19).

Various prophylactic or therapeutic sublingual immunization approaches in BALB/c mice with ovalbumin-induced asthma have been studied. Prophylactic sublingual administration of ovalbumin completely prevents airways hyperresponsiveness as well as IL-5 secretion and IgE induction. This effect can be due to prevention of allergen sensitization or induction of oral tolerance. However, therapeutic administration of ovalbumin in solution via either the sublingual or oral route has limited efficacy in already sensitized mice. In contrast, sublingual application of ovalbumin formulated with maltodextrin to enhance mucosal

adhesion results in a major reduction of established airways hyperresponsiveness, lung inflammation, and IL-5 production in splenocytes. This mucoadhesive formulation significantly enhances ovalbumin-specific T-cell proliferation in cervical, but not mesenteric, lymph nodes and IgA production in the lungs. The availability of a murine SLIT model allows for the investigation of immune mechanisms associated with SLIT (20).

The immunological mechanisms of SLIT seem to be similar to SCIT, although the magnitude of the change in most parameters is modest or no change is observed. It seems likely that the contact of the allergen with the oral mucosa is critical for the success of SLIT (21). It is postulated that most likely oral Langerhans cells are critically involved in this process (22). During SLIT, the allergen is captured within the oral mucosa by oral Langerhans cells and subsequently these cells mature and migrate to proximal draining lymph nodes. Those local lymph nodes may favor the production of blocking IgG antibodies and the induction of T lymphocytes with suppressive function (23,24). Most studies using SLIT report increased levels of serum IgG4 with a relatively modest increase compared with injection immunotherapy (25). There is no consistency in T cell and IgE and effector cell responses, and a significant number of studies fail to detect systemic immunological changes (15,25). This may be related to the different doses of allergen administered in various studies or to the development of more localized immunological changes.

Peripheral blood mononuclear cells (PBMCs) were stimulated with pollen allergen extract after one and two years of SLIT in another study. The expression of IL-10 mRNA increased in both high and low doses and showed a positive correlation with TGF-β expression. IL-5 was suppressed with high dose, which negatively correlated with TGF-β (26). Birch pollen (Bet v 1) extract SLIT patients showed improved nasal provocation scores to birch pollen; however, cross-reactive apple-induced oral allergy syndrome was not significantly reduced. Bet v 1–specific T-cell tolerance to all epitopes and those cross-reactive with Mal d 1 from apple was shown. However, neither Mal d 1–specific IgE and IgG4 levels nor Mal d 1–induced T-cell proliferation changed significantly, probably due to non-cross-reactive epitopes (27). These results may explain why pollen-associated food allergy is frequently not ameliorated by any form of pollen immunotherapy. Although SLIT is increasingly being utilized, several points need further investigation, such as its efficacy in asthma, its mechanisms of action, the optimal dose and time to be administered, its combination with injection immunotherapy, age of onset for its safe use in young children, and its preventive role in the development of allergy.

SEQUENTIAL EVENTS IN ALLERGEN-SIT AND THEIR UNDERLYING MECHANISMS

Very Early Immunotherapy Effect

The underlying immunological mechanisms of allergen-SIT are under investigation. Very early effects are related to mast cell and basophil desensitization. Intermediate effects are related to changes in allergen-specific T cells and late effects are related to B cells, IgE, as well as mast cells, basophils, and eosinophils. Although a definite decrease in IgE antibody levels and IgE-mediated skin sensitivity normally requires years of SCIT, most patients are protected against bee stings during the early stages of SCIT. An important observation is the decrease in mast cell and basophil degranulation and tendency for systemic anaphylaxis within hours (Fig. 1) (28). Although the response seems similar to the rapid desensitization to a drug, the mechanism for this effect is unknown. Acute oral desensitization to penicillin V in the mice demonstrates that antigen-specific mast cell desensitization is one of the main underlying mechanisms for oral desensitization (28). Mediators of anaphylaxis (histamine and leukotrienes) are released during SIT and sting challenges without inducing a systemic anaphylactic response (29). Their piecemeal release, not enough to cause anaphylaxis, may decrease the granule content of mediators and also may affect the threshold of activation of mast cells and basophils. Decreased mediator release in these cells is a demonstrated feature in in vitro analysis shortly after beginning allergen-SIT (29–31). Although there are fluctuations and risks for developing systemic anaphylaxis during the course of allergen-SIT, the suppression of mast cells and basophils continues to be affected by changes in other immune parameters such as the generation of allergen-specific Treg cells and decreased specific IgE.

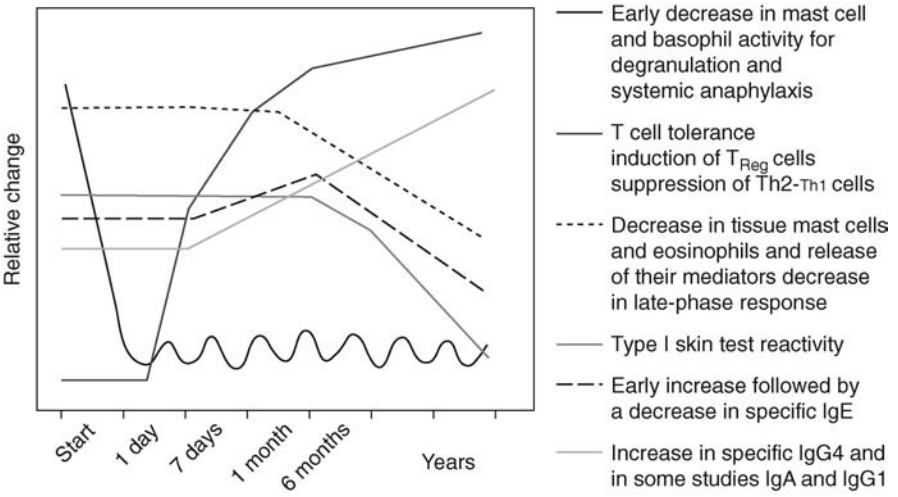

Figure 1 Immunological changes during the course of allergen-SIT. Although there is significant variation between individuals and protocols, with the first injection, an early decrease in mast cell and basophil degranulation and tendency for systemic anaphylaxis is observed. This is followed by generation of allergen-specific Treg cells and suppression of both allergen-specific Th1 and Th2 cells. An early increase of specific IgE and late decrease is observed. This is in parallel to an increase particularly of IgG4 and in some studies IgG1 and IgA. A significant decrease in allergen-specific IgE/IgG4 ratio occurs after several months. A significant decrease in type I skin test reactivity is also observed relatively late in the course of SIT. A decrease in tissue mast cells and eosinophils and release of their mediators and decrease in late-phase response is observed after a few months. The listed effects demonstrated in SLIT were relatively weak compared with injection SIT. *Abbreviations*: SIT, specific immunotherapy; Treg, T regulatory.

Generation of Treg Cells and Peripheral T-Cell Tolerance

The induction of a tolerant state in peripheral T cells represents an essential step in allergen-SIT (Fig. 2). Peripheral T-cell tolerance is characterized by generation of allergen-specific Treg cells and suppressed proliferative and cytokine responses against the major allergen (32–34). Subsets of Treg cells with distinct phenotypes and mechanisms of action include the naturally occurring, thymic-selected CD4+CD25+ Treg cells and the inducible type 1 Treg cells (Tr1) (35). In allergen-SIT, peripheral T-cell tolerance is initiated by the autocrine action of IL-10 and TGF-β, which is increasingly produced by the antigen-specific T cells (33,36,37). The suppression by these cells could partially be blocked by the use of neutralizing antibodies against secreted or membrane-bound IL-10 and TGF-β (34). These cells do, however, express CD4 and CD25, raising the question whether these are inducible Tr1 cells that have upregulated CD25 or naturally occurring CD4+CD25+ Treg cells that produce suppressive cytokines (35). In agreement, CD4+CD25+ Treg cells from atopic donors have a reduced capacity to suppress the proliferation of CD4+CD25− T cells (38). Therefore, upregulation of CD4+CD25+ Treg cells may play a role in allergen-SIT. TGF-β plays a dual role in allergic disease. It suppresses allergen-specific T cells and plays a role in remodeling of the tissues. It remains to be determined whether the remodeling and suppressive role of TGF-β in allergic inflammation show an imbalance, which aggravates instead of controlling the immune response (39).

Studies on the mechanisms by which immune responses to nonpathogenic environmental antigens lead to either allergy or nonharmful immunity demonstrate that Treg cells are dominant in healthy individuals (5,40). If a detectable immune response is mounted, Tr1 cells specific for common environmental allergens, consistently represent the dominant subset in healthy individuals. They use multiple suppressive mechanisms, IL-10 and TGF-β as secreted cytokines, and cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD1) as surface molecules. Healthy and allergic individuals exhibit all three, i.e., Th1, Th2, Tr1 type allergen-specific subsets in different proportions (40). Accordingly, a change in the dominant subset and the balance between Th2 and Treg cells may lead to either allergy development or recovery.

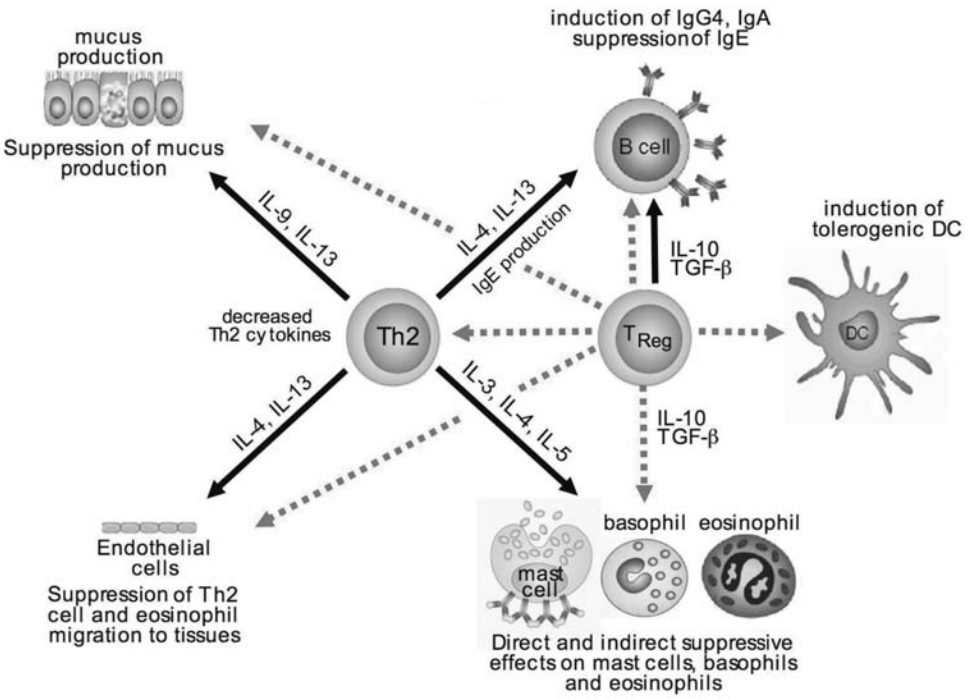

Figure 2 (*See color insert.*) Immune deviation toward Treg-cell response is an essential step in SIT and natural allergen exposure of nonallergic individuals. Treg cells utilize multiple suppressor factors, which influence the final outcome of SIT. Treg cells suppress proliferation, tissue infiltration, proinflammatory cytokine production, and injury/apoptosis of epithelial cells by both Th1 and Th2 cells. IL-10 and TGF-β induce IgG4 and IgA, respectively, from B cells as noninflammatory Ig isotypes and suppress IgE production. These two cytokines directly or indirectly suppress effector cells of allergic inflammation such as mast cells, basophils, and eosinophils. In addition, IL-10 induces tolerogenic dendritic cells in experimental models (*solid line*: activation, *dotted line*: suppression). *Abbreviations*: SIT, specific immunotherapy; Treg, T regulatory.

Although based on a limited number of studied individuals, a longitudinal study demonstrates that successful SLIT induces IL-10-producing Treg cells and that different immune mechanisms are operative during early and later phases of treatment. In this study, lower allergen doses administered during the early phase might promote the induction of allergen-specific IL-10-producing CD4+CD25+ Treg cells, whereas allergen doses cumulating during the further course of SLIT might foster the induction of deletion/anergy and immune deviation of allergen-reactive T cells, respectively. Active suppression provided by IL-10-producing Treg cells is predominant during the early course of therapy followed by other tolerance mechanisms during the later phase of therapy. Thus, SLIT modulates the allergen-specific T-cell response in a similar way as SCIT (41).

Another study on healthy immune response to allergens demonstrated that CD4+CD25+ Treg cells are associated with the spontaneous remission of cow's milk allergy. Children who outgrow their allergy (tolerant children) have higher numbers of circulating CD4+CD25+ T cells and decreased in vitro proliferative responses to bovine β-lactoglobulin in PBMCs compared with children who maintained clinically active allergy (42). Several studies have been reported in other diseases along the same line. The in vitro proliferative response of human CD4+ T cells to nickel from healthy, nonallergic individuals is strongly augmented when CD4+CD25+ Treg cells are depleted (43). Furthermore, a human in vivo study on immunotherapy of rheumatoid arthritis also showed a marked increase in the number of FoxP3+CD4+CD25+ Treg cells in peripheral blood (44). CD25+ Treg cells are characterized by the expression of the transcriptional regulator *Foxp3* (*FOXP3* in humans), which appears to be master switch gene for Treg cell development and function. The spontaneous development of allergic airway inflammation, hyper IgE, and eosinophilia in addition to various autoimmune diseases in *Foxp3* mutant mice provide compelling evidence for its importance in allergic

inflammation (45). Humans suffering from immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome are similarly affected and mostly develop hyper IgE and eczema due to mutations in the *FOXP3* gene (45). Also, a dysregulation of disease-causing effector T cells is observed in atopic dermatitis lesions, in association with an impaired CD4(+) CD25(+)FoxP3(+) T-cell infiltration in the dermis (46).

Allergen-specific T-cell suppression by IL-10, a known suppressive cytokine of T-cell proliferation and cytokine production, is essential in peripheral tolerance to allergens, autoantigens, transplantation antigens, and tumor antigens. The mechanism of how IL-10 directly inhibits T cells is reported (47). IL-10 suppresses T cells by blocking CD28 and inducible costimulator (ICOS) costimulatory signals in a rapid signal transduction cascade. IL-10 binds its receptor and activates two tyrosine kinases, Jak1 and Tyk2. Tyk2 acts as a constitutive reservoir for Src homology 2 domain–containing protein tyrosine phosphatase 1 (SHP-1) in resting T cells and then tyrosine phosphorylates SHP-1 on activation. SHP-1 rapidly binds to CD28 and ICOS costimulatory receptors and dephosphorylates them within minutes. As a consequence, the binding of phosphatidylinositol-3 kinase to either costimulatory receptor no longer occurs and downstream signaling is inhibited. Accordingly, spleen cells from SHP-1-deficient mice show increased proliferation with CD28 and ICOS stimulation in comparison to wild-type mice, which is not suppressed by IL-10. Generation of dominant-negative SHP-1-overexpressing T cells or silencing of the SHP-1 gene by small inhibitory RNA both alter SHP-1 functions and abolish the suppressive effect of IL-10. In conclusion, the rapid inhibition of the CD28 or ICOS costimulatory pathways by SHP-1 represents the mechanism for direct T-cell suppression by IL-10 (47).

The role of Treg cells is not limited to suppression of Th2 cells. Peripheral tolerance utilizes multiple mechanisms to suppress allergic inflammation. Apparently, Treg cells contribute to the control of allergen-specific immune responses in five major ways: suppression of antigen-presenting cells that support the generation of effector Th2 and Th1 cells; suppression of Th2 and Th1 cells; suppression of allergen-specific IgE and induction of IgG4 and/or IgA; suppression of mast cells, basophils, and eosinophils; interaction with resident tissue cells and remodeling (35).

Regulation of Allergen-Specific IgE and IgG Subtype Responses During Allergen-SIT

Specific IgE in serum and on the surface of mast cells and basophils bound to FcεRI in allergic patients is a hallmark of atopic disease. Although peripheral T-cell tolerance is rapidly induced during SIT, there is no evidence for B-cell tolerance in the early course (32). Natural exposure to a relevant allergen is often associated with an increase in the IgE synthesis. Similarly, SIT frequently induces a transient increase in serum-specific IgE, however, followed by gradual decrease over months or years of treatment (48,49). In pollen-sensitive patients, allergen-SIT prevents elevation of the serum-specific IgE during the pollen season (50). However, the changes in IgE levels cannot explain the diminished responsiveness to specific allergen due to SIT, since the decrease in serum IgE is relatively late and does not correlate with clinical improvement from SIT.

Antibody responses induced during allergen-SIT are functionally heterogeneous, which may account for the conflicting data in relation to the protective effects of IgG (34,48,49). Subclasses of IgG antibodies, especially IgG4, are thought to capture the allergen before reaching the effector cell–bound IgE and thus prevent the activation of mast cells and basophils. However, the relationship between the efficacy of SIT and the induction of allergen-specific IgG subgroups remains a controversial issue, with serum concentrations of allergen-specific IgG correlating with clinical improvement in some studies, but not others (51,52). Allergen-specific IgG may be directed against the same epitopes as IgE, resulting in direct competition for allergen binding and a "blocking" effect. By contrast, induction of IgG specific for other epitopes may result in a failure of the IgG response to compete with IgE, even when IgG is present in molar excess. The concept of blocking antibodies is being revaluated. Analysis of the IgG subtypes induced by SIT shows specific increases in IgG1 and, in particular, IgG4, with levels increasing 10- to 100-fold (53,54). There is cumulating evidence that SIT also influences the blocking activity on IgE-mediated responses by IgG4, and cellular assays are commonly used to investigate these changes (55).

A novel assay, which detects allergen-IgE binding using flow cytometry, has been used to detect "functional" SIT-induced changes in IgG antibody activity. Results suggest that

successful SIT is associated with an increase in IgG blocking activity that is not solely dependent on the quantity of IgG antibodies (56,57). It seems to be relevant rather to measure the blocking activity of allergen-specific IgG or IgG subsets, particularly IgG4 and also IgG1, instead of measuring their levels in sera. In this context, the role of anti-IgE treatment in the induction phase of allergen-SIT on safety and efficacy has been questioned. Anti-IgE mAb pretreatment enhances the safety of SIT for allergic rhinitis and may be an effective strategy to permit more rapid and higher doses of allergen immunotherapy (58). Its function on long-term efficacy is still under investigation.

The noninflammatory role for IgG4 may be because the IgG4 hinge region has unique structural features that result in a lower affinity for certain Fcγ receptors and the ability to separate and repair leading to bispecific antibodies that are functionally monomeric (59). Furthermore, IgG4 does not fix complement and is capable of inhibiting immune-complex formation by other isotypes, giving this isotype anti-inflammatory characteristics.

By using well-defined recombinant allergen mixtures, all treated subjects developed very strong allergen-specific IgG4 and also increased IgG1 antibody responses. Some patients who are not initially sensitized to Phl p 5 develop strong specific IgG4, but not IgE antibody responses to Phl p 5 (53). This demonstrates that extract-based antibody measurements may provide wrong information, and studies on mechanisms of allergen-SIT should be performed with single allergens. Nevertheless, IgG4 antibodies can be viewed as a marker of introduced allergen dose, and they have the ability to modulate the immune response to allergen and the potential to influence the clinical response to allergen. In addition, the affinity of newly produced IgG4 and decreasing IgE to allergens has not been intensely studied and may have a very decisive role. Affinity maturation of specific antibodies in allergen immunotherapy and preseasonal versus postseasonal changes in their affinity remain to be elucidated (60).

For SLIT, a significant increase in specific IgG and IgG4 antibodies has not been consistently demonstrated. This has been investigated in a study related to the evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow-up of allergic patients undergoing SCIT and SLIT. Preliminary data indicate that preseasonal high-dose SLIT without a build-up phase is safe and well-tolerated by allergic patients. Compared with IgG4 levels induced by SCIT, only a high-dose SLIT regimen results in an appreciable serum-specific IgG4 increase (61).

IL-10, levels of which are enhanced by SIT, appears to counterregulate antigen-specific IgE and IgG4 antibody synthesis (33). It is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production. Thus, IL-10 not only generates tolerance in T cells, it also regulates specific isotype formation and skews the specific response from an IgE- to an IgG4-dominated phenotype (Fig. 2). The healthy immune response to Der p 1 increases specific IgA and IgG4, small amounts of IgG1, and almost undetectable IgE antibodies in serum (34). House dust mite–SCIT does not significantly change specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1, and IgG4 is observed (34). The increase of specific IgA and IgG4 in serum coincides with increased TGF-β and IL-10, respectively. This may account for the role of IgA and TGF-β as well as IgG4 and IL-10 in peripheral mucosal immune responses to allergens in healthy individuals (33,62). Most probably the decrease in IgE/IgG4 ratio during allergen-SIT is because of the skew of the predominant allergen-specific T-cell subset from Th2 cells to Treg cells. However, although Treg-cell generation happens within days, a significant decrease in IgE/IgG4 ratio occurs after years. The reason for the long-time gap between the change in T-cell subsets, but not IgE/IgG4 levels, is not easily explained by the half-life of antibodies. The role of bone marrow–residing IgE-producing plasma cells with very long life span remains to be investigated (63).

Mechanisms of Suppression of Effector Cells and Inflammatory Responses During Allergen-SIT

Peripheral T-cell tolerance to allergens, characterized by functional inactivation of the cell to antigen encounter, can overcome both acute and chronic events in allergic reactions (Fig. 2). Allergen-SCIT efficiently modulates the thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release (64). In addition, IL-10 reduces proinflammatory cytokine release from mast cells (65). Furthermore, IL-10 downregulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 cells (66).

Moreover, although demonstrated in a model of myocarditis, IL-10 gene transfer significantly reduces mast cell density, local histamine concentration, mast cell growth, and prevents mast cell degranulation (67).

Long-term SCIT is associated with reduction of the immediate and the late-phase reactions (LPRs) to allergen provocation in the nasal and bronchial mucosa or the skin. The mechanism of LPR is different from the mast cell–mediated immediate reaction and involves the recruitment, activation, and persistence of eosinophils, and activated T cells at the sites of allergen exposure (3). Successful SIT results not only in the increase of allergen concentration necessary to induce immediate or LPR in the target tissue but also in the decreased responses to nonspecific stimulation. Bronchial, nasal, and conjunctival hyperreactivity to nonspecific stimuli, which seems to reflect underlying mucosal inflammation, decreases after SCIT and correlates with clinical improvement (68). During birch pollen SCIT, reduced plasma levels of eosinophil cationic protein (ECP), a marker of eosinophil activation, as well as chemotactic factors for eosinophils and neutrophils correlated with decreased bronchial hyperreactivity and clinical improvement (69). Inhibition by SCIT of the seasonal increase in eosinophil priming has also been demonstrated (70). In biopsies obtained during grass pollen SCIT, decreased eosinophil and mast cell infiltration in nasal and bronchial mucosa after SCIT correlates with the anti-inflammatory effect (71). Similar to these findings, SLIT achieved a significant clinical benefit in birch pollinosis, reduced the eosinophil infiltration in nasal mucosa, and significantly improved pulmonary function during the pollen seasons (72).

UNDERLYING MECHANISMS OF NOVEL AND EMERGING SIT VACCINES AND NOVEL ADJUVANTS FOR THE IMPROVEMENT OF INJECTION AND SUBLINGUAL SIT

Intensive studies to improve efficacy and safety of allergen-SIT are underway. A basic requirement for an allergen vaccine in achieving successful SIT without the risk of anaphylaxis is to express T-cell epitopes, which induce T-cell tolerance and lack antibody-binding sites that mediate IgE cross-linking (73). Conformation dependence of B-cell epitopes and linearity of T-cell epitopes may induce a different regulation of allergen-specific T-cell cytokine toward a nonallergic phenotype. Native allergens utilize IgE-facilitated antigen presentation by dendritic cells and B cells, which activates T cells to produce Th2-type cytokines and B cells to produce further IgE in a secondary response. In contrast, B-cell epitope deleted allergens, which do not bind IgE, do not initiate effector cell degranulation. They utilize phagocytotic or pinocytic antigen uptake mechanisms in dendritic cells, macrophages, and B cells (74). T cells may be subsequently induced to generate a balanced Th0/Th1-type cytokine pattern in lower quantities as well as T-cell tolerance, which involves Treg cells. Accordingly, targeting T cells and bypassing IgE by modified allergens will enable the administration of higher doses of allergens, which is required to induce T-cell tolerance without the risk of anaphylaxis (74).

Therefore, immunotherapy using peptides (PIT) is an attractive approach for safe SIT. Induction of T-cell tolerance and increased IL-10 production has been demonstrated both in cat Fel d 1 and bee venom Api m 1 PITs (75). A potential barrier to PIT is the apparent complexity of the allergen-specific T-cell response in terms of epitope usage and dominant epitopes in humans and stability of peptides.

Genetically engineered recombinant hybrid molecules that span the whole T-cell repertoire, but do not bind IgE, have been developed to overcome these problems. Folding is complicated by the formation of intra- and intermolecular disulfide bond formation in cysteine-containing proteins, whereas any formed disulfide bond can fix the conformation and limits the freedom of further folding. The probability of a correct or native-like folding rapidly decreases due to the increasing probability of incorrect disulfide bond formation with increasing numbers of cysteines. A fusion protein consisting of the two major allergens of bee venom, Api m 1 and Api m 2, has been generated to investigate this concept. Destroyed conformational B-cell epitopes, but intact T-cell epitopes of the two allergens, characterize this protein. By providing decreased allergenicity with preserved T-cell tolerance inducing capacity, the Api m [1/2] fusion protein represents a novel vaccine

prototype for allergen-SIT (76). Another interesting approach is to cut the major allergens to fragments and fuse them in a different order without missing any T-cell epitopes in one reassembled mosaic allergen (77). In this study, two fragments of Api m 1, three fragments of Api m 2, and Api m 3 are reassembled in a different order with overlapping residues, in order to not miss any T-cell epitopes. Single injection of both vaccines, which only target T cells, demonstrates a preventive effect on IgE generation in mice. The advantage of these two approaches is that only one molecule has to be produced and purified instead of several recombinant allergens. T-cell epitopes are preserved and B-cell epitopes can be deleted or preserved depending on the type of the fusion molecule.

Another interesting approach is to use fragments and a trimer of major birch pollen allergen, Bet v 1, to treat birch pollen allergy. A double-blind, placebo-controlled study has been completed in three centers. It demonstrates increases in IgG1, IgG2, IgG4, and IgA and suppression of seasonal increases of IgE (78). In a different approach, the effectiveness of a mixture of five recombinant grass pollen allergens in reducing symptoms and the need for symptomatic medication in grass pollen–allergic patients was demonstrated. All treated subjects developed strong allergen-specific IgG1 and IgG4 antibody responses (53). Allergen-SIT vaccines are generally administered subcutaneously, intradermally, or sublingually, from where they must reach secondary lymphatic organs to induce an immune response. In a mouse study, an MHC class I–binding peptide from the lymphocytic choriomeningitis virus enhanced immunogenicity by as much as 106 times when compared with subcutaneous and intradermal vaccination. Intralymphatic administration induced CD8 T-cell responses with strong cytotoxic activity and interferon-γ (IFN-γ) production that conferred long-term protection against viral infections and tumors (79). The efficacy of allergen-SIT vaccines administered directly into inguinal lymph nodes of humans is currently being investigated.

Aluminium hydroxide is used as an adjuvant for allergen-SIT. These preparations are efficacious and have a good safety profile, but might be improved in efficacy. A new class of adjuvants—so-called immune response modifiers—act on antigen-presenting cells through the Toll-like receptors (TLRs). These recognize pathogen-associated molecular patterns on microorganisms, and depending on the type of TLR, different types of antigen-presenting cells can be targeted. TLR-triggering compounds that can control the overexpression of Th2 cytokines or skew the Th1:Th2 balance toward a Th1 profile are effective in murine models of allergy (73). Oligodeoxynucleotides, containing immunostimulatory CpG motifs that trigger TLR 9, linked to the allergen in ragweed allergy in humans have been utilized. Amb a 1–immunostimulatory DNA sequence conjugate SIT led to a prolonged shift from Th2 immunity toward Th1 immunity and appears to be safe (80). The same Amb a 1 CpG conjugate was effective for the treatment of allergic rhinitis for two consecutive seasons. Although early increases in Amb a 1–specific IgE occurred during the injection phase, a seasonal increase in Amb a 1–specific IgE did not occur. A reduction in the number of IL-4-positive basophils was reported (81). In another study, vaccination with a peptide antigen covalently coupled to highly repetitive virus-like particles induced high IgG antibody titers in humans. This suggests that allergens could be coupled to virus-like particles for allergen-SIT (82). As a different immunological approach, the fusion of allergens with human Fcγ is reported to inhibit allergen-induced basophil and mast cell degranulation by cross-linking Fcγ and FcεRI receptors (83). In addition, IL-10-inducing adjuvants enhance the efficacy of allergy vaccines in establishing allergen-specific tolerance in mice models (84).

On the basis of protein transduction domain (PTD) technology, modular antigen translocation (MAT) molecules aimed to enhance antigen presentation through intracellular targeting of the MHC II presentation pathway have been engineered (85). MAT vaccines consist of a cloning cassette, which fuses transactivator of transcription (TAT) peptide to a truncated Ii (invariant chain), which is able to target antigens to the nascent MHC II molecules in the trans-Golgi compartment. MAT-conjugated allergens have the ability to stimulate T-cell proliferation and cytokine production in human PBMC cultures derived from allergic individuals and to elicit protective immune responses in mice. MAT vaccines induce a strong proliferation of PBMC at a low concentration and a Th2/Treg cell shift in the cytokine profile. In allergic mouse models, we showed that MAT vaccines are highly efficient in desensitizing mice and protecting them from anaphylactic shock. This technology is applicable not only for the treatment of allergies but also for the development of preventive vaccines (85).

ROLE OF HISTAMINE IN IMMUNE REGULATION

In addition to its dominant role in type I hypersensitivity reactions, histamine influences several immune/inflammatory and effector functions (86,87). Histamine can selectively recruit the major effector cells into tissue sites and affect their maturation, activation, polarization, and other functions leading to chronic inflammation (88). Histamine also regulates monocytes, dendritic cells, T cells, and B cells, as well as related antibody isotype responses. The diverse effects of histamine on immune regulation appear to be due to differential expression and regulation of four types of histamine receptors and their distinct intracellular signals (86,87). In addition, differences in affinities of these receptors for histamine are highly decisive for the biological effects of histamine and drugs that target histamine receptors.

Histamine possesses all the properties of a classic leukocyte chemoattractant, including agonist-induced actin polymerization, mobilization of intracellular calcium, alteration in cell shape, and upregulation of adhesion molecule expression. In vivo, allergen-specific wild-type but not H1R-deficient CD4+ T cells were recruited to the lungs of naive recipients following inhaled allergen challenge (88). Histamine inhibits neutrophil chemotaxis due to H2R triggering, which is mimicked by impromidine (H2R agonist) but not by betahistine (H1R agonist) (89). Histamine contributes to the progression of allergic-inflammatory responses by enhancement of the secretion of proinflammatory cytokines such as IL-1α, IL-1β, IL-6, as well as chemokines such as RANTES or IL-8, in several cell types and local tissues (90–93). Histamine induces the CC chemokines, monocyte chemotactic protein 1 and 3, RANTES, and eotaxin in explant cultures of human nasal mucosa via H1R, suggesting a prolonged inflammatory cycle in allergic rhinitis between the cells that release histamine and their enhanced migration to nasal mucosa (94).

Immunoregulatory effects of histamine on antigen-presenting cells such as dendritic cells and monocytes are known. In monocytes stimulated with TLR-triggering bacterial products, histamine inhibits the production of proinflammatory IL-1-like activity, tumor necrosis factor (TNF)-α, IL-12, and IL-18, but enhances IL-10 secretion, through H2R stimulation (90,95–97). Histamine also downregulates CD14 expression via H2R on human monocytes (98). The inhibitory effect of histamine via H2R appears through the regulation of ICAM-1 and B7.1 expression, leading to the reduction of innate immune responses stimulated by lipopolysaccharide (99). Histamine induces intracellular Ca^{2+} flux, actin polymerization, and chemotaxis in immature dendritic cells due to stimulation of H1R and H3R subtypes. Maturation of dendritic cells results in loss of these responses. In maturing dendritic cells, however, histamine dose dependently enhances intracellular cAMP levels and stimulates IL-10 secretion, while inhibiting production of IL-12 via H2R (100). Although human monocyte-derived dendritic cells express both H1 and H2Rs and can induce CD86 expression by histamine, human epidermal Langerhans cells express neither H1 nor H2Rs, mainly because of the effect of TGF-β (101).

The balance between production and death is important in the control of cell numbers within physiological ranges. Cell accumulation in the tissues may be a consequence of either increased cell production or decreased cell death. Because apoptosis of cells is a powerful mechanism for deleting the cells, it raises the interesting possibility that unequal apoptosis of effector cells may lead to preferential deletion of one subset over another (102). The effect of histamine on the life span of immune-competent cells has not been investigated so far. Soga and colleagues show that histamine affects monocyte life span via the H2R. These findings provide additional evidence supporting the immunomodulatory effect of histamine on monocytes. Histamine prevents monocytic apoptosis in a dose- and time-dependent manner and this effect is mediated by the H2R and cAMP pathway. (103). This phenomenon results in part from the histamine-induced endogenous production of IL-10. These results may indicate that the H2R signals prolong the life span of monocytes and infiltration to the site of inflammation in modalities such as chronic allergic disorders, including asthma and atopic dermatitis. In addition, studies suggest that histamine may play an important role in the modulation of the cytokine network in the lung via H2R, H3R, and H4R that are expressed in distinct cells and cell subsets (104,105). Apparently, due to the same signal transduction patterns, β_2-adrenergic receptors may function similarly to H2R in humans (106).

Endogenous histamine is actively synthesized during cytokine-induced dendritic cell differentiation (107). Histamine actively participates in functions and activity of dendritic cell

precursors as well as their immature and mature forms. Dendritic cells express all four histamine receptors (108–110). They mature from monocytic and lymphoid precursors and acquire dendritic cell 1 and dendritic cell 2 phenotypes, which in turn facilitates the development of Th1 and Th2 cells, respectively. In the differentiation process of monocyte-derived dendritic cells, H1R and H3R act as positive stimulants that increase antigen-presentation capacity and Th1-priming activity. In contrast, H2R acts as a suppressive molecule for antigen-presentation capacity, enhances IL-10 production, and induces IL-10-producing T cells or Th2 cells (96,100,111).

Differential patterns of histamine receptor expression on Th1 and Th2 cells determine reciprocal T-cell responses following histamine stimulation (112). Th1 cells show predominant but not exclusive expression of H1R, while Th2 cells show increased expression of H2R. Histamine enhances Th1-type responses by triggering the H1R, whereas both Th1- and Th2-type responses are negatively regulated by H2R, due to activation of different biochemical intracellular signals (112). In mice, deletion of H1R results in suppression of IFN-γ and dominant secretion of Th2 cytokines (IL-4 and IL-13). H2R-deleted mice show upregulation of both Th1 and Th2 cytokines. In addition, IL-3 stimulation significantly increases H1R expression on Th1 but not on Th2 cells. Furthermore, histamine stimulation induces IL-10 secretion through H2R (113). Increased IL-10 production in both dendritic cells and T cells may account for an important regulatory mechanism in the control of inflammatory functions by histamine (Fig. 3). In conclusion, histamine and four different histamine receptors constitute a multifaceted system with distinct functions of receptor types due to their differential

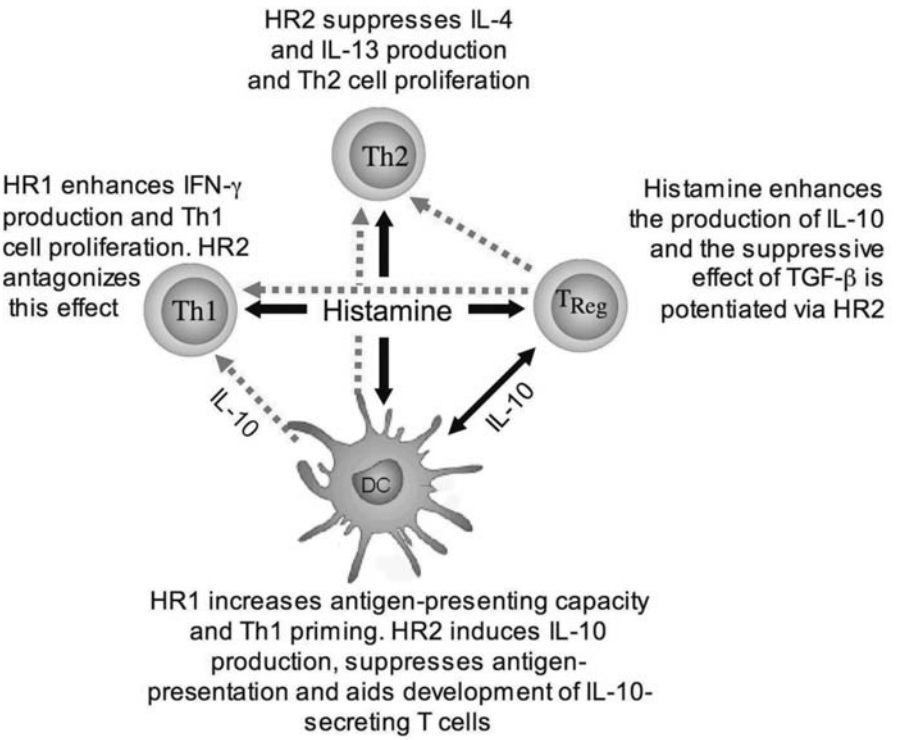

Figure 3 Histamine regulates the inflammatory functions of antigen-presenting cells and T cells in lymphatic organs and subepithelial tissues. The controlled release of histamine from effector cells of allergy induces IL-10 in DC and suppresses both Th1 and Th2 responses through the HR2. Furthermore, IL-10 affects the maturation of DC to IL-10-producing DC, which may further contribute to Treg cell generation. DC expresses all known histamine receptors. HR1 and HR3 induce proinflammatory activity and increased APC capacity, whereas HR2 plays a suppressive role. Th1 cells show predominant expression of HR1, whereas Th2 cells show a higher expression of HR2. HR1 induces increased proliferation and IFN-γ production in Th1 cells. HR2 acts as a negative regulator of proliferation, suppresses IL-4 and IL-13 production in Th2 cells. HR2 negatively regulates both Th1 and Th2 responses, induces IL-10 production, and potentiates the suppressive effect of TGF-β (*solid line*: activation, *dotted line*: suppression). *Abbreviations*: DC, dendritic cells; HR2, histamine receptor 2; IL, interleukin; IFN-γ, interferon-γ; TGF-β, transforming growth factor β.

expression, which changes according to the stage of cell differentiation and influences of the microenvironment. These mechanisms are open to be used in the search for new adjuvants for SLIT.

CONCLUSION

There is growing evidence supporting the role for Treg cells and/or immunosuppressive cytokine, IL-10, and TGF-β as a mechanism by which SCIT, SLIT, and healthy immune response to allergens is characterized by suppression of Th2 responses, ensuring a well-balanced immune response and a switch from IgE to IgG4 antibody production. More studies are required to clarify mechanisms of action of SLIT. Nevertheless, the currently known mechanisms such as generation of Treg cells can be better used for the improvement of current treatment modalities using recombinant allergens or peptide therapy. The elaborations of more efficacious methods, including rapid protocols and targeting histamine receptors, also hold a promise for further development.

SALIENT POINTS

- Generation of Treg cells and peripheral T-cell tolerance is an essential event in allergen tolerance.
- Regulation of allergen-specific IgE and IgG subtype responses occurs during allergen-SIT.
- Immunosuppressive cytokines, IL-10, and TGF-β play a role in the mechanisms by which SCIT and SLIT respond to allergens. This is characterized by suppression of Th2 responses and ensuring a well-balanced immune response and a switch from IgE to IgG4 antibody production.
- Novel adjuvants are required to improve efficacy of SCIT and SLIT.
- Understanding the role of histamine in immune regulation opens a window for future therapies.
- Known mechanisms such as generation of Treg cells can be used to improve current treatment modalities using recombinant allergens or peptide immunotherapy.
- The elaborations of more efficacious methods, including rapid protocols, hold promise for future development.

REFERENCES

1. Akdis CA. Mechanisms of allergic disease. Curr Opin Immunol 2006; 18(6):718–726.
2. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 2004; 113(3):395–400.
3. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol 2006; 6(10):761–771.
4. Akdis CA, Blaser K, Akdis M. Apoptosis in tissue inflammation and allergic disease. Curr Opin Immunol 2004; 16(6):717–723.
5. Akdis M. Healthy immune response to allergens: T regulatory cells and more. Curr Opin Immunol 2006; 18(6):738–744.
6. Trautmann A, Schmid-Grendelmeier P, Krüger K, et al. T cells and eosinophils cooperate in the induction of bronchial epithelial apoptosis in asthma. J Allergy Clin Immunol 2002; 109:329–337.
7. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 2007; 119(4):780–791.
8. Black J. The oral administration of pollen. J Lab Clin Med 1927; 12:1156.
9. Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. J Allergy Clin Immunol 2003; 111(3):437–448.
10. Tari MG, Mancino M, Monti G. Immunotherapy by inhalation of allergen in powder in house dust allergic asthma—a double-blind study. J Investig Allergol Clin Immunol 1992; 2(2):59–67.
11. Crimi E, Voltolini S, Troise C, et al. Local immunotherapy with Dermatophagoides extract in asthma. J Allergy Clin Immunol 1991; 87(3):721–728.

12. Malling HJ. Is sublingual immunotherapy clinically effective? Curr Opin Allergy Clin Immunol 2002; 2(6):523–531.
13. Passalacqua G, Guerra L, Pasquali M, et al. Efficacy and safety of sublingual immunotherapy. Ann Allergy Asthma Immunol 2004; 93(1):3–12.
14. Passalacqua G, Guerra L, Compalati E, et al. New insights in sublingual immunotherapy. Curr Allergy Asthma Rep 2006; 6(5):407–412.
15. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005; 60(1):4–12.
16. Bahceciler NN, Arikan C, Taylor A, et al. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. Int Arch Allergy Immunol 2005; 136(3): 287–294.
17. Arikan C, Bahceciler NN, Deniz G, et al. Bacillus Calmette-Guerin-induced interleukin-12 did not additionally improve clinical and immunologic parameters in asthmatic children treated with sublingual immunotherapy. Clin Exp Allergy 2004; 34(3):398–405.
18. Ippoliti F, De Santis W, Volterrani A, et al. Immunomodulation during sublingual therapy in allergic children. Pediatr Allergy Immunol 2003; 14(3):216–221.
19. Dahl R, Kapp A, Colombo G, et al. Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 118(2):434–440.
20. Razafindratsita A, Saint-Lu N, Mascarell L, et al. Improvement of sublingual immunotherapy efficacy with a mucoadhesive allergen formulation. J Allergy Clin Immunol 2007; 120(2):278–285.
21. Passalacqua G, Albano M, Fregonese L, et al. Randomized controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. Lancet 1998; 351:629–632.
22. Allam JP, Niederhagen B, Bucheler M, et al. Comparative analysis of nasal and oral mucosa dendritic cells. Allergy 2006; 61(2):166–172.
23. Samsom JN, van Berkel LA, van Helvoort JM, et al. Fc gamma RIIB regulates nasal and oral tolerance: a role for dendritic cells. J Immunol 2005; 174(9):5279–5287.
24. van Helvoort JM, Samsom JN, Chantry D, et al. Preferential expression of IgG2b in nose draining cervical lymph nodes and its putative role in mucosal tolerance induction. Allergy 2004; 59(11):1211–1218.
25. Moingeon P, Batard T, Fadel R, et al. Immune mechanisms of allergen-specific sublingual immunotherapy. Allergy 2006; 61(2):151–165.
26. Savolainen J, Jacobsen L, Valovirta E. Sublingual immunotherapy in children modulates allergen-induced in vitro expression of cytokine mRNA in PBMC. Allergy 2006; 61(10):1184–1190.
27. Kinaciyan T, Jahn-Schmid B, Radakovics A, et al. Successful sublingual immunotherapy with birch pollen has limited effects on concomitant food allergy to apple and the immune response to the Bet v 1 homolog Mal d 1. J Allergy Clin Immunol 2007; 119:937–943.
28. Woo HY, Kim YS, Kang NI, et al. Mechanism for acute oral desensitization to antibiotics. Allergy 2006; 61(8):954–958.
29. Eberlein-Konig B, Ullmann S, Thomas P, et al. Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitization. Clin Exp Allergy 1995; 25(8):704–712.
30. Jutel M, Muller UR, Fricker M, et al. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. Clin Exp Allergy 1996; 26(10):1112–1118.
31. Plewako H, Wosinska K, Arvidsson M, et al. Basophil interleukin 4 and interleukin 13 production is suppressed during the early phase of rush immunotherapy. Int Arch Allergy Immunol 2006; 141(4): 346–353.
32. Akdis CA, Akdis M, Blesken T, et al. Epitope specific T cell tolerance to phospholipase A_2 in bee venom immunotherapy and recovery by IL-2 and IL-15 *in vitro*. J Clin Invest 1996; 98:1676–1683.
33. Akdis CA, Blesken T, Akdis M, et al. Role of IL-10 in specific immunotherapy. J Clin Invest 1998; 102:98–106.
34. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-b cooperate in regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003; 33:1205–1214.
35. Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol 2005; 116(5):961–968.
36. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003; 33:1205–1214.
37. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. J Allergy Clin Immunol 2003; 111(6):1255–1261.
38. Ling EM, Smith T, Nguyen XD, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet 2004; 363 (9409):608–615.
39. Schmidt-Weber CB, Blaser K. Regulation and role of transforming growth factor-beta in immune tolerance induction and inflammation. Curr Opin Immunol 2004; 16(6):709–716.

40. Akdis M, Verhagen J, Taylor A, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 2004; 199(11):1567–1575.
41. Bohle B, Kinaciyan T, Gerstmayr M, et al. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. J Allergy Clin Immunol 2007; 120(3):707–713.
42. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med 2004; 199(12):1679–1688.
43. Cavani A, Nasorri F, Ottaviani C, et al. Human CD25+ regulatory T cells maintain immune tolerance to nickel in healthy, nonallergic individuals. J Immunol 2003; 171(11):5760–5768.
44. Prakken BJ, Samodal R, Le TD, et al. Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. Proc Natl Acad Sci U S A 2004; 101(12):4228–4233.
45. Chatila TA. Role of regulatory T cells in human diseases. J Allergy Clin Immunol 2005; 116(5): 949–959.
46. Verhagen J, Akdis M, Traidl-Hoffmann C, et al. Absence of T-regulatory cell expression and function in atopic dermatitis skin. J Allergy Clin Immunol 2006; 117(1):176–183.
47. Taylor A, Akdis M, Joss A, et al. IL-10 inhibits CD28 and ICOS costimulations of T cells via src homology 2 domain-containing protein tyrosine phosphatase 1. J Allergy Clin Immunol 2007; 120(1): 76–83.
48. Van Ree R, Van Leeuwen WA, Dieges PH, et al. Measurement of IgE antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. Clin Exp Allergy 1997; 27(1):68–74.
49. Gleich GJ, Zimmermann EM, Henderson LL, et al. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. J Allergy Clin Immunol 1982; 70(4):261–271.
50. Bousquet J, Maasch H, Martinot B, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. J Allergy Clin Immunol 1988; 82(3 pt 1):439–446.
51. Golden DB, Meyers DA, Kagey-Sobotka A, et al. Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. J Allergy Clin Immunol 1982; 69(6):489–493.
52. Müller UR, Helbling A, Bischof M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. Allergy 1989; 44:412–418.
53. Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005; 116(3):608–613.
54. Reisinger J, Horak F, Pauli G, et al. Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. J Allergy Clin Immunol 2005; 116(2):347–354.
55. Klunker S, Saggar LR, Seyfert-Margolis V, et al. Combination treatment with omalizumab and rush immunotherapy for ragweed-induced allergic rhinitis: inhibition of IgE-facilitated allergen binding. J Allergy Clin Immunol 2007; 120(3):688–695.
56. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol 2004; 4(4):313–318.
57. Till SJ, Francis JN, Nouri-Aria K, et al. Mechanisms of immunotherapy. J Allergy Clin Immunol 2004; 113(6):1025–1034.
58. Casale TB, Busse WW, Kline JN, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2006; 117(1): 134–140.
59. Aalberse RC, Schuurman J. IgG4 breaking the rules. Immunology 2002; 105(1):9–19.
60. Poirier MP, Ahlstedt S, Ford J, et al. Use of thiocyanate elution to estimate relative avidity of allergen specific IgE antibodies. Allergy Asthma Proc 1997; 18(6):359–362.
61. Rossi RE, Monasterolo G, Coco G, et al. Evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow up of allergic patients undergoing subcutaneous and sublingual immunotherapy. Vaccine 2007; 25(5):957–964.
62. Sonoda E, Matsumoto R, Hitoshi Y, et al. Transforming growth factor beta induces IgA production and acts additively with interleukin 5 for IgA production. J Exp Med 1989; 170:1415–1420.
63. Radbruch A, Muehlinghaus G, Luger EO, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol 2006; 6(10):741–750.
64. Pierkes M, Bellinghausen I, Hultsch T, et al. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-gamma production of T cells. J Allergy Clin Immunol 1999; 103(2 pt 1):326–332.
65. Marshall JS, Leal-Berumen I, Nielsen L, et al. Interleukin (IL)-10 Inhibits long-term IL-6 production but not preformed mediator release from rat peritoneal mast cells. J Clin Invest 1996; 97:1122–1128.
66. Schandane L, Alonso-Vega C, Willems F, et al. B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. J Immunol 1994; 152:4368–4374.

67. Palaniyandi SS, Watanabe K, Ma M, et al. Inhibition of mast cells by interleukin-10 gene transfer contributes to protection against acute myocarditis in rats. Eur J Immunol 2004; 34(12):3508–3515.
68. Rak S, Lowhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. J Allergy Clin Immunol 1988; 82(3 pt 1):470–480.
69. Rak S, Hakanson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. J Allergy Clin Immunol 1990; 86(5):706–713.
70. Hakansson L, Heinrich C, Rak S, et al. Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: effect of immunotherapy. J Allergy Clin Immunol 1997; 99(4):551–562.
71. Creticos PS, Adkinson NF Jr., Kagey-Sobotka A, et al. Nasal challenge with ragweed pollen in hay fever patients. Effect of immunotherapy. J Clin Invest 1985; 76(6):2247–2253.
72. Marogna M, Spadolini I, Massolo A, et al. Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: a 3-year randomized controlled study. J Allergy Clin Immunol 2005; 115(6):1184–1188.
73. Crameri R, Rhyner C. Novel vaccines and adjuvants for allergen-specific immunotherapy. Curr Opin Immunol 2006; 18(6):761–768.
74. Akdis CA, Blaser K. Bypassing IgE and targeting T cells for specific immunotherapy of allergy. Trends Immunol 2001; 22:175–178.
75. Larche M. Immunoregulation by targeting T cells in the treatment of allergy and asthma. Curr Opin Immunol 2006; 18(6):745–750.
76. Kussebi F, Karamloo F, Rhyner C, et al. A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy. J Allergy Clin Immunol 2005; 115:323–329.
77. Karamloo F, Schmid-Grendelmeier P, Kussebi F, et al. Prevention of allergy by a recombinant multi-allergen vaccine with reduced IgE binding and preserved T cell epitopes. Eur J Immunol 2005; 35 (11):3268–3276.
78. Niederberger V, Horak F, Vrtala S, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. Proc Natl Acad Sci U S A 2004; 101(suppl 2):14677–4682.
79. Johansen P, Haffner AC, Koch F, et al. Direct intralymphatic injection of peptide vaccines enhances immunogenicity. Eur J Immunol 2005; 35(2):568–574.
80. Simons FE, Shikishima Y, Van Nest G, et al. Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA. J Allergy Clin Immunol 2004; 113(6): 1144–1151.
81. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006; 355(14):1445–1455.
82. Kundig TM, Senti G, Schnetzler G, et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. J Allergy Clin Immunol 2006; 117(6):1470–1476.
83. Zhu D, Kepley CL, Zhang M, et al. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. Nat Med 2002; 8(5):518–521.
84. Van Overtvelt L, Lombardi V, Razafindratsita A, et al. IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. Int Arch Allergy Immunol 2007; 145(2):152–162.
85. Rhyner C, Kundig T, Akdis CA, et al. Targeting the MHC II presentation pathway in allergy vaccine development. Biochem Soc Trans 2007; 35(pt 4):833–834.
86. Jutel M, Watanabe T, Akdis M, et al. Immune regulation by histamine. Curr Opin Immunol 2002; 14(6):735–740.
87. Akdis CA, Simons FE. Histamine receptors are hot in immunopharmacology. Eur J Pharmacol 2006; 533(1–3):69–76.
88. Bryce PJ, Mathias CB, Harrison KL, et al. The H1 histamine receptor regulates allergic lung responses. J Clin Invest 2006; 116(6):1624–1632.
89. Seligmann BE, Fletcher MP, Gallin JI. Histamine modulation of human neutrophil oxidative metabolism, locomotion, degranulation, and membrane potential changes. J Immunol 1983; 130(4): 1902–1909.
90. Vannier E, Dinarello CA. Histamine enhances interleukin (IL)-1-induced IL-1 gene expression and protein synthesis via H2 receptors in peripheral blood mononuclear cells. Comparison with IL-1 receptor antagonist. J Clin Invest 1993; 92(1):281–287.
91. Meretey K, Falus A, Taga T, et al. Histamine influences the expression of the interleukin-6 receptor on human lymphoid, monocytoid and hepatoma cell lines. Agents Actions 1991; 33(1–2):189–191.
92. Jeannin P, Delneste Y, Gosset P, et al. Histamine induces interleukin-8 secretion by endothelial cells. Blood 1994; 84(7):2229–2233.
93. Bayram H, Devalia JL, Khair OA, et al. Effect of loratadine on nitrogen dioxide-induced changes in electrical resistance and release of inflammatory mediators from cultured human bronchial epithelial cells. J Allergy Clin Immunol 1999; 104(1):93–99.
94. Fujikura T, Shimosawa T, Yakuo I. Regulatory effect of histamine H1 receptor antagonist on the expression of messenger RNA encoding CC chemokines in the human nasal mucosa. J Allergy Clin Immunol 2001; 107(1):123–128.

95. Elenkov IJ, Webster E, Papanicolaou DA, et al. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. J Immunol 1998; 161(5):2586–2593.
96. van der Pouw Kraan TC, Snijders A, Boeije LC, et al. Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. J Clin Invest 1998; 102(10):1866–1873.
97. Takahashi HK, Iwagaki H, Mori S, et al. Histamine inhibits lipopolysaccharide-induced interleukin (IL)-18 production in human monocytes. Clin Immunol 2004; 112(1):30–34.
98. Takahashi HK, Morichika T, Iwagaki H, et al. Histamine downregulates CD14 expression via H2 receptors on human monocytes. Clin Immunol 2003; 108(3):274–281.
99. Morichika T, Takahashi HK, Iwagaki H, et al. Histamine inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in an intercellular adhesion molecule-1- and B7.1-dependent manner. J Pharmacol Exp Ther 2003; 304(2):624–633.
100. Mazzoni A, Young HA, Spitzer JH, et al. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. J Clin Invest 2001; 108(12):1865–1873.
101. Ohtani T, Aiba S, Mizuashi M, et al. H1 and H2 histamine receptors are absent on Langerhans cells and present on dermal dendritic cells. J Invest Dermatol 2003; 121(5):1073–1079.
102. Akdis M, Trautmann A, Klunker S, et al. T helper (Th) 2 predominance in atopic disease is due to preferential apoptosis of circulating memory/effector Th1 cells. Faseb J 2003; 17:1026–1035.
103. Soga F, Katoh N, Kishimoto S. Histamine prevents apoptosis in human monocytes. Clin Exp Allergy 2007; 37(3):323–330.
104. Gantner F, Sakai K, Tusche MW, et al. Histamine h(4) and h(2) receptors control histamine-induced interleukin-16 release from human CD8(+) T cells. J Pharmacol Exp Ther 2002; 303(1):300–307.
105. Sirois J, Menard G, Moses AS, et al. Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. J Immunol 2000; 164(6):2964–2970.
106. Benovic J. Novel b2-adrenergic receptor signaling pathways. J Allergy Clin Immunol 2002; 110:229–235.
107. Szeberenyi JB, Pallinger E, Zsinko M, et al. Inhibition of effects of endogenously synthesized histamine disturbs in vitro human dendritic cell differentiation. Immunol Lett 2001; 76(3):175–182.
108. Idzko M, la Sala A, Ferrari D, et al. Expression and function of histamine receptors in human monocyte-derived dendritic cells. J Allergy Clin Immunol 2002; 109(5):839–846.
109. Gutzmer R, Langer K, Lisewski M, et al. Expression and function of histamine receptors 1 and 2 on human monocyte-derived dendritic cells. J Allergy Clin Immunol 2002; 109(3):524–531.
110. Caron G, Delneste Y, Roelandts E, et al. Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. J Immunol 2001; 167(7):3682–3686.
111. Caron G, Delneste Y, Roelandts E, et al. Histamine induces CD86 expression and chemokine production by human immature dendritic cells. J Immunol 2001; 166(10):6000–6006.
112. Jutel M, Watanabe T, Klunker S, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 2001; 413(6854):420–425.
113. Osna N, Elliott K, Khan MM. Regulation of interleukin-10 secretion by histamine in TH2 cells and splenocytes. Int Immunopharmacol 2001; 1(1):85–96.

6 Tree Pollen Allergens

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INTRODUCTION

IgE-mediated allergy affects more than 25% of the world's population, and proteins from trees, grasses, and mites are the most important causes of this disease (1,2). In trees, allergenic proteins can occur in pollen, fruits, or seeds. Pollen released in great abundance during the flowering season of certain trees represents a major trigger of respiratory manifestations of allergy (e.g., rhinitis and asthma), whereas tree fruits and seeds can elicit various symptoms of food allergy.

Trees recognized as major pollen allergen sources belong to certain botanical orders with defined geographical distributions and flowering periods (3). Allergenic tree species are predominantly wind pollinated, whereas insect-pollinated trees rarely elicit allergic symptoms. This is likely due to the process of allergic sensitization, which leads to the production of allergen-specific IgE antibodies (4), preferentially occurring via the respiratory mucosa (5,6) and requiring certain threshold levels of pollen (7–9).

In the temperate climate zone, thus in countries of northern and middle Europe, Northwest Africa, East Asia, from North America to the Andes, as well as in certain areas of Australia (2,3,10,11), trees belonging to the order Fagales (e.g., birch, alder, hazel) represent the predominant allergen sources. Whereas in Mediterranean countries and in areas with a Mediterranean climate (e.g., parts of North and South America, South Africa, and Australia), olive trees, which are members of the order Scrophulariales, are the most important sources of airborne allergens (12–15). Other important trees capable of inducing pollen allergy belong to two closely related plant families of the "nonflowering" plants (Gymnospermae), the Cupressaceae (e.g., cypress, cedar) and the Taxodiaceae. Cupressaceae trees represent important causes of pollinosis, especially in areas characterized by a Mediterranean climate where up to 30% of atopic individuals might be sensitized to Cupressaceae pollen (16–20). Within the Taxodiaceae, one species, the Japanese cedar (*Cryptomeria japonica*), is of increasing allergological relevance and represents the most common cause of seasonal allergy in Japan (21).

The most important allergens of these allergenic trees have been identified and characterized. Many of them have been cloned and produced as defined recombinant proteins, which can be used as tools to study the immunopathology of allergic disease (22). These defined proteins also form the basis for the development of novel strategies for diagnosis, treatment, and prevention of allergies (4,23,24). Tree pollen allergens characterized to date represent predominantly low-molecular-weight intracellular proteins or glycoproteins that are rapidly released after contact of pollen with aqueous solutions (25,26). Carbohydrate moieties may represent cross-reactive IgE epitopes occurring in tree pollen and several unrelated allergen sources, but seem to have little clinical relevance (27,28).

An exciting discovery was that the cross-reactivity observed among certain closely related species (e.g., trees belonging to the order Fagales) can be attributed to the structural and immunological similarity of relevant cross-reactive allergens (29–31). This finding implies that diagnosis and immunotherapy may be performed with a few cross-reactive marker allergens that harbor a large proportion of the cross-reactive epitopes (32–35).

TAXONOMY AND DISTRIBUTION OF ALLERGENIC TREES

Among the 250,000 well-described pollen-producing plant species, less than 100 are considered as potent causes of pollen allergy (36–39). Figure 1 displays the phylogenetic relationship between trees that are relevant sources of allergenic pollen and shows that trees of both plant divisions, the Angiospermae (commonly known as "flowering plants") and the

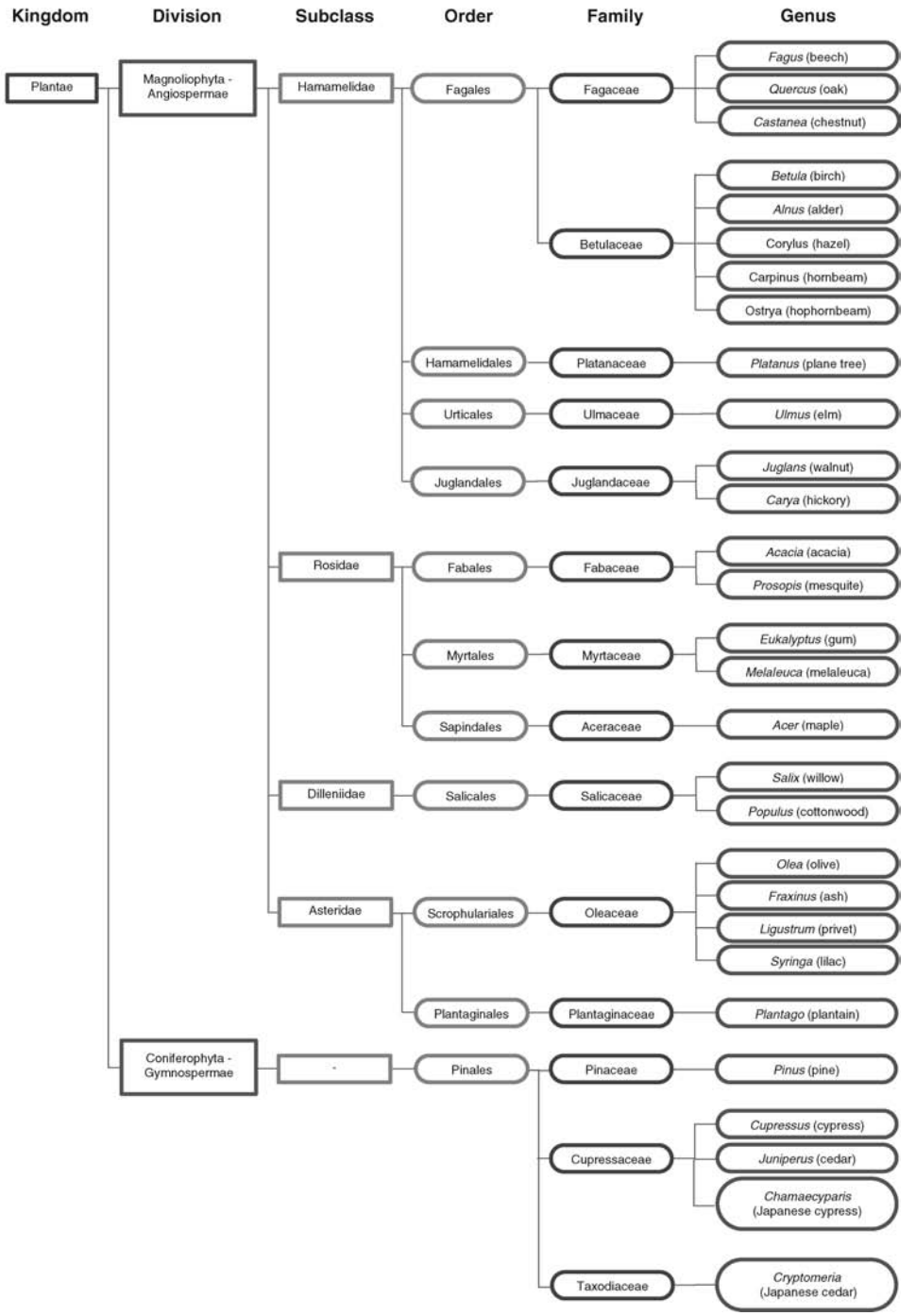

Figure 1 Taxonomy of trees producing allergenic pollen. Plants are listed following taxonomical guidelines of the Integrated Taxonomic Information System (www.itis.gov). For further information see also Ref. 172.

Gymnospermae (nonflowering plants), have an impact on eliciting allergic symptoms in patients. Knowledge about taxonomical relationship between trees is of importance, since pollen of closely related trees contain cross-reactive allergen molecules (e.g., order Fagales with the major birch pollen allergen, Bet v 1), which are absent in pollen from unrelated trees. As mentioned above, overall wind pollination appears to be a prerequisite for an allergenic tree.

Looking at the plant kingdom, the orders Fagales (e.g., birch, alder, hazel, oak, beech), Scrophulariales (e.g., olive, ash), and Pinales (e.g., cypress, cedar) comprise the most potent allergenic trees. Within these orders, the trees birch, olive, and cypress are known as the most potent causes of pollen allergy, whereas plants like alder, hazel, chestnut (members of the Fagales), privet (Scrophulariales), and pine (Pinales) have lesser allergenic potential. Other plant orders contain, as a whole, predominantly trees of low or uncertain importance in eliciting allergic symptoms: these are the orders Sapindales (e.g., maple tree), Hamamelidales (e.g., plane trees), and Myrtales (e.g., gum tree, melaleuca). So far, little is also known about the sensitization potency of pollen allergens from trees belonging to the orders Juglandales (e.g., walnut), Fabales (e.g., acacia), and Salicales (e.g., willow, cottonwood). In the case of melaleuca, the low allergenic potential can be explained by the fact that this tree is not wind pollinated (40). However, other less allergenic trees, like maples, acacias, and walnuts, are wind pollinated, which indicates that the sensitization potency of a plant cannot only be explained by wind pollination.

It is interesting to note that the plant subclasses Hamamelidae and Asteridae not only comprise important allergenic trees but also the most relevant allergenic weeds: within the Hamamelidae it is the genus *Parietaria* (from the order Urticales) and within the Asteridae these are the genera *Artemisia* (mugwort, order Asterales) and *Ambrosia* (ragweed, order Asterales), which represent the most potent elicitors of weed pollen allergy. On the other hand, the subclass Rosidae includes trees (within the order Rosales) that are considered as the most relevant sources of allergenic fruits (e.g., apple, cherry).

Regarding the geographic distribution of allergenic trees, we can discriminate areas with a preferential occurrence of certain trees from areas with mixed vegetation. The geographic distribution influences the sensitization profiles of allergic patients toward certain allergenic molecules (41). Trees belonging to the order Fagales prefer the temperate climate zone and grow in Europe, Northwest Africa, East Asia, and from North America to the Andes. In contrast, olive trees, the most allergenic trees of the order Scrophulariales, occur in the Mediterranean countries and in areas with a Mediterranean climate of North and South America, South Africa, and Australia. These Mediterranean climate zones are also the preferred areas of the most allergenic trees of the order Pinales, cypress and cedar, which grow in the Mediterranean countries, Australia, New Zealand, South America, and parts of Asia (China, India). Ash, the other important pollen allergen source of the Scrophulariales, occurs in middle Europe and North America, often in the same area as the Fagales trees.

Two papers investigating the sensitization profiles of allergic patients from different parts of the world have revealed interesting differences depending on geographic areas (41,42). Birch pollen–allergic patients from the northern parts of Europe are mainly sensitized against the major birch pollen allergen, Bet v 1, which therefore may be considered as a genuine marker for birch sensitization (41). By contrast, patients from the more southern parts of Europe appear positive in a birch pollen extract–based diagnostic test, but when tested with pure recombinant allergens are more frequently positive to cross-reactive allergens (e.g., profilins, calcium-binding allergens). It is therefore likely that these patients are sensitized against other allergen sources and, due to cross-reactivity, appear positive in the birch pollen extract test. Similar results were obtained when an allergic population from Central Africa was tested with recombinant allergens, indicating that the IgE reactivity profile reflects the local pollen exposure (42). These studies and another study performed with recombinant *Parietaria* allergens (43) emphasize the importance of diagnostic testing with recombinant allergens for the accurate diagnosis of the sensitizing allergen source.

TREE POLLEN IDENTIFICATION

Pollen grains are single cells that contain within themselves the male reproductive cells, the sperm cells. Their task is to deliver these sperm cells to the female parts of the flower, where the process of fertilization takes place. Pollen grains are enclosed within an inner wall,

the intine, and an outer wall, the exine, which protect the pollen from harmful environmental influences, such as desiccation and irradiation, during distribution. The outer wall consists of very elaborate, three-dimensional patterns and is interrupted by openings called apertures. The number, distribution, and architecture of the apertures vary between plants and can be used to classify and identify tree pollen by light microscopy (36).

The collection of air samples and the analysis and identification of pollen are of importance for physicians and patients. The allergist needs to know which species of allergenic pollen are present in the atmosphere, the number of allergenic pollen grains in a given volume of air, and the time and spatial variations of concentrations of airborne allergenic pollen. Measurements of pollen loads during certain periods of the year permit prediction of allergen exposure, and such information distributed to allergic patients can help them to avoid exposure (44). Knowledge of pollen loads during certain periods and in certain countries also allows allergic patients to plan their vacations and traveling schedules (45). However, since there may be variations of the allergen contents in pollen grains and since allergens may also be released via submicronic particles, it is important to measure and quantify not only the pollen grains but also the concentrations of the released allergenic molecules (46,47).

An interesting correlation between date of birth and sensitization against certain pollen has been described. Children who were born in early spring and summer are more frequently sensitized against birch and grass pollen, respectively (48,49). There is also compelling evidence that sensitization to certain pollen (e.g., birch) is more frequent in children exposed to heavy pollen exposure early in life than in children who have experienced mild pollen exposure (50).

A number of methods are used to collect and quantify pollen in the air (51). The most widely used technique is the Rotorod® Sampler, a rotating-arm impactor that recovers airborne particles on two rapidly moving plastic collector rods, which are coated with silicon grease. The Rotorod system measures the average pollen concentration during the sampling period but fails to detect variations in concentrations within this period. In contrast to this, volumetric traps allow continuous isokinetic sampling and record variations in the concentrations of pollen during the sample period. The collected samples may be counted on the basis of pollen morphology. Unfortunately, morphological counting usually does not allow discrimination between very closely related pollen species. Alternatively, collected samples can be analyzed with antibodies for quantitative determination of the allergenic molecules. As mentioned above, the latter has the advantage that, in addition to pollen-associated allergens, allergens that are released from pollen and become adsorbed to other carrier particles (e.g., aerosols) can also be measured. In this context it has been reported that pollen from birch and related trees can release allergens by a process of artificial pollen germination, which occurs when pollen are exposed to humidity (Fig. 2) (26). Furthermore, it

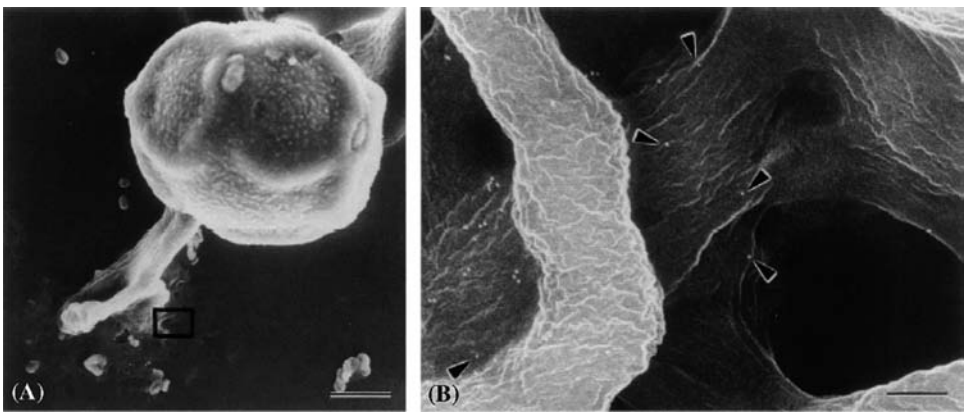

Figure 2 Humidity induced release of allergens from abortively germinated alder pollen. On contact with rain water allergen-containing particles are liberated from ruptured pollen tubes. Field emission scanning electron micrographs after immunogold labeling for Aln g 1. The rectangle in (**A**) marks an area comparable to the area shown in higher magnification in (**B**). Bound Aln g 1–specific antibodies were detected with secondary antibodies coupled to colloidal gold particles, which appear as white dots [bars represent 5 μm in (**A**) and 0.25 μm in (**B**)]. *Source*: From Ref. 26.

was found that certain carrier molecules (diesel exhaust particles) might act as adjuvants by driving the allergen-specific immune response into a preferential TH2 pathway that is accompanied by increased production of IgE antibodies (52). On the basis of such findings, it appears that the actual measurement of allergenic molecules using antibody assays can give more accurate information about true allergen exposure than mere pollen counting. Another argument for antibody-based measurement of allergen exposure is the observation that pollen may contain greatly varying amounts of allergens depending on the maturation state of the pollen or depending on the cultivar (53,54).

CLONING OF TREE POLLEN ALLERGENS

Diagnosis and specific immunotherapy of pollinosis are currently performed with allergen extracts obtained by simple extraction procedures in aqueous buffers. Many attempts have been made to improve the quality of the extracts, since it has been recognized that extracts may lack important allergens, may contain nonallergenic materials, and may vary greatly in their composition (55,56). Furthermore, it is technically impossible to purify all of the major and minor allergens of a natural allergen source to obtain adequate, pure components for diagnostic testing. The application of molecular biology techniques to the field of allergen characterization has enabled the recombinant production of the most relevant allergens from the common allergen sources (55).

In principle, there are two strategies that can be applied to obtain cDNA coding for allergens (57). The first approach uses IgE antibodies of patients for the isolation of allergen-encoding cDNAs from expression cDNA libraries that have been constructed from the allergen source (Fig. 3). For this approach, mRNA is first isolated from the allergen source and converted into a cDNA by reverse transcription. This cDNA is then inserted into a vector (usually a phage vector) suitable for construction of an expression cDNA library. After infection of appropriate host cells (usually *Escherichia coli* cells), clones expressing allergens can be located with patients' serum IgE using immunoscreening technology. DNA from the positive clones is then isolated, purified, and subjected to sequence analysis. The second approach for the isolation of allergen-encoding cDNAs involves DNA-based screening technologies [e.g., DNA-based screening of libraries, polymerase chain reaction (PCR), or reverse transcription PCR (RT-PCR) strategies]. Once allergen-encoding cDNAs have been obtained using either approach, they can be inserted into expression vectors and recombinant allergens can be produced in large amounts and high purity (Fig. 3).

The first isolated cDNA coding for a tree pollen allergen was for Bet v 1, the major birch pollen allergen (58). The Bet v 1 cDNA was obtained following the first strategy by IgE

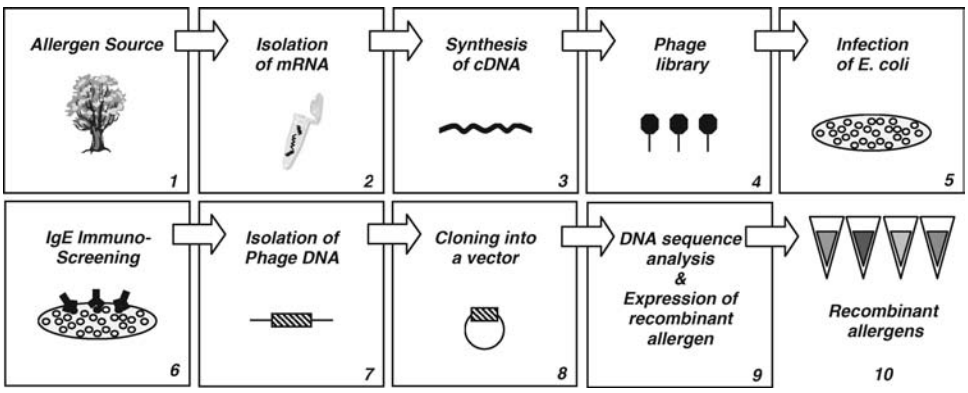

Figure 3 Cloning of tree pollen allergens and production of recombinant allergens. The different steps of the procedure, from mRNA isolation to the production of recombinant allergens, are displayed. The mRNA is isolated from the allergen source (2) and converted into a cDNA (3), which is then ligated into a phage vector (4). Expression of the inserted cDNA is achieved after infection of *Escherichia coli* cells (5) and allergen expressing phage clones can be located with IgE antibodies from allergic patients using immunoscreening technology (6). After the isolation of phage DNA, allergen-encoding cDNAs can be inserted in suitable vector systems (8) and recombinant allergens can be produced in various host organisms (e.g., prokaryotic, eukaryotic organisms).

immunoscreening of an expression cDNA library that had been constructed from mature birch pollen. Using the same approach, a series of tree cDNAs were isolated, including Bet v 2 (profilin), a highly cross-reactive birch pollen allergen and the first known plant actin-binding protein (59); Bet v 3 and Bet v 4, both calcium-binding birch pollen allergens (60–62); and Aln g 4, a calcium-binding pollen allergen from alder pollen (63).

Using the second (the DNA-based) strategy, oligonucleotides constructed according to a previously determined amino acid sequence of an allergen are applied either for PCR cloning or for screening of cDNA libraries. The PCR approach was used to clone the major pollen allergens from alder (Aln g 1), hornbeam (Car b 1), and hazel (Cor a 1) (64–66) and to isolate cDNAs coding for the major olive pollen allergen, Ole e 1; olive pollen profilin, Ole e 2; and the major privet pollen allergen, Lig v 1 (67–69). Further application of this strategy nearly completed the spectrum of olive pollen allergens (70). In contrast to this, Cry j 1 and Cry j 2, the major allergens of Japanese cedar, were obtained by DNA-based screening of cDNA libraries (71,72). On the basis of sequence similarity at the protein and nucleic acid level with the major birch pollen allergen, Bet v 1, an RT-PCR approach was used to isolate cDNAs coding for Mal d 1, the major apple allergen; Api g 1, the major celery allergen; and Pru av 1, the major cherry allergen. These important food allergens were produced as recombinant proteins and their cross-reactivity to Bet v 1 was demonstrated (73–75).

Table 1 gives an overview of tree pollen allergens, their biological functions, and characteristics. The spectrum of tree pollen and tree nut allergens has further been reviewed in several publications and there are several allergen databases that are continuously updated regarding new allergens (70,123–126). The homepage of the World Health Organisation/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Subcommittee (www.allergen.org) summarizes those allergens that have been submitted to the allergen nomenclature subcommittee by researchers for approval and registration. The Structural Database of Allergenic Molecules (SDAP) from the University of Texas Medical Branch offers structural data about allergens. The Allergome database (www.allergome.org) represents a frequently updated and well-kept allergen database that contains published allergen sequences and published studies using allergen molecules. A useful summary of currently available allergen databases can be found in a review article by Mari (126).

The rapid progress in the field of recombinant allergens holds promise that most of the traditional allergen raw extracts will be replaced by recombinant allergens, which cover the complete epitope repertoire of the extracts (32,127,128).

BIOLOGICAL FUNCTIONS AND STRUCTURAL CHARACTERISTICS OF TREE POLLEN ALLERGENS

Application of molecular biology techniques to allergen characterization has permitted the determination of the molecular characteristics of most common environmental allergens during the last two to three decades (127). The DNA and deduced amino acid sequences can be obtained by sequencing of the allergen-encoding cDNAs and thus allow comparisons with sequences deposited in databases. Using this approach, the biological functions of various allergens can be deduced. For example, it was found that the cDNA and the amino acid sequence of the major birch pollen allergen, Bet v 1, showed significant sequence homology with a group of proteins that were found to be upregulated when plants were wounded, infected, or subjected to stressful conditions, and accordingly these proteins were designated pathogenesis-related proteins (PR proteins) (58). Although, to date, there are no definitive experimental data to support that the family of Bet v 1–related allergens contribute to the plant defense system, it is possible that they have protective functions (129). Other functions (e.g., RNAse activity, lipid carrier) have been claimed for the Bet v 1 allergen family on the basis of in vitro experiments and structural data (130–132).

Numerous Bet v 1–homologous allergens have been identified in pollen of trees belonging to the order Fagales (e.g., Aln g 1, alder; Cor a 1, hazel; Car b 1, hornbeam; Que a 1, white oak; and Cas s 1, chestnut) (see www.allergen.org). Figure 4 displays the relationship among Bet v 1–related plant allergens on the basis of sequence identities. Almost all of the proteins contain cross-reactive IgE epitopes. However, it is also known that even birch pollen contains proteins with high sequence identity to Bet v 1 but without relevant allergenic

Table 1 Tree Pollen Allergens Cloned and/or Characterized to Date

Species	Common name	Allergen	Function and similarity	MW (kDa)	References
Fagales					
Betula verrucosa	Birch	Bet v 1	Pathogenesis-related protein (PR10)	17	58
		Bet v 2	Profilin	15	59
		Bet v 3	Ca^{2+}-binding protein	23.7	60
		Bet v 4	Ca^{2+}-binding protein	9.3	61,62
		Bet v 5	Isoflavone reductase	35	76
		Bet v 6	Isoflavone reductase	33.5	77
		Bet v 7	Cyclophilin	18	78,79
		Bet v 8	Pectin esterase	66	80
Alnus glutinosa	Alder	Aln g 1	PR10[a]; Bet v 1 related	17	64
		Aln g 4	Ca^{2+}-binding protein; Bet v 4 related		63
Corylus avellana	Hazel	Cor a 1	PR10; Bet v 1 related		66
		Cor a 2	Profilin; Bet v 2 related	14	b
		Cor a 10	Luminal-binding protein	70	81
Carpinus betulus	Hornbeam	Car b 1	PR10; Bet v 1 related	17	65
Castanea sativa	Chestnut	Cas s 1	PR10; Bet v 1 related	22	82
Quercus alba	White oak	Que a 1	PR10; Bet v 1 related	17	83
Hamamelidales					
Platanus acerifolia	London plane tree	Pla a 1	Invertase inhibitor	18	84,85
		Pla a 2	Polymethylgalacturonase	43	86
		Pla a 3	Lipid transfer protein	10	87
Scrophulariales					
Olea europea	Olive tree	Ole e 1		16	67
		Ole e 2	Profilin, Bet v 2 related	15–18	68
		Ole e 3	Ca^{2+}-binding protein; Bet v 4 related	9.2	88,89
		Ole e 4		32	90
		Ole e 5	Superoxide dismutase	16	90,91
		Ole e 6		5.8	92,93
		Ole e 7			94
		Ole e 8	Ca^{2+}-binding protein	21	95
		Ole e 9	β-1,3-Glucanase	46.4	96,97
		Ole e 10	Glycosyl hydrolase	10.8	98
Fraxinus excelsior	Ash	Fra e 1	Ole e 1 related	20	99,100,101
Ligustrum vulgare	Privet	Lig v 1	Ole e 1 related	20	69
Syringa vulgaris	Lilac	Syr v 1	Ole e 1 related	20	102
		Syr v 3	Ca^{2+}-binding protein; Bet v 4 related	8.9	103
Plantaginales					
Plantago lanceolata	English plantain	Pla l 1		18	104
Pinales					
Cryptomeria japonica	Japanese cedar	Cry j 1	Pectate lyase	41–45	71,105,106
		Cry j 2	Polymethylgalacturonase	46.6	72,107,108
		Cry j 3	PR5; thaumatin-like protein	27.3	109,110
Chamaecyparis obtusa	Japanese cypress	Cha o 1	Pectate lyase; Cry j 1 related	40.2	111
		Cha o 2	Polymethylgalacturonase; Cry j 2 related	46	112
Cupressus arizonica	Arizona cypress	Cup a 1	Pectate lyase; Cry j 1 related	43	113
		Cup a 3	PR5; thaumatin-like protein	21	114
Cupressus sempervirens	Italian cypress	Cup s 1	Pectate lyase; Cry j 1 related	43	AAF72629[c]
		Cup s 3	PR5; thaumatin-like protein	34	115

(*Continued*)

Table 1 Tree Pollen Allergens Cloned and/or Characterized to Date (*Continued*)

Species	Common name	Allergen	Function and similarity	MW (kDa)	References
Juniperus ashei	Mountain cedar	Jun a 1	Pectate lyase; Cry j 1 related	43	116,117,121
		Jun a 2	Polymethylgalacturonase; Cry j 2 related	55.7	118
		Jun a 3	PR5; thaumatin-like protein	30	119
Juniperus oxycedrus	Prickly juniper	Jun o 4	Ca^{2+}-binding protein; Bet v 4 related	18	120
Juniperus virginiana	Eastern red cedar	Jun v 1	Pectate lyase; Cry j 1 related	43	122
		Jun v 3	PR5; thaumatin-like protein		122

Allergenic molecules are listed according to their taxonomical orders (underlined). Allergen sources (species and common name), designations according to the World Health Organization/International Union of Immunological Societies Allergen Nomenclature Subcommittee (WHO/IUIS), functions and similarities, molecular weights (in kDa), and references or accession numbers are displayed.
[a]PR, pathogenesis-related protein.
[b]WHO/IUIS (www.allergen.org) database.
[c]AllergenOnline (FARRP) (www.allergenonline.com) database.

	Asparagus	Carrot	Celery	Tomato	Soybean	Pea	Medicago	Pear	Apricot	Cherry	Apple	Beech	Hornbeam	Alder	Hazel	Birch
Birch	39	38	40	47	49	54	55	58	60	59	66	69	79	81	83	100
Hazel	40	41	40	46	53	53	55	59	63	59	67	69	92	82	100	
Alder	41	39	40	40	50	50	52	55	61	57	64	67	79	100		
Hornbeam	39	42	40	46	51	51	56	56	62	58	66	68	100			
Beech	39	41	42	49	57	54	55	70	74	74	71	100				
Apple	39	40	45	47	50	51	54	70	79	76	100					
Cherry	37	40	43	49	55	51	51	84	76	100						
Apricot	37	44	50	50	53	51	52	73	100							
Pear	35	39	40	43	54	50	50	100								
Medicago	33	36	40	42	66	83	100									
Pea	29	33	39	39	70	100										
Soybean	34	37	40	41	100											
Tomato	43	32	40	100												
Celery	32	51	100													
Carrot	33	100														
Asparagus	100															

Figure 4 Sequence identities (%) between Bet v 1–homologous proteins from different sources. The percentage of sequence identity between Bet v 1–related allergens from various sources is displayed.

activity (133). The existence of these hypoallergenic Bet v 1 isoforms and of nonallergenic proteins with high sequence homology to Bet v 1 (134) demonstrates that sequence homology per se cannot predict with certainty whether a protein is allergenic or not. The latter aspect is also important because it is impossible to predict with certainty the allergenic potential of genetically modified plants exclusively on the basis of sequence homologies of the transgene with genes coding for known allergens (135).

Table 1 gives an overview of tree pollen allergens grouped according to botanical classifications. Each of the different trees contains a spectrum of allergens. However, it appears that certain allergenic molecules occur in different trees as proteins with significant sequence homology and cross-reactive epitopes. In general, it is possible to identify certain groups of

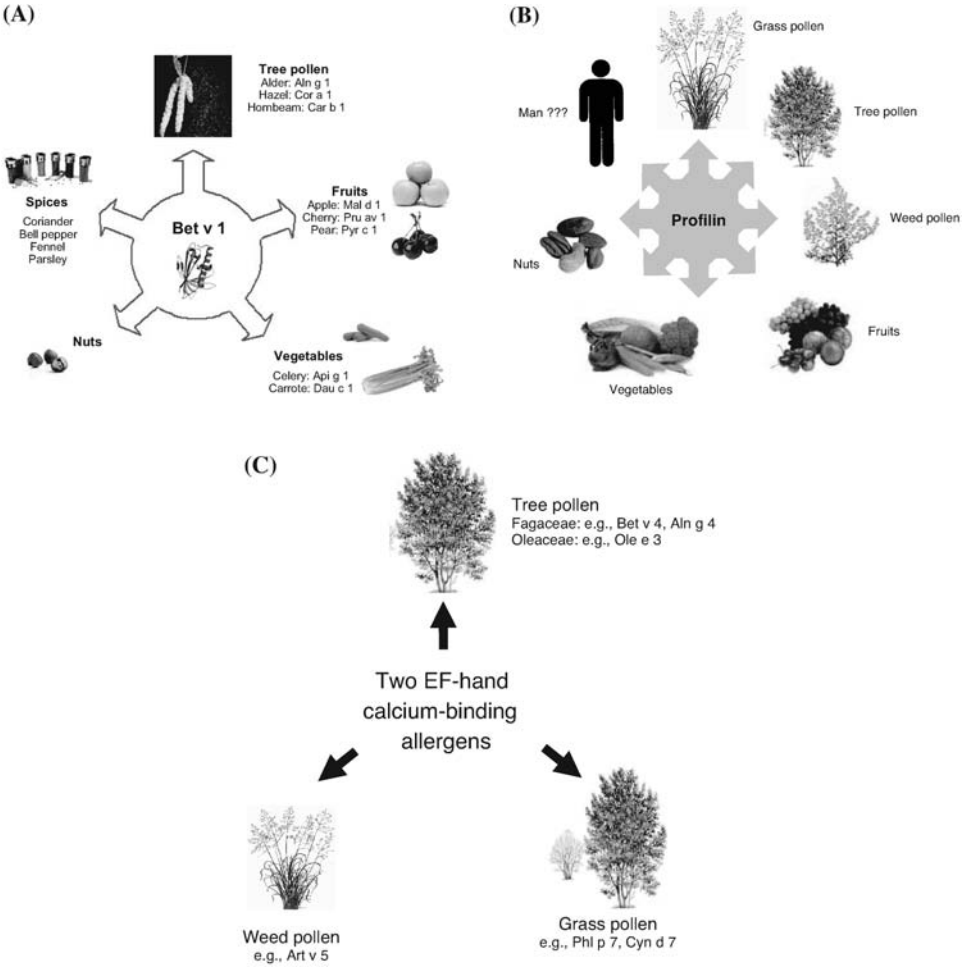

Figure 5 (*See color insert.*) (**A**) Bet v 1 cross-reactive allergens can be found in pollen of trees belonging to the order Fagales as well as in fruits, vegetables, nuts, and spices. (**B**) Profilins, the most cross-reactive allergens described to date, occur in pollen of botanically unrelated plants (trees, grasses, weeds), in plant-derived food (fruits, vegetables, nuts), and even in man. (**C**) Two EF-hand calcium-binding allergens can be found in pollen from trees, grasses, and weeds.

cross-reactive allergens. For example, there are the Bet v 1–related allergens, Aln g 1, Cor a 1, Car b 1, and Cas s 1, which can be found in pollen of trees belonging to the order Fagales. These allergens are also expressed in nuts of trees of the order Fagales and in fruits of unrelated trees belonging to the order Rosales (within the subclass Rosidae), and due to cross-reactivity might elicit symptoms of food allergy in pollen-allergic patients (Fig. 5A) (136).

A second group of highly cross-reactive allergens are the profilins. These are actin-binding proteins, expressed in all eukaryotic cells, which link signal transduction processes with the reassembling of the cytoskeleton (59,137–141). They are structurally conserved low-molecular-weight (12–15 kDa) proteins and represent probably the most widely distributed and conserved allergens described so far (59). They include proteins from pollen of botanically unrelated plants (trees, grasses, weeds), for instance, birch pollen profilin (Bet v 2) or olive pollen profilin (Ole e 2), proteins of plant-derived foods (fruits, vegetables, nuts), and even human proteins (Table 1 and Fig. 5B).

The birch pollen allergens Bet v 3 and Bet v 4 (60–62) as well as the olive pollen allergens Ole e 3 (88) and Ole e 8 (95) belong to the group of calcium-binding proteins (142). Sequence analysis of the cDNA coding for these allergens revealed the presence of typical calcium-binding motifs (i.e., binding sites for calcium), termed EF-hands (142). Bet v 3, an allergen

highly expressed in mature pollen (60), contains three EF-hands, the olive pollen allergen Ole e 8 and the cypress pollen allergen Jun o 4 contain four EF-hands (95,120), whereas Bet v 4 and Ole e 3 contain two EF-hand calcium-binding motifs (61,62). The calcium-binding allergens with two EF-hands have been found in a variety of pollen from botanically unrelated trees, grasses, and weeds, and represent another family of highly cross-reactive allergens (Fig. 5C) (143). It is important to note that calcium-binding allergens are predominantly expressed in pollen but not in other plant tissues and are therefore responsible only for pollen, but not for food cross-reactivity. IgE inhibition experiments indicate that there is also extensive IgE cross-reactivity between members with different numbers of EF-hands (143). IgE recognition of calcium-binding allergens is enhanced in the presence of calcium, and calcium binding causes a conformational change resulting in higher thermal stability of the allergens (144). The calcium dependence of IgE binding suggests that patients are preferentially sensitized against the calcium-bound allergens (144). The first three-dimensional structure of a two EF-hand allergen, namely of the two EF-hand allergen from timothy grass (Phl p 7), has been resolved by X-ray crystallography. This three-dimensional analysis provides further insight into the structure and conformational changes of these highly cross-reactive allergens and suggests a ligand-binding function (145).

Another group of pollen allergens is represented by the major olive pollen allergen, Ole e 1, which shares high sequence identity and cross-reactive epitopes with allergens from closely related trees of the Oleaceae family, ash (Fra e 1) (99–101), privet (Lig v 1) (69), and lilac (Syr v 1) (102), but lacks cross-reactivity with homologous allergens from other plants. The Ole e 1 pollen allergens are glycosylated proteins and their glycan moieties seem to be involved in the antigenic and allergenic properties of these allergens. However, so far no functional role is assigned to these allergens (70).

In the division of Gymnospermae, two separate groups of pollen allergens have been identified (124): the pectate lyases and the polymethylgalacturonases. The major Japanese cedar pollen allergen Cry j 1, the most thoroughly studied member of the pectate lyases (105,106), displays high sequence homology and IgE cross-reactivity with major allergens of other trees from the order Pinales (e.g., Japanese cypress, Arizona cypress, Italian cypress, mountain cedar). Interestingly, sequence identities of nearly 50% were also found with the major ragweed allergens, the pectate lyases Amb a 1 and Amb a 2 (124,146), but no cross-reactivity with these allergens was described. The second major allergen from Japanese cedar pollen, Cry j 2, has been classified as a polymethylgalacturonase, with cross-reactivity to homologous pollen allergens from other members of the Pinales (107,108). Database searches further revealed significant sequence homologies ($\sim$40%) of Cry j 2 with polygalacturonases known as grass pollen allergens (Phl p 13) (147) and with polygalacturonases associated with fruit ripening in tomato (124), but no relevant IgE cross-reactivity seems to exist between Cry j 2 and these enzymes.

Table 1 gives an overview of so far characterized tree pollen allergens and provides information about their sources, biological functions, molecular weights (kDa), and references about their description. Features that all tree pollen allergens have in common are that they represent low-molecular-weight proteins or glycoproteins that rapidly elute from pollen after contact with aqueous solutions (148). The use of immunogold electron microscopy reveals that these allergens are mainly intracellular proteins, which either elute from pollen or, under certain conditions, are expelled from pollen by rupture or abortive pollen germination (25,26). Analysis of the three-dimensional structures of important pollen allergens does not identify structural motifs that are common among all allergens. However, these studies show that cross-reactivity between allergens is based on structural similarities (149).

CROSS-REACTIVITY BETWEEN TREE POLLEN ALLERGENS

During the last decade the most common allergens have been identified by molecular cloning and produced as recombinant allergens (22). In this context, IgE inhibition studies performed with purified recombinant allergens have greatly enhanced our understanding of cross-reactivity at the molecular level (22).

Figure 5A illustrates as an example the cross-reactivity within the group of Bet v 1–related allergens. Allergens containing cross-reactive IgE epitopes have been described in pollen,

fruits, vegetables, nuts, and seeds (136). Accordingly, Bet v 1–sensitized patients frequently suffer from an oral allergy syndrome caused by ingestion of food containing cross-reactive allergens. Because of extensive cross-reactivity among the Bet v 1–related allergens, it is not surprising that immunotherapy with birch pollen vaccine alone also improves allergy to pollens of related trees and food allergy (150–152).

It appears that cross-reactivity has in principle two facets that can be applied to diagnosis and therapy. Certain allergens/epitopes are restricted to certain allergen sources and thus can be used as marker molecules to confirm sensitization to these sources (153). For example, Bet v 1 cross-reacts mainly with pollen allergens of trees belonging to the Fagales order. The major olive pollen allergen, Ole e 1, cross-reacts with pollen allergens of trees belonging to the Oleaceae order, including ash (35,154). The major timothy grass pollen allergens (e.g., Phl p 1, Phl p 2, Phl p 5 from timothy grass) cross-react with allergens from other grasses, and certain weed allergens (e.g., Par j 2 from *Parietaria*) cross-react with allergens only present in weeds (43). On the basis of this observation it has been proposed to use such species-specific marker allergens to confirm sensitization to certain allergen sources. These marker allergens can thus be used as diagnostic gatekeepers to confirm suitability of patients for immunotherapy with a given allergen extract (Fig. 6) (35,153). Another argument for using major species-specific marker allergens as an inclusion criterion for immunotherapy is that the currently used allergen extracts are mainly standardized regarding these major allergens.

However, allergens have been identified that exhibit very broad cross-reactivity and thus indicate polysensitization. These allergens include for example the group of profilins (Fig. 5B) and two EF-hand calcium-binding (Fig. 5C) allergens. Patients who are sensitized to profilin (e.g., Bet v 2 or Phl p 12, the timothy grass pollen profilin) cross-react in most cases with profilins from various unrelated plants and suffer from pollen and plant food polysensitization (Fig. 5B) (153). Patients who are sensitized to calcium-binding allergens (e.g., Bet v 4, Phl p 7, the two EF-hand calcium-binding allergen from timothy grass) suffer in most cases from multiple pollen sensitization to trees, grasses, and weeds (Fig. 5C) (153). Such patients may benefit less from allergen vaccine–based immunotherapy because the currently used therapeutic vaccines are not standardized regarding these molecules, and patients with polysensitization seem to benefit less from allergen-specific immunotherapy (155).

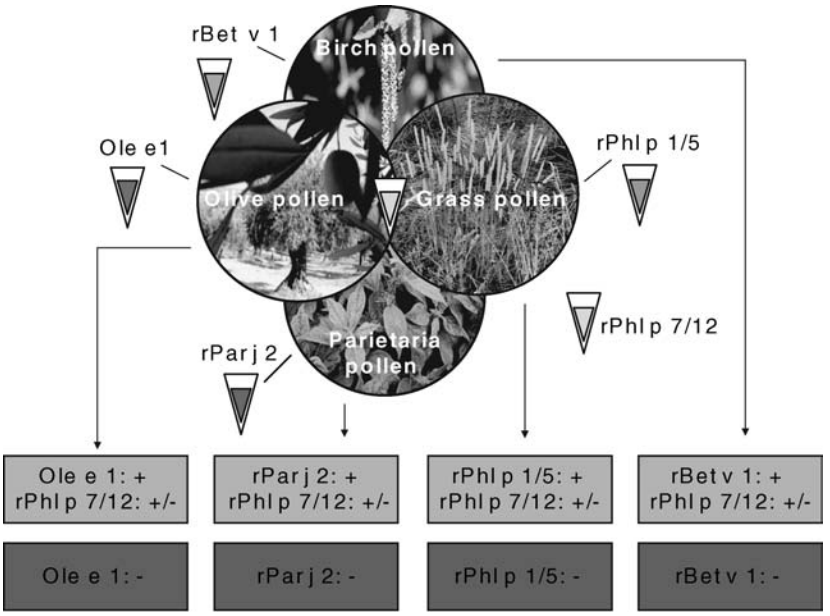

Figure 6 Marker allergens for the diagnosis of pollen allergies. Marker allergens for genuine sensitization to birch pollen, olive and ash pollen, *Parietaria* pollen, and grass pollen are indicated. The marker allergens that occur as cross-reactive allergens in each of these pollen sources are Phl p 7 and Phl p 12. A suggestion for prescription of immunotherapy based on test results obtained with the marker allergens is given at the bottom of the figure. *Source*: From Ref. 35.

In vitro diagnostic tests equipped with recombinant marker allergens to facilitate the selection of patients for immunotherapy with birch pollen and grass pollen extracts are available from diagnostic companies and can currently be used by clinicians (www .meduniwien.ac.at/allergy-research-christian-doppler; Publications).

TRANSITION FROM ALLERGEN EXTRACT–BASED DIAGNOSIS AND THERAPY TO RECOMBINANT ALLERGEN–BASED DIAGNOSIS AND THERAPY

The rapid progress of allergen characterization through the application of molecular cloning techniques has provided recombinant allergens covering most allergen sources, including trees. Recombinant allergens allow determination of the individual sensitization profiles of allergic patients, a process that has been designated component-resolved diagnosis (CRD) (33). The diagnostic information obtained by CRD is more precise than diagnosis based on extract-based methodology. Extract-based diagnosis will only identify potential allergen sources but does not provide any information regarding the disease-eliciting allergens within the given allergen source. In order to utilize the full spectrum of recombinant allergens for allergy diagnosis, novel forms of multiallergen tests are under development (156). Some of the new tests combine chip and microarray technology whereas others simply utilize nitrocellulose-based test systems for the elucidation of a patient's reactivity profile in a single test (156–158). In addition, recombinant allergens have been incorporated into established quantitative and automated in vitro allergy test systems, where they allow a more precise quantitative measurement of specific IgE and IgG.

Using these recombinant allergen–based tests, it has been possible to dissect the sensitization profiles of patients from various populations (41,42,159), to monitor the development of allergies from early childhood to adulthood (160), to investigate the development of IgE profiles during the natural course of allergic disease (161), and to study the effects of allergen-specific immunotherapy (161–164).

Recombinant allergen–based tests and CRD have resulted in several interesting observations regarding the pathogenesis of allergic diseases and the potential mechanisms for allergen-specific immunotherapy. The monitoring of IgE and IgG responses during allergen-specific immunotherapy has reemphasized the importance of specific blocking antibodies for the success of allergen-specific immunotherapy (161,162,164). The finding that allergen vaccines induce a highly heterogeneous immune response against the individual components in the vaccine has underlined the need for improvement of therapeutic allergen preparations (164). Moreover, it appears that injection of allergen vaccines may induce IgE reactivity against new allergens in treated patients (163,165). Although the clinical relevance of these findings has not been confirmed, these data support the idea that patients would benefit from treatment according to their individual sensitization profiles.

The concept of treating allergic patients according to their sensitization profile with purified recombinant allergens, termed component-resolved immunotherapy (CRIT), has therefore been proposed (33). During the last few years, several candidate molecules have been developed by recombinant DNA technology (22,166). These molecules are characterized by strongly reduced allergenic activity, while T-cell epitopes and immunogenicity (i.e., capacity to induce protective IgG responses) are maintained (166–168). The recombinant hypoallergenic allergen derivatives have been evaluated in vitro in experimental animal models and in in vivo provocation testing in patients (22,166). The human work verifies reduced allergenic activity. The first immunotherapy study with recombinant allergen derivatives was performed with hypoallergenic derivatives of the major birch pollen allergen, Bet v 1 (151,152,169–171), and subsequently several other successful immunotherapy studies have been performed with recombinant allergens (24).

SALIENT POINTS

- The most relevant tree pollen allergens are derived from wind-pollinated trees belonging to the order Fagales (e.g., birch), Scrophulariales (e.g., olive), and Pinales (e.g., cedar and cypress).

- The most common and important tree pollen allergens have been produced as recombinant allergens. Panels of recombinant allergens resembling the epitope complexity of natural allergen extracts are becoming available.
- The molecular characterization of tree pollen allergens reveals that there are families of cross-reactive allergens that are characterized by high sequence homology and immunological cross-reactivity.
- Recombinant allergen–based diagnostic tests are available or will be available in the near future for clinical use to determine the sensitization profiles of patients and to improve the selection of the most accurate treatment forms. This technique has been used successfully for research to establish sensitization profiles of patients, to reveal pathogenesis underlying allergic diseases and to study the effects of allergen-specific immunotherapy.
- Recombinant allergen derivatives with reduced allergenic activity have been developed and evaluated. The first immunotherapy trials are underway with the new molecules to study the mechanisms, efficacy, and safety of CRIT with recombinant allergen molecules.

REFERENCES

1. Kay AB. Allergy and Allergic Diseases. Oxford, U.K.: Blackwell Science, 2008.
2. Wuthrich B, Schindler C, Leuenberger P, et al. Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. Int Arch Allergy Immunol 1995; 106:149–156.
3. D'Amato G, Bonini S, Bousquet J, et al. Pollenosis 2000 Global Approach. Naples, Italy: JGC Editions, 2001.
4. Valenta R. The future of antigen-specific immunotherapy of allergy. Nat Rev Immunol 2002; 2:446–453.
5. Durham SR, Gould HJ, Hamid QA. Local IgE production in nasal allergy. Int Arch Allergy Immunol 1997; 113:128–130.
6. Niederberger V, Ring J, Rakoski J, et al. Antigens drive memory IgE responses in human allergy via the nasal mucosa. Int Arch Allergy Immunol 2007; 142:133–144.
7. Florido JF, Delgado PG, de San Pedro BS, et al. High levels of *Olea europaea* pollen and relation with clinical findings. Int Arch Allergy Immunol 1999; 119:133–137.
8. Zwick H, Popp W, Jäger S, et al. Pollen sensitization and allergy in children depend on the pollen load. Allergy 1991; 46:362–366.
9. Ridolo E, Albertini R, Giordano D, et al. Airborne pollen concentrations and the incidence of allergic asthma and rhinoconjunctivitis in northern Italy from 1992 to 2003. Int Arch Allergy Immunol 2007; 142:151–157.
10. Dvorin DJ, Lee JJ, Belecanech GA, et al. A comparative, volumetric survey of airborne pollen in Philadelphia, Pennsylvania (1991–1997) and Cherry Hill, New Jersey (1995–1997). Ann Allergy Asthma Immunol 2001; 87:394–404.
11. Kosisky SE, Carpenter GB. Predominant tree aeroallergens of the Washington, DC area: a six year survey (1989–1994). Ann Allergy Asthma Immunol 1997; 78:381–392.
12. Bousquet J, Cour P, Guerin B, et al. Allergy in the Mediterranean area. I. Pollen counts and pollinosis of Montpellier. Clin Allergy 1984; 14:249–258.
13. D'Amato G, Mullins J, Nolard N, et al. City spore concentrations in the European Economic Community (EEC). VII. Oleaceae (Fraxinus, Ligustrum, Olea). Clin Allergy 1988; 18:541–547.
14. Kerneman SM, McCullough J, Green J, et al. Evidence of cross-reactivity between olive, ash, privet, and Russian olive tree pollen allergens. Ann Allergy 1992; 69:493–496.
15. Liccardi G, D'Amato M, D'Amato G. Oleaceae pollinosis: a review. Int Arch Allergy Immunol 1996; 111:210–217.
16. Guerra F, Daza JC, Miguel R, et al. Sensitivity to Cupressus: allergenic significance in Cordoba (Spain). J Investig Allergol Clin Immunol 1996; 6:117–120.
17. Mari A, Di Felice G, Afferni C, et al. Assessment of skin prick test and serum specific IgE detection in the diagnosis of Cupressaceae pollinosis. J Allergy Clin Immunol 1996; 98:21–31.
18. Panzani RC, Ariano R. Allergenic significance of Cupressaceae and Taxodiaceae pollen in some parts of the Mediterranean and all over the world. Indian J Aerobiol 1999; 12:1–10.
19. Papa G, Romano A, Quaratino D, et al. Prevalence of sensitization to *Cupressus sempervirens*: a 4-year retrospective study. Sci Total Environ 2001; 270:83–87.
20. Di Felice G, Barletta B, Tinghino R, et al. Cupressaceae pollinosis: identification, purification and cloning of relevant allergens. Int Arch Allergy Immunol 2001; 125:280–289.

21. Kaneko Y, Motohashi Y, Nakamura H, et al. Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. Int Arch Allergy Immunol 2005; 136:365–371.
22. Valenta R. Biochemistry of allergens, recombinant allergens. In: Kay AB, Kaplan A, Bousquet J, Holt B, eds. Allergy and Allergic Diseases. 2nd edition. Oxford, England: Blackwell Publishing Ltd, 2008.
23. Valenta R, Ball T, Focke M, et al. Immunotherapy of allergic disease. Adv Immunol 2004; 82:105–153.
24. Valenta R, Niederberger V. Recombinant allergens for immunotherapy. J Allergy Clin Immunol 2007; 119:826–830.
25. Grote M, Vrtala S, Valenta R. Monitoring of two allergens, Bet v 1 and profilin, in dry and rehydrated birch pollen by immunogold electron microscopy and immunoblotting. J Histochem Cytochem 1993; 41:745–750.
26. Grote M, Valenta R, Reichelt R. Abortive pollen germination: a mechanism of allergen release in birch, alder, and hazel revealed by immunogold electron microscopy. J Allergy Clin Immunol 2003; 111:1017–1023.
27. van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. Int Arch Allergy Immunol 2002; 129:189–197.
28. Altmann F. The role of protein glycosylation in allergy. Int Arch Allergy Immunol 2007; 142:99–115.
29. Valenta R, Steinberger P, Duchene M, et al. Immunological and structural similarities among allergens: prerequisite for a specific and component-based therapy of allergy. Immunol Cell Biol 1996; 74:187–194.
30. Ferreira F, Hawranek T, Gruber P, et al. Allergic cross-reactivity: from gene to the clinic. Allergy 2004; 59:243–267.
31. Radauer C, Breiteneder H. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. J Allergy Clin Immunol 2006; 117:141–147.
32. Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. Curr Opin Immunol 1995; 7:751–756.
33. Valenta R, Lidholm J, Niederberger V, et al. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999; 29:896–904.
34. Kazemi-Shirazi L, Niederberger V, Linhart B, et al. Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. Int Arch Allergy Immunol 2002; 127:259–268.
35. Valenta R, Twaroch T, Swoboda I. Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. J Investig Allergol Clin Immunol 2007; 17(suppl 1):36–40.
36. Accorsi CA, Bandini-Mazzanti M, Romano B, et al. Allergenic pollen: morphology and microscopic photographs. In: D'Amato G, Spieksma FThM, Bonini S, eds. Allergenic Pollen and Pollenosis in Europe. Oxford: Blackwell, 1991; 24–44.
37. D'Amato G, Spieksma FThM, Liccardi G, et al. Pollen related allergy in Europe. Position Paper of the European Academy of Allergology and Clinical Immunology. Allergy 1998; 53:567–578.
38. Gregory PH. The Microbiology of the Atmosphere. Aylesbury: L. Hill 1973.
39. Thommen AA. Etiology of hay fever: studies in hay fever. N Y State J Med 1930; 437–441.
40. Stablein JJ, Buchholtz GA, Lockey RF. Melaleuca tree and respiratory disease. Ann Allergy Asthma Immunol 2002; 89:523–530.
41. Moverare R, Westritschnig K, Svensson M, et al. Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization. Int Arch Allergy Immunol 2002; 128:325–335.
42. Westritschnig K, Sibanda E, Thomas W, et al. Analysis of the sensitization profile towards allergens in central Africa. Clin Exp Allergy 2003; 33:22–27.
43. Stumvoll S, Westritschnig K, Lidholm J, et al. Identification of cross-reactive and genuine *Parietaria judaica* pollen allergens. J Allergy Clin Immunol 2003; 111:974–979.
44. Rantio-Lehtimäki A. Sampling airborne pollen and pollen antigens. In: D'Amato G, Spieksma FTM, Bonini S, eds. Allergenic Pollens and Pollinosis in Europe. Oxford, England: Blackwell Scientific, 1991.
45. Jäger S, Emberlin J, Gallop R, et al. The European pollen information service center in the internet. Allergy Suppl 1997; 52:32.
46. Buters JT, Kasche A, Weichenmeier I, et al. Year-to-year variation in release of Bet v 1 allergen from birch pollen: evidence for geographical differences between West and South Germany. Int Arch Allergy Immunol 2007; 145:122–130.
47. Taylor PE, Jacobson KW, House JM, et al. Links between pollen, atopy and the asthma epidemic. Int Arch Allergy Immunol 2007; 144:162–170.
48. Aalberse RC, Nieuwenhuys EJ, Hey M, et al. "Horoscope effect" not only for seasonal but also for non-seasonal allergens. Clin Exp Allergy 1992; 22:1003–1006.
49. Graf N, Johansen P, Schindler C, et al. Analysis of the relationship between pollinosis and date of birth in Switzerland. Int Arch Allergy Immunol 2007; 143:269–275.
50. Kihlstrom A, Lilja G, Pershagen G, et al. Exposure to birch pollen in infancy and development of atopic disease in childhood. J Allergy Clin Immunol 2002; 110:78–84.

51. Rantio-Lehtimäki A, Helander ML, Pessi AM. Significance of sampling height of airborne particles for aerobiological information. Allergy 1991; 46:68–76.
52. Diaz-Sanchez D, Tsien A, Fleming J, et al. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2 type pattern. J Immunol 1997; 158:2406–2413.
53. Mittermann I, Swoboda I, Pierson E, et al. Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): increased profilin expression during pollen maturation. Plant Mol Biol 1995; 27:137–146.
54. Castro AJ, de Dios Alche J, Cuevas J, et al. Pollen from different olive tree cultivars contains varying amounts of the major allergen Ole e 1. Int Arch Allergy Immunol 2003; 131:164–173.
55. Valenta R, Lidholm J, Niederberger V, et al. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999; 29:896–904.
56. Focke M, Marth K, Flicker S, et al. Heterogeneity of commercial grass-pollen extracts. Clin Exp Allergy 2008 (in press).
57. Valenta R, Vrtala S, Laffer S, et al. Recombinant allergens. Allergy 1998; 53:552–561.
58. Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen, Bet v 1, is highly homologous to a pea disease resistance response gene. EMBO J 1989; 8:1935–1938.
59. Valenta R, Duchene M, Pettenburger K, et al. Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. Science 1991; 253:557–560.
60. Seiberler S, Scheiner O, Kraft D, et al. Characterization of a birch pollen allergen, Bet v III, representing a novel class of Ca^{2+} binding proteins; specific expression in mature pollen and dependence of patients' IgE binding on protein-bound Ca^{2+}. EMBO J 1994; 13:3481–3486.
61. Twardosz A, Hayek B, Seiberler S, et al. Molecular characterization, expression in *Escherichia coli* and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. Biochem Biophys Res Commun 1997; 239:197–204.
62. Engel E, Richter K, Obermeyer G, et al. Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. J Biol Chem 1997; 272:8630–8637.
63. Hayek B, Vangelista L, Pastore A, et al. Molecular and immunological characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergens, Aln g 4. Structural basis for calcium-modulated IgE recognition. J Immunol 1998; 161:7031–7039.
64. Breiteneder H, Ferreira F, Reikerstorfer A, et al. cDNA cloning and expression in Escherichia coli of Aln g I, the major allergen in pollen of alder (*Alnus glutinosa*). J Allergy Clin Immunol 1992; 90:909–917.
65. Larson JN, Stroman P, Ipsen H. PCR based cloning and sequencing of isogenes encoding the tree pollen major allergen Car b I from *Carpinus betulus*, hornbeam. Mol Immunol 1992; 1992; 29:703–711.
66. Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, et al. Four recombinant isoforms of Cor a I, the major allergen of hazel pollen, show different IgE-binding properties. Eur J Biochem 1993; 212:355–362.
67. Villalba M, Batanero E, Monsalve RI, et al. Cloning and expression of Ole e 1, the major allergen from olive tree pollen. Polymorphism analysis and tissue specificity. J Biol Chem 1994; 269:15217–15222.
68. Asturias JA, Arilla MC, Gomez-Bayon N, et al. Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. J Allergy Clin Immunol 1997; 100:365–372.
69. Batanero E, Gonzalez de la Pena MA, Villalba M, et al. Isolation, cDNA cloning and expression of Lig v 1, the major allergen from privet pollen. Clin Exp Allergy 1996; 26:1401–1410.
70. Rodriguez R, Villalba M, Monsalve RI, et al. The spectrum of olive pollen allergens. Int Arch Allergy Immunol 2001; 125:185–195.
71. Sone T, Komiyama N, Shimizu K, et al. Cloning and sequencing of cDNA coding for Cry j I, a major allergen of Japanese cedar pollen. Biochem Biophys Res Commun 1994; 199:619–628.
72. Komiyama N, Sone T, Shimizu K, et al. cDNA cloning and expression of Cry j II the second major allergen of Japanese cedar pollen. Biochem Biophys Res Commun 1994; 201:1021–1028.
73. Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, et al. Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. Biochem Biophys Res Commun 1995; 214:538–551.
74. Breiteneder H, Hoffmann-Sommergruber K, O'Riordain G, et al. Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relation-ships to a group of 17 kDa tree pollen allergens. Eur J Biochem 1995; 233:484–489.
75. Scheurer S, Pastorello EA, Wangorsch A, et al. Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the in vitro diagnosis of cherry allergy. J Allergy Clin Immunol 2001; 107:724–731.
76. Karamloo F, Schmitz N, Scheurer S, et al. Molecular cloning and characterization of a birch pollen minor allergen, Bet v 5, belonging to a family of isoflavone reductase-related proteins. J Allergy Clin Immunol 1999; 104:991–999.

77. Karamloo F, Wangorsch A, Kasahara H, et al. Phenylcoumaran benzylic ether and isoflavonoid reductases are a new class of cross-reactive allergens in birch pollen, fruits and vegetables. Eur J Biochem 2001; 268:5310–5320.
78. Cadot P, Diaz JF, Proost P, et al. Purification and characterization of an 18-kd allergen of birch (*Betula verrucosa*) pollen: identification as a cyclophilin. J Allergy Clin Immunol 2000; 105:286–291.
79. Cadot P, Nelles L, Srahna M, et al. Cloning and expression of the cyclophilin Bet v 7, and analysis of immunological cross-reactivity among the cyclophilin A family. Mol Immunol 2006; 43:226–235.
80. Mahler V, Fischer S, Heiss S, et al. cDNA cloning and characterization of a cross-reactive birch pollen allergen: identification as a pectin esterase. Int Arch Allergy Immunol 2001; 124:64–66.
81. Gruehn S, Suphioglu C, O'hehir RE, et al. Molecular cloning and characterization of hazel pollen protein (70 kD) as a luminal binding protein (BiP): a novel cross-reactive plant allergen. Int Arch Allergy Immunol 2003; 131:91–100.
82. Kos T, Hoffmann-Sommergruber K, Ferreira F, et al. Purification, characterization and N-terminal amino acid sequence of a new major allergen from European chestnut pollen, Cas s 1. Biochem Biophys Res Commun 1993; 196:1086–1092.
83. Ipsen H, Hansen OC. The NH2-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) Aln g I, birch (*Betula verrucosa*) Bet v I, hornbeam (*Carpinus betulus*) Car b I and oak (*Quercus alba*) Que a I pollens. Mol Immunol 1991; 28:1279–1288.
84. Asturias JA, Ibarrola I, Eraso E, et al. The major *Platanus acerifolia* pollen allergen Pla a 1 has sequence homology to invertase inhibitors. Clin Exp Allergy 2003; 33:978–985.
85. Asturias JA, Ibarrola I, Bartolome B, et al. Purification and characterization of Pla a 1, a major allergen from *Platanus acerifolia* pollen. Allergy 2002; 57:221–227.
86. Ibarrola I, Arilla MC, Martinez A, et al. Identification of a polygalacturonase as a major allergen (Pla a 2) from *Platanus acerifolia* pollen. J Allergy Clin Immunol 2004; 113:1185–1191.
87. Lauer I, Miguel-Moncin MS, Abel T, et al. Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid-transfer protein, Pru p 3. Clin Exp Allergy 2007; 37:261–269.
88. Batanero E, Villalba M, Ledesma A, et al. Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. Eur J Biochem 1996; 241:772–778.
89. Ledesma A, Villalba M, Rodriguez R. Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of calcium-binding proteins (polcalcins) involved in allergy. Eur J Biochem 1998; 258:454–459.
90. Boluda L, Alonso C, Fernandez-Caldas E. Characterization of two new allergens of Olea europea, Ole e 4 and Ole e 5. Allergy 1997; (suppl):52–81.
91. Butteroni C, Afferni C, Barletta B, et al. Cloning and expression of the Olea europaea allergen Ole e 5, the pollen Cu/Zn superoxide dismutase. Int Arch Allergy Immunol 2005; 137:9–17.
92. Batanero E, Ledesma A, Villalba M, et al. Purification, amino acid sequence and immunological characterization of Ole e 6, a cysteine-enriched allergen from olive tree pollen. FEBS Lett 1997; 410:293–296.
93. Barral P, Tejera ML, Trevino MA, et al. Recombinant expression of Ole e 6, a Cys-enriched pollen allergen, in Pichia pastoris yeast: detection of partial oxidation of methionine by NMR. Protein Expr Purif 2004; 37:336–343.
94. Tejera ML, Villalba M, Batanero E, et al. Identification, isolation, and characterization of Ole e 7, a new allergen of olive tree pollen. J Allergy Clin Immunol 1999; 104:797–802.
95. Ledesma A, Villalba M, Rodriguez R. Cloning, expression and characterization of a novel four EF-hand Ca(2+)-binding protein from olive pollen with allergenic activity. FEBS Lett 2000; 466:192–196.
96. Huecas S, Villalba M, Rodriguez R. Ole e 9, a major olive pollen allergen is a 1,3-beta-glucanase. Isolation, characterization, amino acid sequence, and tissue specificity. J Biol Chem 2001; 276:27959–27066.
97. Palomares O, Villalba M, Rodriguez R. The C-terminal segment of the 1,3-beta-glucanase Ole e 9 from olive (Olea europaea) pollen is an independent domain with allergenic activity: expression in Pichia pastoris and characterization. Biochem J 2003; 369:593–601.
98. Barral P, Batanero E, Palomares O, et al. A major allergen from pollen defines a novel family of plant proteins and shows intra- and interspecies cross-reactivity. J Immunol 2004; 172:3644–3651.
99. Niederberger V, Purohit A, Oster JP, et al. The allergen profile of ash (*Fraxinus excelsior*) pollen: cross-reactivity with allergens from various plant species. Clin Exp Allergy 2002; 32:933–941.
100. Hemmer W, Focke M, Wantke F, et al. Ash (Fraxinus excelsior) pollen allergy in Central Europe: specific role of pollen pan allergens and the major allergen of ash pollen, Fra e 1. Allergy 2000; 55:923–930.
101. Barderas R, Purohit A, Papanikolaou I, et al. Cloning, expression, and clinical significance of the major allergen from ash pollen, Fra e 1. J Allergy Clin Immunol 2005; 115:351–357.
102. Batanero E, Villalba M, Lopez-Otin C, et al. Isolation and characterization of an olive allergen-like protein from lilac pollen. Sequence analysis of three cDNA encoding protein isoforms. Eur J Biochem 1994; 221:187–193.

103. Ledesma A, Barderas R, Westritschnig K, et al. A comparative analysis of the cross-reactivity in the polcalcin family including Syr v 3, a new member from lilac pollen. Allergy 2006; 61:477–484.
104. Calabozo B, Barber D, Polo F. Studies on the carbohydrate moiety of Pla l 1 allergen. identification of a major N-glycan and significance for the immunoglobulin E-binding activity. Clin Exp Allergy 2002; 32:1628–1634.
105. Taniai M, Ando S, Usui T, et al. N-terminal amino acid sequence of a major allergen of Japanese cedar pollen (Cry j I). FEBS Lett 1988; 239:329–332.
106. Griffith IJ, Lussier A, Garman R, et al. The cDNA cloning of Cry j I, the major allergen of Cryptomeria japonica (Japanese cedar). J Allergy Clin Immunol 1993; 91:339.
107. Sakaguchi M, Inouye S, Taniai M, et al. Identification of the second major allergen of Japanese cedar pollen. Allergy 1990; 45:309–312.
108. Tamura Y, Kawaguchi J, Serizawa N, et al. Analysis of sequential immunoglobulin E-binding epitope of Japanese cedar pollen allergen (Cry j 2) in humans, monkeys and mice. Clin Exp Allergy 2003; 33:211–217.
109. Futamura N, Mukai Y, Sakaguchi M, et al. Isolation and characterization of cDNAs that encode homologous of a pathogenesis-related protein allergen from *Cryptomeria japonica*. Biosci Biotechnol Biochem 2002; 66:2495–2500.
110. Fujimura T, Futamura N, Midoro-Horiuti T, et al. Isolation and characterization of native Cry j 3 from Japanese cedar (*Cryptomeria japonica*) pollen. Allergy 2007; 62:547–553.
111. Suzuki M, Komiyama N, Itoh M, et al. Purification, characterization and molecular cloning of Cha o 1, a major allergen of *Chamaecyparis obtusa* (Japanese cypress) Pollen Mol Immunol 1996; 33:451–460.
112. Mori T, Yokoyama M, Komiyama N, et al. Purification, identification and cDNA cloning of Cha o 2, the second major allergen of Japanese cypress pollen. Biochem Biophys Res Commun 1999; 263: 166–171.
113. Aceituno E, del Pozo V, Minguez A, et al. Molecular cloning of major allergen from *Cupressus arizonica* pollen: Cup a 1. Clin Exp Allergy 2000; 30:1750–1758.
114. Cortegano I, Civantos E, Aceituno E, et al. Cloning and expression of a major allergen from *Cupressus arizonica* pollen, Cup a 3, a PR-5 protein expressed under polluted environment. Allergy 2004; 59:485–490.
115. Togawa A, Panzani RC, Garza MA, et al. Identification of Italian cypress (*Cupressus sempervirens*) pollen allergen Cup s 3 using homology and cross-reactivity. Ann Allergy Asthma Immunol 2006; 97:336–342.
116. Liu D, Midor-Horiuti T, White MA, et al. Crystallization and preliminary X-ray diffraction analysis of Jun a 1, the major allergen isolated from pollen of the mountain cedar Juniperus ashei. Acta Crystallogr D Biol Crystallogr 2003; 59:1052–1054.
117. Midoro-Horiuti T, Goldblum RM, Kurosky A, et al. Molecular cloning of the mountain cedar (Juniperus ashei) pollen major allergen, Jun a 1. J Allergy Clin Immunol 1999; 104:613–617.
118. Yokoyama M, Miyahara M, Shimizu K, et al. Purification, identification, and cDNA cloning of Jun a 2, the second major allergen of mountain cedar pollen. Biochem Biophys Res Commun 2000; 275:195–202.
119. Midoro-Horiuti T, Goldblum RM, Kurosky A, et al. Variable expression of pathogenesis-related protein allergen in mountain cedar (Juniperus ashei) pollen. J Immunol 2000; 164:2188–2192.
120. Tinghino R, Barletta B, Palumbo S, et al. Molecular characterization of a cross-reactive Juniperus oxycedrus pollen allergen, Jun o 2: a novel calcium-binding allergen J Allergy Clin Immunol 1998; 101:772–777.
121. Gross GN, Zimburean JM, Capra JD. Isolation and partial characterization of the allergen in mountain cedar pollen. Scand J Immunol 1978; 8:437–441.
122. Midoro-Horiuti T, Goldblum RM, Brooks EG. Identification of mutations in the genes for the pollen allergens of eastern red cedar (*Juniperus virginiana*). Clin Exp Allergy 2001; 31:771–778.
123. Mothes N, Horak F, Valenta R. Transition from a botanical to a molecular classification in tree pollen allergy: implications for diagnosis and therapy. Int Arch Allergy Immunol 2004; 135:357–373.
124. Di Felice G, Barletta B, Tinghino R, et al. Cupressaceae pollinosis: identification, purification and cloning of relevant allergens. Int Arch Allergy Immunol 2001; 125:280–289.
125. Roux KH, Teuber SS, Sathe SK. Tree nut allergens. Int Arch Allergy Immunol 2003; 131:234–244.
126. Mari A. Importance of databases in experimental and clinical allergology. Int Arch Allergy Immunol 2005; 138:88–96.
127. Valenta R, Kraft D. From allergen structure to new forms of allergen-specific immunotherapy. Curr Opin Immunol 2002; 14:718–727.
128. Asturias JA, Ibarrola I, Amat P, et al. Purified allergens vs. complete extract in the diagnosis of plane tree pollen allergy. Clin Exp Allergy 2006; 36:1505–1512.
129. Hoffmann-Sommergruber K. Plant allergens and pathogenesis-related proteins. What do they have in common? Int Arch Allergy Immunol 2000; 122:155–166.

130. Bufe A, Spangfort MD, Kahlert H, et al. The major birch pollen allergen, Bet v 1, shows ribonuclease activity. Planta 1996; 199:413–415.
131. Markovic-Housley Z, Degano M, Lamba D, et al. Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. J Mol Biol 2003; 325:123–133.
132. Mogensen JE, Wimmer R, Larsen JN, et al. The major birch allergen, Bet v 1, shows affinity for a broad spectrum of physiological ligands. J Biol Chem 2002; 277:23684–23592.
133. Ferreira F, Hirtenlehner K, Jilek A, et al. Dissection of immunglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. J Exp Med 1996; 183:599–609.
134. Laffer S, Hamdi S, Lupinek C, et al. Molecular characterization of recombinant T1, a non-allergenic periwinkle (*Catharanthus roseus*) protein, with sequence similarity to the Bet v 1 plant allergen family. Biochem J 2003; 373:261–269.
135. Spök A, Gaugitsch H, Laffer S, et al. Suggestions for the assessment of the allergenic potential of genetically modified organisms. Int Arch Allergy Immunol 2005; 137:167–180.
136. Hoffmann-Sommergruber K, O'Riordain G, Ahorn H, et al. Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. Clin Exp Allergy 1999; 840–847.
137. Valenta R, Ferreira F, Grote M, et al. Identification of profilin as an actin-binding protein in higher plants. J Biol Chem 1993; 268:22777–22781.
138. van Ree R, Voitenko V, van Leeuwen WA, et al. Profilin is a cross-reactive allergen in pollen and vegetable foods. Int Arch Allergy Immunol 1992; 98:97–104.
139. Vallier P, DeChamp C, Valenta R, et al. Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. Clin Exp Allergy 1992; 22:774–782.
140. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992; 175:377–385.
141. Staiger CJ, Gibbon, Kovar DR, et al. Profilin and actin-depolymerizing factor: modulators of actin organization in plants. Trends Plant Sci 1997; 2:275–281.
142. Valenta R, Hayek B, Seiberler S, et al. Calcium-binding allergens: from plants to man. Int Arch Allergy Immunol 1998; 117:160–166.
143. Tinghino R, Twardosz A, Barletta B, et al. Molecular, structural, and immunologic relationships between different families of recombinant calcium-binding pollen allergens. J Allergy Clin Immunol 2002; 109:314–320.
144. Valenta R, Twardosz A, Swoboda I, et al. Calcium-binding proteins in type I allergy: elicitors and vaccines. In: Pochet R, Donato R, Haiech J, et al., eds. Calcium: The Molecular Basis of Calcium Action in Biology and Medicine. Netherlands: Kluwer Academic Publishers, 2000:365–377.
145. Verdino P, Westritschnig K, Valenta R, et al. The cross-reactive calcium-binding pollen allergen, Phl p 7, reveals a novel dimer assembly. EMBO J 2002; 21:5007–5016.
146. Rafnar T, Friffith IJ, Kuo M, et al. Cloning of Amb a I (antigen E), the major allergen family of short ragweed pollen. J Biol Chem 1995; 95:970–978.
147. Swoboda I, Grote M, Verdino P, et al. Molecular characterization of polygalacturonases as grass pollen-specific marker allergens: expulsion from pollen via submicronic respirable particles. J Immunol 2004; 172:6490–6500.
148. Vrtala S, Grote M, Duchene M, et al. Properties of tree and grass pollen allergens; reinvestigation of the linkage between solubility and allergenicity. Int Arch Allergy Immunol 1993; 102:160–169.
149. Valenta R, Kraft D. Recombinant allergen molecules: tools to study effector cell activation. Immunol Rev 2001; 179:119–127.
150. Asero R. Fennel, cucumber, and melone allergy successfully treated with pollen-specific injection immunotherapy. Ann Allergy Asthma Immunol 2000; 84:460–462.
151. Niederberger V, Horak F, Vrtala S, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. Proc Natl Acad Sci U S A 2004; 101:14677–14682.
152. Niederberger V, Reisinger J, Valent P, et al. Vaccination with genetically modified birch pollen allergens: immune and clinical effects on oral allergy syndrome. J Allergy Clin Immunol 2007; 119:1013–1016.
153. Kazemi-Shirazi L, Niederberger V, Linhart B, et al. Recombinant marker allergens: diagnostic gate keepers for therapy of allergy. Int Arch Allergy Immunol 2002; 127:259–268.
154. Palomares O, Swoboda I, Villalba M, et al. The major allergen of olive pollen Ole e 1 is a diagnostic marker for sensitization to Oleaceae. Int Arch Allergy Immunol 2006; 141:110–118.
155. Bousquet J, Becker WM, Hejjaoui A, et al. Differences in clinical and immunologic reactivity of patients allergic to grass pollens and to multiple-pollen species. II. Efficacy of a double-blind, placebo-controlled, specific immunotherapy with standardized extracts. J Allergy Clin Immunol 1991; 88:43–53.

156. Hiller R, Laffer S, Harwanegg C, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J 2002; 16:414–416.
157. Suck R, Nandy A, Weber B, et al. Rapid method for arrayed investigation of IgE-reactivity profiles using natural and recombinant allergens. Allergy 2002; 57:821–824.
158. Harwanegg C, Laffer S, Hiller R, et al. Microarrayed recombinant allergens for diagnosis of allergy. Clin Exp Allergy 2003; 33:7–13.
159. Laffer S, Spitzauer S, Susani M, et al. Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. J Allergy Clin Immunol 1996; 98:652–658.
160. Niederberger V, Niggemann B, Kraft D, et al. Evolution of IgM, IgE and IgG(1-4) antibody responses in early childhood monitored with recombinant allergen components: implications for class switch mechanisms. Eur J Immunol 2002; 32:576–584.
161. Ball T, Fuchs T, Sperr WR, et al. B cell epitopes of the major timothy grass pollen allergen, Phl p 1, revealed by gene fragmentation as candidates for immunotherapy. FASEB J 1999; 13:1277–1290.
162. Ball T, Sperr WR, Valent P, et al. Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. Eur J Immunol 1999; 29:2026–2036.
163. Moverare R, Elfman L, Vesterinen E, et al. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. Allergy 2002; 57:423–430.
164. Mothes N, Heinzkill M, Drachenberg KJ, et al. Allergen-specific immunotherapy with a Monophosphoryl Lipid A-adjuvanted vaccine: reduced seasonally boosted IgE production and inhibition of basophil histamine release by therapy-induced blocking antibodies. Clin Exp Allergy 2003; 33:1–11.
165. van Hage-Hamsten M, Valenta R. Specific immunotherapy—the induction of new IgE-specificities? Allergy 2002; 57:375–358.
166. Valenta R. The future of antigen-specific immunotherapy of allergy. Nat Rev Immunol 2002; 2:446–453.
167. Larché M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol 2006; 6:761–771.
168. Linhart B, Valenta R. Molecular design of allergy vaccines. Curr Opin Immunol 2005; 17:646–655.
169. Gafvelin G, Thunberg S, Kronqvist M, et al. Cytokine and antibody responses in birch-pollen-allergic patients treated with genetically modified derivatives of the major birch pollen allergen Bet v 1. Int Arch Allergy Immunol 2005; 138:59–66.
170. Reisinger J, Horak F, Pauli G, et al. Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. J Allergy Clin Immunol 2005; 116:347–354.
171. Pree I, Reisinger J, Focke M, et al. Analysis of epitope-specific immune responses induced by vaccination with structurally folded and unfolded recombinant Bet v 1 allergen derivatives in man. J Immunol 2007; 179:5309–5316.
172. Watson L, Dallwitz MJ. The families of flowering plants. Descriptions, Illustrations, Identification, and Information Retrieval (1992 Onwards). Version: 1st June 2007. Available at: www.delta-intkey.com.

7 Grass Pollen Allergens

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INTRODUCTION

Grass pollens represent a major component of the airborne allergen load during the spring and summer months in most parts of the world. They are responsible for the symptoms in the majority of allergic rhinitis patients and can also trigger asthma. The diagnosis and treatment of grass pollen allergy with grass pollen allergen extracts/vaccines is nearly a hundred years old and their use for immunotherapy is unequaled by any other allergen vaccine. Since Charles Blackley's initial investigations (1) during the 1870s that led to the identification of grass pollen as the cause of his own illness, the study of grass pollen allergens has continued to fascinate botanists, allergists/immunologists and more recently, molecular biologists. In this chapter, the grass family (Poaceae), their ecology, and pollen allergens will be described. Special attention will be given to the molecular characteristics of grass pollen allergens with regard to their cross-reactivities.

Classification and Taxonomy

The grasses belong to the family Poaceae (Gramineae) and are grouped with the sedges, rushes, and other monocots belonging to the order Poales. The family Poaceae is the fourth largest family of flowering plants with more than 600 genera and 10,000 species. The family has historically been divided into two major groups, the pooids and the panicoids, on the basis of the structure of the spikelet, the basic unit of inflorescence (2). The pollen antigens of the pooids and panicoids are immunochemically distinct, as are other characteristics including leaf anatomy, embryo anatomy, and karyotype. These and additional morphological, physiological, biochemical, and cytological comparisons have led to the recognition of up to nine subfamilies and as many as 60 tribes. Most agrostologists today recognize five or six subfamilies although some recognize up to sixteen (3). A taxonomic grouping of common grass genera is presented in Table 1. The classification system is on the basis of that of Watson and Dallwitz (4) with minor modifications. Over 95% of the allergenically important grass species belong to the three subfamilies Pooideae, Chloridoideae and Panicoideae.

THE GRASS FLOWER AND POLLEN

Flowers of the allergenic grasses have obvious characteristics for wind pollination: reduced perianth, small and smooth pollen grains, high pollen-ovule ratio, and feathery stigmas. The flower head, known as the inflorescence (Fig. 1), is made up of spikelets that are highly modified branches consisting of a pair of bracts called glumes. They protect the immature spikelet and a rachilla, on which are borne one to several florets. There is a wide variation in spikelet structure, size and shape, and this is of great value in identification and classification of grasses.

Pollination in grasses is of short duration, and it regularly occurs at a certain time of day or night. The breeding systems of the grasses are extremely varied. Some grasses are cleistogamous (self-fertile) or entomophilous (insect pollinated) and therefore arenot allergenically important. Polyploidy is common among the grasses, and hybridization is known to contribute to the adaptation and evolution of many grass groups, especially among the tribe Triticeae, the cereal grasses.

The pollen structure is unique to the family, but they are too uniform to be useful taxonomically (Fig. 2). The pollen is more or less spheroidal to ovoid, 20 to 55 μm in diameter.

Table 1 Taxonomic Relationships Between Common Grasses

Subfamily	Tribe	Genus and species	Common name
Bambusoideae	Oryzeae	*Oryza sativa*	Cultivated rice
		Zizania aquatica	Wild rice
		Ehrharta erecta	Panic, veldt grass
Arundinoideae	Arundineae	*Phragmites communis*	Common reed
		Cortoderia	Pampas grass
	Aristideae	*Aristida* spp.	Three-awns
	Stipeae	*Stipa* spp.	Needlegrass
Panicoideae	Paniceae	*Digitaria sanguinalis*	Crabgrass
		Paspalum notatum	Bahia grass
		Panicum miliaceum	Common millet
		Panicum virgatum	Switch grass
		Stentaphrum secundatum	Buffalo grass, Saint Augustine grass
	Andropogoneae	*Eremochloa ophiuroides*	Centipede grass
		Saccharum officinarum	Sugar cane
		Sorghum halepense	Johnson grass
		Sorghum sudanense	Sudan grass
		Zea mays	Corn, maize
Chloridoideae	Chlorideae	*Bouteloua* spp.	Grama grass
		Buchloë dactyloides	Buffalo grass
		Choris spp.	Finger grass
		Cynodon dactylon	Bermuda, couch grass
	Aeluropodeae	*Distichlis spicata*	Salt grass
	Eragrosteae	*Eragrostis* spp.	Love grass
		Eleusine indica	Goose grass
		Tridens flavus	Purpletop
Pooideae	Poaceae	*Bromus inermis*	Smooth brome
		Dactylis glomerata	Orchard grass, cocksfoot
		Festuca elatior	Meadow fescue
		Lolium multiforme	Italian rye
		Lolium perenne	Perennial rye
		Poa compressa	Canada bluegrass
		Poa pratensis	Kentucky bluegrass (June grass)
	Avenae (including Agrostideae and Phalarideae)	*Agrostis alba*	Redtop, bent grass
		Anthoxanthum odoratum	Sweet vernal
		Avena sativa	Cultivated oat
		Holcus lanatus	Velvet grass
		Koeleria cristata	June grass
		Phalaris arundinacea	Reed canary
		Phalaris canariensis	Canary
		Phleum pratense	Timothy grass
	Triticeae	*Agropyron repens*	Quack, wheat grass
		Elymus spp.	Wild rye
		Hordeum vulgare	Barley
		Secale cereale	Cultivated rye
		Triticum aestivum	Wheat

The pollen grain wall consists of two layers, the exine (outer wall) and the intine (inner wall), and a single germination aperture or pore. Pollen antigens are stored in both the exine and intine walls, most being localized in the intine. A wide range of pollen antigens, including those that are allergenic, undoubtedly have a major role in the recognition of a suitable reproductive partner and thus may be expected to be species specific. Many grass pollen antigens also have wide taxonomic spans. On moistening, exine- and intine-associated components are released into the medium (Fig. 3). The kinetics of antigen release from grass pollen suggests minimal structural compartmentalization as compared to pollen derived from other plant families (5).

Variations in a patient's allergic symptoms during the year depend, in part, on the pattern of seasonal pollen exposure. The expected seasonal levels of grass pollen for a given

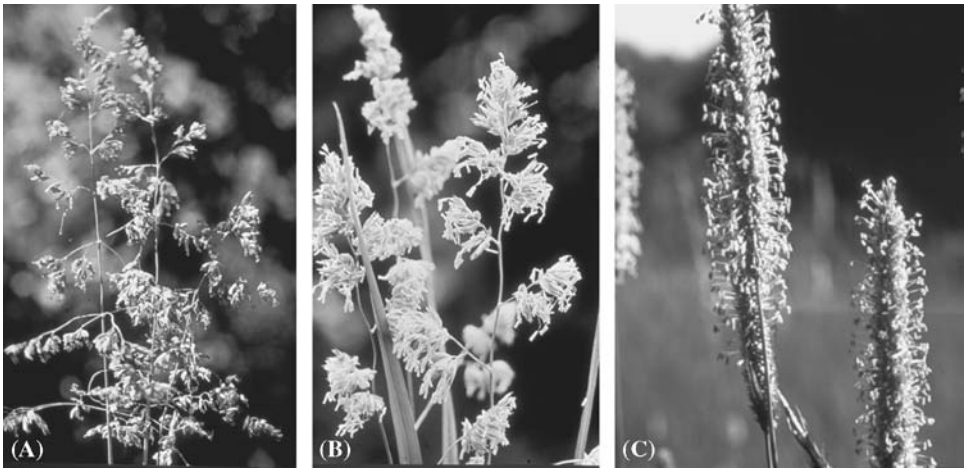

Figure 1 Grass Inflorescence, the arrangement of the flowers on the stem, is illustrated by the three pooids (**A**) Kentucky bluegrass with panicles, a compound inflorescence, bearing flowers along slender, spreading branches; (**B**) orchard grass with panicles bearing clusters of flowers near the ends of stout branches; and (**C**) timothy grass with spikes, or cylindrical clusters of flowers with no stalks.

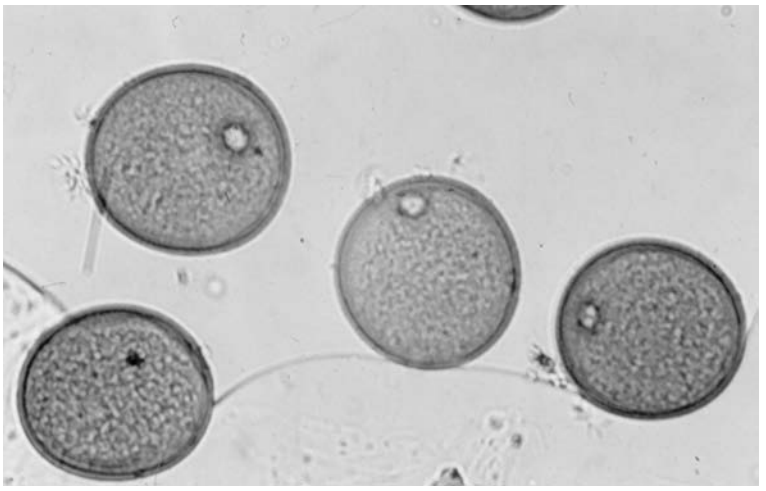

Figure 2 The pollen grains of the grasses are remarkably uniform. They are spheroidal, and in most allergenic species they range from about 20 μm to less than 50 μm in diameter. The exine is thin and has a characteristically granular texture without adornments of any kind. The most distinctive characteristic is the single germ pore, consisting of a small aperture surrounded by a thickened rim of the exine and covered by a transparent membrane.

geographic locality in the U.S. can be obtained from various sources including the American Academy of Allergy Asthma and Immunology's (AAAAI) Aerobiology Committee's Annual Pollen and Spore Reports (6). Grass pollen are most abundant during the spring and summer months and account for a significant portion of the total pollen count during this time. Because whole pollen grains are too large to be respirable, it has been difficult to explain how grass pollen provoke asthmatic symptoms. Several possibilities, including the presence of submicronic particles possessing allergenic activity, have been suggested as the trigger of asthma attacks. The existence of such particles has been confirmed by specialized airborne sampling and immunochemical detection methods (7,8) and has been shown to correlate to weather (e.g., thunderstorms) and epidemics of asthmas (9). A primary source of such particles has been identified as starch granules (0.6–2.5 μm in diameter) that are released from grass pollen on contact with moisture. Other sources, including pollen fragments (10,11), orbicules (12), and allergen-adsorbed aerosols, remain to be investigated.

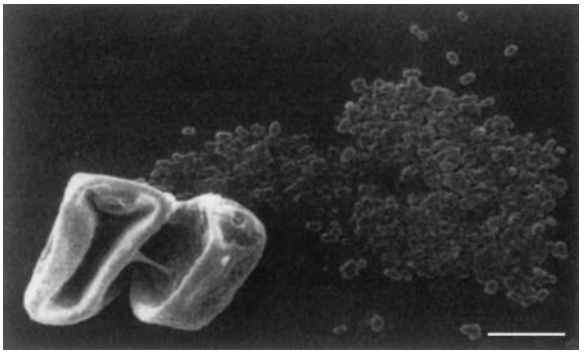

Figure 3 Ryegrass pollen ruptures after slight wetting with sedimenting mist droplets. The cytoplasmic debris from the ruptured pollen forms an aerosol of respirable particles that are loaded with allergens. *Source*: From Ref. 10.

ECOLOGY AND HABITAT

Grasses occur on all continents, from desert to polar regions and in freshwater to marine habitats and account for about 25% to 35% of the earth's vegetation. The steppes of Eurasia, the prairies and plains of central and western North America, and the Pampa of Argentina represent the most extensive grassland areas of the temperate zone. Less extensive grasslands are found in the velds of South Africa and in Australia and New Zealand. Tropical and subtropical grasslands are located in central Africa and in central South America. In the grasslands, drought, fire and grazing by animals are the major ecological challenges for a plant's survival. The growth tissue in most plants is located at the tip of the leaf or shoot and once clipped, it will not grow back. In contrast, the growth tissue in the grasses is located near the base of the leaf or the shoot, and growth continues even after the grass plant is cropped, burned, or grazed. This and other distinctive features including basal tillering, protection of the flower and fruit within the spikelet, a great diversity of habitats, alternative photosynthetic pathways, breeding systems, and dispersal mechanisms allow them to survive and dominate in areas where other plants cannot.

The distribution of grass species are delimited by conditions of soil, moisture, temperature, exposure, and altitude. Some species are restricted in habitat, being found only in salt marshes or alpine summits. Their geographical range, however, may be extensive. A species found on one mountain range may also be found at the same altitude on another mountain range. Other more tolerant species, such as Festuca rubra, can be found in meadows, bogs, marshes and hills of North America, Eurasia, and North Africa.

Seventy percent of the world's farmland is planted in crop grasses with sugar cane (Saccharum officinarum), wheat (Triticum aestivum), rice (Oryza sativa), and maize (Zea mays) being the most widely cultivated. Bamboos are a critical part of the economy of many tropical areas because they contribute young shoots for food, fiber for paper, and stems for construction. Grasses are cultivated for livestock feed, erosion control and as ornamentals. Many grasses introduced into cultivation escape and become established over wide areas. Their seeds may be carried long distances in cattle cars as impurities in the seed of crop plants and by birds and insects. Often, they may become troublesome weeds. The turfgrasses are planted to cover lawns, parks, roadsides, cemeteries, golf courses, and sporting fields. Considerable energy is spent maintaining turfgrasses in areas where they would normally not survive. The lawn industry, which accounts for more than a billion dollars of sales of seed, fertilizers, chemicals, paraphernalia, and services, supports the maintenance of grasses in regions that would otherwise be deciduous forests and deserts. Of the hundreds of genera of grasses recognized, only a few are known to cause allergic disease. The major grass species responsible for inducing allergic symptoms are usually those that are cultivated and, therefore, are prevalent where people live (Table 1).

Bambusoideae

The Bambusoideae are the most primitive extant grasses and are associated with forest and aquatic habitats. Bamboos are distributed on the continents of Asia, Africa, North and South America. They are the least understood in terms of their classification and evolution among the

grasses. Bamboos are not allergenically important due to the infrequency of flowering, with up to 120 years elapsing between pollination in many species. The woody bamboos inhabit the tropical regions as well as temperate regions in Asia. The most primitive grasses are represented among the herbaceous bamboos that inhabit the tropical rain forests. Due to the relative lack of wind in their habitats, they have developed animal pollination. The more advanced climbing and herbacious bamboos have adapted to colder climates of the Himalaya and Andes mountains. The only native bamboos in North America are the two species of Arundinaria tecta and A. gigantea (giant or switch cane) that grow in moist ground from southern Maryland and Ohio to Florida and Texas. The rices, a herbaceous and mainly aquatic group, are in the Bambusoideae subfamily on the basis of their leaf blade anatomy and the presence of six stamens. The Asian species Oryza sativa and the wild rice of North America, Zinania aquatica, are the best known species.

Arundineae

The Arundineae are thought to represent the direct descendants of the earliest grasses, which moved into the open savanna ecosystem. This subfamily is a heterogeneous group of unrelated genera and tribes that do not fit into the other relatively well-defined subfamilies. As a group, they are distributed mainly in the tropical and temperate regions of the southern hemisphere. Of the some 75 genera represented in the subfamily, only about five are native in North America. This group includes giant reed (Arundo) and the common reed (Phragmites communis), which are frequently planted to control erosion. The female plants of the South American pampasgrass (Cortaderia), with their large, plumose panicles, are commonly grown as ornamentals in warmer regions of the world. The some 250 species of Aristida (three-awns), having adapted to the semiarid habitats of South Africa and northern Mexico, are one of the more successful genera of this subfamily.

Pooideae

The temperate zones are dominated by grasses belonging to the subfamily Pooideae. The major tribes, consisting of about 155 genera, are distributed across the world in relatively well-defined latitudinal belts with the majority of genera found in the northern hemisphere. The center of pooid distribution is the Mediterranean area, and they have adapted to cool and cold climates of the open steppe or meadows. They are virtually absent at low elevations in both humid and dry tropical areas. Species of Bromus, Poa, Festuca, and Agropyron can be found only at high altitudes in mountainous regions of tropical latitudes. The pooids account for approximately 70% to 85% of the grasses in Canada and northwestern United States, 40% to 50% in the middle latitudes, and less 15% to 25% in the southern United States. The cool-season turfgrasses representing this subfamily include the genera Poa (bluegrasses), Agrostis (bent grasses), Festuca (fescues) and Lolium (ryegrasses). These represent the major allergenic grass genera along with Dactylis glomerata (orchard grass), Phleum pratense (timothy grass), and Anthoxanthum odoratum (vernal grass), which are common in meadows, pastures, and waste places. The subfamily also includes the important cultivated cereals Triticum aestivum (wheat), Secale cereale (rye), and Hordeum vulgare (barley).

Chloridoideae

The members of the subfamily Chloridoideae are well distributed over the North American, African and Australian continents. The chloridoids have adapted to a wide range of ecotypes, especially the warm and arid habitats, with high winter temperatures and summer or nonseasonal rainfall. Over 50% of the grass species in the southwestern United States are chloridoid, compared with less than 10% of the total in the northwestern United States. The centers of distribution are in the savannas of southern Africa and in the open grasslands of Queensland. Their success in the warm, arid environments is due to the distinct physiological and anatomical features of their C_4 dicarboxylic acid pathway of photosynthesis, referred to as the Kranz syndrome. The popular southern turf grass Cynodon dacylon (Bermuda grass) is widespread throughout the warmer regions of the world and is a major allergenic species. Several species of Bouteloua (grama grass) and Buchloë (buffalo grass) are the outstanding range forage grasses and occur widely in the central and western United States.

Panicoideae

The subfamily Panicoideae dominates the humid, tropical to subtropical environments of the savannas of Indochina and Africa as well as the moist New World tropics, especially northeastern South America. Over 75% of the grasses in the Panama Canal Zone are panicoid, 50% in the southern United States, but only about 5% of the species belong to this subfamily in the northwestern United States. The subfamily includes the largest of the grass genera, Panicum, with about 600 species distributed throughout the warmer parts of the world and the cultivated species Saccharum officinarum (sugar cane) and Sorghum vulgare (sorghum). Allergenically important species include Paspalum notatum (Bahia grass), an important forage and erosion control grass in the Gulf Coast states of the United States, and Sorghum halepense (Johnson grass), a forage grass and frequently a troublesome weed in the warmer and tropical regions of both hemispheres.

MOLECULAR CHARACTERISTICS AND CROSS-REACTIVITIES OF GRASS POLLEN ALLERGENS

Since the pioneering work of David Marsh and coworkers (13–15) with the perennial ryegrass groups 1, 2, and 3 allergens during the 1960s and 1970s, a number of new allergens have been identified, isolated, and characterized. The International Union of Immunological Societies (IUIS) allergen nomenclature subcommittee's current official list identifies 13 grass pollen allergen groups (Table 2). The techniques of molecular biology and protein chemistry have contributed to the increased knowledge regarding the structure and possible function of grass pollen allergens. Murine monoclonal antibodies raised against specific allergens have been used to define allergenically important and cross-reactive B-cell epitopes as well as to develop specific assays for their detection and quantitation in allergen extracts. Cloning of cDNA and nucleic acid sequencing has accelerated the availability of primary structure data. Recombinant allergen fragments, mutated recombinant proteins, and synthetic peptides have been useful in delineating determinants involved in B- and T-cell recognition. High resolution protein separation and immunoblotting techniques and more recently, the

Table 2 Grass Pollen Allergen Groups

Allergen group	Biochemical name/characteristics	IgE reactivity
1	β-expansins; 27 to 35 kDa major grass pollen allergen produced by every grass species examined so far.	>90%
2	Acidic protein (11 kDa); highly homologous to group 3 and C-terminal portion of group 1 allergens	35% to 50%
3	Basic protein (11–14 kDa); highly homologous to group 2 and C-terminal portion of group 1 allergens	35% to 70%
4	High molecular weight (50–60 kDa) basic glycoprotein; member of the berberine bridge enzyme family, plant pathogen response system.	50% to 75%
5/9	Heterogeneous proteins (27–35 kDa) found in pooid grass species; ribonuclease activity; associated with submicronic cytoplasmic starch particles.	65% to 85%
6	Phl p 6 (12–13 kDa), associated with submicronic cytoplasmic P-particles; homologous to internal Phl p 5 sequences	60 to 70
7	Calcium-binding protein (8–12 kDa) with novel dimer assembly; cross-reactive with birch (Bet v 4), olive (Ole e 3) and rape (Bra r 1) pollen allergens.	10% to 35%
10	Cytochrome c (11 kDa)	<20%
11	Glycoprotein (16–20 kDa); trypsin inhibitor; structurally similar to pollen allergens from olive tree (Ole e 1) and lamb's quarter (Che a 1).	35%
12	Profilin (13–14 kDa); possible association with pollen-plant food cross-sensitization.	20%–50%
13	High molecular weight glycoprotein (45–60 kDa); polygalacturonase; highly susceptible to protease degradation; associated with submicronic cytoplasmic P-particles	30% to 40%
22	Cyn d 22; enolase (42 kDa)	Not determined
24	Cyn d 24; pathogenesis-related protein (21 kDa)	65%

application of proteomic approaches have led to the identification of new allergen groups and the detection of microheterogeneity (isoallergens or isoforms) within the grass allergen groups.

Group 1 Antigens

The grass group 1 allergens are acidic or basic glycoproteins with molecular weights (MW) in the 27 to 35 kDa range and exist in multiple isoallergenic forms or isoforms distinguished by their respective isoelectric points (pIs) and amino acid sequences. Histochemical examination has localized this glycoprotein in the exine and cytoplasm of the pollen grain (16). The protein can account for 6% to 8% of the total extractable pollen protein and has been detected in every grass species examined to date. The complete group 1 amino acid sequences from perennial ryegrass (Lol p 1), timothy grass (Phl p 1), velvet grass (Hol l 1), corn (Zea m 1), Johnson grass (Sor h 1), and Bermuda grass (Cyn d 1) have been determined and the sequences of internal peptide fragments or N-terminal sequences from other several other grass species have been reported (17–21). The degree of glycosylation varies between 2% and 7% and the monosaccharides fucose, arabinose, xylose, mannose and *N*-acetylglucosamine have been detected. The carbohydrate moiety does not appear to play an important role in the allergenicity of the group 1 allergens although IgE antibodies toward the carbohydrate structures have been detected in a select group of subjects (22,23).

The group 1 allergens belong to a subfamily of structurally related proteins called β-expansins, which are cell wall loosening proteins. Their activity shows specificity to grass cell walls, suggesting that they act on the matrix polymers specific to grasses, for example, glucuronoarabinoxylan or mixed-linked 1,3: 1:4-β-glucan (24). The expansin activity of grass group 1 allergens has been attributed to a papain-like proteinase activity (25–27), but this proposed mechanism of action has been challenged (28). The characteristics of Zea m 1 suggest a specialized role of β-expansins in pollen function (29,30). A function of group 1 allergens is to facilitate growth of the pollen tube and penetration of the ovule to achieve fertilization and thus, breeding success under conditions of pollen competition (31). Structural modeling of the presumed cellulose-binding domain of Lol p 1 suggests a close structural relationship between its cellulose-binding and allergenic properties (32).

Allergens homologous to Lol p 1 have been detected in pollen extracts from all grass species examined to date and in each case greater than 90% of allergic subjects were highly reactive to the respective group 1 allergens. Patient sensitivity to the group 1 allergens has been found to correlate with their sensitivity to the whole pollen extract as measured by both skin test and histamine release assays. Extensive immunological cross-reactivity among the group 1 allergens from taxonomically related grasses is also firmly established. For these reasons, a potency assay on the basis of the group 1 content of grass pollen extracts has been proposed as an approach to grass pollen allergen extract standardization (33).

Two important cross-reactive allergenic determinants or sites have been localized on the pooid grass group 1 allergen molecule with the aid of murine monoclonal antibodies that were selected for their ability to inhibit human IgE binding to grass group 1 allergens. One site has been localized on a 28-mer located at the C-terminus (34) (amino acid residues 213–240) of the molecule and the second site has been localized within amino acid residues 23–35 (35). Continuous B-cell epitopes on Phl p 1 that represent five major IgE-reactive regions of the allergen molecule have been identified by a gene fragmentation approach. The IgE-binding fragments, generated from a random fragment expression library of Phl p 1 (36) and Hol l 1 (20) cDNA, represented regions localized at C-terminus, N-terminus, and in the center of the allergen sequence. The C-terminal portion of the molecule accounts for the majority of the IgE-binding sites of Phl p 1 and they cluster in a sterically oriented manner that may facilitate the cross-linking of effector cell-bound IgE antibodies (37). The importance of correct folding and a stable secondary structure for its allergenic activity is demonstrated by the difficulty to express active, soluble grass group 1 allergens as recombinant molecules. This is not unexpected considering that group 1 proteins possess seven cysteine residues and three disulfide bridges. The replacement of a single cysteine residue at position 77 of Lol p 1 with a serine residues using site-directed mutagenesis was sufficient to reduce the IgE-binding activity by more than 60% (38). Insect-cell expressed Phl p 1 (39) and purified natural Dac g 1 (40) have been shown to possess superior allergenic activity as compared to recombinant molecules expressed in bacteria. A recombinant variant of Phl p 1 introducing a cysteine residue to promote the formation of an additional disulfide bridge yielded a product that was stable, soluble, and

comparable in activity to native Phl p 1 (41). The allergenic epitopes of the group 1 allergen of Bermuda grass (Cyn d 1) appear to be different from those defined for the pooid grasses (42) in spite of a 70% to 75% sequence homology. Nonconservative amino acid substitutions in allergenically important regions may explain the lack of cross-reactivity between Cyn d 1 and the other grass group 1 allergens (19).

A major human T cell determinant has been localized within amino acid residues 191 to 210 utilizing overlapping peptides spanning the entire Lol p 1 molecule (17). Subsequent studies revealed multiple T-cell determinants distributed throughout the molecule including cross-reactive T-cell epitopes shared with grass group 2, group 3, and group 5 allergens (43–47). An immunodominant epitope within residues 126 to 134 was identified in a study employing class II Major Histocompatibility Complex (MHC) tetramers in grass pollen-allergic individuals (48). DR*0401 tetramers loaded with this peptide consistently detected CD4+ T cells expressing a Th2 cytokine profile in allergic but not nonallergic subjects. Nonallergic subjects exhibited very low frequency of such tetramer-specific cells even after allergen stimulation and cell expansion, suggesting a fundamental difference in T-cell responses to group 1 allergens in allergic and nonallergic individuals.

Group 2 Antigens

The grass group 2 allergens are acidic proteins (MW = 11,000) toward which 35 to 50% of grass-allergic subjects are sensitive. The perennial ryegrass group 2 antigens exist in at least 2 immunochemically indistinguishable isoforms, Lol p 2A (pI = 5.0) and Lol P 2B (pI = 5.1–5.3). The complete primary structure of Lol p 2A was determined by peptide sequencing of the purified protein and found to contain 97 amino acids without evidence of glycosylation sites (47). The complete primary structures of Lol p 2, Dac g 2, and Phl p 2 was deduced from the nucleotide sequence of the cDNA encoding the protein and was found homologous to the Lol p 2 sequence (49–51).

Mapping of IgE-reactive epitopes was attempted using a model on the basis of the solution structure of Phl p 2 and recombinant Phl p 2 fragments spanning the entire molecule (52). Only relatively long fragments representing the N-terminal and C-terminal regions of the molecule showed strong IgE reactivity. No reactivity could be detected when synthetic dodecapeptides spanning the complete Ph1 p 2 sequence were evaluated. These results indicate that grass group 2 IgE epitopes are highly conformation dependent.

The amino acid sequences of group 2 and 3 allergens are also highly homologous. Lol p 2A and Lol p 3 possess 59% identical amino acids and this percentage increased to 67% when similar amino acids are equated. A similar homology was found for Dac g 2 and Dac g 3 with 66% sequence identity and 79% sequence similarity. This sequence homology translates to a high degree of cross-reactivity at the B- and T-cell levels (43,53). The grass group 2 and group 3 allergens also show sequence similarity with the C-terminal portion of the group 1 allergens, although the clinical significance of this has not been thoroughly investigated. It has been suggested that the three allergen groups may share a common origin, with groups 2 and 3 being generated by partial gene duplication of the C-terminal portion of group 1 (54).

Group 3 Antigens

The grass group 3 allergens are basic proteins (MW = 11–14 kDa, pI = 8.9–9.4) with a reported frequency of sensitization of 35% to 70% among grass pollen-allergic subjects. The group 3 allergens from perennial ryegrass, orchard grass and timothy grass pollen have been isolated and characterized at the molecular level (55–57). The complete primary structures of Lol p 3 and Dac g 3 revealed a 92.6% similarity and 84.2% identity and the mature proteins lack cysteine and show no evidence of glycosylation. In spite of this high degree of homology, computer analyses detected differences in their predicted secondary structure and antigenic sites.

Group 4 Antigens

The grass group 4 allergens are high molecular weight basic glycoproteins (MW = 50–60 kDa, pI = 8.6–10.4) with sequence similarity to the berberine bridge enzymes, a member of a flavoprotein oxidoreductase superfamily. The initial report by Marsh found only a 20%

sensitization rate toward Lol p 4 among grass-allergic subjects, but other studies suggest that group 4 allergens from timothy, orchard grass, and Bermuda grass may be responsible for sensitization rates of 50%–75%. The complete primary structure of group 4 allergens was only recently elucidated (58,59). On the basis of the reported mass determination of native Phl p 4 and that deduced from the sequence, approximately 10% of its total mass appears to be made up of carbohydrate. Two potential N-glycosylation sites can be identified from the Phl p 4 sequence. A flavin-binding domain, first identified in BG60, the basic 60 kD allergen from Bermuda grass pollen, is consistent with other proteins of the BBE family thought to be involved in the plant pathogen response system (60). Using monoclonal antibodies raised against Dac g 4, related proteins from a various pooid and chlorodoid grass species, can be detected on SDS-PAGE immunoblots. ELISA inhibition experiments, however, revealed cross-reactivity only among the pooid grasses (61). Allergenic determinants have been localized on two Lol p 4 peptide fragments (MW = 17.4 and 11.0 kDa) by CNBr cleavage of the purified protein. Fragmentation of these Lol p 4 fragments with trypsin or chymotrypsin completely destroyed their IgE-binding capacity, hampering further resolution, and delineation of the allergenic sites (62). A decapeptide sequence of Ph1 p 4 shows significant sequence similarity to peptides from the major allergen family of ragweed pollen, Amb a 1 and Amb a 2 (63). Ph1 p 4–specific monoclonal antibody and human IgE antibody binding could be inhibited by preadsorption with Amb a 1. Rabbit and human antibodies directed toward Ph1 p 4 react with allergens present in various tree and weed pollens as well as vegetables and fruits (64,65). Together, these findings suggest the possibility of common IgE-binding epitopes on grass group 4 allergens and various unrelated pollen and plant foods. Studies with the group 4 allergen from Bermuda grass (66) suggest that the carbohydrate moiety, accounting for about 7.5% of the mass, may be important allergenic determinants of the molecule. Periodate oxidation reduced the IgE-binding activity of the allergen by approximately 50%. The predominant N-linked oligosaccharides of the molecule are unique among plant glycoproteins in that they possess a-(1,3)-linked fucose without any xylose (67). The recombinant forms of this allergen will most likely play a role in delineating allergenically important epitopes and cross-reacting carbohydrate determinants.

Group 5 Antigens (Includes Group 9 Antigens)

The group 5 allergens are a heterogeneous group of proteins with multiple isoforms varying in pIs and primary sequences (68–71). Comparison of the deduced amino acid sequences of the three group 5 allergens shows a high degree of homology (80–90%), which is consistent with the high degree of cross-reactivity observed. Their having similar molecular weights to the group 1 allergens (27–35 kDa for the group 1 and 27–38 kDa for group 5) probably explains why traditional protein fractionation methods based on molecular size failed to establish the identity of the group 5 allergens. The group 5 allergens, together with the group 1 allergens, account for the majority of the IgE-binding reactivity of most grass-allergic sera. In contrast to the group 1 allergens, group 5 allergens have been identified only among the subfamily Pooideae, and polyclonal antibodies raised against group 5 allergens failed to detect cross-reactive antigens outside of the subfamily. Furthermore, Northern analysis with Poa p V probes could only identify homologous transcripts among the pooids (72). Thus, it appears that group 5 allergens are restricted to a single subfamily of grasses, and if similar proteins are produced by the panicoid, chloridoid and arundinoid grasses, they are immunochemically and genetically unique.

By using recombinant and synthetic allergen fragments, or site-directed mutagenesis, investigators have localized IgE-binding determinants in the central and C-terminal regions of the Lol p 5 molecule (73,74) to both the N-terminal and C-terminal ends of Ph1 p 5 (75–77) and predominantly on a C-terminal fragment of Poa p 5 (Poa p 9) (78). At least four continuous and five discontinuous IgE-binding sites were localized on the group 5 allergen from velvet grass pollen (Hol l 5), and each were differentially recognized by individual patient IgE antibodies (79). Conformational IgE epitopes of Phl p 5 were mapped using grass-allergic patient sera and a random peptide-phage display library (80). Peptide alignment with the solvent-accessbile amino acids revealed at least three sequence sections on Phl p 5a. Taken together, these studies suggest either an extremely heterogeneous human B-cell response to group 5 allergens or a marked difference in the epitope structures of group 5 proteins derived from the different grass species. Nevertheless, a few point mutations in the group 5 sequence can yield mutants with significantly reduced allergenic activity (81,82). In one study, a single deletion (175–198)

in the Phl p 5 sequence caused a large reduction in IgE reactivity in a subset of allergic sera and a double deletion (175–198 and 94–113) further reduced IgE reactivity in all sera tested. The hypoallergenic double mutant retained its T-cell reactivity, an important attribute for immunotherapy. A more subtle approach utilizing site-direct mutagenesis involving amino acid exchanges in highly conserved sequence domains may also be good candidates for hypoallergen immunotherapy.

A remarkable characteristic of the group 5 allergens is their association with intracellular starch granules within the pollen grain. The cDNA sequence of Lol p 5 revealed the flanking transit peptide sequences typical of chloroplast-targeted proteins and thus, it has been proposed that the Lol p 5 is synthesized as a preallergen in the cytosol and transported to the amyloplast for posttranslational modification (83). This model may explain the existence of the multiple isoforms and the molecular weight heterogeneity of group 5 allergens isolated from pollen extracts. The size of the starch granules (0.6–2.5 μm in diameter) and their sudden appearance in air samples following rainfall is suggestive of a role in triggering asthmatic reactions. Ph1 p 5, has been shown to possess ribonuclease activity and the homologous group 5 allergens from the other grass species may be expected to possess this activity as we1l (84). It is interesting to speculate on the role of ribonuclease activity at the level of pollen-stigma interaction: Its release during hydration and stigma contact might facilitate the reproductive responses of the stigma.

T-cell determinants have been localized on Lol p 5 and Ph1 p 5 allergens by generating specific T cell lines or clones and measuring proliferative responses to a series of overlapping group 5 synthetic peptides spanning the entire sequence of the molecule (45,46,85,86). T-cell determinates were spread throughout the allergen molecule. Regions of high reactivity were found in specific patients but they differed among patients. Isoform-specific T cell epitopes were also detected with Ph1 p 5a and Ph1 p 5b fragments. A HLA-DR ligand prediction software (TEPITOPE) identified DR ligands within the Lol p 5a sequence that proved to be novel T-cell epitopes (87). Most of these ligands were clustered at the C-terminal part of a repeated 32-amino acid sequence motif, which occupies about 50% of the Lol p 5 sequence. A similar structural organization is shared by other group 5 allergens suggesting that this repeated motif may be a molecular signature of group 5 allergens. The observed diversity of the human T-cell response and specificity shown by individual patient responses suggest that immunotherapy with allergen peptides is not feasible.

Group 6 Antigens

The group 6 allergens from timothy grass pollen, Ph1 p 6, are polypeptides (MW = 12–13 kDa, pI = 5.2–5.5) toward which a majority of timothy grass pollen-sensitive subjects react (88,89). The group 6 allergens have so far been detected only in timothy pollen extracts. Ph1 p 6 is a pollen-specific protein localized on the polysaccharide-containing wall-precursor bodies or P-particles (88). The amino acid sequence deduced from the cDNA sequence revealed no cysteines and one potential glycosylation site, although no carbohydrate structures were detected (89). The N-terminal sequence of Ph1 p 6 is highly homologous to internal Ph1 p 5 sequences and epitope mapping studies with rPhl p 6 fragments indicated that the N-terminus of the molecule is required for IgE recognition. Sequence analysis of a cDNA encoding the complete Ph1 p 6 allergen provides evidence for an independent gene family arising from gene duplication. Comparison of the complete Ph1 p 6 and Ph1 p 5 sequences showed only a 55% to 60% match even though the N- and C-termini of Ph1 p 6 showed about a 95% similarity to the internal Ph1 p 5 protein sequence. Both unique and shared epitopes have been identified on Ph1 p 5 and 6 allergens using antibodies raised against Ph1 p 5 and Ph1 p 6, and allergenic crossreactivity has been detected by immunoadsorption studies (90). Epitope mapping studies with rPhl p 6 fragments indicated that the N-terminus of the molecule is required for IgE recognition.

Group 7 Antigens

The group 7 allergens (MW = 8–12 kDa) from Bermuda (Cyn d 7) and Timothy grass pollen (Ph1 p 7) were identified and isolated from a cDNA expression library using serum IgE from a grass-allergic individuals (91,92). The group 7 allergens belong to a family of Ca^{2+}-binding proteins, characterized by the presence of two potential EF-hand calcium-binding domains (93). Approximately 35% of grass pollen–allergic subjects possessed IgE antibodies toward the recombinant Cyn d 7. In addition, approximately 10% of pollen-allergic patients possessed IgE

antibodies toward to group 7-homologous allergens present in pollen of monocotyledonic and dicotyledonic plants. The deduced amino acid sequence of this protein showed significant sequence similiarity with a variety of Ca^{2+}-binding proteins, including the pollen allergens Bet v 4 from birch, Aln g 4 from alder, Ole e 3 from olive, Bra r 1 from oilseed rape, and calmodulin from the fungus Fusarium oxysporum. A three-dimensional model of Ph1 p 7 with two calcium-binding domains (EF hands) shows a novel dimer assembly adopting a barrel-like structure with an extended hydrophobic cavity providing a ligand-binding site (94). Structural similarities with other pollen allergens with two EF hands (Bet v 4, Aln g 4, Ole e 3, Cyn d 7 and Bra r 1), three EF hands (Bet v 3), and four EF hands (Jun o 4 and Ole e 8) has also been suggested by molecular modeling studies (95). Cross-reactivity with Bet v 4 has been established and a cross-reactive allergenic epitope was localized to the region representing the Ca^{2+}-binding domain II of the molecule, which shows an 83.3% amino acid sequence identity between Bet v 4 and Cyn d 7. The Cyn d 7 clone hybridized to transcripts in 13 other grass pollen using RNA gel blot analysis, suggesting that homologous proteins with similar allergenic activity may be present in other grass pollens. In addition, pollen extracts derived from 16 unrelated genera exhibited cross-reactivity with Aln g 4 and Jun o 4, suggesting that calcium-binding allergens are widely distributed in pollen from various plants. Disruption of the structure essential for calcium binding reduced the allergenic activity of Phl p 7 without affecting its ability to induce blocking antibodies (96). It is interesting to note that Phl p 7, unlike Bet v 4 and all the other EF-hand proteins appears to occur exclusively as a domain-swapped dimer (97). Presumably, the structural similarities between monomeric and dimeric forms explain the IgE cross-reactivity and that the two forms are allergenically similar.

Group 10 Antigens (Cytochrome c)

Cytochrome c (MW = 11 kDa, pI = 10) from timothy grass, perennial ryegrass, and Bermuda grass pollen have been demonstrated to be allergenic in humans having allergies to the respective pollen. Their importance as allergens on the basis of sensitization rates has not been thoroughly documented. This allergen group has been proposed as a model system for studying the molecular basis of cross-reactivity because a vast knowledge base exists for cytochrome structure and function (98).

Group 11 Antigens

The group 11 allergens are a group of glycoproteins (MW = 16–20 kDa, pI = 5.0–6.0) structurally similar to the Kunitz soy bean trypsin inhibitor, but lack the active site and appear not to possess inhibitory activity. Proteins that are homologous to Lo1 p 11 have been reported in other grass and non-grass pollen, most notably Ole e 1, the major allergen of olive tree pollen (99–101). This allergen may have eluded detection in conventional immunoblotting techniques using SDS-PAGE under reducing conditions because there are three potential disulfide bridges present that may be required for maintaining the IgE-binding peptide epitopes.

Among individuals with IgE antibodies against grass pollen, approximately 65% possessed IgE antibodies toward Lol p 11 and of these, about 35% possessed IgE antibodies against the carbohydrate moiety. Monosaccharide analysis suggested that *N*-glycan (mannose-type) or arabinoxylan substitutions may represent the IgE-binding carbohydrate group(s). The *N*-glycan structures appear to be involved in IgE binding as is bee venom phospholipase A_2, an allergen with known *N*-glycan IgE-binding epitopes, which is a potent inhibitor of IgE binding to Lol p 11. The recombinant form of Ph1 p 11, expressed as a soluble fusion protein in E. coli, induced histamine release from basophils and skin reactivity in grass pollen-sensitized subjects. The unglycosylated rPhl p 11 showed a reduced prevalence of IgE reactivity among grass pollen-positive sera and little or no cross-reactivity with other members of this allergen family, suggesting its diagnostic utility in identifying the primary sensitizer in allergic individuals.

Group 12 Antigens (Profilin)

Profilin, purified from grass pollen, has been suggested as an important allergen because anti-profilin IgE antibodies can be detected in 20% to 50% of grass-sensitive subjects (102). Because profilin is a highly conserved protein present in all organisms, the potential role of this allergen as a "panallergen" has been proposed (103). The cDNA sequences of timothy grass, Bermuda grass, and birch (Betula verrucosa) pollen encoding for the respective profilins are 80%

homologous but, profilins from unrelated sources are much more variable and typically are less than 50% homologous (104,105). Allergic patients with multiple pollen and plant-derived food sensitizations frequently possess IgE antibodies toward profilin. Evidence for both cross-reactive and unique IgE-binding epitopes exist and potential conformational epitopes have been predicted by structural analyses (106). However, the clinical relevance of the cross-reacting or species-specific IgE antibodies could not be established (107).

Group 13 Antigens

The group 13 allergens (MW = 50–60 kDa, pI = 6.0–7.5) have similar molecular weights to the grass group 4 allergens and are difficult to distinguish by one-dimensional SDS-PAGE. This and the finding that group 13 allergens are highly susceptible to proteolytic degradation may explain why they were not identified earlier. Approximately 42% to 75% of grass-sensitive subjects possess IgE antibodies to the group 13 allergens and allergenic proteins homologous to the Ph1 p 13 have been detected in all grass pollen extracts examined to date. The deduced amino acid sequence from the cloned cDNA of Ph1 p 13, consisting of 394 residues, indicated homology with pollen-specific polygalacturonases (108,109). IgE-binding epitopes have been mapped to the less-conserved regions of the C-terminus of the molecule and cross-reactivity was restricted to polygalacturonases from grass pollens. The carbohydrate moiety of Phl p 13 has been shown to cross-link IgE receptors on basophils and induce mediator release (23). This has been attributed to the presence of multiple *N*-glycan epitopes on the molecule. In contrast, Phl p 1, which has at most a single N-glycosylation site, bound IgE antibodies but did not cause mediator release. Phl p 13 has been localized on submicronic particles released from hydrated timothy pollen grains and like Phl p 5, may be considered as environmental markers for grass pollen exposure (110,111).

Group 22 and 24 Antigens

The group 22 (enolase) and group 24 (PR-1) antigens were identified in Bermuda grass pollen extract using a proteomic approach combining two-dimensional electrophoretic separations, immunoblotting, MALDI-MS, LC-MS/MS, and bioinformatics to identify these novel pollen allergens (112). Both proteins have been purified from whole Bermuda grass pollen extracts and shown to possess allergenic activity on the basis of histamine release or skin test responses in allergic patients. Cyn d 24 is a glycoprotein (MW = 19–21 kDa, pI = 5.9) structurally similar to the plant pathogenesis-related proteins (PR-1). Sequence identity ranged from 45% to 50% with PR-1s from barley, wheat, maize, and rice (113). The carbohydrate moiety, which makes up about 6% of the total weight of the glycoprotein resembles the structure possessed by Cyn d 4 (BG60): An L-fucose a-(1,3)-linked to an *N*-acetyl glucosamine without xylose linked to the branching mannose. As with Cyn d 4, there is evidence that the carbohydrate moiety is involved with IgE binding.

GRASS POLLEN ALLERGEN CROSS-REACTIVITY

Grass-allergic subjects almost always display multiple grass pollen sensitivities. Because many grass species coexist in the same geographical area, simultaneous sensitization to pollen from multiple grass species is expected. Various approaches have been used to investigate grass pollen allergen cross-reactivities and most of these studies support the hypothesis that sensitization to one grass pollen species leads to multiple pollen sensitivities. RAST and ELISA inhibition assays have revealed extensive allergenic cross-reactivity among taxonomically related grasses (114). Much data have been generated using whole pollen extracts but more precise information about cross-reactivity, i.e., relative affinity, can only be obtained by using individual specific allergens. Structural details of homologous grass pollen allergens may also reveal potential cross-reactive epitopes (115,116). These studies may be on the basis of the primary amino acid sequence data or on more sophisticated 3-dimensional structural data of surface exposed residues.

The pattern of allergenic cross-reactivity among the pollen species follows closely their taxonomic relationships (Fig. 4). All studies found the highest degree of allergenic cross-reactivity among pollen extracts derived from grasses of the same subfamily. Martin et al. (117), using a human serum pool from allergic North American subjects with pollen extracts

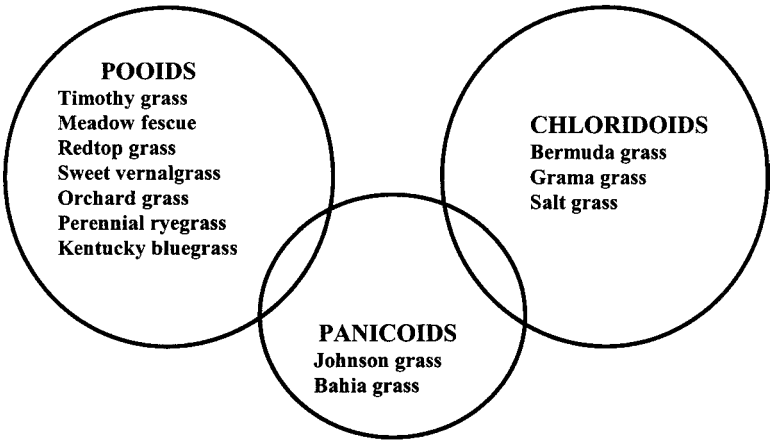

Figure 4 A Venn diagram representing cross-relativity grouping of major allergenic grasses based on their taxonomic and immunological relationship. The grass species within each subfamily group are highly cross-reactive and are difficult to differentiate immunologically. The two groups pooids and chloridoids are allergenically distinct and require separate diagnoses and immunotherapies. The panicoids, Johnson and Bahia grass, are allergenically cross-reactive with both the pooids and chloridoids.

from the three subfamilies Pooideae (brome, meadow fescue, perennial ryegrass, timothy, sweet vernal, redtop, Kentucky bluegrass, and Western wheatgrass), Chloridoideae (salt grass, Bermuda grass, and grama grass) and Panicoideae (Bahia grass and Johnson grass), detected little or no cross-reactivity between pooid and chloridoid pollen while the panacoid grasses showed moderate cross-reactivity with both the pooids and chloridoids. In a study using IgE antibodies from Australian subjects and selected for reactivity to Lol p 1 and Lol p 5, no cross-reactivity was observed between ryegrass and Bahia or Bermuda grass pollen allergens. However, in reciprocal experiments using antibodies selected for reactivity to Bahia pollen allergens, grass pollens from ryegrass, Timothy, Johnson grass, and Bermuda were cross-reactive (118). Gonzalez et al. (119), employing sera from European subjects selected for reactivity toward each pollen group, detected little or no allergenic cross-reactivity between the pooids (perennial ryegrass, timothy and cultivated rye) and Bermuda grass. P. communis (common reed), an arundinoid, showed moderate cross-reactivity with both the pooids and chloridoids. In a study with 209 individual sera with reactivity toward grass pollen, extensive cross-reactivity was detected among 14 different species of pooids with the highest responses directed toward P. pratensis, F. rubra, P. pratense, and D. glomerata. Excluding P. communis, an arundinoid, C. dactylon, a chloridoid, and Z. mays, a panicoid, any one grass species was sufficient for in vitro diagnosis of grass pollen allergy (120). No studies have examined possible allergenic cross-reactivities between arundinoid and panicoid pollen.

The availability of purified grass pollen allergens, allergen fragments, and recombinant allergens has allowed for more refined studies that detect cross-reactivity among allergens derived from the same species as well as among homologous allergens from different species. Of particular interest is the strong homology between the C-terminal end of the group 1 molecule (amino acid residues 145–240) and the entire 97–amino acid sequence of the grass group 2 and group 3 allergens. The human immune response to the three ryegrass allergens is associated with histocompatibility leukocyte antigen (HLA) DR3 and concordant reactivity to all three allergens is common. These observations may be explained by cross-reactivity among Lol p 1, Lol p 2, and Lol p 3, which has been detected at both the B-cell level and at the T-cell level (43,53). Some human T-cell clones are reactive to the purified protein of the three allergen groups and the group 5 allergen. Stretches of homologous segments with an amphipathic nature suggest the presence of structural similarities that may account for this cross-reactivity.

The cross-reactive nature of the ubiquitous protein profilin is illustrated by the serological cross-reactivity between grass pollen profilins and profilins from other pollen and vegetable foods (121). Human anti-profilin IgE antibodies have been shown to cross-react with almost all plant profilins. The presence of highly conserved structures among plant

profilins may explain the reports of coincident oral allergies to fruits and vegetables eaten by grass-allergic subjects (102,122,123) as well as allergenic cross-reactivities occasionally detected between unrelated pollen. The group 4 and group 7 allergens may also be useful diagnostic markers to identify patients with multiple sensitizations caused by cross-reactivity. For example, grass group 4-related proteins have been identified in unrelated plant foods including peanut, apple, celery, and carrot root as well as mugwort and birch pollen. Approximately 20% of polysensitized individuals possess IgE antibodies toward calcium-binding pollen proteins. Among the calcium-binding proteins, Ph1 p 7 contained most of the relevant IgE epitopes in the population studied and may be a useful molecule for detecting sensitization to this group of cross-reactive pollen allergens.

Cross-reactive carbohydrate determinants of grass pollen allergens have also been implicated in serological cross-reactions among a variety of pollen and vegetable foods (124–126). The β(1-2)-xylose- and α(1-3)-fucose-containing glycans on glycoproteins from several plants, mollusks, and insects have been shown to be highly cross-reactive. The clinical significance of anti-carbohydrate IgE antibodies has not been established and the role of CCD in allergic sensitization remains to be explored. In this regard, the grass group 11 allergens, Cyn d 4 (BG60), and Cyn d 24 may present a unique opportunity to investigate the role of both carbohydrate and peptide epitopes in allergenic cross-reactivity among glycoproteins from related and unrelated sources. The presence of conserved amino acids and cysteine positions in the primary structure of Lol p 11 suggests homology with pollen glycoproteins from maize, rice, tomato, olive tree, and privet.

Since grass pollen immunotherapy was pioneered by Freeman and Noon (127) almost a century ago, the specificity of grass immunotherapy has been questioned. Freeman advocated the use of only extracts/vaccines from timothy grass pollen for the diagnosis and treatment of grass allergy in Great Britain as did Cooke and Vander Veer (128) in the United States. Leavengood et al. (129) selected a group of patients showing multiple sensitivities to pooid, panicoid, and chloridoid grasses and treated them with vaccines prepared only from timothy and Bermuda grass pollen. The treatment significantly reduced the skin test responses to all of the grass pollen, suggesting that treatment with the two grass pollen vaccines may be sufficient for effective treatment in these grass-allergic patients. The current practice parameters for allergen immunotherapy established by both the American Academy of Allergy Asthma and Immunology and the American College of Allergy Asthma and Immunology specifically states that information regarding allergen cross-reactivity should be used in the selection of relevant allergens for immunotherapy because limiting the number of allergens in a treatment vial may be necessary to attain optimal therapeutic doses for the individual patient (130). The molecular and clinical evidence for cross-reactivity among grass pollen allergens supports the premise that effective diagnosis and immunotherapy can be accomplished with a limited number of grass pollen extracts/vaccines. The use of representative extracts/vaccines from the major grass subfamilies appears to be a reliable strategy, and the selection of the species should be based on their prevalence. For example, timothy grass (or any of the pooids), Bermuda grass and Johnson grass, which represent the three major allergenic grass subfamilies should be sufficient for clinical practice.

CONCLUSION

Grasses are ubiquitous and their pollens are important aeroallergens in most parts of the world. Only a few grass species have been positively identified as important sources of allergens, but less conspicuous grass species may add to the aeroallergen load due to their cross-reactivity. The degree of allergenic cross-reactivity tends to correlate with their taxonomic grouping and the treatment of grass allergy with vaccines derived from representative species have been shown to be efficacious. Cross-reactivities between homologous proteins from different grass species and their clinical relevance have long been established. There is evidence of cross-reactivities between grass pollen allergens and proteins derived from diverse plant sources including other pollen, fruits, and vegetables. Clinical investigations to establish the relevance of such cross-reactivities are still needed.

New immunochemical and molecular biological approaches to the study of grass pollen allergens have greatly increased the knowledge about this important group of pollen allergens.

Due to their worldwide importance, grass allergens are a subject of great interest among researchers from virtually every continent and more than 100 grass pollen allergen structures have been identified, purified, and sequenced. Two groups independently sequenced and assembled more than 90% of the rice (Oryza sativa) genome (131,132) and the U.S. government agencies are funding a $40 million effort to sequence the corn genome (133). Because the members of the grass family are closely related and their genomes share extensive synteny, this genetic information will undoubtedly have a great impact on grass pollen allergen research. For example, the complexity of grass group 1 allergen genes and their pattern of expression (134) suggests that attempts to silence their expression as has been attempted with the group 5 allergen genes (135) will not be successful. Only a few minor grass pollen allergen groups are yet to be identified and it is expected that in the next few years the molecular structure of all of the major grass allergen groups will have been established. The human immune responses at both the B-cell and T-cell levels are being studied to define relevant structures on the grass allergen molecules and novel diagnostic and therapeutic approaches based on their immunological activities are in progress. Together with advances being made with allergens from other environmental sources, the study of grass pollen will contribute to a better understanding of allergic responses to these ubiquitous allergens.

SALIENT POINTS

- Grasses are ubiquitous and grass pollen allergens are of worldwide importance.
- Of the hundreds of grass genera and thousands of species, only a small number are allergenically important. Most of these species are cultivated.
- The pooids account for most grass species in Canada and northwestern United States and are prevalent in the cooler regions of the world; the chloridoids are well established especially in the warm and arid habitats with high winter temperatures such as the southwestern United States; and the panicoids dominate the humid tropical environments including the southern United States and northeastern South America.
- Allergic symptoms depend on pollen exposure. Particles that are significantly smaller than the size of grass pollen grains and capable of entering the lower airway have been implicated as major causes of allergic reactions.
- Eleven groups of structurally related allergens have been identified and characterized across multiple grass subfamilies.
- Grass-allergic patients often display multiple sensitivities. Simultaneous sensitization to multiple species are expected on the basis of the numerous grass species pollinating at a given time and place and on the high degree of cross-reactivity among them.
- Allergenic cross-reactivities have been documented among homologous allergens produced by taxonomically related grasses and between allergens produced in a single grass species.
- The presence of conserved structures among the proteins and various carbohydrate determinants of pollen and vegetable foods is consistent with coincident allergic reactions to fruits and vegetables as well as unrelated pollen in grass pollen-allergic patients.
- Diagnosis and immunotherapy with a limited number of grass allergen vaccines representing the subfamilies Pooideae, Chloridoideae and Panicoideae may be effective in most grass-allergic patients.
- Advances made with the rice and corn genome projects should be leveraged to increase understanding of the structure, expression, and function of grass pollen allergens.

REFERENCES

1. Waite KJ. Blackley and the development of hay fever as a disease of civilization in the nineteenth century. Med Hist 1995; 39:186–196.
2. Hitchcock AS. Manual of the Grasses of the United States. 2nd ed. Revised by Chase A. Washington D.C.: USDA Misc Publ 200, 1951.

3. Freeling M. Grasses as a single genetic system. Plant Physiol 2001; 125:1191–1197.
4. Watson L, Dallwitz MJ. The Grass Genera of the World. 2nd ed. Wallingford: CAB International, 1994.
5. Barunuik IN, Bolick M, Esch R. Buckely CE Quantification of pollen solute release using pollen grain column chromatography. Allergy 1992; 47:411–417.
6. Aeroallergen Monitoring Network. 1996 Pollen and Spore Report. Milwaukee: AAAA&I, 1997.
7. Habenicht HA, Burge HA, Muilenburg ML, et al. Allergen carriage by atmospheric aerosol II. Ragweed-pollen determinants in submicronic atmospheric fractions. J Allergy Clin Immunol 1984; 74:64–67.
8. Schumacher MJ, Griffith RD, O'Rourke MK. Recognition of pollen and other particulate aeroantigens by immunoblot microscopy. J Allergy Clin Immunol 1988; 82:608–616.
9. Knox RB. Grass pollen, thunderstorms and asthma. Clin Exp Allergy 1993; 23:354–359.
10. Taylor PE, Flagan RC, Valenta R, et al. Release of allergens as respirable aerosols: a link between grass pollen and asthma. J Allergy Clin Immunol 2002; 109:51–56.
11. Taylor PE, Jacobson KW, House JM, et al. Links between pollen, atopy and the asthma epidemic. Int Arch Allergy Immunol 2007; 144:162–170.
12. Vinckier S, Smets E. The potential role of orbicules as a vector of allergens. Allergy 2001; 56: 1129–1136.
13. Johnson P, Marsh DG. Allergens from common rye grass pollen (Lolium perenne): I. Chemical composition and structure. Immunochemistry 1966; 3:91–100.
14. Johnson P, Marsh DG. Isoallergens from rye grass pollen. Nature 1965; 206:935–937.
15. Marsh DG, Haddad ZH, Campbell DH. A new method for determining the distribution of allergenic fractions in biological materials: its application to grass pollen extracts. J Allergy 1970; 46:107–121.
16. Howlett BJ, Vithanage HIMV, Knox RB. Immunofluorescence localization of two water soluble glycoproteins, including the major allergen of rye-grass, Lolium perenne. Histochem J 1981; 13: 461–480.
17. Perez M, Ishioka GY, Walker LE, et al. cDNA cloning and immunological characterization of the rye grass allergen Lol p I. J Biol Chem 1990; 265:16210–16215.
18. Laffer S, Valenta R, Vrtala S, et al. Complementary DNA cloning of the major allergen Phl p 1 from timothy grass (Phleum pratense); recombinant Phl p 1 inhibits IgE binding to group 1 allergens from eight different grass species. J Allergy Clin Immunol 1994; 94:689–698.
19. Smith PM, Suphioglu C, Griffith IJ, et al. Cloning and expression in yeast Pichia pastoris of a biologically active form of Cyn d 1, the major allergen of Bermuda grass pollen. J Allergy Clin Immunol 1996; 98:331–343.
20. Schramm G, Bufe A, Petersen A, et al. Mapping of IgE-binding epitopes on the recombinant major group I allergen of velvet grass pollen, rHol l 1. J Allergy Clin Immunol 1997; 99:781–789.
21. Broadwater AH, Rubinstein AL, Chay CH, et al. Zea ml, the maize homolog of the allergen-encoding Lol p I gene of rye grass. Gene 1993; 131:227–230.
22. Petersen A, Becker W-M, Moll H, Blumke M, et al. Studies on the carbohydrate moieties of the timothy grass pollen allergen Phl p 1. Electrophoresis 1995; 16:869–875.
23. Wicklein D, Lindner B, Moll H, et al. Carbohydrate moieties can induce mediator release: a detailed characterization of two major timothy grass pollen allergens. Biol Chem 2004; 385:397–407.
24. Cosgrove DJ. Loosening of plant cell walls by expansins. Nature 2000; 407:321–326.
25. Grobe K, Becker WM, Schlaak M, et al. Grass group 1 allergens (beta-expansins) are novel, papain-related proteinases. Eur J Biochem 1999; 263:33–40.
26. Grobe K, Poppelmann M, Becker WM, et al. Properties of group 1 allergens from grass pollen and their relation to cathepsin B, a member of the C1 family of cysteine proteinases. Eur J Biochem 2002; 269:2083–2092.
27. Raftery MJ, Saldanha RG, Geczy CL, et al. Mass spectrometric analysis of electrophoretically separated allergens and proteases in grass pollen diffusates. Respir Res 2003; 4:10.
28. Li LC, Cosgrove DJ. Grass group 1 pollen allergens (beta-expansins) lack proteinase activity and do not cause wall loosening via proteolysis. Eur J Biochem 2001; 268:4217–4226.
29. Crosgrove DJ, Bedinger P, Durachko DM. Group 1 allergens of grass pollen as cell wall-loosening agents. Proc Natl Acad Sci U S A 1997; 94:6559–6564.
30. Li LC, Bedinger PA, Volk C, et al. Purification and characterization of four beta-expansins (Zea m 1 isoforms) from maize pollen. Plant Physiol 2003;132:2073–2085.
31. Valdivia ER, Wu Y, Li LC, et al. A group 1 grass pollen allergen influences the outcome of pollen competition in maize. PLoS One 2007; 2:e154.
32. Barre A, Rouge P. Homology modeling of the cellulose-binding domain of a pollen allergen from rye grass: structural basis for the cellulose recognition and associated allergenic properties. Biochem Biophys Res Commun 2002; 296:1346–1351.
33. Baer H, Maloney CJ, Norman P, et al. The potency and group I antigen content of six commercially prepared grass pollen extracts. J Allergy Clin Immunol 1974; 54:157–164.

34. Esch RE, Klapper DG. Isolation and characterization of a major cross-reactive grass group I allergenic determinant. Mol Immunol 1989; 26:557–561.
35. Hiller KM, Esch RE, Klapper DG. Mapping of an allergenically important determinant of grass group 1 allergens. J Allergy Clin Immunol 1997; 100:335–340.
36. Ball T, Fuchs T, Sperr R, et al. B cell epitopes of the major timothy grass pollen allergen, Phl p 1, revealed by gene fragmentation as candidates for immunotherapy. FASEB J 1999; 13:1277–1290.
37. Flicker S, Steinberger P, Ball T, et al. Spatial clustering of the IgE epitopes on the major timothy grass pollen allergen Phl p 1: importance for allergenic activity. J Allergy Clin Immunol 2006; 117:1336–1343.
38. de Weerd N, Bhalla PL, Singh MB. Effect of cysteine mutagenesis on human IgE reactivity of recombinant forms of the major rye grass pollen allergen Lol p 1. Allergol Int 2003; 52:183–190.
39. Ball T, Ekstrom W, Mauch L, et al. Gain of structure and IgE epitopes by eukaryotic expression of the major timothy grass pollen allergen, Phl p 1. FEBS J 2005; 272:217–227.
40. van Oort E, de Heer PG, Dieker M, et al. Characterization of natural Dac g 1 variants: an alternative to recombinant group 1 allergens. J Allergy Clin Immunol 2004; 114:1124–1130.
41. Suck R, Kamionka T, Schaffer B, et al. Bacterially expressed and optimized recombinant Phl p 1 is immunobiochemically equivalent to natural Phl p 1. Biochim Biophys Acta 2006; 1764:1701–1709.
42. Han S, Chang Z, Chang H, et al. Identification and characterization of epitopes on Cyn d 1, the major allergen of Bermuda grass pollen. J Allergy Clin Immunol 1993; 91:1035–1041.
43. Baskar S, Parronchi P, Mohapatra S, et al. Human T cell responses to purified pollen allergens of the grass, Lolium perenne. Analysis of relationship between structural homology and T cell recognition. J Immunol 1992; 148:2378–2383.
44. Mohapatra SS, Mohapatra S, Yang M, et al. Molecular basis of cross-reactivity among allergen-specific human T cells: T-cell receptor Vα gene usage and epitope structure. Immunology 1994; 81:15–20.
45. Mulller WD, Karamfilov T, Bufe A, et al. Group 5 allergens of timothy grass (Ph1 p 5) bear cross-reacting T cell epitopes with group 1 allergens of rye grass (Lol p 1). Int Arch Allergy Immuno1 1996; 109:352–355.
46. Burton MD, Papalia L, Eusebius NP, et al. Characterization of the human T cell response to rye grass pollen allergens Lol p 1 and Lol p 5. Allergy 2002; 57:1136–1144.
47. Ansari AA, Shenbagamurthi P, Marsh DG. Complete primary structure of a Lolium perenne (perennial rye grass) pollen allergen, Lol p II. J Biol Chem 1989; 265: 11181–11185.
48. Macaubas C, Wahlstrom J, Galvao da Silva AP, et al. Allergen-specific MHC class II tetramer+ cells are detectable in allergic, but not in nonallergic individuals. J Immunol 2006; 176:5069–5077.
49. Roberts AM, Bevan LJ, Flora PS, et al. Nucleotide sequence of cDNA encoding the group II allergen cocksfoot/orchard grass (Dactylis glomerata), Dac g II. Allergy 1993; 48:615–623.
50. Tomborini E, Brandazza A, De Lalla C, et al. Recombinant allergen Lol p II: expression, purification and characterization. Mol Immunol 1995; 32:505–513.
51. Dolecek C, Vrtala S, Laffer S, et al. Molecular characterization of Phl p II, a major timothy grass (Phleum pratense) pollen allergen. FEBS Lett 1993; 335:299–304.
52. De Marino S, Castiglione Morelli MA, Fratemali F, et al. An immunoglobulin-like fold in a major plant allergen: the solution structure of Phl p 2 from timothy grass pollen. Structure 1999; 7:943–952.
53. Ansari AA, Kihara TK, Marsh DG. Immunochemical studies of Lolium perenne (rye grass) pollen allergens Lol p I, II, and III. J Immuno1 1987; 139:4034–4041.
54. Petersen A, Suck R, Lindner B, et al. Phl p 3: structural and immunological characterization of a major allergen of timothy grass pollen. Clin Exp Allergy 2006; 36:840–849.
55. Ansari AA, Shenbagamurthi P, Marsh DG. Complete primary structure of a Lo/ium perenne (perennial rye grass) pollen allergen, Lol p III: comparison with known Lol p I and II sequences. Biochemistry 1989; 28:8665–8670.
56. Guerin-Marchand C, Senechal H, Bouin A-P, et al. Cloning, sequencing and immunological characterization of Dac g 3, a major allergen from Dactylis glomerata pollen. Mol Immunol 1996; 33:797–806.
57. Devanaboyina V, Keller W, Nandy A, et al. Crystallization and structure solution of major grass pollen allergen Phl p 3.
58. Nandy A, Petersen A, Wald M, et al. Primary structure, recombinant expression, and molecular characterization of Phl p 4, a major allergen of timothy grass (Phleum pratense). Biochem Biophys Res Comm 2005; 305:563–570.
59. DeWitt AM, Andersson K, Peltre G, et al. Cloning, expression and immunological characterization of full-length timothy grass pollen allergen Phl p 4, a berberine bridge enzyme-like protein with homology to celery allergen Api g 5. Clin Exp Allergy 2006; 36:77–86.
60. Dittrich H, Kutchan TM. Molecular cloning, expression and induction of berberine bridge enzyme, an enzyme essential to the formation of benzophenanthridine alkaloids in the response of plants to pathogenic attack. Proc Natl Acad Sci U S A 1991; 88:9969–9973.

61. Leduc-Brodard V, Inacio F, Jaquinod M, et al. Characterization of Dac g 4, a major basic allergen from Dactylis glomerata pollen. J Allergy Clin Immunol 1996; 98:1065–1072.
62. Jaggi KS, Ekramoddoullah AKM, Kisil FT. Allergenic fragments of rye grass (Lolium perenne) pollen allergen Lol p IV. Int Arch Allergy Appl Immunol 1989; 89:342–348.
63. Fischer S, Grote M, Fahlbusch B, et al. Characterization of Ph1 p 4, a major timothy grass (Phleum pratense) pollen allergen. J Allergy Clin Immunol 1996; 98:189–198.
64. Grote M, Stumvoll S, Reichelt R, et al. Identification of an allergen related to Ph1 p 4, a major timothy grass pollen allergen, in pollen, vegetables, and fruits by immunogold electron microscopy. Biol Chem 2002; 383:1441–1445.
65. Stumvoll S, Lidholm J, Thunberg R, et al. Purification, structural and immunological characterization of a timothy grass (Phleum pratense) pollen allergen, Ph1 p 4, with cross-reactive potential. Biol Chem 2002; 383:1383–1396.
66. Su S-N, Shu P, Gai-Xuong L, et al. Immunologic and physicochemical studies of Bermuda grass pollen antigen BG60. J Allergy Clin Immunol 1996; 98:486–494.
67. Ohsuga H, Su S-N, Takahashi N, et al. The carbohydrate moiety of the Bermuda grass antigen BG60: new oligosaccharides of plant origin. J Biol Chem 1996; 271:26653–26658.
68. Matthiesen F, Lowenstein H. Group V allergens in grass pollen. 1. Purification and characterization of group V allergen from Phleum pratense pollen, Ph1 p V. Clin Exp Allergy 1991; 21:297–307.
69. Silvanovich A, Astwood J, Zhang L, et al. Nucleotide sequence analysis of three cDNAs coding for Poa p IX isoallergens of Kentucky bluegrass pollen. J Biol Chem 1991; 266:1204–1210.
70. Ong EK, Griffith IJ, Knox RB, et al. Cloning of a cDNA encoding a group V (group IX) allergen isoform from rye-grass pollen that demonstrates specific antigenic immunoreactivity. Gene 1993; 134:235–240.
71. Suphioglu C, Mawdsley D, Schappi G, et al. Molecular cloning, expression and immunological characterization of Lol p 5C, a novel allergen isoform of rye grass pollen demonstrating high IgE reactivity. FEBS Lett 1999; 462:435–441.
72. Smith PM, Ong EK, Knox RB, et al. Immunological relationships among group I and group V allergens from grass pollen. Mol Immunol 1994; 31:491–498.
73. Ong EK, Knox RB, Singh MB. Mapping of the antigenic and allergenic epitopes of Lol p VB using gene fragmentation. Mol Immunol 1995; 32:295–302.
74. Suphioglu C, Blaher B, Rolland JM, et al. Molecular basis of IgE-recognition of Lol p 5, a major allergen of ryegrass pollen. Mol Immunol 1998; 35:293–305.
75. Bufe A, Becker W-M, Schramm G, et al. Major allergen Ph1 p Va (timothy grass) bears at least two different IgE-reactive epitopes. J Allergy Clin Immunol 1994; 94:173–181.
76. Flicker S, Vrtala S, Steinberger P, et al. A human monoclonal IgE antibody defines a highly allergenic fragment of the major timothy grass pollen allergen, Phl p 5: molecular, immunological, and structural characterization of the epitope-containing domain. J Immunol 2000; 165:3849–3859.
77. Gehlhar K, Rajashankar KR, Hofmann E, et al. Lysine as a critical amino acid for IgE binding in Phl p 5b C terminus. Int Arch Allergy Immunol 2006; 140:285–294.
78. Zhang L, Olsen E, Kisil FT, et al. Mapping of antibody binding epitopes of a recombinant Poa p IX allergen. Mol Immunol 1992; 29:1383–1389.
79. Schramm G, Bufe A, Petersen A, et al. Discontinuous IgE-binding epitopes contain multiple continuous epitope regions: results of an epitope mapping on recombinant Hol l 5, a major allergen from velvet grass pollen. Clin Exp Allergy 2001; 31:331–341.
80. Hantusch B, Krieger S, Untersmayr E, et al. Mapping of conformational IgE epitopes on Phl p 5a by using mimotopes from a phage display library. J Allergy Clin Immunol 2004; 114:1294–1300.
81. Wald M, Kahlert H, Weber B, et al. Generation of a low immunoglobulin E-binding mutant of the timothy grass pollen major allergen Phl p 5a. Clin Exp Allergy 2007; 37:441–450.
82. Swoboda I, De Weerd N, Bhalla PL, et al. Mutants of the major ryegrass pollen allergen, Lol p 5, with reduced IgE-binding capacity: candidates for grass pollen-specific immunotherapy. Eur J Immunol 2002; 32:270–280.
83. Singh MB, Hough T, Theerakulpisut P, et al. Isolation of cDNA encoding a newly identified major allergenic protein of rye-grass pollen: intracellular targeting to the amyloplast. Proc Natl Acad Sci U S A 1991; 88:1384–1388.
84. Bufe A, Uhlig U, Scholzen T, et al. A nonspecific, single-stranded nuclease activity with characteristics of a topisomerase found in a major grass pollen allergen: possible biological significance. Biol Chem 1999; 380:1009–1016.
85. Blaher B, Suphioglu C, Knox RB, et al. Identification of T-cell epitopes of Lol p 9, a major allergen of rye grass (Latium perenne) pollen. J Allergy Clin Immunol 1996; 98:124–132.
86. Wurtzen P, Wissenbach M, Ipsen H, et al. Highly heterogeneous Ph1 p 5-specific T cells from patients with allergic rhinitis differentially recognize recombinant Ph1 p 5 isoallergens. J Allergy Clin Immunol 1999; 104:115–122.

87. de Lalla C, Sturniolo T, Abbruzzese L, et al. Identification of novel T cell epitopes in Lol p 5a by computational prediction. J Immunol 1999; 163:1725–1729.
88. Vrtala S, Fischer S, Grote M, et al. Molecular, immunological and structural characterization of Ph1 p 6, a major allergen and P-particle-associated protein from Timothy grass (Phleum pratense) pollen. J Immunol 1999; 163:5489–5496.
89. Petersen A, Bufe A, Schramm G, et al. Characterization of the allergen group VI in timothy grass pollen (Ph1 p 6) II. cDNA cloning of Ph1 p 6 and structural comparison to group V. Int Arch Allergy Immunol 1995; 108:55–59.
90. Petersen A, Bufe A, Schlaak M, et al. Characterization of the allergen group VI in timothy grass pollen (Ph1 p 6) I. Immunological and biochemical studies. Int Arch Allergy Immunol 1995; 108:49–54.
91. Suphioglu C, Ferreira F, Knox RB. Molecular cloning and immunological characterisation of Cyn d 7, a novel calcium-binding allergen from Bermuda grass pollen. FEBS Letters 1997; 402:167–172.
92. Niederberger V, Hayek B, Vrtala S, et al. Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a cross-reactive two EF-hand timothy grass pollen allergen, PhI p 7. F ASEB J 1999; 13:843–856.
93. Wopfner N, Dissertori O, Ferreira F, et al. Calcium-binding proteins and their role in allergic disease. Immunol Allergy Clin North Am 2007; 27:29–44.
94. Verdino P, Westritschnig K, Valenta R, et al. The cross-reactive calcium-binding pollen allergen Ph1 p 7, reveals a novel dimer assembly. EMBO J 2002; 21:5007–5016.
95. Tinghino R, Twardosz A, Barletta B, et al. Molecular, structural and immunological relationships between different families of recombinant calcium-binding pollen allergens. J Allergy Clin Immunol 2002; 109:314–320.
96. Westritschnig K, Focke M, Verdino P, et al. Generation of an allergy vaccine by disruption of the three-dimensional structure of the cross-reactive calcium-binding allergen, Phl p 7. J Immunol 2004; 172:5684–5692.
97. Neukecker P, Nerkamp J, Eisenmann A, et al. Solution structure, dynamics, and hydrodynamics of the calcium-bound cross-reactive birch pollen allergen Bet v 4 reveal a canonical monomeric two EF-hand assembly with a regulatory function. J Mol Biol 2004; 336:1141–1157.
98. Ekramoddoullah AKM, Kisil FT, Sehon AH. Allergenic cross-reactivity of cytochromes c of Kentucky bluegrass and perennial ryegrass pollen. Mol Immunol 1982; 19:1527–1534.
99. van Ree R, Hoffman DR, Vandijk W, et al. Lol p XI, a new major grass pollen allergen, is a member of a family of soybean trypsin inhibitor-related proteins. J Allergy Clin Immunol 1995; 95:970–978.
100. Marknell DeWitt A, Niederberger V, Lehtonen P, et al. Molecular and immunological characterization of a novel timothy grass (Phleum pratense) pollen allergen, PhI p 11. Clin Exp Allergy 2002; 32:1329–1340.
101. Barderas R, Villalba M, Lombardero M, Rodriguez R. Identification and characterization of Che a 1 allergen from Chenopodium album pollen. Int Arch Allergy Immunol 2002; 127:47–54.
102. van Ree R, Voitenko V, van Leeuwen WA, et al. Profilin is a cross-reactive allergen in pollen and vegetable foods. Int Arch Allergy Immunol 1992; 98:97–104.
103. Valenta R, Duchene M, Vrtala S, et al. Profilin, a novel plant pan-allergen. Int Arch Allergy Immunol 1992; 99:271–273.
104. Valenta R, Ball T, Vrtala S, et al. cDNA cloning and expression of timothy grass (Phleum pratense) pollen profilin in Escherichia coli: comparison with birch pollen profilin. Biochem Biophys Res Commun 1994; 199:106–118.
105. Asturias JA, Arilla MC, Gomez-Bayon N, et al. Cloning and high level expression of Cynodon dactylon (Bermuda grass) pollen profilin (Cyn d 12) in Escherichia coli: purification and characterization of the allergen. Clin Exp Allergy 1997; 27:1307–1313.
106. Asturias JA, Arilla MC, Gomez-Bayon N, et al. Recombinant DNA technology in allergology: cloning and expression of plant profilins. Allergol Immunopathol (Madr) 1997; 25:127–134.
107. Wensing M, Akkerdaas JH, van Leeuwen WA, et al. IgE to Bet v 1 and profilin: Crossreactivity patterns and clinical relevance. J Allergy Clin Immunol 2002; 11:435–442.
108. Suck R, Petersen A, Hagen S, et al. Complementary DNA cloning and expression of a newly recognized high molecular mass allergen Ph1 p 13 from timothy grass (Phleum pratense). Clin Exp Allergy 2000; 30:324–332.
109. Petersen A, Suck R, Hagen S, et al. Group 13 grass allergens: structural variability between different grass species and analysis of proteolytic stability. J Allergy Clin Immunol 2001; 107:856–862.
110. Swoboda I, Grote M, Verdino P, et al. Molecular characterization of polygalacuronases as grass pollen-specific marker allergens: expulsion from pollen via submicronic respirable particles. J Immunol 2004; 172:6490–6500.
111. Grote M, Swoboda I, Valenta R, et al. Group 13 allergens as environmental and immunological markers for grass pollen allergy: studies by immunogold field emission scanning and transmission electron microscopy. Int Arch Allergy Immunol 2005; 136:303–310.

112. Kao SH, Su SN, Huang SW, et al. Sub-proteome analysis of novel IgE-binding proteins from Bermuda grass pollen. Proteomics 2005; 5:3805–3813.
113. Chow LP, Chiu LL, Khoo KH, et al. Purification and structural analysis of the novel glycoprotein allergen Cyn d 24, a pathogenesis-related protein PR-1, from Bermuda grass pollen. FEBS J 2005; 272:6218–6227.
114. Weber RW. Cross-reactivity of plant and animal allergens. Clin Rev Allergy Immunol 2001; 21:153–202.
115. Aalberse RC, Akkerdaas JH, van Ree R. Cross-reactivity of IgE antibodies to allergens. Allergy 2001; 56:478–490.
116. Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol 2000; 106:228–238.
117. Martin BG, Mansfield LE, Nelson HS. Cross-allergenicity among the grasses. Ann Allergy 1985; 54:99–104.
118. Davies JM, Bright ML, Rolland JM, et al. Bahia grass pollen specific IgE is common in seasonal rhinitis patients but has limited cross-reactivity with ryegrass. Allergy 2005; 60:251–255.
119. Gonzales RM, Cortes C, Conde J, et al. Cross-reactivity among five major pollen allergens. Ann Allergy 1987; 59:149–154.
120. van Ree R, van Leeuwen WA, Aalberse RC. How far can we simplify in vitro diagnostics for grass pollen allergy? A study with 17 whole pollen extracts and purified natural and recombinant major allergens. J Allergy Clin Immunol 1998; 102:184–190.
121. Radauer C, Willerroider M, Fuchs H, et al. Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. Clin Exp Allergy 2006; 36:920–929.
122. De Martino M, Novembre E, Cozza G, et al. Sensitivity to tomato and peanut allergens in children monosensitized to grass pollen. Allergy 1988; 43:206–213.
123. Petersen A, Vieths S, Aulepp H, et al. Ubiquitous structures responsible for IgE cross-reactivity between tomato fruit and grass pollen allergens. J Allergy Clin Immuno1 1996; 98:805–813.
124. Calkhoven PG, Aalbers M, Koshte VL, et al. Cross reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. Allergy 1987; 42:382–390.
125. Mari A. Multiple pollen sensitization: a molecular approach to the diagnosis. Int Arch Allergy Immunol 2001; 125:57–65.
126. Aalberse RC, van Ree R. Crossreactive carbohydrate determinants. Clin Rev Allergy Immunol 1997; 15:375–387.
127. Freeman J, Noon L. Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. Lancet 1911; 2:814–817.
128. Cooke RA, Vander Veer A. Human sensitization. J Immuno1 1916; 1:201–237.
129. Leavengood DC, Renard RL, Martin BG, et al. Cross allergenicity among grasses determined by tissue threshold changes. J Allergy Clin Immunol 76:789–794.
130. Joint Task Force on Practice Parameters (AAAAI, ACAAI, JCAAI). Allergen immunotherapy: A practice parameter, second update. J Allergy Clin Immunol 2007; 120:S25–S85.
131. Yu J, Hu S, Wang J, et al. A draft sequence of the rice genome (Oryza sativa L. spp indica). Science 2002;296:79–92.
132. Goff SA, Ricke D, Lan TH, et al. A draft sequence of the rice genome (Orysa saliva L. spp japonica). Science 2002; 296:92–100.
133. Pennisi E. The greening of plant genomics. Science 2007; 317:317.
134. Valdivia ER, Sampedro J, Lamb JC, et al. Recent proliferation and translocation of pollen group 1 allergen genes in the maize genome. Plant Physiol 2007; 143:1269–1281.
135. Bhalla PL, Swoboda I, Singh MB. Antisense-mediated silencing of a gene encoding a major ryegrass pollen allergen. Proc Natl Acad Sci U S A 1999; 96:11676–11680.

8 Weed Pollen Allergens

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INTRODUCTION

Pollens derived from various ragweed species are one of the most important sources of allergenic proteins in different parts of the world. Although pollination typically begins in midsummer and extends to late autumn, the pollinating seasons vary by geographic location. Immunotherapy with ragweed vaccines is effective therapy for ragweed-allergic patients. There are many other allergic weeds, both related and unrelated; however, ragweed species are by far the most important clinically and, hence, have been studied thoroughly. Using molecular biological techniques, investigators have advanced the knowledge of the allergenic constituents of different ragweed species and provided information on the molecular structure of their allergens and their potential cross-reactivity. Special attention is given in this chapter to the integration of morphological, taxonomical, and aerobiological aspects, as well as, the biochemical and clinically relevant aspects of weed pollen allergens.

TAXONOMY OF WEEDS

Of the families of weeds that have been identified, some have been implicated in pollen allergy. As for others, their importance as allergens is unknown. Table 1 provides a comprehensive list of the most prevalent weeds and their botanical classification (taxonomic family and genus), common names, and relative clinical importance (1). A botanist and/or an aerobiologist should be consulted for detailed information on the prevalent weeds in the surrounding geographical areas. The exposure records, as collected and compiled by the American Academy of Allergy, Asthma, and Immunology, can be obtained by calling the Pollen Hotline, 1-800-POLLEN, or by writing to the American Academy of Allergy, Asthma, and Immunology, 611 East Wells Street, Milwaukee, Wisconsin.

WEED AND POLLEN IDENTIFICATION

Although there are many species of ragweed, their morphologies are strikingly similar (2). Some of the important weeds of the United States are shown in Figure 1. The characteristic features of short ragweed (*Ambrosia artemisiifolia*) include a finely divided leaf, each leaf being subdivided into three or more further divided segments, giving the weed a fernlike appearance. The pyramidal-shaped plants grow in the thousands, mostly on land that has been disturbed. They branch extensively, with a ground spread spanning 3 to 4 ft and a height of 4 to 5 ft, producing greenish-yellow flower spikes. In contrast to short ragweed, giant ragweed (*A. trifida*) features broad leaves, which may be three or five lobed or undivided, with blades of the leaf carried down the leaf stalk as narrow wings on each side. Giant ragweed grows above the ground as a columnar bush frequently attaining a height between 10 and 15 ft. Western ragweed is similar to short ragweed in size and habit. However, these weeds typically have inconsistently divided leaves and grow each spring from the roots rather than from seeds. Western giant ragweed differs from its eastern counterpart by its wingless leaves and its tendency to branch well above the ground, rising from the root on a central stalk. Southern ragweed has slender, lance-shaped leaves with one or more large teeth near its base and is

Table 1 Botanical Classification of Important Allergenic Weeds

Family	Genus	Common name	Clinical importance
Compositae	*Ambrosia*	Ragweed	+++
	Franseria	False ragweed	−
	Iva	Marsh elder	+
	Xanthicum	Cocklebur	−
	Artemisia	Sage, mugwort	++
	Chrysanthemum	Daisy	+
	Taraxacum	Dandelion	−
	Parthemum	Guayule	+
	Parthenium	American feverfew	++
Amaranthaceae	*Amaranthus*	Pigweed, amaranthus	+
	Atriplex	Orache, scale	+
	Beta	Beet	+
	Chenopodium	Lamb's quarters Burning bush Russian thistle Pellitory	+
	Kochica	Nettle	+
	Salsola	Patterson's curse	+
Urticaceae	*Parietaria*	Ram	++
	Urtica	Plantain	+
Boraginaceae	*Echium*	Sorrel, dock	+
Brassicaceae	*Brassica*	Mustard	+
Plantaginaceae	*Plantago*	English plantain	+
Polygonaceae	*Rumex*	Unknown	+

Relative clinical importance of the weeds is as follows: +++, major; ++, moderate; +, minor; and −, no or little importance. *Source*: From Ref. 2.

much smaller than other ragweed. All of the above ragweed species are to be distinguished from clinically less important species that are essentially similar in their general appearance but distinct by their shinier seedpods.

Toward the end of summer, most ragweed produce extremely small flowers borne in immense numbers on small heads and arranged in long spikes at the top of the plant and at the ends of side branches. The spikes stand out in the ragweed plant, and these spike-like structures produce pollen. The pollen grains of the large number of different ragweed species vary considerably in size and form, but they are all light, buoyant, and shed in large quantities over prolonged periods. Table 2 summarizes the anatomical features of ragweed pollen (3). Ragweed pollen causes allergic rhinitis, but its relatively large size (~20 pm) suggests that ragweed pollen may be less likely to penetrate past the glottis to cause an asthma exacerbation (4). However, a study by Agarwal et al. indicates that airborne ragweed allergens are present in particles of less than 10 μm in diameter. Ragweed plant debris exists in different-sized particles both before and after the ragweed season. These allergenic particles may contribute to out-of-pollination-season symptoms in many ragweed-sensitive subjects (5).

Other weeds which also produce pollens and cause hay fever, include mugwort, plantain, pellitory, sunflower, marsh elder, cocklebur, sagebrush, chenopods, amaranths, and Russian thistle. Each of these has morphological features distinct from ragweed (Fig. 1). Most of these weeds are suspected to cause allergic asthma, allergic rhinitis, and conjunctivitis; however, except for a small number of species, few studies have been conducted to confirm their allergenic importance or to characterize their allergens.

Mugwort (*Artemisia vulgaris*), which also belongs to the family Compositae, is an aromatic, perennial weed, with stalks of 2 to 4 ft long, petiolate leaves, and small and inconspicuous flower heads. It preferably grows on disturbed ground often along roadways in urban, suburban, and rural areas, and pollinates in late summer (6). Pollen grains of mugwort are oblate spheroidal, 18 to 22 μm in diameter, and normally tricolporate.

The genus *Plantago* belongs to the family Plantaginaceae and comprises about 250 species. One of the most common species is *Plantago lanceolata* (English plantain or ribwort) and has been associated with hay fever since the beginning of last century (7). English plantain is a biennial or perennial herbaceous weed. It has a basal rosette of ribbed lanceolate leaves and

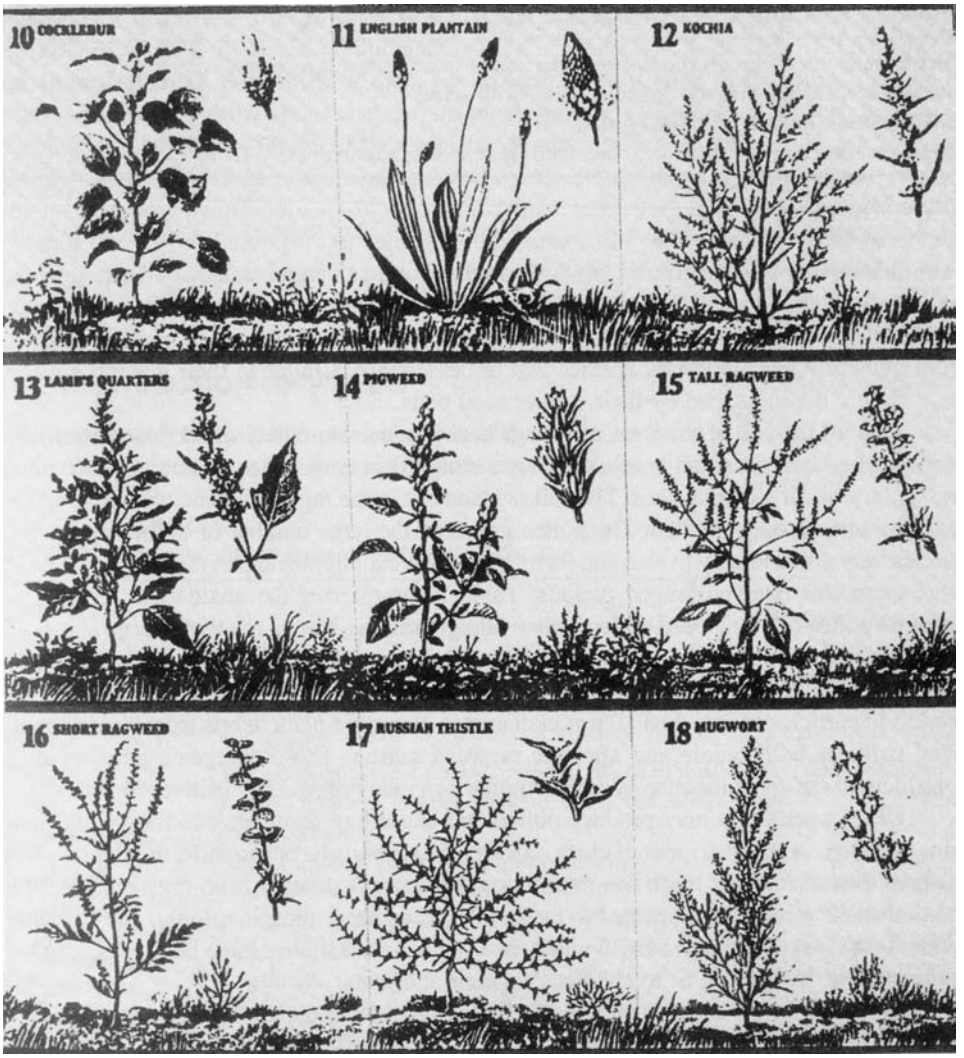

Figure 1 Illustration of some common allergenic weeds in the United States. *Source*: Poster on Center Laboratories Guide to Allergenic Trees, Weeds, and Grasses of the United States.

Table 2 The Characteristic Features of Ragweed Pollens

Tricolporate: three furrows (boat-shaped portion of grain surface), each enclosing a pore
Subechinate: surface coarsely granular, beset with spines
Small: greatest diameter 18–20 μm
Exine: medium size, subechinate, beset with short spines, approximately 1.7–3.4 μm apart

Source: From Ref. 2.

leafless stems up to 2 ft long bearing dense spikes of hermaphrodite flowers protected by oval bracts. It grows in moist soils, waste places, fields, and pastures. Spheroidal, multipored, with six to eight pores, pollen grains (24–28 μm) are airborne during the pollination period that occurs in spring and summer.

The genus *Parietaria* (pellitory), which belongs to the family Urticaceae, is composed of a number of allergenically related species. The most common and best-studied species are *P. judaica* and *P. officinalis*. They are perennial weeds with a barely branched hairy stem of 1 to 3 ft height. They possess oval or lanceolate leaves and axillary agglomerated flowers. The preferred habitat has been defined for some *Parietaria* species. *P. officinalis* is mainly found in mountainous zones; whereas, *P. judaica* grows on walls in urban coastal areas. Nevertheless,

there are no clear limits to their distribution, and two or more different species may grow simultaneously in most regions. *Parietaria* plants produce large amounts of small (12–18 μm), spheroidal tricolporate pollen grains, which are released through a mechanism of propulsion operated by the elastic filament of the anther, ensuring the success of wind-borne pollination. *Parietaria* has a very long pollen season, which extends over several months. Depending on the climate, the pollination may last nearly the whole year, as is the case in southern Italy, where it starts in February and persists until December and has two cycles of flowering (8).

DISTRIBUTION OF WEEDS

The important and clinically relevant weeds of the world are listed in Table 3 (4,9,10). Ragweed is the most important weed in terms of its allergenic pollen worldwide. There are nearly 60 species included in the ragweed family. In the eastern United States, the common or short ragweed and its relative, the giant ragweed, grow in great profusion in roadside ditches and disturbed areas. These two species are the major ragweed found in the northeastern United States, including parts of Canada, but they are much less abundant in the western states and on the pacific coast. Common ragweed is also widespread in China. Since the 1930s, *A. artemisiifolia* along with *Humulus scandens* (Japanese hops) have become the main source of pollen allergens in China (9). Studies in Croatia also identify common ragweed as an important allergen in Central and Southern Croatia (11–13). Western ragweed prevails in the states west of the Rocky Mountains, on the pacific coast, and in the southern and southwestern states from Louisiana to Arizona. Southern ragweed, with typical lance-shaped leaves, is an important cause of hay fever, but it is mainly confined to the central United States. The slender and bur ragweed are among the false ragweeds and consist of about 25 species in the United States. These are common in the southwest and in Colorado; the latter also ranges northward into Canada, where it is abundant in most arid regions.

Seasonal pollen dispersal is important since it suggests the critical period of avoidance for ragweed-sensitive individuals in various locations. Frenz and associates (14) compared

Table 3 Allergenically Relevant Weeds of the World

Country	Weeds
Argentina	Short ragweed
Australia	Capeweed, wattle, plantain, dock, goosefoot family, Paterson's curse, wild mustard
Brazil	Ragweed
Canada	Ragweed, goosefoot family, pigweed, Russian thistle, sage, dock, plantain
Chile	Lambs quarters, English plantain, sweet clover, cocklebur, dandelion, dock, sorrell
China	Japanese hop, common ragweed
Colombia	Pigweed family
Cuba	Short ragweed, goosefoot, pigweed family
Ecuador	Goosefoot and pigweed families
Egypt	Goosefoot and pigweed families
France	Ragweed, sagebrush, plantain, Pellitory, goosefoot, and pigweed families
Russia	Sagebrush, Ragweed
Germany	Dock, nettle, plantain, goosefoot family, sagebrush
Great Britain	Sunflower family, plantain, sorrels and docks, nettle, goosefoot, mugwort
Hawaii	Goosefoot and pigweed families
Hungary	Plantain, dock, goosefoot, hemp, ragweed, sagebrush, pigweed, cocklebur
India	Cocklebur, hemp, sagebrush, dock, goosefoot and pigweed family, castor bean, mugwort
Italy	Pellitory, mugwort, sunflower, warnwood, burweed
Japan	Sagebrush, ragweed, English plantain
The Netherlands	Ragweed, sagebrush, sedges, cattails
New Zealand	Plantain, dock, goosefoot family
Portugal	Goosefoot and pigweed families, cocklebur, plantain, sage, pellitory
Romania	Goosefoot, sages, ragweed
Sweden	Dock, sagebrush, goosefoot family, pellitory
United States	Ragweeds (short, giant, western, southern, slender, and false ragweeds), burweed, marsh elder, cocklebur, sages, pigweed, Russian thistle, firebush
Yugoslavia	Hemp, pellitory, dock, goosefoot and pigweed families, ragweed, plantain, castor bean

Source: From Ref. 1.

ragweed pollen dispersal in the United States using volumetric techniques and recorded the date of first and final pollen capture and the date of maximum airborne pollen concentration at locations ranging from 30° to 45° north latitude. Sixteen cities located 38° N have similar peak dates, achieving maximal pollen concentration in late August or early September. Four cities, located south of 38° N, experienced later peak dates, reaching maximum pollen levels in mid-October.

Mugwort is widely spread, especially in the temperate and humid zones of the northern hemisphere and along the Mediterranean basin. Mugwort is considered the most important cause of pollinosis at the end of summer and the beginning of autumn in Europe. Pollination occurs at the end of July and through August in Central Europe and takes place mostly in September and beginning of October (15) in the Mediterranean areas.

English plantain is distributed in the temperate zones of both hemispheres. It was introduced from Europe into North America and is now found throughout Canada and the United States. Furthermore, English plantain is widespread in Asia, Australia, and Japan. The pollen season begins in April or early May, peaking in May–July, depending on the latitude and climatic conditions, and continues throughout the remainder of summer and early fall. The clinical importance of pollinosis caused by English plantain has been underestimated for a long time because of the low frequency of monosensitized patients and the overlap of the pollen season with that of grasses. Nevertheless, a high incidence of allergy to English plantain pollen has been reported in the last two decades in different geographic areas, particularly in the Mediterranean basin, Australia, and Japan (10,16,17).

Parietaria is the most important allergenic plant in most regions of the Mediterranean surrounding countries (8). The abundance of this plant, and hence its allergenic relevance, decreases in Europe as the latitude increases, although sensitization to *Parietaria* has been reported to occur as far north as southern England (18). Only one report identifying *Parietaria* as a possible cause of respiratory allergy in the United States has been published so far, in which 8 out of 100 sequential patients with seasonal respiratory allergy referred to the allergist practice were prick tested positive to *Parietaria* (19). One important characteristic of *Parietaria* is the very long pollination period, resulting in multiseasonal or almost perennial symptoms shown by allergic patients, ranging from mild rhinoconjunctivitis to severe asthma. The prevalence of asthma is very high (40–60%) in regions in which this plant is prevalent, such as central and southern Italy (20).

MOLECULAR CHARACTERISTICS OF RAGWEED AND OTHER WEED POLLEN ALLERGENS

Ragweed

Although a number of allergens from diverse weeds have been studied, the most extensively investigated is short ragweed. The allergens described from various weeds are listed in Table 4 (16–19,21,22). Crossed-radioimmunoelectrophoresis of aqueous short ragweed pollen extract detected 22 distinct proteins which bound to specific human IgE antibodies (23,24). However, not all of these allergens are fully characterized.

Amb a 1 is the predominant allergen from short ragweed pollen. Amb a 1(formerly known as antigen E) first purified and identified in 1964 (23–25), consists of an alpha and a beta chain. Amb a 1 constitutes about 6% of the protein content of short ragweed pollen, and about 90% of ragweed-sensitive subjects have Amb a 1-specific antibodies. Using site-specific monoclonal antibodies, a small number of major antigenic determinants were found on the native Amb a 1 molecule (26). The cloning of Amb a 1 polypeptide subunits reveal that short ragweed pollen contains four isoforms of Amb a 1, designated as 1.1, 1.2, 1.3, and 1.4 (25). The IgE binding of these isoforms indicates that they differ in their capacity to bind human IgE with a ranking of 1.1 > l.3 > I.4 > I.2. T-cell responses to these individual isoforms of Amb a 1, measured by the stimulation index (SI), which is an index of T-cell proliferation compared with control cells of T-cell lines, show a different ranking: 1.1 (SI 25), 1.2 (SI 4.2), 1.3 (SI 9.1), and 1.4 (SI 8.3). Together, these studies confirm that Amb a 1 is the dominant allergen of short ragweed pollen (25).

Amb a 2 is the second most important short ragweed allergen and is closely related to Amb a 1 (65% amino acid identity). Amb a 1 is present in both the pollen and flower heads of

Table 4 Weed Pollen Allergens

Botanical name (common name)	Allergens	Molecular weight (kDa)
Ambrosia artemisiifolia (short ragweed)	Amb a 1 (antigen E)	38
	Amb a 2 (antigen K)	38
	Amb a 3 (Ra 3)	11
	Amb a 5 (Ra 5)	5
	Amb a 6 (Ra 6)	10
	Amb a 7 (Ra 7)	12
	Amb a	11
	Amb a 8	14
	Amb a 9	9
Ambrosia trifida (giant ragweed)	Amb t 5 (Ra 5G)	4.4
Artemisia vulgaris (mugwort)	Art v 1	27–29
	Art v 2 (Ag 7)	35
	Art v 3 (LTP)	12
	Art v 4 (profilin)	14
	Art v (unclassified)	60
	Art v 5	9
	Art v 6	42
Parietaria judaica (pellitory of the wall)	Par j 1	10–15
	Par j 2	11.3
	Par j 3 (profilin)	14
Parietaria officinalis	Par o 1	11–15
Plantago lanceolata (English plantain)	Pla l 1	17–20
Chenopodium album (Lamb's quarter)	Che a 1	17
Salsola kali (Russian thistle)	Sal k 1	43
Helianthus annuus (sunflower)	Hel a 1	34
	Hel a 2 (profilin)	15.7
Mercurialis annua	Mer a 1 (profilin)	14–15

short ragweed, while Amb a 2 is detectable only in flower heads. *Escherichia coli* recombinant and native Amb a 2 differ in their ability to bind human 1gE antibodies (27), indicating that the recombinant protein is not as allergenic as the native protein. More than 50% of the T-cell lines could be stimulated with Amb a 2, exhibiting an average SI of 14 (25).

Amb a 3 is a basic glycoprotein, having a single polypeptide chain composed of 101 amino acid residues (28). Clinical testing shows that Amb a 3 is highly allergenic in about 30% to 50% of short ragweed-sensitive patients (29) and therefore, is a minor allergen. The antibody and T lymphocyte recognition regions on short ragweed allergen Amb a 3 (Ra3) are characterized (30).

Amb a 5 is one of the most studied among the minor ragweed allergens. About 10% to 20% of short ragweed-allergic subjects are sensitized to this allergen (31,32). The Amb a 5 allergens are cloned and sequenced from different species of ragweed and are characterized with respect to their B- and T-cell epitopes (33). The three-dimensional structures of Amb a 1 and Amb a 5 were also derived by two-dimensional spectroscopy (34,35). The HLA association study of the human allergic immune response demonstrates that all Amb a 5 allergen immune responses are restricted by the same DR molecule (36).

Amb a 6 and four other minor allergens have also been defined in the pollens of short ragweed. By Radioallergosorbent test (RAST) analysis, between 17% and 51% of ragweed-allergic patients exhibit IgE antibodies that bind to these minor allergens (21,37,38). Amb a 7 is highly basic, blue protein and is postulated to be a plastocyanin. Approximately 15% to 20% of ragweed-allergic individuals have antibodies that react to Amb a 7 (39). Three additional allergens have been identified by molecular weight (Table 4). One of these allergens with molecular weight 11 kDa may actually be an isoform of Amb a 3. Two additional allergens have been tentatively identified as Amb a 8 and Amb a 9 in Table 4.

Other Weed Pollen Allergens

Allergens from other weeds are important in different geographic regions of the world. These include mugwort (40–47), English plantain (48–51), *Parietaria* (52–61), sunflower (62,63), lamb's quarter (64), Russian thistle (65), and parthenium (66).

Mugwort pollens contain approximately 40 extractable proteins of which 10 appear to be allergens (40). Five allergens from mugwort are characterized although none of them are yet included in the official list of allergens of the International Union of Immunological Societies because no sequence information is available. The first allergen isolated from mugwort is termed Art v 1 in the article dealing with its purification (41). It is a monomeric, acidic glycoprotein of 60 kDa in SDS-PAGE that is recognized by the IgE from 73% of mugwort allergic patients. The allergen, Art v 1, in the official list of allergens, is a different glycoprotein, with 108 amino acid residues and high sugar content (30–40%), which 95% of the individuals allergic to mugwort have specific IgE. The official Art v 1 is a modular glycoprotein with an N-terminal cysteine-rich domain homologous to plant defensins and a C-terminal domain rich in hydroxyproline residues, some of which are O-glycosylated (42). The carbohydrate moiety is highly heterogeneous (two major series of peaks centered around 13.4 and 15.6 kDa are observed in mass spectra of the natural allergen), and the carbohydrate greatly influences the electrophoretic mobility of the allergen, since the apparent molecular weight in SDS-PAGE is as large as 27 to 29 kDa. The carbohydrate moiety is likely important in the allergenicity of the Art v (42). A single immunodominant T-cell epitope is recognized by 81% of affected patients (43).

Art v 2 is also a glycosylated protein (10% carbohydrate content) that consists of two identical polypeptide chains covalently linked by disulfide bridges. It exists in at least six different isoforms, and it cannot be considered a major allergen, since it binds IgE from only 33% of sera from mugwort allergic patients (44).

Two plant panallergens (a panallergen is an allergen present in multiple unrelated species), lipid-transfer protein (LTP) and profilin, are present in mugwort pollen. Art v 3 belongs to the LTP family. The N-terminal amino acid sequence of this allergen, covering more than one-third of its complete sequence, has a 40% to 50% sequence identity with LTPs from the family Rosaceae fruits (40). A positive skin prick test to Art v 3 is present in 40% of mugwort allergic patients (46). Art v 4 is mugwort profilin, and 36% of mugwort-sensitive patients have IgE antibodies against this allergen (47). Two additional mugwort allergens have been tentatively listed as Art v 5 and Art v 6 because of their distinct molecular weights (Table 4).

English plantain pollen contains 5 to 10 allergenic proteins (48–50). The prevalence of specific IgE to the major allergen, Pla l 1, in plantain allergic patients is about 90%. Pla l 1 is a mixture of isoforms that may occur in glycosylated and unglycosylated forms (45,46). Three Pla l 1 variants are sequenced and display about 40% sequence identity with the major *Olea europaea* pollen allergen, Ole e 1 (51). Although authors differ about the number of allergens present in *Parietaria* pollen, all agree that a highly heterogeneous glycoprotein with a molecular weight in the range 10 to 15 kDa is the main allergen, inducing an IgE response in at least 95% of *Parietaria* allergic patients (52,53). The major allergens from *P. judaica* and *P. officinalis*, Par j 1 and Par o 1, isolated from their respective pollens, exhibit very similar physicochemical and immunochemical properties (54–56). Different Par j 1 isoforms and variants have been isolated both from the natural source and through recombinant expression (57–59). Another allergen, Par j 2, sharing 45% sequence identity and an immunodominant IgE epitope with Par j 1, has been produced as a recombinant protein (60,67). Both Par j 1 and Par j 2 are related to the plant LTP family. The panallergen profilin has been identified in *P. judaica* pollen and named Par j 3 (61). A calcium-binding protein (see chap. 6) in *Parietaria* has also been identified as a panallergen (68).

WEED POLLEN ALLERGEN CROSS-REACTIVITY

Plants with a close taxonomic relationship will most likely have pollen proteins with homologous sequences. Clinical studies reveal that skin test-positive, ragweed-allergic patients are also positive to pollen proteins derived from several distinct plant families (69). Cross-reactivity among geographically distant pollen allergens also occurs (70). The cross-reactivity among weed pollen allergens may be categorized to between species (interspecies) and in different strains within a species (intraspecies). Table 5 summarizes the western blotting analyses of pollen proteins from different ragweeds that demonstrate both intra- and interspecies cross-reactivity (25). The results of these studies show that the Amb a 1 and Amb a 2 allergens of short ragweed not only share significant homologies with each other but also

Table 5 Cross-Reactivity Among Weed Pollen Allergens

	Anti-Amb a 1	Anti-Amb a 2	Anti-Amb a 2
Ragweed species	pAbs	PAbs	mAb
False ragweed (*Franseria acanthicarpa*)	Yes	Yes	No
Slender ragweed (*F. ensifolia*)	Yes	Yes	No
Wooly ragweed (*F. tomentosa*)	Yes	Yes	No
Short ragweed (*A. artemisiifolia*)	Yes	Yes	Yes
Southern ragweed (*A. bidentata*)	Yes	Yes	No
Western ragweed (*A. psilostachya*)	Yes	Yes	Yes
Western giant ragweed (*A. aptera*)	Yes	Yes	No
Giant ragweed (*A. trifida*)	Yes	Yes	No

Abbreviations: pAb, polyclonal antibodies; mAb, monoclonal antibody.

Table 6 Interspecies Cross-Reactivity of Weed Pollen

Species (common name)	Ragweed allergens	Remarks
Phleum pratense (Phl p 4, timothy grass)	Amb a 1	Basis for cross-reactivity between grass and weed allergens
Chamaecyparis obtusa (*Cha a I*, Japanese cypress)	Amb a 1 Amb a 2	46–49% sequence identity
Cryptomeria japonica (Cry j 1, Japanese cedar)	Amb a 1 Amb a 2	46–49% sequence identity
Zea mays (corn)	Amb a 1	Sequence homology
Parthenium histerophorus (American feverfew)	Amb a	82–94% cross-inhibition

with the equivalent allergens from other ragweed species (25). Thus, these two allergens have not diverged significantly throughout the evolution of different ragweed species. Similarly, Amb a 5 and Amb t 5 (*A. trifida*) share 49% identity in their amino acid sequence (33).

In addition to the cross-reactivity among related ragweeds, the cross-reactivity of ragweed allergens and the allergens of other plants have been reported. Some of these studies are listed in Table 6. Analysis by RAST and immunoblotting inhibition reveals cross-reactivity between sunflower pollen and other pollens of the Compositae family (mugwort, marguerite, goldenrod, and short ragweed). Mugwort pollen exhibits the greatest degree of allergenic homology with sunflower pollen; whereas, at the other end of the spectrum, short ragweed shows less cross-reactive epitopes (71). One study shows that there is no cross-reactivity between mugwort and ragweed pollens (72); whereas, another indicates that the pollens contain a number of cross-reactive allergens, including the major mugwort allergen Art v 1 and profilin (73). Another study by Asero et al. demonstrates that mugwort reactivity on skin prick test was strongly associated with ragweed hypersensitivity (74).

Skin tests and tests for IgE antibodies of ragweed-sensitive subjects are usually positive to a number of different pollens, frequently from taxonomically diverse species, which are assumed to be allergenically not cross-reactive (75–78). Cross-reactivity occurs between ragweed and a number of vegetables, including fennel, parsley, and carrot (79). Several other weed pollen food syndromes include mugwort-celery-spice (80,81) and ragweed-melon-banana syndromes, the former being the best characterized (81). Parthenium, a weed introduced from the United States into India, is the major aeroallergen in southern India. Parthenium allergens are cross-reactive with short ragweed pollen (82). Similarly, recombinant proteins from *H. scandens* in Japan react with sera from patients allergic to *A. artemisiifolia* (9). The panallergens, profilin and calcium-binding proteins, present in weed pollen may be responsible for the extensive cross-reactivity among pollen-sensitized patients (83). One study demonstrates that villin-related proteins (cytoskeletal proteins) occur in pollens of weeds, namely, mugwort, trees, and grasses; thus, villin-related proteins may represent a novel family of cross-reactive allergens (84). In effect, the presence of pollen-reactive IgE antibodies may not necessarily reflect the sensitizing pollen species. This information is clinically important in view of the increased and rapid migration of people throughout the world.

In vitro and in vivo studies suggest that sensitization to the cross-reactive mugwort LTP, Art v 3, may extend the recognition pattern of these patients to more distantly related species (45,46). Although Par j 1 and Par j 2 are also related to the LTP family, an association between sensitization to *Parietaria* and Rosaceae fruits has not been demonstrated. It is worth mentioning that sensitization to *Parietaria* normally means sensitization to several species of this genus, since a strong cross-reactivity occurs among the major allergens from different species within the genus (85). The major allergens implicated in cross reactivity among the species are Par j 1 and Par j 2 (86).

A 30-kDa allergen in English plantain cross-reacts with the grass Group 5 allergens, yet this cross-reactivity shows little or no clinical relevance (49). In the same way, there is a rather limited allergenic cross-reactivity between Pla l 1 and Ole e 1, despite structural similarity (51).

WEED IMMUNOTHERAPY

The effectiveness of ragweed immunotherapy was established in the 1960s, and simultaneously the allergenic composition of the extract was determined (87–89). There have been attempts, however, to investigate the efficacy and safety of variations in the approach to immunotherapy. In an effort to increase the safety of allergen immunotherapy, some clinical studies have been performed using chemically modified (90) peptide fragments of ragweed vaccine (91), or encapsulated allergens (92). Studies involve the use of immunostimulatory adjuvants, such as monophosphoryl lipid A (MPL), a detoxified form of lipid A from the lipopolysaccharide of *Salmonella minnesota*; a ragweed-Toll-like receptor ligand; and other forms of chemical modification that entail the use of immunostimulatory DNA sequences (93–95). Allergens modified with immunostimulatory DNA mask IgE epitopes and stimulate a Th-1 immune response (95). The goal of these studies is to produce safer and more efficacious immunotherapy; however, none of these modified products are utilized currently in clinical practice. The original immunotherapy protocols for ragweed-allergic subjects remain unchanged, except that ragweed allergens used today are standardized in the United States with respect to the content of Amb a 1, the major ragweed allergen (88,92). Similarly, methods to determine the concentration of the major allergens have been applied to *Parietaria*, mugwort, and English plantain pollens, and some companies market allergenic products of these species that are standardized with their own "in-house" process of standardization (85,96–98).

Sublingual immunotherapy experience with weeds is limited. Evidence exists regarding safety and efficacy of sublingual immunotherapy for ragweed rhinoconjunctivitis (99).

Short and giant ragweeds are largely cross-reactive, and usually one species is used for skin testing and immunotherapy (100). However, in a small group of patients, Asero et al. demonstrated that short and giant ragweeds are not totally allergenically equivalent. They suggest that diagnostic and therapeutic procedures be conducted using both species (101).

Rush subcutaneous ragweed immunotherapy, used in conjunction with the humanized monoclonal anti-IgE, omalizumab, demonstrates enhanced safety of immunotherapy, presumably by downregulating the expression of the IgE receptors on mast cells and basophils as well as reducing ragweed-specific IgE (102,103). Studies have also been conducted to produce a safer and more efficacious immunotherapy against *Parietaria* allergens. Reduced IgE binding and reduced allergenic activity have been observed using the hybrid proteins Par j 1 and Par j 2 (104). Clinical studies are underway for evaluating the effectiveness of these proteins in *Parietaria* allergic patients.

SALIENT POINTS

- A large number of weed species contribute to the seasonal increases in weed pollen allergens in the air. The most important allergenic pollens are derived from ragweed and its relatives, mugwort, and pellitory.
- The identification of local and regional weed plants and weed pollens is important for clinical practice.
- The cross-reactivity of weed allergens should be considered in the management of weed-allergic subjects.

- The most important allergens of short ragweed are the major allergens, Amb a 1 and Amb a 2. These two major allergens and other minor short ragweed allergens have been characterized in terms of their molecular structure and cross-reactivity.
- Many weed pollen allergens are cross-reactive. Amb a 1, the major allergen of ragweed, cross-reacts with other allergens of ragweed pollen and also cross-reacts with allergens from taxonomically diverse genera and species. On the basis of cross-reactivity, weed allergens can be categorized into three classes: (*i*) ragweed and related plants, including parthenium; (*ii*) mugwort and sunflower; and (*iii*) *Parietaria*.
- The immunotherapy of weed-allergic subjects in the United States is conducted with ragweed allergen vaccines standardized with respect to Amb a 1 content.

REFERENCES

1. Holm L, Doll J, Holm E, et al. World Weeds: Natural Histories and Distributions. New York: John Wiley & Sons, 1997.
2. Wodenhouse R. Pollengrains: Their Structure, Identification, and Significance in Science and Medicine. New York: McGraw-Hill Book Co. Inc., 1935.
3. Agriculture, Agricultural Research Service and the US Department of Agriculture. Common Weeds of the United States. New York: Dover Publications, Inc., 1971.
4. Gutman A, Bush RK. Allergens and other factors important in atopic disease. In: Patterson R, Grammer LC, Greenberger PA, Zeiss CR, Eds. Allergic Diseases: Diagnosis and Management. 4th ed. Philadelphia: Lippincott, 1993:93–158.
5. Agarwal MK, Swanson MC, Reed CE, et al. Airborne ragweed allergens: association with various particle sizes and short ragweed plant parts. J Allergy Clin Immunol 1984; 74(5):687–693.
6. Spieksma FTM, PC Von Waill, et al. Allergenic Significance of Artemesia (Mugwort) Pollen. Oxford: Blackwell Scientific Publications, 1991:121–124.
7. Bernton H. Plantain hay fever and asthma. JAMA 1925; 84:944–946.
8. D'Amato G, et al. Parietaria pollinosis: a review. Allergy 1992; 47(5):443–449.
9. Tao AL, He SH. Cloning, expression, and characterization of pollen allergens from Humulus scandens (Lour) Merr and Ambrosia artemisiifolia L. Acta Pharmacol Sin 2005; 26(10):1225–1232.
10. Nakamaru Y, et al. Plantago lanceolata (English plantain) pollinosis in Japan. Auris Nasus Larynx 2005; 32(3):251–256.
11. Cvitanovic S, et al. Allergic rhinitis and asthma in southern Croatia: impact of sensitization to Ambrosia elatior. Croat Med J 2007; 48(1):68–75.
12. Peternel R, et al. Variation in ragweed (Ambrosia artemisiifolia L.) pollen concentration in central Croatia, 2002 to 2003. Ann Agric Environ Med 2005; 12(1):11–16.
13. Prus V, Culjak Z. [Ragweed hypersensitivity in Osijek-Baranja region]. Arh Hig Rada Toksikol 2004; 55(2–3):155–158.
14. Frenz DA, et al. Seasonal characteristics of ragweed pollen dispersal in the United States. Ann Allergy Asthma Immunol 1995; 75(5):417–422.
15. Spieksma FT, et al. City spore concentrations in the European Economic Community (EEC). IV. Summer weed pollen (Rumex, Plantago, Chenopodiaceae, Artemisia), 1976 and 1977. Clin Allergy 1980; 10(3):319–329.
16. Subiza J, et al. Allergenic pollen pollinosis in Madrid. J Allergy Clin Immunol 1995; 96(1):15–23.
17. Krilis S, Baldo BA, Basten A. Analysis of allergen-specific IgE responses in 341 allergic patients. Associations between allergens and between allergen groups and clinical diagnoses. Aust N Z J Med 1985; 15(4):421–426.
18. Holgate ST, et al. Sensitivity to Parietaria pollen in the Southampton area as determined by skin-prick and RAST tests. Clin Allergy 1988; 18(6):549–556.
19. Kaufman HS. Parietaria: an unrecognized cause of respiratory allergy in the United States. Ann Allergy 1990; 64(3):293–296.
20. D'Amato G, Lobefalo G. Allergenic pollens in the southern Mediterranean area. J Allergy Clin Immunol 1989; 83(1):116–122.
21. Lubahn BK, Klapper DG. Cloning and characterization of ragweed allergen Amb a VI. J Allergy Clin Immunol 1993; 91:338.
22. Roebber M, et al. Isolation and properties of a new short ragweed pollen allergen, Ra6. J Immunol 1983; 131(2):706–711.
23. King TP, Norman PS, Connell JT. Isolation and characterization of allergens from ragweed pollen. II. Biochemistry 1964; 3:458–468.
24. King TP, Norman PS, Lichtenstein LM. Isolation and characterization of allergens from ragweed pollen. IV. Biochemistry 1967; 6(7):1992–2000.

25. Rogers BL, Bond JF, Morgenstern IP, et al. Immunochemical characterization of the major ragweed allergens Amba I and Amba II. In: Mohapatra SS, Knox RB, eds. Pollen Biotechnology, Gene Expression, and Allergen Characterization. New York: Chapman & Hall, 1996:13.
26. Smith JJ, Olson JR, Klapper DG. Monoclonal antibodies to denatured ragweed pollen allergen Amb a I: characterization, specificity for the denatured allergen, and utilization for the isolation of immunogenic peptides of Amb a I. Mol Immunol 1988; 25(4):355–365.
27. Rogers BL, et al. Complete sequence of the allergen Amb alpha II. Recombinant expression and reactivity with T cells from ragweed allergic patients. J Immunol 1991; 147(8):2547–2552.
28. Klapper DG, Goodfriend L, Capra JD. Amino acid sequence of ragweed allergen Ra3. Biochemistry 1980; 19(25):5729–5734.
29. Adolphson C, Goodfriend L, Gleich GJ. Reactivity of ragweed allergens with IgE antibodies. Analyses by leukocyte histamine release and the radioallergosorbent test and determination of cross-reactivity. J Allergy Clin Immunol 1978; 62(4):197–210.
30. Atassi H, Atassi MZ. Mapping of allergen epitopes by antibody and T-cells. In: Sehon AH, Kraft D, Kunkel G, eds. Epitopes of atopic allergens. Brussels: The UCB Institute of Allergy, 1990:33.
31. Roebber M, et al. Immunochemical and genetic studies of Amb.t. V (Ra5G), an Ra5 homologue from giant ragweed pollen. J Immunol 1985; 134(5):3062–3069.
32. Goodfriend L, et al. Ra5G, a homologue of Ra5 in giant ragweed pollen: isolation, HLA-DR-associated activity and amino acid sequence. Mol Immunol 1985; 22(8):899–906.
33. Ghosh B, et al. Cloning and expression of immunologically active recombinant Amb a V allergen of short ragweed (Ambrosia artemisiifolia) pollen. J Immunol 1993; 150(12):5391–5399.
34. Metzler WJ, et al. Determination of the three-dimensional solution structure of ragweed allergen Amb t V by nuclear magnetic resonance spectroscopy. Biochemistry 1992; 31(22):5117–5127.
35. Metzler WJ, et al. Proton resonance assignments and three-dimensional solution structure of the ragweed allergen Amb a V by nuclear magnetic resonance spectroscopy. Biochemistry 1992; 31(37): 8697–8705.
36. Rafner T, Metzler WJ, Marsh DG. The Amb V allergens from Ragweed. In: Mohapatra SS, Knox B, eds. Pollen Biotechnology: Gene Expression and Allergen Characterization. New York: Chapman & Hall, 1996:9.
37. Pilyavskaya A, et al. Isolation and characterization of a new basic antigen from short ragweed pollen (Ambrosia artemisiifolia). Mol Immunol 1995; 32(7):523–529.
38. Rogers BL, et al. Sequence of the proteinase-inhibitor cystatin homologue from the pollen of Ambrosia artemisiifolia (short ragweed). Gene 1993; 133(2):219–221.
39. Wopfner N, et al. The spectrum of allergens in ragweed and mugwort pollen. Int Arch Allergy Immunol 2005; 138(4):337–346.
40. Ipsen H, et al. Immunochemical and biological characterization of a mugwort (Artemisia vulgaris) pollen extract. Allergy 1985; 40(4):289–294.
41. de la Hoz F, et al. Purification of Art v I, a relevant allergen of Artemisia vulgaris pollen. Mol Immunol 1990; 27(7):651–657.
42. Himly M, et al. Art v 1, the major allergen of mugwort pollen, is a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain. FASEB J 2003; 17(1):106–108.
43. Jahn-Schmid B, et al. The T cell response to Art v 1, the major mugwort pollen allergen, is dominated by one epitope. J Immunol 2002; 169(10):6005–6011.
44. Nilsen BM, et al. Structural analysis of the glycoprotein allergen Art v II from the pollen of mugwort (Artemisia vulgaris L.). J Biol Chem 1991; 266(4):2660–2668.
45. Diaz-Perales A, et al. Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of Artemisia pollen, Castanea nut and Rosaceae fruits, with different IgE-binding capacities. Clin Exp Allergy 2000; 30(10):1403–1410.
46. Garcia-Selles FJ, et al. Patterns of reactivity to lipid transfer proteins of plant foods and Artemisia pollen: an in vivo study. Int Arch Allergy Immunol 2002; 128(2):115–122.
47. Wopfner N, et al. Molecular and immunological characterization of profilin from mugwort pollen. Biol Chem 2002; 383(11):1779–1789.
48. Baldo BA, et al. Allergens from plantain (plantago lanceolata). Studies with pollen and plant extracts. Int Arch Allergy Appl Immunol 1982; 68(4):295–304.
49. Asero R, et al. Detection of allergens in plantain (Plantago lanceolata) pollen. Allergy 2000; 55(11): 1059–1062.
50. Calabozo B, Barber D, Polo F. Purification and characterization of the main allergen of Plantago lanceolata pollen, Pla l 1. Clin Exp Allergy 2001; 31(2):322–330.
51. Calabozo BD-P, Salcedo A, Barber G, et al. Structural and antigenic similarity between Pla 11 and Ole e 1, the major allergens of English plantain olive pollens. J Allergy Clin Immunol 2001; 107(2):S15 (abstr.).
52. Corbi AL, Carreira J. Identification and characterization of Parietaria judaica allergens. Int Arch Allergy Appl Immunol 1984; 74(4):318–323.

53. Ford SA, et al. Identification of Parietaria judaica pollen allergens. Int Arch Allergy Appl Immunol 1986; 79(2):120–126.
54. Polo F, Ayuso R, Carreira J. HPLC purification of the main allergen of Parietaria judaica pollen. Mol Immunol 1990; 27(2):151–157.
55. Oreste U, et al. Purification and characterization of Par o I, major allergen of Parietaria officinalis pollen. Int Arch Allergy Appl Immunol 1991; 96(1):19–27.
56. Kahlert H, et al. Characterization of major allergens of Parietaria officinalis. Int Arch Allergy Immunol 1996; 109(2):141–149.
57. Ayuso R, et al. Isolation by mAb based affinity chromatography of two Par j I isoallergens. Comparison of their physicochemical, immunochemical and allergenic properties. Mol Immunol 1993; 30(15):1347–1354.
58. Costa MA, et al. cDNA cloning, expression and primary structure of Par jI, a major allergen of Parietaria judaica pollen. FEBS Lett 1994; 341(2–3):182–186.
59. Duro G, et al. Isolation and characterization of two cDNA clones coding for isoforms of the Parietaria judaica major allergen Par j 1.0101. Int Arch Allergy Immunol 1997; 112(4):348–355.
60. Colombo P, et al. Identification of an immunodominant IgE epitope of the Parietaria judaica major allergen. J Immunol 1998; 160(6):2780–2785.
61. Asturias JA, et al. Recombinant DNA technology in allergology: cloning and expression of plant profilins. Allergol Immunopathol (Madr) 1997; 25(3):127–134.
62. de la Hoz F, et al. Isolation and partial characterization of allergens from Helianthus annuus (sunflower) pollen. Allergy 1994; 49(10):848–854.
63. Jimenez A, et al. Sensitization to sunflower pollen: only an occupational allergy? Int Arch Allergy Immunol 1994; 105(3):297–307.
64. Barderas R, et al. Identification and characterization of Che a 1 allergen from Chenopodium album pollen. Int Arch Allergy Immunol 2002; 127(1):47–54.
65. Carnes J, et al. Immunochemical characterization of Russian thistle (Salsola kali) pollen extracts. Purification of the allergen Sal k 1. Allergy 2003; 58(11):1152–1156.
66. Gupta N, et al. Identification of a novel hydroxyproline-rich glycoprotein as the major allergen in Parthenium pollen. J Allergy Clin Immunol 1996; 98(5 pt 1):903–912.
67. Duro G, et al. cDNA cloning, sequence analysis and allergological characterization of Par j 2.0101, a new major allergen of the Parietaria judaica pollen. FEBS Lett 1996; 399(3):295–298.
68. Asturias JA, et al. PCR-based cloning and immunological characterization of Parietaria judaica pollen profilin. J Investig Allergol Clin Immunol 2004; 14(1):43–48.
69. Weber RW, Nelson HS. Pollen allergens and their interrelationships. Clin Rev Allergy 1985; 3(3): 291–318.
70. Sharma S, et al. Evaluation of cross-reactivity between Holoptelea integrifolia and Parietaria judaica. Int Arch Allergy Immunol 2005; 136(2):103–112.
71. Fernandez C, et al. Analysis of cross-reactivity between sunflower pollen and other pollens of the Compositae family. J Allergy Clin Immunol 1993; 92(5):660–667.
72. Park HS, Kim MJ, Moon HB. Antigenic relationship between mugwort and ragweed pollens by crossed immunoelectrophoresis. J Korean Med Sci 1994; 9(3):213–217.
73. Hirschwehr R, et al. Identification of common allergenic structures in mugwort and ragweed pollen. J Allergy Clin Immunol 1998; 101(2 pt 1):196–206.
74. Asero R, et al. Artemisia and Ambrosia hypersensitivity: co-sensitization or co-recognition? Clin Exp Allergy 2006; 36(5):658–665.
75. Fischer S, et al. Characterization of Phl p 4, a major timothy grass (Phleum pratense) pollen allergen. J Allergy Clin Immunol 1996; 98(1):189–198.
76. Astwood JD, et al. Pollen allergen homologues in barley and other crop species. Clin Exp Allergy 1995; 25(1):66–72.
77. Mohapatra SS. Determinant spreading: implications in allergic disorders. Immunol Today 1994; 15(12): 596–597.
78. Turcich MP, Hamilton DA, Mascarenhas JP. Isolation and characterization of pollen-specific maize genes with sequence homology to ragweed allergens and pectate lyases. Plant Mol Biol 1993; 23(5): 1061–1065.
79. Bonnin JP, et al. [A very significant case of allergy to celery cross-reacting with ragweed]. Allerg Immunol (Paris) 1995; 27(3):91–93.
80. Wuthrich B, Dietschi R. [The celery-carrot-mugwort-condiment syndrome: skin test and RAST results]. Schweiz Med Wochenschr 1985; 115(11):258–264.
81. Egger M, et al. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy 2006; 61(4):461–476.
82. Sriramarao P, Rao PV. Allergenic cross-reactivity between Parthenium and ragweed pollen allergens. Int Arch Allergy Immunol 1993; 100(1):79–85.
83. Gadermaier G, et al. Biology of weed pollen allergens. Curr Allergy Asthma Rep 2004; 4(5):391–400.

84. Mittermann I, et al. Identification of a villin-related tobacco protein as a novel cross-reactive plant allergen. FEBS Lett 2005; 579(17):3807–3813.
85. Ayuso R, Carreira J, Polo F. Quantitation of the major allergen of several Parietaria pollens by an anti-Par 1 monoclonal antibody-based ELISA. Analysis of crossreactivity among purified Par j 1, Par o 1 and Par m 1 allergens. Clin Exp Allergy 1995; 25(10):993–999.
86. Bonura A, et al. Cross-reactivity between Parietaria species using the major rParj1 and rParj2 allergens. Allergy Asthma Proc 2006; 27(5):378–382.
87. Creticos P. Efficacy Parameters. Milwaukee, WI: American Academy of Allergy and Immunology, 1994.
88. Helm RM, et al. Production and testing of an international reference standard of short ragweed pollen extract. J Allergy Clin Immunol 1984; 73(6):790–800.
89. Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hay fever with ragweed antigen E: comparisons with whole pollen extract and placebos. J Allergy 1968; 42(2):93–108.
90. Grammer LC, et al. A double-blind, placebo-controlled trial of polymerized whole ragweed for immunotherapy of ragweed allergy. J Allergy Clin Immunol 1982; 69(6):494–499.
91. Litwin A, et al. Regulation of the human immune response to ragweed pollen by immunotherapy. A controlled trial comparing the effect of immunosuppressive peptic fragments of short ragweed with standard treatment. Clin Exp Allergy 1991; 21(4):457–465.
92. Litwin A, et al. Immunologic effects of encapsulated short ragweed extract: a potent new agent for oral immunotherapy. Ann Allergy Asthma Immunol 1996; 77(2):132–138.
93. Baldrick P, et al. Pollinex Quattro Ragweed: safety evaluation of a new allergy vaccine adjuvanted with monophosphoryl lipid A (MPL) for the treatment of ragweed pollen allergy. J Appl Toxicol 2007; 27(4):399–409.
94. Creticos PS, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006; 355(14):1445–1455.
95. Ferreira F, et al. Modified recombinant allergens for safer immunotherapy. Inflamm Allergy Drug Targets 2006; 5(1):5–14.
96. Garcia Villalmanzo I, et al. Immunotherapy with a mass unit Parietaria judaica extract: a tolerance study with evidence of immunological changes to the major allergen Par j 1. J Investig Allergol Clin Immunol 1999; 9(5):321–329.
97. Jimeno L, et al. Monoclonal antibody-based ELISA to quantify the major allergen of Artemisia vulgaris pollen, Art v 1. Allergy 2004; 59(9):995–1001.
98. Calabozo B, et al. Monoclonal antibodies against the major allergen of Plantago lanceolata pollen, Pla l 1: affinity chromatography purification of the allergen and development of an ELISA method for Pla l 1 measurement. Allergy 2001; 56(5):429–435.
99. Bowen T, et al. Canadian trial of sublingual swallow immunotherapy for ragweed rhinoconjunctivitis. Ann Allergy Asthma Immunol 2004; 93(5):425–430.
100. White JF, Bernstein DI. Key pollen allergens in North America. Ann Allergy Asthma Immunol 2003; 91(5):425–435; quiz 435-6, 492.
101. Asero R, et al. Giant ragweed specific immunotherapy is not effective in a proportion of patients sensitized to short ragweed: analysis of the allergenic differences between short and giant ragweed. J Allergy Clin Immunol 2005; 116(5):1036–1041.
102. Casale TB, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2006; 117(1):134–140.
103. Klunker S, et al. Combination treatment with omalizumab and rush immunotherapy for ragweed-induced allergic rhinitis: Inhibition of IgE-facilitated allergen binding. J Allergy Clin Immunol 2007; 120(3):688–695.
104. Gonzalez-Rioja R, et al. Genetically engineered hybrid proteins from Parietaria judaica pollen for allergen-specific immunotherapy. J Allergy Clin Immunol 2007; 120(3):602–609.

9 Fungal Allergens

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INTRODUCTION

Fungi are eukaryotic, nonchlorophyllous, mostly spore-bearing organisms that exist as saprophytes or as parasites of animals and plants (1). Fungi constitute unicellular to multicellular organisms, and their presence in the environment depends on the climate, vegetation, and other ecological factors. The presence and prevalence of fungi indoors depends on the moisture content, ventilation, and the presence and absence of carpets, pets, and houseplants (2). Spores of *Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium*, and *Fusarium* are universally present in the indoor and outdoor environments (Fig. 1). The development of allergies to fungi follows the same biological phenomena as allergies to other environmental agents.

Fungi are associated with a number of allergic diseases in humans. The prevalence of respiratory allergy to fungi is estimated to be 20% to 30% of atopic individuals and up to 6% in the general population (2–6). The major allergic manifestations induced by these agents are allergic asthma, rhinoconjunctivitis, bronchopulmonary mycoses, and hypersensitivity pneumonitis (7–12). These diseases result from exposure to spores, vegetative cells, or metabolites of the fungi. Spores from some fungi are shown in Figure 1. The conidia and spores associated with IgE immediate-type hypersensitivity are usually larger than 5 μm, while those associated with delayed-type hypersensitivity are considerably smaller and can penetrate into the smaller airways (7). The site of deposition of spores also depends on whether spores enter the respiratory tract as individual propagules or as aggregates. For example, the clusters of small conidia of *Aspergillus* and *Penicillium* are usually deposited in the upper respiratory tract, while the smaller individual spores reach the lower airways. Spore and fungal extracts both cause early and late-phase allergic asthmatic reactions in patients. More than 80 genera of the major fungal groups have been associated with symptoms of respiratory tract allergy (7,13). Ascomycetes and Deuteromycetes include the largest number of fungal species; however, only a few of them, all in the Deuteromycetes, such as *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium*, have been investigated systematically for causing allergic diseases (2,14–17). Exposure to fungi such as *A. flavus* and *Stachybotrys chartarum*, often present in food and agricultural products, moldy vegetables, and water damaged or moist buildings, also has been reported to cause allergy (17,18).

The allergens of fungi are highly heterogeneous and complex and are partly or completely shared by a number of fungal species. Therefore, accurate interpretation of skin tests and serological test results can be ascertained only by understanding the cross-reactivity of different mold allergens. Even though a number of fungal allergens have been isolated and characterized, no standardized extracts (vaccines) are available at this time to more reliably diagnose and treat allergic diseases.

CLASSIFICATION OF FUNGI

Molds belong to the fungal kingdom that includes yeasts, mildews, and mushrooms (19). Classification schemes for fungi are continually being revised to develop a more acceptable and comparatively easier system (20–22). Fungi constitute a very large and diverse group of organisms with a complex taxonomy (23). The hyphae are the basic structural unit for most fungi and typically are branched with tubular filaments possessing a defined cell wall

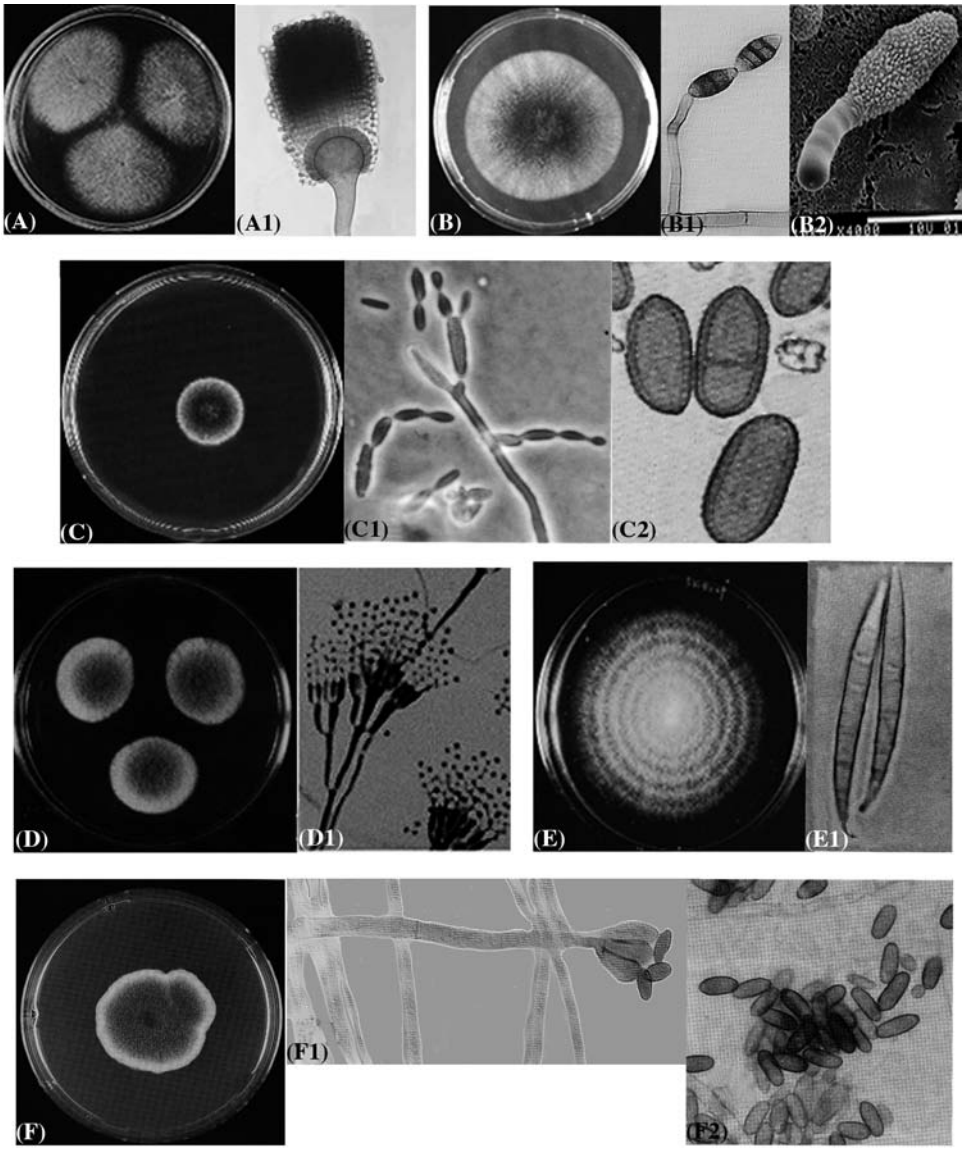

Figure 1 Colonies of *Aspergillus fumigatus* (**A**), *Alternaria alternata* (**B**), *Cladosporium herbarum* (**C**), *Penicillium chrysogenum* (**D**), *Fusarium solani* (**E**), and *Stachybotrys chartarum* (**F**). Conidiospores of *A. fumigatus* (**A1**), *Alt. alternata* showing vertical and horizontal septa (**B1**), scanning electronmicrograph of *Alt. alternata* (**B2**), conidiophores and conidia of *C. herbarum* (**C1**, **C2**), broom-shaped sporophores of *Penicillium* sp. (**D1**), spores (macroconidia) of *Fusarium* sp. (**E1**), and conidiophores and conidia of *S. chartarum* (**F1**, **F2**).

composed of chitin and other complex carbohydrates. These hyphae may be divided into individual cells by cross-walls called "septa." Some fungi exist exclusively as single-cell yeast forms, while others demonstrate extensive hyphae. Mushrooms belong to the group Basidiomycetes, where aggregation of mycelium results in the development of large macroscopic structures of diverse color and shape. The pleomorphism of fungi further complicates the classification, antigenicity, and poses problems for accurate identification (24,25).

Fungi are usually heterotrophic because they lack chlorophyll. The various modes of fungal reproduction include fragmentation, fission, budding, and spore production, and most produce both sexual and asexual spores. The taxonomy of fungi is based primarily on spore size, shape, color, surface ornamentation, and ontogeny (26). They are named in accordance with guidelines of the International Code of Botanical Nomenclature (ICBN) and are eukaryotic, unicellular, or multicellular organisms with absorptive nutrition. They have been traditionally

Table 1 Taxonomic Distribution of Allergenic Fungi

Phycomycetes	Deuteromycetes (fungi imperfecti)
Phytophthora	*Acremonium*
Plasmophora	*Alternaria*
Mucor	*Aspergillus*
Rhizopus	*Aureobasidium*
Ascomycetes	*Botryotrichum*
Chaetomium	*Botrytis*
Claviceps	*Cephalosporium*
Daldinia	*Chrysosporium*
Didymella	*Cladosporium*
Erysiphe	*Coniosprium*
Eurotium	*Curvularia*
Microsphaera	*Cylindrocarpon*
Zylaria	*Drechslera*
Yeasts	*Epicoccum*
Candida	*Fusarium*
Rhodotorula	*Gliocladium*
Saccharomyces	*Helminthosporium*
Basidiomycetes	*Monilia*
Agaricus	*Neurospora*
Calvatia	*Nigrospora*
Cantharellus	*Paecilomyces*
Cyathus	*Penicillium*
Ganoderma	*Phoma*
Geastrum	*Pyrenochaeta*
Lentinus	*Scopulariopsis*
Merulius	*Sporotrichum*
Phollogaster	*Stachybotrys*
Pleurotus	*Stemphylium*
Polyporus	*Torula*
Psilocybe	*Trichoderma*
Puccinia	*Trichophyton*
Tilletia	*Ulocladium*
Urocystis	*Wallemia*
Ustilago	
Xylobolus	

classified as members of the plant kingdom and now are reclassified under a new kingdom, Myceteae.

Myceteae are divided into standard taxonomic categories, including division, class, order, family, genus, and species, and each of these categories may contain further subdivisions, subclasses, and suborders. The kingdom Myceteae is divided into three major divisions, namely Gymnomycota, Mastigomycota, and Amastigomycota (27,28). The organisms belonging to Gymnomycota are referred as the "true plasmodium slime molds." The fungi belonging to Mastigomycota produce flagellated cells at some part in their life cycle while Amastigomycota produce extensive well-developed mycelia, consisting of either septate or aseptate hypha (29). Some single-celled organisms are also included in Amastigomycota.

In another classification, fungi that produce airborne spores are grouped into three divisions, Dikaryomycota, Zygomycota, and Oomycota. Three classes, Ascomycetes, Basidiomycetes, and Deuteromycetes, are included in Dikaryomycota. The fungi associated with allergic diseases are listed in Table 1. The fungi belonging to the class Deuteromycetes are of considerable interest and importance in human allergic diseases (30).

The organisms belonging to Deuteromycetes are also designated as "fungi imperfecti," which, as the name indicates, are an artificial group consisting of fungi known to reproduce only by asexual means. The conidial stages of many Duteromycetes fungi are similar to those of Ascomycetes and, in some cases, to those of Basidiomycetes. The members of the group fungi imperfecti are also believed to represent Ascomycetes and Basidiomycetes whose sexual stages have not been identified or have been excluded from the life cycle during their evolution.

IDENTIFICATION OF FUNGI

The most important group of air-disseminated fungi that causes respiratory allergic diseases in humans is the conidial fungi, which comprise the class Deuteromycetes. Most spores produced by the imperfect fungi vary in shape, size, texture, color, number of cells, thickness of the cell wall, and methods by which they are attached to each other and to their conidiophores. The identification of the common fungi is difficult as their fungal colony characteristics and even microscopic characteristics vary according to the medium on which they are grown, the incubation temperature, and the strain variation and pleomorphic nature of the spores (31).

Within the Hyphomycetes (a class of fungi in the phylum Deuteromycota), two principal classifications have been proposed. The first is based on spore morphology using the characteristics of color and septation (Fig. 1). Thus, *Alternaria* has dark "dictyospores," with both horizontal and vertical septa (Fig. 1B1, B2). *Fusarium* has colorless "phragmospores" (horizontal septa) (Fig. 1E1) and *Aspergillus* and *Penicillium* have bright-colored "amerospores" (Fig. 1A1, D1), with no septation at all. Some fungi, however, have several different methods of spore production within each life cycle. The second approach emphasizes details of asexual spore production as in *Alternaria*, where the porospores are formed by extrusion of protoplasm through the tiny pores of special spore-bearing hyphae or sporophores, and the phialospores of *Penicillium* and *Fusarium* are formed within a specialized hyphal cell called the phialide (Fig. 1D1) (32). The chemical composition of the cell wall may help to classify different fungal allergens. The cell wall of yeasts is mostly composed of a chitin-glucan combination, in contrast with chitin, predominantly present in mycelial fungi. Some fungi can change from yeast to mycelial form, depending on environmental conditions (33). Another aspect of vegetative morphology commonly used for identification purposes is color. The allergenic fungi have been mainly classified into two large groups on the basis of whether the mycelium and asexual spores are brown (Dematiaceae) or colorless (Moniliaceae).

FUNGAL ALLERGENS

Fungi spores are ubiquitous in nature and at least one million fungal species are found on earth (26). *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium* are found throughout the world. The airborne spores of these fungi are important causes of allergic diseases (allergic rhinitis, asthma, bronchopulmonary mycoses, and hypersensitivity pneumonitis) (7,34,35).

The accurate in vivo and in vitro diagnoses of fungal allergies depend on the availability of well-characterized allergen preparations. Aerobiological identification and assessment of fungi in outdoor and indoor environments is necessary to determine their role in causing allergic diseases. Such surveys conducted in different parts of the world and skin and in vitro tests for specific mold allergy have led to the identification of relevant mold allergens. On the basis of such data, extracts from *Alt. alternata*, *C. herbarum*, *A. fumigatus*, *Epicoccum purpurascens*, *Fusarium roseum*, and *Penicillium chrysogenum*, and others are commercially available. Selection of species and strains of fungi is crucial for obtaining a representative allergen. As the prevalence of fungi and their allergenicity varies, relevant fungi need to be identified for consistent and reproducible results, both for diagnosis and treatment of allergic diseases.

Because of the variability among strains and species in morphology, biochemistry, and allergenicity, it is difficult to obtain relevant antigens with consistent allergenic activity. In addition, there is considerable immunological cross-reactivity among various taxonomically and antigenically related strains, species, and even genera. It is almost impossible to grow two consecutive cultures with similar antigenic profiles with some fungi (36). Factors contributing to the differences of commercial and laboratory extracts are (*i*) variability in the proper identification of stock cultures used to prepare allergenic extracts, (*ii*) the use of mycelial rich material as the source of allergens, (*iii*) conditions under which molds are grown and extracts prepared, (*iv*) the stability of the extracts, and (*v*) the quality control measures used. It is now possible to grow allergenic fungi in synthetically defined medium rather than in complex media containing macromolecules. These allergenic extracts show less variability and demonstrate more specific reactivity in allergic patients (37,38). However, for the production of certain relevant allergens, complex media components still are essential.

The extraction procedures for inhalant allergens should reflect the pattern by which the allergens are released under natural conditions. The extraction procedure should be optimized for consistent results by the use of a suitable extraction buffer, length of extraction, appropriate cell disruption, and the use of protease inhibitors and preservatives (39,40). The allergenic activity of an extract, or fraction, can be evaluated either by prick or intradermal skin testing. The intradermal method, however, is more quantitative and sensitive than prick testing (41,42). The most common in vitro tests are RAST (radioallergosorbent test) and ELISA (enzyme-linked immunosorbent assay), both of which correlate with allergen-specific IgE in the sera (43). Semiautomated specific IgE assays, such as Immuno-CAP, are available for a number of allergens, including mold allergens (44).

Antibody response to allergens and their specificity can also be studied by competitive inhibition assays. Patients' sera are incubated with varying dilutions of the allergens before adding the sera to the solid-phase bound reference allergens. Immunoassays, namely RAST or ELISA, can be performed and the percent inhibition of binding of the preadsorbed sera to the reference allergen determined. A 50% inhibition in binding of the patient's IgE to the reference allergen is interpreted as a measure of potency of the test allergen. Direct challenge of allergic patients by inhalation of small doses of various fungal extracts has been used in patient evaluation studies; however, the use of mold allergens for inhalation studies is controversial because of possible late-phase reactions and other adverse effects, causing new sensitizations.

The stability of allergenic extracts depends on the type and quality of the allergen, the storage temperature, and the presence of preservatives and other nonallergic materials in the mixture. For most extracts, lyophilization is the best method to maintain the allergenic potency, but some allergens may be permanently altered and inactivated by this process. The loss of potency of any extract may be due to degradation of a specific allergen rather than a general reduction in activity of all allergens. Moreover, reconstituted extract must contain a stabilizer such as human serum albumin, glycerol, phenol, or aminocaproic acid to preserve the integrity of allergenic extracts (45).

DISTRIBUTION OF INDOOR AND OUTDOOR FUNGAL ALLERGENS

Fungi grow on almost any material if sufficient moisture is available. Large numbers of airborne spores are usually present in outdoor air throughout the year, frequently exceeding pollen population by 100- to 1000-fold, depending on environmental factors, such as water, nutrients, temperature, and wind (8,46). Most fungi commonly considered allergenic, such as *Alternaria*, *Cladosporium*, *Epicoccum*, or *Ganoderma*, have a seasonal spore-releasing pattern (2,47).

Indoor fungi are a mixture of those that have entered from outdoors and those that grow and multiply indoors (48,49). *Aspergillus* and *Penicillium* usually are less common outdoors and are primarily considered to be indoor fungi. *Alternaria* can be found in house dust samples in the absence of environmental mold spores (50). Some investigators find good correlation between outdoor spore counts and allergic symptoms; however, little information is available on the effects of indoor spore concentrations and allergic symptoms (47,51). Dampness, excess moisture, and mold growth in buildings are associated with an increased prevalence of asthma and bronchitis. The indoor versus the outdoor air fungal flora may differ, both quantitatively and qualitatively, and most of the time outdoor concentrations of fungal spores outnumber those of indoor environments. The ratio of indoor to outdoor concentration (I/O) of spores is usually less than 1. The intramural sources of fungi result in a different composition of indoor airborne fungi compared with the outdoor air (52). The health effects caused by fungal propagules are primarily allergic, but can be infectious and possibly irritative. These effects can be caused by viable and nonviable fungal spores and hyphal particles. Therefore, the overall concentration of both viable and nonviable propogules may give a more accurate estimate of the actual exposure.

Most studies of indoor and mold spores in the air have been performed with a discontinuous viable sampler. Surveys on outdoor mold spores are mostly done with continuous nonviable techniques (53). The spectrum of airborne mold spores in homes, offices, and other work places differs from place to place due to the influx of spores from outdoor air

Table 2 Distribution of Indoor and Outdoor Allergenic Molds

		Range spores/m^3			
	Indoor[a]	Indoor summer[b]	Indoor winter[b]	Outdoor summer[b]	Outdoor summer[c]
Penicillium	0–4,737	0–7,900	0–480	0–95	15,000
Cladosporium	12–4,637	0–160	0–160	11–430	600,000
Botrytis	0–54	—	—	—	12,000
Yeasts	0–5	0–74	0–78	0–790	10,000
Aspergillus	0–306	0–76	0–19	0–11	15,000
Alternaria	0–282	—	—	—	7,500
Rhizopus	0–24	—	—	—	—
Nonsporulating mycelium	0–14,194	0–1,700	0–200	19–9,300	—
Epicoccum	0–155	—	—	—	—
Fusarium	0–47	—	—	—	7,500

[a]Ref. 55; studies carried out in Southern California homes.
[b]Ref 53; studies carried out in Finnish homes.
[c]Ref. 56; studies carried out in European homes.

through ventilations and air exchangers. Hence, it is difficult to arrive at any significant conclusion on the role of the indoor mold spore in the allergic response. Spieksma (54) reported that the 10 most common types of outdoor atmospheric mold spores are present in all distant regions of Europe. Distribution of indoor and outdoor mold spore counts reported from different parts of the world is provided in Table 2 (53,55–57). The fungal spore count in outdoor air is usually about 230/m^3 while the indoor count may vary from 100 to 1000/m^3 (53,55). A spore count of 10 to 100/m^3 is a substantially high antigen load to exposed individuals. Garrett and colleagues (58) found that most common fungal genera/groups were *Cladosporium*, *Penicillium*, and yeast, both indoor and outdoor in winter and late spring, in their studies with airborne fungal spores in southeastern Australian homes. Outdoor versus indoor levels were higher throughout the year, and significant seasonal variation in spore levels occurred indoors and outdoors with maximum levels found during the summer. To the contrary, the levels of *Aspergillus*, *Cephalosporium*, *Gliocladium*, and yeasts were higher in winter. *Penicillium* was detected more commonly indoors than outdoors. Outdoor spore levels do have significant influence on their indoor levels of spores.

CROSS-REACTIVITY OF FUNGAL ALLERGENS

The term cross-reactivity refers to the antigenic determinants shared by different molecules from different fungi (59). Studies of cross-reactivity with techniques such as immunoprecipitation, immunoblotting, and RAST inhibition have contributed to the understanding of this phenomenon. Cross-reactivity should be distinguished from parallel, independent sensitization to multiple fungal allergens (59). The degree of cross-reactivity among different species and strains of fungi depends on the number of antigenic components that cross-react, the immunogenicity of epitopes, and the method used to detect the reactivity (60). The presence of cross-reactive epitopes among allergens is advantageous to diagnose allergic diseases because it reduces the number of antigens required in the panel of extracts for testing (16). However, this may lack specificity and necessitate secondary testing to determine the specific sensitizing mold. Cross-reactive antigens are more advantageous for allergen immunotherapy due to their broad-spectrum effect.

There are shared allergenic and antigenic components from cytoplasmic and cell wall antigens of a number of fungi. The cell wall antigens usually contain carbohydrates, which may contribute to the cross-reactivity. Several of the related genera of fungi share similar proteins. For example, *Aspergillus* and *Penicillium* species share a number of proteases and these proteins cross-react. Even unrelated fungi share some of these antigens with low to high levels of cross-reactivity.

Allergens from unrelated sources can also cross-react. Mold-latex allergy is such an example. A number of minor and major allergens from *Hevea brasiliensis* (the source of latex) share partial homology with fungal allergens (61). These allergens show some degree of cross-reactivity and thereby complicate the specificity of test results. However, further research is necessary to establish the importance and degree of allergen cross-reactivity. As fungal extracts are variable, several batches of antigens should be used for cross-reactivity studies to prevent inaccurate results. Cross-reactivity among fungal allergens can be understood more precisely by the use of monoclonal antibodies (MAbs) and recombinant allergens. A better understanding of cross-reactivity between different fungi is important as it may be relevant for diagnosis, treatment, and devise prevention control measures.

ISOLATION AND CHARACTERIZATION OF FUNGAL ALLERGENS

Although a large number of fungi cause allergic diseases, the understanding of fungal allergens is limited only to a few species. In the present discussion, only the most predominant fungi associated with IgE-mediated allergy are discussed. These include a number of allergens derived from *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Malasezzia*, *Trichophyton*, and species belonging to Basidiomycetes fungi and yeasts, which have been isolated and characterized.

Allergens of *Alt. alternata*

Alt. alternata, a member of the imperfect fungi, is one of the most important allergenic fungi (62). Most of the spores produced by imperfect fungi vary in shape, size, texture, color, number of cells, and thickness of the cell wall. This species is known to be an important cause of bronchospasm. (63,64). Hypersensitivity pneumonitis, a condition associated with IgG-precipitating antibodies, may also be caused by *Alternaria* (65). Most fungi, including *Alt. alternata* and *C. herbarum*, have a seasonal spore-releasing pattern. Also, *Alternaria*, an outdoor fungus, is found in house dust samples in the absence of mold spores of this species (50). Although other *Alternaria* species are probably also clinically relevant, most research has been done with *Alt. alternata* (60).

The first allergen of *Alt. alternata* (ATCC 6663) is a mycelial allergen partially purified by gel chromatography. This glycoprotein fraction, named Alt-1, has a molecular weight between 25 and 50 kDa and contains at least five isoelectric variants between pI 4.0 and 4.5 (66). The two variants of Alt-1, Ag 1 and Ag 8, have molecular masses of 60 and 35 to 40 kDa and pIs of 4.0 and 4.3 to 4.65, respectively (67). Hybridoma technology has been employed to produce murine MAbs to *Alt. alternata*. Vijay et al. (68) reported on the purification of a 31-kDa protein of *Alt. alternata* using MAb affinity chromatography. Using immunoblots, this protein reacts with human IgE. Sanchez and Bush (69) reported purification of *Alternaria* allergens of 62 kDa by IgE immunoblot using MAbs. Similarly, Portnoy et al. (70) purified an allergen of 70 kDa (gp 70) using MAbs. Of the 16 *Alternaria* skin test positive subjects, 11 reacted to gp70, although purified allergen was less potent than the crude extract in producing positive skin test results. Lepage et al. (71) produced 11 MAbs with antigenic determinants at 200-, 65-, and 45-kDa regions that reacted with IgE. Vijay et al. (72) reported the detection and quantitation of *Alt. alternata* using MAbs and polyclonal antibodies (PAbs) to the native and cloned Alt a 1. Similar results have been reported by Barnes et al. (73), with enzyme immunoassays and polyclonal antibodies against purified Alt a 1. Also, detection of Alt a 1, using two-site, IgM-based sandwich ELISA, has been reported by Abebe et al. (74).

The major allergenic component of *Alt. alternata* has been isolated. Two groups of investigators used anion exchange chromatography to purify Alt a l from mycelium (75,76). Paris and coworkers (76) designated the allergen Alt a 1 (31 kDa, pI 4.0–4.5) and determined that it is a heat-stable glycoprotein containing 20% carbohydrate. Deards and Montague designated this allergen as Alt a BD 29k (pI 4.2, 29 kDa) and determined that it is composed of 15-kDa subunits (75). Matthiesen et al. and Curran et al. purified Alt a l of molecular weights 28 kDa and 29 kDa, respectively (77,78). These authors showed that a reduced form of Alt a l produced a doublet pattern on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with molecular weights of 14.5 and 16 kDa. This doublet was confirmed as being allergenic in immunoblots with human atopic serum (a pool prepared from equal amounts of 10 atopic sera). These polypeptide chains are closely related since their N-terminal sequences are virtually

identical. A 29-kDa protein and its reduced form reacted with 92% of the atopic human sera tested as determined by immunoblots (78). Bush and Sanchez (79) determined the amino acid sequence of 60-kDa *Alt. alternata* allergen and established the partial cDNA sequence for another *Alt. alternata* allergen. There is a high variability of Alt a 1 expression in different *Alt. alternata* strains (80). Another partially purified allergen, designated as a basic peptide (pI 9.5, 6 kDa), is able to induce a wheal-and-flare skin reaction in sensitized subjects. Eighteen of 20 (90%) skin test–positive subjects reacted to this basic peptide, which is designated as Alt a II d (81).

Recombinant Allergens from Alt. alternata

Alt. alternata allergens that have been cloned and expressed as IgE-binding protein include a subunit of the major allergen, Alt a l (82,83). Recombinant Alt a 1 secreted into the media of *Pichia pastoris* cultures appeared as a dimer, similar to the natural allergen from *Alt. alternata* culture medium or mycelium. Recombinant Alt a l, like the natural allergen in *Alt. alternata*, is reactive with serum IgE antibodies from *Alt. alternata*–sensitive patients (82). Several groups have isolated and characterized minor allergens of *Alt. alternata*: Alt a 2 (25 kDa), Alt a 3 (hsp 70), Alt a 4 (57 kDa), Alt a 6 (ribosomal P2 protein, 11 kDa), Alt a 7 (22 kDa), Alt a l0 (aldehyde dehydrogenase, 53 kDa), Alt a 11 (45 kDa), and Alt a l2 (11 kDa) (Table 3) (79,82–84). Alt a 7, a 22-kDa allergen, has a 70% sequence homology with YCP4 protein of *Saccharomyces cerevisiae* while Alt a 6, the 11-kDa protein, has homology with ribosomal P2 protein. They also have homology with *C. herbarum* allergens. Alt a 5, which was identified as a peptide with a length of 69 amino acids (peptide 9), has been isolated by screening a complementary DNA (cDNA) expression library with a *C. herbarum* enolase (Cla h 6) DNA probe (85). This peptide binds IgE from eight of eight patients indicating that the epitope or epitopes on peptide 9 constitute a major cross-reacting epitope or epitopes on the enolases from *C. herbarum* and *Alt. alternata*. Studies of Alt a l have also been carried out (86) for its IgE-binding linear epitopes using overlapping decapeptides spanning the whole Alt a 1 sequence. The reactivity of the synthesized peptides was studied using serum IgE from *Alternaria*-allergic patients. The two peptides, K41-P50 and Y54-K63, reacted strongly by the in vitro test, ELISA, in all the patients studied. It has also been reported that mannitol dehydrogenase (Alt a 8) is recognized by 41% of *Alt. alternata*–allergic patients (87).

Allergens of *Cladosporium*

Spores of *Cladosporium* spp. probably occur more abundantly worldwide than any other spore type and are the dominant airborne spores in many areas, especially in temperate climates (88). Although *C. cladosporioides* can be the most prevalent airborne species, *C. herbarum* frequently dominates indoor and outdoor air and is a major source of fungal inhalant allergens (88–90). Its mycelial allergens have been studied intensively (90). The allergenic composition of the extracts from this organism is complex and difficult to characterize (91,92). There are at least 17 different allergens in an extract of *C. herbarum* isolate, IMI 96220 (93).

Recombinant Allergens of C. herbarum

About 60 antigens from *C. herbarum* have been identified by crossed immunoelectrophoresis (CIE), and 36 of them react with IgE antibodies from patients' sera by CIE (92). Three major *C. herbarum* allergens are purified and characterized (Table 3). Cla h 1 is a small 13-kDa acidic allergen composed of five isoallergens (pI 3.4–4.4) (91), and Cla h 2, a slightly larger molecule with a size of 23-kDa glycoprotein, is less acidic (pI 5.0) and contains 50% carbohydrates (91,94,95). The protein part retains the IgE-binding property even after the carbohydrate moieties are removed and the binding is stronger than that shown by the native Cla h 2. Cla h 4, a ribosomal P2 protein, is a low molecular weight (11 kDa) acidic allergen (pI 3.94) with high alanine and serine content and shares 60% sequence homology with other ribosomal P2 protein (96). Breitenbach et al. (97) reported that purified recombinant *Cladosporium* enolase (Cla h 6, 48 kDa) strongly binds the IgE by immunoblots in 20% of patients allergic to *Alternaria*. Enolase is a highly conserved major allergen in most fungi and may contribute to allergen cross-reactivity in mold allergy. About 20% of the serum IgE from patients allergic to *Alternaria* and *Cladosporium* binds to enolase. An allergenic heat shock protein (HSP) 70 has also been isolated from the organism (97).

Table 3 Mold Allergens Approved by the Allergen Nomenclatural Committee of the International Union of Immunological Societies[a]

Fungus	Mol. size (kDa)	Biological activity	Sequence accession number
Alternaria alternata			
Alt a 1	28		U82633
Alt a 2	25		U62442
Alt a 3		Heat shock protein 70	U87807
Alt a 4	57	Prot. disulfidisomerase	X84217
Alt a 6	11	Acid. ribosomal protein P2	X-78222
Alt a 7	22	YCP4 protein	X-78225
Alt a 10	53	Aldehyde dehydrogenase	X-78227
Alt a 11	45	Enolase	U82437
Alt a 12	11	Acid. ribosomal protein P1	X84216
Cladosporium herbarum			
Cla h 1	13		
Cla h 2	23		
Cla h 3	53	Aldehyde dehydrogenase	X-78228
Cla h 4	11	Acid. ribosomal protein P2	X-78223
Cla h 5	22	YCP4 protein	X-78224
Cla h 6	46	Enolase	X-78226
Cla h 12	11	Acid. ribosomal protein P1	X85180
Aspergillus flavus			
Asp fl 13	34	Alkaline serine protease	
Aspergillus fumigatus			
Asp f 1	18	Ribonuclease mitogillin	AAB07779
Asp f 2	37		AAC69357
Asp f 3	19	Peroxisomal protein	AAB95638
Asp f 4	30		CAA04959
Asp f 5	40	Metalloproteinase	CAA83015
Asp f 6	26.5	Mn superoxide dismutase	AAB60779
Asp f 7	12		CAA11255
Asp f 8	11	Ribosomal protein P2	CAB64688
Asp f 9	34		CAA11266
Asp f 10	34	Aspartic proteinase	CAA59419
Asp f 11	24	Peptidyl prolyl isomerase	CAB44442
Asp f 12	90	Heat shock protein P90	AAB51544
Asp f 13	34	Alkaline serine proteinase	CAA77666
Asp f 15	16		CAA05149
Asp f 16	43		AAC61261
Asp f 17			CAA12162
Asp f 18	34	Vacuolar serine proteinase	CAA73782
Asp f 22	46	Enolase	AAK49451
Asp f 23	44	Ribosomal protein L3	AAM43909
Asp f 27	18	Cyclophilin	
Asp f 28	12	Thioredoxin	
Asp f 29	12	Thioredoxin	
Aspergillus niger			
Asp n 14	105	β-Xylosidase	AF108944
Asp n 18	34	Vacuolar serine protease	
Asp n ?	85		Z84377
Asp n 25	66–100	3-Phytase B	P34754
Aspergillus oryzae			
Asp o 13	34	Alkaline serine protease	X17561
Asp o 21	53	TAKA-amylase A	D00434
Penicillium brevicompactum			
Pen b 13	33	Alkaline serine protease	
Pen b 26	11	Acidic ribosomal P1 protein	AY786077
Penicillium chrysogenum			
Pen ch 13	34	Alkaline serine proteinase	
Pen ch 18	32	Vacuolar serine proteinase	
Pen ch 20	68	*N*-acetyl glucosaminidase	

(*Continued*)

Table 3 Mold Allergens Approved by the Allergen Nomenclatural Committee of the International Union of Immunological Societies[a] (*Continued*)

Fungus	Mol. size (kDa)	Biological activity	Sequence accession number
Penicillium citrinum			
Pen c 3	18	Peroxisomal membrane protein	
Pen c 13	33	Alkaline serine proteinase	
Pen c 19	70	Heat shock protein P70	U64207
Pen c 22	46	Enolase	AF254643
Penicillium oxalicum			
Pen o 18	34	Vacuolar serine protease	
Fusarium culmorum			
Fus c 1	11	Ribosomal protein P2	AY077706
Fus c 2	13	Thioredoxin-like protein	AY077707
Trichophyton rubrum			
Tri r 2			
Tri r 4		Serine protease	
Trichophyton tonsurans			
Tri t 1	30		
Tri t 4	83	Serine protease	
Candida albicans			
Cand a 1	40		
Cand a 3	29	Peroxisomal protein	AY136739
Candida boidinii			
Cand b 2	20		J04984
Psilocybe cubensis			
Psi c 1			
Psi c 2	16	Cyclophilin	
Coprinus comatus			
Cop c 1	11	Leucine zipper protein	AJ132235
Cop c 2			AJ242791
Cop c 3			AJ242792
Cop c 5			AJ242793
Cop c 7			AJ242794
Rhodotorula mucilaginosa			
Rho m 1	47	Enolase	
Malassezia furfur			
Mala f 2	21	MF1, peroxisomal membrane protein	AB011804
Mala f 3	20	MF2, peroxisomal membrane protein	AB011805
Mala f 4	35	Mitochondrial malate dehydrogenase	AF084828
Malassezia sympodialis			
Mala s 1			X96486
Mala s 5	18		AJ011955
Mala s 6	17		AJ011956
Mala s 7			AJ011957
Mala s 8	19		AJ011958
Mala s 9	37		AJ011959
Epicoccum purpurascens			
Epi p 1	30	Serine protease	P83340

[a]http://www.allergen.org (132) IUIS Allergen List.

Cloning, expression, and characterization of NADP-dependent mannitol dehydrogenase of *C. herbarum* (Cla h 8), which is recognized by IgE antibodies of 57% of all *Cladosporium*-allergic patients, had been reported (98). This protein appears to be a new major allergen of *C. herbarum*.

Allergens of *Aspergillus*

A. fumigatus is one of the predominant fungi implicated in the pathogenesis of allergic diseases in humans and the principal etiological agent of allergic bronchopulmonary aspergillosis (ABPA). Other species, such as *A. nidulans*, *A. oryzae*, *A. terreus*, *A. flavus*, and *A. niger* also cause allergic

diseases in humans (7,99,100). All these organisms are freely distributed in most environments, although in certain conditions they grow much faster and liberate numerous spores.

A. fumigatus antigens are diverse in their physiochemical and immunological characteristics (101). A number of protein and glycoprotein antigens react with specific antibodies in the sera from patients with allergic aspergillosis (102). Four antigens (Ag 3, Ag 5, Ag 7, and Ag 13) were purified by size exclusion chromatography (103–105). Ag 7, 150 to 200 kDa, and Ag 13, 70 kDa, bind to Con-A and react with sera from ABPA patients. Ag 5 and Ag 3, the thermolabile peptides with molecular masses of 35 and 18 kDa, respectively, are also useful to detect antibodies in patients with ABPA. Two allergens, 18 and 20 kDa, purified by conventional purification techniques, were compared with other allergens of *A. fumigatus*. The crossed immunoelectrophoretic pattern of 18 kDa is similar to that of Ag 3 or Ag 10 described earlier, whereas the 20-kDa allergen is a Con-A nonbinding glycoprotein and appears to be different from the other known allergens of *A. fumigatus*. Another glycoprotein allergen designated as gp 55 is sensitive to protease treatment but not to deglycosylation (106). The amino terminal sequence of protein gp 55 does not show sequence homology with other allergens. Two nonglycosylated 18 kDa (Asp f 1) and 24 kDa allergens of *A. fumigatus*, purified using MAb affinity chromatography, demonstrated strong IgE binding with sera from ABPA patients (107,108).

Recombinant Allergens of Aspergillus

Several recombinant allergens from *A. fumigatus* have been identified and purified from cDNA and the phage display library of *A. fumigatus* (Table 3). The majority of these proteins show specific binding to IgE from asthmatic and ABPA patients. The molecular structures cover a wide range of functional proteins, including toxins, enzymes, heat shock proteins, and several unique proteins lacking homology to any of the known proteins. Asp f 1, a ribotoxin, which inhibits protein translation, is toxic to EBV-transformed PHA-stimulated peripheral blood mononuclear cells (PBMCs). IgE antibody to this allergen is present in 68% to 83% of patients with skin test positivity to *Aspergillus* allergens (109,110). Th1 and Th2 Asp f 1–specific epitopes also were demonstrated when studied in a murine model of allergic aspergillosis (111,112).

Another major allergen, a 37-kDa protein of *A. fumigatus* (Asp f2), has been cloned, expressed, and characterized (113). Recombinant Asp f 2 exhibits specific IgE binding with sera of ABPA patients and discriminates ABPA with serological confirmation and no evidence of central bronchiectasis (ABPA-S) from ABPA with definitive central bronchiectasis (ABPA-CB).

The Af gene encoding a polypeptide fragment of an HSP 90 family has been expressed and its allergenicity confirmed (114). The heat shock protein Asp f 12 has homologous counterparts in *Candida albicans*, *Saccharomyces*, *Trypanasoma*, housefly, mouse, and humans because of the extremely conserved HSP gene. Asp f 16 has no known biological functions and strongly binds to IgG from ABPA patients (115). This antigen shows sequence homology with Asp f 9 and a membrane protein from *Saccharomyces*. There are a few other minor allergens isolated from *A. fumigatus* and related *Aspergillus* species that demonstrate binding to IgE antibody from ABPA and allergic asthma patients (Table 3). Several of these *A. fumigatus* allergens also exhibit high sequence homologies with the known functional proteins and enzymes of other fungi (116–119). Alkaline serine proteinases with allergenic properties, such as Asp f 13, Asp f 1 13, and Asp o 13 from *A. fumigatus*, *A. flavus*, and *A. oryzae*, respectively, have been reported (119,120). Similar serine proteinases, Pen b 13, Pen c 13, and Pen ch 13, with sequence homology to *Aspergillus* proteinases, have also been identified from various species of *Penicillium* (121,122). Another group of homologous vacuolar serine proteinases, Asp f 18, Asp n 18, Pen ch 18, and Pen o 18, with conserved sequence, have been reported from *Aspergillus* and *Penicillium* (117,123). *A. flavus* extracts demonstrate IgE antibody binding in 44% of asthmatic patients studied by immunoblotting (124). A 34-kDa alkaline serine proteinase, Asp f 1 13, with significant IgE antibody binding was purified and its enzyme activity ascertained (124).

A phage display method has been used to express allergenic proteins from Af (125). The expressed proteins from a cDNA library from Af have been displayed on the surface of filamentous phage M13 and screened with sera from ABPA patients for IgE-binding antibodies to the phage surface protein. The Af proteins selected from the phage display library, which binds IgE, is in the range of 20 to 40 kDa. A 26.7-kDa manganese superoxide dismutase, cloned and expressed from Af, reacts with IgE antibodies in serum from patients with allergic aspergillosis and stimulates their peripheral blood lymphocytes (126).

Allergens of *Penicillium* Species

Species belonging to the genus *Penicillium* are prevalent indoor fungi (7,8). Inhalation of *Penicillium* spores in quantities comparable with those encountered by natural exposure can induce both immediate and late asthma in sensitive persons (49). Among more than a hundred different *Penicillium* species, *P. citrinum* together with *P. chrysogenum* (*P. notatum*), *P. oxalicum*, *P. brevicompactum*, and *P. spinulosum* are the five most frequently recovered species of *Penicillium* in the United States, while *P. citrinum* is the most prevalent *Penicillium* species reported from Taiwan (127,128).

About 12 antigens from *P. citrinum* and 11 antigens from *P. chrysogenum* react with IgE from patients with sera by immunoblotting (129). Several *Penicillium* allergens also have been characterized at the molecular level (Table 3). Among the *Penicillium* allergens, the 32- to 34-kDa alkaline and/or vacuolar serine proteases were identified as the major allergens of *P. citrinum*, *P. brevicompactum*, *P. chrysogenum*, and *P. oxalicum* (130). They have been designated as group 13 for alkaline serine protease and group 18 for vacuolar serine protease allergens as recommended by the Allergen Nomenclature Subcommittee (131,132). Immunoblotting data show that IgE antibodies against components of these prevalent *Penicillium* species could be detected in the sera of about 16% to 26% of asthmatic patients (131). The majority of the positive serum samples tested show IgE binding to the 32/34-kDa serine proteinase(s), with a frequency >80% in different fungal species tested. Vijay et al. studied 14 *Penicillium* species and found that *P. viridicatum*, *P. janthinellum*, *P. oxalicum*, *P. brevicompactum*, and *P. italicum* are highly immunogenic as well as allergenic and possibly good candidates for allergen cloning studies (133). Sevinc et al. isolated Pen b 26 clone from *P. brevicompactum* characterized and expressed in *Escherichia coli* (134). This allergenic protein of molecular weight 11 kDa appears to recognize IgE antibodies from 25% of patients allergic to *P. brevicompactum*. The cDNAs of the alkaline serine protease allergens from *P. citrinum* (Pen c 13) and *P. chrysogenum* (Pen ch 13), and the vacuolar serine proteases from *P. citrinum* (Pen c 18), *P. oxalicum* (Pen o 18), and *P. chrysogenum* (Pen ch 18) are now cloned (135–137). The mature Pen ch 13 allergens are formed by the removal of the pre-prosequence of the precursor molecule (135). Besides N-terminal cleavage, the mature Pen c 18 and Pen o 18 also undergo C-terminal processing (136). IgE cross-reactivity between the allergens in *Penicillium* and *Aspergillus* species has been detected (129,135,136,138). In addition to the reactivity with IgE antibody, serine proteases (Pen ch 13) also demonstrate histamine-releasing activity from peripheral blood leukocytes of asthmatic patients (135).

Besides the serine protease allergens, a 68-kDa allergen *N*-acetylglucosaminidase and an allergenic heat shock protein belonging to the HSP 70 family also have been identified from *P. chrysogenum* and in *P. citrinum*, respectively (139). The Allergen Nomenclature Subcommittee has designated them as Pen ch 20 and Pen c 19, respectively (132) (Table 3). The 18-kDa peroxisomal membrane protein (Pen c 3), an important allergen of *P. citrinum*, is similar to Asp f 3 (140). Novel enolase allergens from *P. citrinum* (Pen c 22) and *A. fumigatus* (Asp f 22) have been identified. Cross-reacting IgE antibodies have been reported against these allergens (141).

Allergens of Basidiomycetes

Basidomycetes are physically the largest and most morphologically complex fungi. Most are considered as microfungi. Basidiomycetes fungi number over 20,000 species, including mushrooms, puffballs, bracket fungi, rusts, and smuts. Although microfungi unquestionably are important allergen sources, reports now indicate that basidiospores occur in the air in high concentrations in many parts of the world, and positive skin tests, RAST, and bronchial reactivity to their extracts have been detected in hypersensitive subjects (142,143).

Calvatia species are seasonally occurring puffballs that produce a large number of spores. Immunoprints of crude and fractionated extracts of *Calvatia cyathiformis* indicate that allergens (pI 9.3 and 6.6) react with 68% and 63%, respectively, of serum samples from 19 patients with positive skin tests to this mold antigen (144). These allergens are designated as Cal c Bd q3 and Cal c Bd 6.6 (132).

For *Coprinus quadrifidus* spores and *Cop. comatus* mycelium extracts, skin tests and RAST demonstrate that most reactive fractions of each extract are in the same size range (10.5–12 kDa) (145).

Ganoderma

Ganoderma are important wood-decaying fungi that produce large shelf-like fruiting bodies called brackets or conks. Spores of *Ganoderma* occur widely and are easily demonstrable in air-sampling surveys (146,147). The allergenicity of *Ganoderma* has been studied by more laboratories than that of other Basidiomycetes. Despite the fact that several extracts are reasonably well characterized, no allergens have been isolated. Western blots of *Ganoderma meredithae* spore and cap extracts with atopic serum reveal 10 allergens (14 to $>$ 66 kDa and pI $<$ 3.5–6.6). *G. applanatum* spore and fruiting body extracts tested by crossed line immunoelectrophoresis (CLIE) also demonstrate common antigens (148). In another study of *G. applanatum* spores, 14 antigens have been detected by CIE and immunoblots (149). This study also reveals that IgE-binding bands are mostly between 18 and 82 kDa. However, no purified antigens have been obtained.

Allergens of *Can. albicans*

Ten of 120 *Candida* species cause significant human infections, and *Can. albicans* is the most frequently isolated pathogenic species (30). Although IgE reactivity of *Can. albicans* allergens has been reported on several occasions, the view that *Can. albicans* is a major inhalant allergen remains controversial. A 40-kDa *Can. albicans* allergen has been cloned and sequence identity reveals 70% homology with alcohol dehydrogenase (150,151). A 29-kDa IgE-reacting component (Cand a 3) from *Can. albicans* has been cloned and MAbs produced against this clone. These reagents could be used in the standardization of diagnostic extracts (152).

Yeasts

Yeasts are true fungi belonging to the group Ascomycetes. Most yeasts are single celled and reproduce by budding. Various species within Ascomycetes, Basidiomycetes, and fungi imperfecti have yeast forms (30,153).

Malassezia furfur

M. furfur (as *Pityrosporum orbiculare*) extracts induce positive skin tests and leukocyte histamine release in subjects with atopic dermatitis (154). SDS-PAGE immunoblots of *M. furfur* extracts demonstrate dominant allergens at 9, 15, 25, and 72 kDa (155). The 9- and 15-kDa components are mostly carbohydrates. MAbs have been produced against 67-kDa allergen of *M. furfur* (156).

Trichophyton spp.

Trichophyton species induce classic delayed-type or cell-mediated hypersensitivity. A possible role of *Trichophyton* spp. in IgE-mediated urticaria, asthma, and rhinitis has been suggested; however, the relevance of these species in causing allergy remains controversial. IgE antibodies to *Trichophyton tonsurans* have been found in skin test–positive subjects (157). A 30-kDa hydrophobic major allergen of *T. tonsurans* (Tri t 1) has been purified by gel filtration and hydrophobic interaction chromatography and the sequence of 30 N-terminal amino acids determined (158). The MAbs that recognize distinct epitopes on Tri t 1 have been prepared, and studies with these MAbs should help understand the importance of *Trichophyton* spp. as an allergen.

Other Fungi

Aerobiological studies performed in different countries demonstrate the presence of *Botrytis*, *Phoma*, *Helminthosporium*, *Fusarium*, *Epicoccum*, *Puccinia*, *Ustilago*, *Cephalosporium*, and *Saccharomyces*, and these fungi have been implicated in allergic disorders in humans (Table 1). However, careful evaluation has not been carried out due to the lack of appropriate, reliable antigens and diagnostic methods to determine the results.

CONCLUSIONS AND FUTURE DIRECTIONS

Significant progress in fungal allergen standardization, particularly since 1990 as a result of the availability of partially purified and well-characterized antigens, has occurred. MAbs serve as useful immunoprobes for studying epitopes responsible for allergic diseases. These antibodies also help to understand the cross-reactivity between the antigens of different fungi. Most importantly, MAbs are extremely useful to obtain pure antigenic and allergenic proteins for

diagnosis and immunotherapy. Several IgE-binding allergens of *Alt. alternata*, *C. herbarum*, *A. fumigatus*, and *Penicillium* spp. have been obtained using molecular cloning techniques. The complete amino acid and DNA sequences of these allergens have been reported. Large quantities of these purified allergens can be produced in appropriate expression systems. Two epitopes of Asp f 2, a major allergen of *A. fumigatus*, show strong IgE binding and cross-reactivity with related species of *Aspergillus*, but not with allergens from unrelated taxa. Two major epitopes of Alt a 1 show strong IgE binding and no identity with any of the known allergens. Hence, these epitopes can be safely and efficiently used as immunotherapeutic agents for managing fungal allergies. Similarly, mutants engineered from allergens may be of value for allergen immunotherapy.

The availability of well-characterized recombinant allergens may lead to the development of standardized allergens. Production of more well-characterized allergens at the molecular level for immunological evaluation of patients, combined with engineered allergens, synthetic peptides, conjugated allergens with CpG-motif and DNA vaccines, will lead to better understanding of the mechanisms of allergy as well as information for improved management of allergic diseases.

SALIENT POINTS

- Progress in standardization of mold vaccines has been impeded by the wide variation in biological potency among mold allergen extracts.
- Fungi may mutate, producing morphologically different forms. Once the fungal isolate is correctly identified, the question arises as to whether spores, mycelia, or culture filtrate should be used for the preparation of the antigen. Most extracts are prepared from mycelia and contain little or no spore material, and their inherent variability is a major problem.
- Fungal spores are structurally very different from pollens since inhaled particles consist of entire living cells, capable of growing and secreting allergens in vivo.
- Apart from *Alternaria*, *Aspergillus*, *Penicillium*, *Cladosporium*, and a few other species of the fungi, purified and standardizable antigens are not available from other fungal species. Hence the use of fungal antigens for diagnosis or for use as vaccines may not be comparable due to their variability.
- Many common fungi still await clinical evaluation and testing.
- Cloning of allergen genes will facilitate desirable epitope identification and produce allergens that could provide safer and more effective treatment of mold allergy.
- Some mold allergens, such as glycopeptides, share common antigenic determinants with related and, at times, with unrelated species.
- Although the fungal spores in the outdoor air are seasonal, in cold climates some mold-sensitive patients have perennial symptoms, possibly as a result of growth and sporulation of fungi in the indoor environment.
- Production of more well-characterized allergens at the molecular level for immunological evaluation of patients, combined with engineered allergens, synthetic peptides imitating T- and B-cell epitopes of allergen, will lead to better understanding of the mechanisms of allergy as well as information for improved management of the disease.

ACKNOWLEDGMENTS

This study was supported by the Department of Pediatrics, Medical College of Wisconsin. The editorial assistance of Drs Makonnen Abebe and Serdal Sevinc is gratefully acknowledged.

REFERENCES

1. Ainsworth GC. Ainsworth and Bisby's Dictionary of the Fungi. 6th ed. Kew, England: Commonwealth Mycological Institute, 1971.
2. Portnoy J, Chapman J, Burge H, et al. *Epicoccum* allergy skin reaction patterns and spore/mycelium disparities recognized by IgG and IgE ELISA inhibition. Ann Allergy 1987; 59:39–43.

3. Wuethrich B. Epidemiology of allergic diseases: are they really on the increase. Int Arch Allergy Appl Immunol 1989; 90:3–10.
4. Hagy GW, Settipane GA. Bronchial asthma allergic rhinitis, and allergy skin tests among college students. J Allergy 1969; 44:323–332.
5. Salo PM, Arbes SJ Jr., Sever M, et al. Exposure to *Alternaria alternata* in US homes is associated with asthma symptoms. J Allergy Clin Immunol 2006; 118:892–898.
6. Reijula K, Leino M, Mussalo-Rauhamma H, et al. IgE mediated allergy to fungal allergens in Finland with special reference to *Alternaria alternata*. Ann Allergy Asthma Immunol 2003; 91:280–287.
7. Kurup VP, Fink JN. Fungal allergy. In: Murphy JW, Friedman H, Bendinelli M, eds. Fungal Infection and Immunity Responses. New York: Plenum Press, 1993:393–404.
8. Burge HA. Airborne-allergenic fungi. Immunol Allergy Clin North Am 1989:9:307–319.
9. Greenberger PA. Allergic bronchopulmonary aspergillosis and fungoses. Clin Chest Med 1988; 9: 599–608.
10. Fink JN. Allergic bronchopulmonary aspergillosis. Hosp Pract 1988; 23:105–128.
11. Katzenstein A, Sale SR, Greenberger PA. Allergic aspergillus sinusitis: a newly recognized form of sinusitis. J Allergy Clin Immunol 1983; 72:89–98.
12. Kurup VP. Hypersensitivity pneumonitis due to sensitization with thermophilic actinomycetes. Immunol Allergy Clin North Am 1989; 9:285–306.
13. Bierman WC, Van Arsdel PP Jr. Clinical evaluation of the patient with allergic and immunologic disease. In: Lockey RF, Bukantz SC, eds. Principles of Immunology and Allergy. Philadelphia: WB Saunders, 1987:1–26.
14. Kurup VP, Kumar A. Immunodiagnosis of aspergillosis. Clin Microbiol Rev 1991; 4:439–459.
15. Patterson R, Greenberger PA, Roberts ML. Allergic Bronchopulmonary Aspergillosis. Providence RI: Oceanside Publications, Inc., 1996.
16. Horner WE, Helbling A, Salvaggio JE, et al. Fungal allergens. Clin Microbiol Rev 1995; 8:161–179.
17. Ganassini A, Cazzadori A. Invasive pulmonary aspergillosis complicating allergenic bonchopulmonary aspergillosis. Respir Med 1995; 89:143–145.
18. Johannine E, Morev PR, Jarvis BB. Clinical-epidemiological investigation of health effects caused by Stachybotrys atra building contamination. In: Jaakola JJK, Ilmarinen R, Sepanen S, eds. Indoor Air '93. Proceedings of the 6th International Conference on Indoor Air Quality and Climate. Vol I: Health Effects. Helsinki: Institute of Occupational Health, 1993:225–230.
19. Margulis L, Schwartz KV. Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth. 2nd ed. New York: W. H. Freeman and Company, 1988.
20. Hawksworth, DL, Sutton BC, Ainsworth GC. Ainsworth and Bisby's Dictionary of Fungi. International Mycological Institute. Cambridge: University Press, 1994.
21. Miller OK, Fair DF. An Index of the Common Fungi of North America (Synonyms and Common Names). Vaduz Liechtenstein: J. Cramer, 1990.
22. Alexopoulos C, Mims C, Blackwell M. Introductory Mycology. New York: Wiley & Sons, 1996.
23. Ainsworth GC. Introduction and keys to higher taxa. In: Ainsworth GC, Sparrow FK, Sussman AS, eds. The Fungi. New York: Academic Press, 1973.
24. Carmichael JW. Pleomorphism. In: Cole GT, Kendrick B, eds. Biology of Conidial Fungi. New York: Academic Press, 1981.
25. Weresub LK, Pirozynski KA. Pleomorphism of fungi as treated in the history of mycology and nomenclature. In: Kendrick B, ed. The Whole Fungus. Ottawa: National Museums of Canada, 1979.
26. Bold HC, Alexopoulos CJ, Delevoryas T. Morphology of Plants and Fungi. New York: Harper and Row, 1973.
27. Alexopoulos CJ, Mims CW. Introductory Mycology. 3rd ed. New York: John Wiley and Sons, 1979.
28. Talbot PHB. Principles of Fungal Taxonomy. New York: St. Martin's Press, 1971.
29. Loomis WF. Dictyostelium discoideum, A Developmental System. New York: Academic Press, 1975.
30. Kendrick B. The Fifth Kingdom. Waterloo, Canada: Mycologue Publications, 1985.
31. Kendrick B. The history of conidial fungi. In: Cole GT, Kendrick B, eds. Biology of Conidial Fungi. New York: Academic Press, 1981.
32. Kendrick B, Nag Raj TR. Morphological terms in fungi imperfecti. In: Kendrick B, ed. The Whole Fungus. Ottawa: National Museums of Canada, 1979.
33. Wicklow ET, Carroll GC. The Fungal Community: Its Organization and Role in the Ecosystem. New York: Marcell Dekker, 1981.
34. Latge JP, Paris S. The fungal spore: reservoir of allergens. In: Cole GT, Moch HC, eds. The Fungal Spore and Disease Initiation in Plants and Animals. New York: Plenum Press, 1991:379–401.
35. Yunginger JW, Jones RT, Gleich GJ. Studies on *Alternaria* allergens. II. Measurement of the relative potency of commercial *Alternaria* extracts by the direct RAST and by RAST inhibition. J Allergy Clin Immunol 1976; 58:405–413.
36. Aukrust L. Cross radioimmunoelectrophoretic studies of distinct allergens in two extracts of *Cladosporium herbarum*. Int Arch Allergy Appl Immunol 1979; 58:375–390.

37. Kim SJ, Chaparas SD. Characterization of antigens from Aspergillus fumigatus. I. Preparation of antigens from organism grown in completely synthetic medium. Am Rev Respir Dis 1978; 118:547–551.
38. Kurup VP, Fink JN, Scribner GH, et al. Antigenic variability of *Aspergillus fumigatus* strains. Microbios 1977; 19:191–204.
39. Chapman MD. Monoclonal antibodies as structural probes for mite, cat and cockroach allergens. In: Shami AE, Merrett TG, eds. Advances in Biosciences, Allergy and Molecular Biology. New York: Pergamon Press, Inc., 1989:281–295.
40. Lehrer SB, Salvaggio JE. Allergens: standardization and impact of biotechnology—a review. Allergy Proc 1990; 11:197–208.
41. Aas K, Backman A, Belin L, et al. Standardization of allergen extracts with appropriate methods. Allergy 1978; 33:130–137.
42. Dreborg S, Agrell B, Foucard T, et al. A double-blind, multicenter immunotherapy trial in children, using a purified and standardized *Cladosporium herbarum* preparation. Allergy 1986; 41:131–140.
43. Turner KJ, Stewart GA, Sharp AH, et al. Standardization of allergen extracts by inhibition of RAST, skin test and chemical composition. Clin Allergy 1980; 10:441–450.
44. Kurup VP, Banerjee B, Hemmann S, et al. Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. Clin Exp Allergy 2000; 30:988–993.
45. Weber RW. Allergen immunotherapy and standardization and stability of allergen extracts. J Allergy Clin Immunol 1989; 84:1093–1095.
46. Lehrer SB, Aukrust L, Salvaggio JE. Respiratory allergy induced by fungi. Clin Chest Med 1983; 4: 23–41.
47. Beaumont F, Kauffman HF, Sluiter HJ, et al. Sequential sampling of fungal air spores inside and outside the homes of mold sensitive, asthmatic patients: a search for a relationship to obstructive reactions. Ann Allergy 1985; 55:740–746.
48. Burge HA. Fungus allergens. Clin Rev Allergy 1983; 3:319–329.
49. Licorish K, Novey HS, Kozak P, et al. Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. J Allergy Clin Immunol 1985; 76:819–825.
50. Becker AB, Muradia G, Vijay HM. Immunoreactive Alternaria allergens in house dust in the absence of environmental mold. (abstr 151). J Allergy Clin Immunol 1996; 97(suppl 1):220.
51. Malling HJ. Diagnosis and immunotherapy of mold allergy. IV. Relation between asthma symptoms, spore counts and diagnostic tests. Allergy 1986; 41:342–350.
52. Lehtonen M, Reponen T, Nevalainen A. Every day activities and variation of fungal spore concentrations in indoor air. Int Biodeterior Biodegradation 1993; 31:25–39.
53. Nevalainen A, Rautiala S, Hyvarinen A, et al. Exposure to fungal spores in mouldy houses. Effect of remedial work. In: Agashe SN, ed. Recent Trends in Aerobiology, Allergy and Immunology. New Delhi, Bombay, and Calcutta: Oxford & IBH Publishing Co. Pvt., Ltd., 1994:99–107.
54. Spieksma FTh M. Aerobiology of common environmental allergens: sizes of allergen carrying particles. Asian Pac J Allergy Immunol 1993; 11(suppl 1):93–94.
55. Kozak PP Jr., Gallup J, Cummins LH, et al. Factors of importance in determining the prevalence of indoor molds. Ann Allergy 1979; 43:88–94.
56. D'Amato G, Spieksma FTh M. Aerobiologic and clinical aspects of mould allergy in Europe. Allergy 1995; 50:870–877.
57. Solomon WR. A volumetric study of winter fungus prevalence in the air of midwestern homes. J Allergy Clin Immunol 1976; 57:46.
58. Garrett MH, Hooper BM, Cole FM, et al. Airborne fungal spores in 80 homes in Latrobe Valley, Australia: levels, seasonality and indoor-outdoor relationship. Aerobiologia 1997; 13:121–126.
59. Vijay HM, Hughes DH, Young NM. The allergens of *Alternaria* species. J Palynology 1991; 27:387–397.
60. Aukrust L, Borch SM. Cross-reactivity of moulds. Allergy 1985; 40:57–60.
61. Wagner S, Sowka S, Mayer C, et al. Identification of a *Hevea brasiliensis* latex manganese superoxide dismutase (Hev b 10) as a cross-reactive allergen. Int Arch Allergy Immunol 2001; 125:120–127.
62. Durham OC. Incidence of air-borne fungus spores. I. *Alternaria*. J Allergy 1937; 8:480–490.
63. Aas K. Bronchial provocation tests in asthma. Arch Dis Child 1970; 45:221–228.
64. Bronsky EA, Ellis EF. Inhalation bronchial challenge testing in asthmatic children. Pediatr Clin North Am 1969; 16:85–94.
65. Schlueter DP, Fink JN, Hensley GT. Wood-pulp worker's disease: a hyper-sensitivity pneumonitis caused by *Alternaria*. Ann Intern Med 1972; 77:907–914.
66. Yunginger JW, Jonen RT, Nesheim ME, et al. Studies on Alternaria allergens. III. Isolation of major allergenic fraction (ALT-1). J Allergy Clin Immunol 1980; 66:138–147.
67. Nyholm L, Lowenstein H, Yunginger JW. Immunochemical partial identity between two independently identified and isolated major allergens from *Alternaria alternata* (ALT-1 and Ag1). J Allergy Clin Immunol 1983; 71:461–467.

68. Vijay HM, Muradia G, Young NM, et al. Characterization of monoclonal antibodies of *Alternaria alternata* (abstr). J Allergy Clin Immunol 1993; 91(suppl 1):273.
69. Sanchez H, Bush RK. Purification of *Alternaria* (ALT) allergens by use of monoclonal antibody. J Allergy Clin Immunol 1988; 81:184 (abstr).
70. Portnoy J, Olson I, Pacheco F, et al. Affinity purification of a major *Alternaria* allergen using a monoclonal antibody. Ann Allergy 1990; 65:109–114.
71. Lepage D, Boutin Y, Vrancken ER. Allergenic determinants of *Alternaria* extracts defined by monoclonal antibodies (abstr). J Allergy Clin Immunol 1989; 83:216.
72. Vijay HM, Kumar V, Abebe M, et al. Polyclonal and monoclonal antibodies to cloned and native Alt a1. J Allergy Clin Immunol 2003, 111:242 (abstr 694).
73. Barnes C, Portnoy J, Sever M, et al. Comparison of enzyme immunoassay-based assays for environmental *Alternaia alternata*. Ann Allergy Asthma Immunol 2006; 97:350–356.
74. Abebe M, Kumar V, Rajan S, et al. Detection of recombinant *Alt a* 1 in a two-site, IgM based, sandwich ELISA open up possibilities of developing alternative assays for the allergen. J Immunol Methods 2006; 312:111–117.
75. Deards MJ, Montague AE. Purification and characterization of a major allergen of *Alternaria alternata*. Mol Immunol 1991; 28:409–415.
76. Paris S, Debeaupuis JP, Prevost MC, et al. The 31 kD major allergen, ALT a 1,1563, *Alternaria alternata*. J Allergy Clin Immunol 1991; 88:902–908.
77. Matthiesen F, Olsen M, Lowenstein H. Purification and partial sequenization of the major allergen of *Alternaria alternata Alt a*1. J Allergy Clin Immunol 1992; 89:241 (abstr 386).
78. Curran IHA, Young NM, Burton M, et al. Purification and characterization of Alt a-29 from *Alternaria alternata*. Int Arch Allergy Immunol 1993; 102:267–275.
79. Bush RK, Sanchez H. cDNA sequence of an *Alternaria* allergen. In: Kraft D, Sehon A, eds. Biology and Immunology of Allergens. Boca Raton, FL: CRC Press, Inc., 1993:271–273.
80. Martinez-Quesada J, Gutierrez A, Postigo I, et al. Variability of Alt a 1 expression by different strains of *Alternaria alternata*. J Investig Allergol Clin Immunol 2006; 16:279–282.
81. Budd TW, Kuo CY, Yoo TJ, et al. Antigens of Alternaria. I. Isolation and partial characterization of a basic peptide allergen. J Allergy Clin Immunol 1983; 71:277–282.
82. DeVouge MW, Thaker AJ, Curran IHA, et al. Isolation and expression of cDNA clone encoding an *Alternaria alternata* Alt a1 subunit. Int Arch Allergy Immunol 1996; 111:385–395.
83. DeVouge MW, Thaker AJ, Zhang L, et al. Molecular cloning of IgE-binding fragments of *Alternaria alternata* allergens. Int Archs Allergy Immunol 1998; 116:261–268.
84. Achatz G, Oberkofler H, Lechenauer E, et al. Molecular cloning of major and minor allergens of *Alternaria alternata* and *Cladosporium herbarum*. Mol Immunol 1995; 32(suppl 3):213–227.
85. Kurup VP, Vijay HM, Kumar V, et al. IgE binding synthetic peptides of Alt a1, allergen of Alternaria alternata peptides. Peptides 2003; 24:179–185.
86. Simon-Nobbe B, Probst G, Kajava AV, et al. IgE-binding epitopes of enolases, a class of highly conserved fungal allergens. J Allergy Clin Immunol 2000; 106:887–895.
87. Schneider PB, Denk U, Brietenbach M, et al. *Alternaria alternata* NADP 8-dependent mannitol dehydrogenase is an important fungal allergen. Clin Exp Allergy 2006; 36:1513–1524.
88. Solomon WR. Matthews KP. Aerobiology and inhalant allergens. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, et al., eds. Allergy: Principles and Practice. 3rd ed. St. Louis: The C.V. Mosby Co., 1989:312–372.
89. Wickman M, Gavesen S, Nordvall SL, et al. Indoor viable dust-bound microfungi in relation to residential characteristics, living habits, and symptoms in atopic and control children. J Allergy Clin Immunol 1993; 89:752–759.
90. Lacey J. The aerobiology of conidial fungi. In: Cole GT, Kendrick B, eds. Biology of Conidial Fungi. New York: Academic Press, Inc., 1981:373–416.
91. Aukrust L, Borch SM. Partial purification and characterization of two *Cladosporium herbarum* allergens. Int Arch Allergy Appl Immunol 1979; 60:68–79.
92. Aukrust L. Cross radioimmunoelectrophoretic studies of distinct allergens in two extracts of *Cladosporium herbarum*. Int Arch Allergy 1979; 58:375–390.
93. Zhang L, Curran I, Muradia G, et al. Two dimensional immunoblot analysis of allergens of Cladosporium herbarum. Clin Exp Allergy 1994; 24:263–269.
94. Sward-Nordmo M, Wold JK, Paulsen BS, et al. Purification and partial characterization of the allergen Ag54 from *Cladosporium herbarum*. Int Arch Allergy Appl Immunol 1985; 78:249–255.
95. Sward-Nordmo M, Paulsen BS, Wold JK. Immunological studies of glycoprotein allergen Ag-54 (Cla h II) in *Cladosporium herbarum* with special attention to the carbohydrate and protein moieties. Int Arch Allergy Appl Immunol 1989; 90:155–161.
96. Zhang L, Muradia G, Curran IHA, et al. A cDNA clone coding for a novel allergen, Cla h III, of *Cladosporium herbarum* identified as a ribosomal P2 protein. J Immunol 1995; 154:710–716.

97. Breitenbach M, Simon B, Probst G, et al. Enolases are highly conserved fungal allergens. Int Arch Allergy Immunol 1997; 113:114–117.
98. Simon Nobbe B, Denk U, Schneider PB, et al. NADP-dependent mannitol dehydrogenase, a major allergen of *Cladosporium herbarum*. J Biol Chem 2006; 281:16354–16360.
99. Shimada, Matsumura K. A case of probable allergic bronchopulmonary aspergillosis due to *Aspergillus niger*. Nippon Kyobu Shikkan Zasshi 1995; 33:336–341.
100. Laham MN, Allen RC, Greene JC. Allergic bronchopulmonary aspergillosis (ABPA) caused by *Aspergillus terreus*: specific lymphocyte sensitization and antigen-directed serum opsonic activity. Ann Allergy 1981; 46:74–80.
101. Hearn VM, Wilson EV, Latge JP, et al. Immunochemical studies of *Aspergillus fumigatus* mycelial antigens by polyacrylamide gel electrophoresis and Western blotting techniques. J Gen Microbiol 1990; 136:1525–1535.
102. Kurup VP, Ramasamy M, Greenberger PA, et al. Isolation and characterization of a relevant *Aspergillus fumigatus* antigen with IgG and IgE binding activity. Int Arch Allergy Appl Immunol 1988; 86:176–182.
103. Longbottom JL, Austwick PKC. Antigens and allergens of Aspergillus fumigatus. 1. Characterization by quantitative immunoelectrophoretic techniques. J Allergy Clin Immunol 1986; 78:9–17.
104. Longbottom JL, Harvery C, Taylor ML, et al. Characterization of immunologically important antigens and allergens of *Aspergillus fumigatus*. Int Arch Allergy Appl Immunol 1989; 88:185–186.
105. Taylor ML, Longbottom JL. Partial characterization of a rapidly released antigenic/allergenic component (Ag 5) of *Aspergillus fumigatus*. J Allergy Clin Immunol 1988; 81:548–557.
106. Teshima R, Ikebuchi H, Sawader J, et al. Isolation and characterization of a major allergenic component (gp 55) of *Aspergillus fumigatus*. J Allergy Clin Immunol 1993; 92:698–706.
107. Kumar A, Kurup VP, Greenberger PA, et al. Production and characterization of a monoclonal antibody to a major Concanavalin A-nonbinding antigen of *Aspergillus fumigatus*. J Lab Clin Med 1993; 121:431–436.
108. Arruda KL, Platts-Mills TAE, Fox JW, et al. *Aspergillus fumigatus* allergen 1. a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. J Exp Med 1990; 172:1529–1532.
109. Arruda LK, Mann BJ, Chapman MD. Selective expression of a major allergen and cytotoxin, *Asp f1* in *Aspergillus fumigatus*. Implications for the immunopathogenesis of *Aspergillus* related diseases. J Immunol 1992; 149:3354–3359.
110. Moser M, Crameri R, Menz G, et al. Cloning and expression of recombinant *Aspergillus fumigatus* allergen I/a (rAsp f I/a) with IgE binding and type I skin test activity. J Immunol 1992; 149:454–460.
111. Kurup VP, Vijay H, Guo J, et al. *Aspergillus fumigatus* peptides differentially express Th1 and Th2 cytokines. Peptides 1996; 17:183–190.
112. Kurup VP, Banerjee B, Murali PS, et al. Immunodominant peptide epitopes of allergen Asp f I from the fungus *Aspergillus fumigatus*. Peptides 1998; 19:1469–1477.
113. Banerjee B, Kurup VP, Phadnis S, et al. Molecular cloning and expression of a recombinant *Aspergillus fumigatus* protein Asp f2 with significant Immunoglobulin E reactivity in allergic bronchopulmonary aspergillosis. J Lab Clin Med 1996; 127:253–262.
114. Kumar A, Reddy LV, Sochanik A, et al. Isolation and characterization of a recombinant heat shock protein of A. fumigatus. J Allergy Clin Immunol 1993; 91:1024–1030.
115. Banerjee B, Kurup VP, Greenberger PA, et al. Cloning and expression of *Aspergillus fumigatus* allergen Asp f 16 mediating both humoral and cell-mediated immunity in allergic bronchopulmonary aspergillosis (ABPA). Clin Exp Allergy 2001; 31:761–770.
116. Chou H, Lai HY, Tarn MF, et al. cDNA cloning biological and immunological characterization of the alkaline serine protease major allergen from *Penicillium chrysognum*. Int Arch Allergy Immunol 2002; 127:15–26.
117. Shen HD, Wang CW, Lin WL, et al. CDNA cloning and immunological characterization of *Pen o* 18, the vacuolar serine protease major allergen of *Penicillium oxalicum*. J Lab Clin Med 2001; 137:115–124.
118. Su NY, Yu CJ, Shen HD, et al. Pen c I, a novel enzymic allergen protein from *Penicillium citrinum*. Purification, characterization, cloning and expression. Eur J Biochem 1999; 261:115–123.
119. Shen HD, Lin WL, Tarn MF, et al. Alkaline serine proteinase: a major allergen of *Aspergillus oryzae* and its cross-reactivity with Penicillium citrinum. Int Arch Allergy Immunol 1998; 116:29–35.
120. Shen HD, Tarn MF, Chou H, et al. The importance of serine proteinases as aeroallergens associated with asthma. Int Arch Allergy Immunol 1999; 119:259–264.
121. Shen HD, Au LC, Lin WL, et al. Molecular cloning and expression of a. *Penicillium citrinum* allergen with sequence homology and antigenic cross-reactivity to a hsp 70 human heat shock protein. Clin Exp Allergy 1997; 27:682–690.
122. Shen HD, Lin WL, Tsai JJ, et al. Allergenic components in three different species of *Penicillium*: cross-reactivity among major allergens. Clin Exp Allergy 1996; 26:444–451.

123. Shen HD, Lin WL, Tarn MF, et al. Identification of vacuolar serine proteinase as a major allergen of *Aspergillus fumigatus* by immunoblotting and N- terminal amino acid sequence analysis. Clin Exp Allergy 2001; 31:295–302.
124. Lai HY, Tarn MF, Tang RB, et al. cDNA cloning and immunological characterization of a newly identified enolase allergen from *Penicillium citrinum* and *Aspergillus fumigatus*. Int Arch Allergy Immunol 2002; 127:181–190.
125. Crameri R, Jaussi R, Menz G, et al. Display of expression products of cDNA libraries on phage surfaces. Eur J Biochem 1994; 226:53–58.
126. Crameri R, Faith A, Hemmann S, et al. Humoral and cell-mediated autoimmunity in allergy to *Aspergillus fumigatus*. J Exp Med 1996; 184:265–270.
127. Muilenberg M, Burge H, Sweet T, et al. *Penicillium* species in and out of doors in Topeka KS. J Allergy Clin Immunol 1990; 85:247.
128. Wei DL, Chen JH, Jong SC, et al. Indoor airborne *Penicillium* species in Taiwan. Curr Microbiol 1993; 26:137–140.
129. Shen HD, Lin WL, Tsai JJ, et al. Allergenic components in three different species of *Penicillium*: cross-reactivity among major allergens. Clin Exp Allergy 1996; 26:444–451.
130. Shen HD, Lin WL, Liaw SF, et al. Characterization of the 33-kilodalton major allergen of *Penicillium citrinum* by using MoAbs and N-terminal amino acid sequencing. Clin Exp Allergy 1997; 27:79–86.
131. Shen HD, Tarn MF, Chou H, et al. The importance of serine proteinases as aeroallergens associated with asthma. Int Arch Allergy Immunol 1999; 119:259–264.
132. Official List of Allergens. IUIS Allergen Nomenclature Subcommittee. Available at: http://www.allergen.org.
133. Vijay HM, Abebe M, Kumar V, et al. Allergenic and mutagenic characterization of 14 *Penicillium* species. Aerobiologia 2005; 21:95–103.
134. Sevinc MS, Kumar V, Abebe M, et al. Isolation and characterization of a cDNA clone encoding one IgE-binding fragment of *Penicillium brevicompactum*. Int Archs Allergy Immunol 2005; 138:12–20.
135. Chou H, Lai HY, Tarn MF, et al. cDNA cloning biological and immunological characterization of the alkaline serine protease major allergen from *Penicillium chrysognum*. Int Arch Allergy Immunol 2002; 127:15–26.
136. Shen HD, Wang CW, Lin WL, et al. CDNA cloning and immunological characterization of Pen o 18, the vacuolar serine protease major allergen of *Penicillium oxalicum*. J Lab Clin Med 2001; 137:115–124.
137. Su NY, Yu CJ, Shen HD, et al. Pen c I, a novel enzymic allergen protein from *Penicillium citrinum*. Purification, characterization, cloning and expression. Eur J Biochem 1999; 261:115–123.
138. Shen HD, Lin WL, Tarn MF, et al. Identification of vacuolar serine proteinase as a major allergen of *Aspergillus fumigatus* by immunoblotting and N-terminal amino acid sequence analysis. Clin Exp Allergy 2001; 31:295–302.
139. Shen HD, Au LC, Lin WL, et al. Molecular cloning and expression of a Penicillium citrinum allergen with sequence homology and antigenic cross-reactivity to a hsp 70 human heat shock protein. Clin Exp Allergy 1997; 27:682–690.
140. Lai HY, Tam MF, Tang RB, et al. cDNA cloning and immunological characterization of a newly identified enolase allergen from *Penicillium citrinum* and *Aspergillus fumigatus*. Int Arch Allergy Immunol 2002; 127:181–119.
141. Shen HD, Wang CW, Chou H, et al. cDNA cloning and immunological characterization of a new *Penicillium citrinum* allergen (Pen c 3). J Allergy Clin Immunol 2000; 105:827–833.
142. Vijay HM, Thaker AJ, Banerjee B, et al. Mold allergens. In: Lockey RF, Bukantz C, eds. Allergens and Allergen Immunotherapy. New York: Marcel Dekker, Inc., 1998:133–154.
143. Kurup VP, Shen H-D, Banerjee B. Respiratory fungal allergy. Microbes Infect 2000; 2:1101–1110.
144. Horner WE, Ibanez MD, Lehrer SB. Immunoprint analysis of Calvatia cyathiformis allergens. I. Reactivity with individual sera. J Allergy Clin Immunol 1989; 83:784–792.
145. Davis WE, Horner WE, Salvaggio JE, et al. Basidiospore allergens: analysis of Coprinus quadrifidus spore, cap, and stalk extracts. Clin Allergy 1988; 18:261–267.
146. Tarlo SM, Bell B. Srinivasan J, et al. Human sensitization to *Ganoderma* antigen. J Allergy Clin Immunol 1979; 64:43–49.
147. Cutteon AEC, Hasnain SM, Segedin BP, et al. The basidiomycete *Ganoderma* and asthma: collection, quantitation and immunogenicity of the spores. N Z Med J 1988; 101:361–363.
148. Horner WE, Helbling A, Lehrer SB. Basidiomycete allergens. Comparison of three *Ganoderma* species. Allergy 1993; 48:110–116.
149. Vijay HM, Comtois P, Sharma R, et al. Allergenic components of Ganoderma applanatum. Grana 1991; 30:167–170.
150. Ishiguro A, Homma M, Torii S, et al. Identification of Candida albicans antigens reactive with immunoglobulin E antibody of human sera. Infect Immunol 1992; 60:1550–1557.

151. Shen HD, Choo KB, Lee HH, et al. The 40 kD allergen of Candida albicans is an alcohol dehydrogenase molecular cloning and immunological analysis using monoclonal antibodies. Clin Exp Allergy 1991; 21:675–681.
152. Santos MO, Pereira M, Felipe MSS, et al. Molecular cloning and characterization of cDNA encoding the N-acetyl-b-D-glucosaminidase homologue of Paracoccidioides brasiliensis. Med Mycol 2004; 42:247–253.
153. Baldo BA, Baker RS. Inhalant allergies to fungi: reactions to baker's yeast and identification of baker's yeast enolase as an important allergen. Int Arch Allergy Appl Immunol 1998; 86:201–208.
154. Jensen-Jarolim E, Poulsen LK, With H, et al. Atopic dermatitis of the face, scalp, and neck: type I reaction to the yeast Pityrosporum ovale? J Allergy Clin Immunol 1992; 89:44–51.
155. Zargari A, Doekes G, van Ieperen-van Dijk AG, et al. Influence of culture period on the allergenic composition of Pityrosporum orbiculare extracts. Clin Exp Allergy 1995; 25:1235–1245.
156. Zargari A, Harfast AB, Johansson S, et al. Identification of allergen components of the opportunistic yeast Pityrosporum orbiculare by monoclonal antibodies. Allergy 1994; 49:50–56.
157. Woodfolk JA, Wheatley LM, Piyasena RN, et al. Trichophyton antigens associated with lgE antibodies and delayed type hypersensitivity. Sequence homology to two families of serine proteinases. J Biol Chem 1998; 273:489–496.
158. Deuell B, Arruda LK, Hayden ML, et al. Tr*ichophyton tonsurans* allergen. I. Characterization of a protein that causes immediate but not delayed hypersensitivity. J Immunol 1991; 147:96–101.

10 Mite Allergens

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INTRODUCTION

From the early 1960s, considerable literature has been published concerning the taxonomy, biology, immunochemistry, and control of mites implicated in allergic reactions. Numerous species have been described as the source of allergens capable of sensitizing and inducing allergic symptoms in sensitized and genetically predisposed individuals. Allergic diseases triggered by mite allergens include allergic rhinoconjunctivitis, asthma, atopic dermatitis, and other skin diseases. The most studied species, because of their abundance and allergenic importance, belong to the family Pyroglyphidae, especially *Dermatophagoides pteronyssinus*, *D. farinae,* and *Euroglyphus maynei*. These mites are called house-dust mites. House-dust mites are commonly present in human dwellings and are especially abundant in mattresses, sofas, carpets, and blankets. Other species, such as *D. microceras*, *D. siboney,* and *Gymnoglyphus longior*, and other genera, such as *Hirstia* and *Malayoglyphus*, are also considered allergenic, although their study is limited.

Numerous mite allergens have been purified, sequenced, and cloned. An important group of mites, referred to as "storage mites," comprises mainly members of the Acaridae and Glycyphagidae families that live in stored food and grains. All mite species present in the home environment and capable of inducing IgE-mediated sensitization are currently called "domestic mites" (1). Approximately 150 storage mite species are known (2); approximately 20 can be considered to be important from an economic and sanitary perspective. The most studied species are *Blomia tropicalis*, because of its abundance in tropical and subtropical regions of the world (3), and *Lepidoglyphus destructor*, because of its frequent presence in barns. Storage mite species can be present in kitchen floor dust, cupboards, and pantries. In humid homes, storage mites can also be found in mattress dust. They can be an important plague with economical consequences and cause occupational respiratory allergies in farmers and other occupationally exposed individuals. The most important genera are *Lepidoglyphus* (family Glycyphagidae), *Glycyphagus* (family Glycyphagidae), *Acarus* (family Acaridae), *Tyrophagus* (family Acaridae), *Aleuroglyphus* (family Acaridae), *Suidasia* (family Suidasidae), *Chortoglyphus* (family Chortoglyphidae), and *Cheyletus* (family Cheyletidae).

ALLERGENS FROM HOUSE-DUST MITES

The main source of allergens in house dust in the United States are the mite species *D. pteronyssinus, D. farinae, E. maynei,* and *B. tropicalis* (4). Most ecological studies in temperate climates have demonstrated that *D. pteronyssinus* (originally known as the European house-dust mite) and *D. farinae* (American house-dust mite) are the predominant house-dust mites worldwide. In tropical and subtropical areas of the world, *B. tropicalis* occurs with a very high frequency and, in some regions, it is present at a similar rate as *D. pteronyssinus* (5).

Mite allergens are capable of inducing an IgE-mediated immune response. These allergens can be extracted and isolated by conventional biochemical methods, molecular

cloning, or by using proteomic approaches. Mite allergens are present in mite bodies, secreta, and excreta. Fecal particles contain a great proportion of mite allergen (6). Mite allergens can be detected in many areas of the home, including beds, carpets, upholstered furniture, and clothing. Leather-covered couches, wood furniture, and bare floors contain fewer mites than the above-mentioned locations. Beds are the ideal habitat for mites, since they provide the ideal temperature, food and moisture for their proliferation, and allergens they produce accumulate deep inside old mattresses and pillows. They can also be detected airborne. Studies using volumetric samples equipped with sizing devices have shown that mite allergens remain airborne for a short period of time. Allergenic activity has been detected in particles smaller than 1 μm and in particles larger than 10 μm. Mite allergens settle more rapidly than cat allergens, which remain airborne for longer periods of time and can be detected in air samples collected in homes under disturbed and undisturbed conditions. Mite fecal pellets may occasionally enter the lung and cause inflammation and bronchoconstriction. Fergusson and Brodie (7) demonstrated the presence of Der p 1 in bronchial alveolar lavage fluids of asthmatic children after an overnight exposure to 13.4 and 27.3 μg of Der p 1 in carpets and mattresses, respectively. Exposure to 2 μg of Der p 1 and/or Der f 1/g of dust is considered a risk factor for sensitization; exposure to 10 μg/g of dust is considered a major risk factor for sensitization and asthma in genetically predisposed individuals. Allergen levels in excess of 10 μg/g of dust have been identified in many parts of the world, and there seems to be no difference between mite allergens levels in homes of mite-allergic asthmatic and nonallergic control individuals (1).

CHARACTERIZED HOUSE-DUST MITE ALLERGENS

Most of the isolated allergens have been placed in groups on the basis of their chronological characterization and/or homology with previously purified *Dermatophagoides* spp. allergens (Table 1). Purified allergens are named according to the first three letters of the genus, the first letter of the species and a number indicating the group in which they are placed. Thus, the first identified allergen of *D. pteronyssinus* was named Der p 1 and belongs to group 1.

Table 1 Biological Function, Molecular Weight and Prevalence of Specific IgE Binding of Several Mite Allergens

Group	Biological function	Molecular weight (kDa)	Mite species	% IgE binding
1	Cystein protease	25	Bt, Dp, Df, Dm, Em	80–100
2	Cholesterol-binding proteins	14	Bt, Dp, Df, Ds, Em, Ld, Tp, Gd, As	80–100
3	Tripsina	25 to 30	Bt, Dp, Df, Ds, Em	16–100
4	α-Amylase	57	Bt, Dp, Dm, Em	40–46
5	Unknown	15	Bt, Dp, Ld	50–70
6	Chymotrypsin	25	Bt, Dp, Df	40
7	Unknown	25,31,29,26	Dp, Df, Ld	50
8	Glutathion-S-tranpherase	26	Dp	40
9	Colagenolytic serin protease	30	Dp	90
10	Tropomyosin	37	Bt, Dp, Df, Ld	50–95
11	Paramyosin	96,92,98	Bt, Dp, Df	80
12	Unknown	14	Bt	50
13	Fatty acid-binding proteins	15	Bt, Df, Ld, Tp	10–23
14	Vitellogenin	117	Df, Dp, Em	90
15	Chitinase	62.5,98,105	Df	70
16	Gelsolin	55	Df	35
17	Calcium-binding proteins	33	Df	35
18	Chitinase	60	Df	60
19	Antimicrobial peptide	7	Bt	10
20	Argininquinase		Dp	
21	Unknown	13.2	Dp, Bt	
22	Unknown		Dp	

Abbreviations: As, *Acarus siro*; Bt, *B. Tropicalis*; Df, *Dermatophagoides farinae*; Dm, *Dermatophagoides microceras*; Dp, *Dermatophagoides pteronyssinus*; Em, *Euroglyphus mayne*; Gd, *Glycyphagus domesticus*; Ld, *L. Destructor*; Tp, *T. Putrescentiae.*
Source: http://www.allergen.org/Allergen.aspx.

Group 1: Der p 1 and Der f 1, and group 2 (Der p 2 and Der f 2) are considered major allergens on the basis of the frequency of patients sensitized, amount of specific IgE, and content in mite extract. Der p 1 is a glycoprotein with sequence homology and thiol protease functions similar to the enzymes papain, actinidin, bromelain and cathepsins B and H (8). Der p 1 and Der f 1 are synthesized as proenzymes and removal of the propiece is necessary to unmask their proteolytic activity. A study suggests that group 1 mite allergens are a new subgroup among C1 family cysteine peptidases, and that the rapid inactivation of Der p 1 prodomain is a newly identified mechanism that may contribute to the potency of this allergen (9). Several mechanisms have been demonstrated to be involved in the hyperresponsiveness and airway inflammation associated with this allergen; Der p 1 upregulates the IgE synthesis by cleaving the low-affinity IgE receptor (CD23) from the surface of human B-cell lymphocytes (10). Der p 1 cleaves the α subunit of the IL-2 receptor (IL-2R or CD25) from the surface of human peripheral blood T cells and, as a result, these cells show markedly diminished proliferation and interferon γ secretion, which consequently shifts the immune response toward Th2 cells. The cysteine protease activity of Der p 1 seems to selectively enhance the IgE response, and the proteolytic activity of Der p 1 conditions T cells to produce more IL-4 and less IFN-gamma (11,12). The enzymatic activity for Der p 1, and other mite allergens, may also contribute to their immunogenicity by increasing mucosal permeability. The peptidase activity creates conditions that favor delivery of any allergen to antigen-presenting cells by a process that involves cleavage of tight junctions that regulate paracellular permeability (13). Studies using A549 epithelial cells demonstrate that Der p 1 produces both damage and activation of airway epithelial cells. Der p 1 activates the release of cytokines from this cell line in a protease-activated receptor (PAR)-independent manner, in agreement with another study, which shows that Der p 3, but not Der p 1, activates the PAR2 signaling cascade (14). Conflicting reports have emerged in the literature regarding Der p 1 and PAR activation (15,16). A functional PAR2 has been demonstrated on A549 cells by a specific PAR2 agonists, which induces the production of IL-6 and IL-8. However, the mouse fibroblasts expressing the human PAR1, PAR2, or PAR4 demonstrate that Der p 1 does not affect intracellular calcium mobilization in these cells, providing evidence against a PAR-mediated mechanism. Der p 1 and Der f 1 cleave the lung surfactant protein A (SP-A) and the lung surfactant protein D (SP-D), lung collectins, which have protective roles in allergy, in a time- and concentration-dependent manner at multiple sites. Cleavage of these collectins abrogates their lectin activity and lectin-associated functions such as bacterial agglutination and allergen binding. The cleavage and consequent inactivation of SP-A and SP-D may be a novel mechanism to account for the potent allergenicity of Der p 1 and Der f 1 (17). Work in mice suggests that the cysteine protease activity of Der p 1 crucially contributes to in vivo immune responses, including the production of not only IgE but also IgGs. In this study, intranasal administration of proteolytically active Der p 1 to sensitized mice leads to enhanced inflammatory cellular infiltration of the lungs and systemic production of IgE, in comparison to inactive Der p 1, which has no effect (18).

Eur m 1 is an important allergen of *E. mainey* and has an amino acid (aa) sequence homology of approximately 85% with Der p 1 and Der f 1 (19). Der s 1, a major allergen of *D. siboney*, purified using cross-reacting monoclonal antibodies directed against group 1 allergen from *Dermatophagoides* spp., has an 89% frequency of specific IgE binding (20).

Because of its high prevalence in house dust, and worldwide distribution, the group 1 allergen is used as a standard to estimate environmental exposure to *Dermatophagoides* spp. in the indoor environment.

Group 2: Der p 2 and Der f 2 are heat- and pH-stable proteins of 14 kDa (21,22). These allergens have 88% sequence similarity. In their native stage and, expressed as a fusion protein, both have an 83% frequency of specific IgE recognition (23). The aa sequences of Der p 2 and Lep d 2 have 28% and 26.4% similarity, respectively, with the human epididymis 1 (HE1) gene product. These proteins seem to arise from secretions of the male mite reproductive tract (24). Der p 2 and Der f 2 show a significant degree of sequence polymorphism. The polymorphic residues are also found in regions containing T-cell epitopes (25). Crystallographic studies suggest that Der p 2 is a lipid-binding protein (26). The existence of Eur m 2 in *E maynei* has also been demonstrated (27).

The aa sequence of Der f 2 is homologous to other group 2 allergens from different mite species and the Niemann-Pick type C2 disease protein (NPC2/ HE1) with cholesterol-binding activity. The structural relationship between Der f 2 and the myeloid differentiation type 2

molecule (MD2), which associates with Toll-like receptor 4 on the cell surface and is part of the mammalian innate immune system involved in the recognition of lipopolysaccharide from gram-negative bacteria, was indicated by sequence homology search at http://expasy.org/tools/blast (28).

In Central Europe, more than 95% of mite-allergic patients are sensitized to Der p 1 and Der p 2. Diagnostic tests containing these allergens plus the highly cross-reactive allergen, Der p 10, may improve the diagnostic selection of patients for immunotherapy with *D. pteronyssinus* extracts (29). Suzuki M et al. demonstrated that the conformation of Der f 2 was critical in the determination of Th1/Th2 shift on the basis of physicochemical and immunological analyses (30). This study suggests that the rigidly folded and singly dispersed structure is required for the generation of Th2 type cells by the allergen, while conformational variant protein leads to Th1 skewing, irrespective of the same aa sequence.

Groups 3 and 4: Group 3 has a trypsin-like serine protease activity and 50% sequence similarity with other serine proteases, including chymotrypsin (31). The sequence of Der p 3 has 81% sequence identity with Der f 3 and both have a 41% sequence identity with bovine trypsin. A frequency of IgE binding between 51% and 90% for Der p 3 and between 42% and 70% for Der f 3 has been described (32). Der p 4 is an enzyme similar to carbonic anhydrases, which shows significant homology with mammalian α-amylase (33). It is recognized as an allergen by 25% to 46% of mite-allergic individuals.

Group 5: Der p 5 is a 15 kDa allergen, which has an estimated IgE binding prevalence of 50% (34). Studies with epithelial cells demonstrate that Der p 5 also induces the secretion of IL-6 and IL-8, even to a higher extent than Der p 1. This effect of Der p 5 is dose-dependent, not blocked by protease inhibitors, and is specific.

Groups 6 and 7: Der p 6 is a chymotrypsin-like serine protease, which shows a 40% to 60% frequency of IgE binding. It has 37% similarity with Der p 3 (35). Der p 7 and Der f 7 have 86% of similarity in aa sequences. Recombinant Der f 7 reacts with 46% of sera from asthmatic children (36). The allergenicity of r Der p 7 is demonstrated by direct specific IgE binding and skin testing; about 50% of mite-allergic individuals analyzed are sensitized to this allergen (37).

Groups 8 and 9: Der p 8 is a 26 kDa allergen with strong homology with rat and mouse glutathione-S-transferase (GST). Approximately 40% of mite-allergic subjects tested with recombinant Der p 8 bind specific IgE to this allergen (38). At least eight isoforms of native Der p 8 were detected by two-dimensional gel and immunoblot analyses. Sera from Taiwanese asthmatics show 96% and 84% IgE reactivity to native Der p 8 and recombinant Der p 8, respectively. Native Der p 8 shows 75% and 65% IgE reactivity with sera from Malaysia and Singapore, respectively. Native Der p 8 exists in multiple isoforms and inclusion of these isoforms for diagnostic and therapeutic purposes may be necessary. The presence of cross-reactive IgE between Der p 8 and GST in *Periplaneta americana* cockroach suggests that GST in mites and cockroach may be considered a pan-allergen (39).

Der p 9 is a 24 kDa protein, as indicated by mass spectroscopy, with collagenolytic serine protease activity and a frequency of IgE reactivity higher than 80% (40).

Groups 10 and 11: These groups are comprised of tropomyosin and paramyosin, respectively. They are involved in muscle contraction in invertebrates and are present in low concentrations in mite extracts. The invertebrate tropomyosins are allergenic in man with high IgE cross-reactivity and therefore, have been referred to as pan-allergens. Der f 10 is a 32 kDa allergen with significant homology with tropomyosins from different species (41). Der p 10 may be involved in the cross-reactivity process between mites, shrimp, and insects in shrimp-allergic patients (42,43). Allergenic cross-reactivity has been reported between Der p 10 and Blo t 10. Although Blo t 10 and Der p 10 are highly conserved and significantly cross-reactive, unique IgE epitopes do exist (44).

Der f 11 has 34% to 60% sequence identity with other known paramyosins (45); 62% of mite-sensitive asthmatic patients react when skin tested with recombinant Der f 11 and 50% have a positive specific IgE determination to Der f 11 (46). rDer p 11 has a frequency of specific IgE binding in the sera of allergic patients of 60%, suggesting that Der p 11 is also an important allergen (47). The aa sequence of Der p 11 shares over 89% identity with aa sequence of Der f 11 and Blo t 11 (48).

Group 12: Group 12 has only been described by cDNA cloning from *B. tropicalis* Blo t 12 having a mature sequence of 14 kDa, which binds specific IgE with a 50% frequency and does

not show homology with other known proteins (49). The aa sequence of Blo t 12 had no significant homology with known proteins. However, a limited homology with the C-terminal region of the putative chitinase allergen from *D. farinae* was observed. Recombinant Blo t 12 has been expressed in both *Escherichia coli* and *Pichia pastoris* expression systems (50). An isoform of Blo t 12 was isolated by molecular cloning and showed 14.4% specific IgE reactivity in the sera of allergic subjects from Singapore (51).

The IgE reactivity of this isoform has been tested in Colombian mite-allergic patients and shows 23.4% and 17% reactivity by ELISA and skin prick tests (SPT), respectively (52).

Groups 13 and 14: The group 13 allergen belongs to the family of fatty acid–binding proteins (FABPs) and has been cloned and characterized from a number of mites of clinical importance (53). Der f 13 shares a medium to high sequence homology with human FABPs, with the closest one being human brain FABP, having 39.1% aa identity and 58.6% similarity. A solution structure study reveals that Der f 13 adopts the typical β barrel fold of FABPs, which is very similar to that of other human FABPs (54). ELISA inhibition assays with monoclonal antibody specific for Blo t 13 suggest that the homologous allergen Der s 13 is also present in *D. siboney* (55).

Eleven percent of patients with asthma in Colombia show IgE reactivity to Blo t 13. In the mite-allergic population from Cuba, the frequency of IgE reactivity to this allergens is 53% (56). Blo t 13 has approximately 35.3% sequence identity with FABPs of human, bovine, mouse, and rat (57). Tyr p 13 was isolated from a cDNA library of the mite *Tyrophagus putrescentiae*, showing 62.3% of identity in the aa sequence with Blo t 13. The recombinant allergen showed 6.4% of reactivity in allergic individuals from Korea (58). In the sheep scab mite, *Psoroptes ovis*, a genomic sequence encoding for a FABP with 55% similarity with Blo t 13 has been identified (59).

Group 14 is an apolipophorin-like lipid transport protein, isolated by molecular cloning from *Dermatophagoides* spp. (60,61).

Group 15: Der f 15 is homologous to insect chitinases. It is a major allergen recognized by dogs and cats and by the sera of approximately 70% of mite-allergic humans (62). McCall et al. isolated a pair of natural Der f 15 proteins with apparent molecular weight masses of 98 and 109 kDa. The proteins were heavily O-glycosylated leading to the discrepancy between the calculated (61.2 kDa) and apparent molecular mass. It was concluded that the 98 and 109 kDa proteins reflected glycosylation variants of the same protein.

Two variants of Der p 15 have been isolated, encoding mature proteins of 58.8 and 61.4 kDa. Their aa sequences had 90% identity with Der f 15. Der p 15-specific IgE was detected in 70% of a panel of 27 human allergic sera (63). They are, therefore, potentially important allergens for humans as well as dogs.

Groups 16 and 17: Der f 16 polypeptide sequence has similarity to gelsolin, a Ca2+- and polyphosphoinositide 4,5-biphosphate (PIP2)-regulated actin filament severing and capping protein. In allergic individuals, IgE reactivity by skin and serological tests are between 62% and 50%, respectively (64). Der f 17 is a calcium-binding protein, which binds IgE in 35% of the sera from mite-allergic patients (65).

Group 18: Der f 18 is a 60,000 kDa molecular weight chitinase, which is a strong allergen for dogs and also reacts with 60% of mite-allergic humans (66). The cDNA for Der p 18 encodes a mature protein of 49.2 kDa with 88% sequence identity to Der f 18. Specific IgE to this allergen has been reported in 63% of a panel of 27 human allergic sera (67).

Groups 19 and 20: The allergen list from International Union of Immunological Societies Allergen Nomenclature Sub-Committee (http://www.allergen.org/Allergen/, updated/09/Jan/2008) shows that recombinant Blo t 19, under the biochemical name of antimicrobial peptide homologue, binds IgE in 10% of mite-allergic subjects. Der p 20 and Der f 20 are mite arginine kinase. Its sequence is highly conserved amongst invertebrates, showing 80% identity to crustaceans and 75% to insects compared with 45% for mammalian enzymes (68). Recombinant Der p 20 tested in allergic children showed a prevalence of specific IgE reactivity between 44.2% and 12.2%, with IgE levels lower than those obtained for Der p 1, Der 2, Der p 3, Der p 4, Der p 5, Der p 6, and Der p 7 (69).

Group 21: A novel allergen from *B. tropicalis*, Blo t 21, has been described. It is a 129-aa protein with an alpha-helical secondary structure and localizes to midgut and hindgut contents of *B. tropicalis*, as well as fecal particles. The gene encoding this novel antigen could be a duplicate (paralogous gene) of the Blo t 5 gene in the *B. tropicalis* genome. Blo t 21 shares 41%

and 39% aa identity with Der p 21 (UniProtKD/TrEMBL Q2L7C5) and Blo t 5, respectively. Blo t 21 shares some primary and secondary structural similarities with Blo t 5. However, there seems to be little cross-reactivity between these allergens. Positive responses to Blo t 21 were shown in 93% by means of ELISA and 95% by means of skin prick testing when assayed in 43 adult patients with allergic rhinitis (70).

Group 22: A sequence of 155 aa and 16,896 Da has been registered in GenBank (http://www.ncbi.nlm.nih.gov) and termed Der f 22.

ALLERGENS FROM STORAGE MITES

Several storage mite allergens have been purified, cloned, and sequenced (71). Some of these allergens can be considered as pan-allergens. The allergenicity of *B. tropicalis*, *L. destructor*, *Glycyphagus domesticus*, *T. putrescentiae, Acarus siro*, *Aleuroglyphus ovatus*, *Suidasia medanensis,* and *Thyreophagus entomophagus* has been demonstrated. Table 2 shows a list of the main families and species of storage mite that have been described as allergenic (72). Several allergens from these species have been purified, sequenced, and cloned. Some of these allergens have shown sequence homology and biological function similar to those previously described in *Dermatophagoides* spp. The main allergens described in storage mites include FABP, tropomysin, and paramyosin homologues, apolipophorine-like proteins, alfa-tubulines, and other, such as group 2, 5, and 7 allergens. The allergenicity of other species such as *A. farris*, *Austroglycyphagus malaysiensis*, *B. kulagini, B. tjibodas*, *Cheyletus eruditus*, *Chortoglyphus arcuatus*, *Gohieria fusca*, *Th. entomophagus* and *T. longior* has been investigated (Table 2).

Allergens from *Blomia tropicalis*

Several allergens of *B. tropicalis* are cloned and sequenced (73–80). Allergen sequence identity between *B. tropicalis* and *D. pteronyssinus* or *D. farinae* mite allergens ranges from moderate (30–45%) to high (50–70%) and to highly conserved (80–95%).

B. tropicalis allergens show variable degrees of sequence homology with purified allergens of *D. pteronyssinus,* such as Blo t 5, a homologue of Der p 5; Blo t 13, with homology to FABPs; Blo t 11, a homologue of paramyosin; Blo t 10, a homologue of tropomyosin and Der p 10; Blo t 3, a trypsin-like protease; Blo t 4, homologous to Der p 4 (amylase); and Blo t 1, homologous to Der p 1, a cysteine protease. Blo t 1, with an estimated molecular weight of

Table 2 Main Families and Species of Storage Mite That Have Been Described as Allergenic

Family	Species
Glycyphagidae	*Glycyphagus domesticus*
	G. privatus
	Gohieria fusca
	Lepidoglyphus destructor
Echimyopodidae	*Blomia tropicalis*
	B. kulagini
	B. tjibodas
Chortoglyphidae	*Chortoglyphus arcuatus*
Ebertiidae	*Suidasia medanensis*
Acaridae	*Tyrophagus putrescentiae*
	T. longior
	Acarus siro
	A. farris
	Thyreophagus entomophagus
	Aleuroglyphus ovatus
Cheyletidae	*Cheyletus eruditus*
	Ch. tenuipilis
	Ch. malaccensis

26 kDa, is an important allergen. Recombinant Blo t 1 reacted positively with IgE in 90% and 65% of sera from asthmatic children and adults from Singapore, respectively. Furthermore, there is a low correlation between IgE reactivity to Blo t 1 and Der p 1 (81).

Several studies have focused on the in vitro cross-reactivity of purified Blo t 5 and Der p 5 (82,83) and Blo t 10 and Der p 10 (tropomyosin). Most group 5 studies demonstrate low to moderate cross-reactivity at the molecular level. Yi et al. demonstrated that Blo t 10 and Der p 10 are highly conserved molecules, sharing 95% aa identity, and that they are cross-reactive allergens, although unique IgE epitopes do exist (84). Blo t 5 is recognized by 60% to 70% of *B. tropicalis*-sensitive patients, especially those residing in tropical areas (75,85).

Unlike *D. pteronyssinus*, where the group 1 and 2 allergens are the major allergens, the group 5 allergen of the *B. tropicalis*, Blo t 5, is the dominant major allergen (86). The recombinant Blo t 5 shows up to 70% of IgE reactivity in sensitized asthmatic patients, whereas the homologous Der p 5 reacts with 40% to 50% of sera from mite-allergic asthmatic individuals (87). Despite the sequence homology between the group 5 allergens, the IgE cross-reactivity of the major allergen Blo t 5 and the minor allergen Der p 5 is surprisingly low (88). The three-dimensional structure of Blo t 5 is a triple-helical bundle fold not described previously in other identified major allergens (89). Therefore, the molecular function of group 5 allergen remains unknown.

In vivo studies using inhalation tests to investigate the clinical significance of sensitivity to *B. tropicalis* and its cross-reactivity with *D. pteronyssinus* are scarce. Stanaland et al. (90) demonstrated that 83% of *B. tropicalis*-sensitive patients in Florida had a positive nasal challenge with a *B. tropicalis* extract. Therefore, a positive skin test to *B. tropicalis* is a good indicator of possible allergic symptoms after inhalation of *B. tropicalis* allergens. Other studies in Brazil and Singapore have demonstrated the allergenicity of *B. tropicalis*, in vivo, also using nasal challenges (91,92). In Brazil, a group of *D. pteronyssinus*- and *B. tropicalis*-sensitive patients was evaluated; 90% of the patients had a positive nasal challenge to *D. pteronyssinus* and 60% to *B. tropicalis*. The study conducted in Singapore included 20 adults with persistent allergic rhinitis, five of whom had a history of asthma. Significant increases in subjective and objective nasal symptoms, together with a significant increase of tryptase and LTC4 concentrations in nasal secretion, were found in all patients after each challenge with *B. tropicalis*. A study conducted by García Robaina et al. (93) demonstrated that patients who are sensitized to two mite species (*D. pteronyssinus* and *B. tropicalis*) may only react to one of them. This had been previously suggested for even more closely related species such as *D. pteronyssinus* and *D. farinae* (94). In some cases, it may, therefore, be necessary to conduct challenge tests to better define the relevant allergen from a clinical point of view and to start the appropriate specific immunotherapy treatment. The study by García Robaina et al. confirms previous in vivo and in vitro cross-reactivity observations using whole extracts as well as purified allergens. It suggests that although there is some in vitro and in vivo allergenic cross-reactivity between *B. tropicalis* and *D. pteronyssinus*, clinical symptoms induced by the inhalation of *B. tropicalis* and *D. pteronyssinus* allergens seem to be species specific, although some patients may react to common allergens.

Standardized extracts of *B. tropicalis* are only available in some European and Asian countries, in Cuba and in some African and South American countries. However, *B. tropicalis* extracts are only available under experimental conditions in the united states and many other countries throughout the world. There is a definitive need to use standardized extracts of *B. tropicalis* in countries with tropical, subtropical, and temperate climates of the world where *Blomia* species are endemic. Because of its wide distribution, the high rate of sensitization in many countries and the specificity of the allergic reactions, the inclusion of *B. tropicalis* for the diagnosis and treatment of specific IgE-mediated allergic reactions to this mite is warranted. A list of the main *B. tropicalis* allergens is shown in Table 3.

Allergens from *Lepidoglyphus destructor*

The most important storage mite species by distribution and abundance, excluding *B. tropicalis*, is *L. destructor*. At least 20 allergenic proteins have been identified in the extract of *L. destructor* (95) The major allergen, Lep d 2, formerly named Lep d 1, is a protein of 141 aa and molecular weight of 14 to 18 kDa by SDS-PAGE (96). It is present in the digestive tract of the mite (97). Lep d 2 has been cloned, sequenced, and expressed as a recombinant protein (rLep d 2) (98).

Table 3 Described Allergens in *B. tropicalis*

Allergens	*Dp*/*Df* allergens	Identity (identical residues/total residues)	MW (kDa)	Molecular function	IgE reactivity	
					Bt	*Dp*/*Df*
Blo t 1	Der p 1	32% (108/333)	26	cysteine protease	62–90%	80%
Blo t 2[b]	Der p 2	39% (57/146)	14	unknown	ND	80%
Blo t 3	Der p 3	49% (131/266)	25	trypsin	50–57%	16–100%
Blo t 4[a]	Der p 4	65% (335/515)	56	alpha amylase	<15%	40%
Blo t 5	Der p 5	42% (56/134)	14	unknown	43–92%	50–70%
Blo t 6[b]	Der p 6	58% (164/281)	25	chymotrypsin	<10%	40%
Blo t 10	Der p 10	95% (270/284)	33	tropomyosin	29%	50–95%
Blo t 11	Der f 11	89% (781/875)	110	paramyosin	12–52%	80%
Blo t 12	ND	ND	14	unknown	50%	ND
Blo t 13	Der f 13	80% (105/131)	15	Fatty acid–binding protein	11%	ND
Blo t 19	ND	ND	7.2	antimicrobial peptide	3%	ND

[a]http://www.ncbi.nlm.nih.gov.
[b]http://www.allergen.org.
Source: Adapted from Ref. 87
Abbreviations: MW, molecular weight; ND, not determined; Bt, *B. tropicalis*; Dp, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*.

This allergen possesses high IgE reactivity in vitro (99) and in vivo (100). Lep d 2 presents a high degree of polymorphism, with two distinct isoforms, Lep d 2.01 and Lep d 2.02. Lep d 2.02 has two variants and Lep d 2.01, three, two of which (Lep d 2.0101a and Lep d 2.0101b) have identical aa sequences. The rest of variants differ only in a few aa. The frequency of these variants may differ between wild and cultured mites (101).

Lep d 5 has been partially cloned and expressed. This partial clone of 110 aa and a molecular mass of 12.5 kDa is recognized by 9% of the sera from patients sensitized to *L. destructor* (102). Two isoforms have been sequenced, Lep d 5.02 with 171 aa and 19.5 kDa and Lep d 5.04, with 169 aa and 19.3 kDa. The allergen Lep d 7 has been sequenced and cloned; the calculated molecular mass is 22 kDA, without N-glycosylation sites. The recombinant protein rLep d 7 was recognized by 62% of the sera of *L. destructor*-positive subjects. The biochemical function of the group 7 mite allergens is unknown, and Lep d 7 does not show significant homologies to proteins other than to the group 7 mite allergens (102).

An allergenic protein homologous to tropomyosin Lep d 10 was identified as an allergen from a phage display cDNA library. The molecule of 284 aa is formed by two polypeptide chains. The sequence exhibits a prominent seven-residue periodicity. The IgE-binding frequencies of the recombinant Lep d 10 is estimated at 13% among subjects with IgE reactivity to mites and/or crustaceans (103).

The allergen Lep d 13 is sequenced and cloned. Its length is 131 aa with a calculated molecular weight mass of 14.6 kDa. It is involved in the intracellular transport of lipids, belonging to the family of FABP. The recombinant protein rLep d 13 is recognized by approximately 13% of the sera of *L. destructor* sensitized patients (102).

Other allergens have been sequenced, including the proteases Lep d 3 UniProtKB/Swiss-Prot (http://www.expasy.uniprot.org) (Q1M2L7), a GST Lep d 8 (Q1M2L6) and Lep d 12 (Q1M2L5) (104). An α-tubulin was identified as a putative allergen from a phage display *L. destructor* cDNA library. The IgE-binding frequency to the recombinant allergen is 12% among subjects with IgE reactivity to mites and/or crustaceans (103).

Allergens from *Glycyphagus domesticus*

G. domesticus is a phylogenetically closely related species with *L. destructor*. A 15 kDa allergen, belonging to group 2, and termed Gly d 2, is cloned and expressed as a recombinant protein. Gly d 2 shows a high degree of homology with Lep d 2. Three isoforms of Gly d 2 have been isolated; 16 out of 17 sera of sensitized patients recognize this recombinant protein (105). Other proteins homologous to group 3 (Q1M2M8), 5 (Q1M2M7), 7 (Q1M2M5), 8 (Q1M2M4), 10 (Q1M2L8) and 13 (Q1M2M3) are sequenced for this mite species (104).

Allergens from *Tyrophagus putrescentiae*

T. putrescentiae is one of the most important pest mite species on stored products. The presence of at least 14 allergens has been demonstrated in this mite species by means of crossed radioimmunoelectrophoresis (106). The major allergen is a 16 kDa protein recognized by 80% of sera from sensitized patients (107). This allergen, Tyr p 2, is cloned (108), sequenced, and expressed as a recombinant protein (rTyr p 2). This recombinant protein has demonstrated high IgE reactivity in vitro (99) and in vivo (100). Another allergen, Tyr p 13, homologous to FABPs, has also been identified, sequenced, and cloned. The recombinant allergen was detected by 6.4% of sensitized patients. An α-tubulin has been identified as a putative allergen from a phage display cDNA library of *T. putrescentiae*. The IgE-binding frequency of the recombinant allergen was 29.3% among subjects with IgE reactivity to mites and/or crustaceans (109). Tyr p 10 also has been cloned and shows 64% to 94% shared aa identity with other allergenic tropomyosins. This recombinant allergen is recognized by 12.5% of sera from sensitized patients (110).

Allergens from *Acarus siro*

A. siro belongs to the Acaridae family. A protein of 15 kDa with homology to other FABPs was identified, isolated, cloned, sequenced, and expressed as a recombinant protein. This allergen, Aca s 13, is recognized by 23% of the sera of patients sensitized to this mite (111).

Allergens from *Aleuroglyphus ovatus* and *Chortoglyphus arcuatus*

Numerous IgE-binding bands have been described with layer isoelectric focusing immunoblots in extracts of the brown-legged mite, *A. ovatus.* This species exhibits minimal to moderate cross-reactivity with house-dust mites (112,113). The most frequently detected allergens in the tropical mite, *S. medanensis*, have molecular weights of 21, 24.5, 30, 31, 47, and 50 kDa. Sui m 2, with a molecular weight of 15 kDa (Q2TUH5), has been described (104). There is a moderate degree of cross-reactivity among *S. medanensis*, *B. tropicalis*, and *D. farinae* (114). Anaphylaxis, after the ingestion of flour contaminated with *S. medanensis* has been reported (115). The mite species *Th. entomophagus* also has been implicated in cases of anaphylaxis after the ingestion of contaminated flour (116).

Several bands are recognized in extracts of *C. arcuatus* by specific IgE of sensitized patients. The most predominant bands have between 14 and 25 kDa, between 30 and 45 kDa, and between 46 and 65 kDa. There is minimal cross-reactivity between this mite species and *D. pteronyssinus* and moderate cross-reactivity with other storage mites (117). A tropomyosin allergen, Cho a 10, has been cloned and sequenced form a *C. arcuatus* expression library. The homology between Cho a 10 and Der p 10 is 94% and between Cho a 10 and Lep d 10, 95% (118). *Ch. eruditus* is a predator mite species frequently identified in house dust, especially in rural environments, where it feeds on storage mites. The existence of numerous allergens by immunoblotting (119) has been identified with a prominent band at approximately 16 kDa. There is a variable degree of cross-reactivity of this mite with the other domestic mites.

ALLERGENS OF MITES PRESENT IN AGRICULTURAL SETTINGS AND OF PARASITIC IMPORTANCE

The spider mites are main pests of fruit and horticultural crops and are common sensitizing allergens that are related to the prevalence of allergic diseases (120). Epidemiologic studies have also demonstrated high rates of sensitization in the surrounding population, which is not occupationally exposed to orchard trees (121). Major allergens with 10, 14, 19, 29, 67 and 75 kDa have been described by immunoblotting extracts of the two-spotted spider mite, *Tetranychus urticae* (122). Three allergens have been identified in *Panonychus ulmi*, the apple spider mite, with molecular weights of 33, 41, and 51 kDa. Specific IgE-binding against *Tet. urticae* and *Panonychus ulmi* was partially inhibited by extracts of *D. pteronyssinus* and *T. putrescentiae* (123). In the case of the citrus spider mite, *P. citri*, two allergens of 24 and 35 kDa have been identified. The N-terminal aa sequences of these major allergens of the spider mites are not homologous with any characterized allergens (124).

Allergens of mites used as biological control agents against spider mites and other pests, such as the Phytoseiidae, *Phytoseiulus persimilis* and *Amblyseius cucumeris,* or the

Table 4 Other Mite Families and Species of Allergenic Mites, Excluding House Dust and Storage Mites

Family	Parasites of Plants
Tetranychidae	*Tetranychus urticae*
	Panonychus ulmi
	P. citri
Tydeidae	*Pronematus davisi*
	Predator mites
Phytoseiidae	*Amblyseius cucumeris*
	Phytoseiulus persimilis
Hypoaspidae	*Hypoaspis miles*
Hemisarcoptidae	*Hemisarcoptes cooremani*
	Parasites of animals
Varroaidae	*Varroa* spp.
Sarcoptidae	*Sarcoptes scabiei*
Analgidae	*Diplaegidia columbae*
Ixodidae	*Ixodes pacificus*
	I. holociclus
	I. ricinus
	Riphicephalus spp.
Argasidae	*Argas reflexus*

Dermanyssidae, *Hypoaspis miles*, have been described (125,126). These predator mites have species specific as well as common antigens that are cross-reactive with *D. pteronyssinus* (127).

Several parasitic mite species are in frequent contact with humans and domestic animals. The itch mite, *Sarcoptes scabiei*, causes skin lesions and IgE-mediated sensitization in parasitized individuals (128–130). Allergens homologous to serine proteases (group 3) (131), GST (group 8) (132), paramyosin (group 11) (133) and apolipoprotein (134) have been identified. Ticks (Ixodida) belonging to the families Ixodidae and Argasidae have several proteins in their saliva that can induce IgE-mediated reactions after biting. Several cases of anaphylaxis after tick bites are reported (135). The allergenic composition of these mites has been analyzed, and an important allergen of *Argas reflexus*, the European pigeon tick, has been cloned (136). Arg r 1 is a protein belonging to the lipocalin family (137). In the case of the paralysis tick, *Ixodes holocyclus*, an allergen of 28 kDa from the salivary gland has been identified (138). Other allergenic proteins with molecular masses of 51, 38, 35 kDa from *I. pacificus*, *I. ricinus*, *Haemaphysalis punctata* and *Rhipicephalus* spp. have been described (139–142).

Other parasitic mites that have been involved in allergic reactions in humans are the bee parasite, *Varroa jacobsoni*, with an allergenic protein of 13 kDa (143) and the feather mite of domestic birds, *Diplaegidia columbae*, with 20 IgE-binding components ranging from 22 to 200 kDa (144). Two allergens from *Hemisarcoptes cooremani*, a predator of scale insects, of 16 kDa and 19 kDa have been described (145). Other mite families and species of allergenic mites, excluding house dust and storage mites, are shown in Table 4 (72).

CROSS-REACTIVITY OF MITE ALLERGENS

Cross-reactivity is a common feature among mite allergens, especially in those from taxonomically related species. The fact that mite-allergic individuals may be sensitized by various species could be due, in part, to cross-reactivity. Originally, cross-reactivity was studied using whole extracts and radioallergosorbent test (RAST) inhibition techniques. More recently, purified native or recombinant allergens, epitope mapping, and T-cell proliferation techniques have been used (146). Small peptides, with eight to 15 aa, are responsible for specific IgE binding and, to a large extent, of cross-reactivity between different allergens. It is evident that cross-reactivity studies to a great extent depend on the serum pool used and if the patients are mono- or poly-sensitized to different mite allergens.

The allergenic cross-reactivity between *L. destructor* and *B. tropicalis* was initially demonstrated by specific IgE inhibition studies using whole allergen extracts. Puerta et al. (147) demonstrated a greater degree of cross-reactivity between *B. tropicalis* and *L. destructor* than

between *B. tropicalis* and *Dermatophagoides* spp. The participation of group 2 in the cross-reactivity between these two species has been suggested (95). The sequence identity between Lep d 2 y and Gly d 2 is high (79%), but only 40 % in Tyr p 2 and Der p 2 (105). However, the cross-reactivity among group 2 allergens from storage mites, *L. destructor*, *T. putrescentiae*, and *G. domesticus*, is high, whereas there is only limited cross-inhibition between Der p 2 and the non-pyroglyphid mite allergens (105). This lack of cross-reactivity between Der p 2 and the group 2 of storage mites is a result of the multiple aa substitutions across the surface (148). Other studies have shown limited cross-reactivity between *D. pteronyssinus, L. destructor,* and *T. putrescentiae* (149,150), but others have reported a greater cross-reactivity between *Dermatophagoides* spp. and *T. putresentiae* (151). A low degree of cross-reactivity between *D. pteronyssinus* and *A. siro* and *T. putrescentiae* was described in one study, whereas cross-reactivity between *L. destructor* and *A. siro* was high (152).

Individuals allergic to the *Dermatophagoides* spp. may experience allergic symptoms after the consumption of crustacean and mollusks (153). Der f 10 and Der p 10 proteins with homology to tropomyosin from various animals are involved in the cross-reactivity among *Dermatophagoides* spp., mollusks, and crustaceans. The cross-reactive tropomyosin present in mites, chironomids, mosquitoes, cockroaches, shrimps (42), oysters (154), crabs (155), lobsters (156), and squids (157) could be responsible for cross-reactivity among different arthropods. Immunochemical studies demonstrate that allergens from snails, crustaceans, cockroaches, and chironomids cross-react with house-dust mite allergens. However, house-dust mites are usually the primary source of sensitizing allergens. The nematode, *Anisakis simplex*, a common fish parasite, can act as a hidden food allergen, inducing IgE-mediated reactions. Allergic cross-reactivity between this nematode and the domestic mites, *A. siro*, *L. destructor*, *T. putrescentiae*, and *D. pteronyssinus*, have been reported, in which tropomyosin seems to be involved. The clinical relevance of this cross-reactivity needs to be further investigated (158).

The feather mite, *Dip. columbae*, is a major source of clinically relevant allergens for pigeon breeders. The results of RAST inhibition experiments suggest that this feather mite cross-reacts with *D. pteronyssinus* (144). Arlian et al. (159) demonstrated that antigens of the parasitic mite, *Sar. scabiei*, cross-react with antigens of *D. pteronyssinus*. Proteins with homology to different groups of mite allergens have been identified by molecular cloning in the parasitic mites, *Sar. scabiei* (133) and *P. ovis* (160).

ENZYMATIC ACTIVITY IN MITE EXTRACTS

Important airborne allergens may possess hydrolytic enzymatic activities, such as proteases, glycosidases, and ribonucleases. Several of these enzymes are important allergens, including cysteine and serine proteases and glycosidases (amylase) in mites and molds and ribonucleases in grass and tree pollens and molds. Allergenic mite extracts have enzymes capable of degrading a wide range of substances, including other proteins and allergens and could have negative effects on the efficacy and stability of therapeutic vaccines. House-dust mite allergen extracts contain more than 20 IgE-binding molecules (161–163). Some of them with enzymatic activity included groups 1, 3, 4, 6, 8, 9, and 15 (164,165). Other mite species, such as *G. domesticus* (166), *A. farris* (167), *T. putrescentiae* (168), *B. tropicalis* (169,170), *P. cuniculi* (171), and *A. ovatus* (172) also contain allergens with enzymatic activity. Serine proteases (trypsin and chymotrypsin) seem to be more abundant in fecal than in whole body extracts (173,174). Enzyme allergens are widely present in other allergen sources, such as honey-bee venom (175), imported fire ant (176), vespids (177), and *Candida albicans* (178–180).

Comparative studies have been conducted to analyze the presence of enzymes in mite bodies and spent media. It seems that serine proteases (trypsin and chymotrypsin) are more abundant in fecal than in whole body extracts (181,182). The presence of proteolytic activity in feces extracts is of great clinical importance, since fecal pellets are more likely to become airborne and inhaled, thus reaching the respiratory mucosa. This enzymatic activity seems to play an important role in the allergenicity of certain mites, since it can facilitate the access of the allergen to the immune system and has an adjuvant proallergic role influencing its immunogenicity (183). The enzymatic activity is present in body and mainly in fecal extracts suggesting their role in digestion. The presence or absence of certain digestive enzymes may reflect the trophic specialization, showing different "enzyme patterns" among house-dust and storage mites.

The protease activity of allergens may induce a range of inflammatory effects modulating the adaptative immune system by cleavage of CD23 and CD25, including induction of vascular permeability, edema, activation of neural reflexes leading to bronchoconstriction and stimulation of gland secretion and cough (184,185). Der p 1 additionally may effect the innate defense mechanisms of the lung by inactivating the elastase inhibitors. Der p 1 may also increase the susceptibility to infection of patients with allergic inflammation (186). Der p 1 can damage bronchial epithelium, increasing their permeability and detaching cells in vitro. The resulting breach of the epithelial barrier may increase exposure to antigen-presenting cells and increase the likelihood that IgE will be raised against proteins in mite fecal pellets. Allergens, such as Der p 3 and Der p 9, with serine protease activity, may also induce a nonallergic inflammatory response in the airways through the release of proinflammatory cytokines from the bronchial epithelium (187).

Patients with mite sensitization and allergic asthma often react to a variety of allergens present in mite bodies, feces, and secretions (188,189). The proteolytic activity of mite allergens may play a proallergic role, influencing their immunogenicity by enabling allergens to breach the integrity of the airway epithelial barrier. As a consequence, allergic inflammatory responses are promoted. Furthermore, by disrupting epithelial tight junctions, the enzymatic activity facilitates the transport of allergen across the epithelium (190).

An influence on the innate mechanism of defense has also been described. The proteolytic activity of mite feces interacts with pulmonary elastase, inactivating a human elastase inhibitor in vivo and ex vivo. Because these elastase inhibitors have antimicrobial, as well as antielastase activity, the inactivation of these innate components of the lung defense system by proteolytic enzymes present in mite feces may increase the susceptibility of patients with allergic inflammation to infection and, therefore, produce a possible exacerbation of allergic respiratory diseases (191). Following exposure of the bronchial mucosa to house-dust mite fecal pellete, the mite proteases can access the lung interstitium and promote Th2 responses.

Chronic exposure to mites is an important risk factor for the development of sensitization, allergic respiratory diseases, and chronic inflammation of the lungs (192). While this relationship was primarily established for *Dermatophagoides* spp., other mite species are also the source of potent enzymes (193).

GENETICS OF IGE RESPONSES TO MITE ALLERGENS

High total IgE and specific IgE immune response to mite allergens are known risk factors for asthma and other allergic diseases. There is evidence that IgE synthesis, which involves a great number of metabolic pathways, is under genetic control. Several candidate genes influencing this process are identified. Some of them are also associated with asthma, probably because atopy is a main feature of this disease. The field of immunogenetics of IgE responses has been mainly supported by the late Dr. David Marsh's work on the association of ragweed allergens and the human major histocompatibility complex (HLA) alleles. Since then, a variety of work has been done in this field. Today most data showing genetic influence (linkage or association) on mite-specific IgE responses are related to HLA. In 1990, Caraballo et al., using affected sib pair analysis, showed that IgE hyperresponsiveness to *D. farinae* in patients with allergic asthma was linked to HLA (194). Eight years later, a genome-wide search found strong evidence of linkage between the specific IgE responsiveness to *D. pteronyssinus* and chromosomes 6p21 (HLA-D region), 2q21-q23, 8p23-p21, 13q32-q34, and 5q23-q33 in Caucasians families (195). The role of HLA on the IgE response to this mite was further analyzed by the same authors, evaluating the IgE responses to several allergen components of *D. pteronyssinus* extract (196). Linkage studies involving HLA loci and mite IgE responses have been replicated in other populations (197,198).

Associations between HLA class II genes and IgE hyperresponsiveness to mite allergens have been positive and negative, involving different alleles and loci, and often showing no replication among different studies. This could be explained by the large number of mite allergenic epitopes, the variable level of exposure to each epitope, the ability of each HLA class II allele to present more than one peptide, and the participation of genes from other chromosome regions. In a case-control study, the frequency of allele HLA-DPB1*0401 was remarkably decreased in patients with IgE hyperresponsiveness to mite allergens, suggesting

that it could be suppressing this phenotype in the nonallergic population (199). This apparent protective role of DPB1 locus has been also observed in an Asian population where DPB1*0201 was negatively associated with the IgE responsiveness to Der p 1 (200).

Using BtM, a short allergen peptide of Blo t 5, it was found that IgE hyper-responsiveness of asthmatic patients was positively associated with HLA-DRB1*03 (*0301 plus *0302) and negatively associated with HLA-DQB1*0601 (201). A positive association between HLA-DRB1*03 and IgE responsiveness to Der p 2 was also found in a study involving a large group of subjects from 76 nuclear and 7 extended families (202). These findings suggest that the group of alleles conforming DRB1*03 may be involved in controlling specific IgE to particular mite allergens, although the role of other HLA alleles also has been documented (203–205).

As suggested by genome-wide linkage searches, associations between mite IgE responsiveness and non-HLA genes have been found, including interleukin-18 (IL18) (206), leukotriene C4 synthase (LTC4S) (207), nitric oxide synthase 1 (NOS1) (208), and dendritic cell–associated nuclear protein 1 (DCNP1) (209), all of them participating in any of the multiple steps of IgE synthesis. For example, there is evidence that cysteinyl leukotrienes can enhance IgE and IgG production in human B cells and a LTC4S knockout mouse has markedly reduced antigen-induced Th2 pulmonary inflammation. Therefore, the finding of Acevedo et al., describing an association between the A-444C polymorphism of LTC4S and low IgE response to *D. pteronyssinus*, supports a role of this gene in the regulation of human IgE responses to mite allergens (207).

The genetics of nitric oxide synthase 1 function in asthma pathogenesis has been investigated for several years and polymorphisms in the NOS1 gene have been associated with asthma and total IgE levels. Nitric oxide, a mediator of innate immunity, also influences the adaptative response in both mouse and humans by exerting a regulatory role in Th1/Th2 generation, and it could contribute to asthma pathogenesis by selective down regulation of the Th1 response or promoting the Th2 response. The results of another investigation extend this hypothesis to mite-specific IgE response in asthmatics patients living in the tropics. Allele 16 of the cytosine adenine (CA) dinucleotide repeat polymorphism in exon 29 of *NOS1* was found associated with low levels of specific IgE to *D. pteronyssinus* and *B. tropicalis*, suggesting that nitric oxide synthase 1 and nitric oxide could act through different ways in asthma pathogenesis, one of them by controlling specific IgE response (208).

Because dendritic cells can influence polarization of T cells, the specific IgE response against mite allergens could be regulated by polymorphisms in non-HLA genes related with the mechanisms of allergen recognition and processing. Kim et al. found a single nucleotide polymorphism (SNP) in the promoter region (C-1289T) of the DCNP1 gene; allele T was associated with low levels of specific IgE against Der p 1 in asthmatic patients, while other SNP in the 3′UTR (C1718T) was more frequent in patients with high levels of specific IgE. Dendritic cell–associated nucleoprotein 1 expression seems to be regulated in a differentiation stage-dependent manner. Functional analyses indicates that the promoter allele T would affect the gene expression level by altering DNA–protein interaction, and it was postulated that the genetic polymorphism of DCNP1 may influence production of specific IgE by altering DC functions in the mite allergen presenting and/or processing (209).

Although the mechanisms of HLA influence on specific humoral immune response are partially elucidated, further investigation is needed to understand the role of genes in other chromosomal regions regulating IgE response and conferring susceptibility to allergic disorders. Advances in genomics and molecular biology of allergens will improve the ability to dissect the genetics of specific IgE response, especially because the latter can now be investigated at the epitope level, and genome-wide scans for association studies are available.

SALIENT POINTS

- Numerous mite species are the source of allergens capable of sensitizing and inducing allergic symptoms in sensitized and genetically predisposed individuals. Allergic diseases triggered by mite allergens include allergic rhinoconjunctivitis, asthma, atopic dermatitis, and other skin diseases.

- The main source of allergens in house dust worldwide are the house-dust mite species, *D. pteronyssinus*, *D. farinae*, *E. maynei*, and the storage mites, *B. tropicalis*, *L. destructor*, and *T. putrescentiae*.
- Several storage mite allergens have been purified, cloned, and sequenced. Some of these allergens can be considered as pan-allergens.
- The immune response to mite allergens is very complex, exhibiting marked geographical and racial differences. Human-specific IgE binds to several allergens in variable degrees of frequency and intensity. However, in *Dermatophagoides* spp., group 1 and 2 seem to be the most important.
- The main allergens described in storage mites include FABPs, tropomysin, and paramyosin homologues, apolipophorine-like proteins, alfa-tubulines and others, such as group 2, 5, and 7 allergens.
- The spider mites are main pests of fruit and horticultural crops and are common sensitizing allergens that are related to the prevalence of allergic diseases.
- Allergens from several parasitic mite species, which are in frequent contact with humans and domestic animals, have been isolated and characterized.
- Cross-reactivity has been traditionally studied using whole extracts and RAST-inhibition techniques. Currently, purified native or recombinant allergens, epitope mapping, and T-cell proliferation techniques are being used. Because of cross-reactivity, individuals allergic to the *Dermatophagoides* spp. may experience allergic symptoms after the consumption of crustacean and mollusks.
- Mite allergen extracts contain enzymes capable of degrading a wide range of substances, including other proteins and allergens, and could have negative effects on the efficacy and stability of therapeutic extracts. Most mite allergens are potent enzymes.
- Advances in genomics and molecular biology of mite allergens will improve the ability to dissect the genetics of specific IgE response, especially since investigation at the epitope level and genome-wide scans for association studies are available.

REFERENCES

1. Platts-Mills TA, Vervloet D, Thomas WR, et al. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol 1997; 100:S2–S24.
2. Smiley RL. The ordinal and subordinal names of mites with a list of mite pests of stored food products. Proceedings of the International Working Conference on Stored Product Entomology, 1983, 3:37–43.
3. Fernández-Caldas E, Lockey RF. *Blomia tropicalis*, a mite whose time has come. Allergy 2004; 59 (11):1161–1164.
4. Arlian L, Bernstein D, Bernstein L, et al. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. J Allergy Clin Immunol 1992; 90: 292–300.
5. Fernández-Caldas E, Fox R, Bucholtz G, et al. House dust mite allergy in Florida. Mite survey in households of mite sensitive individuals in Tampa, Florida. Allergy Proc 1990; 11:263–267.
6. Tovey ER, Chapman MD, Platts-Mills TAE. Mite feces are a major source of house dust allergens. Nature 1982; 289:592–593.
7. Fergusson P, Broide DH. Environmental and bronchoalveolar lavage *D. pteronyssinus* antigen levels in tropic asthmatics. Am J Respir Crit Care Med 1995; 151:71–74.
8. Chua KY, Stewart GA, Thomas WR, et al. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p I homology with cysteine proteases. J Exp Med 1988; 167:175–182.
9. Zhang J, Hamilton JM, Garrod DR, et al. Interactions between mature Der p 1 and its free prodomain indicate membership of a new family of C1 peptidases Allergy 2007; 62:1302–1309.
10. Hewitt CRA, Brown AP, Hart BJ, et al. A major house dust mite allergen disrupts the immunoglobulin E network by selectively clearing CD23: innate protection by antiproteases. J Exp Med 1995; 182:1537–1544.
11. Gough L, Sewell HF, Shakib F. The proteolytic activity of the major dust mite allergen Der p 1 enhances the IgE antibody response to a bystander antigen. Clin Exp Allergy 2001; 31:1594–1598.
12. Ghaemmaghami AM, Robins A, Gough L, et al. Human T cell subset commitment determined by the intrinsic property of antigen: the proteolytic activity of the major mite allergen Der p 1 conditions T cells to produce more IL-4 and less IFN-gamma. Eur J Immunol 2001; 31:1211–1216.

13. Wan H, Winton HL, Soeller C, et al. The transmembrane protein occluding of epithelial tight junctions is a functional target for serine peptidases from faecal pellets of *Dermatophagoides pteronyssinus*. Clin Exp Allergy 2001; 31:279–294.
14. Kauffman HF, Tamm M, Timmerman JA, et al. House dust mite major allergens Der p 1 and Der p 5 activate human airway-derived epithelial cells by protease-dependent and protease-independent mechanisms. Clinical and Molecular Allergy 2006; 4:5.
15. Asokananthan N, Graham PT, Stewart DJ, et al. House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease activated receptor (PAR)-2 and inactivates PAR-1. J Immunol 2002; 169:4572–4578.
16. Adam E, Hansen KK, Astudillo Fernandez O, et al. The house dust mite allergen DER P 1, unlike DER P 3, stimulates the expression of IL-8 in human airway epithelial cells via a proteinase-activated receptor-2 (PAR2) independent mechanisms. J Biol Chem 2006; 281:6910–6923.
17. Deb R, Shakib F, Reid K, et al. Major house dust mite allergens Der p 1 and Der f 1 degrade and inactivate lung surfactant proteins-A and -D. J Biol Chem 2007; 282:36808–36819.
18. Kikuchi Y, Takai T, Kuhara T, et al. Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p 1 to sensitization toward IgE and IgG responses. J Immunol 2006; 177:1609–1607.
19. Kent NA, Hill MR, Keen NJ, et al. Molecular characterization of group I allergen Eur m I from house dust mite Euroglyphus meynei. Int Arch Allergy Immunol 1992; 99:150–152.
20. Ferrándiz R, Casas R, Dreborg S, et al. Characterization of allergenic components from house dust mite Dermatophagoides siboney. Purification of Der s I and Der s 2 allergens. Clin Exp Allergy 1995; 25:922–928.
21. Ovsyannikova IG, Vailes LD, Li Y, et al. Monoclonal antibodies to group II Dermatophagoides spp. allergens: murine immune response, epitope analysis, and development of a two-site ELISA. J Allergy Clin Immunol 1994; 94:537–546.
22. Lombardero M, Heymann PW, Platts-Mills TA, et al. Conformational stability of B cell epitopes on group I and group II Dermatophagoides spp. allergens. Effect of thermal and chemical denaturation on the binding of murine IgG and human IgE antibodies. J Immunol 1990; 144:1353–1360.
23. Chua KY, Doyle CR, Simpson RJ, et al. Isolation of cDNA coding for the major mite allergen Der p 2 by IgE plaque immunoassay. Int Arch Allergy Appl Immunol 1990; 91:118–123.
24. Thomas WR, Chua KY. The major mite allergen Der p 2 a secretion of the male mite reproductive tract? Clin Allergy 1995; 25:666–669.
25. Chua KY, Huang CH, Shen HD, et al. Analysis of sequence polymorphism of a major mite allergen, Der p 2. Clin Exp Allergy 1996; 26:829–837.
26. Derewenda U, Li J, Derewenda Z, et al. The crystal structure of a major dust mite allergen Der p 2, and its biological implications. J Mol Biol 2002; 318:189–197.
27. Morgan MS, Arlian LG, Barnes KC, et al. Characterization of the allergens of the house dust mite Euroglyphus maynei. J Allergy Clin Immunol 1997; 100:222–228.
28. Gruber A, Mancek M, Wagner H, et al. Structural model of MD-2 and functional role of its basic amino acid clusters involved in cellular lipopolysaccharide recognition. J Biol Chem 2004; 279: 28475–28482.
29. Pittner G, Vrtala S, Thomas WR, et al. Component-resolved Diagnosis of house dust mite allergy with purified natural and recombinant purified allergens. Clin Exp Allergy 2004; 34:597–603.
30. Suzuki M, Tanaka Y, Korematsu S, et al. Crystal structure and some properties of a major house dust mite allergen, Der f 2. Biochem Biophys Res Commun 2006; 339:679–686.
31. Stewart GA, Ward LD, Simpson RJ, et al. The group III allergen form the house dust mite Dermatophagoides pteronyssinus is a trypsin-like enzyme. J Immunol 1992; 75:29–35.
32. Stewart GA, Thompson PJ. The biochemistry of common aeroallergens. Clin Exp Allergy 1996; 26:1020–1044.
33. Lake FR, Ward LD, Simpson RJ, et al. House dust mite derived amylase: allergenicity and physicochemical characterization. J Allergy Clin Immunol 1991; 87:1035–1042.
34. Lin KL, Hsieh KH, Thomas WR, et al. Characterization of Der p 5 allergen, cDNA analysis and IgE-mediated reactivity of the recombinant protein. J Allergy Clin Immunol 1995; 94:989–996.
35. Bennett BJ, Thomas WR. Cloning and sequencing of the group 6 allergen of Dermatophagoides pteronyssinus. Clin Exp Allergy 1996; 26:1150–1154.
36. Shen HD, Chua KY, Lin WL, et al. Molecular cloning and immunological characterization of the house dust mite allergen Der f 7. Clin Exp Allergy 1995; 25:1000–1006.
37. Shen HD, Chua KY, Lin KL, et al. Molecular cloning of a house dust mite allergen with common antibody binding specificities with multiple components in mite extracts. Clin Exp Allergy 1993; 23:934–940.
38. O'Neill GM, Donavan GR, Baldo BA. Cloning and characterization of a major allergen of the house dust mite, Dermatophagoides pteronyssinus, homologous with glutathione-S-transferase. Biochim Biophys Acta 1994; 1219:521–528.

39. Huang CH, Liew LM, Mah KW, et al. Characterization of glutathione S-transferase from dust mite, Der p 8 and its immunoglobuling E cross-reactivity with cockroaches glutathione S-transferase. Clin Exp Allergy 2006; 36:369–376.
40. King C, Simpson RJ, Moritz RL, et al. The isolation and characterization of a novel collagenolitic serine protease allergen (Der p 9) from the dust mite Dermatophagoides pteronyssinus. J Allergy Clin Immunol 1996; 98:739–747.
41. Aki T, Kodama T, Fujikawa A, et al. Immunochemical characterization of recombinant and native tropomyosin as a new allergen from the house dust mite, Dermatophagoides farinae. J Allergy Clin Immunol 1995; 96:74–83.
42. Witteman AM, Akkerdaas JH, van Leeuwen J, et al. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. Int Arch Allergy Immunol 1994; 105:56–61.
43. Asturias JA, Arilla MC, Gómez-Bayón N, et al. Sequencing and high level expression in Escherichia coli of the tropomyosin allergen (Der p 10) from Dermatophagoides pteronyssinus. Biochim Biophys Acta 1998; 1397(1):27–30.
44. Yi FC, Cheong N, Shek PC, et al. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy 2002; 32:1203–1210.
45. Tsai LC, Chao P, Hung MW, et al. Protein sequence analysis and mapping of IgE and IgG epitopes of an allergenic 98-kDa Dermatophagoides farinae paramyosin, Der f 11. Allergy 2000; 55:141–147.
46. Tsai LC, Sun YC, Chao PL, et al. Sequence analysis and expression of a cDNA clone encoding a 98-KDa allergen in *Dermatophagoides farinae*. Clin Exp Allergy 1999; 29:1606–1613.
47. Lee CS, Tsai LC, Chao PL, et al. Protein sequence analysis of a novel 103-kDa Dermatophagoides pteronyssinus mite allergen and prevalence of serum immunoglobulin E reactivity to rDer p 11 in allergic adult patients. Clin Exp Allergy 2004; 34(3):354–362.
48. Tsai LC, Peng HJ, Lee CS, et al. Molecular cloning and characterization of full-length cDNAs encoding a novel high-molecular-weight Dermatophagoides pteronyssinus mite allergen, Der p 11. Allergy 2005; 60:927–937.
49. Puerta L, Caraballo L, Fernández-Caldas E, et al. Nucleotide sequence analysis of a complementary DNA coding for a Blomia tropicalis allergen. J Allergy Clin Immunol 1996; 98:932–937.
50. Sanjuan H, Puerta L, Caraballo L. Expression of a Blomia tropicalis allergen in the yeast Pichia pastoris. J Allergy Clin Immnol 1998; 101:S155 (abstr).
51. Lim D, Shek P, Shaikh W, et al. Pattern of sensitization to Blomia tropicalis and its recombinant allergens in four tropical Asian populations. J Allergy Clin Immunol 2002; 109: S179.
52. Zakzuck J, Jiménez S, Chua KY, et al. IgE immune response to Blo t 12 isoallergens from Blomia tropicalis in asthmatic patients living in the tropics collection of papers from 13th International Congress of Immunology, Rio de Janeiro, August 2007. In: Kalil J, Cunha-Neto, Rizzo L, eds. Medimond International Proceedings, Italy, 2007.
53. Angus AC, Ong ST, Chew, FT. Sequence tag catalogs of dust mite-expressed genomes: utility in allergen and acarologic studies. Am J Pharmacogenomics 2004; 4:357–369.
54. Siew LC, Seow TO, Su YO, et al. Nuclear magnetic resonance structure-based epitope mapping and modulation of dust mite group 13 allergen as a hypoallergen. J Immunol 2006; 176:4852–4860.
55. Labrada M, Uyema K, Sewer M, et al. Monoclonal antibodies against Blo t 13, a recombinant allergen from Blomia tropicalis. Int Arch Allergy Immunol 2002; 129:212–218.
56. Puerta L, Labrada A, Labrada M, et al. Blo t 13 allergen from *Blomia tropicalis* shows high frequency of IgE binding in allergic Cuban patients and cross-reactivity with Dermatophagoides siboney extract. J Allergy Clin Immunol 2003; 111:S325 (abstr).
57. Caraballo L, Puerta L, Jiménez S, et al. Cloning and IgE binding of a recombinant allergen from the mite Blomia tropicalis, homologous with fatty acid-binding proteins. Int Arch Allergy Immunol 1997; 112:341–347.
58. Jeong KY, Kim WK, Lee JS, et al. Immunoglobulin E reactivity of recombinant allergen Tyr p 13 from Tyrophagus putrescentiae homologous to fatty acid binding protein. Clin Diagn Lab Immunol 2005; 12:581–585.
59. Kenyon F, Welsh M, Parkinson J, et al. Expressed sequence tag survey of gene expression in the scab mite Psoroptes ovis allergens, proteases and free-radical scavengers. Parasitology 2003; 126:451–460.
60. Epton MJ, Dilworth RJ, Smith W, et al. Sensitisation to the lipid-binding apolipophorin allergen Der p 14 and the peptide Mag-1. Int Arch Allergy Immunol 2001; 124:57–60.
61. Epton MJ, Dilworth RJ, Smith W, et al. High-molecular-weight allergens of the house dust mite: an apolipophorin-like cDNA has sequence identity with the major M-177 allergen and the IgE-binding peptide fragments Mag1 and Mag3. Int Arch Allergy Immunol 1999; 120:185–191.
62. McCall C, Hunter S, Stedman K, et al. Characterization and cloning of a major high molecular weight house dust mite allergen (Der f 15) for dogs. Vet Immunol Immunopathol 2001; 78:231–247.
63. O'Neil SE, Heinrich TK, Hales BJ, et al. The chitinase allergens Der p 15 and Der p 18 from Dermatophagoides pteronyssinus. Clin Exp Allergy 2006; 36:831–839.

64. Kawamoto S, Suzuki T, Aki T, et al. Der f 16: a novel gelsolin-related molecule identified as an allergen from the house dust mite, Dermatophagoides farinae. FEBS Lett 2002; 516:234–238.
65. Wopfner N, Dissertori O, Ferreira F, et al. Calcium-binding proteins and their role in allergic diseases. Immunol Allergy Clin North Am 2007; 27(1):29–44.
66. Weber E, Hunter S, Stedman K, et al. Identification, characterization, and cloning of a complementary DNA encoding a 60-kd house dust mite allergen (Der f 18) for human beings and dogs. J Allergy Clin Immunol 2003; 112:79–86.
67. O'Neil SE, Heinrich TK, Hales BJ, et al. The chitinase allergens Der p 15 and Der p 18 from Dermatophagoides pteronyssinus. Clin Exp Allergy 2006; 36:831–839.
68. Thomas WR, Heinrich TK, Smith WA, et al. Pyroglyphid house dust mite allergens. Protein Pept Lett 2007; 14(10):943–953.
69. Hales BJ, Martin AC, Pearce LJ, et al. IgE and IgG anti–house dust mite specificities in allergic disease. J Allergy Clin Immunol 2006; 118:361–367.
70. Yun Feng G, De Yun W, Tan Ching O, et al. Identification and characterization of a novel allergen from *Blomia tropicalis*: Blo t 21. J Allergy Clin Immunol 2007; 120:105–112.
71. Fernández-Caldas E, Iraola V, Carnés J. Molecular and biochemical properties of storage mites (except Blomia species). Protein Pept Lett 2007; 14(10):954–959.
72. Fernández-Caldas E, Iraola Calvo V. Mite allergens. Curr Allergy Asthma Rep 2005; 5(5):402–410.
73. Puerta L, Caraballo L, Fernández-Caldas E, et al. Nucleotide sequence analysis of a complementary DNA coding for a Blomia tropicalis allergen. J Allergy Clin Immunol 1996; 98(5 pt 1):932–937.
74. Caraballo L, Puerta L, Jimenez S, et al. Cloning and IgE binding of a recombinant allergen from the mite Blomia tropicalis, homologous with fatty acid-binding proteins. Int Arch Allergy Immunol 1997; 112(4):341–347.
75. Caraballo L, Mercado D, Jimenez S, et al. Analysis of the cross-reactivity between BtM and Der p 5, two group 5 recombinant allergens from Blomia tropicalis and Dermatophagoides pteronyssinus. Int Arch Allergy Immunol 1998; 117(1):38–45.
76. Puerta L, Kennedy MW, Jiménez S, et al. Structural and ligand binding analysis of recombinant Blo t 13 allergen from *Blomia tropicalis* mite, a fatty acid binding protein. Int Arch Allergy Immunol 1999; 119(3): 181–184.
77. Ramos JD, Cheong N, Lee BW, et al. cDNA cloning and expression of Blo t 11, the Blomia tropicalis allergen homologous to paramyosin. Int Arch Allergy Immunol 2001; 126(4):286–293.
78. Yi FC, Cheong N, Shek PC, et al. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy 2002; 32(8):1203–1210.
79. Flores I, Mora C, Rivera E, et al. Cloning and molecular characterization of a cDNA from Blomia tropicalis homologous to dust mite group 3 allergens (trypsin-like proteases). Int Arch Allergy Immunol 2003; 130(1):12–16.
80. Mora C, Flores I, Montealegre F, et al. Cloning and expression of Blo t 1, a novel allergen from the dust mite Blomia tropicalis, homologous to cysteine proteases. Clin Exp Allergy 2003; 33(1):28–34.
81. Cheong N, Soon SC, Ramos JD, et al. Lack of human IgE cross-reactivity between mite allergens Blo t 1 and Der p 1. Allergy 2003; 58(9):912–920.
82. Simpson A, Green R, Custovic A, et al. Skin test reactivity to natural and recombinant Blomia and Dermatophagoides spp. allergens among mite allergic patients in the UK. Allergy 2003; 58(1):53–56.
83. Kuo IC, Cheong N, Trakultivakorn M, et al. An extensive study of human IgE cross-reactivity of Blo t 5 and Der p 5. J Allergy Clin Immunol 2003; 111(3):603–609.
84. Yi FC, Shek LP, Cheong N, et al. Molecular cloning of Blomia tropicalis allergens–a major source of dust mite allergens in the tropics and subtropics. Inflamm Allergy Drug Targets 2006; 5(4):261–266.
85. Arruda K, Vailes LD, Platts-Mills AE, et al. Sensitization to Blomia tropicalis in patients with asthma and identification of allergen Blo t 5. Am J Respir Crit Care Med 1997; 155:343–350.
86. Jiménez S, Puerta L, Mendoza D, et al. IgE antibody responses to recombinant allergens of Blomia tropicalis and Dermatophagoides pteronyssinus in a tropical environment. Allergy Clin Immunol Int J World Allergy Org. 2007; 19(6):1–6.
87. Chua KY, Cheong N, Kuo IC, et al. The Blomia tropicalis allergens. Protein Pept Lett 2007; 14(4): 325–333.
88. Arruda LK, Vailes LD, Platts-Mills TAE, et al. Sensitisation to Blomia tropicalis in patients with asthma: molecular cloning and expression of a major allergen (Blo t 5) with homology to Dermatophagoides pteronyssinus Der p 5. Am J Respir Crit Care Med 1997; 155:343–350.
89. Naik MT, Chang CF, Kuo IC, et al. Roles of structure and structural dynamics in the antibody recognition of the allergen proteins: an NMR study on Blomia tropicalis major allergen. Structure 2008; 16(1):125–136.
90. Stanaland BE, Fernández-Caldas E, Jacinto CM, et al. Positive nasal challenge response to Blomia tropicalis. J Allergy Clin Immunol 1996; 97:1045–1049.

91. Barreto BA, Daher S, Naspitz CK, et al. Specific and non-specific nasal provocation tests in children with perennial allergic rhinitis. Allergol Immunopathol (Madr) 2001; 29(6):255–263.
92. Wang DY, Goh DY, Ho AK, et al. The upper and lower airway responses to nasal challenge with house-dust mite Blomia tropicalis. Allergy 2003; 58(1):78–82.
93. Garcia Robaina JC, Sanchez Machin I, Fernandez-Caldas E, et al. Skin tests and conjunctival and bronchial challenges with extracts of Blomia tropicalis and Dermatophagoides pteronyssinus in patients with allergic asthma and/or rhinoconjunctivitis. Int Arch Allergy Immunol 2003; 131(3): 182–188.
94. Niggemann B, Kleinau I, Schou C, et al. Discrepancies between in vitro and in vivo tests for house dust mite allergy: is domestic exposure a better predictor than sensitization? Clin Exp Allergy 1994; 24(10):946–948.
95. Johansson E, Schmidt M, Johansson SG, et al. Allergenic crossreactivity between Lepidoglyphus destructor and Blomia tropicalis. Clin Exp Allergy 1997; 27(6):691–699.
96. Ventas P, Carreira J, Polo F. Purification and characterization of Lep d I, a major allergen from the mite Lepidoglyphus destructor. Clin Exp Allergy 1992; 22(4):454–460.
97. Van Hage-Hamsten M, Olsson S, Emilson A, et al. Localization of major allergens in the dust mite Lepidoglyphus destructor with confocal laser scanning microscopy. Clin Exp Allergy 1995; 25: 536–542.
98. Olsson S, van Hage-Hamsten M, Whitley P, et al. Expression of two isoforms of Lep d 2, the major allergen of Lepidoglyphus destructor, in both prokaryotic and eukaryotic systems. Clin Exp Allergy 1998; 28:984–991.
99. Johansson E, Eriksson TL, Olsson S, et al. Evaluation of specific IgE to the recombinant group 2 mite allergens Lep d 2 and Tyr p 2 in the Pharmacia CAP system. Int Arch Allergy Immunol 1999; 120:43–49.
100. Kronqvist M, Johansson E, Magnusson CG, et al. Skin prick test and serological analysis with recombinant group 2 allergens of the dust mites L. destructor and T. putrescentiae. Clin Exp Allergy 2000; 30:670–676.
101. Kaiser L, Gafvelin G, Johansson E, et al. Lep d 2 polymorphisms in wild and cultured Lepidoglyphus destructor mites. Eur J Biochem 2003; 270(4):646–653.
102. Eriksson TL, Rasool O, Huecas S, et al. Cloning of three new allergens from the dust mite Lepidoglyphus destructor using phage surface display technology. Eur J Biochem 2001; 268:287–294.
103. Saarne T, Kaiser L, Rasool O, et al. Cloning and characterisation of two IgE-binding proteins, homologous to tropomyosin and alpha-tubulin, from the mite Lepidoglyphus destructor. Int Arch Allergy Immunol 2003; 130:258–265.
104. Mari A, Scala E, Palazzo P, et al. Bioinformatics applied to allergy: allergens database, from collecting sequence information to data integration. The Allergome platform as a model. Cell Immunol 2006; 244:97–100.
105. Gafvelin G, Johansson E, Lundin A, et al. Crossreactivity studies of a new group 2 allergen from the dust mite *G. domesticus*, Gly d 2, and group 2 allergens from Dermatophagoides pteronyssinus, Lepidoglyphus destructor, and Tyrophagus putrescentiae with recombinant allergens. J Allergy Clin Immunol 2001; 107:511–518.
106. Arlian LG, Vyszenski-Moher DL, Johansson SG, et al. Allergenic characterization of Tyrophagus putrescentiae using sera from occupationally exposed farmers. Ann Allergy Asthma Immunol 1997; 79:525–529.
107. Johansson E, Johansson SG, Van Hage-Hamsten M. Allergenic characterization of Acarus siro and Tyrophagus putrescentiae and their crossreactivity with Lepidoglyphus destructor and Dermatophagoides pteronyssinus. Clin Exp Allergy 1994; 24:743–751.
108. Eriksson TL, Johansson E, Whitley P, et al. Cloning and characterisation of a group II allergen from the dust mite Tyrophagus putrescentiae. Eur J Biochem 1998; 251(1–2):443–447.
109. Jeong KY, Lee H, Lee JS, et al. Immunoglobulin E binding reactivity of a recombinant allergen homologous to alpha-tubulin from Tyrophagus putrescentiae. Clin Diagn Lab Immunol 2005; 12:1451–1454.
110. Jeong KY, Lee H, Lee JS, et al. Molecular cloning and the allergenic characterization of tropomyosin from Tyrophagus putrescentiae. Protein Pept Lett 2007;14(5):431–436.
111. Eriksson TL, Whitley P, Johansson E, et al. Identification and characterisation of two allergens from the dust mite Acarus siro, homologous with fatty acid-binding proteins. Int Arch Allergy Immunol 1999; 119:275–281.
112. Silton RP, Fernández-Caldas E, Trudeau WL, et al. Prevalence of specific IgE to the storage mite, Aleuroglyphus ovatus. J Allergy Clin Immunol 1991; 88:595–603.
113. Puerta L, Fernández Caldas E, Lockey R, et al. Sensitization to Chortoglyphus arcuatus and Aleuroglyphus ovatus in Dermatophagoides spp. allergic individuals. Clin Exp Allergy 1993; 23:117–123.
114. Puerta L, Lagares A, Mercado D, et al. Allergenic composition of the mite Suidasia medanensis and crossreactivity with Blomia tropicalis. Allergy 2005; 60:41–47.

115. Sánchez-Borges M, Capriles-Hulett A, Fernández-Caldas E, et al. Mite-contaminated foods as a cause of anaphylaxis. J Allergy Clin Immunol 1997; 99(6 pt 1):738–743.
116. Blanco C, Quiralte J, Castillo R, et al. Anaphylaxis after ingestion of wheat flour contaminated with mites. J Allergy Clin Immunol 1997; 99(3):308–313.
117. Boquete M, Carballas C, Carballada F, et al. In vivo and in vitro allergenicity of the domestic mite Chortoglyphus arcuatus. Ann Allergy Asthma Immunol 2006; 97:203–208.
118. Fenández-Caldas E, Vailes L, Troubina O, et al. Molecular cloning of Chortoglyphus arcuatus allergens. J Allergy Clin Immunol 2007; 119(1):S106.
119. Fernández-Caldas E, Lafosse Marin S, Ochoa C, et al. Allergenicity of the predator mite Cheyletus eruditus. In: Abstract Book of XXIII EAACI Congress, Amsterdam, 12–16 June 2004:183.
120. Kim YK, Lee MH, Jee YK, et al. Spider mite allergy in apple-cultivating farmers: european red mite (Panonychus ulmi) and two-spotted spider mite (Tetranychus urticae) may be important allergens in the development of work-related asthma and rhinitis symptoms. J Allergy Clin Immunol 1999; 104:1285–1292.
121. Lee MH, Cho SH, Park HS, et al. Citrus red mite (Panonychus citri) is a common sensitizing allergen among children living around citrus orchards. Ann Allergy Asthma Immunol 2000; 85:200–204.
122. Park HS, Jee YK, Kim YK, et al. Identification of immunoglobulin E binding components of the two-spotted spider mite Tetranychus urticae: allergenic relationships with the citrus red mite and house-dust mite. Allergy Asthma Proc 2002; 23(3):199–204.
123. Kim YK, Oh SY, Jung JW, et al. IgE binding components in Tetranychus urticae and Panonychus ulmi-derived crude extracts and their crossreactivity with domestic mites. Clin Exp Allergy 2001; 31:1457–1463.
124. Kim HY, Park HS, Kim YK, et al. Identification of IgE-binding components of citrus red mite in sera of patients with citrus red mite-induced asthma. J Allergy Clin Immunol 2001; 107:244–248.
125. van Hage-Hamsten M, Kolmodin-Hedman B, et al. Predatory mites, Phytoseiulus persimilis and Amblyseius cucumeris, used for biological crop protection, cause sensitization among greenhouse workers. Allergy 2000; 55:30.
126. Kronqvist M, Johansson E, Kolmodin-Hedman B, et al. IgE-sensitization to predatory mites and respiratory symptoms in Swedish greenhouse workers. Allergy 2005; 60:521–526.
127. de Jong NW, Groenewoud GC, van Ree R, et al. Immunoblot and radioallergosorbent test inhibition studies of allergenic cross-reactivity of the predatory mite Amblyseius cucumeris with the house dust mite Dermatophagoides pteronyssinus. Ann Allergy Asthma Immunol 2004; 93:281–287.
128. Falk ES, Bolle R. IgE antibodies to house dust mites in patients with scabies. Br J Dermatol 1980; 103:283–288.
129. Morgan MS, Arlian LG, Estes SA. Skin test and radioallergosorbent test characteristics of scabietic patients. Am J Trop Med Hyg 1997; 57:190–196.
130. Arlian LG, Morgan MS, Estes SA, et al. Circulating IgE in patients with ordinary and crusted scabies. J Med Entomol 2004; 41(1):74–77.
131. Holt DC, Fischer K, Allen GE, et al. Mechanisms for a novel immune evasion strategy in the scabies mite Sarcoptes scabiei: a multigene family of inactivated serine proteases. J Invest Dermatol 2003; 121(6):1419–1424.
132. Dougall A, Holt DC, Fischer K, et al. Identification and characterization of Sarcoptes scabiei and Dermatophagoides pteronyssinus glutathione S-transferases: implication as a potential major allergen in crusted scabies. Am J Trop Med Hyg 2005; 73:977–984.
133. Fischer K, Holt DC, Harumal P, et al. Generation and characterization of cDNA clones from Sarcoptes scabiei var. hominis for an expressed sequence tag library: identification of homologues of house dust mite allergens. Am J Trop Med Hyg 2003; 68:61–64.
134. Harumal P, Morgan M, Walton SF, et al. Identification of a homologue of a house dust mite allergen in a cDNA library from Sarcoptes scabiei var. hominis and evaluation of its vaccine potential in a rabbit/S. scabiei var. canis model. Am J Trop Med Hyg 2003; 68(1):54–60.
135. Kleine-Tebbe J, Heinatz A, Graser I, et al. Bites of the European pigeon tick (argas reflexus): risk of IgE-mediated sensitizations and anaphylactic reactions. J Allergy Clin Immunol 2006; 117:190–195.
136. Rolla G, Nebiolo F, Marsico P, et al. Allergy to pigeon tick (argas reflexus): demonstration of specific IgE-binding components. Int Arch Allergy Immunol 2004; 135:293–295.
137. Hilger C, Bessot JC, Hutt N, et al. IgE-mediated anaphylaxis caused by bites of the pigeon tick Argas reflexus: cloning and expression of the major allergen Arg r 1. J Allergy Clin Immunol 2005; 115: 617–622.
138. Gauci M, Loh RK, Stone BF, et al. Evaluation of partially purified salivary gland allergens from the Australian paralysis tick Ixodes holocyclus in diagnosis of allergy by RIA and skin prick test. Ann Allergy 1990; 64(3):297–299.
139. Van Wye JE, Hsu YP, Terr AI, et al. Anaphylaxis from a tick bite. N Engl J Med 1991; 324:777–778.
140. Fernández-Soto P, Dávila I, Laffond E, et al. Tick-bite-induced anaphylaxis in Spain. Ann Trop Med Parasitol 2001; 95:97–103.

141. Acero S, Blanco R, Bartolomé B. Anaphylaxis due to a tick bite. Allergy 2003; 58:824–825.
142. Miadonna A, Tedeschi A, Leggieri E, et al. Anaphylactic shock caused by allergy to the venom of Argas reflexus. Ann Allergy 1982; 49:293–294.
143. Rudeschko O, Machnik A, Dorfelt H, et al. A novel inhalation allergen present in the working environment of beekeepers. Allergy 2004; 59:332–337.
144. Colloff MJ, Merrett TG, Merret J, et al. Feather mites are potentially an important source of allergens for pigeon and budgerigar keepers. Clin Exp Allergy 1998; 27:60–67.
145. Arlian LG, Morgan MS, Houck MA. Allergenicity of the mite Hemisarcoptes cooremani. Ann Allergy Asthma Immunol 1999; 83:401–409.
146. Hales BJ, Thomas WR. T-cell sensitization to epitopes from the house dust mites Dermatophagoides pteronyssinus and Euroglyphus maynei. Clin Exp Allergy 1997; 27:868–875.
147. Puerta L, Fernández-Caldas E, Caraballo Gracia LR, et al. Sensitization of Blomia tropicalis and Lepidogyphus destructor in Dermatophagoides spp. Allergic individuals. J Allergy Clin Immunol 1991; 88:943–950.
148. Smith AM, Benjamin DC, Hozic N, et al. The molecular basis of antigenic cross-reactivity between the group 2 mite allergens. J Allergy Clin Immunol 2001; 107(6):977–984.
149. Luczynska CM, Griffin P, Davies RJ, et al. Prevalence of specific IgE to storage mites (A. siro, L. destructor and T. longior) in an urban population and crossreactivity with the house dust mite (D. pteronyssinus). Clin Exp Allergy 1990; 20(4):403–406.
150. van Hage-Hamsten M, Johansson SG, Johansson E, et al. Lack of allergenic cross-reactivity between storage mites and Dermatophagoides pteronyssinus. Clin Allergy 1987; 17:23–31.
151. Park JW, Ko SH, Yong TS, et al. Cross-reactivity of Tyrphagus putrescentiae with Dermatophagoides farinae and Dermatophagoides pteronyssinus in urban areas. Ann Allergy Asthma Immunol 1999; 83:533–539.
152. Johansson E, Johansson SG, Van Hage-Hamsten M. Allergenic characterization of Acarus siro and Tyrophagus putrescentiae and their crossreactivity with Lepidoglyphus destructor and Dermatophagoides pteronyssinus. Clin Exp Allergy 1994; 24:743–751.
153. van Ree R, Antonicelli L, Akkerdaas JH, et al. Asthma after consumption of snails in house-dust-mite-allergic patients: a case of IgE cross-reactivity. Allergy 1996; 51(6):387–393.
154. Leung PS, Chu KH. cDNA cloning and molecular identification of the major oyster allergen from the Pacific oyster Crassostrea gigas. Clin Exp Allergy 2001; 31(8):1287–1294.
155. Leung PS, Chen YC, Gershwin ME, et al. Identification and molecular characterization of Charybdis feriatus tropomyosin, the major crab allergen. J Allergy Clin Immunol 1998; 102(5):847–852.
156. Leung PS, Chen YC, Mykles DL, et al. Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. Mol Mar Biol Biotechnol 1998; 7(1):12–20.
157. Miyazawa H, Fukamachi H, Inagaki Y, et al. Identification of the first major allergen of a squid (Todarodes pacificus). J Allergy Clin Immunol 1996; 98(5 pt 1):948–953.
158. Johansson E, Aponno M, Lundberg M, et al. Allergenic cross-reactivity between the nematode Anisakis simplex and the dust mites Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae, and Dermatophagoides pteronyssinus. Allergy 2001; 56:660–666.
159. Arlian LG, Vyszenski-Moher DL, Ahmed SG, et al. Cross-antigenicity between the scabies mite, Sarcoptes scabiei, and the house dust mite, Dermatophagoides pteronyssinus. J Invest Dermatol 1991; 96:349–354.
160. Temeyer KB, Soileau LC, Pruett JH. Cloning and sequence analysis of a cDNA encoding Pso II, a group II mite allergen of the sheep scab mite (Acari: Psoroptidae). J Med Entomol 2002; 39:384–391.
161. Thomas WR, Smith WA, Hales BJ, et al. Characterization and immunobiology of house dust mite allergens. Int Arch Allergy Immunol 2002; 129:1–18.
162. Thomas WR. Mite allergens groups I-VII. A catalogue of enzymes. Clin Exp Allergy 1993; 23: 350–353.
163. Stewart GA, Boyd SM, Bird CH, et al. Immunobiology of the serine protease allergens from house dust mites. Am J Ind Med 1994; 25:105–107.
164. Fernández-Caldas E, Puerta L, Caraballo L, et al. Mite allergens. Clin Allergy Immunol 2004; 18: 251–270.
165. Stewart GA, Kollinger MR, King CM, et al. A comparative study of three serine proteases from Dermatophagoides pteronyssinus and D. farinae. Allergy 1994; 49:553–560.
166. Bowman CE. Comparative enzymology of economically important astigmatid mites. In: Griffiths DA, Bowman CE, eds. Acarology VI. Chichester: Ellis Horwood 1984; 2:993.
167. Sánchez-Ramos I, Hernandez CA, Castanera P, et al. Proteolytic activities in body and faecal extracts of the storage mite, Acarus farris. Med Vet Entomol 2004; 18:378–386.
168. Ortego F, Sánchez-Ramos I, Ruiz M, et al. Characterization of proteases from a stored product mite, Tyrophagus putrescentiae. Arch Insect Biochem Physiol 2000; 43:116–124.
169. Montealegre F, Quinones C, Torres N, et al. Detection of serine proteases in extracts of the domestic mite Blomia tropicalis. Exp Appl Acarol 2002; 26:87–100.

170. Flores I, Mora C, Rivera E, et al. Cloning and molecular characterization of a cDNA from Blomia tropicalis homologous to dust mite group 3 allergens (trypsin-like proteases). Int Arch Allergy Immunol 2003; 130:12–16.
171. Nisbet AJ, Billingsley PF. Hydrolytic enzymes of Psoroptes cuniculi (Delafond). Insect Biochem Mol Biol 1999; 29:25–32.
172. Edwards TB, Trudeau WL, Fernández-Caldas E, et al. Proteinases in extracts of the storage mite, Aleuroglyphus ovatus. J Allergy Clin Immunol 1992; 90:129–131.
173. Ando T, Homma R, Ino Y, et al. Trypsin-like protease of mites: purification and characterization of trypsin-like protease from mite faecal extract Dermatophagoides farinae. Relationship between trypsin-like protease and Der f III. Clin Exp Allergy 1993; 23:777–784.
174. Stewart GA, Lake FR, Thompson PJ. Faecally derived hydrolytic enzymes from Dermatophagoides pteronyssinus: physicochemical characterisation of potential allergens. Int Arch Allergy Appl Immunol 1991; 95:248–256.
175. Kemeny DM, Harries MG, Youlten LJF, et al. Antibodies to purified bee venom proteins and peptides. I. Development of a highly specific RAST for bee venom antigens and its application to bee sting allergy. J Allergy Clin Immunol 1983; 71:505.
176. Hoffman DR. Allergens in Hymenoptera venom. XVII. Allergenic components of Solenopsis invicta (imported fire ant) venom. J Allergy Clin Immunol 1987; 80:300.
177. Hoffman DR. Allergens in Hymenoptera venom. XIV. IgE binding activities of venom proteins from three species of vespids. J Allergy Clin Immunol 1985; 75:606.
178. Baldo BA, Baker RS. Inhalant allergies to fungi: reactions to baker's yeast (saccharomyces cerevisiae) and identification of baker's yeast enolase as an important allergen. Int Arch Allergy Appl Immunol 1988; 86:201.
179. Baur X, Weiss W, Sauer W, et al. Baking ingredients as a contributory cause of baker's asthma. Deut Mediz Woch 1988; 113:1275.
180. Shen HD, Choo KB, Lee HH, et al. The 40-KiloDalton allergen of Candida albicans is an alcohol dehydrogenase: molecular cloning and immunological analysis using monoclonal antibodies. Clin Exp Allergy 1991; 21:675–681.
181. Ando T, Homma R, Ino Y, et al. Trypsin-like protease of mites: purification and characterization of trypsin-like protease from mite faecal extract Dermatophagoides farinae. Relationship between trypsin-like protease and Der f III. Clin Exp Allergy 1993; 23(9):777–784.
182. Stewart GA, Lake FR, Thompson PJ. Faecally derived hydrolytic enzymes from Dermatophagoides pteronyssinus: physicochemical characterisation of potential allergens. Int Arch Allergy Appl Immunol 1991; 95(2–3):248–256.
183. Kikuchi Y, Takai T, Kuhara T, et al. Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p1 to sensitization toward IgE and IgG responses. J Immunol 2006; 177(3):1609–1617.
184. Caughey GH. Of mites and men: Trypsin–like proteases in the lungs. Am J Respir Cell Mol Biol 1997; 16:621–628.
185. Hewitt CR, Brown AP, Hart BJ, et al. A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. J Exp Med 1995; 182:1537–1544.
186. Brown A, Farmer K, MacDonald L, et al. House dust mite Der p 1 downregulates defenses of the lung by inactivating elastase inhibitors. Am J Respir Cell Mol Biol 2003; 29:381–389.
187. Sun G, Stacey MA, Schmidt M, et al. Interaction of mite allergens Der p 3 and Der p 9 with protease-activated receptor-2 expressed by lung epithelial cells. J Immunol 2001; 167:1014–1021.
188. Weghofer M, Thomas WR, Pittner G, et al. Comparison of purified Dermatophagoides pteronyssinus allergens and extract by two-dimensional immunoblotting and quantitative immunoglobulin E inhibitions. Clin Exp Allergy 2005; 35(10):1384–1391.
189. Pittner G, Vrtala S, Thomas WR, et al. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. Clin Exp Allergy 2004; 34(4):597–603.
190. Sehgal N, Custovic A, Woodcock A. Potential roles in rhinitis for protease and other enzymatic activities of allergens. Curr Allergy Asthma Rep 2005; 5(3):221–226.
191. Brown A, Farmer K, MacDonald L, et al. House dust mite Der p 1 downregulates defenses of the lung by inactivating elastase inhibitors. Am J Respir Cell Mol Biol. 2003; 29(3 pt 1):381–389. [Epub 2003, Apr 10].
192. Custovic A, Simpson A, Woodcock A. Importance of indoor allergens in the induction of allergy and elicitation of allergic disease. Allergy 1998; 53:115–120.
193. Carnés J, Boquete M, Carballada FJ, et al. Enzymatic activity in body and fecal extracts of the storage mite Chortoglyphus arcuatus. Int Arch Allergy Immunol 2007; 145(3):207–212.
194. Caraballo LR, Hernandez M. HLA haplotype segregation in families with allergic asthma. Tissue Antigens 1990; 35(4):182–186.

195. Hizawa N, Freidhoff LR, Chiu YF, et al. Genetic regulation of Dermatophagoides pteronyssinus-specific IgE responsiveness: a genome-wide multipoint linkage analysis in families recruited through 2 asthmatic sibs. Collaborative Study on the Genetics of Asthma (CSGA). J Allergy Clin Immunol 1998; 102(3):436–442.
196. Hizawa N, Collins G, Rafnar T, et al. Linkage analysis of Dermatophagoides pteronyssinus-specific IgE responsiveness with polymorphic markers on chromosome 6p21 (HLA-D region) in Caucasian families by the transmission/disequilibrium test. Collaborative Study on the Genetics of Asthma (CSGA). J Allergy Clin Immunol 1998; 102(3):443–448.
197. Stephan V, Kuehr J, Seibt A, et al. Genetic linkage of HLA-class II locus to mite-specific IgE immune responsiveness. Clin Exp Allergy 1999; 29(8):1049–1054.
198. Torres-Galvan MJ, Quiralte J, Blanco C, et al. Linkage of house dust mite allergy with the HLA region. Ann Allergy Asthma Immunol 1999; 82(2):198–203.
199. Caraballo L, Marrugo J, Jimenez S, et al. Frequency of DPB1*0401 is significantly decreased in patients with allergic asthma in a mulatto population. Hum Immunol 1991; 32(3):157–161.
200. Hu C, Hsu PN, Lin RH, et al. HLA DPB1*0201 allele is negatively associated with immunoglobulin E responsiveness specific for house dust mite allergens in Taiwan. Clin Exp Allergy 2000; 30(4):538–545.
201. Caraballo L, Martinez B, Jimenez S, et al. HLA-DR3 is associated with the IgE immune responsiveness to a recombinant allergen from Blomia tropicalis (BT). Adv Exp Med Biol 1996; 409:81–83.
202. Young RP, Dekker JW, Wordsworth BP, et al. HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. Clin Exp Allergy 1994; 24(5):431–439.
203. Lara-Marquez ML, Yunis JJ, Layrisse Z, et al. Immunogenetics of atopic asthma: association of DRB1*1101 DQA1*0501 DQB1*0301 haplotype with Dermatophagoides spp.-sensitive asthma in a sample of the Venezuelan population. Clin Exp Allergy 1999; 29(1):60–71.
204. Blumenthal MN. Positive association between HLA-DRB1*07 and specific IgE responses to purified major allergens of D. pteronyssinus (Der p 1 and Der p 2). Ann Allergy Asthma Immunol 2002; 88(2): 147–149.
205. Kim YK, Oh HB, Oh SY, et al. HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of a sensitization to house dust mite Dermatophagoides pteronyssinus. Clin Exp Allergy 2001; 31(1):110–115.
206. Shin HD, Kim LH, Park BL, et al. Association of interleukin 18 (IL18) polymorphisms with specific IgE levels to mite allergens among asthmatic patients. Allergy 2005; 60(7):900–906.
207. Acevedo N, Vergara C, Mercado D, et al. The A-444C polymorphism of leukotriene C4 synthase gene is associated with IgE antibodies to Dermatophagoides pteronyssinus in a Colombian population. J Allergy Clin Immunol 2007; 119(2):505–507.
208. Martinez B, Barrios K, Vergara C, et al. A NOS1 gene polymorphism associated with asthma and specific immunoglobulin E response to mite allergens in a Colombian population. Int Arch Allergy Immunol 2007; 144(2):105–113.
209. Kim Y, Park CS, Shin HD, et al. A promoter nucleotide variant of the dendritic cell-specific DCNP1 associates with serum IgE levels specific for dust mite allergens among the Korean asthmatics. Genes Immun 2007; 8(5):369–378.

11 Cockroach and Other Inhalant Insect Allergens

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INTRODUCTION

Insect inhalant allergy is a health problem all over the world due to the cosmopolitan existence of these arthropods. The variety and distribution of insects and the accumulation of debris associated with heavy infestations vary significantly with time and geographic location. Insect inhalant allergens are found indoors, outdoors, in homes, and at the workplace. Sensitization can be due to involuntary exposure to windborne insect emanations in house dust or to occupational exposures, encountered by professionals such as research entomologists. The concept of inhalant insect allergies was developed by the early observations of Figley (1929), Parlato (1930, 1932), and Kern (1938) of asthma associated to sensitization to the mayfly, sandfly, mushroom fly, moth, and butterflies (1,2). In the animal kingdom, the phylum Arthropoda constitutes 75% of known animal species that can contribute significant organic material for airborne dispersal. Three major taxonomic groups, Insecta, Crustacea, and Arachnida, are of major concern as allergen producers. Some of the allergens from the three groups share amino acid sequence homology that manifests in allergenic cross-reactivity.

This chapter focuses on the class Insecta, which includes more than 80% of species from the three groups combined. Insects have three pairs of legs and bodies divided into head, thorax, and abdomen, and they are either wingless or have one or two pairs of wings. Cockroaches, mayflies, caddisflies, moths, butterflies, flies, fleas, midges, ants, bees, and vespids are representative members of this class. Insect allergy (i.e., IgE-mediated sensitivity) may be induced by a wide variety of insect-derived allergens in the environment either on a seasonal (vast aquatic insect emergences, such as caddisflies, mayflies, and midges) or perennial basis (terrestrial pests, such as cockroaches). In certain places, inhalant insect dust is clearly visible and associated with the emergence of caddisflies in May, June, and July. In Japan, documented sensitization to moths and butterflies is as common as sensitization to house-dust mite. Chironomidae larvae and midges cause allergic reactions in approximately 20% of environmentally exposed workers with insect larvae and subjects living in affected areas. Exposure to large numbers of the "green nimitti" midge in Sudanese communities is associated with an increased incidence of both asthma and allergic rhinitis. Honeybees produce "bee dust" that causes inhalant allergy in beekeepers, and subjects extracting bee venom can develop inhalant allergy to phospholipase C. Wherever allergenic exposure (onset, intensity, and frequency) and adjuvant forces (ozone, NO_2, tobacco smoke, viruses, etc.) are present in the environment, allergic symptoms can develop, particularly in those with a genetic atopic predisposition.

Cockroach allergy is especially important for the development of asthma in inner cities among lower socioeconomic groups (3–7). Over 4000 species of cockroach are described worldwide, the majority of which are not directly associated with humans in their home and work environments. Cockroaches can be categorized as domestic, peridomestic, or feral. Feral species are those that survive independent of humans and represent 95% of all species worldwide. Seventy-four species occur in the United States, some of which have been introduced from other parts of the world. Domestic species include the German and brown-banded cockroaches that live almost exclusively indoors and depend on human refuse (harborage food and water) for survival. Their ideal environment is warm and humid, making indoor households primary dwelling places; however, some species live outdoors. Peridomestic species include those that survive in or around domestic environments. This group is represented by American, Australian, brown and smoky brown, Oriental, and woods cockroaches. These were introduced into North America over the past two centuries and have been very successful in establishing habitats throughout the world including industrialized/highly developed countries where insect infestation is better controlled.

The desire to control the indoor climate with air conditioning units to mitigate extremes of temperature, moisture, and airflow sets the stage for several cockroach species to infest and inhabit homes. The presence of some domestic species in dwellings, such as the German or brown-banded cockroaches, is often a sign of poor sanitation or substandard housekeeping. Survival of these species is enhanced by crowded living, as in apartment complexes, where associated clutter and accumulation of organic debris is often present. An overpopulation of the peridomestic American or Oriental cockroaches in their native habitats, such as municipal sewage systems and septic tank areas, facilitates their entrance into nearby homes through crawl spaces, construction joints, and attic vents, causing infestation of even the best kept homes and workplaces. The species that infest household structures typically have a high reproduction potential, which results in accumulation of relatively high dust levels of cockroach airborne allergenic proteins derived from shed exoskeletons (cast skins) and feces.

ORIGIN AND TAXONOMY OF COCKROACHES

Some fossils attributed to cockroaches date back to the Carboniferous period from the Paleozoic era (over 350 million years ago), representing some of the oldest and most primitive of insects. However, such an early origin of cockroaches cannot be dated with reasonable confidence, and the idea of an ancient origin of cockroaches is been revised. The only obvious character in these fossils (a long ovipositor) is inconsistent with an hypothesis of relationship with cockroaches, and the first fossils of "modern" cockroaches with short ovipositors appear later, in the early Cretaceous, Mesozoic era, like most other insects (8).

Cockroaches belong to the phylum Arthropoda, class Insecta, and there are six cockroach families in the order Blattaria: Blaberidae, Blattellidae, Blattidae, Cryptocercidae, Nocticolidae, and Polyphagidae. The second and third families contain the most common peridomestic pests found throughout the world (Table 1). A more detailed taxonomy of cockroaches can be found in the publications by Atkinson and Koehler (9,10).

COCKROACH IDENTIFICATION

Cockroaches have an exoskeleton, a segmented body (head, thorax, and abdomen), three pairs of legs, and one or two pairs of wings or none. The possession of an exoskeleton gives the insect its form, attachment points for muscles, and provides a hardened protective covering (exoskeleton) that requires molting for growth. The old exoskeleton is discarded as exuviae (cast skins), allowing the insect to enlarge before a new exoskeleton hardens. Cockroaches are omnivorous and will consume any organic material, including fresh and processed foods, stored products, and even bookbindings and paste found on stamps and in wallpaper. In times of food shortage, some species will become cannibalistic to maintain a colony.

Table 1 Taxonomy of Cockroaches

Phylum:	Arthropoda	
Class:	Insecta	
Order:	Blattaria	
Family	**Genus/species**	**Common name**
Blaberidae	*Leucophaea maderae*	Madeira
Blattellidae	*Blattella germanica*	German
	Blattella asahinai	Asian
	Supella longipalpa	Brown-banded
Blattidae	*Periplaneta americana*	American
	Periplaneta australasiae	Australian
	Periplaneta brunnea	Brown
	Periplaneta fuliginosa	Smoky brown
	Blatta orientalis	Oriental

Table 2 Cockroach Identification

Name	Morphology	Features	Habitat
German *Blattella germanica*	16 mm long, brown, parallel dark bands along axis of body	Incapable of flight, nocturnal. Varying degrees of pesticide resistance. Most prominent pest. Strictly domestic	Kitchens, pantries, bathrooms, bedrooms
Asian *Blattella asahinai*	16 mm long, light brown, requires taxonomist for differentiation from German	Capable of flight, attracted to light. Wild and peridomestic. Introduced in Tampa and Lakeland, Florida, 1986. Interbreed with German	Rich ground cover, citrus grooves of Florida leaf litter: manicured lawns
American *Periplaneta americana*	34–53 mm long reddish brown with variation light	Capable of flight; mostly cosmopolitan. Peridomestic	Landfills, crawlspaces, sewage systems, storm drains, septic tanks, attics, and dark tree holes, caves, mines
Oriental *Blatta orientalis*	25 to 35 mm long, black or dark brown	May or may not fly. Commonly known as water bug	Dark, damp conditions, water meter boxes, garbage chutes
Somky brown *Periplaneta fuliginosa*	25 to 33 mm long, dark brown	Major southern U.S. pest. Peridomestic, majority are wild	Tree holes, palm trees, loose mulch (pine bark, straw), firewood piles, soffits, panel walls, block wall interstices, false ceilings
Brown-banded *Supella longipalpa*	13–14.5 mm long, dark band across abdomen	Capable of flight, attracted to light	Nonfood areas, bedrooms, closets, living rooms

Infestations of cockroaches in primary dwellings and workplaces represent one of the most intimate and chronic associations of pests with humans. All cockroach species are adept crawlers; however, flight ability varies. The two most common species are the American (*Periplaneta americana*) and German (*Blattella germanica*) cockroaches. Table 2 describes the five major cockroach species associated with humans and their immediate environment.

PUBLIC HEALTH IMPORTANCE OF COCKROACHES

Cockroaches may adversely affect human health in several ways through biting, psychological stress and contamination of food with excrement (causing vomiting and diarrhea), associated pathogens, and allergy. Bernton and Brown made the first reports of cockroach sensitization in the 1960s (1,11). Kang and Sulit showed that 60% of patients with asthma in the Chicago area had positive skin tests, serum IgE antibodies, or positive bronchial challenge tests to *B. germanica* allergens (12). The National Cooperative Inner-City Asthma Study found that exposure and sensitization to cockroach allergens were associated with asthma morbidity in children from eight major inner-city areas in the United States. Of 476 children with asthma (age 4–7 yr), 36.8% were allergic to cockroach allergens, followed by dust mite (34.9%) and cat (22.7%) (6). The same association was found in children for cockroaches, and not for mite, cat and dog, in another Inner-City Asthma Study, reporting a 69% prevalence of sensitization to cockroach allergens in 937 children, reaching the highest value of 81% in the Bronx (7). Other parts of the world have reported an important association between cockroach infestations and asthma, including occupational asthma. Infestations by domiciliary cockroaches are largely dependent on housing conditions, and high-rise apartments have higher levels of cockroach allergens (7,13,14).

Americans now spend more than 95% of their time indoors in homes that are better insulated and temperature controlled while outdoor air exchange has been drastically reduced, creating conditions that support both pest growth and associated dust accumulation in the

home (6,7,11,12,15,16). Cockroach allergy can result from initial sensitization to the allergen though inhalation, ingestion, dermal abrasion, or injection. Potential sources of relevant cockroach allergens in the environment include whole bodies, cast skins, secretions, egg casings, and/or fecal material. Airborne cockroach allergens are associated primarily with amorphous and larger particles (10 μm) (which settle after disturbance) than are animal allergens. The threshold levels of allergen exposure above which susceptible individuals are at increased risk for sensitization or asthma symptoms are 2 and 8 U/g of dust for Bla g 1 or Bla g 2 (equivalent to 80 and 160 ng/g for Bla g 2) (4). In a nationally representative sample, cockroach allergen (Bla g 1) concentrations exceeded 2 U/g in 11% of U.S. living room floors and 13% of kitchen floors, and exceeded 8 U/g in 3% of living room floors and 10% of kitchen floors. Detectable concentrations of >0.4 U/g were found in 27.4% of U.S. homes (14). Mild-to-moderate symptoms induced by cockroach allergen inhalation include sneezing and rhinorrhea, skin reactions (mild dermatitis), and eye irritation, with difficulty in breathing and anaphylactic episodes occurring in more severely allergic individuals.

EARLY IDENTIFICATION OF COCKROACH ALLERGENS AND ASSESSMENT OF ENVIRONMENTAL EXPOSURE

Several groups used conventional physicochemical techniques to identify and characterize cockroach allergens. Allergenic proteins, with molecular weights ranging from 6 to 120 kDa, were initially identified from cockroach extracts using serum IgE from cockroach-sensitive individuals (17–19). A wide variation in their IgE-binding patterns to extracts of crude whole-body German cockroaches was observed. A high correlation of radioallergosorbent test (RAST) activity between German whole-body and fecal extracts was observed, and five allergens (MWs of 67, 60, 50, 45, and 36 kDa) showed IgE-binding reactivity in 50% to 80% of 37 human sera tested (19). Twarog et al. found three major allergens with molecular weights of 25, 63 to 65, and <10 kDa (20). Helm et al. identified a 36-kDa protein as a principal allergen of German cockroach whole-body extracts, not present in extracts of armyworm, caddisfly, lakefly, and other insects, and a 55-kDa protein also present in true armyworm, honeybee, and lakefly extracts (21). Crude extracts of whole-body American cockroach contain at least 29 antigenic components from which 18 were allergens (22,23). Two of these are major allergens (78 and 72 kDa) and cause T-cell proliferation of peripheral blood cells from cockroach-allergic patients (24). Zwick et al. localized, by immunohistochemistry, proteins derived from the epithelial cells of the intestinal tract that are present in the feces as well as in whole-body sections. They could represent important cockroach allergens (25).

Visual assessment of cockroach infestations correlates with skin test results. However, the best way to assess environmental concentration of cockroach allergens is by using enzyme-linked immunoassays. Two allergens from the German cockroach, Bla g 1 and Bla g 2, were purified using monoclonal antibodies (26). Bla g 1 is a 25 kDa acidic, cross-reacting allergen previously identified by Twarog et al., and Bla g 2, a 36 kDa species-specific allergen (20). Specific immunoassays for both allergens are used to monitor environmental cockroach exposure (26–28). A sandwich ELISA, based on a monospecific rabbit antibody reactive with Per a 1 and Bla g 1, had also been suggested for use in environmental assays (28). Other monoclonal antibodies to American cockroach allergens have also been produced that could be used to measure allergen levels (23,29).

MOLECULAR STRUCTURE AND BIOLOGICAL FUNCTION OF COCKROACH ALLERGENS

In the last 12 years, several cockroach allergens have been cloned, and their biochemical activities and biological roles investigated. American and German cockroach cDNA expression libraries have been screened with human IgE or murine monoclonal antibodies to identify clones expressing allergens. A list of the cockroach allergens reported by the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Subcommittee is shown in Table 3.

Table 3 Nomenclature and Function of Cockroach Allergens (WHO/IUIS)

Allergen	MW[a]	Function/homology (kDa)	Accession number	References
B. germanica				
Bla g 1.0101	46,21	Midgut microvilli protein-homolog	AF072219, AF072221	31
Bla g 1.0102	90	Midgut microvilli protein-homolog	L47595	30
Bla g 1.0201	56	Midgut microvilli protein-homolog	AF072220	31
Bla g 2[b]	36	Unusual aspartic protease	U28863	37,39,42
Bla g 4	21	Lipocalin	U40767	44
Bla g 5	23	Glutathione S-Transferase	U92412	50
Bla g 6.0101	17	Troponin C	DQ279092	51
Bla g 6.0201	17	Troponin C	DQ279093	51
Bla g 6.0301	17	Troponin C	DQ279094	51
Bla g 7	33	Tropomyosin	AF260897	54
Bla g 8	21	Myosin light chain	DQ389157	51
P. americana				
Per a 1.0101	26	Midgut microvilli protein-homolog	AF072222	32
Per a 1.0102	26	Midgut microvilli protein-homolog	U78970	33
Per a 1.0103	45	Midgut microvilli protein-homolog	U69957	104
Per a 1.0104	31	Midgut microvilli protein-homolog	U69261	33
Per a 1.0201	51	Midgut microvilli protein-homolog	U69260	105
Per a 3.0101	79	Arylphorin/hemocyanin	L40818	56
Per a 3.0201	75	Arylphorin/hemocyanin	L40820	56
Per a 3.0202	56	Arylphorin/hemocyanin	L40819	57
Per a 3.0203	46	Arylphorin/hemocyanin	L40821	57
Per a 6	17	Troponin C	AY792950	51
Per a 7.0101	33	Tropomyosin	Y14854	52
Per a 7.0102	33	Tropomyosin	AF106961	53
Per a 9	43	Arginine kinase	AY563004	72
Per a 10	28	Serine protease	–	–

[a]Molecular weight calculated from amino acid sequence.
[b]The X-ray crystal structure of Bla g 2 is accessible in the Protein Data Bank under the accession number 1YG9.

Helm et al. identified the first Bla g 1 clone that contains a 4 kb insert encoding for a protein with an apparent molecular weight of 90 kDa (Bla g 90 kDa or Bla g 1.0102) (30). The DNA encoding for Bla g 90 kDa consists of seven 576 bp tandem repeats with a shorter unique region at both ends. Other Bla g 1 and Per a 1 isoforms were identified in subsequent studies (31–33). Each of the tandem nucleotide repeats encodes for two consecutive amino acid repeats of approximately 100 residues each. DNA sequence analysis shows that Group 1 cockroach allergens originated by gene duplication and subsequent mutagenesis of a mitochondrial energy transfer domain (31). These allergens are thought to be involved in digestion, although the specific function is unknown. Bla g 1 is most prevalent in the midgut, and the Bla g 1 gene is exclusively expressed in midgut cells (31,34). Adult females produce and excrete significantly more Bla g 1 in their feces than males and nymphs, most likely because females process more food than males and other life stages of the cockroach, and production is related to food intake (35).

Bla g 2 is the most important among other cockroach allergens (rBla g 1, rBla g 4, rBla g 5, rPer a 7) (36) and has the highest prevalence of IgE antibody binding (54.4%), which is approximately twofold higher than the prevalence to any of the other four allergens tested using 118 sera from cockroach-sensitized subjects. Among sera with high IgE antibody levels to cockroach extract (3.5–100 IU/mL), the prevalence of IgE antibodies to Bla g 2 and Bla g 5 is 71% and 58%, respectively (36). These results confirm previous findings that Bla g 2 elicits IgE responses in 42% to 70% of cockroach-allergic patients. The prevalence of IgE antibody binding to Bla g 1 and Bla g 2 is 30% and 58%, respectively, using 106 sera from cockroach-allergic patients (37). Bla g 2 is homologous to aspartic proteases, which are a widely distributed group of digestive enzymes. Several studies support the idea that allergens with proteolytic activity may achieve access to antigen-presenting cells in the absence of inflammation by damaging the epithelium and facilitating their own access and penetration into the mucosa (38). For example, proteolytic activity of mite allergens (Der p 1, Der p 3, Der p 6) may contribute to allergenicity. However, Bla g 2 is a proteolytically inactive and potent allergen, inducing sensitization at

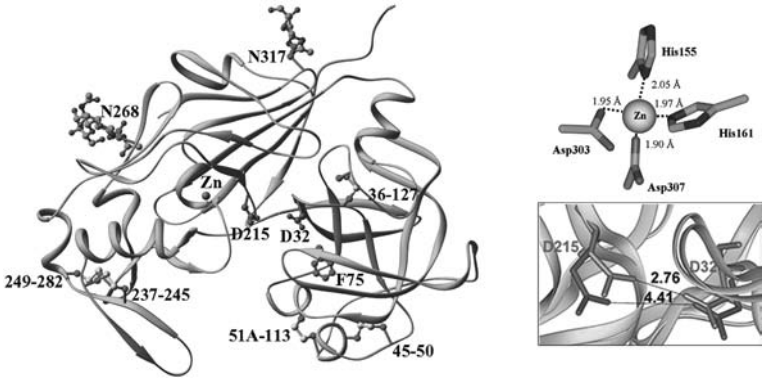

Figure 1 (*See color insert.*) Crystal structure of the cockroach allergen Bla g 2. (*Left*) Bilobal structure of Bla g 2 showing residues at the region of the catalytic site (D215, D32) and F75 (previous to insertion F75a), carbohydrates and the five disulfide bonds (1yg9.pdb). (*Right*) The zinc ion (*sphere*) with coordinating residues and interatomic distances (*top*); and aspartate positions in Bla g 2 (*dark grey aspartates*) and in pepsin (*light grey aspartates*) (*bottom*). *Source*: Reprinted with permission from Ref. 39.

exposure levels that are one or two orders of magnitude lower than other allergens such as Der p 1 (39,40).

The X-ray crystallographic structure of Bla g 2 has been solved, revealing interesting features that contribute to allergenicity and explain why Bla g 2 is not an active aspartic protease (Fig. 1, in color insert) (39). Aspartic proteases have a bilobal molecular structure, and the catalytic activity depends on a couple of amino acid triads (DTG) at the bottom of a cleft between the two lobes. However, Bla g 2 has important amino acid substitutions in the catalytic site, especially at the level of the triads (DST and DTS instead of DTG) that make this molecule enzymatically inactive (41,42). Distortions in the catalytic site, due to these substitutions, lead to a bigger distance of the catalytic aspartates, which is expected to impair enzymatic activity (Fig. 1, bottom right). In addition, there is an insertion (F75a) that interferes with the typical substrate-binding site, providing an autoinhibitory mechanism of enzymatic activity (39). These structural studies indicate that proteolytic activity is not necessary for allergenicity (41–43).

Two unique structural characteristics of Bla g 2 may contribute to allergenicity by conferring stability to the molecule: (*i*) the allergen binds one atom of zinc (Fig. 1, top right) and (*ii*) Bla g 2 has five disulfide bonds in contrast to only two or three in active aspartic proteases (Fig. 1, left) (39). Chronic exposure to low doses (1–10 μg/yr) of this stable cockroach allergen may explain why sensitization and exposure to Bla g 2 is associated with asthma.

Bla g 4 is another *B. germanica* allergen that belongs to the family of proteins called lipocalins (44). Apart from another insect lipocalin allergen identified and cloned from the biting reduviid *Triatoma protracta,* most of the known lipocalin allergens are from mammalian origin: Bos d 2 (cow), Can f 1 and Can f 2 (dog), Equ c 1 (horse), Fel d 4 (cat), Mus m 1 (mouse) and Rat n 1 (rat) (38,45,46). The milk allergen β-lactoglobulin (Bos d 5) is also a lipocalin. The molecular structure of these allergens is very stable and consists of a C-terminal α-helix and a β-barrel enclosing an internal hydrophobic cavity that binds small ligands such as retinoids, glucocorticosteroids, and pheromones (Fig. 2) (47). The homology of Bla g 4 (calycin) with rodent urinary proteins raises the possibility of pheromone or lipid-transport proteins being potential families of inhalant arthropod allergens. Immunohistochemical localization studies show that Bla g 4 is only expressed in the accessory glands of the male cockroach reproductive system (conglobate gland and utricles) and is transferred to the female during copulation. The study suggests that Bla g 4 has a reproductive function, and the allergen could be released from dried seminal secretions or spermatophores into the environment (48).

Additional cloned allergens are the glutathione S-transferase (Bla g 5), which could have a detoxifying function, and two proteins involved in muscle contraction: troponin C (Bla g 6) and tropomyosin (Group 7) (49–54). Troponin C has a calcium-dependent regulatory function, and calcium binding induces changes in the molecular structure that affect IgE antibody binding (51). Troponins and tropomyosins include a diverse group of proteins with distinct

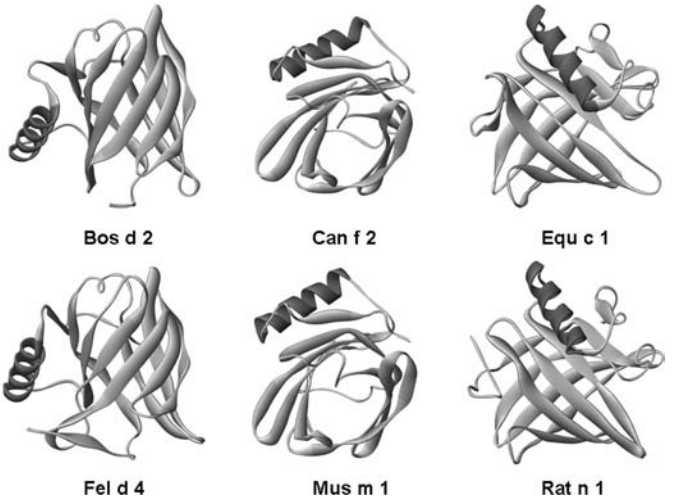

Figure 2 Ribbon representation of the molecular structure of Bla g 4 homolog allergens from mammals: Bos d 2 (cow), Equ c 1 (horse), Mus m 1 (mouse), and Rat n 1 (rat), and molecular models of Can f 1 (dog) and Fel d 4 (cat) obtained using Swiss-Model (106–108). The C-terminal α-helix is shown in dark gray. Three different orientations of the molecules are shown.

isoforms found in muscle, brain, and non-muscle tissue. Structurally, tropomyosins are elongated two-stranded proteins wound around each other with dimeric α-helical coiled structures along their length (55). The considerable homology of tropomyosins from different species explains part of the allergenic cross-reactivity among arthropods and mollusks (section Extra Species Cross-Reactivity).

Per a 3 shows high homology to insect hemolymph proteins (56). Isoallergenic variants Per a 3.0202 (C13) and Per a 3.0203 (C28) of the allergen Per a 3.0201 (C20) display significant differences in skin reactivity, suggesting a high degree of polymorphism among the allergens (57). Hemoglobins of the Diptera (insect) family of Chironomidae are causative agents in asthmatic patients living in regions where large swarms of nonbiting midges occur. Chi t 1, the hemoglobin from the European midge species (*Chironomus thummi*), represents the major allergenic component causing rhinitis, conjunctivitis, and bronchial asthma in exposed populations. There is considerable immunological cross-reactivity between hemoglobins of the same and closely related Chironomidae species, suggesting that hemoglobins and hemocyanins of insects may also represent an important source of arthropod allergens (58).

OTHER SOURCES OF INSECT ALLERGENS

Arthropods that have been most studied as sources of allergens include crustaceans (mussels, snails, squids), insects (caddisflies or sedges, mayflies, moths and butterflies, chironomid midges, and cockroaches), and arachnids (mites). A number of other arthropods, including the houseflies, ants, spiders, locusts and grasshoppers, bees and silverfish (in this case it is a single genus, species; each of the other groups consists of several known species producing allergens), cause sensitization either in the home or occupational settings. Table 4 shows a summary of insect species and their association to inhalant allergies.

The role of insects as providing inhalant allergens is further supported by data showing positive bronchial or nasal challenge. Airborne insect-derived particles include shed hairs, scales, excreta, and disintegrated body parts that contribute to amorphous dust. The composition of dust is influenced by geographical location, diligence and thoroughness of cleaning, use of insecticides, and both qualitative and quantitative sampling. The widespread incidence of swarming insects outdoors and the presence of cockroaches in house-dust samples and their related allergenicity are firmly established. Less certainty exists for other insect allergens serving as allergen source material in dust samples.

Dogs and cats contribute dander, hair, and body secretions to allergenic loads in household dust. Not widely known is the contribution of the common flea. When dogs and

Table 4 Inhalant Insect Allergies

Species	Common name	Order	Place of exposure	
Apis mellifera	Honeybee	Hymenoptera	Environment of beekeepers (O)	Rudeschko, 2004; Bousquet, 1982
Bombyx mori	Silkworm	Lepidoptera	Bedding, clothes; silk workers (O)	Kino, 1987; Wen, 1990; Uragoda, 1991; Suzuki, 1995; Komase, 1997; Hirabayashi, 1997
Chironomus sp.	Midges, chironomids	Diptera	Hypereutrophic lake in Japan Outdoors	Kino, 1987; Eriksson, 1989 Baur, 1992; Liebers, 1993; Ree, 1996; Komase, 1997; Hirabayashi, 1997; Yong, 1999; Morsy, 2000
Ctenocephalides felis felis	Cat flea	Siphonaptera	House dust	Trudeau, 1993
Ephestia kuehniella	Flour moth	Lepidoptera	Bakers (O)	Makinen-Kiljunen, 2001
Harmonia axyridis	Asian lady beetle	Coleoptera	Infested homes	Albright, 2006; Goetz, 2007; Nakazawa, 2007
Lepisma saccharina	Silverfish	Zygentoma	Infested homes	Witteman, 1996; Barletta, 2002; Barletta, 2005
Locusta migratoria	Grasshopper	Orthoptera	Research laboratories (O)	Soparkar, 1993; Lopata, 2005
Lucilia cuprina	Blowfly	Diptera	Entomological research laboratory (O)	Kaufman, 1986
Monomorium pharaonis	Pharaoh ant	Hymenoptera	Indoor infested environments	Kim, 2005; Kim, 2007
Musca domestica	Common housefly	Diptera	Closed breeding rooms; livestock stables, barns; pharmaceutical industry; workers (O)	Wahl, 1997; Tas, 2007; Focke, 2003; Barletta, 2003; Smith, 2005
Sitophilus oryzae	Rice weevil	Coleoptera	Infested building	Kleine-Tebbe, 1992
Tenebrio molitor	Mealworm (beetle larvae)	Coleoptera	Warehouse fishing bait handling (O)	Bernstein, 1983; Siracusa, 1994
Thaumetopoea pityocampa	Pine processionary (caterpillar)	Lepidoptera	Airborne urticating hairs	Werno, 1993; Fuentes, 2006
Triatoma infestans	Reduviid	Hemiptera	Indoor and outdoor environments	Alonso, 1996
Several species	Caddisflies	Trichoptera	Outdoor environments; hydroelectric plant workers (O)	Kino, 1987; Koshte, 1989; Warrington, 2003; Smith, 2005
Several species	Mayflies	Ephemeroptera	Outdoor rural environment	Smith, 2005

Occupational exposures are indicated by (O). Usually housefly, caddisfly, and mayfly implies plural species. Studies are cited by first author and year of publication.

cats are present in the house, the dog flea, *Ctenocephalides canis,* and the cat flea, *C. felis*, can reach pest proportions. Most flea allergenicity has been attributed to bites from these insects. However, Trudeau et al. were able to detect IgE antibodies to cat flea allergens in 33.3% (16 of 48) of cat flea skin test–positive sera of individuals in the Tampa Bay area of Florida, and the allergens were different from the antigenic components of the salivary cat flea (59). These results suggest a mode of sensitization other than by bite. Furthermore, using their in-house flea extract, flea allergens were quantified in eight house-dust samples using RAST inhibition assays. Increasing evidence such as this indicates that insects are a significant source of both indoor and outdoor inhalant allergens.

A new inhalant allergy is associated with the Asian lady beetle or ladybug (*Harmonia axyridis*). This insect was introduced in 1916 into the United States and released until 1990 as an ecological control for aphids. The first report of allergy to Asian lady beetle was published in 1999 by Yarbrough et al. and describes two cases of allergic rhinoconjunctivitis. *H. axyridis* seeks refuge in homes during fall and winter, leading to patient sensitization and allergic symptoms including rhinitis, wheezing, urticaria, conjunctivitis, chronic cough, and asthma (60,61). Reports on ladybug allergy come from St. Louis (Missouri), Appleton (Wisconsin), Louisville (Kentucky), West Virginia, and Georgia, and a survey reveals positive responses in North Central, Mid-Atlantic, and New England states (60,61). Five ladybug proteins, with molecular weights of approximately 8.6, 21, 28, 31, and 75 kDa, bind IgE antibody in Western blots (60). Partial protein sequences of purified Har a 1 (10 kDa) and Har a 2 (55 kDa), as well as the complete cDNA sequence encoding for two additional ladybug allergens (16.6 and a 30 kDa), have been reported (61,62). Further analysis of the allergens involved in Asian lady beetle allergy will help to better understand this novel inhalant allergy.

COCKROACH ALLERGEN CROSS-REACTIVITY

Inter-Cockroach Species Cross-Reactivity

Allergen cross-reactivity refers to the concordance of skin or RAST reactivity between two or more crude extracts due to the presence in the extracts of homologous proteins from different species that share IgE antibody–binding epitopes. Skin test or in vitro test panels are unlikely to identify primary sources of sensitization without adequate histories and evidence of exposure. In the attempt to control allergic disease by reducing allergen exposure, it is necessary to minimize exposure to all sources of the sensitizing and the cross-reacting allergens. Cross-reactivity studies clarify exposure patterns that are reflected in skin or in vitro test results and define important shared or unique allergens for further study.

Although some of the cloned cockroach allergens from *B. germanica* (i.e., Bla g 5) and *P. americana* (i.e., Per a 3) are species specific, cross-reactivity occurs among American and German cockroach allergens (63–66). Early clinical studies confirm cross-reactivity between both cockroach species (20,63,67,68). Stankus et al. identified two major acidic cockroach allergens from *P. americana* and *B. germanica* that share allergenic activity using physicochemical techniques and immunoprinting studies (65). Allergenic cross-reactivity is proven between the Madagascar hissing cockroach (*Gromphadorhina portentosa*), a popular pet, and other four cockroach species (69). An analysis of 45 antigens in *P. americana* and 29 antigens in *B. germanica* by crossed immunoelectrophoresis and immunoblots identified Per a 1 and Bla g 1 as cross-reactive homologous allergens from *P. americana* and *B. germanica,* respectively (64). Subsequent molecular cloning reveals that the Group 1 cockroach allergens is a mixture of allergenic proteins of different sizes (6, 21, 32, 43 kDa up to 90 kDa) (31,70). A 70% to 72% amino acid identity between Bla g 1 and Per a 1 reveals the molecular basis of allergenic cross-reactivity between both allergens (30–33).

Other allergens are also responsible for the cross-reactivity among different cockroach species. As mentioned, tropomyosins constitute the Group 7 cockroach allergens (52–54). Bla g 7 shares approximately 98% amino acid sequence identity with Per a 7. The dusky brown cockroach (*P. fuliginosa*) also produces a tropomyosin with 100% and 98.2% amino acid identity to Per a 7 and Bla g 7, respectively (71). Since tropomyosin is a common protein in arthropods, cross-reactivity among species in this big taxonomic group has been described (section Extra Species Cross-Reactivity). However, a comparative study of the relative importance of five cockroach allergens reveals that the prevalence of sensitization to tropomyosin among

cockroach-sensitized patients is low (~13% to Per a 7) compared to other cockroach allergens (54% to rBla g 2, 26% to rBla g 1, 17% to rBla g 4, and 37% to rBla g 5) (36).

Other cockroach allergens have been cloned that share homology between German and American cockroach and most likely also contribute to inter-cockroach species cross-reactivity. The Group 6 of cockroach allergens (troponin C), for example, shows a 14% prevalence of IgE antibodies among cockroach-sensitized patients (51). Additionally, an arginine kinase from *P. americana* has been described as a major allergen for cockroach-allergic Thai patients and cloned (accession number AY563004) (72). A German cockroach homolog is included in the GenBank (accession number ABC86902). Continued recognition and identification of cockroach allergens will help to understand and guide the proper management of cockroach-induced atopic disease.

Extra Species Cross-Reactivity

Several reports in the literature prove cross-reactivity between allergens from cockroach and other species. Witteman et al. showed that IgE antibodies in patients' sera react with silverfish, cockroach, and/or chironomid extracts in 30% of house-dust mite-allergic patients in the Netherlands (73). RAST inhibition studies identified a cross-reactive allergen among members of the groups Crustacea, Arachnida (*Dermatophagoides pteronyssinus*), and Insecta (*B. germanica*) (74).

Tropomyosin, a protein involved in muscle contraction, is also a cross-reactive allergen among members of the phyla Arthropoda and Mollusca (75–77). The Arthropoda producing allergenic tropomyosin include species from Crustacea (shrimp, crab, lobster, crawfish), Arachnida (dust mites), and Insecta (cockroaches, chironomids, silverfish). The Mollusca include Bivalvia (oysters, mussels, scallops, clams, pen shells), Gastropoda (snails, abalones, whelks), and Cephalopoda (squids, octopus, and cuttlefish). For example, tropomyosin could be the cross-reactive allergen in IgE-binding components between boiled Atlantic shrimp and German cockroach in the studies performed by Crespo et al. (78). An insect tropomyosin from *Lepisma saccharina*, Lep s 1, is also immunologically characterized and shows cross-reactivity with rPer a 7, the dust mite rDer p 10, and natural shrimp tropomyosin (79). These invertebrate tropomyosins share an approximately 80% amino acid sequence identity, whereas they are only approximately 45% homologous to human and edible meat (chicken, beef, pork, lamb, etc.) tropomyosins. This difference in homology may explain why humans do not develop allergies to edible meat tropomyosin (38). Interesting observations illustrate the clinical relevance of tropomyosin cross-reactivity. Exposure and sensitization to a particular food tropomyosin (dietary source) may lead to reactivity to aeroallergen exposure, and vice versa; increased exposure to aeroallergens (such as mite tropomyosin during immunotherapy) may result in reactivity to cross-reacting seafood tropomyosin (80). Immunoglobulin E antibody reactivity to the major shrimp allergen, tropomyosin, occurs in unexposed Orthodox Jews who observe Kosher dietary laws that prohibit eating shellfish. This reactivity is due to sensitization to tropomyosin from other origin (cockroach, mite) (81).

In investigations of crustacean foods and stinging insects, Koshte et al. found IgE antibodies to cross-reacting carbohydrate determinants (CCD) and other cross-reacting antibodies to homologous proteins in extracts of mussels, oysters, shrimps, crabs, and honeybee and yellow jacket venoms (82). An IgE-reactive determinant has been proposed to be the α(1,3)-fucosylation site of the innermost *N*-acetyl glucosamine residue of *N*-glycoproteins, which are frequent in insects and plants. IgE antibodies against nonmammalian *N*-glycans, α(1,3)-fucose, and β(1,2)-xylose can result in extensive cross-reactivity among plants and invertebrates (83). Whether these glycans play a prominent clinical role as dominant IgE epitopes or in the synthesis of allergen-specific IgE in vivo is not yet determined.

Indoor, outdoor, and workplace exposure to large numbers of insect species in different geographic regions make it extremely difficult to determine whether multiple sensitivities are explained by multiple exposures or by insect allergen cross-reactivity. From clinical and immunological observations, allergy to a single arthropod is uncommon, and cross-reactivity can extend to foods and other arthropods. The term "pan allergy," sensitization to one or a few insects with allergenic similarities that may extend to other noninsect member of the phylum Arthropods, defines this phenomenon (84).

Other cockroach allergens show cross-reactivity with arthropod homologs. The above-mentioned arginine kinase shares homology with Pen m 2 from black tiger shrimp (*Penaeus monodon*), Der p 20 from house-dust mite (reported in the WHO/IUIS list of allergens), and

Plo i 1 from the indianmeal moth, *Plodia interpunctella* (85,86). Inhibition experiments using dust mite, cockroach, king prawn, lobster, and mussel extracts suggest that arginine kinase is an invertebrate panallergen (86). The glutathione S-transferase from dust mite Der p 8 shows cross-reactivity with a cockroach homolog (87). Other proteins from *P. americana* are cloned and share homology to the cockroach groups 2 and 4 (AY792947 and AY792948) and to the mite allergens from groups 2, 3 (trypsin), and 13 (fatty acid–binding protein) (GenBank accession numbers AY792953, AY792954, and AY792955). Additional cross-reactivities among some of these proteins, not yet characterized as allergens, would be expected.

MECHANISMS RELATED TO COCKROACH ALLERGEN SENSITIZATION

Although little is known about the underlying mechanisms of cockroach sensitization, few studies report effects that may contribute to cockroach allergy. Antony et al. observed that American cockroach extracts (lacking serine and aspartic proteinase activity) induce the release of a vascular permeability factor (VEGF) to bronchial airway epithelial cells causing endothelial barrier abnormalities and increased microvascular permeability (88). The authors proposed a mechanism for increased sensitization to cockroach allergens by suggesting that this barrier breakdown facilitates allergen entry into the bronchial airways causing both sensitization and the allergic response. In contrast, Bhat et al. demonstrated that German cockroach extracts contain a serine protease activity, which has a direct inflammatory effect upon airway epithelial cells (89). Serine protease activity in German cockroach extract has been reported (41). Cockroach allergen extracts stimulate protease-activated receptor-2 (PAR-2), which is upregulated in respiratory epithelium from asthmatic patients (90,91). A serine protease (Per a 10) has been added to the WHO/IUIS list of Allergen Nomenclature, but the allergen needs further characterization (Table 3).

Using cultured human epithelial cells, German cockroach extracts synergistically increase TNF-α-induced transcription from the IL-8 promoter (89). Moreover, the IL-8 expression is dependent on a serine protease activity, sensitive to protease inhibitors, but it is not induced by the endotoxin at levels present in cockroach extracts. Rullo et al. investigated the levels of endotoxin, mite, and cockroach allergens in schools and suggested that endotoxin may be capable of inducing airway inflammation and worsening asthma (92). Thus, environmental control of both allergen and endotoxin levels may modify sensitization and allergic response. At the molecular level, the characterization of B- and T-cell epitopes on cockroach allergens will permit a better understanding of the immunopathogenic mechanisms involved in insect hypersensitivity.

DIAGNOSIS AND IMMUNOTHERAPY

Diagnosis

Skin testing using crude whole-body extracts is the gold standard to diagnose cockroach allergy. RAST, basophil histamine release, and total IgE are poor predictors of subsequent bronchial provocation results. RAST has an approximately 50% false-negative rate. At present, cockroach extracts used for skin testing are not standardized, and those commercially marketed are prepared from whole-body extracts of the three most common species: American, German, and Oriental. Assays, using allergen-specific monoclonal antibodies, showed up to 200-fold differences in Bla g 1 levels in six commercial extracts, ranging from 4.7 to 1085 units/mL (U/mL), whereas only two extracts contained detectable Bla g 2 (248 and 324 U/mL) (26). Serological studies suggest that a cocktail of *B. germanica* allergens, Bla g 1, Bla g 2, Bla g 4, and Bla g 5, would diagnose 95% of U.S. patients with cockroach allergy (93). The use of recombinant allergens that can be produced as pure solutions, using in vitro expression systems, should allow diagnosis of sensitization to specific allergens in the future.

Measurement of cockroach allergen exposure may allow prediction of sensitization. As with other indoor aeroallergens, airborne particles carrying allergens cannot be readily identified or counted. There is no equivalent of a pollen count. Counting numbers of cockroaches and mites may be a reasonable guide to the quantity of allergen; however, the best measurements are obtained using immunochemical assays of major allergens in extracts of dust collected from natural sources. Emergency room studies show that individuals with a

positive RAST to cockroach of >40 U/mL have Bla g 2 levels of >2 U/g in house-dust samples. As mentioned in section Public Health Importance of Cockroaches, current evidence suggests that >2 U/g Bla g 2 or Bla g 1 is the "threshold" allergen level for cockroach allergen sensitization (40,94). The risk levels for asthmatic symptoms are 8 U/g Bla g 1 (6).

Immunotherapy

Allergen immunotherapy is effective for patients with insect sting hypersensitivity. However, at present, cockroach immunotherapy is not considered efficacious (93). In a single study, allergen immunotherapy using cockroach vaccines in sensitive individuals decreased symptom scores and medication requirements. It also increased specific IgG levels and decreased basophil histamine release in response to cockroach antigen (95). The use of standardized cockroach extracts could improve cockroach immunotherapy efficacy. However, the U.S. FDA has reported variability of commercially available cockroach allergen extracts in protein content, electrophoretic banding patterns, relative potency, and Bla g 2 levels (96). Efforts to standardize cockroach extracts have been reported and will be necessary to properly develop effective immunotherapy for cockroach allergy (97,98).

As with any other allergy, cockroach allergy therapy should be based on three approaches: (*i*) environmental control (avoidance) (section Environmental Control), (*ii*) pharmacotherapy, and (*iii*) immunotherapy with the appropriate allergens. The use of recombinant cockroach allergens is envisioned as a way to improve therapy of cockroach hypersensitivity. Benefits include better control of batch-to-batch variability and the assurance of representation of minor allergens in standard amounts. Additionally, immunotherapy with specific hypoallergenic recombinant allergens or peptides lacking IgE-binding epitopes rather than crude allergen vaccine mixtures could prove to be a more effective regimen to avoid anaphylactic reactions. Specific immunotherapy with recombinant cockroach allergens, such as performed with cat and mite allergens, has yet to be performed.

ENVIRONMENTAL CONTROL

Advances in integrated pest management include preventing or minimizing populations within structures. Manipulations of microclimates in discrete areas of new homes can and does reduce infestation. Methods include incorporation of nontoxic repellents in structures to deny access, such as beneath sinks in kitchens and bathrooms. As in any management scheme, environmental control and reduction in allergen levels are the main objectives for asthma-related illness management. Additionally, monitoring allergen levels by individuals in their own homes should improve their understanding of the role of allergens in asthma and improve compliance with future avoidance measures.

For most inhalant allergens, the actual amount of allergen inhaled during natural exposures is low, but the inhaled particles can have very high allergen content. Thus, for environmental control, it is mandatory, not only to remove cockroaches but to remove dust-containing particles carrying the allergen. Control measures should include removal of food and water sources from the natural habitat areas. Increased airflow, maintenance of dryness, and removal of any potential food sources will facilitate environmental control in kitchen cabinets, under sinks and kitchen floors where high concentrations of cockroach allergen are found.

Although these recommendations are sound for some dwellings, heavily infested homes and buildings that contain multiple apartments will be more difficult to control. Re-infestation from neighboring apartments, inadequate eradication and prevention measures, and poor construction and sanitation are sometimes obstacles that may be difficult to control. A significant problem is the determination of allergen levels. Assays currently being used must be standardized to define the relationship between different cockroach species and other cross-reacting allergen sources.

Cockroaches can be controlled by using a variety of chemicals, including organophosphates, carbamates, and botanicals, such as pyrethrins and pyrethroids, which disrupt

the insect's nervous system, causing locomotion and respiratory failure. Other materials, including wettable powders, emulsified concentrates, aerosols and baits, have been added to the pesticide management of these pests. Ingestion of boric acid leads to damage of the epithelial cells in the gut, precluding nutrient absorption that leads to subsequent starvation. Newer formulations containing active ingredients that interfere with metabolic activity and growth regulation are being used as baits for foraging cockroaches. Although currently available pesticide use (abamectin, hydramethylnon) can reduce populations by 93% to 100%, cockroach allergen found in feces, cast skins, and body parts remain in accumulated dust. Sarpong et al., using several rooms in a college dormitory as a model for home extermination studies, showed that Bla g 2 allergen levels of dust of 5.2 U/g could be reduced to 0.95 U/g following an extermination regimen and regular vacuuming (99). Cockroach control alone can significantly reduce cockroach allergens in infested homes (100). However, sustained decrease of cockroach allergens is difficult to achieve, even after successful extermination of cockroach populations, and the levels may remain above those reported to be clinically significant (reviewed in Ref. 93). Pesticide treatment should be rotated to reduce the risk of resistant strains, and careful cleaning and maintenance are essential to remove and/or reduce the allergen load. Despite limited evidence, reduced exposure to cockroach allergens in infested structures could lead to improvements in asthma morbidity among cockroach-sensitized patients (reviewed in Refs. 101 and 102).

The current recommendations for cockroach control include both physical and chemical measures. Table 5 identifies several cockroach control techniques. Approaches of biological control have been tried or are under development, such as the use of the hymenopter parasite of the ootheca, *Comperia merceti*, against *Supella longipalpa*, or the potential use of a *P. fuliginosa* densovirus as a biopesticide (103). However, these biological approaches to environmental control are still far from being commercially developed. An integrated pest management strategy consisting of sanitation, landscape management, and a perimeter insecticide treatment applied according to label directions is the best control measure possible. Although extensive measures are available to control cockroach populations, neither control procedures for reducing allergen levels nor the extent of cockroach allergen stability and allergen persistence in the environment following cockroach eradication measures are known.

Table 5 Cockroach Control Measures

I. Physical measures
  A. Reduce access to food
    1. Store food in sealed containers
    2. Eliminate sources of organic debris
  B. Reduce access to water
    1. Repair leaking faucets
    2. Wrap pipes to prevent condensation
    3. Eliminate damp areas beneath sinks
    4. Repair damp, damaged wood
  C. Improve ventilation by eliminating clutter beneath sinks
  D. Eliminate hiding places and access points
    1. Caulk and seal cracks and crevices in foundations
    2. Caulk around water pipes entry into house and beneath sinks
    3. Eliminate clutter within household (e.g., remove all newspaper and magazine storage areas)

II. Chemical measures

Aerosol sprays of organophosphates like chlorpyrifos were banned (i.e. pesticide diazinon was banned on June 8, 2000; EPA banned indoor use in December 2002)

  1. Pyrethrum or pyrethroids
  2. Boric acid powders and baits
  3. Orange guard (D-limonene)
  4. Bait stations
    a. Hydramethylnon (Combat)
    b. Abamectin (Roach Ender)
    c. Fipronil (Maxforce Roach Bait Station)
    d. Roach Free™ System (food source with boric acid)
    e. Baits with other active ingredients (sulfuramid, xanthines, oxypurinol)

SALIENT POINTS

- Sensitization to indoor inhalant allergens is strongly associated with the development of asthma. In urban and inner-city areas, up to 80% of children with asthma may have IgE antibody to cockroach allergens.
- Infestations of domiciliary cockroaches are largely dependent on housing conditions. The average American spends approximately 95% of the time indoors in controlled environments that lead to continued low-dose allergen exposure, which may lead to sensitization in predisposed individuals.
- Amorphous cockroach particles containing allergens are recognized as an important source of indoor allergens, together with dust mite particles.
- Cross-reactivity of arthropod allergens can be identified among members of the taxonomic groups Crustacea, Arachnida, and Insecta, described as "pan allergy."
- The cloning of several cockroach and other insect allergens has been accomplished using molecular biology techniques. These studies offer the basis for investigating the relationship between allergen function/structure and allergenicity.
- The X-ray crystal structure of Bla g 2 reveals features that contribute to allergenicity. The unusual structure of the area corresponding to the catalytic site explain why this potent allergen does not have standard aspartic protease activity, which is not necessary for allergenicity.
- Recombinant cockroach allergens that retain IgE antibody–binding capacity are new tools that can be used in the future to improve the diagnosis and treatment of cockroach hypersensitivity.
- Environmental control of cockroach and other insect infestations is essential to control inhalant insect-allergic diseases.
- The composition of environmental dust includes a wide range of components from the biosystem and, given the widespread distribution of insects in the world, their involvement in allergic reactions will continue to be of major social, economic, and medical importance.
- Future directions for research should include the study of cockroach reduction strategies, development of specific assays to detect clinically relevant insect inhalant allergens, measures to reduce exposure to environmental allergens, including patient education for pest management and the safe use of insecticides and nontoxic traps, and the study of the mechanisms of cockroach allergy.

ACKNOWLEDGMENTS

Thanks to Dr. Ricki M. Helm for his contributions to cockroach allergy and to the previous versions that evolved into the present chapter. Part of the research described in this chapter was supported by Indoor Biotechnologies, Inc.

REFERENCES

1. Bernton HS, Brown H. Insect allergy—Preliminary studies of the cockroach. J Allergy Clin Immunol 1964; 35:506–513.
2. Barletta B, Pini C. Does occupational exposure to insects lead to species-specific sensitization? Allergy 2003; 58(9):868–870.
3. Pollart SM, Chapman MD, Fiocco GP, et al. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83(5):875–882.
4. Arruda LK, Vailes LD, Ferriani VP, et al. Cockroach allergens and asthma. J Allergy Clin Immunol 2001; 107(3):419–428.
5. Huss K, Adkinson NF Jr., Eggleston PA, et al. House dust mite and cockroach exposure are strong risk factors for positive allergy skin test responses in the Childhood Asthma Management Program. J Allergy Clin Immunol 2001; 107(1):48–54.
6. Rosenstreich DL, Eggleston P, Kattan M, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997; 336(19):1356–1363.

7. Gruchalla RS, Pongracic J, Plaut M, et al. Inner City Asthma Study: relationships among sensitivity, allergen exposure, and asthma morbidity. J Allergy Clin Immunol 2005; 115(3):478–485.
8. Grandcolas Ph. Domestic and non-domestic cockroaches: facts versus received ideas. Rev Fr Allergol 1998; 38(10):833–838.
9. Atkinson TH, Koehler PG, Paterson RS. Catalog and atlas of the cockroaches (*Dictyoptera*) of North America and Mexico. Misc Pub Entomol Soc Am 1991; 78:1–86.
10. Koehler PG, Patterson RS, Brenner RJ. Cockroaches. In: Storey K, ed. Handbook of Pest Control: The Behavior, Life, History and Control of Household Pests. 7th ed. Cleveland: Franzak & Foster Co., 1995:100–175.
11. Bernton HS, Brown H. Cockroach allergy II: the relation of infestation to sensitization. South Med J 1967; 60(8):852–855.
12. Kang B, Sulit N. A comparative study of prevalence of skin hypersensitivity to cockroach and house dust antigens. Ann Allergy 1978; 41(6):333–336.
13. Chew GL, Higgins KM, Gold DR, et al. Monthly measurements of indoor allergens and the influence of housing type in a northeastern US city. Allergy 1999; 54(10):1058–1066.
14. Cohn RD, Arbes SJ Jr., Jaramillo R, et al. National prevalence and exposure risk for cockroach allergen in U.S. households. Environ Health Perspect 2006; 114(4):522–526.
15. Kang B, Jones J, Johnson J, et al. Analysis of indoor environment and atopic allergy in urban populations with bronchial asthma. Ann Allergy 1989; 62(1):30–34.
16. Gelber LE, Seltzer LH, Bouzoukis JK, et al. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. Am Rev Respir Dis 1993; 147(3):573–578.
17. Richman PG, Khan HA, Turkeltaub PC, et al. The important sources of German cockroach allergens as determined by RAST analyses. J Allergy Clin Immunol 1984; 73(5 pt 1):590–595.
18. Lehrer SB, Horner WE, Menon P, et al. Comparison of cockroach allergenic activity in whole body and fecal extracts. J Allergy Clin Immunol 1991; 87(2):574–580.
19. Musmand JJ, Horner WE, López M, et al. Identification of important allergens in German cockroach extracts by sodium dodecylsulfate-polyacrylamide gel electrophoresis and western blot analysis. J Allergy Clin Immunol 1995; 95(4):877–885.
20. Twarog FJ, Picone FJ, Strunk RS, et al. Immediate hypersensitivity to cockroach. Isolation and purification of the major antigens. J Allergy Clin Immunol 1977; 59(2):154–160.
21. Helm RM, Bandele EO, Swanson MC, et al. Identification of a German cockroach-specific allergen by human IgE and rabbit IgG. Int Arch Allergy Appl Immunol 1988; 87(3):230–238.
22. Lan JL, Lee DT, Wu CH, et al. Cockroach hypersensitivity: preliminary study of allergic cockroach asthma in Taiwan. J Allergy Clin Immunol 1988; 82(5 pt 1):736–740.
23. Wu CH, Chiang BT, Fann MC, et al. Production and characterization of monoclonal antibodies against major allergens of American cockroach. Clin Exp Allergy 1990; 20(6):675–681.
24. Jeng KC, Liu MT, Wu CH, et al. American cockroach Cr-PI allergen induces lymphocyte proliferation and cytokine production in atopic patients. Clin Exp Allergy 1996; 26(3):349–356.
25. Zwick H, Popp W, Sertl K, et al. Allergenic structures in cockroach hypersensitivity. J Allergy Clin Immunol 1991; 87(3):626–630.
26. Pollart SM, Mullins DE, Vailes LD, et al. Identification, quantitation, and purification of cockroach allergens using monoclonal antibodies. J Allergy Clin Immunol 1991; 87(2):511–521.
27. Pollart SM, Smith TF, Morris EC, et al. Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. J Allergy Clin Immunol 1991; 87(2):505–510.
28. Schou C, Fernández-Cladas E, Lockey RF, et al. Environmental assay for cockroach allergens. J Allergy Clin Immunol 1991; 87(4):828–834.
29. Wu CH, Hsieh MJ, Huang JH, et al. Identification of low molecular weight allergens of American cockroach and production of monoclonal antibodies. Ann Allergy Asthma Immunol 1996; 76(2):195–203.
30. Helm R, Cockrell G, Stanley JS, et al. Isolation and characterization of a clone encoding a major allergen (Bla g Bd90K) involved in IgE-mediated cockroach hypersensitivity. J Allergy Clin Immunol 1996; 98(1):172–180.
31. Pomés A, Melén E, Vailes LD, et al. Novel allergen structures with tandem amino acid repeats derived from German and American cockroach. J Biol Chem 1998; 273(46):30801–30807.
32. Melén E, Pomés A, Vailes LD, et al. Molecular cloning of Per a 1 and definition of the cross-reactive Group 1 cockroach allergens. J Allergy Clin Immunol 1999; 103(5 pt 1):859–864.
33. Wu CH, Wang NM, Lee MF, et al. Cloning of the American cockroach Cr-PII allergens: evidence for the existence of cross-reactive allergens between species. J Allergy Clin Immunol 1998; 101(6 pt 1): 832–840.
34. Gore JC, Schal C. Gene expression and tissue distribution of the major human allergen Bla g 1 in the German cockroach, *Blattella germanica* L. (Dictyoptera: Blattellidae). J Med Entomol 2004; 41(5):953–960.
35. Gore JC, Schal C. Expression, production and excretion of Bla g 1, a major human allergen, in relation to food intake in the German cockroach, *Blattella germanica*. Med Vet Entomol 2005; 19(2): 127–134.

36. Satinover SM, Reefer AJ, Pomés A, et al. Specific IgE and IgG antibody-binding patterns to recombinant cockroach allergens. J Allergy Clin Immunol 2005; 115(4):803–809.
37. Arruda LK, Vailes LD, Mann BJ, et al. Molecular cloning of a major cockroach (*Blattella germanica*) allergen, Bla g 2. Sequence homology to the aspartic proteases. J Biol Chem 1995; 270(33):19563–19568.
38. Pomés A, Smith AM, Grégoire C, et al. Functional properties of cloned allergens from dust mite, cockroach and cat - are they relevant to allergenicity? Allergy Clin Immunol Int 2001; 13:162–169.
39. Gustchina A, Li M, Wünschmann S, et al. Crystal structure of cockroach allergen Bla g 2, an unusual zinc binding aspartic protease with a novel mode of self-inhibition. J Mol Biol 2005; 348(2):433–444.
40. Sporik R, Squillace SP, Ingram JM, et al. Mite, cat, and cockroach exposure, allergen sensitisation, and asthma in children: a case-control study of three schools. Thorax 1999; 54(8):675–680.
41. Pomés A, Chapman MD, Vailes LD, et al. Cockroach allergen Bla g 2: structure, function, and implications for allergic sensitization. Am J Respir Crit Care Med 2002; 165(3):391–397.
42. Wünschmann S, Gustchina A, Chapman MD, et al. Cockroach allergen Bla g 2: an unusual aspartic proteinase. J Allergy Clin Immunol 2005; 116(1):140–145.
43. Pomés A. Intrinsic properties of allergens and environmental exposure as determinants of allergenicity. Allergy 2002; 57(8):673–679.
44. Arruda LK, Vailes LD, Hayden ML, et al. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses. J Biol Chem 1995; 270(52):31196–31201.
45. Paddock CD, McKerrow JH, Hansell E, et al. Identification, cloning, and recombinant expression of procalin, a major triatomine allergen. J Immunol 2001; 167(5):2694–2699.
46. Smith W, Butler AJ, Hazell LA, et al. Fel d 4, a cat lipocalin allergen. Clin Exp Allergy 2004; 34(11): 1732–1738.
47. Flower DR. The lipocalin protein family: structure and function. Biochem J 1996; 318(pt 1):1–14.
48. Fan Y, Gore JC, Redding KO, et al. Tissue localization and regulation by juvenile hormone of human allergen Bla g 4 from the German cockroach, *Blattella germanica* (L.). Insect Mol Biol 2005; 14(1):45–53.
49. Arruda LK, Vailes LD, Benjamin DC, et al. Molecular cloning of German cockroach (*Blattella germanica*) allergens. Int Arch Allergy Immunol 1995; 107(1–3):295–297.
50. Arruda LK, Vailes LD, Platts-Mills TA, et al. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (*Blattella germanica*). J Biol Chem 1997; 272(33):20907–20912.
51. Hindley J, Wünschmann S, Satinover SM, et al. Bla g 6: a troponin C allergen from *Blattella germanica* with IgE binding calcium dependence. J Allergy Clin Immunol 2006; 117(6):1389–1395.
52. Asturias JA, Gómez-Bayon N, Arilla MC, et al. Molecular characterization of American cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. J Immunol 1999; 162(7): 4342–4348.
53. Santos AB, Chapman MD, Aalberse RC, et al. Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. J Allergy Clin Immunol 1999; 104(2 pt 1):329–337.
54. Jeong KY, Lee J, Lee IY, et al. Allergenicity of recombinant Bla g 7, German cockroach tropomyosin. Allergy 2003; 58(10):1059–1063.
55. Smillie LB. Structure and functions of tropomyosins from muscle and non-muscle sources. Trends Biochem Sci 1979; 4:151–155.
56. Wu CH, Lee MF, Liao SC, et al. Sequencing analysis of cDNA clones encoding the American cockroach Cr-PI allergens. Homology with insect hemolymph proteins. J Biol Chem 1996; 271 (30):17937–17943.
57. Wu CH, Lee MF, Wang NM, et al. Sequencing and immunochemical characterization of the American cockroach per a 3 (Cr-PI) isoallergenic variants. Mol Immunol 1997; 34(1):1–8.
58. Baur X, Liebers V, Mazur G, et al. Immunological cross-reactivity of hemoglobins in the Diptera family Chironomidae. Allergy 1991; 46(6):445–451.
59. Trudeau WL, Fernández-Caldas E, Fox RW, et al. Allergenicity of the cat flea (*Ctenocephalides felis felis*). Clin Exp Allergy 1993; 23:377–383.
60. Albright DD, Jordan-Wagner D, Napoli DC, et al. Multicolored Asian lady beetle hypersensitivity: a case series and allergist survey. Ann Allergy Asthma Immunol 2006; 97(4):521–527.
61. Nakazawa T, Satinover SM, Naccara L, et al. Asian ladybugs (Harmonia axyridis): a new seasonal indoor allergen. J Allergy Clin Immunol 2007; 119(2):421–427.
62. Knuffman JE, Opsahl MA, Gern BH, et al. Identification of an allergen of *Harmonia axyridis* (multicolor Asian lady beetle). J Allergy Clin Immunol 2007; 119(1):S106.
63. Helm RM, Squillace DL, Jones RT, et al. Shared allergenic activity in Asian (*Blattella asahinai*), German (*Blattella germanica*), American (*Periplaneta americana*), and Oriental (*Blatta orientalis*) cockroach species. Int Arch Allergy Appl Immunol 1990; 92(2):154–161.
64. Schou C, Lind P, Fernández-Caldas E, et al. Identification and purification of an important cross-reactive allergen from American (*Periplaneta americana*) and German (*Blattella germanica*) cockroach. J Allergy Clin Immunol 1990; 86(6 pt 1):935–946.

65. Stankus RP, Horner WE, Lehrer SB. Identification and characterization of important cockroach allergens. J Allergy Clin Immunol 1990; 86(5):781–787.
66. Wu CH, Luo SF, Wong DW. Analysis of cross-reactive allergens from American and German cockroaches by human IgE. Allergy 1997; 52(4):411–416.
67. Menon P, Menon V, Hilman B, et al. Skin test reactivity to whole body and fecal extracts of American (*Periplaneta americana*) and German (Blatella germanica) cockroaches in atopic asthmatics. Ann Allergy 1991; 67(6):573–577.
68. Chaudhry S, Jhamb S, Chauhan UP, et al. Shared and specific allergenic and antigenic components in the two sexes of American cockroach-*Periplaneta americana*. Clin Exp Allergy 1990; 20(1):59–65.
69. Morgan MS, Arlian LG, Bernstein JA, et al. Allergenicity of the Madagascar hissing cockroach. Ann Allergy Asthma Immunol 2007; 98(3):258–261.
70. Pomés A, Vailes LD, Helm RM, et al. IgE reactivity of tandem repeats derived from cockroach allergen, Bla g 1. Eur J Biochem 2002; 269(12):3086–3092.
71. Jeong KY, Hwang H, Lee J, et al. Allergenic characterization of tropomyosin from the dusky brown cockroach, *Periplaneta fuliginosa*. Clin Diagn Lab Immunol 2004; 11(4):680–685.
72. Sookrung N, Chaicumpa W, Tungtrongchitr A, et al. *Periplaneta americana* arginine kinase as a major cockroach allergen among Thai patients with major cockroach allergies. Environ Health Perspect 2006; 114(6):875–880.
73. Witteman AM, Van den OS, Van LJ, et al. IgE antibodies reactive with silverfish, cockroach and chironomid are frequently found in mite-positive allergic patients. Int Arch Allergy Immunol 1995; 108(2):165–169.
74. Witteman AM, Akkerdaas JH, Van LJ, et al. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. Int Arch Allergy Immunol 1994; 105(1):56–61.
75. Leung PS, Chow WK, Duffey S, et al. IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. J Allergy Clin Immunol 1996; 98(5 pt 1): 954–961.
76. Ayuso R, Reese G, Leong-Kee S, et al. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Int Arch Allergy Immunol 2002; 129(1):38–48.
77. Reese G, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate pan-allergen. Int Arch Allergy Immunol 1999; 119(4):247–258.
78. Crespo JF, Pascual C, Helm R, et al. Cross-reactivity of IgE-binding components between boiled Atlantic shrimp and German cockroach. Allergy 1995; 50(11):918–924.
79. Barletta B, Butteroni C, Puggioni EM, et al. Immunological characterization of a recombinant tropomyosin from a new indoor source, *Lepisma saccharina*. Clin Exp Allergy 2005; 35(4):483–489.
80. van Ree R, Antonicelli L, Akkerdaas JH, et al. Possible induction of food allergy during mite immunotherapy. Allergy 1996; 51(2):108–113.
81. Fernandes J, Reshef A, Patton L, et al. Immunoglobulin E antibody reactivity to the major shrimp allergen, tropomyosin, in unexposed Orthodox Jews. Clin Exp Allergy 2003; 33(7):956–961.
82. Koshte VL, Kagen SL, Aalberse RC. Cross-reactivity of IgE antibodies to caddis fly with arthropoda and mollusca. J Allergy Clin Immunol 1989; 84(2):174–183.
83. van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. Int Arch Allergy Immunol 2002; 129(3):189–197.
84. Baldo BA, Panzani RC. Detection of IgE antibodies to a wide range of insect species in subjects with suspected inhalant allergies to insects. Int Arch Allergy Appl Immunol 1988; 85(3):278–287.
85. Yu CJ, Lin YF, Chiang BL, et al. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. J Immunol 2003; 170(1):445–453.
86. Binder M, Mahler V, Hayek B, et al. Molecular and immunological characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate pan-allergen. J Immunol 2001; 167(9):5470–5477.
87. Huang CH, Liew LM, Mah KW, et al. Characterization of glutathione S-transferase from dust mite, Der p 8 and its immunoglobulin E cross-reactivity with cockroach glutathione S-transferase. Clin Exp Allergy 2006; 36(3):369–376.
88. Antony AB, Tepper RS, Mohammed KA. Cockroach extract antigen increases bronchial airway epithelial permeability. J Allergy Clin Immunol 2002; 110(4):589–595.
89. Bhat RK, Page K, Tan A, et al. German cockroach extract increases bronchial epithelial cell interleukin-8 expression. Clin Exp Allergy 2003; 33(1):35–42.
90. Kondo S, Helin H, Shichijo M, et al. Cockroach allergen extract stimulates protease-activated receptor-2 (PAR-2) expressed in mouse lung fibroblast. Inflamm Res 2004; 53(9):489–496.
91. Knight DA, Lim S, Scaffidi AK, et al. Protease-activated receptors in human airways: upregulation of PAR-2 in respiratory epithelium from patients with asthma. J Allergy Clin Immunol 2001; 108(5): 797–803.

92. Rullo VE, Rizzo MC, Arruda LK, et al. Daycare centers and schools as sources of exposure to mites, cockroach, and endotoxin in the city of Sao Paulo, Brazil. J Allergy Clin Immunol 2002; 110(4):582–588.
93. Arruda LK, Ferriani VP, Vailes LD, et al. Cockroach allergens: environmental distribution and relationship to disease. Curr Allergy Asthma Rep 2001; 1(5):466–473.
94. Eggleston PA, Rosenstreich D, Lynn H, et al. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. J Allergy Clin Immunol 1998; 102(4 pt 1):563–570.
95. Kang BC, Johnson J, Morgan C, et al. The role of immunotherapy in cockroach asthma. J Asthma 1988; 25(4):205–218.
96. Patterson ML, Slater JE. Characterization and comparison of commercially available German and American cockroach allergen extracts. Clin Exp Allergy 2002; 32(5):721–727.
97. Slater JE, James R, Pongracic JA, et al. Biological potency of German cockroach allergen extracts determined in an inner city population. Clin Exp Allergy 2007; 37(7):1033–1039.
98. Thangam Sudha V, Arora N, Sridhara S, et al. Biopotency and identification of allergenic proteins in *Periplaneta americana* extract for clinical applications. Biologicals 2007; 35(2):131–137.
99. Sarpong SB, Wood RA, Eggleston PA. Short-term effects of extermination and cleaning on cockroach allergen Bla g 2 in settled dust. Ann Allergy Asthma Immunol 1996; 76(3):257–260.
100. Arbes SJ Jr, Sever M, Mehta J, et al. Abatement of cockroach allergens (Bla g 1 and Bla g 2) in low-income, urban housing: month 12 continuation results. J Allergy Clin Immunol 2004; 113(1):109–114.
101. Gore JC, Schal C. Cockroach allergen biology and mitigation in the indoor environment. Annu Rev Entomol 2007; 52:439–463.
102. Morgan WJ, Crain EF, Gruchalla RS, et al. Inner-City Asthma Study Group. Results of a home-based environmental intervention among urban children with asthma. N Engl J Med 2004; 351(11):1068–1080.
103. Jiang H, Zhang JM, Wang JP, et al. Genetic engineering of *Periplaneta fuliginosa* densovirus as an improved biopesticide. Arch Virol 2007; 152(2):383–394.
104. Yang CY, Wu JD, Wu CH. Sequence analysis of the first complete cDNA clone encoding an American cockroach Per a 1 allergen. Biochim Biophys Acta 2000; 1517(1):153–158.
105. Wang NM, Lee MF, Wu CH. Immunologic characterization of a recombinant American cockroach (*Periplaneta americana*) Per a 1 (Cr-PII) allergen. Allergy 1999; 54(2):119–127.
106. Schwede T, Kopp J, Guex N, et al. SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res 2003; 31(13):3381–3385.
107. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 1997; 18(15):2714–2723.
108. Peitsch MC. Protein modeling by e-mail. Bio/Technology 1995; 13:658–660.

12 Mammalian Allergens

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INTRODUCTION

People come into contact with animals in many different occupations and activities. Household animals are significant sources of allergens in the indoor environment. Cat and dog allergens are especially recognized as being associated with allergic disorders, including asthma. Exposure to these allergens is perennial and not limited to immediate contact with the animals. It is not surprising that sensitization is common, since people in industrialized countries spend some 90% of their time indoors.

Almost all important mammalian respiratory allergens belong to the lipocalin family of proteins, with the exception of cat allergen, Fel d 1 (1). This naturally raises the question whether lipocalin allergens have intrinsic properties that explain this phenomenon (2). However, the allergenicity of lipocalin allergens cannot be explained by simple physicochemical characteristics.

TAXONOMY OF MAMMALS

Figure 1 shows the condensed taxonomy of eight mammals emitting allergens identified at the sequence level. Sensitization also occurs to other members of the order Cetartiodactyla (e.g., reindeer, *Rangifer tarandus*, family Cervidae, and pig, *Sus scrofa domestica*, family Suidae). Several members of the family Felidae, in addition to the house cat, are also possible sources of allergens. Their hair extracts contain allergens similar to and immunoglobulin E (IgE) cross-reactive with the house cat allergen, Fel d 1. Although mouse, rat and guinea pig are the only rodents included in the current list of allergens (http://www.allergen.org, July 25, 2007), e.g., hamsters (family Muridae) are known to be significant causes of allergy both in home and in occupational environments (Table 1).

HUMAN CONTACT WITH OTHER MAMMALS

People come into direct contact with mammalian animals in many ways. Household pets, especially cats and dogs, are found in many (30–50%) homes in industrialized countries. Consequently, high levels of Can f 1 or Fel d 1 allergens occur in the homes of dog and cat owners, although variations of several orders of magnitude between houses are observed.

The effects of the exposure depend on a complex array of environmental and genetic factors. In this context, it is of interest to note that high levels of exposure to animal allergens may lead to tolerance. The highest levels of sensitization in children exposed to Fel d 1 are observed with "intermediate" levels of the allergen (1.7–23.0 μg/g of dust) (3). This suggests that the dose-response relationship between exposure to cat and sensitization could have a bell-shaped curve. The protective effect is hypothesized to be due to a modified T-helper type 2 (Th2) response involving the synthesis of specific IgG4. In an experimental human study of nasal neoantigen exposure, specific IgE demonstrated a bell-shaped dose response, while the specific IgG response went up with the increasing exposure (4). There are also studies that provide no evidence for the protective effect of high exposure to mammalian allergens. Several factors, such as the age of the subjects or the nature of the exposure, may account for these conflicting results.

High exposure to mites does not protect against sensitization. It can be speculated that allergens of different origin have distinct optimal concentrations that favor tolerance; perhaps, this level for mite allergens is very high. On the other hand, it has been found that the level of

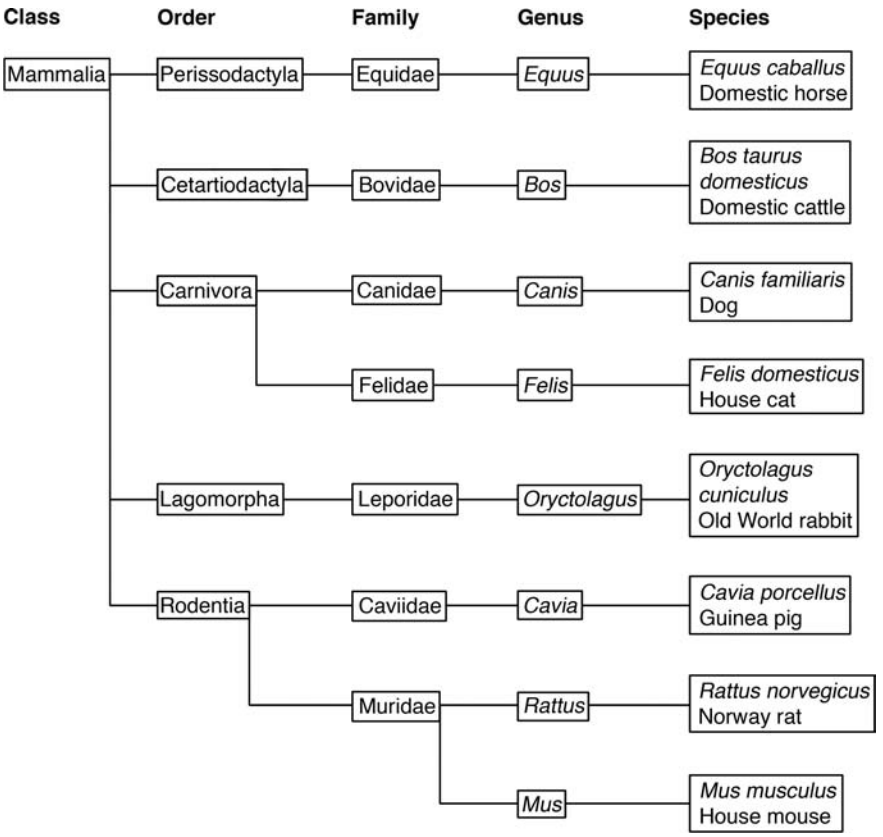

Figure 1 Condensed presentation of the taxonomical location of eight mammals emitting allergens.

Table 1 Internet Resources Referred to in the Text

Description	Web site address
Lipocalin family, documentation in the PROSITE protein database	http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00187
Lipocalins, general information and the list of lipocalins in the PROSITE protein database	http://www.expasy.org/cgi-bin/nicesite.pl?PS00213
List of allergens by Allergen Nomenclature Sub-Committee of International Union of Immunological Societies	http://www.allergen.org
PDB, a databank for three-dimensional biological macromolecular structure data	http://www.rcsb.org/pdb/
Serum albumin family, documentation in the PROSITE protein database	http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00186
SWISS-PROT protein database	http://www.expasy.org/sprot/

Abbreviation: PDB, Protein Data Bank.

exposure to mite allergens is essentially lower than that to cat allergens (5,6). Therefore, both high (or "intermediate")-dose (cat) and low-dose (mite) allergen exposures seem to induce sensitization. One possibility is that both types of exposures result in a suboptimal stimulation of specific T-helper cells (see section "Lipocalins"). With cat allergens, suboptimal stimulation would be due to the homology with human proteins (evolutionary relatedness), whereas with mite allergens, it would be due to the low concentration of the allergen (low epitope density on antigen-presenting cells). Other factors, such as the enzyme activity of mite Der p 1 or factors not directly related to allergenic molecules themselves, such as associated substances with adjuvant properties, are probably implicated in the sensitization to cat and mite allergens. For example, it appears that pathogen-associated molecular patterns (PAMPs), such as endotoxin,

are responsible for the apparent beneficial effect of the farming environment against sensitization (7).

Exposure to pet allergens is not limited only to direct contact. Dog and cat allergens stick to clothing, and they are consistently found in homes without pets as well as in public buildings, including schools and day care centers and public transport vehicles. The concentrations are low, but may be high enough to cause sensitization and symptoms in sensitized persons.

Mice, hamsters, guinea pigs, and gerbils are also popular household pets, and handling the pets and cleaning their cages expose their owners to allergens. The presence of rodent allergens in the home depends not only on the presence of pets but also on the living conditions: mouse Mus m 1 and rat Rat n 1 are detectable in apartments infested with these animals. In U.S. studies, approximately 20% of inner-city children with asthma appear to be sensitized to mouse and rat allergens. Keeping guinea pigs as pets is associated with a more than a threefold increased risk of atopic eczema, an effect not seen with other pets such as cats, dogs, or hamsters. Horse allergy is not a very common health problem, but horseback riding as a hobby causes sensitization and clinical illness.

Sensitization associated with the handling of laboratory animals is a worldwide occupational problem. The exposure occurs through the respiratory tract and conjunctiva and by skin contact. One review of seven studies found that 15.6% of workers in laboratory animal facilities had work-related symptoms and 22.5% were skin prick test–positive for animal allergens (8). The common laboratory animals (mouse, rat, guinea pig, hamster, rabbit, and dog) appear to be equal sensitizers. The level of exposure varies according to the task concerned. The highest concentrations of airborne allergens are encountered during the emptying and cleaning of the cages. However, several factors in exposure to laboratory animal allergens are incompletely understood, for example, whether mean or peak exposures are the most relevant for sensitization (9). The highest prevalence of sensitization to laboratory animals is generally found in subjects with moderate exposure.

An example of work-related allergy caused by domestic animals is occupational asthma in Finnish dairy farmers. An interesting feature is the prolonged exposure time (median, 22 years) before cattle asthma becomes clinically evident. In contrast, symptoms of laboratory animal allergy appear within two to three years of exposure in 70% to 80% of cases.

MOLECULAR CHARACTERISTICS OF MAMMALIAN ALLERGENS

Protein Families of Mammalian Allergens

Lipocalins

Lipocalins are a large protein group (http://www.expasy.org/cgi-bin/nicesite.pl?PS00213) comprising proteins from vertebrate and invertebrate animals, plants, and bacteria. In addition to mammalian respiratory allergens, a milk allergen, Bos d 5 (β-lactoglobulin), a cockroach allergen, Bla g 4, a "kissing bug" (*Triatoma protracta)* allergen, Tria p 1, and a pigeon tick (*Argas reflexus*) allergen, Arg r 1, belong to the lipocalin group. Together, with fatty acid–binding proteins, avidins, a group of metalloproteinase inhibitors, and triabin, lipocalins form the calycin superfamily (10). A protein should fulfill the requirements for sequence homology, biological function, and structural similarity to be included in the family (http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00187).

Although the overall amino acid identity between lipocalins is usually below 20%, they contain one to three characteristic conserved sequence motifs [structurally conserved regions (SCR)] (10). The first motif, containing the triplet glysine-x-tryptophane, is present in all lipocalins (Fig. 2). While kernel lipocalins contain all three motifs, outlier lipocalins contain only one or two. In some cases, the sequential identity over animal species can be well above 20%. For example, dog Can f 1 exhibits a 57% identity with human tear lipocalin (von Ebner's gland protein), and human epididymal-specific lipocalin-9 exhibits a 40% to 50% identity with rodent lipocalins [the SIB BLAST network service (SBNS) at the Swiss Institute of Bioinformatics, Jul. 25, 2007 (11)]. Lipocalins exist as both monomers and dimers, and they can be either glycosylated or nonglycosylated (Table 2).

Despite the low sequential identity, lipocalins share a common three-dimensional structure (Fig. 3) (10). The central β-barrel of lipocalins is composed of eight antiparallel

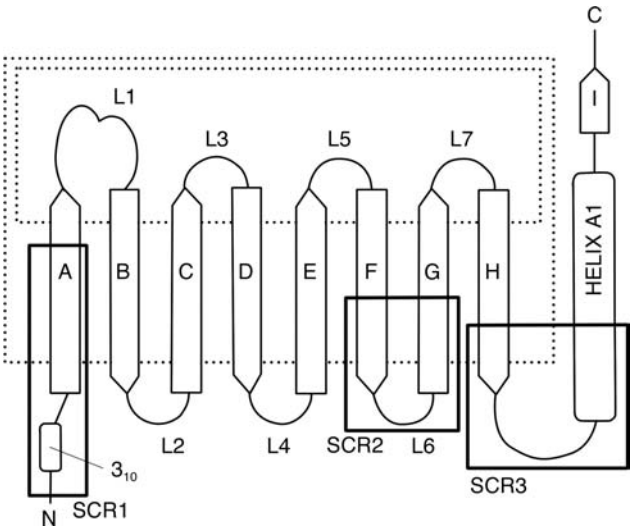

Figure 2 Schematic structure of the lipocalin fold. The nine β-strands of the antiparallel β-sheet are shown as arrows and labeled A-I. The C-terminal α-helix A1 and N-terminal 3_{10}-like helix are also marked. Connecting loops are shown as solid lines and labeled L1–L7. A pair of dotted lines indicates the hydrogen-bonded connection of two strands. One end of the lipocalin β-barrel has four loops (L1, L3, L5, and L7); the opening of the internal ligand-binding site is here, and so it is called the open end of the molecule. The other end has three β-hairpin loops (L2, L4, and L6); the N-terminal polypeptide chain crosses this end of the barrel to enter strand A via a conserved 3_{10} helix, closing this end of the barrel, the closed end of the molecule. Those parts that form the three main structurally, and sequence, conserved regions (SCRs) of the fold (SCR1, SCR2, and SCR3) are marked as heavy boxes. SCR3 corresponds closely with the sequence conserved region rather than the structurally conserved region. *Abbreviation*: SCR, structurally conserved region. *Source*: From Ref. 10.

β-strands, and it encloses an internal ligand-binding site (Figs. 2 and 3). At the N-terminus, there is a 3_{10} helix, whereas at the C-terminus, there is an α-helix. The three-dimensional structures of several lipocalin allergens are known (Table 2).

Lipocalins are typically small, extracellular proteins with the capacity to bind small, principally hydrophobic molecules, to attach to specific cell-surface receptors, and to form covalent and noncovalent complexes with soluble macromolecules (10). Most of the mammalian lipocalins are produced in the liver or secretory glands. Although they were originally characterized as transport proteins for diverse molecules, such as odorants, steroids, and pheromones, they are involved in a wide range of other biological functions.

Some lipocalins show immunomodulatory activity. One such protein, glycodelin (placental protein 14), exerts its anti-inflammatory activity by elevating the T-cell activation threshold and possibly in this way favors the Th2 deviation of immune response (12). Some lipocalins can also be enzymes, such as glutathione-independent prostaglandin D_2 synthase. Two other lipocalins, β-lactoglobulin (Bos d 5) and tear lipocalin, have been reported to have nonspecific endonuclease activity. The glutamic acid at position 128 in tear lipocalin, important for the enzyme activity, is present in several lipocalin allergens, such as Bos d 2, Mus m 1, Rat n 1, Equ c 1, and Can f 1. The amino acid is situated at or adjacent to the immunodominant T-cell epitope in Bos d 2 (13). Can f 1 has also been proposed to act as a cysteine proteinase inhibitor because of its sequential homology with tear lipocalin. Whether this is the case is not known, but the motifs crucial for the function are only partially conserved in Can f 1. Lipocalins also participate in the regulation of cell growth and proliferation.

Why Th2 responses arise against inert inhaled antigens is unknown. One property associated with the allergenicity of a protein is that it is effectively dispersed in the environment. Lipocalin allergens appear to fulfill this requirement, since they are found in animal dander and excretions. However, the crucial element in the sensitization to a protein is its recognition by the immune system. In this respect, lipocalin allergens seem to differ from infectious agents, since they are not known to contain PAMPs, the recognition of which favors the Th1 (or Th2) deviation of immune response. It is not known whether some intrinsic biologic property of lipocalin allergens, e.g., enzyme activity (Bos d 5), would favor the Th2 immunity.

Table 2 Physicochemical Characteristics of Mammalian Lipocalin Allergens Causing Respiratory Sensitization

Allergen	Animal	MM[a], kDa	Amino acids	Isoelectric point	Glycosylation	Oligomeric state	SWISS-PROT accession #[b]	Structure, PDB ID code[c]	Key reference
Bos d 2	Cow	20	156	4.2	No	M	Q28133	1BJ7	54
Can f 1	Dog	22–25	156	5.2	Putative	D	O18873		42
Can f 2	Dog	22–27	162	4.9	Putative	D	O18874		42
Cav p 1[d]	Guinea pig	20		4.3	No	M/D	P83507		66
Cav p 2[d]	Guinea pig	17		4.3–4.5	No		P83508		58
Equ c 1	Horse	22	172	3.9	Yes	D	Q95182	1EW3	49
Equ c 2[d]	Horse	16		3.4–3.5	No		P81216 P81217		52
Fel d 4	Cat	20	171	4.6	Putative		Q5VFH6		39
Mus m 1	Mouse	18–21	162	4.6–5.3	No	M	P02762	1MUP	62
Ory c 1[d]	Rabbit	17–18			Yes				68
Ory c 2[d,e]	Rabbit	21							68
Rat n 1	Rat	17–21	162	4.2–5.5	Yes	M	P02761	2A2U	62

[a]Molecular mass.
[b]SWISS-PROT data base, http://www.expasy.org/sprot/.
[c]Protein Data Bank, http://www.rcsb.org/pdb/.
[d]Only N-terminus known.
[e]Tentatively named.

Figure 3 Molecular structure of three lipocalin allergens, bovine Bos d 2, horse Equ c 1, and mouse Mus m 1. *Source*: Courtesy of Juha Rouvinen, Department of Chemistry, University of Joensuu, Joensuu, Finland.

It is possible that evolutionary relatedness between human proteins and animal allergens is implicated in the allergenicity of the latter (2,6). In line with this view, we have observed an unexpected characteristic in lipocalin allergens: when the peripheral blood mononuclear cells (PBMCs) from allergic patients sensitized to cow Bos d 2 (13), dog Can f 1 (14,15) or horse Equ c 1 (16) are stimulated with the allergens, the cells proliferate very weakly. The stimulation indices, in general, are below two. The PBMC response to rat Rat n 1 is also weak (17). In parallel with these findings, Bos d 2 is a weak immunogen for several inbred mouse strains (18). A weak stimulatory capacity is also a characteristic of another animal-derived (nonlipocalin) allergen, cat Fel d 1 (19,20). Consistent with the hypothesis is the report indicating that the sequences of allergenic proteins have few or no bacterial homologues, in contrast to those of randomly selected control proteins (nonallergens from the same species as the allergen) (21). This observation suggests that allergens may not be clearly recognized as foreign substances by the immune system.

The human T-cell response is directed to a few epitope regions in Bos d 2, Can f 1, and Equ c 1 (13,15,16). For example, the total number of epitopes detected in Bos d 2 is seven, and the maximal number an individual's T cells can recognize is five. Both Bos d 2 and Equ c 1 contain one immunodominant epitope region at the carboxy-terminal end of the molecule. It is of interest that several of the epitope regions in these three allergens colocalize (Fig. 4), including those of Rat n 1 (17), since this points to the possibility that the T-cell epitopes of lipocalin proteins, in general, colocalize. Of all the lipocalin allergens examined, the first epitope region is found to be colocalizing. This region contains the signature motif G-x-W present in all lipocalin proteins, including human endogenous lipocalins.

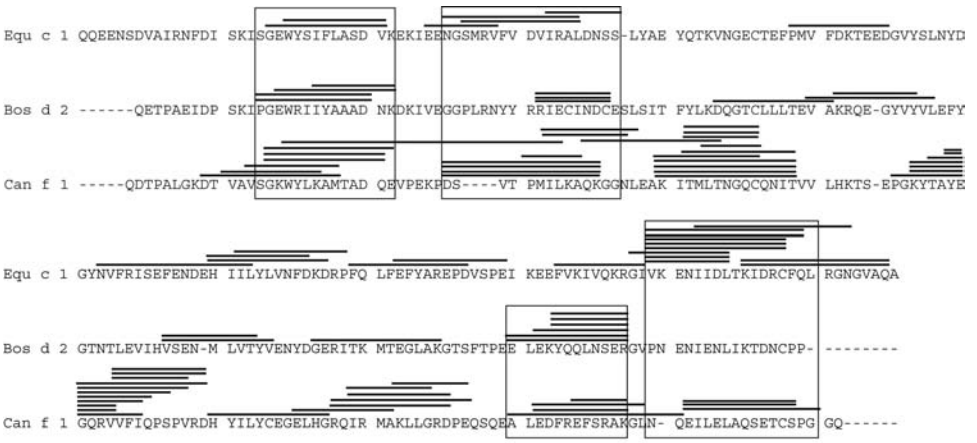

Figure 4 Alignment of the amino acid sequences of the major allergens of horse, Equ c 1, cow, Bos d 2, and dog, Can f 1. Lines above the sequences represent the cores of the epitopes recognized by individual T cell lines and clones. The core sequence is defined as those amino acids that are shared by two to five consecutive peptides able to stimulate the T cells. The most colocalizing epitope regions between the allergens are indicated by boxes. *Source*: From Ref. 16.

It has been observed in studies with peptide analogues (altered peptide ligands) that the outcome of T-cell response is influenced by the extent of T-cell receptor (TCR) ligation. Weak stimulation favors Th2-type responses, whereas stronger stimulation favors Th1-type responses (22). As lipocalins can exhibit considerable amino acid identity between species, it is possible that high-avidity lipocalin allergen-reactive T cells are deleted during thymic maturation (1,2), as is the case with high-avidity self-reactive T cells. The remaining T-cell population, with low-avidity TCRs, might recognize exogenous lipocalin allergens in a suboptimal way. Therefore, it is noteworthy that the immunodominant epitope of Bos d 2 (23) and at least one T-cell epitope of Can f 1 (R. Juntunen, personal communication) are found to be suboptimal. Their optimal peptide analogues can stimulate human T-cell clones at 10- to 100-fold lower concentrations than the natural ligands. Importantly, the optimal peptide analogue of the immunodominant epitope of Bos d 2 is able to give rise to novel T-cell populations with the Th0/Th1 phenotype in vitro (24). However, further studies are needed to assess whether T-cell recognition plays a role in the allergenicity of lipocalins.

Others

Albumins constitute another protein family (http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00186) containing respiratory allergens from several mammals. Albumin is produced in the liver, and it is a major constituent of plasma. It is involved in transporting various molecules and in maintaining the colloidal osmotic pressure of blood. The molecular mass of albumins is around 67 kDa (25). Albumins show about 80% amino acid identity among mammals (25). For cross-reactivity among albumins, see section "Allergenic cross-reactivity among mammals."

Allergenic Proteins from Mammals

Cat

Fel d 1. Cat dander contains several IgE-binding components, the most important being Fel d 1 [P30438 and P30440 at SWISS-PROT (http://www.expasy.org/sprot/)]. Fel d 1, formerly cat-1, is a potent allergen sensitizing over 90% of cat-allergic individuals (26). It is also responsible for 80% to 90% of the IgE-binding capacity of cat allergen extracts (26). The removal of Fel d 1 from a dander extract decreases the histamine-releasing capacity of the preparation by 200- to 300-fold.

Fel d 1 is a glycoprotein with a molecular mass of 38 kDa (27). It is a tetramer composed of two noncovalently linked heterodimers, each with a molecular mass of about 19 kDa. These dimers comprise 8-kDa chain 1 (α-chain) and 10-kDa chain 2 (β-chain), which are linked together covalently by three disulfide bonds. Chain 1 contains 70 and chain 2 contains 90 to 92 amino acids (28,29). Chain 1 exhibits about 25% amino acid identity with uteroglobins, anti-inflammatory proteins, but considerably higher identities (up to 50%) can be observed with other proteins, mostly rodent androgen-binding proteins (SBNS). Chain 1 is classified as a member of the secretoglobin (uteroglobin) family. Chain 2 shows various degrees of amino acid identity with several proteins, up to 39% with a human protein in a segment of 41 amino acids (SBNS). It belongs to the secretoglobin protein family. Fel d 1 exists in several isoforms (27). It can be produced in a recombinant form. The three-dimensional structure of Fel d 1 is strikingly similar to that of uteroglobin (30).

Genes encoding Fel d 1 chains are expressed in the salivary glands and in the skin (29). Fel d 1 is found in hair roots and sebaceous glands, in dander and saliva, and in high concentrations in anal glands. Male cats appear to produce more Fel d 1 than female cats. The biological function of Fel d 1 is unknown, but it may be related to the protection of epithelia (28). It has been speculated that Fel d 1 could have an intrinsic capacity to promote allergy by sequestering calcium ions from phospholipase A_2 (30). Fel d 1 does not have enzymatic activity (30).

Most of the IgE-binding epitopes on Fel d 1 are conformational, and glycosylation present in chain 2 does not play a major role in IgE binding (31). Analyses with overlapping synthetic peptides suggest that IgE-binding epitopes are localized at residues 25 to 38 and 46 to 59 in chain 1 and at residues 15 to 28 in chain 2 (32). Among the sera tested, the highest percentage of positive reactions (46%) is against peptide 25 to 38.

The proliferative response of PBMC induced by Fel d 1 is, in general, not strong (19,20). In contrast, Fel d 1–specific T-cell clones and lines proliferate vigorously upon stimulation with Fel d 1 (33,34). In two studies, T-cell responses against Fel d 1 exhibited no correlation with human leukocyte antigen (HLA) phenotypes (19,33). A third study found a possible HLA-DR1

excess (odds ratio = 2, $p = 0.002$) among subjects with Fel d 1–specific IgE (35). In another study, no association was found between specific IgE and the alleles of the loci examined (including HLA-DRB1) (36). Human T-cell epitopes are present in several regions of Fel d 1, but T-cell reactivity is more pronounced against chain 1 than against chain 2 of the molecule (19,34). In chain 1, most of the reactivity is concentrated in the N-terminal half of the molecule, in amino acids 18 to 42, while in chain 2, the most reactive region is the C-terminus, amino acids 74 to 92. Two peptides, Fel-1 (IPC-1) and Fel-2 (IPC-2), amino acids 7 to 33 and 29 to 55 of chain 1, respectively, stimulate T cells but bind IgE only at low levels, which suggests that they could be suitable for peptide-based allergen immunotherapy (19). It appears that T-cell epitope recognition does not distinguish Fel d 1–allergic from nonallergic subjects. However, distinct Fel d 1 epitopes may be able to induce qualitatively different T-cell responses (37).

Fel d 2. Fel d 2, cat serum albumin (P49064 at SWISS-PROT), is a minor allergen with IgE reactivity in about 20% of cat-allergic individuals (26), although higher figures are also reported. Its role in cat allergy is unclear, in that dominant IgE response against it is found only in 2% of cat-allergic individuals (26). Moreover, the significance of cat albumin as a primary sensitizer is difficult to assess (26), since albumins exhibit cross-reactivity across animal species (see section "Allergenic cross-reactivity among mammals".). In accordance with IgE determinations, polyclonal T-cell lines, raised with cat dander extract, proliferated only weakly upon stimulation with cat albumin, whereas the response was strong against Fel d 1 (33).

Fel d 3. Fel d 3, cystatin (Q8WNR9 at SWISS-PROT), was cloned from cat skin (38). The prevalence of IgE reactivity among cat-allergic subjects is about 10% when measured using *Escherichia coli*-produced recombinant protein in a solid-phase enzyme-linked immunosorbent assay (ELISA) (38).

Fel d 3 is an 11-kDa protein containing 98 amino acids. There is one potential N-linked glycosylation site in the sequence. Fel d 3 exhibits nearly 80% amino acid identity with bovine and human cystatin A. As endogenous protease inhibitors, cystatins control the function of cysteine proteases. Fel d 3 contains the signature motif conserved in cysteine protease inhibitors. Dog allergens, Can f 1 and Can f 2, which are lipocalins, exhibit some degree of conservation with the sequence motif.

Fel d 4. The sole cat allergen known to belong to the lipocalin family of proteins is Fel d 4 (Q5VFH6 at SWISS-PROT). It is the second major allergen of cat, since 63% of cat-allergic subjects have IgE against it (39), on the basis of measurements using recombinant Fel d 4. In general, the level of the antibody is low compared to that induced by Fel d 1, but about half of the Fel d 4–sensitized subjects have higher Fel d 4 than Fel d 1 IgE levels. The physicochemical characteristics of Fel d 4 are shown in Table 2.

Fel d 4 was cloned from submandibular salivary glands (39). It seems that the expression of Fel d 4 is limited to this tissue, since mRNA for it was not found in several other tissues examined. Isoallergens were not detected. Fel d 4 exhibits a considerable amino acid identity with several mammalian lipocalins; the highest level is observed with Equ c 1, the horse allergen (67%). Among human proteins, epididymal-specific lipocalin-9 shows the highest amino acid identity (38%) with Fel d 4 (SBNS).

Other cat allergens. Cat IgA (Fel d 5w), IgM (Fel d 6w), and IgG (Fel d 7w) have been identified as respiratory allergens. Thirty-eight percent of cat-sensitized patients have IgE to cat IgA (40). It is of interest that the IgE reactivity is mainly directed to the carbohydrates of the α-chain. Further studies are needed to clarify the clinical significance of cat immunoglobulins as aeroallergens.

Dog

Can f 1. The major allergen of dog, Can f 1 (O18873 at SWISS-PROT), formerly called Ag 8, Ag 13, or Ag X, sensitizes 50% to 75% of dog-allergic subjects (41–43). It accounts for about 50% of the IgE-binding capacity of dog hair and dander extract (41) and for 60% to 70% of the IgE-binding capacity of dog saliva preparation (44). Can f 1 belongs to the lipocalin family of proteins (42). Its physicochemical characteristics are shown in Table 2.

Can f 1 is primarily found in dog saliva, but it is also present in dog dander (44). It is absent or in very low concentrations in serum, urine, and feces. The allergen is detected in the hair extracts of all dog breeds examined, with variable amounts among individual dogs within a breed (44,45). Male dogs produce Can f 1 more than female dogs (45). Can f 1 has been cloned from the parotid gland, and it has been produced in a recombinant form (42). It is homologous to human tear lipocalin (see section "Lipocalins"). Can f 1 mRNA is present in the parotid and mandibular glands, tongue epithelial tissue, and skin, but not in the liver or kidney (42,46).

Human cellular immune responses to Can f 1 have been examined (see section "Lipocalins"). In one more study, it was observed that the TCR Vβ5.1$^+$ CD4$^+$ T cells and the DR4-DQ8 haplotype may be protective against allergy to Can f 1 (14). In two studies, no association between the Can f 1–specific IgE response and the HLA class II genotype was observed (35,36).

Can f 2. Can f 2 (O18874 at SWISS-PROT), formerly called dog allergen 2 or Dog 2, is a minor allergen sensitizing 25% to 30% of dog-allergic subjects (42,43). Dog-allergic subjects' average IgE response against Can f 2 is estimated to be 23% of that against dog dander extract (44). Can f 2 belongs to the lipocalin family of proteins (42). Its physicochemical characteristics are illustrated in Table 2.

Can f 2 is found in dog dander and in saliva, whereas urine or feces contain very little of the allergen (44). The amount of Can f 2 in the hair extracts of nine dog breeds varied widely. It has been cloned from the parotid gland and produced as a recombinant protein (42). Can f 2 exhibits the highest level of amino acid identity, 36% with trichosurin, a milk-derived lipocalin from the brush-tailed possum (SBNS). Identities at the level of 30% are observed with rodent urinary proteins (42). Can f 2 mRNA is predominantly expressed in parotid and mandibular glands and to a lesser extent in skin and tongue (42,46). It is not found in the kidney or liver. The immunological properties of Can f 2 have not been studied in detail.

Can f 3. Thirty-five percent of dog-allergic patients have IgE against Can f 3, dog serum albumin (P49822 at SWISS-PROT) (47), although both lower and higher figures are also reported. In individual patients, a major part of dog-specific IgE is directed to Can f 3 (47). Can f 3 has been cloned from dog liver and produced as a recombinant protein (25).

Other dog allergens. Dog can be a source of up to 20 allergens. In an analysis of hair and dander extract with sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, a total of 11 allergens in the molecular mass range of 14 to 68 kDa were detected. One of these is an immunoglobulin. In another study, an 18-kDa protein (Can f 4) was recognized by 60% of dog-allergic subjects in IgE immunoblotting (43). The aminoterminal sequencing of Can f 4 (P84494 at SWISS-PROT) suggests that it is a lipocalin.

Horse

Equ c 1. IgE against Equ c 1 (Q95182 at SWISS-PROT), probably previously named Ag 6, is found in 76% of horse-allergic subjects' sera (48). According to one study, Ag 6 accounts for 55% of skin prick test reactivity of horsehair and dandruff extract. The physicochemical characteristics of Equ c 1, a lipocalin allergen (49), are shown in Table 2.

In addition to horse dander (48), Equ c 1 is found in a high concentration in saliva, while urine contains little of the allergen. Equ c 1 mRNA expression is about 100-fold higher in sublingual salivary glands than in submaxillary salivary glands or the liver (49). The allergen has been cloned from sublingual salivary glands and produced in a recombinant form (49). As mentioned above, Equ c 1 exhibits a considerable amino acid identity with cat Fel d 4 (67%). Its amino acid identity with a pig salivary lipocalin is 61% (SBNS) and with rodent major urinary proteins about 50% (49). The amino acid identity with human epididymal-specific lipocalin-9 is 37% (SBNS). There are several isoforms of the allergen. It has been proposed to bind histamine. Unlike some other allergens, such as Equ c 2, Equ c 1 has a surfactant-like property (50).

An analysis of the IgE-binding epitopes of Equ c 1 suggests that the dominant epitopes are localized in a restricted region of the molecule (51). Carbohydrates may have some impact on IgE binding (50,51). Human cellular immune response to Equ c 1 has been examined (see section "Lipocalins").

Equ c 2. The N-terminal sequences of two horse dander allergens, with slightly different isoelectric points (pI), were shown to be identical and the allergens were named Equ c 2.0101 and Equ c 2.0102 (P81216 and P81217 at SWISS-PROT) (52). A 29 amino acid fragment exhibits a 44% identity with bovine Bos d 2 and also contains the highly conserved G-X-W motif of lipocalins. Analyses of the amino acid compositions of the allergens also suggest that they are lipocalins (Table 2). Up to 50% of horse-allergic patients have IgE against Equ c 2 (52).

Equ c 3. As with albumins from other mammals, the significance of horse serum albumin (P35747 at SWISS-PROT) as an allergen is not clear. The prevalence of IgE reactivity against it is between 20% (53) and 50%.

Other horse allergens. Horse dander contains more than 10 IgE-binding proteins (52). Equ c 4 and Equ c 5 are partially characterized (50). Like Equ c 1, these allergens have surfactant-like properties. Equ c 4 was originally described as a 19-kDa glycoprotein with a pI of 3.8 (50). Its partial sequence is 100% identical with the sequence of horse latherin, a surfactant protein (SBNS). About 30% of horse-allergic individuals have IgE against Equ c 4. Equ c 5 is a 17-kDa nonglycosylated protein with a pI of 5.3 (50). Its peptide fragments show almost complete homology with latherin (SBNS). In one study, 77% of horse-allergic patients had IgE against this allergen (50). In the SWISS-PROT database, Equ c 4 and Equ c 5 are contained in a single entry, horse latherin (P82615). It is a 208 amino acid protein with a molecular mass of 24 kDa. The nomenclature of horse allergens is discussed in the publication by Goubran Botros et al. (50).

Cow

Bos d 2. Bos d 2 (Q28133 at SWISS-PROT), a lipocalin allergen (54), also known as Ag 3 or BDA20, is the major respiratory allergen in cow dander (Table 2). About 90% of dairy farmers with asthma of bovine origin react against Bos d 2, as analyzed by IgE immunoblotting (55) or by bronchial allergen challenge. Both Ag 3 (Bos d 2) and Ag 1 account for about 70% of the IgE-binding capacity of cow hair and dander extract. Together they bind about 80% of the IgE.

Bos d 2 is found in cow skin (56), although the same or an immunologically related allergen is present in urine (55). In skin, Bos d 2 is localized in the secretory cells of the apocrine sweat glands and the basement membranes of the epithelium and hair follicles. It is probably a pheromone carrier (56). There are several isoforms of Bos d 2. It has been cloned from cow skin (54) and produced as a recombinant protein (57). Bos d 2 exhibits the highest amino acid identity with a probable bovine odorant-binding protein (68%) from the mammary gland (SBNS). The amino acid identity with other odorant-binding proteins and lipocalins from other species are at the level of 30% to 40%. Sequencing the 15 aminoterminal residues of Cav p 2, a guinea pig allergen, revealed a 69% identity with the bovine allergen (58).

To reduce its IgE-binding capacity, Bos d 2 has been produced in fragments and in mutated forms. IgE binding is highly dependent on an intact three-dimensional structure. The epitopes responsible for IgE binding appear to be localized in the C-terminal part of Bos d 2. Most of the studies on cellular immune response to lipocalin allergens have been performed with Bos d 2 (see section "Lipocalins").

Bos d 3. Bos d 3 (Q28050 at SWISS-PROT), known also as BDA11, is a minor bovine respiratory allergen (59). According to the immunoblotting analysis with recombinant Bos d 3, about 40% of patients with cow dust-induced asthma have IgE against the allergen (59).

Bos d 3 is an 11-kDa protein with a predicted pI of 5.19 (59). In addition to bovine skin, it is found in amniotic fluid. This 101 amino acid long allergen belongs to the S-100 family of proteins and exhibits more than 60% amino acid identity with horse and human psoriasins (SBNS). Human psoriasin (or S100A7) is a calcium-binding keratinocyte protein found in normal skin. It is highly upregulated in psoriatic skin. The calcium-binding motif containing the so-called EF hand is located in psoriasin in the segment that is almost identical to that of Bos d 3. It is probable that Bos d 3 is a bovine homologue of psoriasin. The expression of psoriasin is not limited to psoriasis, and it has chemokine-like properties selective for $CD4^+$ T cells and neutrophils. Human psoriasin also has antimicrobial activity against *E. coli* (60).

Other bovine respiratory allergens. Using crossed radioimmunoelectrophoresis, serum proteins, including albumin (Bos d 6; P02769 at SWISS-PROT) and IgG (Bos d 7), have been

found to be allergens in cow hair and dander. By immunoblotting, up to 10 IgE-binding components are detected in the bovine epithelial extract and four in the urine preparation in the molecular mass range of 16 kDa to over 100 kDa (55). Two of the allergens with the molecular masses of 20 kDa (Bos d 2) and 22 kDa are major allergens (55). In another study, an 11-kDa protein showing almost complete homology with the bovine oligomycin sensitivity-conferral protein of the mitochondrial adenosine triphosphate synthase complex (P13621 at SWISS-PROT) was identified as a minor allergen in cow dander.

Mouse

Mus m 1. Mus m 1, a major allergen, known also as Ag 1, prealbumin, or mouse allergen 1 (MA1) (61), is the mouse major urinary protein MUP6 (P02762 at SWISS-PROT). It accounts for the major part of the IgE-binding capacity of the crude male urine (61). Mus m 1 belongs to the lipocalin family of proteins (62). The physicochemical characteristics of Mus m 1 are shown in Table 2.

Mus m 1 is found in mouse urine, serum, pelt, and especially in the liver (61), where it is primarily produced (62). The production of MUPs is under hormonal control and influenced by androgens (62). Forms of MUPs are also expressed constitutively in the exocrine glands of mice and rats (62). Mus m 1 is found in about fourfold higher concentration in male than in female urine (61). Mouse MUPs are encoded by about 35 genes, and 15 forms of MUPs are detected in male urine. Mouse MUP has been produced as a recombinant protein. The amino acid identity between mouse and rat MUPs is about 65% (62). The amino acid identity of Mus m 1 with Fel d 4 is 49%, with Equ c 1, 46%, and with human epididymal-specific lipocalin-9, 39% (SBNS).

Other mouse allergens. The other major allergen of mouse, Ag 3, tentatively named Mus m 2, is a glycoprotein (63). It is found in mouse dander and fur. It is localized in the hair follicles, coating the hairs, and on the skin (63). Mouse albumin is also an allergen.

Rat

Rat n 1. Rat n 1, also known as rat MUP (P02761 at SWISS-PROT), prealbumin, or α_{2u}-globulin (α_2-euglobulin), is closely related to the major urinary proteins of mouse (see section "Mus m 1") and belongs to the lipocalin group (62). Its physicochemical characteristics are shown in Table 2. Sixty-six percent of laboratory workers with asthma and rhinitis, on exposure to rats, had IgE against Rat n 1 (64). Adult female rats excrete in urine about one-sixth of the amount of MUPs of male rats. As Mus m 1, Rat n 1 exhibits considerable amino acid identities with Fel d 4 (55%), Equ c 1 (47%), and human epididymal-specific lipocalin-9 (42%) (SBNS).

Rat urinary prealbumin and α_{2u}-globulin were considered distinct allergens in the 1980s. Later analyses of these strongly cross-reactive proteins (64) showed that prealbumin is an isoform of α_{2u}-globulin. Therefore, a more appropriate name for prealbumin is Rat n 1.01 and for α_{2u}-globulin, Rat n 1.02 (1). α_{2u}-globulin has been cloned and produced as a recombinant protein. One study suggests that the IgE-binding epitopes of Rat n 1.02 tend to be clustered toward the N- and C-terminal parts of the allergen.

Human cellular immune response to Rat n 1 has been examined (see section "Lipocalins"). In one more study, HLA-DR7 was positively associated and HLA-DR3 negatively associated with sensitization to rat urinary proteins (65).

Other rat allergens. Male rat urine contains a total of eight allergens in the molecular mass range of 17 to 75 kDa. About 20 allergens have been observed in rat fur and in saliva. Rat serum proteins, including albumin, transferrin, and IgG, are allergens.

Guinea Pig

Cav p 1. Cav p 1 (P83507 at SWISS-PROT) is a major guinea pig allergen. The prevalence of guinea pig-allergic subjects' IgE reactivity against Cav p 1 in hair extract is 70% compared with 87% in urine (66). The allergen was purified from the hair extract. The physicochemical characteristics of Cav p 1 are shown in Table 2. Analysis of the 15 aminoterminal residues

shows that Cav p 1 is a lipocalin with a 57% amino acid identity with the major mouse allergen, Mus m 1 (66).

Cav p 2. Cav p 2 (P83508 at SWISS-PROT) is another major guinea pig allergen and belongs to the lipocalin group. About 55% of guinea pig-allergic subjects have IgE against it (66). The physicochemical characteristics of Cav p 2 are shown in Table 2. Analysis of the 15 aminoterminal residues of the allergen shows a 69% amino acid identity with the major cow dander allergen, Bos d 2 (58).

Other guinea pig allergens. In addition to Cav p 1 and 2, guinea pig hair extract and urine contain at least 8 IgE-binding components in the molecular mass range between 8 and 70 kDa (66). Sixty-five percent of guinea pig-allergic individuals have IgE against an 8-kDa allergen, whereas IgE reactivity to the other allergens is below 33%. Guinea pig serum albumin was previously considered a major allergen, but only 8% of guinea pig-allergic patients are found to exhibit IgE reactivity to it (66).

Rabbit

Ory c 1. The major allergen Ory c 1, also known as Ag R1, is found in saliva and, to a slightly lesser extent, in fur (67). It is present in dander in small amounts but not in urine. The physicochemical characteristics of Ory c 1 are shown in Table 2. The sequence of the 20 N-terminal amino acids suggests that the allergen is a lipocalin with a 72% amino acid identity with rabbit odorant-binding protein-II (68).

Other rabbit allergens. Rabbit urine, fur, and saliva extracts contain several allergens with molecular masses from 8 to 80 kDa (68). Saliva, which contains 12 allergens, is the most potent of the extracts, according to radioallergosorbent test (RAST) inhibition experiments (68).

The N-terminus of a 21-kDa allergen exhibits an even higher amino acid identity with the odorant-binding protein-II than Ory c 1 (68). This protein could be the minor allergen Ag2, found in several source materials, and is also referred to as Ory c 2 (Table 2). Rabbit serum albumin is of minor importance, although in individual cases sensitization can be strong (68).

Human Autoallergens

IgE antibodies against human proteins are found in patients with chronic allergic conditions. These are evolutionary conserved proteins, and some of them are homologues of recognized exogenous allergens.

In one study, using extracts from a human epithelial cell line, IgE autoantibodies against a variety of human proteins were found in 43% of patients with atopic dermatitis (69). Recombinant forms of several of the autoantigens induced histamine release from basophils and produced a positive skin prick test. The levels of IgE antibodies to the autoantigens were found to correlate with the severity of atopic dermatitis. However, the role of autoantibodies in the pathogenesis of allergic conditions remains unclear.

Hom s 1. Hom s 1 (O43290 at SWISS-PROT) is one of the five autoallergens listed in the allergen nomenclature. Six out of 65 sera from atopic dermatitis patients had IgE antibodies against Hom s 1 (70). Deduced from the cDNA sequence, it has a molecular mass of 73 kDa. However, a rabbit antiserum against Hom s 1 detected proteins with varied sizes in extracts of human tissues (70). Immunohistochemistry reveals that Hom s 1 is a cytoplasmic protein, although SART-1, a protein with an almost complete sequence identity with Hom s 1, is located in nuclei of normal and malignant cells.

Hom s 2-5 Like Hom s 1, these four autoallergens were found by screening a cDNA library from a human epithelial cell line with IgE antibodies from patients with atopic dermatitis (69). The presence of IgE antibodies was restricted to a few dermatitis patients. The cDNAs of Hom s 2-5 were shown to code for fragments of intracellular proteins. Hom s 2 displays sequence identity with a portion of the α-nascent polypeptide-associated complex (α-NAC) (Q13765 at SWISS-PROT). An isoform of Hom s 2 (Hom s 2.01), containing 21 amino acid exchanges

compared with α-NAC, has been identified (71). Hom s 3 displays sequence identity with the oncoprotein BCL7B (Q13845 at SWISS-PROT). Hom s 4 (O75785 at SWISS-PROT) is a 54-kDa basic protein with a pI of 8.7 (72). It appears to belong to a subfamily of calcium-binding proteins and displays IgE cross-reactivity with exogenous calcium-binding allergens from plants (Phl p 7) and fish (Cyp c 1). Hom s 5 is identical to a portion of cytokeratin type II (P02538 at SWISS-PROT).

Human homologues of exogenous allergens. Several fungal allergens are phylogenetically highly conserved, and the corresponding human proteins react with IgE antibodies from patients with severe fungal allergies. Asp f 6 is a manganese superoxide dismutase (MnSOD) allergen of *Aspergillus fumigatus*. Recombinant human MnSOD, which displays a 48% amino acid identity with Asp f 6 (SBNS), reacts with IgE and stimulates T cells from patients with chronic *A. fumigatus* allergy (73). Similarly, IgE cross-reactivity between several other fungal allergens, such as acidic ribosomal phosphoprotein type 2 (P2 protein) (74), thioredoxin (75), and cyclophilins (76), and their human homologues has been described. Profilins are another group of conserved proteins identified as allergens of several plants, e.g., Bet v 2 of birch. IgE from sera of patients sensitized to plant profilins cross-react with human profilin (77).

ALLERGENIC CROSS-REACTIVITY AMONG MAMMALS

IgE cross-reactivities among mammalian allergens have been characterized to some extent. Earlier analyses were based on inhibiting IgE binding to an allergen extract by another extract. The inhibition was usually found to be individually variable. Moreover, the extracts often showed an unequal inhibitory capacity, which suggests that only a part of the IgE-binding epitopes were common. Such experiments pointed to the possibility that there are IgE-cross-reacting allergens, for example, in cat, dog, and horse allergen extracts (53), including the components representing the major cat and dog allergens (78). Some studies suggest that the taxonomical relationship among animals is probably a factor contributing to the cross-reactivity of IgE antibodies.

More specific results have been obtained by using pure allergen molecules. rFel d 1 (100 μg/mL) inhibits the binding of IgE from Fel d 1–sensitized cat-allergic patients to a dog allergen preparation by an average of 41% (79). An inhibition of more than 50% was detected with 25% of the sera. The probable IgE-cross-reactive dog allergen of 20 kDa was not characterized further. The IgE cross-reactivity of another cat allergen, Fel d 4, a lipocalin, also has been characterized (39). Allergen extracts from cow (mean 66%), and to a lesser extent, from horse and dog, inhibited IgE binding to rFel d 4. Since cow Bos d 2 shows a low amino acid identity with Fel d 4, the inhibition was supposed to be due to an unidentified bovine homologue. In a study with the guinea pig allergen, Cav p 1, a lipocalin, allergen preparations from cat, mouse, and rat in a hundred-fold excess were able to induce only a weak inhibition (less than 10%) of IgE binding to Cav p 1 (66). A further study suggests that there is IgE cross-reactivity between Cav p 1 and Cav p 2 (58). In another study, a monoclonal antibody raised against Bos d 5 (β-lactoglobulin), a bovine food allergen of the lipocalin family, reacted against human serum retinol-binding protein, another lipocalin (80). The core of the antibody-binding epitope, DTDY, is localized in the second structurally conserved region of lipocalins. The sequence is found, for example, in human glycodelin (SBNS).

Studies on IgE cross-reactivity between pure mammalian allergens are largely missing. One limited study, exploiting *E. coli*-produced rCan f 1 and 2, found IgE cross-reactivity between these two dog allergens (81). This observation has been confirmed by another group, using *Pichia pastoris*-produced rCan f 1 and 2 (82). The cross-reactivity was found, however, to be weaker than that reported by Kamata et al. (81). In the same study, IgE cross-reactivity was also found between Can f 1 and human tear lipocalin and between Equ c 1 and Mus m 1. The cross-reactivities were probably due to sequential and structural similarities of the proteins (82).

The clinical significance of IgE cross-reactivity between mammalian nonserum-derived respiratory allergens is unclear. It seems that the cross-reactivity is mostly weak to moderate. It is possible that IgE cross-reactivity, including to human homologues, could be implicated in the regulation of allergic sensitization.

Animal-allergic patients may have IgE antibodies against a number of albumins from different mammalian species (83). Inhibition experiments show that albumin-specific IgE is often cross-reactive, although patients exhibit individual variation in this respect (53,83). As pointed out for Fel d 2, the primary sensitizer can be difficult to identify (26). A study with three tryptic peptides from horse serum albumin identified regions involved in IgE cross-reactivity with dog albumin (84). Inhibition of a monoclonal antihuman albumin antibody with cat or dog albumin indicated that cat, dog, and human albumins have similar epitopes (25). In another study with monoclonal antibodies specific to cat or dog albumin, antibodies recognized the albumin of both species (85). The study also suggests that the monoclonal antibodies and human IgE recognize identical or closely related epitopes on cat and dog albumin.

Subjects sensitized to cat IgA have IgE cross-reactive with carbohydrate moieties, for example, in cat IgM (40). The IgE cross-reactivity of human autoallergens is discussed above.

ENVIRONMENTAL CONTROL

Exposure to indoor allergens can be reduced by control measures. It is reasonable to assume that the primary prevention of avoiding contact with pets during childhood would restrain sensitization and the clinical manifestations of allergy. This paradigm has been questioned, however, in view of several studies pointing to the protective effect of a high-level exposure to cat- and dog-derived dust (3,86). As a consequence, recommendations about pets and children in the same household, as far as primary sensitization is concerned, may need to be reconsidered.

The guidelines are more straightforward for persons who are already sensitized against mammalian allergens. Avoidance, or reduction of the exposure load when total avoidance is not possible, is the primary strategy to prevent or to alleviate allergic symptoms.

Allergen concentrations in homes with pets are 10 to 1000 times higher than in homes without pets (87). Removing the pet from the household gradually reduces the allergen levels over time (87). In practice, families often try to keep their pets, and various measures have been proposed to reduce the exposure in those circumstances. These include keeping the pet out of the main living area, using vacuum cleaners with high-efficiency particulate air (HEPA) filters, and frequent washings of the pet. Although a reduction in the allergen levels can be achieved, the effect on clinical outcomes is not documented (87).

As the first line of prevention against laboratory animal allergy, persons with an atopic background, especially if they are already allergic to animals, should be discouraged from doing these jobs (88). Within laboratory animal facilities, the aims of preventive measures are to reduce the airborne allergen levels and make use of personal protection against exposure. Ideally, a comprehensive plan should be used, starting from the appropriate designing of the facilities and ventilation system.

The use of curtains in front of cage racks prevents the spread of rodent allergens to the animal room. In one study, individually ventilated cage systems decreased ambient rodent allergen levels 250-fold or more under optimal conditions. To reduce the exposure to persons emptying and cleaning soiled cages, automated cage-handling machines have been developed. Handling animals during experimental procedures in class II ventilated cabinets results in a greater than 10-fold protection factor. The most effective personal protection against airborne allergens is achieved by the use of ventilated, motorized helmets in which inhaled air is pumped through type P2 or P3 filters. Although somewhat inconvenient to use, the helmet allows even asthmatic persons to continue to work with animals.

SALIENT POINTS

- Mammalian respiratory allergens are primarily dispersed in dander, saliva, and urine.
- Exposure to mammalian allergens is not limited to immediate contacts with animals; these allergens are widely present in indoor environments.
- Almost all important mammalian aeroallergens belong to the lipocalin family of proteins. Factors accounting for the allergenicity of lipocalins remain to be identified.

- Environmental control measures can help symptomatic individuals, although avoidance of exposure is preferable.
- High exposure to pets in early childhood may be protective against sensitization.
- IgE cross-reactivity between animal serum albumins is established. Lipocalin allergens also exhibit IgE cross-reactivity.

ACKNOWLEDGMENT

The work was supported by the Academy of Finland (contract #115160).

REFERENCES

1. Virtanen T, Zeiler T, Mäntyjärvi R. Important animal allergens are lipocalin proteins: Why are they allergenic? Int Arch Allergy Immunol 1999; 120:247–258.
2. Virtanen T, Zeiler T, Rautiainen J, et al. Allergy to lipocalins: a consequence of misguided T-cell recognition of self and nonself? Immunol Today 1999; 20:398–400.
3. Platts-Mills T, Vaughan J, Squillace S, et al. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001; 357:752–756.
4. Riedl MA, Landaw EM, Saxon A, et al. Initial high-dose nasal allergen exposure prevents allergic sensitization to a neoantigen. J Immunol 2005; 174:7440–7445.
5. Erwin EA, Wickens K, Custis NJ, et al. Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. J Allergy Clin Immunol 2005; 115:74–79.
6. Platts-Mills TA. The role of indoor allergens in chronic allergic disease. J Allergy Clin Immunol 2007; 119:297–302.
7. Heederik D, Sigsgaard T. Respiratory allergy in agricultural workers: recent developments. Curr Opin Allergy Clin Immunol 2005; 5:129–134.
8. Bush RK, Wood RA, Eggleston PA. Laboratory animal allergy. J Allergy Clin Immunol 1998; 102:99–112.
9. Pacheco KA. New insights into laboratory animal exposures and allergic responses. Curr Opin Allergy Clin Immunol 2007; 7:156–161.
10. Flower DR, North AC, Sansom CE. The lipocalin protein family: structural and sequence overview. Biochim Biophys Acta 2000; 1482:9–24.
11. Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997; 25:3389–3402.
12. Rachmilewitz J, Riely GJ, Huang JH, et al. A rheostatic mechanism for T-cell inhibition based on elevation of activation thresholds. Blood 2001; 98:3727–3732.
13. Zeiler T, Mäntyjärvi R, Rautiainen J, et al. T cell epitopes of a lipocalin allergen colocalize with the conserved regions of the molecule. J Immunol 1999; 162:1415–1422.
14. Kinnunen T, Taivainen A, Partanen J, et al. The DR4-DQ8 haplotype and a specific TCR Vbeta T-cell subset are associated with absence of allergy to Can f 1. Clin Exp Allergy 2005; 35:797–803.
15. Immonen A, Farci S, Taivainen A, et al. T cell epitope-containing peptides of the major dog allergen Can f 1 as candidates for allergen immunotherapy. J Immunol 2005; 105:3614–3620.
16. Immonen A, Kinnunen T, Sirven P, et al. The horse major allergen Equ c 1 contains one immunodominant region of T cell epitopes. Clin Exp Allergy 2007; 37:939–947.
17. Jeal H, Draper A, Harris J, et al. Determination of the T cell epitopes of the lipocalin allergen, Rat n 1. Clin Exp Allergy 2004; 34:1919–1925.
18. Saarelainen S, Zeiler T, Rautiainen J, et al. Lipocalin allergen Bos d 2 is a weak immunogen. Int Immunol 2002; 14:401–409.
19. Counsell CM, Bond JF, Ohman JL, et al. Definition of the human T-cell epitopes of Fel d 1, the major allergen of the domestic cat. J Allergy Clin Immunol 1996; 98:884–894.
20. Marcotte GV, Braun CM, Norman PS, et al. Effects of peptide therapy on ex vivo T-cell responses. J Allergy Clin Immunol 1998; 101:506–513.
21. Emanuelsson C, Spangfort MD. Allergens as eukaryotic proteins lacking bacterial homologues. Mol Immunol 2007; 44:3256–3260.
22. Brogdon JL, Leitenberg D, Bottomly K. The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naive CD4+ T cells. J Immunol 2002; 168:3825–3832.
23. Kinnunen T, Buhot C, Närvänen A, et al. The immunodominant epitope of lipocalin allergen Bos d 2 is suboptimal for human T cells. Eur J Immunol 2003; 33:1717–1726.
24. Kinnunen T, Jutila K, Kwok WW, et al. Potential of an altered peptide ligand of lipocalin allergen Bos d 2 for peptide immunotherapy. J Allergy Clin Immunol 2007; 119:965–972.

25. Pandjaitan B, Swoboda I, Brandejsky-Pichler F, et al. *Escherichia coli* expression and purification of recombinant dog albumin, a cross-reactive animal allergen. J Allergy Clin Immunol 2000; 105:279–285.
26. van Ree R, van Leeuwen WA, Bulder I, et al. Purified natural and recombinant Fel d 1 and cat albumin in in vitro diagnostics for cat allergy. J Allergy Clin Immunol 1999; 104:1223–1230.
27. Kristensen AK, Schou C, Roepstorff P. Determination of isoforms, N-linked glycan structure and disulfide bond linkages of the major cat allergen Fel d 1 by a mass spectrometric approach. Biol Chem 1997; 378:899–908.
28. Morgenstern JP, Griffith IJ, Brauer AW, et al. Amino acid sequence of Fel d I, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. Proc Natl Acad Sci U S A 1991; 88:9690–9694.
29. Griffith IJ, Craig S, Pollock J, et al. Expression and genomic structure of the genes encoding FdI, the major allergen from the domestic cat. Gene 1992; 113:263–268.
30. Kaiser L, Velickovic TC, Badia-Martinez D, et al. Structural characterization of the tetrameric form of the major cat allergen Fel d 1. J Mol Biol 2007; 370:714–727.
31. Duffort OA, Carreira J, Nitti G, et al. Studies on the biochemical structure of the major cat allergen *Felis domesticus I*. Mol Immunol 1991; 28:301–309.
32. van Milligen FJ, van't Hof W, van den Berg M, et al. IgE epitopes on the cat (*Felis domesticus*) major allergen Fel d I - a study with overlapping synthetic peptides. J Allergy Clin Immunol 1994; 93:34–43.
33. van Neerven RJ, van de Pol MM, van Milligen FJ, et al. Characterization of cat dander-specific T lymphocytes from atopic patients. J Immunol 1994; 152:4203–4210.
34. Mark PG, Segal DB, Dallaire ML, et al. Human T and B cell immune responses to Fel d 1 in cat-allergic and non-cat-allergic subjects. Clin Exp Allergy 1996; 26:1316–1328.
35. Young RP, Dekker JW, Wordsworth BP, et al. HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. Clin Exp Allergy 1994; 24:431–439.
36. Howell WM, Standring P, Warner JA, et al. HLA class II genotype, HLA-DR B cell surface expression and allergen specific IgE production in atopic and non-atopic members of asthmatic family pedigrees. Clin Exp Allergy 1999; 29:35–38.
37. Reefer AJ, Carneiro RM, Custis NJ, et al. A role for IL-10-mediated HLA-DR7-restricted T cell-dependent events in development of the modified Th2 response to cat allergen. J Immunol 2004; 172:2763–2772.
38. Ichikawa K, Vailes LD, Pomes A, et al. Molecular cloning, expression and modelling of cat allergen, cystatin (Fel d 3), a cysteine protease inhibitor. Clin Exp Allergy 2001; 31:1279–1286.
39. Smith W, Butler AJ, Hazell LA, et al. Fel d 4, a cat lipocalin allergen. Clin Exp Allergy 2004; 34: 1732–1738.
40. Adedoyin J, Grönlund H, Öman H, et al. Cat IgA, representative of new carbohydrate cross-reactive allergens. J Allergy Clin Immunol 2007; 119:640–645.
41. Schou C, Svendsen UG, Løwenstein H. Purification and characterization of the major dog allergen, Can f I. Clin Exp Allergy 1991; 21:321–328.
42. Konieczny A, Morgenstern JP, Bizinkauskas CB, et al. The major dog allergens, Can f 1 and Can f 2, are salivary lipocalin proteins: cloning and immunological characterization of the recombinant forms. Immunology 1997; 92:577–586.
43. Saarelainen S, Taivainen A, Rytkönen-Nissinen M, et al. Assessment of recombinant dog allergens Can f 1 and Can f 2 for the diagnosis of dog allergy. Clin Exp Allergy 2004; 34:1576–1582.
44. de Groot H, Goei KG, van Swieten P, et al. Affinity purification of a major and a minor allergen from dog extract: serologic activity of affinity-purified Can f I and of Can f I-depleted extract. J Allergy Clin Immunol 1991; 87:1056–1065.
45. Ramadour M, Guetat M, Guetat J, et al. Dog factor differences in Can f 1 allergen production. Allergy 2005; 60:1060–1064.
46. Kamata Y, Miyanomae A, Nakayama E, et al. Characterization of dog allergens Can f 1 and Can f 2. 1. Preparation of their recombinant proteins and antibodies. Int Arch Allergy Immunol 2007; 142:291–300.
47. Spitzauer S, Schweiger C, Sperr WR, et al. Molecular characterization of dog albumin as a cross-reactive allergen. J Allergy Clin Immunol 1994; 93:614–627.
48. Dandeu JP, Rabillon J, Divanovic A, et al. Hydrophobic interaction chromatography for isolation and purification of Equ.c1, the horse major allergen. J Chromatogr B Biomed Appl 1993; 621:23–31.
49. Gregoire C, Rosinski-Chupin I, Rabillon J, et al. cDNA cloning and sequencing reveal the major horse allergen Equ c 1 to be a glycoprotein member of the lipocalin superfamily. J Biol Chem 1996; 271:32951–32959.
50. Goubran Botros H, Poncet P, Rabillon J, et al. Biochemical characterization and surfactant properties of horse allergens. Eur J Biochem 2001; 268:3126–3136.
51. Lascombe M-B, Gregoire C, Poncet P, et al. Crystal structure of the allergen Equ c 1—a dimeric lipocalin with restricted IgE-reactive epitopes. J Biol Chem 2000; 275:21572–21577.
52. Bulone V, Krogstad-Johnsen T, Smestad-Paulsen B. Separation of horse dander allergen proteins by two-dimensional electrophoresis - molecular characterisation and identification of Equ c 2.0101 and Equ c 2.0102 as lipocalin proteins. Eur J Biochem 1998; 253:202–211.

53. Cabanas R, Lopez-Serrano MC, Carreira J, et al. Importance of albumin in cross-reactivity among cat, dog and horse allergens. J Investig Allergol Clin Immunol 2000; 10:71–77.
54. Mäntyjärvi R, Parkkinen S, Rytkönen M, et al. Complementary DNA cloning of the predominant allergen of bovine dander: a new member in the lipocalin family. J Allergy Clin Immunol 1996; 97:1297–1303.
55. Ylönen J, Mäntyjärvi R, Taivainen A, et al. IgG and IgE antibody responses to cow dander and urine in farmers with cow-induced asthma. Clin Exp Allergy 1992; 22:83–90.
56. Rautiainen J, Rytkönen M, Syrjänen K, et al. Tissue localization of bovine dander allergen Bos d 2. J Allergy Clin Immunol 1998; 101:349–353.
57. Rouvinen J, Rautiainen J, Virtanen T, et al. Probing the molecular basis of allergy - Three-dimensional structure of the bovine lipocalin allergen Bos d 2. J Biol Chem 1999; 274:2337–2343.
58. Fahlbusch B, Rudeschko O, Schlott B, et al. Further characterization of IgE-binding antigens from guinea pig hair as new members of the lipocalin family. Allergy 2003; 58:629–634.
59. Rautiainen J, Rytkönen M, Parkkinen S, et al. cDNA cloning and protein analysis of a bovine dermal allergen with homology to psoriasin. J Invest Dermatol 1995; 105:660–663.
60. Gläser R, Harder J, Lange H, et al. Antimicrobial psoriasin (S100A7) protects human skin from *Escherichia coli* infection. Nat Immunol 2005; 6:57–64.
61. Lorusso JR, Moffat S, Ohman JLJ. Immunologic and biochemical properties of the major mouse urinary allergen (Mus m I). J Allergy Clin Immunol 1986; 78:928–937.
62. Cavaggioni A, Mucignat-Caretta C. Major urinary proteins, α_{2U}-globulins and aphrodisin. Biochim Biophys Acta 2000; 1482:218–228.
63. Price JA, Longbottom JL. Allergy to mice. II. Further characterization of two major mouse allergens (AG 1 and AG 3) and immunohistochemical investigations of their sources. Clin Exp Allergy 1990; 20:71–77.
64. Platts-Mills TA, Longbottom J, Edwards J, et al. Occupational asthma and rhinitis related to laboratory rats: serum IgG and IgE antibodies to the rat urinary allergen. J Allergy Clin Immunol 1987; 79:505–515.
65. Jeal H, Draper A, Jones M, et al. HLA associations with occupational sensitization to rat lipocalin allergens: a model for other animal allergies? J Allergy Clin Immunol 2003; 111:795–799.
66. Fahlbusch B, Rudeschko O, Szilagyi U, et al. Purification and partial characterization of the major allergen, Cav p 1, from guinea pig *Cavia porcellus*. Allergy 2002; 57:417–422.
67. Price JA, Longbottom JL. Allergy to rabbits. II. Identification and characterization of a major rabbit allergen. Allergy 1988; 43:39–48.
68. Baker J, Berry A, Boscato LM, et al. Identification of some rabbit allergens as lipocalins. Clin Exp Allergy 2001; 31:303–312.
69. Natter S, Seiberler S, Hufnagl P, et al. Isolation of cDNA clones coding for IgE autoantigens with serum IgE from atopic dermatitis patients. FASEB J 1998; 12:1559–1569.
70. Valenta R, Natter S, Seiberler S, et al. Molecular characterization of an autoallergen, Hom s 1, identified by serum IgE from atopic dermatitis patients. J Invest Dermatol 1998; 111:1178–1183.
71. Mossabeb R, Seiberler S, Mittermann I, et al. Characterization of a novel isoform of alpha-nascent polypeptide-associated complex as IgE-defined autoantigen. J Invest Dermatol 2002; 119:820–829.
72. Aichberger KJ, Mittermann I, Reininger R, et al. Hom s 4, an IgE-reactive autoantigen belonging to a new subfamily of calcium-binding proteins, can induce Th cell type 1-mediated autoreactivity. J Immunol 2005; 175:1286–1294.
73. Fluckiger S, Scapozza L, Mayer C, et al. Immunological and structural analysis of IgE-mediated cross-reactivity between manganese superoxide dismutases. Int Arch Allergy Immunol 2002; 128:292–303.
74. Mayer C, Appenzeller U, Seelbach H, et al. Humoral and cell-mediated autoimmune reactions to human acidic ribosomal P2 protein in individuals sensitized to *Aspergillus fumigatus* P2 protein. J Exp Med 1999; 189:1507–1512.
75. Limacher A, Glaser AG, Meier C, et al. Cross-reactivity and 1.4-Å crystal structure of Malassezia sympodialis thioredoxin (Mala s 13), a member of a new pan-allergen family. J Immunol 2007; 178:389–396.
76. Flückiger S, Fijten H, Whitley P, et al. Cyclophilins, a new family of cross-reactive allergens. Eur J Immunol 2002; 32:10–17.
77. Valenta R, Duchene M, Pettenburger K, et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. Science 1991; 253:557–560.
78. Spitzauer S, Pandjaitan B, Mühl S, et al. Major cat and dog allergens share IgE epitopes. J Allergy Clin Immunol 1997; 99:100–106.
79. Reininger R, Varga EM, Zach M, et al. Detection of an allergen in dog dander that cross-reacts with the major cat allergen, Fel d 1. Clin Exp Allergy 2007; 37:116–124.
80. Reddy BM, Karande AA, Adiga PR. A common epitope of β-lactoglobulin and serum retinol-binding proteins: elucidation of its core sequence using synthetic peptides. Mol Immunol 1992; 29:511–516.

81. Kamata Y, Miyanomae A, Nakayama E, et al. Characterization of dog allergens Can f 1 and Can f 2. 2. A comparison of Can f 1 with Can f 2 regarding their biochemical and immunological properties. Int Arch Allergy Immunol 2007; 142:301–308.
82. Saarelainen S, Rytkönen-Nissinen M, Rouvinen J, et al. Animal-derived lipocalin allergens exhibit immunoglobulin E cross-reactivity. Clin Exp Allergy 2008; 38:374–381.
83. Spitzauer S, Pandjaitan B, Söregi G, et al. IgE cross-reactivities against albumins in patients allergic to animals. J Allergy Clin Immunol 1995; 96:951–959.
84. Goubran Botros H, Gregoire C, Rabillon J, et al. Cross-antigenicity of horse serum albumin with dog and cat albumins: study of three short peptides with significant inhibitory activity towards specific human IgE and IgG antibodies. Immunology 1996; 88:340–347.
85. Boutin Y, Hebert J, Vrancken ER, et al. Mapping of cat albumin using monoclonal antibodies: identification of determinants common to cat and dog. Clin Exp Immunol 1989; 77:440–444.
86. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA 2002; 288:963–972.
87. Eggleston PA. Improving indoor environments: reducing allergen exposures. J Allergy Clin Immunol 2005; 116:122–126.
88. Cullinan P, Cook A, Gordon S, et al. Allergen exposure, atopy and smoking as determinants of allergy to rats in a cohort of laboratory employees. Eur Respir J 1999; 13:1139–1143.

13 Food Allergens

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INTRODUCTION

Food allergy is an immune system–mediated adverse reaction to food proteins that can affect multiple organs. Organ/systemic involvement includes cutaneous, gastrointestinal, respiratory, oral, and generalized reactions, including potentially lethal anaphylaxis (1,2). Reports of anaphylactic episodes are updated in a registry of the American Academy of Allergy Asthma & Immunology and the Food Allergy and Anaphylaxis Network (3). This registry has been maintained continuously to represent a systematic, although incomplete, accounting of fatal food-induced allergic reactions in the United States.

Similar reports from the United Kingdom stress the importance of identifying and reporting fatal food reactions (4). Risk factors include a family history of atopic disorders with environmental factors seemingly modulating the expression of food allergies (5,6). In young children, the most common food allergies are cow's milk, egg, peanut, wheat, soy, tree nuts, fish, and shellfish. Early childhood allergies to milk, egg, soy, and wheat usually resolve by school age (7). Adult food allergies primarily include shellfish, peanut, tree nuts, and fish. However, individuals with known food allergies experience food-induced anaphylaxis primarily because they are unaware that the food they eat contains the allergen to which they are allergic (8). In the British Anaphylaxis Campaign, foods were implicated in 112 of 126 reported cases in children (aged 1–15.9 years) and adults (aged 16–72 years); 14 of these reactions were to unidentified substances. There was a similar distribution among the two groups for common allergenic foods; however, adults reported more reactions to more unusual but internationally well-recognized allergenic foods (citrus foods, peas, bananas, kiwi, sesame, mustard, and food additives) (9). In a multicenter airway research collaboration of 21 North American emergency departments, a chart review of a randomly selected cohort of 678 out of 5396 cases, documentation of a known food allergy was identified in 41% of patients using food allergy or ICD-9 codes (10). Biphasic reactions, a recurrence of symptoms after resolution of the initial presentation, occur in up to 25% of fatal or near-fatal anaphylactic food reactions (11).

Absorption of dietary protein is important in the development and elicitation of food-induced allergic reactions. Investigations by Dirks et al. (12) were performed to determine the absorption and kinetics of allergenic peanut proteins and a possible local uptake from the oral cavity in healthy adults. In brief, donor mononuclear cells containing basophils from nonallergic individuals were stripped of cell-bound IgE, subsequently sensitized by adding serum IgE from double-blind, placebo-controlled food challenged (DBPCFC) positive peanut–allergic subjects and challenged with peanut allergen to establish a histamine-releasing standard curve. Individuals with negative case histories for peanut allergy, negative skin prick test responses, negative specific IgE assay results for peanuts, soy, and pollen, and fasted eight hours were asked to chew peanuts for two minutes and spit out the contents without swallowing. Plasma samples were collected at various time points and used to determine histamine-releasing capacity of the IgE-sensitized basophils. The histamine released was determined by fluorometric analyses and expressed as the percentage of the total cellular histamine content. Histamine release greater than 10% above negative serum controls was considered positive. Assay sensitivity was determined to be 5 pg of peanut protein per milliliter of serum. Results showed that peanut allergens with biologic activity can be measured in the plasma of subjects as early as 10 minutes after chewing peanuts and spitting

them out without swallowing. These findings demonstrate allergen absorption from the buccal mucosa, which is regarded to explain the early onset of many systemic reactions (12).

In another sensitization scheme, Strid et al. (13) demonstrated that the epicutaneous exposure to peanut proteins in a murine model prevents oral tolerance and enhances allergic sensitization. In experiments to determine transepithelial transport of peanut proteins in a murine model, ileal loops containing Peyer's patches were filled with solutions containing digested peanuts. Combined immunohistochemical analysis reveals both peanut allergens and M cells at the highly inductive Peyer's patch sites of the normal, presensitized mucosal immune system (14).

In a discussion of the classification of digestion resistance of food allergens, Jensen-Jarolim and Untersmayr (15) reported that both the quality and extent of clinical reactivity to food allergens correlate with the following classification of food allergens. Class 1 food allergens both sensitize and trigger allergic reactions via the oral route because they are quickly absorbed and distributed to the systemic immune system. However, class 2 food allergens do not sensitize orally because they are easily digested into small peptides and lose their sensitization potential. They elicit allergy indirectly when ingested by the fact that they cross-react with other allergens, typically inhalant allergens. Examples include birch pollen, Bet v 1, and its numerous homologues in apple, Mal d 1; celery, Api g 1; and carrot, Dau c 1.

Not all proteins in foods are allergenic of the more than 70 foods that cause food allergies (16). Historically, major food allergens are water-soluble glycoproteins with molecular weights ranging from 10 to 60 kDa. However, structural characteristics are important for a protein's allergenicity, and many food allergens occur naturally as dimers or trimers with molecular weights of 150 to 200 kDa (17). These oligomeric forms might have a higher allergenic potential than monomers because larger molecules have additional epitopes for IgE-mediated histamine release. For example, inhibition experiments demonstrate extensive IgE cross-reactivity of recombinant mugwort profilin and profilin from various pollens and food extracts (18). The parvalbumin allergen, Gad m 1, of the Atlantic cod (*Gadus morhua*) forms oligomers consisting of multiples of a single 12.5-kDa protein, in native (gel filtration) and under reducing conditions that does not completely dissociate the oligomeric structure consisting of 24, 38, and 51 kDa (19). This is considered a novel finding as no aggregation of fish parvalbumins is previously described, and work to determine whether oligomerization of this protein has an influence on allergenicity is underway.

Using size exclusion chromatography, Ara h 1, a major peanut allergen, appears to exist in an oligomeric structure rather than its stable trimeric state (20). Hyrodrophobic interaction chromatography causes the oligomers to partly dissociate into trimers, which leads to a change in tertiary structure of the monomeric subunits of the allergen. Monomers of Ara h 1 oligomers have a more compact tertiary structure compared with monomers in Ara h 1 trimers. Thus, as indicated in both Gad m 1 and Ara h 1, structural characteristics of oligomerization may be important for protein allergenicity.

There are no known unique biochemical or immunochemical characteristics for food allergens versus other allergens. Comparisons of primary structure (amino acid sequences) of allergenic proteins do not reveal typical patterns that could be related to allergenicity. Food allergens tend to be resistant to the usual food processing and preparation conditions and are comparatively resistant to heat and acid treatment, proteolysis, and digestion. For example, treatment of food allergens with acid concentrations simulating stomach acid typically has little effect on the specific IgE binding of the class 1 food allergens. There are, however, important exceptions, such as the major IgE-binding allergens in fresh fruits and some vegetables (class 2 allergens), which are affected by these physical conditions, resulting in dissociation into smaller peptides and loss of allergenicity. For example, Mal d 1, Api g1, and Dau c 1 are rapidly digested (15).

IgE immunogenicity is determined by factors that can be unrelated to the primary structure (amino acid sequence) of the protein and must take into account secondary and tertiary structure (folding, disulfide bonding, and oligomerization) and the food source from which the allergen has been characterized, e.g., Ara h 1 from peanuts. Additionally, the way allergens enter the body, epicutaneously or by ingestion, may have a significant impact on the immune response. Essentially, the protein must induce B cells to produce IgE to be an allergen and on reexposure induce an allergic response in sensitized individuals.

Table 1 Plant and Animal Food Allergens

Plant food allergens	
Prolamin superfamily	2S albumins, nsLTP
2S albumins	Ber e 1 (walnut), Ses i 2 (sesame)
Nonspecific lipid transfer proteins	Pru av 2 (cherry), Mal d 2 (apple)
Cereal α-amylase/trypsin inhibitors	Tri a 19 (wheat), Sec c 20 (rye), STI (soybean)
Cupin superfamily	7S and 11S seed storage proteins
Vicilins	Ara h 1 (peanut), Jug r 2 (walnut)
Legumes	Ara h 3/4 (peanut), Cor a 8 (hazelnut)
Profilin family	Api g 4 (celery), Pru av 1 (cherry)
Bet v 1 family	Gly m 4 (soybean), Ara h 8 (peanut), Pru av 1 (cherry)
Others	Gly m Bd 30K (soybean), Act c 1 (kiwi)
Animal food allergens	
Mammalian milk	α-lactalbumin, β-lactoglobulin, casein
Egg	Gal d 1 (ovomucoid, hen's egg)
Seafood	Pen a 1 (tropomysins, shrimp), Gad c 1 (calcium-binding parvalbumins, cod)

Source: From Refs. 21,25,26.

TAXONOMY OF FOOD ALLERGENS

Food allergens, found in plants and animals, are classified as to their biologic function or protein family group (21). Plant food allergens are contained in 31 of 8296 protein families (22) with the most important animal food allergens present in milk, egg, and seafoods. Examples of animal groups include birds (chicken and duck), crustaceans (crab and lobster), and red meats (beef and veal). Examples of plant groups include the apple family (apple and pear), grass family (corn and wheat), legume family (lentil and peanut), and walnut family (black walnut and pecan). Allergy, because of cross-reacting allergens, to one member of some food groups may result in a variable degree of clinical reactivity to other members of the same group.

Food allergens are named using the first three letters of the genus, followed by a single letter for the species and a number indicating the chronologic order of allergen purification (23). Standardization of food allergen nomenclature was proposed by the World Allergy Organization and refers to food hypersensitivity reactions as "food allergy" only when immunologic mechanisms are demonstrated. "The term *hypersensitivity* should be used to describe *objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons.* An allergen is an antigen causing allergic disease. If IgE is involved in a food-related reaction, the term IgE-mediated food allergy is appropriate." As a consequence, entities within the field of environmental medicine, such as multiple chemical sensitivity, multisymptomatic reactions (amalgam in tooth fillings), and electrical waves do not fulfill these criteria. All other reactions are described as "nonallergic food hypersensitivity" (24). How nomenclature and structural biology play a crucial role in defining allergens for research studies and for the development of new clinical products is reviewed in Ref. 25. An abbreviated listing of the families and examples of plant and animal proteins is presented in Table 1. A format with listed allergens is also available in the Allergome e-mail Newsletter (Table 2).

MAJOR AND MINOR FOOD ALLERGENS

The most common foods that cause IgE-mediated reactions in childhood are cow's milk, eggs, peanut, soybean, wheat, fish, and tree nuts (Table 3). Approximately 80% of these reactions are secondary to milk, eggs, and peanut alone. In adulthood, the most common food allergens are peanut, tree nuts, fish, and shellfish. Worldwide, there are some differences in which foods cause problems in both children and adults primarily based on different diets (29).

Cow's Milk

A number of milk proteins are allergenic and patients react to various cow's milk proteins by either skin prick tests or challenge. Caseins and β-lactoglobulin are the major allergens in cow's

Table 2 AllFam Allergen Family Chart

Protein family name	Number of allergens
Prolamin superfamily	56
Profilins	44
EF hand domain	32
Tropomysins	27
Cupin superfamily	25
Expansins, C-terminal domain	23
Bet v 1–related proteins	22
PR-1 proteins	21
Lipocalins	20
Subtilisin-like serine proteases	17

Source: From Refs. 27,28.

Table 3 Major Food Allergens in Children and Adults

Children	Adults
Milk	Peanut
Egg	Tree nuts
Peanut	Fish
Soybean	Shellfish
Wheat	
Fish	
Tree nuts	

milk. Casein, a phosphoprotein, is the major protein of bovine milk that exists in equilibrium of soluble and complex colloidal aggregates (micelles). Its heterogeneity has long been recognized as consisting of α-, β-, γ-caseins (75%, 22%, and 3%, respectively). The major α- and β-caseins have molecular weights of approximately 23 kDa, and there are several genetic variants of each. β-Lactoglobulin (17 kDa), the most abundant whey protein, also has several genetic variants. α-Lactalbumin (14 kDa) and bovine serum albumin (67 kDa), both whey proteins, are minor cow's milk allergens. The IgE-binding epitopes on the milk caseins (30), lactalbumin and lactoglobulin, are identified (31). So too are specific IgE-binding epitopes that may differentiate between patients with persistent and transient cow's milk allergy (32,33).

Confusion can arise in the determination of cow's milk allergy because of the different forms of cow's milk used in challenges, e.g., liquid cow's milk, nonfat dry milk, and infant formula. Similarly, reported incidences of inadvertent or unexpected exposure to different milk proteins, e.g., casein in sausage equivalent to 60 mg of casein, have led to fatal anaphylaxis (34). Reliable analytical results for milk allergens (casein, lactalbumin, and lactoglobulin) in nondairy allergic reactions are needed for them to be temporally associated with milk allergy. Data from Morisset et al. (35) suggest that in patients allergic to egg, peanut, and milk, detection tests should show a sensitivity of 10 parts per million (ppm) for egg, 24 ppm for peanut, and 30 ppm for cow's milk, respectively, when 110 g of a food source is consumed. Wal's (36) update on the biochemistry and immunochemistry of milk proteins indicates that no single allergen or structure accounts for milk allergenicity or provides for a predicted allergic response. The great variability in the polysensitization and IgE responses to cow's milk and potential immunologic cross-reactions to milk of other species, such as buffalo's, goat's, ewe's, and camel's milk, will vary according to the characteristics of the population studied. Any milk product that contains native or denatured milk proteins or fragments derived thereof may trigger an allergic reaction, even those present in nondairy foods.

Egg

Several studies have identified the major egg allergens (37,38). Ovomucoid (Gal d 1), a glycoprotein with a molecular weight of 28 kDa and an acidic isoelectric point, is the major egg allergen (39). In a study of 18 children with egg allergy, ovomucoid was found to be a more potent allergen than purified ovalbumin as determined by skin prick test and radio-allergosorbent test (RAST). While previous studies indicate that ovalbumin was the major egg

allergen, this study demonstrates ovomucoid contamination of the ovalbumin, accounting for this discrepancy. Ovalbumin (Gal d 2) is a monomeric phosphoglycoprotein with a molecular weight of 43 to 45 kDa and an acidic isoelectric point. Purified ovalbumin has three primary variants, A_1, A_2, and A_3. It is difficult to determine the exact role of this allergen because of ovomucoid contamination of ovalbumin (39). Ovotransferrin (Gal d 3) or conalbumin has a molecular weight of 77 kDa and an acidic isoelectric point. It has antimicrobial activity and iron-binding properties. Lysozyme (Gal d 4) is a lower molecular weight allergen (14.3 kDa) that in some studies appears to be a major allergen, but in other studies is thought to be a minor allergen. Other minor allergens in eggs include apovitellin, ovomucin, and phosvitin. Additional studies show that the carbohydrate portion of the glycoproteins in eggs, particularly in ovomucoid, does not play a primary role in specific IgE binding. B- and T-cell epitopes for ovalbumin and ovomucoid are mapped in a limited way, as are the major IgE- and IgG-binding epitopes of ovomucoid (40).

Four sets of distinct egg allergic groups (A, lysozyme and ovalbumin; B, ovomucoid; C, ovomucin; and D, ovotransferrin and the yolk proteins apovitelenins I and VI and phosvitin) were identified in 40 subjects that react to four discrete hen's egg protein sets (1, egg white proteins lysozyme and ovalbumin; 2, egg white ovomucoid; 3, egg white ovomucin; and 4, egg yolk proteins apovitelenins I and IV and egg white ovotransferrin) (41). Both lysozyme and ovomucin bind significant amounts of IgE in the sera of patient groups A and C. Lysozyme was statistically correlated with ovalbumin and was a significant allergen for group A. Other differences in IgE binding were found that may explain why various investigators report different allergens to be important in egg hypersensitivity.

Allen et al. (42) reviewed and identified key points of egg allergy. It primarily affects preschool children. Life-threatening reactions are less common in egg than in peanut/tree nut allergy, and heat and digestion alters the allergenicity of egg proteins. Heating reduces the allergenicity of ovomucoid and ovalbumin, but does not affect lysozyme. Ovomucoid allergenicity may also be reduced by gastric pH. It is possible that the age and/or the use of inhibitors of gastric acid secretion in young children may be a factor that promotes egg protein food sensitization.

Peanut

Peanut allergy in 14 of 22 preschool children in Southampton and South Manchester with a history of mild-to-moderate peanut allergy spontaneously resolved with time (43). However, the recurrence rate in patients who outgrow peanut allergy is 7.9%, and the risk is significantly higher in patients who avoid known peanut foods following resolution of their peanut allergy (44). Peanut proteins are customarily classified as albumins (water soluble) and globulins (saline soluble). The globulin proteins are made up of two major fractions, arachin and conarachin, also known as legumin and vicilin, respectively. Arachin, in its native state exists as a molecule of at least 600 kDa and readily dissociates into a 340- to 360-kDa dimer and a monomer of approximately 170 to 180 kDa. Conarachin can be divided by ultracentrifugation into two fractions, 2S and 8.4S.

Historically, peanut-1 and concanavalin A–reactive glycoprotein were some of the first peanut allergens partially characterized using peanut-specific IgE from allergic subjects. With the advent of more refined methods for allergen purification, allergens were named using a systematic nomenclature—abbreviated Linnean genus and species names and an Arabic number to indicate the chronology of allergen purification. Thus, Ara h 1, is a 63.5-kDa glycoprotein identified as the first major peanut allergen using immunoblotting and enzyme-linked immunosorbent assay (ELISA) (45). This allergen has an acidic isoelectric point and is relatively resistant to enzymatic degradation. There are multiple IgE-binding sites in the amino acid sequence of Ara h 1, which has at least 23 specific IgE-binding epitopes. Ara h 1 is a member of the vicilin family of seed storage proteins. Ara h 2 is a 17-kDa allergen with an acidic isoelectric point that has at least 10 specific IgE-binding epitopes along its amino acid sequence and is a member of the conglycinin family of seed storage proteins. Ara h 3 is a glycinin seed storage protein with a molecular weight of 60 kDa. Approximately 45% of patients with peanut allergy have specific IgE to this allergen (46,47).

Ara h 2 is the most potent allergen in a functional IgE cross-linking assay using RBL SX-38 cells with sera from 12 highly sensitive peanut-allergic individuals (48). Mechanistically, soluble peanut antigen (PNAg) and Ara h 1 were prepared from defatted peanut flour and

both induce the Erk1/2 phosphorylation of monocyte-derived dendritic cells consistent with T helper 2 (Th2) adjuvant activity (49). Altogether, eight allergens are identified—Ara h 1 to Ara h 8 (50), with Ara h 1, 2, and 3 considered to be the main allergens (51,52). Advances in peanut allergy are reviewed in Ref. 53.

Soybean

Globulins are the major proteins of soybean. These globulins can be separated into ultracentrifugation fractions 2S, 7S, 11S, and 15S. α-Conglycinin is a primary protein of the 2S fraction while β-conglycinin is the primary fraction of the 7S component. The glycinin fraction is the primary component of the 11S ultracentrifugation fraction.

Soybeans, like peanut, are legumes that contain multiple allergens (54,55). When examining specific IgE to ultracentrifugation components, the 2S or 7S fraction contain the primary allergens. Gly m 1 is a 30-kDa allergen that is a component of the 7S fraction. The majority of patients have soybean-specific IgE to Gly m 1 (56). Gly m 1 has an acidic isoelectric point and sequence homology to a soybean seed 34-kDa oil-body-associated protein, soybean vacuolar protein P34. There are at least 16 distinct soybean-specific IgE-binding epitopes along the amino acid sequence of this allergen. The Kunitz soybean trypsin inhibitor binds soybean-specific IgE in soybean-allergic patients, although only in a minority of patients, making it a minor allergen. A comprehensive review is available for the identification and characterization of soybean allergens with current techniques to reduce allergenicity including thermal, enzymatic, chemical, traditional breeding, and genetic modification of the allergens (57).

Wheat

Wheat and other cereal grains are often implicated as food allergens, particularly in children (58). The proteins of wheat include the water-soluble albumins, the saline-soluble globulins, the aqueous ethanol-soluble prolamins, and the glutelins. Patients with wheat allergy have specific IgE to wheat fractions 47 kDa and 20 kDa, proteins not recognized by the sera of patients with grass allergy. Wheat α-amylase inhibitor (15 kDa) is also a major wheat allergen. This protein does not bind IgE from wheat-tolerant control patients, including those with grass allergy (59,60). Battais et al. (61) identified major wheat allergens in the water/salt soluble, gluten fractions, and isolated wheat fractions by IgE-binding studies. IgE from wheat-dependent exercise-induced anaphylaxis and urticaria reacts with sequential epitopes (QQX1PX2QQ) in the repetitive domain of gliadins whereas IgE from atopic eczema/dermatitis patients recognizes conformational epitopes (62,63).

Fish

Fish and shellfish allergy are extensively reviewed in Ref. 64 with parvalbumins and tropomyosins identified as the major allergens, respectively. The clinical manifestations, diagnosis, immunologic mechanisms, and molecular biology of seafood allergens are discussed in Ref. 65.

The consumption or inhalation of fish allergen is a common cause of IgE-mediated food reactions. The incidence of fish allergy is believed to be much higher in countries where fish consumption is greatest. For example, codfish allergy is common in the Scandinavian countries (66). Red and golden snapper, local species of snapper consumed in Malaysia, commonly cause food allergy with parvalbumin being considered as a minor allergen (67).

One of the most comprehensive descriptions of a food allergen is by Esayed and Apol of codfish allergen, Gad c 1 (originally called Allergen M) (68). Gad c 1 belongs to a group of muscle proteins known as parvalbumins. The parvalbumins control the flow of calcium in and out of cells and are only found in the muscles of amphibians and fish. This allergen has an acidic isoelectric point and a molecular weight of 12 kDa. The tertiary structure of Gad c 1 has three domains. There are at least five IgE-binding sites on the allergen and the carbohydrate moiety does not appear to be important in its allergenicity. Fish consumed in India include pomfret and hilsa, which contain heat-labile allergens, while bhetki and mackerel have more heat-stable allergen characteristics (69).

Shrimp

Shrimp is the most studied of the Crustacea allergens (70,71). The IgE-binding epitopes of the shrimp allergen Pen a 1 are now identified (72,73). Deduced amino acid sequence of 284 amino acids

from recombinant allergens and amino acid sequences from allergenic and nonallergenic vertebrate tropomysins reveal 80% to 99% and 51% to 58% amino acid sequence homology, respectively. Analysis of secondary structure for natural and recombinant Pen a 1 shows that both have α-helical conformation that is typical for tropomysins with essentially the same IgE-binding capacity as determined by RAST (74).

Tree Nuts

Tree nuts cause food-allergic reactions in both children and adults. Just as allergic reactions to fish and peanuts can persist for life, so too can reactions to tree nuts. Hazelnut, walnut, cashew, and almond are the most common tree nuts responsible for allergic reactions with less frequent reactions to pecan, chestnut, Brazil nut, pine nut, macadamia nut, pistachio, coconut, and Nangai (75). Clark and Ewan (76) reviewed the development, sensitization, and clinical impact of tree nut allergens suggesting that multiple nut sensitizations and allergies can take place in utero or soon after birth. Findings from the study reveal that a large proportion of children aged 0 to 1 year were already sensitized (nut-specific IgE) to almond, Brazil, hazel, and walnut. Potential sensitization routes included breast milk, peanut- or soya-containing infant formula, and trace contamination of infant diet and use of peanut- or soya-containing eczema creams. Two major allergens in almonds are identified: 70-kDa heat-labile and 45- to 50-kDa heat-stable proteins. Although several different Brazil nut proteins are allergens, the major one, Ber e 1, is a high-methionin-containing protein (77). This 12-kDa protein has two subunits, a 9-kDa and a 3-kDa protein. A major walnut allergen is a 65-kDa glycoprotein, Jug r 2, similar to other plant vicilins, as well as another walnut allergen, Jug r 1, a 2S albumin seed storage protein (78).

OTHER ALLERGENS

Protein and oil components of sesame seed cause immediate and delayed hypersensitivity reactions (79). Lupine allergens extensively cross-react with other legume species (80,81). Kiwi allergy is increasing (82,83) with kiwellin, a cysteine-rich 28-kDa protein, isolated as the important allergen (84). Apple peel extract from 10 different apple varieties show both antigenic and allergenic activity associated with the oral allergy syndrome (85). Oilseed rape and turnip napin 2S allergens have been identified (86). An N-linked glycan from oranges has specific IgE-binding properties (87). Examples of pollen-food related syndromes are listed in Table 4.

HIDDEN ALLERGENS

Hidden allergens are protein sources that often are not recognized or included on product labels. In a United States study, there was a known allergy to the triggering food in 41% of emergency department visits for allergic reactions to foods (10). Of the 32 food-related fatalities reported in Ref. 95, at least 87% of subjects had a prior history of a reaction to the responsible food allergen. In a retrospective study of the 530 food-allergic reactions, 22.4% were considered to be due to hidden allergens (96) (Table 5). Although food-induced anaphylaxis is well known, continued ongoing research seeks to improve both diagnostic tools and to improve predictors of anaphylaxis risk.

Table 4 Pollen- and Respiratory Food–Related Syndromes

	Reference
Pollen	
Pollen/food allergy syndrome	88
Ficus fruit syndrome	89
Latex fruit syndrome	90,91
Oral allergy syndrome	92
Respiratory	
Flour	93
Seafood	94

Table 5 Source of Hidden Food Allergens

Food group	Food source
Shellfish	Oil, contaminated equipment
Fish	Cooking oil, salmon cream canape
Nuts	Chocolates, cookies, pastries, cakes
Fruits	Ice creams
Legumes	Ham, sausage, cheese puffs
Egg	Ice cream, pastries

Other examples of hidden allergens include uncooked anchovy, fish, and shellfish, contaminated with *Anisakis simplex* larvae, a fish worm truly responsible for the allergic reactions, accounting for the majority of anaphylactic reactions in a Spanish geographical area (96). In another case, a lupine allergen with a molecular weight close to 14 kDa was detected in extracts from cookies, a chicken bouillon cube, and a chicken dehydrated soup (97). Similarly, a human sera–based immunoassay identified the presence of unknown peanut allergens in products belonging to various food categories, such as cereals, cookies, cakes, and snacks (98). In most cases, food producers are able to detect and eliminate this sort of contamination and implement measures to prevent the presence of hidden allergens in their products, thereby increasing food safety for sensitized persons.

DIAGNOSIS

Food allergy often manifests as gastrointestinal symptoms, and patients therefore often consult a family physician or a gastroenterologist who may not be sufficiently knowledgeable to diagnose food allergy (99–101). Allergic reactions to foods are the most common cause of life-endangering anaphylaxis (102). Asero et al. (103) in a review of food allergy identified a major problem in the diagnosis of food allergy, i.e., the relatively poor "clinical specificity" of both skin and in vitro tests. Another major problem in appropriately diagnosing food allergy is the fact that there are no standardized food allergen extracts (104).

A detailed medical history, physical examination, and appropriate laboratory tests are necessary to diagnose food allergy (Table 6). Skin prick test and RAST are sensitive indicators of food-specific IgE; however, they are not very predictive of clinical sensitivity (105). A positive skin test to a food indicates the possibility that the patient has symptomatic reactivity to that specific food, although overall the positive predictive accuracy is less than 50%. A negative skin test confirms the absence of an IgE-mediated reaction with an overall negative predictive accuracy of greater than 95%. The definitive diagnosis of food allergy is based on standardized oral challenges.

On the basis of previously established 95% predictive decision points for egg, milk, peanut, and fish allergy, greater than 95% of food allergies diagnosed in prospective study of 100 children were correctly identified by quantifying serum food-specific IgE concentrations (106). Using allergen-specific IgE values for egg, 6 kUA/L; milk, 32 kUA/L; peanut, 15 kUA/L; and fish, 20 kUA/L as diagnostic decision points, the positive predictive values in this prospective study ranged from 96% to 100%. By using decision points of 100 kUA/L for wheat and 65 kUA/L for soy, the predictive accuracy was 100% and 86%, respectively. Using these predictive values, the authors have been able to reduce DBPCFC by 40% to 50%.

Table 6 Important Information from Medical History

The food suspected to have provoked the reaction
The quantity of the food ingested
The length of time between ingestion and development of symptoms
A description of the symptoms provoked
If similar symptoms developed on other occasions when the food was eaten
If other factors (e.g., exercise) are necessary
The length of time since the last reaction

Over a nine-year period in a tertiary clinic in Australia, infants and young children with a large skin prick test wheal (mean size 8–10 mm) was associated with a >95% likelihood of clinical reactivity to cow's milk, egg, and peanut, as confirmed by an open food challenge (107). For each food it was possible to identify a skin wheal diameter at and above which negative reactions did not occur: cow's milk, 8 mm; egg, 7 mm; and peanut, 8 mm. In contrast, positive reactions could occur even with a skin wheal diameter of 0 mm. Therefore, the DBPCFC remains the gold standard to determine food allergy.

A retrospective chart review of 584 food challenges where 253 (43%) resulted in an allergic reaction, the median food-specific IgE for a failed challenge was 2.0 kUA/L for milk, 1.2 kUA/L for egg, 1.9 kUA/L for peanut, 9.3 kUA/L for soy, and 19.6 kUA/L for wheat (108). Celik-Bilgili S et al. (109) calculated a 90% positive predictive serum-specific IgE level for cow's milk to be 88.8 kU/L and for hen's egg to be 6.3 kU/L that correlated with positive challenge studies. In each case, caveats include the age of the population under investigation and sensitivity and specificity of the allergen-specific IgE.

A newer method to confirm food allergy is by food allergen epitope recognition patterns, conformational versus sequential and number of epitopes recognized, combined with the intensity of IgE binding by microarray analysis. They are considered to be important determinants of severity and duration of food allergy (51).

Food challenges, when necessary, should be administered with the patient in a fasting state, starting with a challenge dose of the food in question unlikely to provoke symptoms, generally 125 mg to 500 mg of lyophilized food. This dose is then increased every 15 to 60 minutes depending on the historical reaction. A similar scheme is followed with the placebo portion of the study. Clinical reactivity can be ruled out when the blinded patient tolerates up to 10 g of lyophilized food in capsules or liquid. If the blinded portion of the challenge is negative, it must be confirmed by an open feeding under observation to rule out rare false-negative challenges (Table 7). Retrospective studies have been investigated to determine serum-specific IgE concentrations in subjects that underwent oral food challenges (Table 8).

Shreffler et al. (51) demonstrated that 97% of subjects with peanut allergy have IgE that reacts to at least one peanut allergen, whereas 77%, 75%, and 77% recognized rAra h 1, rAra h 2, and rAra h 3, respectively. Subsequently, Astier et al. (118) showed that cosensitization with rAra h 1, 2, and 3 is more predictive of clinical reactions and even more severe reactions.

Table 7 Sample Schedule for Double-Blind, Placebo-Controlled Food Challenge[a]

Time (min)	Food	Time (hour of day) (PM)	Placebo
0:00	125–500 mg	3:00	500 mg
0:15	1 g	3:15	1 g
0:30	2 g	3:30	2 g
0:45	3 g	3:45	3 g
60	3.5 g	4:00	3.5 g

[a]For a review of DBPCFC recipe validation of food challenge materials for children, the reader is referred to the Journal of Allergy Online Repository (http://www.jacionline.org/article/S0091-6749(03)02481-3/fulltext).

Table 8 Novel Food Therapies

Therapy	Mode of action	Reference
TNX-901	Anti-IgE antibody	111
Chinese herbal medicine	Unknown	112
Birch pollen immunotherapy	Tolerance	113
Peptide(s)	Tolerance	110
Chimeric antibodies	Tolerance	114
Probiotics and Th adjuvants	Tolerance/Th switch	115
Dietary manipulation	Avoidance	116,117

Source: From Ref. 110.

Table 9 Published Values for Diagnosis (IgE), Exposure, and Threshold Response Concentrations

Food	Diagnosis (kUA/L specific antibody)	Exposure (grams food/protein)	Threshold response (milligrams protein)
Egg	≥7 (120); 6 (121)	43 g (4.3 g protein) (122)	1/20 (2000 mg protein) (123)
Milk	32 (121)	226 g (7.2 g protein) (122)	30 mL(965 mg protein) (123)
Peanut	15 (121)	14 g (3 g protein) (122)	1½ (500 mg protein) (124)
Fish	20 (121)	NA[a]	NA

[a]Not available.

THERAPIES

Nowak-Wegrzyn and Sampson (119) reviewed novel therapies for food allergy (Table 9). The immunotherapeutic strategies to treat peanut allergy suggest that a combination therapy of anti-IgE and other therapies could work (125). In a randomized, uninterrupted avoidance versus oral desensitization protocol of children with IgE-dependent milk or egg allergy, oral desensitization helped children overcome their food allergy while those on avoidance diets often develop a lower threshold of reactivity to the incriminated food as evidenced by single-blind, placebo-controlled food challenge (SBPCFC) (126). A SBPCFC to milk was positive in 11.1% of those following oral desensitization versus 40% after avoidance, whereas for egg allergy, a SBPCFC was positive in 30.6% after oral desensitization versus 48.6% for avoidance.

THRESHOLD DOSES

A better knowledge of clinical reactive thresholds in still needed (123). Small quantities of hidden allergens in foods and their concentrations are calculated to be in the range of 0.003% to 2.3% (wt/wt) (127). Taylor et al. (128) presents a comprehensive review of problems encountered by industry and the rational link with the determination of threshold doses as well as a thorough report of the experience of several clinical groups with a daily practice of standardized food diagnostic challenges. Morisset et al. (129) reports minimal reactive quantities that guarantee a 95% safety for patients who are allergic to egg, peanut, or milk. On the basis of consumption of 100 g of food, the detection tests should ensure a sensitivity of 10 ppm for egg, 24 ppm for peanut, and 30 ppm for milk. Lowest reactive threshold doses from 125 positive oral challenges to egg, 103 to peanut, 59 to milk, and 12 to sesame were 2 mg of egg, 5 mg of peanut, 0.1 mL of milk, and 30 mg of sesame seed, respectively.

Factors that are likely to modify threshold or response levels include the allergen(s) in the food, exercise, simultaneous intake of aspirin, regular treatment with β-blockers or angiotensin-converting enzyme inhibitors, association with other food/pollen allergens, alcohol, and content of fat in the food or meal. Additional problems include processing, storage, and genetically modified food technologies that may alter a protein or food itself exposing neoallergens (130). Guidelines to assist clinicians and allergic individuals continue to be challenging in light of these many variables.

MECHANISMS

Allergen-reactive Th2 cells play a central role in the pathophysiology of allergic diseases. A summary of food allergens mapped for T-cell epitopes indicates that T-cell activating peptides also bind IgE antibodies (131). T-cell subset patterns reveal that the childhood IL-4 cytokine response is predominantly from CD4+ CD45RO+ cells, whereas IL-4 and IFN-γ secretion of nonallergic controls involves CD4+ and CD8+ CD45RO+ cell populations (132). Food-allergic IL-4 cytokine response to relevant allergens is predominantly from a memory population of CD4+ CD45RO+ cells, whereas INF-γ and IL-4 of nonallergic controls is predominantly from mixed CD4+ and CD8+ CD45RO+ cells.

Using carboxyfluorescein succinimidyl ester labeling and cloned T cells to investigate food antigen–specific T-cell responses in peanut-allergic and nonallergic children and children

who outgrew their food allergy, nonallergenic foods (β-lactoglobulin, ovalbumin) induce a Th1-skewed cytokine profile regardless of the allergic status of the donors (133). This Th1-skewing induced by nonallergenic food antigens is characterized by a high production of IFN-γ and TNF-α, almost undetectable production of IL-4 and IL-5, and relatively low production of IL-13. Peanut-allergic donors show a Th2 polarization of cytokine production by peanut-specific cells (IFN-γ^{low}, TNF-α^{low}, IL-4^{high}, IL-5^{high}, IL-13^{high}), confirming that these cells are indeed peanut specific by cloning.

Children, age two to five years, with active egg allergy atopic dermatitis and those who had outgrown their egg allergy, were used to detail their peripheral blood lymphocyte immunologic response (134). A marked increase in IL4 and a decrease in IFN-γ synthesis by peripheral blood lymphocytes following ovalbumin specific in vitro stimulation were observed in active atopic dermatitis. In contrast, ovalbumin-induced IL4 synthesis in patients in remission was comparable to that in normal individuals. Children with clinically resolving milk allergy had a persisting Th2 cytokine response. Th2-cell-dependent, peanut-specific IL-5, IL-13, and CCL22 were common in peanut-tolerant individuals regardless of whether they had a positive or negative skin test (135). This led the authors to suggest that the continuum and spectrum of Th2 responses among individuals with negative and positive skin tests might be more important than the Th1/Th2 balance.

Children who outgrew their milk allergy (tolerant children) had higher frequencies of circulating CD4(+)CD25(+) T cells and decreased in vitro proliferative responses to bovine β-lactoglobulin in peripheral blood mononuclear cells (PBMCs) compared with children who maintained clinically active allergy. Clearly, there is additional research needed to address the significance of Th1/Th2 cytokine balance, T regulatory cells defined as regulatory T (Treg) cells, and allergen-specific IgE and skin test responses that could be related to the allergen and mucosal response.

Comparison of phenotypic and functional characterization of PBMCs before and after in vivo milk challenges from children with clinical active or resolved cow's milk allergy suggests a possible mechanism for mucosal induction of tolerance against β-lactoglobulin (136). Gastrointestinal lymphoid tissue activation and expression of specific effector T cells and CD4+CD25+ Treg are thought to be responsible for the reduced in vitro proliferative response to β-lactoglobulin in children who outgrow their cow's milk allergy.

Factors necessary for a food protein antigen to function as an allergen remain unidentified, even though structural and biochemical characteristics that provoke allergic responses continue to be discovered. Allergen-specific antibody-binding sites are routinely limited to short amino acid sequences of proteins identified as sequential or conformational. Specific cross-reactivity with proteins sharing sufficient sequential and/or conformational homology can exist with the original immunogen/allergen as well as other related proteins (137). Continuous epitopes of allergens consist of amino acids in the primary sequences, while conformational epitopes exist as a consequence of three-dimensional folding of the proteins. Therefore, divergent patterns of cross-reactivity and clinically relevant allergic reactions to foods can occur in individual patients (138) (Table 10).

To trigger an allergic reaction, at least two different epitopes on the surface of the allergen must cross-link IgE to result in a biologic response. Sequential epitopes, consisting of linear amino acid sequences, have been characterized by preparing overlapping peptides derived from the primary allergen sequence (139). Conformational epitopes have nonlinear

Table 10 Food Allergen Cross-Reactivity

	Specific IgE to multiple members of the family	Clinical reactivity
Milk	Common	Common
Legumes	Common	Uncommon
Wheat	Common	Uncommon
Fish	Common	Uncommon
Crustacea—mollusks	Common	Unknown or questionable
Tree nuts	Common	Uncommon
Egg—chicken	Occasional	Rare
Milk—beef	Occasional	Uncommon

amino acid sequences due to secondary and tertiary structure. Evidence for conformational epitopes have been determined using resolution of antigen-antibody complex structures (140), site-directed mutagenesis using mismatched oligonucleotides prepared by polymerase chain reaction to alter the amino acid sequences (141,142), and peptide mimics (individual peptides isolated from peptide libraries that frequently yield peptides mimicking the binding site of the cognate antigen for the specific antibody but does not correspond to the linear sequence) (143). Using a Bet v 1–related food allergy as a model, five patients were investigated by cross-competitive ELISA for specific and cross-reacting IgE to Bet v 1 and its homologues, Gly m 4 (soybean), Ara h 8 (peanut), and Pru av 1 (cherry) (144). By competitive immunoscreening and epitope mapping of these birch pollen–related food, the model revealed patient-specific IgE epitopes; however, one surface binding area was recognized by all patients and two areas were recognized by three patients. Cross-reactivity can further be assessed by structural similarity using an in silico homology search in combination with in vitro IgE antibody assays (145). Using peptides or mimotopes for allergen molecules, epitopes in a three-dimensional format can be studied by crystallographic comparisons. IgE epitopes identified in this manner were all conformational and responsible for high-affinity interactions with specific IgE preferentially forming di-, tri-, or multimers that display repetitive IgE epitopes (146). As B lymphocytes are pattern recognizers, this feature is essential for a memory response, but may also be critical for the very first allergen contact and initiation of the IgE response.

CONCLUSION

The incidence of food-allergic-induced anaphylaxis is increasing. The natural history of food allergy varies; some go away with time while others are lifelong. New food allergens continue to be recognized, identified, and characterized at the molecular level, and the immunologic mechanisms responsible for both food tolerance and hypersensitivity are under investigation. Although several experimental treatments are promising, none are currently clinically available for the practicing physician.

Detailed studies on how foods sensitize will become more feasible as the structure and molecular properties of food allergens are finalized and more standardized food allergens become available for diagnosis. Continuing studies on the patterns of sensitization will give more insight into the development and possible treatment of protein-related food allergy.

SALIENT POINTS

- Food allergens, found in plants and animals, are classified on the basis of their biologic function or protein membership families.
- A complete medical history and physical examination combined with laboratory testing is essential to diagnose food allergy.
- Many food allergens occur naturally as dimers or trimers often making their molecular weight 150 to 200 kDa.
- IgE immunogenicity is accurately determined by factors unrelated to the primary structure of the protein and must take into account the food source and how the allergen enters the body—essentially, the protein must induce B cells to produce IgE.
- Food allergies are different throughout the world because of different foods and diets.
- A retrospective study of food-allergic reactions reveals that 22.4% are due to hidden allergens.

REFERENCES

1. Sampson HA. Update on food allergy. J Allergy Clin Immunol 2004; 113:805–819.
2. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2006; 117:S470–S475.
3. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001–2006. J Allergy Clin Immunol 2007; 119:1016–1018.
4. Pumphrey RSH, Gowland MH. Further fatalities caused by anaphylactic reactions to food, 2001–2006. J Allergy Clin Immunol 119:118–119.

5. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey. J Allergy Clin Immunol 2003; 112:1203–1207.
6. Sicherer SH, Leung DYM. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. J Allergy Clin Immunol 2007; 119:1463–1469.
7. Wood RA. The natural history of food allergy. Pediatrics 2003; 111:1631–1637.
8. Wang J, Sampson HA. Food anaphylaxis. Clin Exp Allergy 2007; 37:651–660.
9. Uguz A, Lack G, Pumphrey R, et al. Allergic reactions in the community: a questionnaire survey of members of the anaphylaxis campaign. Clin Exp Allergy 2005; 35:746–750.
10. Clark S, Bock SA, Gaeta TJ, et al. Multicenter study of emergency department visits for food allergies. J Allergy Clin Immunol 2004; 113:346–352.
11. Golden DB. Patterns of anaphylaxis: acute and late phase features of allergic reactions. Novartis Found Symp 2004; 257:101–115.
12. Dirks CG, Pedersen MH, Platzer MH, et al. Does absorption across the buccal mucosa explain early onset of food-induced allergic systemic reactions. J Allergy Clin Immunol 2005; 115:1321–1323.
13. Strid J, Hourihane J, Kimber I, et al. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. Clin Exp Allergy 2005; 35(6):757–766.
14. Chambers SJ, Wickman MS, Regoi M, et al. Rapid in vivo transport of proteins from digested allergens across pre-sensitized gut. Biochem Biophys Res Commun 2004; 325:1258–1263.
15. Jensen-Jarolim E, Untersmayr E. Food safety: in vitro digestion tests are non-predictive for allergenic potential of food in stomach insufficiency. Immunol Lett 2006; 102:118–119.
16. The InformAll Database. Available at: http://food allergens.ifr.ac.uk.
17. Lemanske RF Jr., Taylor SL. Standardized extracts, foods. Clin Rev Allergy 1987; 5(1):23–36.
18. Wopfner N, Willeroidee M, Hebenstreit D, et al. Molecular and immunological characterization of profilin from mugwort pollen. Biol Chem 2002; 383(11):1779–1789.
19. Das Dores S, Chopin C, Villaume C, et al. A new oligomeric parvalbumin allergen of Atlantic cod (Gad m I) encoded by a gene distinct from that of Gad c I. Allergy 2002; 57(suppl 72):79–83.
20. van Boxtel EL, van Beers MM, Koppelman SJ, et al. Allergen Ara h 1 occurs in peanuts as a large oligomer rather than as a trimer. J Agric Food Chem 2006; 54(19):7180–7186.
21. Breiteneder H, Radauer C. A classification of plant food allergens. J Allergy Clin Immunol 2004; 113:821–830.
22. Jenkins JA, Griffiths-Jones S, Shewry PR, et al. Structural relatedness of plant food allergens with specific reference to cross-reactive allergens: an in silico analysis. J Allergy Clin Immunol 2005; 115:163–170.
23. King TP, Hoffman D, Lowenstein H, et al. Allergen nomenclature. Bull World Health Organ 1994; 72:797–780.
24. Johansson SG, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October, 2003. J Allergy Clin Immunol 2004; 113:832–836.
25. Chapman MD, Pomes A, Breiteneder H, et al. Nomenclature and structural biology of allergens. J Allergy Clin Immunol 2007; 119:414–420.
26. Zuidmeer L, van Rhee R. Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. Curr Opin Allergy Clin Immunol 2007; 7:269–273.
27. AllFAm allergen family chart. Downloaded from AllFam version of May 5, 2007. Available at: http://www.meduniwien.ac.at/allergens/allfam/.
28. Radauer C, Marl A, Breiteneder H. The AllFam database—a resource for classifying allergenic proteins. Allergy 2007; 62(suppl 83):154.
29. Strobel S, Mowat AM. Immune responses to dietary antigens: oral tolerance. Immunol Today 1998; 19(4):173–181.
30. Busse PJ, Jarvinen KM, Vila L, et al. Identification of sequential IgE-binding epitopes on bovine alpha(s2)- casein in cow's milk allergic patients. Int Arch Allergy Immunol 2002; 129:93–96.
31. Jarvinen KM, Chatchatee P, Bardina L, et al. IgE and IgG binding epitopes on alpha-lactalbumin and beta-lactoglobulin in cow's milk allergy. Int Arch Allergy Immunol 2001; 126(2):111–118.
32. Jarvinen KM, Beyer K, Vila L, et al. B-cell epitopes as a screening instrument for persistent cow's milk allergy. J Allergy Clin Immunol 2002; 110(2):293–297.
33. Chatchatee P, Jarvinen KM, Bardina L, et al. Identification of IgE- and IgG-binding epitopes on alpha (s1)-casein: differences in patients with persistent and transient cow's milk allergy. J Allergy Clin Immunol 2001; 107(2):379–383.
34. Taylor SL, Hefle SL, Bindslev-Jensen C, et al. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? J Allergy Clin Immunol 2002; 109:24–30.
35. Morisset M, Moneret-Vautrin DA, Kanny G, et al. Thresholds of clinical reactivity to milk, egg, peanut, and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. Clin Exp Allergy 2003; 33:1046–1051.

36. Wal JM. Bovine milk allergenicity. Ann Allergy Asthma Immunol 2004; 93:2–11.
37. Anet J, Back JF, Baker RS, et al. Allergens in the white and yolk of hen's egg. A study of IgE binding by egg proteins. Int Arch Allergy Appl Immunol 1985; 77(3):364–371.
38. Hoffman DR. Immunochemical identification of the allergens in egg white. J Allergy Clin Immunol 1983; 71(5):481–486.
39. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, et al. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. J Allergy Clin Immunol 1994; 93(6):1047–1059.
40. Mine Y, Wei ZJ. Identification and fine mapping of IgG and IgE epitopes in ovomucoid. Biochem Biophys Res Commun 2002; 292(4):1070–1074.
41. Walsh BJ, Hill DJ, Macoun P, et al. Detection of four distinct groups of hen egg allergens binding IgE in sera of children with egg allergy. Allergol Immunopathol (Madrid) 2005; 33:183–191.
42. Allen CW, Campbell DE, Kemp AS. Egg allergy: are all childhood food allergies the same? J Paediatr Child Health 2007; 43:214–218.
43. Hourihane JO, Roberts SA, Warner JO. Resolution of peanut allergy: case-control study. Br Med J 1998; 316:1271–1275.
44. Fleischer DM, Conover-Walker MK, Christie L, et al. Peanut allergy: recurrence and its management. J Allergy Clin Immunol 2004; 114:1195–1201.
45. Burks AW, Cockrell G, Stanley JS, et al. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. J Clin Invest 1995; 96(4):1715–1721.
46. Rabjohn P, West CM, Connaughton C, et al. Modification of peanut allergen Ara h 3: effects on IgE binding and T cell stimulation. Int Arch Allergy Immunol 2002; 128(1):15–23.
47. Rabjohn P, Helm EM, Stanley JS, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. J Clin Invest 1999; 103(4):535–542.
48. Palmer GW, Dibbern DA, Burks AW, et al. Comparative potency of Ara h 1 and Ara h 2 in immunochemical and functional assays of allergenicity. Clin Immunol 2005; 115:302–312.
49. Shreffler WG, Castro RR, Kucuk ZY, et al. The major glycoprotein form Arachis hyogaea, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. J Immunol 2006; 177:3677–3685.
50. Allergen Nomenclature—The Official "List of Allergy." WHO/IUIS Allergen Nomenclature Sub-Committee, 2006. Available at: http://www.allergen.org.
51. Shreffler WG, Beyer K, Chu TH, et al. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. J Allergy Clin Immunol 2004; 113:776–782.
52. Shreffler WG, Lencer DA, Bardina L, et al. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen Ara h 2. J Allergy Clin Immunol 2005; 116:893–899.
53. Palmer K, Burks W. Current development in peanut allergy. Curr Opin Allergy Clin Immunol 2006; 6:202–206.
54. Burks AW Jr., Brooks JR, Sampson HA. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. J Allergy Clin Immunol 1988; 81(6):1135–1142.
55. Ogawa A, Samoto M, Takahashi K. Soybean allergens and hypoallergenic soybean products. J Nutr Sci Vitaminol (Tokyo) 2000; 46(6):271–279.
56. Ogawa T, Tsuji H, Bando N, et al. Identification of the soybean allergenic protein, Gly m Bd 30K, with the soybean seed 34-kDa oil-body-associated protein. Biosci Biotechnol Biochem 1993; 57(6): 1030–1033.
57. L'Hocine L, Boye JI. Allergenicity of soybean: new developments in identification of allergenic proteins, cross-reactivities and hypoallergenization technologies. Crit Rev Food Sci Nutr 2007; 47:127–143.
58. Sutton R, Hill DJ, Baldo BA, et al. Immunoglobulin E antibodies to ingested cereal flour components: studies with sera from subjects with asthma and eczema. Clin Allergy 1982; 12(1):63–74.
59. Jones SM, Magnolfi CF, Cooke SK, et al. Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity. J Allergy Clin Immunol 1995; 96(3):341–351.
60. James JM, Helm RM, Burks AW, et al. Comparison of pediatric and adult IgE antibody binding to fish proteins. Ann Allergy Asthma Immunol 1997; 79(2):131–137.
61. Battais F, Pineau F, Popineau Y, et al. Food allergy to wheat: identification of immunoglobulin E- and immunoglobulin G-binding proteins with sequential extracts and purified proteins from wheat flour. Clin Exp Allergy 2003; 33:962–970.
62. Battais F, Mothes T, Moneret-Vautrin DA, et al. Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat. Allergy 2005; 60:815–821.
63. Battaiis F, Richard C, Szustakowski G, et al. Wheat flour allergy: an entire diagnostic tool for complex allergy. Eur Annals Allergy Clin Immunol 2006; 38:59–61.

64. Wild LG, Lehrer SB. Fish and shell fish allergy. Curr Allergy Asthma Rep 2005; 5:74–79.
65. Chua KH, Tang CY, Wu A, et al. Seafood allergy: lessons from clinical symptoms, immunological mechanisms and molecular biology. Adv Biochem Eng Biotechnol 2005; 97:205–235.
66. Aas K. Studies of hypersensitivity to fish. A clinical study. Int Arch Allergy Appl Immunol 1966; 29(4): 346–363.
67. Rosmilah M, Shahnaz M, Masita A, et al. Identification of major allergens of two species of local snappers: Lutjanus argentimaculatus (mera/red snapper) and Lutjanus johnii (janahak/golden snapper). Trop Biomed 2005; 22:171–177.
68. Elsayed S, Apol J. Immunochemical analysis of cod fish allergen M: locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. Allergy 1983; 38:449–459.
69. Chatterjee U, Mondal G, Chakraborti P, et al. Changes in the allergenicity during different preparations of pomfret, hilsa, bhetki and mackerel fish as illustrated by enzyme-linked immunosorbent assay and immunoblotting. Int Arch Allergy Immunol 2006; 141:1–10.
70. Lehrer SB, Ibanez MD, McCants ML, et al. Characterization of water-soluble shrimp allergens released during boiling. J Allergy Clin Immunol 1990; 85(6):1005–1013.
71. Daul CB, Morgan JE, Waring NP, et al. Immunologic evaluation of shrimp-allergic individuals. J Allergy Clin Immunol 1987; 80(5):716–722.
72. Ayuso R, Lehrer SB, Reese G. Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomysin). Int Arch Allergy Immunol 2002; 127(1):27–37.
73. Reese G, Ayuso R, Leong-Kee SM, et al. Characterization and identification of allergen epitopes: recombinant peptide libraries and synthetic, overlapping peptides. J Chromatogr B Biomed Sci Appl 2001; 756(1–2):157–163.
74. Reese G, Schicktanz S, Lauraer I, et al. Structural, immunological and functional properties of natural recombinant Pan a 1, the major allergen of Brown shrimp. Penaeus aztecus. Clin Exp Allergy 2006; 36:517–524.
75. Roux KH, Teuber SS, Sathe SK. Tree nut allergens. Int Archives Allergy Immunol 2003; 131:L234–L244.
76. Clark AT, Ewan PW. The development and progression of allergy to multiple nuts at different ages. Pediatr Allergy Immunol 2005; 16:507–511.
77. Nordlee JA, Taylor SL, Townsend JA, et al. Identification of a Brazil-nut allergen in transgenic soybeans. N Engl J Med 1996; 334(11):688–692.
78. Robotham JM, Teuber SS, Sathe SK, et al. Linear IgE epitope mapping of the English walnut (Juglans regia) major food allergen, Jug r 1. J Allergy Clin Immunol 2002; 109(1):143–149.
79. Gangur V, Kelly C, Navulure L. Sesame allergy: a growing allergy of global proportions? Ann Allergy Asthma Immunol 2005; 95:11–13.
80. Guarneri F, Guarneri C, Benvenga S. Identification of potentially cross-reactive peanut-lupine proteins by computer-assisted search of amino acid sequence homology. Int Arch Allergy Immunol 2005; 138:273–277.
81. Magni C, Herndl A, Sironi E, et al. One- and two-dimensional electrophoretic identification of IgE-binding polypeptides of Lupinus albus and other legume seeds. J Agric Food Chem 2005; 53: 4567–4571.
82. Lucas JS, Grimshaw KE, Collins K, et al. Kiwi fruit is a significant allergen and is associated with differing patterns of reactivity in children and adults. Clin Exp Allergy 2004; 34:1115–1121.
83. Lucans JS, Lewis SA, Trewin JB, et al. Comparison of the allergenicity of Actnidia deliciosa (kiwi fruit) and Actinidia chinensis (gold kiwi). Pediatr Allergy Immunol 2005; 16:647–654.
84. Tamburrini M, Cerasuolo I, Carratore V, et al. Kiwellin, a novel protein from kiwi fruit. Purification, biochemical characterization and identification as an allergen. Protein J 2005; 24:423–429.
85. Carnes J, Ferrer A, Fernandez-Caldas E. Allergenicity of 10 different apple varieties. Ann Allergy Asthma Immunol 2006; 96:564–570.
86. Puumalainen TJ, Poikonen S, Kotovuori A, et al. Napins, 2S albumins, is major allergens in oilseed rape and turnip rape. J Allergy Clin Immunol 2006; 117:426–432.
87. Ahrazem O, Ibanez MD, Lopez-Torrejon G, et al. Orange germin-like glycoprotein Cit s 1: an equivocal allergen. Int Arch Allergy Immunol 2006; 139:96–103.
88. Egger M, Mutschlechner S, Wopfner N, et al. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy 2006; 61:461–476.
89. Hemmer W, Focke M, Gotz M, et al. Sensitization to Ficus benjamina: relationship to natural rubber latex allergy and identification of foods implicated in the Ficus-fruit syndrome. Clin Exp Allergy 2004; 34:1251–1258.
90. Blanco C. Latex-fruit syndrome. Curr Allergy Asthma Rep 2003; 3:467–453.
91. Yagami T, Osuna H, Kuono M, et al. Significance of carbohydrate epitopes in a latex allergen with beta-1,3-glucanase activity. Int Arch Allergy Immunol 2002; 129:27–37.
92. Purohit-Sheth TS, Carr WW. Oral allergy syndrome (pollen-food allergy syndrome). Allergy Asthma Proc 2005; 26:229–230.

93. Hoffman HJ, Skjold T, Raithel M, et al. Response of respiratory flour allergics in an ingested flour challenge may involve plasmacytoid dendritic cells, CD25+ and CD152+ T cells. Int Arch Allergy Immunol 2006; 140:252–260.
94. Goetz DW, Whisman BA. Occupational asthma in a seafood restaurant worker: cross-reactivity of shrimp and scallops. Ann Allergy Asthma Immunol 2000; 85:461–466.
95. Bock SA, Munoz-Furlong A, Sampson. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001; 107:191–193.
96. Anibarro B, Seone FJ, Mugica MV. Involvement of hidden allergens in food allergic reactions. J Investig Allergol Clin Immunol 2007; 17:168–172.
97. Rojas-Hijazo B, Garcés MM, Caballero ML, et al. Unsuspected lupine allergens hidden in food. Int Arch Allergy Immunol 2006; 141(1):47–50.
98. Schäppi GF, Konrad V, Imhof D, et al. Hidden peanut allergens detected in various foods: findings and legal measures. Allergy 2001; 56:1216–1220.
99. Bischoff SC. Food allergies. Curr Treat Options Gastroenterol 2007; 10:34–43.
100. Bischoff S, Crowe SE. Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives. Gastroenterology 2005; 128:1089–1013.
101. Sicherer SH. Clinical aspects of gastrointestinal food allergy in childhood. Pediatrics 2003; 111: 1609–1616.
102. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001; 107:191–193.
103. Asero R, Ballmer-Weber BK, Beyer K, et al. IgE-mediated food allergy diagnosis: current status and new perspectives. Mol Nutr Food Res 2007; 51:135–147.
104. Kiel T. Epidemiology of food allergy: what's new? A critical appraisal of recent population-based studies. Curr Op Allergy Clin Immunol 2007; 7:259–263.
105. Nowak-Wergrzyn A. Food allergy to proteins. In: Cooke RJ, Vanderplas Y, Wahn U, eds. Nutrition Support for Infants at Risk. Nestle Nutr Workshop Ser Pediatr Program 2007; 59:17–35.
106. Sampson HA. Utility of food-specific concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001; 107:891–896.
107. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive food challenges to milk, egg, and peanut in children. Clin Exp Allergy 2000; 30:1540–1546.
108. Perry TT, Matsui EC, Conover-Walker M, et al. The relationship of allergen-specific IgE levels and oral food challenge outcome. J Allergy Clin Immunol 2004; 114:144–149.
109. Celik-Bilgili S, Mehl A, Verstege A, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy 2005; 35:268–273.
110. Nowak-Wegrzyn A. Food allergy to proteins. Nestle Nutr Workshop Ser Pediatr Program 2004; 59:17–36.
111. Leung DY, Sampson HA, Yunginger JY, et al. Effect of anti-IgE therapy in patients with peanut allergy. New Eng J Med 2003; 348:986–993.
112. Li XM, Zhang TF, Huang CK, et al. Food allergy herbal formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in a murine model. J Allergy Clin Immunol 2001; 108:639–646.
113. Bolhaar T, Tiemessen MM, Zuidmeer L, et al. Efficacy of birch-pollen immunotherapy on cross-reactive food allergy confirmed by skin tests and double-blind food challenges. Clin Exp Allergy 2004; 34:761–769.
114. Zhu D, Kepley CL, Zhang K, et al. A chimeric human-cat fusion protein blocks cat-induced allergy. Nat Med 2005; 60:11:446–449.
115. Frick OL, Teuber SS, Buchanan BB, et al. Allergen immunotherapy with heat-killed Listeria monocytogenes alleviates peanut and food-induced anaphylaxis in dogs. Allergy 2005; 60:243–250.
116. Muraro A, Dreborg S, Halken S, et al. Dietary prevention of allergic disease in infant and small children. Part III: Critical review of published peer-reviewed observations and interventional studies and final recommendations. Pediatr Allergy Immunol 2003; 15:291–307.
117. American Academy of Pediatrics Committee on Nutrition. Hypoallergenic infant formulas. Pediatrics 2000; 106:346–349.
118. Astier C, Morisset M, Roitel O, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol 2006; 118:250–256.
119. Nowak-Wergrzyn A, Sampson HA. Food allergy therapy. Immunol Allergy Clin North Am 2004; 113:705–725.
120. Sampson HA. Utility of food-specific IgE concentrations in predicting food allergy. J Allergy Clin Immunol 2001; 107:891–896.
121. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997; 100:444–451.
122. McCone KL, Sickles-Wright H, Birch LL, et al. Food portions are positively related to energy intake and body weight in children. J Pediatr 2002; 140:340–347.

123. Moneret-Vautrin DA, Kanny G. Update on threshold doses of food allergens: implications for patients and the food industry. Curr Opin Allergy Clin Immunol 2004; 4:215–219.
124. Leung DYM, Sampson HA, Yunginger JY, et al Effect of anti-IgE therapy in patients with food allergy. NEJM 2003; 348:986–993.
125. Pons L, Palmer K, Burks W. Towards immunotherapy for peanut allergy. Curr Opin Allergy Clin Immunol 2005; 5:558–562.
126. Morriset M, Moneret-Vautrin DA, Guenard L, et al. Oral desensitization in children with milk and egg allergies obtains recovery in significant proportion of cases. A randomized study of 60 children with cow's milk allergy and 90 children with egg allergy. Allerg Immunol (Paris) 2007:39:12–19.
127. Malmheden Yman I, Eriksson A, Everitt G, et al. Analysis of food proteins for verification of contamination or mislabeling. Food Agric Immunol 1994; 6:167–172.
128. Taylor SL, Hefle S, Bindslev-Jensen C, et al. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? J Allergy Clin Immunol 2002; 109:24–30.
129. Morisset M, Moneret-Vautrin DA, Kanny G, et al. Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. Clin Exp Allergy 2003; 33:1046–1051.
130. Thomas K, Herouet-Guicheney C, Ladics G, et al. Evaluation the effect of food processing on the potential human allergenicity on novel proteins: international workshop report. Food Chem Toxicol 2007; 45:1116–1122.
131. Bohle B. T-cell epitopes of food allergens. Clin Rev Allergy Immunol 2006; 30:97–108.
132. Scott-Taylor TH, Hourihane JB, Harper J, et al. Patterns of food allergen-specific cytokine production by T lymphocytes of children with multiple allergies. Clin Exp Allergy 2005; 35:1473–1480.
133. Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children and allergic children who acquired tolerance to peanuts. J Clin Invest 2003; 111:1065–1072.
134. Noma T, Yoshizawa I, Aoki K, et al. Cytokine production in children outgrowing hen egg allergy. Clin Exp Allergy 1996:26:1298–1307.
135. Thottingal TB, Setfura BP, Simons FER, et al. Human subjects without peanut allergy demonstrate T cell-dependent, Th2-based, peanut-specific cytokine and chemokine responses independent of th1 expression. J Allergy Clin Immunol 2006; 118:905–914.
136. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med 2004; 199:1679–1688.
137. Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. Ann N Y Acad Sci 2002; 964:47–68.
138. Holm J, Gajhede M, Ferreras M, et al. Allergy vaccine engineering: epitope modulation of recombinant Bet v 1 reduces IgE-binging but retains protein folding pattern for induction of protective blocking-antibody responses. J Immunol 2004; 173:5258–5267.
139. Reese G, Ayuso R, Leon-Kee SM, et al. Characterization and identification of allergen epitopes: recombinant peptide libraries and synthetic, overlapping peptides. J Chromatogr B Biomed Sci Appl 2001; 756:157–163.
140. Mirza O, Henriksen A, Ipsen H, et al. Dominant epitopes and allergic cross-reactivity: complex formation between a fab fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen. J Immunol 2000; 165:331–338.
141. Wiche R, Gubesch M, Konig H, et al. Molecular basis of pollen-related food allergy: identification of a second cross-reactive IgE epitope on Pru a 1, the major cherry allergen (Prunus avium). Biochem J 2005; 385:319–327.
142. Neudecker P, Lehmann K, Nerkamp J, et al. Mutational epitope analysis of Pru av 1 and Api g 1, the major allergens of cherry (Prunus avium) and celery (Apium graveolens): correlating IgE reactivity with three-dimensional structure. Biochem J 2003; 15; 376:97–107.
143. Suphioglu C, Schäppi G, Kenrick J, et al. A novel grass pollen allergen mimotope identified by phage display peptide library inhibits allergen-human IgE antibody interaction. FEBS Lett 2001; 27:502:46–52.
144. Mittag D, Batori V, Neudecker P, et al. A novel approach for investigation of specific and cross-reactive IgE epitopes on Bet v 1 and homologous food allergens in individual patients. Mol Immunol 2006; 43:268–278.
145. Aalberse RC, Stadler BM. In silica predictability of allergenicity: from amino acid sequence via 3-D structure to allergenicity. Mol Nutr Food Res 2006; 50:625–627.
146. Untersmayr E, Jensen Jarolim E. Mechanisms of type I food allergy. Pharmacol Ther 2006; 112:787–798.

14 Hymenoptera Allergens

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INTRODUCTION

Many insects can cause allergy in man (Table 1) (1). People can be exposed to insect body parts or their secretions by inhalation, to their venoms by stinging, and to their salivary gland secretions by biting. Examples of these routes of sensitization are, respectively, allergies to cockroaches of the order Orthoptera, to ants, bee, and vespids of the order Hymenoptera, and to flies and mosquitoes of the order Diptera.

The importance of venoms as the allergen source in Hymenoptera allergy has been known for some time (2,3). All known insect venom allergens are proteins of 10 to 50 kDa containing 100 to 400 amino acid residues. The one exception is that the bee venom allergen melittin is a 26-residue peptide. But melittin is a minor allergen, active in less than one-third of bee allergic patients (4). Nearly all these allergens have been sequenced and/or cloned. Several of these allergens have been expressed in bacteria, insect, or yeast cells.

This chapter will review the immunochemical properties of known Hymenoptera venom proteins and peptides, and their relevance to our understanding and treatment of insect allergy.

TAXONOMY, GEOGRAPHIC DISTRIBUTION, AND IDENTIFICATION OF HYMENOPTERA INSECTS

Essentially all insects responsible for causing insect sting allergic reactions belong to the order Hymenoptera. This is a large and diverse order composed of over 70 families (5) with over 100,000 species (6). Although many Hymenoptera are capable of stinging, only species belonging to three families sting people with a high degree of frequency. The usual perpetrators are social insects and belong to the Apidae (bees), Formicidae (ants), or Vespidae (wasps). The medically important genera in the United States are outlined in Table 2. Four of these insects are shown in Figure 1. Many of the bees and vespids listed in Table 2 or their closely related species are distributed worldwide. In addition to the fire ants that are in the Americas only, bulldog, jumper, and Samson ants are of medical importance in Asia and Australia.

Accurate identification of social stinging Hymenoptera to species level is a difficult task even for most entomologists. Although not definitive, there are several behavioral characteristics that can help provide clues as to a specimen's identity. For example, honeybees have a unique sting anatomy that causes worker bees to leave their sting apparatuses in the victim's skin. Although sting autotomy is almost exclusively attributed to honeybees, other stinging Hymenoptera will occasionally lose their sting. And conversely, honeybees will occasionally sting without autotomizing (7). Annoying wasps foraging around picnic foods, garbage, or fallen fruit are usually yellow jackets and belong to the genus *Vespula*. Large colonies of wasps living in subterranean nests are also usually of the genus *Vespula* (1). Since there are notable exceptions to the above, the only reliable means of obtaining a positive identification is to collect a specimen and have its identity determined by an entomologist with expertise in the social Hymenoptera.

Table 1 Insects Reported to Cause Allergy in Man

Order Coleoptera—beetles
Order Diptera—flies and mosquitoes
Order Ephermeroptera—mayflies
Order Hemiptera—aphids, bed bugs, and kissing bugs
Order Hymenoptera—ants, bees, and vespids
Order Lepidoptera—moths and caterpillars
Order Orthoptera—cockroaches
Order Siphonatera—fleas
Order Trichoptera—caddis flies

Source: From Ref. 1.

Table 2 Geographic Distribution and Medical Importance of Some Insects of the Order Hymenoptera

Family/subfamily	Genus and species	Common name	Geographic distribution within U.S.	Medical importance
Apidae/Apinae	*Apis mellifera*	Honeybee	Entire U.S.	Major
	Bombus spp.	Bumble bee	Entire U.S.	Moderate
Formicidae/Myrimicinae	*Solenopsis invicta*	Fire ant	SE, SW	Major
	Solenopsis richteri	Fire ant	Mississippi, Alabama	Minor
Vespidae/Vespinae	*Vespa crabro*	European hornet	NE, SE	Minor
	Dolichovespula maculata	Whitefaced hornet (baldfaced hornet)	Entire U.S.	Major
	Dolichovespula arenaria	Yellow hornet (aerial yellow jacket)	NE, NW, SW	Major
	Vespula flavopilosa	Yellow jacket	NE, SE	Major
	Vespula germanica	Yellow jacket	NE, NW	Major
	Vespula maculifrons	Yellow jacket	NE	Major
	Vespula pensylvanica	Yellow jacket	NW, SW	Major
	Vespula vulgaris	Yellow jacket	NE, NW, SW	Major
	Vespula squamosa	Yellow jacket	NE, SE	Major
Polistinae	*Polistes* spp.	Paper wasp	Entire U.S.	Major

Only insects with known venom allergens are listed.
Data for geographic distribution and medical importance are taken from Ref. 1.

BIOCHEMICAL STUDIES OF HYMENOPTERA VENOM PROTEIN ALLERGENS

In Table 3 are listed some of the venom allergens of bees, vespids, and fire ants, which have been sequenced and/or cloned.

Honeybee venom has six allergens of known sequences. Four are proteins, acid phosphatase, hyaluronidase, phospholipase A_2, and protease, the fifth one is a cytolytic peptide melittin, and the sixth one Api m 6 is miniprotein of about 70 amino acid residues. Bumble bee venom has two protein allergens of known sequences: phospholipase A_2 and a protease. Bumble bee and honeybee venom phospholipases A_2 as well as their homologs from Asian bees have high degree of sequence identity with each other.

Each of vespid venoms contains three to four known protein allergens. Three of them have been isolated from all vespids studied, and they are antigen 5, hyaluronidase, and phospholipase A_1. And the fourth one is a protease that has been characterized only from paper wasps. The biological function of antigen 5 is not known, but it may be a protease because of sequence homology with a protease in cone snail (37). Vespid phospholipase A_1 differs from bee phospholipase A_2 in its structure and enzymatic specificity (15,24). Vespid and bee hyaluronidases are homologous with about 55% sequence identity (17,24,38).

Fire ant venom contains four known protein allergens: Sol i 1 to 4. Sol i 1 and 3 are homologous with vespid phospholipase and antigen 5, respectively (12,20).

Only one allergen of ~12kDa is known from jumper ant (*Myrmecia pilosula*) venom, and it was identified by immunoblot with mercaptoethanol-reduced venom (39). The presence of

Figure 1 (*See color insert.*) Common stinging insects. The photos, starting from top left and going clockwise, show respectively honeybee (*Apis mellifera*), yellow jacket (*Vespula maculifrons*), paper wasp (*Polistes fuscatus*), and fire ant (*Solenopsis invicta*). The approximate lengths of these insects in the order given are 16, 10, 19, and 3 mm, respectively. The photos are of different magnifications.

hyaluronidase, phosphatase, phospholipase, and other enzymes in jumper and bulldog ant venoms have been reported (40), and it is possible that these venom proteins also are allergens.

Several venom allergens have partial sequence identity with other proteins from diverse sources, and this is summarized in Table 4. As an example, the sequence identities of three vespid antigen 5s, fire ant antigen 5 (Sol i 3), human and mouse testis proteins, human glioma protein, and proteins from tomato, nematode, and lizard, in their C-terminal 50 residue region, are given in Figure 2. We may note in particular the partial sequence identity of venom allergens with proteins of male reproductive functions; antigen 5s with a mammalian testis protein (18), hyaluronidases with those from mammalian sperm and other tissues, phosphatase with a prostate enzyme (20), and protease with mammalian acrosin (15).

X-ray crystallography was used to determine the structures of honeybee venom hyaluronidase (11) and phospholipase A_2 (9), and those of antigen 5 and hyaluronidase from yellow jacket, *V. vulgaris* (26,27). Vespid phospholipase A_1 has sequence homology with porcine pancreatic lipase (46). As the structure of porcine lipase is known, the structure of vespid phosphlipase can be obtained by modeling. Using the modeling approach, the structures of nearly all the proteins in Table 3 can be obtained.

The structures of a number of allergen proteins from different sources have been determined. No unusual structural features of these protein allergens are known (49).

RECOMBINANT HYMENOPTERA VENOM PROTEIN ALLERGENS

Several of the allergens in Table 3 have been expressed in bacteria, insect, or yeast cells to yield recombinant proteins. The recombinant proteins that are expressed in the cytoplasm of bacteria are usually unfolded as they lack the disulfide bonds of the natural proteins and do not have the native conformation of the natural proteins. The cytoplasm of bacteria is a

Table 3 Some Insect Venom Allergens with Known Sequences and Structures

Allergen name[a]	Common name	Glycoprotein	Molecular size[b]	Structure[c]	Recombinant protein[d]	References
Honeybee, *Apis melifera*						
Api m 1[e]	Phospholipase A_2	Yes	16 kDa	Direct	Unfolded/folded	8,9
Api m 2	Hyaluronidase	Yes	39 kDa	Direct	Folded	10,11
Api m 3	Acid phosphatase		43 kDa			12
Api m 4	Melittin	No	3 kDa	Direct	Folded	4
Api m 5	Protease		28 kDa			13
Api m 6			8kDa			14
Bumble bee, *Bombus pennsylvanicus*						
Bom p 1	Phospholipase A_2		16 kDa	Modeling		15
Bom p 4	Protease		28 kDa			15
White face hornet, *Dolichovespula maculate*						
Dol m 1	Phospholipase A_1	No	34 kDa	Modeling	Unfolded	16
Dol m 2	Hyaluronidase	Yes	38 kDa	Modeling	Unfolded	17
Dol m 5[f]	Antigen 5	No	23 kDa	Modeling	Unfolded/folded	18,19
European hornet, *Vespa crabro*						
Ves c 1	Phospholipase A_1		34 kDa	Modeling		20
Ves c 5	Antigen 5	Yes	23 kDa	Modeling		21
Paper wasp, *Polistes annularis*						
Pol a 1	Phospholipase A_1	No	34 kDa	Modeling	Unfolded	22
Pol a 2	Hyaluronidase	Yes	38 kDa	Modeling	Unfolded	22
Pol a 5	Antigen 5	No	23 kDa	Modeling	Unfolded/folded	18,23
	Protease[g]		28 kDa			13
Yellow jacket, *Vespula vulgaris*						
Ves v 1[h]	Phospholipase A_1	No	34 kDa	Modeling	Unfolded	24
Ves v 2[h]	Hyaluronidase	Yes	38 kDa	Modeling	Unfolded	25,26
Ves v 5	Antigen 5	No	23 kDa	Modeling	Unfolded/folded	23,27
Fire ant, *Solenopsis invicta*						
Sol i 1[i]	Phospholipase A_1	Yes	37 kDa	Modeling		28
Sol i 2			30 kDa			29
Sol i 3[i]	Antigen 5		23 kDa	Modeling		30
Sol i 4			20 kDa			30

[a]Allergen names are designated according to an accepted nomenclature system (31).
[b]Several allergens are glycoproteins, and the molecular size given refers only to the protein portion.
[c]Structures were determined directly or by modeling of structures of homologous proteins.
[d]Availability of recombinant proteins in folded or unfolded forms is indicated.
[e]Sequences of phospholipases A_2 from *A. crena, A. dorsata* (32), and *B. terrestris* (33) are known.
[f]Other vespid antigen 5s with known sequences are *D. arenaria, P. exclamans, P. fuscatas , P. dominulus, V. flavopilosa, V. germanica, V. maculifrons, V. pennsylvanica, V. squamosa, V. vidua,* and *V. mandarinia* (12).
[g]Cloning of proteases from *P. dominulus*, *P. exclamans*, and *P. Gallicus* were reported (34).
[h]Sequences of phospholipase A_1 from *V. germanica, maculifrons, pennsylvanica, flavopilosa* and *squamosa* and of hyaluronidase *V. germanica* are known (35).
[i]Sequences of homologs from *S. invicta* are known (36).

reducing environment and any disulfide bonds that do form are reduced through the action of disulfide reducing enzymes. Several recombinant proteins with disulfide bonds have been obtained in mutants of *Escherichia coli* with decreased disulfide reducing enzymes in their cytoplasm (50). In some cases the unfolded recombinant proteins can be folded and oxidized in vitro into their native conformation, e.g., bee venom phospholipase A_2 (38,51,70) and yellow jacket venom hyaluronidase (26).

Recombinant proteins from insect or yeast cells have the native conformation of the natural proteins as they are folded during secretion into medium, e.g., Api m 2 (10), Sol i 2 (29), and vespid antigen 5s (19,23).

Recombinant allergens have different applications. One obvious application is for use as diagnostic reagents. For example, recombinant yellow jacket antigen 5 was used to show the frequency of patient response to three yellow jacket venom allergens. Ninety percent of the 26 patients tested were positive to antigen 5, and 70% to 80% positive to hyaluronidase and phospholipase (52).

Table 4 Sequence Identity of Insect Allergens and Other Proteins

Insect allergens	Other proteins	Residues compared	% Identity	References
Antigen 5s	Mammalian testis protein	130	35	41
	Human glioma PR protein	124	23	42
	Hookworm protein[a]	130	28	43
	Plant leaf PR protein[b]	130	28	44
	Mexican lizard toxin	130	28	44
	Cone snail protease			37
Hyaluronidase	Mammalian sperm protein[c]	331	50	45
Phosphatase	Mammalian phosphatase	343	16	20
Phospholipase A_1	Mammalian lipases	123	40	46
Phospholipase A_2	Mammalian phospholipases	129	20	
Protease	Mammalian acrosin	243	38	20
	Horseshoe crab enzyme	243	41	20

[a]Homologous worm proteins are present in other nematodes (47).
[b]Homologous plant PR proteins are present in tobacco, tomato, barley and maize (44).
[c]Sperm protein is one of six known mammalian hyaluronidases from different tissues (48).

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Dol m 5    VGHYTQMVWG KTKEIGCGSI KYIE.DNWYT H....YLVCN YGPGGNDFNQ
Pol a 5    IGHYTQMVWG KTKEIGCGSL KYME.NNMQN H....YLICN YGPAGNYLGQ
Ves v 5    TGHYTQMVWA NTKEVGCGSI KYIQ.EKWHK H....YLVCN YGPSGNFMNE
Sol i 3    VEHYTQIVWA KTSKIGCARI MFKEPDNWTK H....YLVCN YGPAGNVLGA
human tpx  VGHYTQLVWY STYQVGCGIA YCPNQDSLKY .....YYVCQ YCPAGNNMNR
mouse tpx  VGHYTQLVWY SSFKIGCGIA YCPNQDNLKY .....FYVCH YCPMGNNVMK
hum glioma CGHYTQVVWA DSYKVGCAVQ FCPKVSGFDA LSNGAHFICN YGPGGNYPTW
tomato pr  CGHYTQVVWR NSVRVGCARV QC....NNGG Y....VVSCN YDPPGNYRGE
hookworm   IGHYTQMAWD TTYKLGCAVV FC....NDFT .....FGVCQ YGPGGNYMGH
lizard     IGHYTQVVWY RSYELGCAIA YCPDQPTYKY .....YQVCQ YCPGGNIRSR
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Figure 2 Sequence identity of vespid antigen 5s and other proteins in their C-terminal region. The sequences shown from top to bottom are for antigen 5s from hornet, paper wasp, yellow jacket and fire ant venoms, human and mouse testis–specific proteins, human glioma protein, tomato leaf pathogenesis–related protein, hookworm protein, and lizard venom protein, respectively. References for these proteins are given in Table 4. Bold characters indicate residues identical to those of vespid antigen 5s and dots indicate blanks added for maximal alignment of sequences. The underlined peptide region was found to contain a dominant T-cell epitope of vespid antigen 5 (see text).

Another application is to prepare allergen hybrids with reduced allergenicity but retaining its immunogencity. The hybrids contain a small segment of the guest allergen of interest and a large segment of a host protein. The host protein is homologous to the guest allergen, and they are poorly cross-reactive as antigens. The host protein functions as a scaffold to hold the segment of the guest allergen in its native conformation as homologous proteins of $>30\%$ sequence identity can have closely similar structures. In this way, the hybrids retain the discontinuous epitopes of the guest allergen but at a reduced density.

The above approach was demonstrated with hybrids of yellow jacket and wasp antigen 5s (53). These two antigens 5s have 59% sequence identity, and they are poorly cross-reactive in patients or in animals. Hybrids with one-fourth of yellow jacket antigen 5 and three-fourth of wasp antigen 5 showed 10^2 to 10^3 fold reduction in allergenicity when tested by histamine release assay in yellow jacket–sensitive patients. These hybrids retained the immunogenicity of antigen 5s for antibody responses specific for the native protein and for T-cell responses in mice. Therefore, the hybrids may be useful vaccines as they may be used at higher doses than the natural allergen.

B-CELL EPITOPES OF HYMENOPTERA VENOM ALLERGENS

The entire accessible surface of a protein is believed to represent a continuum of B-cell epitopes (54). The B-cell epitopes are divided into the continuous and discontinuous types, and their sizes range from 6 to 17 amino acid residues. The continuous type consists of only contiguous amino acid residues in the molecule, while the discontinuous type consists of contiguous as well as noncontiguous residues, which are brought together in the folded molecule. The

Table 5 Cross Reactivity of Native and Reduced Vespid Allergens Detected with Specific Mouse Sera

Solid-phase antigen 5	*Anitgen 5–specific sera* Hornet		Yellow jacket		Wasp	
Hornet	++	++	+	+	+	−
yellow jacket	+	+	++	++	±	−
Wasp	+	+	+	+	++	+
Solid-phase hyaluronidase	*Hyaluronidase-specific sera* Hornet		Yellow jacket		Wasp	
Hornet	++	±	++	+	±	−
yellow jacket	++	−	++	++	±	−
Wasp	++	−	+	+	++	−
Solid-phase phospholipase	*Phospholipase-specific sera* Hornet		Yellow jacket		Wasp	
Hornet	++	±	−	−	−	−
Yellow jacket	±	−	++	±	±	−
Wasp	±	nd	±	nd	++	−

For each sera there are two columns of results. The first column is from ELISA on solid phase natural allergens, and the second column is from immunoblot of reduced allergens.
The ++, +, ±, and − signs refer to relative titer of sera on ELISA, or intensity of bands of immunoblots. "nd" denotes not done.

majority of protein-specific antibodies, 90% or more, are of the discontinuous type. This is the case for venom allergen-specific IgEs in patients by comparative tests with natural or disulfide bond reduced allergens, e.g., bee venom phospholipase A_2 (51) and fire ant Sol i 2 (29). Studies have shown that the same B-cell epitopes can induce both IgE and IgG responses.

Data in agreement with the above generalization were obtained with vespid allergen–specific mouse antisera, which contain mainly specific IgGs. Comparison of the data in Table 5 shows that vespid allergen–specific antisera bind natural allergens, and they bind poorly, if at all, reduced and unfolded allergens that lack the discontinuous epitopes of the folded molecules. This is particularly the case for vespid hyaluronidases and phospholipases (17,24) and to a lesser extent for vespid antigen 5s (18,55). Another general conclusion is that cross-reactivity is readily detectable for homologous venom proteins of >90% sequence identity, and barely detectable for homologous venom proteins of <50% sequence identity, and variable extents of cross-reactivity are detectable for homologous proteins with about 50% to 90% sequence identity. The variable extents of cross-reactivity of homologous proteins probably reflect the degree and the area of identity on the protein surface.

The continuous B-cell epitopes of a protein can be mapped readily by testing polyclonal antibodies with a series of overlapping peptides of 7 to 20 residues in length. Multiple epitopes were found for the 204-residue hornet antigen 5, and only one was found for the 26-residue bee venom melittin (56). No unusual pattern of amino acid sequence was observed for these B-cell epitopes. The discontinuous B-cell epitopes can be mapped with monoclonal antibodies for proteins of known structures (57).

Several insect venom proteins are glycoproteins. Their oligosaccharide side chains have been demonstrated to function as B-cell epitopes for IgE and IgG responses in patients as well as in animals (58,59). In contrast to the multitude of amino acid sequences of different insect allergens, the sequence differences in their carbohydrate side chains are limited. The carbohydrate side chain is N-linked to the asparagine residue of the protein by its innermost *N*-acetylglucosamine residue. The predominant carbohydrate side chain has the sequence of Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc with one or two fucoses (α1,6 and/or α1,3) linked to the innermost *N*-acetylglucosamine residue. This was shown for bee phospholipase (60) and bee and yellow jacket hyaluronidases (25). Similar or identical carbohydrate side chains are present in other plant and animal proteins. The glycan in plant proteins contains, in addition to the fucoses, xylose(β1,2) linked to the mannose in the middle position. Tests with patient or animal sera specific for glycans from different sources suggest that the specificity of the cross-reactive carbohydrate determinant resides mainly in the fucose residues for insect proteins and in the fucose and xylose residues for plant proteins (61). Indeed

horseradish peroxidase can be used to establish whether patient sera cross-reactive to bees and yellow jackets are due to their cross-reactive carbohydrate determinant (62).

T-CELL EPITOPES OF HYMENOPTERA VENOM ALLERGENS

T-cell epitopes are of interest because of the central role of T cells in regulating the antibody class switch event of B cells. They are also of interest as possible reagents for immunotherapy as shown with T-cell peptides of bee venom phospholipase A_2 (63).

T-cell epitopes are peptides of about 15 residues in length formed following intracellular processing of antigens by antigen-presenting cells, and they do not depend on the secondary or tertiary structure of the antigen. This is the case with venom allergens as shown by the identical T-cell stimulating activities of natural or recombinant allergens or reduced allergens; e.g., vespid antigen 5s, hyaluronidases, and phospholipases A_1 (24,55).

Bee venom phospholipase A_2 and hornet antigen 5 are found to have multiple T-cell epitopes distributed throughout the entire molecule by tests with a series of overlapping peptides in patients (64–66) or in mice (67,68). Because of major histocompatibility complex (MHC) class II restriction, patients of different polymorphic background, or mice of different haplotypes, differ in their pattern of peptide recognition. Nonetheless, both insect allergens were found to have several dominant T-cell epitopes recognized by both patients or mice tested.

One T-cell epitope-containing peptide of bee venom phospholipase was found to require the presence of its carbohydrate side chain for its activity (69).

One of the dominant T-cell epitope peptides of hornet antigen 5 was found to cross-react with paper wasp and yellow jacket antigen 5s as well as with a homologous peptide of a mouse testis protein (Fig. 2). The cross-reactivity with mouse protein is not reciprocal as the corresponding peptide from mouse testis protein did not cross-react with hornet antigen 5–specific cells (68).

No unusual features were observed for the dominant T-cell epitope peptides of insect venom allergens. Others have reported similar findings for T-cell epitopes of allergens from grass and tree pollens, cat dander, mites, and chicken ovalbumin (71). Both normal and atopic people were found to recognize the same T-cell epitope peptides of bee venom phospholipase (65) and the major birch pollen allergen (72).

ANTIGENIC CROSS-REACTIVITY OF HYMENOPTERA VENOMS

Insect allergic patients often have sensitivity to multiple insects by skin test or RAST with venoms (3). This multiple sensitivity can be due to exposure to different insects and/or antigenic cross-reactivity of different venoms. This issue of multiple exposure or antigenic cross-reactivity is of importance in the choice of single or multiple venoms for immunotherapy of patients. RAST inhibition carried out with multiple venoms is one possible approach to resolve this issue of multiple sensitivities (73). As noted earlier, the cross-reactivity can be due to their common peptide and carbohydrate determinants.

Bees, fire ants, and vespids each have unique as well as homologous venom allergens. Two of the six known bee allergens are homologous to vespid allergens; hyaluronidase and protease with about 50% sequence identity. Two of the four known fire ant allergens are homologous to vespid allergens, antigen 5 and phospholipase. Fire ant antigen 5 has about 35% sequence identity with vespid antigen 5s. For the vespids, the sequence identity of their homologous antigen 5s, hyaluronidases, and phospholipases ranges from about 40% to 99% for different species of hornets, yellow jackets, and wasps. As noted in section "B-Cell Epitopes of Hymenoptera Venom Allergens," cross-reactivity of B-cell epitopes is readily detectable for homologous venom proteins of >90% sequence identity, and barely detectable for homologous venom proteins of <50% sequence identity, and variable extents of cross-reactivity are detectable for homologous proteins with about 50% to 90% sequence identity.

The above considerations would indicate that patients with sensitivity to multiple insect can be due to cross-reactivity of a single allergen, hyaluronidase, as in the case for bees and vespids, or of multiple allergens in other cases. For cross-reactivity of fire ants and vespids, or

of different vespids, hyaluronidase again has the major role with antigen 5 and phospholipase having secondary and negligent roles, respectively.

Some insect allergens have common peptide and carbohydrate determinants. However, peptide and carbohydrate determinants differ in their epitope density and antibody affinity. For peptide determinants, the entire accessible surface of a protein represents a continuum of epitopes (54). For carbohydrate determinants, the number of epitopes is restricted to a few potential glycosylation sites, and these sites are not necessarily glycosylated. As an example, yellow jacket hyaluronidase has five potential sites but only two to three sites are glycosylated (26).

Mediators are released from IgE-bound mast cells or basophils on allergen challenge. This biological activity requires the allergens to have multiple determinants/epitopes so that they can cross-link the bivalent IgE antibodies. Thus, the low-density carbohydrate determinants are likely to be of less biological importance than are the high-density peptide determinants. Also the biological activity of allergens to cause mediator release depends on the affinity of allergen-specific IgEs (74,75). For these reasons, there is debate as to the importance of glycan-specific IgE in allergic diseases (76).

Several authors have reported that a sizable group of normal people who showed no clinical sensitivity to insects tested positive with insect venoms (77,78). These false-positive results may possibly represent cross-reactivity of insect venoms with other proteins to which people have been exposed. As noted earlier in Table 4, insect allergens have variable extents of sequence identity with proteins from diverse sources.

Investigators have observed that more men than women, in a ratio of about 2 to 1, had insect allergy as judged by their systemic and large local reactions or by their death statistics (79). It has been assumed that these results were primarily due to greater exposure because of work habits of men and women. Whether or not the partial sequence identity of venom allergens with proteins of male reproductive functions (Table 4) plays a role in these observations is not known.

BIOCHEMICAL STUDIES OF HYMENOPTERA VENOM PEPTIDES

In addition to proteins, Hymenoptera venoms contain peptides, biogenic amines such as histamine and dopamine, and other low molecular weight components (80). Table 6 lists the biological activities and the names of these venom peptides (81). These biological activities include mast cell degranulation, chemotaxis, kinin, and others. The most abundant peptides in bee and vespid venoms are melittin and mastoparan, respectively, and they have mast cell degranulating activity. Bee venom contains another mast cell degranulating peptide, known as MCD, with a greater mast cell degranulating activity than melittin. Bee venom induced mast cell degranulation leads to the release of biologically active products: some of these products cause inflammatory responses resulting in morbidity or mortality but some, such as proteases, are in fact of protective value by their action on venom components (82).

Melittin and mastoparan are basic peptides with 26 and 14 to 15 residues, respectively. Both peptides are synthesized in insects as precursor proteins and are released on proteolysis (83). Both peptides are immunogenic in mice for antibody responses (84–86). But melittin, not mastoparan, was reported to be an allergen (4).

Table 6 Bioactive Peptides in Hymenoptera Venoms

Species	Kinin	Chemotaxis	Mast cell degranulation[a]	Others[a]
Apis mellifera	No	No	MCD	Melittin Apamin
Bombus spp.				Bombolitin
Polistes spp.	Yes	Yes	Mastoparan	
Vespa spp.	Yes	Yes	Mastoparan	Crabolin
Vespula spp.	Yes	Yes	Mastoparan	

[a]Peptide names are listed.

Mastoparan was discovered for its activity to induce release of histamine and other mediators from mast cells (87). It binds to cell membranes (88,89), and it can act as a strong secretagogue for different cell types. For examples, mastoparan is reported to stimulate the release of the inflammatory mediators, TNF-α, IL-1β, nitrous oxide, and prostaglandin E_2 into peritoneal exudates of mice (90). These mediator releases are related to its diverse range of biochemical activities. They include stimulation of phospholipases A_2 (91), C (92) and D (93,94), and G-protein activation (95).

Mastoparan was found to have a weak adjuvant activity to enhance IgG1 and IgE responses to yellow jacket antigen 5 in mice (96). This adjuvant action may be related to its activity to induce the release of T_H2 cell–associated mediators from basophils/mast cells, macrophages, and possibly other antigen-presenting cells. Melittin was not found to have adjuvant activity for Ves v 5–specific antibody response, although melittin has biological properties similar to mastoparan. Others found melittin to be an adjuvant for ovalbumin-specific IgE response in mice (84). The two different findings on melittin may reflect the experimental conditions used as IgE responses in mice are antigen, dose, and mice strain dependent.

Yellow jacket venom was found to be lethal in mice when injected intraperitoneally but not subcutaneously (96). The toxic action was shown to require the synergistic action of the venom peptide mastoparan and the venom protein phospholipase A_1.

STING REACTIONS

There are three types of reactions that individuals may experience from a Hymenoptera sting. The normal response is a *local cutaneous reaction* characterized by redness, swelling, and pain confined to the sting site. This is a toxic response. A *large local reaction* is thought to be IgE mediated and involves an extensive area of warmth, redness, and swelling contiguous with the site of the sting. Large local reactions typically develop in one to three days, may involve an entire extremity, and may persist for up to 5 days. An *allergic systemic reaction* usually occurs within half an hour of envenomization and includes symptoms remote from the site of the sting. Systemic allergic reactions may involve the skin, the respiratory system, the vascular system, or any combination thereof.

Minimal treatment is necessary for local cutaneous reactions. The sting site should be kept clean to avoid secondary infections, and ice packs may help to reduce local pain and swelling. Large local reactions may cause considerable discomfort and are frequently treated with analgesics, antihistamines, and glucocorticosteroids. Systemic allergic reactions can be quite serious and occasionally fatal. They can be successfully treated with venom immunotherapy to prevent future reactions (see chap. 24).

SALIENT POINTS

- The medically important stinging insects are fire ants, bees, and vespids (wasps). The vespids include hornets, paper wasps, and yellow jackets.
- Insect venom allergens are proteins of 10 to 50 kDa. Nearly all known venom allergens have been cloned and expressed as recombinant proteins in different systems. However, some recombinant proteins are not properly folded.
- Insect venom allergens have different biochemical functions. Their only known common feature is their partial sequence identity with proteins from other sources in the environment.
- Each of these insect venoms has unique allergen(s) as well as homologous allergen(s) with partial sequence identity.
- Multiple sensitivity of patients to different insects or to more closely related vespids can be due to multiple exposures and/or antigenic cross-reactivity of venom allergen(s).
- Detailed immunochemical knowledge of insect venom allergens are useful for monitoring the quality of insect venoms used for diagnosis and treatment, as well as lead to the development of new immunotherapeutic reagents.

REFERENCES

1. Guralnick, MW, Benton AW. Entomological aspects of insect sting allergy. In: Levine MI, Lockey RL, eds. Monograph on Insect Allergy. Milwaukee, WI: American Academy of Allergy and Immunology, 1995 and 2003.
2. Loveless MH, Fackeler WR. Wasp venom allergy and immunity. Ann Allergy 1959; 14:347–366.
3. Lichtenstein LM, Valentine MD, Sobotka AK. Insect Allergy: the state of the art. J Allergy Clin Immunol 1979; 64:5–12.
4. King, TP, Sobotka AK, Kochoumian L, et al. Allergens of honeybee venom. Arch Biochem Biophys 1976; 172:661–671.
5. Krombein KV, Hurd PV, Smith DR, et al. Catalog of Hymenoptera in America north of Mexico. Washington, D.C.: Smithsonian Institute Press, 1979:v–vii.
6. Borror DJ, White RE. Sawflies, Ichneumons, Chalcids, Ants, Wasps, and Bees: Order Hymenoptera. Boston: Houghton Mifflin Company, 1970:312.
7. Mulfinger LM, Yunginger JW, Styer WE, et al. Sting morphology and frequency of sting autotomy among medically important vespids and the honey bee. J Med Entomol 1992; 29:325–328.
8. Dudler T, Schneider T, Annand RR, et al. Antigenic surface of bee venom allergen phospholipase A2. J Immunol 1994; 152:5514–5522.
9. Scott DL, Otwinowski Z, Gelb MH, et al. Crystal structure of bee venom phospholipase A2 in a complex with a transition-state analogue. Science 1990; 250:1563–1566.
10. Soldatova LN, Crameri R, Gmachl M, et al. Superior biologic activity of the recombinant bee venom allergen hyaluronidase expressed in baculovirus-infected insect cells as compared with *Escherichia coli*. J Allergy Clin Immunol 1998; 101:691–698.
11. Markovic-Housley Z, Miglierini, G, Soldatova L, et al. Crystal structure of hyaluronidase, a major allergen of bee venom. Structure 2000; 8:1025–1035.
12. Hoffman DR. Hymenoptera venom allergens. Clin Rev Allergy Immunol 2006; 30:109–128.
13. Winningham KM, Fitch CD, Schmidt M, et al. Hymenoptera venom protease allergens. J Allergy Clin Immunol 2004; 114:928–933.
14. Kettner A, Hughes GJ, Frutiger S, et al. Api m 6: a new bee venom allergen. J Allergy Clin Immunol 2001; 107:914–920.
15. Hoffman DR, Jacobson RS. Allergens in Hymenoptera venom XXVII: bumble bee venom allergy and allergens. J Allergy Clin Immunol 1996; 97:812–821.
16. Soldatova L, Kochoumian L, King TP. Sequence similarity of a hornet D. maculata venom allergen phospholipase A1 with mammalian lipases. FEBS Lett 1993; 320:145–149.
17. Lu G, Kochoumian L, King TP. Sequence identity and antigenic cross reactivity of white face hornet venom allergen, also a hyaluronidase, with other proteins. J Biol Chem 1995; 270:4457–4465.
18. Lu, G, Villalba M, Coscia MR, et al. Sequence analysis and antigen cross reactivity of a venom allergen antigen 5 from hornets, wasps and yellowjackets. J Immunol 1993; 150:2823–2830.
19. Tomalski MD, King TP, Miller LK. Expression of hornet genes encoding venom allergen antigen 5 in insects. Arch Insect Biochem Physiol 1993; 22:303–313.
20. Hoffman DR. Hymenoptera venom proteins. New York and London: Plenum Publishing Co., 1996:169–186.
21. Hoffman DR. Allergens in Hymenoptera venom XXV: the amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. J Allergy Clin Immunol 1993; 92:707–716.
22. King TP, Lu G. Genbank Accession No. AF174527 and AF174528, 1999.
23. Monsalve RI, Gang L, King TP. Recombinant venom allergen, antigen 5 of yellowjacket (*Vespula vulgaris*) and paper wasp (*Polistes annularis*) by expression in bacteria or yeast. Protein Expr Purif 1999; 16:410–416.
24. King TP, Lu G, Gonzalez M, et al. Yellowjacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross reactivity with their hornet and wasp homologs and possible implications for clinical allergy. J Allergy Clin Immunol 1996; 98:588–600.
25. Kolarich D, Leonard R, Hemmer W, et al. The N-glycans of yellowjacket venom hyaluronidases and the protein sequence of its major isoform in *Vespula vulgaris*. FEBS J 2005; 5182–5190.
26. Skov LK, Seppala U, Coen JJ, et al. Structure of recombinant Ves v 2 at 2.0 Angstrom resolution: structural analysis of an allergenic hyaluronidase from wasp venom. Acta Crystallogr D 2006; 62:595–604.
27. Henriksen A, King TP, Mirza O, et al. Major venom allergen of yellowjackets, Ves v 5: structural characterization of a pathogenesis-related protein superfamily. Proteins 2001; 45:438–448.
28. Hoffman DR, Sakell RH, Schmidt M. Sol i 1, the phospholipase allergen of imported fire ant venom. J Allergy Clin Immunol 2005; 115:611–616.
29. Schmidt M, McConnel TJ, Hofmann DR. Production of a recombinant imported fire ant venom allergen, Sol i 2, in native and immunoreactive form. J Allergy Clin Immunol 1996; 98:82–88.
30. Hoffman DR. Allergens in Hymenoptera venom XXIV: the amino acid sequences of imported fire ant venom allergens Sol i II, Sol i III, and Sol i IV. J Allergy Clin Immunol 1993; 91:71–78.

31. King TP, Hoffman DR, Lowenstein H, et al. Allergen nomenclature. J Allergy Clin Immunol 1995; 96:5–14.
32. Hoffman DR, Schmidt JO. Phopholipases from Asian honeybees. J Allergy Clin Immunol 2000; 105:S56.
33. Hoffmann DR, El-Choufani E, Smith MM, et al. Occupational allergy to bumblebees:allergens of *Bombus terrestris*. J Allergy Clin Immunol 2001; 108:855–860.
34. Pantera B, Hoffman DR, Carresi L, et al. Characterization of the major allergens purified from the venom of the paper wasp *Polistes gallicus*. Biochim Biophys Acta 2003; 1623:72–87.
35. Kolarich D, Loos A, Leonard R, et al. A proteomic study of the major allergens from yellowjacket venoms. Proteomics 2007; 7:1615–1623.
36. Hoffman DR. Reactions to less common species of fire ants. J Allergy Clin Immunol 1997; 100:679–683.
37. Milne TJ, Abbenante G, Tyndall JDA, et al. Isolation and characterization of a cone snail protease with homology to CRISP proteins of the pathogenesis-related protein superfamily. J Biol Chem 2003; 278:31105–31110.
38. Gmachl M, Kreil G. Bee venom hyaluronidase is homologous to a membrane protein of mammalian sperm. Proc Natl Acad Sci U S A 1993; 90:3569–3573.
39. Donovan GR, Baldo BA, Sutherland S. Molecular cloning and characterization of a major allergen (Myr p I) from the venom of the Australian jumper ant, *Myrmecia pilosula*. Biochim Biophys Acta 1993; 1171:272–280.
40. Matuszek MA, Hodgson WC, King RG, et al. Some enzymatic activities of two Australian ant venoms: a jumper ant *Myrmecia pilosula* and a bulldog ant *Myrmecia pyriformis*. Toxicon 1994; 32:1543–1549.
41. Kasahara M, Gutnecht J, Brew K, et al. Cloning and mapping of a testis-specific gene with sequence similarity to a sperm-coating glycoprotein gene. Genomics 1989; 5:527–534.
42. Murphy EV, Zhang Y, Zhu W, et al. The human glioma pathogenesis-related protein is structurally related to plant pathogenesis-related proteins and its gene is expressed specifically in brain tumors. Gene 1995; 159:131–135.
43. Hawdon JM, Jones BF, Hoffmann DR, et al. Cloning and characterization of ancylostoma-secreted protein. J Biol Chem 1996; 271:6672–6678.
44. Morrissette J, Kratzschmar J, Haendler B, et al. Primary structure and properties of helothermine, a peptide toxin that blocks ryanodine receptors. Biophys J 1995; 68:2280–2288.
45. Kreil G. Hyaluronidases—a group of neglected enzymes. Prot Sci 1995; 4:1666–1669.
46. Carriere F, Thirstrup K, Boel E, et al. Structure-function relationships in naturally occurring mutants of pancreatic lipase. Protein Eng 1994; 7:563–569.
47. Ravi V, Ramachandran S, Thompson RW, et al. A characterization of a recombinant immunodiagnostic antigen (NIE) from *Strongyloides stercoralis L3* larvae. Mol Biochem Parasitol 2002; 125:73–81.
48. Lokeshwar VB, Schroeder GL, Carey RL, et al. Regulation of hyaluronidase activity by alternative mRNA splicing. J Immunol 2002; 277:33654–33663.
49. Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol 2000; 106:228–238.
50. Bessette P, Aslund F, Beckwith J, et al. Efficient folding of proteins with multiple disulfide bonds in the Escherichia coli cytoplasm. Proc Natl Acad Sci 1999; 96:13703–13708.
51. Dudler T, Chen W, Wang S, et al. High-level expression in Escherichia coli and rapid purification of enzymatically active honey bee venom phospholipase A2. Biochim Biophys Acta 1992; 1165:201–210.
52. Binder M, Fierlbeck G, King TP, et al. Individual hymenoptera venom compounds induce upregulation of the basophil activation marker ectonuleotide pyrophosphatase/phosphodiesterase 3 (CD203c) in sensitized patients. Int Arch Allergy Immunol 2002; 129:160–168.
53. King TP, Jim SY, Monsalve RI, et al. Recombinant allergens with reduced allergenicity but retaining immunogenicity of the natural allergens: hybrids of yellowjacket and paper wasp venom allergen antigen 5s. J Immunol 2001; 166:6057–6065.
54. Davies DR, Cohen GH. Interaction of protein antigens with antibodies. Proc Natl Acad Sci U S A 1996; 93:7–12.
55. King TP, Kochoumian L, Lu G. Murine T and B cell responses to natural and recombinant hornet venom allergen, Dol m 5.02 and its recombinant fragments. J Immunol 1995; 154:577–584.
56. King TP, Lu G, Agosto G. Antibody responses to bee melittin (Api m 4) and hornet antigen 5 (Dol m 5) in mice treated with the dominant T-cell epitope peptides. J Allergy Clin Immunol 1998; 101:397–403.
57. Henriksen A, Ipsen, H, Larsen JN, et al. Dominant epitopes and allergic cross-reactivity: complex formation between a Fab fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen Bet v 1. J Immunol 2000; 165:331–338.
58. Tretter V, Altmann F, Kubelka V, et al. Fucose alpha 1,3-linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. Int Arch Allergy Immunol 1993; 102: 259–266.
59. Altmann F. The role of protein glycosylation in allergy. Int Arch Allergy Immunol 2007; 142: 99–115.
60. Kubelka V, Altmann F, Staudacher E, et al. Primary structures of the N-linked carbohydrate chains form honeybee venom phospholipase A2. Eur J Biochem 1993; 213:1193–1204.

61. Bencurova M, Hemmer W, Focke-Tejki M, et al. Specificity of IgG and IgE antibodies against plant and insect glycoprotein glycans determined with artificial glycoforms of human transferrin. Glycobiology 2004; 14:457–466.
62. Jappe U, Raulf-Heimsoth M, Hoffmann M, et al. In vitro Hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. Allergy 2006; 61:1220–1229.
63. Muller U, Akdis CA, Fricker M, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell tolerance in bee sting allergic patients. J Allergy Clin Immunol 1998; 101:747–754.
64. Dhillon M, Roberts C, Nunn T, et al. Mapping human T cell epitopes on phospholipase A2: the major bee-venom allergen. J Allergy Clin Immunol 1992; 90:42–51.
65. Carballido JM, Carballido-Perrig N, Kagi MK, et al. T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A2. J Immunol 1993; 150:3582–3591.
66. Bohle B, Zwolfer B, Fischer GF, et al. Characterization of the human T cell response to antigen 5 from *Vespula vulgaris* (Ves v 5). Clin Exp Allergy 2005; 35:367–373.
67. Specht C, Kolsch E. The murine (H-2k) T-cell epitopes of bee venom phospholipase A2 lie outside the active site of the enzyme. Int Arch Allergy Immunol 1997; 112:226–230.
68. King TP, Lu G. Hornet venom allergen antigen 5, Dol m 5: its T-cell epitopes in mice and its antigenic cross-reactivity with a mammalian testis protein. J Allergy Clin Immunol 1997; 99:630–639.
69. Dudler T, Machado DC, Kolbe L, et al. Carbohydrate-dependent, HLA class II-restricted, human T cell response to the bee venom allergen phospholipase A2 in allergic patients. Eur J Immunol 1995; 25:538–542.
70. Schneider T, Dudler T, Annand RR, et al. Comparison of the antibody response to bee venom phospholipase A2 induced by natural exposure in humans or by immunization in mice. J Mol Recognit 1997; 10:93–100.
71. van Neerven RJJ, Ebner C, Yssel H, et al. T-cell responses to allergens: epitope-specificity and clinical relevance. Immunol Today 1996; 17:526–532.
72. Ebner C, Schenk S, Najafian N, et al. Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. J Immunol 1995; 154:1932–1940.
73. Hamilton RG, Wisenauer JA, Golden DB, et al. Selection of Hymenoptera venoms for immunotherapy on the basis of patient's IgE antibody cross-reactivity. J Allergy Clin Immunol 1993; 92:651–709.
74. Fromberg J. IgE as a marker in allergy and the role of IgE affinity. Allergy 2006; 61:1234.
75. Christensen LH, Holm J, Skovsgaard J, et al. The significance of clonality and affinity of individual allergen-specific IgE antibodies for the activation of human basophils. J Allergy Clin Immunol 2006; 117(2):S310.
76. van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. Int Arch Allergy Immunol 2002; 129:189–197.
77. Muller UR. Insect Sting Allergy. Stuttgart: Gustav Fisher, 1990; 54:54.
78. Zora JA, Swanson MC, Yunginger JW. A study of the prevalence and clinical significance of venom-specific IgE. J Allergy Clin Immunol 1988; 81:77–82.
79. Settipane GA, Chafee FH, Klein DE, et al. Anaphylactic reactions to Hymenoptera stings in asthmatic patients. Clin Allergy 1980; 10:659–665.
80. Habermann E. Bee and wasp venoms. Science 1972; 177:314–322.
81. Nakajima T. Biochemistry of vespid venom. In: Tu AT, ed. Handbook of Natural Toxins. New York: Marcel Dekker 1984:109–133.
82. Metz M, Piliponsky AM, Chen CC, et al. Mast cells can enhance resistance to snake and honeybee venoms. Science 2006; 313:525–530.
83. Lee VS, Tu WC, Jinn TR, et al. Molecular cloning of the precursor polypeptide of mastoparan B and its putative processing enzyme, dipeptidyl peptidase IV, from the black-bellied hornet, *Vespa basalis*. Insect Mol Biol 2007; 16:231–237.
84. Kind LS, Ramaika C, Allaway E. Antigenic, adjuvant and permeability enhancing properties of melittin in mice. Allergy 1981; 36:155–160.
85. King TP, Kochoumian L, Joslyn A. Melittin-specific monoclonal and polyclonal IgE and IgG1 antibodies from mice. J Immunol 1984; 133:2668–2673.
86. Ho CL, Lin YL, Chen WC, et al. Immunogenicity of mastoparan B, a cationic tetradecapeptide isolated from the hornet (*Vespa basalis*) venom, and its structural requirements. Toxicon 1995; 33:1443–1451.
87. Hirai Y, Kuwada M, Yasuhara T, et al. A new mast cell degranulating peptide "mastoparan" in the venom of *Vespula lewisii*. Chem Pharm Bull (Tokyo) 1979; 27:1942–1944.
88. Higashijima T, Wakamatus K, Takemitsu M, et al. Conformational change of mastoparan from wasp venom on binding with phospholipid membrane. FEBS Lett 1983; 152:227–230.
89. Whiles JA, Brasseur R, Glover KJ, et al. Orientation and effects of mastoparan X on phospholipid bicelles. Biophys J 2001; 80:280–293.
90. Wu TM, Chou TC, Ding YA, et al. Stimulation of TNF-alpha, IL1-beta and nitrite release from mouse cultured spleen cells and lavaged peritoneal cells by mastoparan M. Immunol Cell Biol 1999; 77:476–482.

91. Agriolas A, Pisano JJ. Facilitation of phospholipase A2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom. J Biol Chem 1983; 258:13697–13702.
92. Okano Y, Takai H, Tohmatsu T, et al. A wasp venom mastoparan-induced polyphosphoinositide breakdown in rat peritoneal mast cells. FEBS Lett 1985; 188:363–366.
93. Mizuno K, Nakahata N, Ohizumi Y. Mastoparan-induced phosphatidylcholine hydrolysis by phospholipase D activation in human astrocytoma cells. Br J Pharmacol 1995; 116:2090–2096.
94. Mizuno K, Nakahata N, Ohizumi Y. Characterization of mastoparan-induced histamine release RBL-2H3 cells. Toxicon 1998; 36:447–456.
95. Higashijima T, Burnier J, Ross EM. Regulation of Gi and Go by mastoparan, related amphiphilic peptides and hydrophobic amines. J Biol Chem 1990; 265:14176–14186.
96. King TP, Sui YJ, Wittkowski KM. Inflammatory role of two venom components of yellowjackets (*Vespula vulgaris*): a mast cell degranulating peptide mastoparan and phospholipase A1. Int Arch Allergy Immunol 2003; 131:25–32.

15 Biting Insect Allergens

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INTRODUCTION

Allergic reactions to insect bites are much less common than reactions to insect stings. Several studies suggest that severe bite reactions occur about 50 times less commonly than severe sting reactions. Many of the clinical aspects of biting insect allergy have been thoroughly discussed in a 2003 review (1). In this chapter, the main focus will be on which insects are important, the known allergens and salivary components, and the appropriate use of immunotherapy. There are more than 14,000 species from 400 genera of blood-feeding arthropods. The most important hematophagous insects belong to the orders Diptera (flies), Hemiptera (bugs), and Siphonaptera (fleas). Ticks of the order Acarina of the class Arachnida will also be considered, although they are not insects. Many other bugs of the order Hemiptera and some beetles, especially aquatic species, of the order Coleoptera occasionally bite humans, but allergic reactions have not been reported. In addition, many larval forms may bite, but again allergic reactions to these bites are extremely rare. Allergic reactions to bites have been ascribed to other arachnids, but definitive evidence is lacking to demonstrate IgE antibodies against centipede and millipede bites. There probably are rare cases of IgE-mediated allergy to spider bites, but there are no published systematic studies.

TAXONOMY OF BITING INSECTS

Diptera, Flies

Many flies are hematophagous. In almost all cases only the females bite, requiring a blood meal to develop eggs. The more common biting flies are outlined in Table 1. A blackfly, deerfly, and horsefly are illustrated in Figures 1 to 3.

Hemiptera, Bugs

There are two important families of biting bugs in North America. The first are variously called kissing bugs, assassin bugs, conenose bugs, vinchucas, or reduviid bugs and are members of the family Reduviidae. There are 39 genera of which the most important are *Triatoma* (Fig. 4) and *Reduvius*. The Latin American genera *Rhodnius* and *Panstrongylus* are important members of this family. The second family of blood-sucking bugs is Cimicidae or bedbugs. There are seven genera, and the species *Cimex lectularius* is the most infamous human bedbug.

Siphonaptera, Fleas

The fleas are almost all parasitic insects with 74% of species associated with rodent hosts, and about 6% with avian hosts. The species associated with humans are members of the superfamily Pulicoidea, family Pulicidae. The most common are the dog and cat fleas *Ctenocephalides canis* and *Cte. felis felis*. *Pulex irritans*, a parasite of carnivores, is sometimes called the human flea. Fleas of the genus *Tunga* are found in Central and South America.

Arachnids

Many species of hard and soft ticks and chiggers bite humans. Allergic reactions to these bites are extremely rare, although they have been reported (2,3) from many regions.

Table 1 Biting Flies (Diptera)

Common name	Family	Genera
Mosquito	Culicidae	*Aedes, Culex, Anopheles*, others
Blackfly	Simuliidae	*Simulium, Prosimulium, Cnephia*
Biting midge	Ceratopogonidae	*Culicoides*, others
Horsefly	Tabanidae	*Tabanus, Hybomitra*
Deerfly, yellow fly	Tabanidae	*Chrysops*
Sand fly	Psychodidae (Phlebotominae)	*Lutzomyia, Phlebotomus*
Botfly and warble fly	Oestridae	*Dermatobia*, others
Stable fly	Muscidae	*Stomoxys, Haematobia*
Tsetse fly	Glossinidae (Muscidae)	*Glossina*

Figure 1 (*See color insert.*) Photograph of a blackfly, *Simulium*; note the humped appearance. *Source*: Courtesy of Jerry F. Butler, University of Florida.

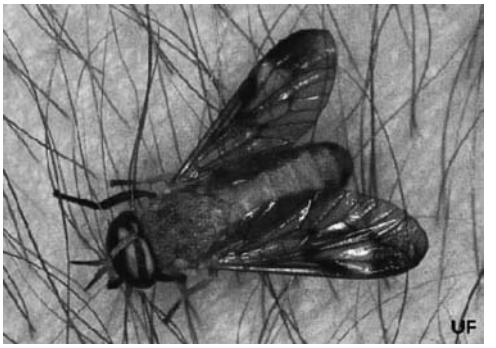

Figure 2 (*See color insert.*) A deerfly, *Chrysops*, in biting position. The insect is usually yellow or green, and the bite is painful. *Source*: Courtesy of Jerry F. Butler, University of Florida.

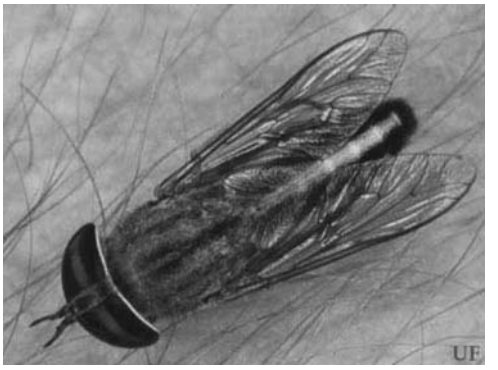

Figure 3 (*See color insert.*) A horsefly, *Tabanus*, biting. Horseflies are typically larger than deerflies, have very noisy flight, and the bites are quite painful. *Source*: Courtesy of Jerry F. Butler, University of Florida.

Figure 4 Scanning electron micrograph of a kissing bug, *Triatoma protracta*. Bites are painless, typically occurring while sleeping. The insect's definitive host is the wood rat. *Source*: Courtesy of C. Demetry and R. Biderman, Worcester Polytechnic Institute.

IDENTIFYING BITING INSECTS

The identification of biting insect can be extremely difficult, even with representative specimens. Deerflies, horseflies (Fig. 1), and stable flies all cause immediate pain when they bite. Mosquitoes can usually be recognized, but identification of species may require an expert. Identification of flea species is the realm of specialists. Kissing bugs typically bite painlessly, most commonly while the victim is sleeping. Useful identification guides with many illustrations are available for hobbyists including Borror and White's A Field Guide to the Insects and the National Audubon Society Field Guides. The much more technical and comprehensive reference to insects of North America by Arnett (4) is recommended for those with a serious interest. Keys to various groups are available in the entomology literature and vary widely in quality and usability. Most states have official entomologists, usually with the Department of Agriculture, who are many times willing to assist in insect identification for medical purposes. There are also entomologists at many land-grant universities, who are willing to assist with insect identification.

GEOGRAPHIC DISTRIBUTION OF SOME BITING INSECTS

Mosquitoes are cosmopolitan with species found in almost all land areas of the world. Fleas are found in most areas of the world, except in very dry climate areas. Blackflies are found in the northern United States, in most of Canada, and in much of Northern and Central Europe and Asia; in tropical areas they require the presence of rapidly running water to breed. Horseflies and deerflies are found in most temperate areas of the world. Tsetse flies are only found in tropical Africa and a few laboratories in the United States and Europe.

Ticks are found around wooded areas and are commonly carried by dogs, birds, and deer. Various species are found distributed in different areas of the world. Sand flies and biting midges are also found in many areas, especially around beaches and livestock.

Although bugs of the reduviid group are found in many areas, almost all cases of allergic reactions to bites are found in the southwestern United States, Hawaii, Mexico, and Central America. These insects are dependent on the distribution of their hosts, for example, the wood rat in California for *Triatoma protracta*. Other species feed on dogs, cats, mice, opossums, and armadillos.

SALIVARY COMPONENTS AND ALLERGENS OF BITING INSECTS

According to Ribeiro (5), blood feeding evolved independently multiple times among hematophagous arthropods. A variety of anticlotting factors, platelet aggregation antagonists, and vasodilators developed to counter the host's hemostatic and immunomodulatory factors (6). In addition, arthropod salivas contain digestive enzymes (7) and hyaluronidase. One unsuspected property of some insect salivas is enhancement of infectivity of parasites carried by the arthropod (8). Sand fly saliva decreases the minimum effective dose of *Leishmania major* in mice by several orders of magnitude. Studies suggest that immune reactions to biting insect

saliva may increase resistance to infection with protozoans (9). In 2002, the first complete genome sequence of a biting insect, the malaria mosquito, *Anopheles gambiae*, became available (9). The set of proteins expressed by the salivary glands have been named the sialome (10) by Ribeiro (11) and have been mapped for mosquitoes (11), kissing bugs (12), and ticks (13).

Mosquitoes

Many of the characterized protein components of mosquito saliva are described in Table 2. A number of the proteins appear to be related to either digestive functions such as maltase, amylase, and esterase or inhibition of hemostasis such as tachykinin, factor Xa inhibitor, purine nucleosidase, and apyrase or adenosine triphosphate diphosphohydrolase, which inhibits ADP-dependent platelet aggregation (11,14–19). Protein D7 contains two insect pheromone/odorant-binding protein domains and is expressed in a number of different sizes (11,19). D7 proteins appear to be major allergens in most species.

There are numerous published studies of IgE-binding components of various mosquito extracts. Some are performed with "saliva," some with salivary gland extract, some with thorax extract, and some with whole-body extract. Numerous species and at least four genera have been investigated. Table 3 is a compendium of the major and shared allergens in

Table 2 Selected Protein Components in *Aedes aegypti* Mosquito Saliva Identified by Two-Dimensional Electrophoresis Followed by Trypsin Digestion and Mass Spectrometry, and Also by cDNA Expression

Component	Genbank identifier (gi)
Protein D7 11	16225992
Protein D7 12	118216
Antigen 5–like protein	18568284
Apyrase	1703351
Purine nucleosidase	21654712
Protein disulfide isomerase	94468800
Heat shock protein 70 kDa	94468818
Adenosine triphosphate phosphatase	
Beta unit	94468834
Alpha unit	94468442
Angiopoietin-like protein	1858298
Salivary serpin 1	18568304
Salivary serpin 2	94469320
62-kDa proteins	18568300 and 2
Amylase	2190949
Adenosine deaminase	18568326
C type lectins	94468370, 18568318
34-kDa protein 1	94468642
34-kDa protein 2	18568296
Angiopoietin-like protein 2	94468352
30.5-kDa salivary protein	61742033
30-kDa salivary antigen	18568322

Source: From Ref. 11.

Table 3 Molecular Weights (in kDa) of IgE-Binding Components in Mosquito Extracts from Various Species

Aedes aegypti	*Aedes vexans*	*Aedes communis*	*Culex tarsalis*	*Culiseta inornata*
Major allergens				
65	65	36	43	65
48	43	30	17	40
34 = D7	38	22	15	34
31				
15				

Minor allergens shared by at least three species 160, 110, 65, 62, 50, 46, 40, 32.5, 24, 17, and 15 kDa.
Allergens have not been purified; data mainly from immunoblot experiments, some, e.g., D7, have been cloned or expressed.
Source: From Refs. 21–26.

immunoblot experiments for five species and thirteen species, respectively. It appears that D7 protein is an important allergen in *Aedes*, *Culex*, and related mosquitoes, and that apyrase may also be an allergen. None of the other IgE-binding bands has been definitively characterized. A 2004 study (20) reported on 14 individuals with acute systemic reactions to mosquito bites; all showed IgE antibodies specific to saliva from at least one of the five species tested.

Blackflies

Studies on the saliva of blackflies are very limited. Cupp et al. (27,28) isolated and cloned a major protein of molecular weight 15,351 Da with strong vasodilator activity manifested by rapid and persistent induction of erythema. The enzyme apyrase is also found in blackfly salivary gland secretions. Wirtz (29) demonstrated high contents of histamine, putrescine, spermine, *N*-monoacetylspermine, and spermidine, as well as the presence of proteins with esterase activity. Almost all reactions to blackfly bites are not IgE mediated, and the dermatologic reactions have been classified into six forms by Farkas (30). These are edematous, erythematous-edematous, "erysipeloid," inflammatory-indurative, hemorrhagic (plaques, nodules, or vesicles), and allergic.

Horseflies and Deerflies

Deerfly saliva contains chrysoptin, an inhibitor of ADP-induced platelet aggregation that inhibits fibrinogen binding to the glycoprotein IIb/IIIa receptor on platelets (31,32). The recombinant protein with a molecular mass of 65 kDa, the same as that of the natural protein, has been expressed in insect cells. This may be a protein similar to the 69-kDa IgE-binding protein found in immunoblots using sera from European patients who experienced anaphylaxis from Chrysops bites (33).

Sand Flies

Sand fly saliva contains a factor that enhances the infectivity of *Leishmania* by inhibiting the ability of interferon-γ to activate macrophages. It also reduces nitric oxide production (34). A delayed-type hypersensitivity reaction to saliva components may also play a role in infectivity and adverse reactions (35). Sand fly saliva is also known to contain a potent vasodilator named maxadilan, apyrase, 5′-nucleotidase, hyaluronidase. Other proteins include an anticlotting protein with a carbohydrate-recognition domain and several proteins of unknown function.

Kissing Bugs and Bedbugs

The major salivary anticoagulant proteins of *Rhodnius prolixus* are named prolixins and consist of four related nitrophorin molecules (36), which are heme proteins that carry nitric oxide. The major component has a molecular weight of 19,689 Da and inhibits factor VIII–mediated activation of factor X. Two proteins have been characterized from the saliva of *T. pallidipennis*, triabin of molecular weight 15,620 Da, an inhibitor of thrombin-based hydrolysis of fibrinogen (37), and pallidipin (38) of molecular weight 19,000 Da, an inhibitor of collagen-induced platelet aggregation. Functional studies of coagulation inhibition suggest that different species of Triatominae have functionally different mechanisms of coagulation inhibition and different sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) profiles of salivary proteins (39). These proteins along with proteins having histamine binding, platelet inhibition, anticoagulation, and nitric oxide transport activities are all members of the lipocalin family (40). The three-dimensional structures of the nitrophorins NP1, 2, and 4 have been determined by X-ray crystallography. Many important vertebrate-derived allergens are also members of the lipocalin family. An activatable serine protease of molecular weight 40,000 Da, named triapsin, with an arginine specificity, has been isolated from saliva of *T. infestans* (41).

Studies to characterize the allergenic proteins of *T. protracta* indicate that the major allergens are of molecular weight 18,000 to 20,000 Da and almost all the allergenic activity was found between pI 6.7 and 7.3 and at pI 8.2 (42). This allergen, a member of the lipocalin family named procalin, has been identified, cloned, and expressed in yeast cells (43). The recombinant procalin reacts in ELISA assays with IgE antibodies from allergic patients and cross-reacts with native allergen. Antiserum against procalin was used in immunohistochemistry to localize procalin to the cytoplasm of cuboidal epithelium and the luminal contents of the salivary glands.

The saliva of the bedbug, *C. lectularius*, contains a nitrophorin (44) and also an inhibitor of activation of factor X to factor Xa in the tenase complex, which does not directly inhibit factor VIII (45). The apparent molecular weight of this factor was 17,000 Da. Bedbug saliva also contains apyrase.

Fleas

Very little work has been done applying contemporary methods to studies of flea saliva. The only characterized proteins in flea saliva are apyrase, which prevents ADP-induced platelet aggregation (46), platelet-activating factor acetylhydrolase, and naphthyl esterases.

Diagnostic studies of allergy to flea bites in humans are complicated by the relatively more common occurrence of inhalant allergy to cat fleas. The major salivary allergen of cat fleas causing allergy in dogs is a protein of molecular weight 18,000 Da and pI 9.3, termed Cte f 1 (47).

Ticks

There has been a great deal of interest in ticks with the recognition of Lyme disease and ehrlichiosis. Allergic reactions to tick bites are usually the result of bites by soft ticks, Ixodidae. Pigeon ticks, *Argas reflexus*, as well as deer ticks and paralysis ticks have all been reported to cause systemic allergic reactions. Tick salivas have been found to contain apyrase and antiplatelet activities (13,48) as well as numerous proteins from 18 to 160 kDa. Several proteins from 15 to 50 kDa were induced by feeding (49).

Five salivary allergens have been isolated from the Australian paralysis tick, *Ixodes holocyclus*, of molecular weights 28, 45, 50, 55, and 355 kDa (50). The allergens at 28 and 355 kDa appear to react with IgE from most patients and SGA1 at 28 kDa is useful for skin prick testing and radioimmunoassay (51).

CROSS-REACTIVITY AMONG BITING INSECTS

There is very limited experimental data on IgE cross-reactivity among biting insects. There are some common antigens exhibiting a limited degree of cross-reactivity among mosquito genera and species (21–23). However, typical clinical reactions, including nonallergic responses, are species dependent for most individuals. There appears to be some cross-reactivity based on RAST testing between horseflies and deerflies and sometimes also blackflies (1). It is not known if this is clinically relevant. There are antigen 5–related proteins in insect salivas (11), which could be cross-reactive with vespid venom proteins.

Allergic reactions to kissing bugs and bedbugs exhibit a strong species dependence, and it is rare to find patients either skin test positive or RAST positive to more than a single species (52).

There are no data on cross-reactivity with fleas in human subjects, but studies on dogs suggest species specificity. Reactions to sand flies, biting midges, ticks, tsetse flies, and other biting arthropods are probably species specific, but experimental data are lacking.

BITING INSECT CONTROL

The control of biting insects is a very difficult problem, as attempts at mosquito vector control in the tropical world have demonstrated. Use of most pesticides, especially large area spraying, is best left to public health authorities. Spraying of yards is usually not recommended, is almost always of limited value, and may involve significant risk of pesticide exposure to children and pets.

Control of biting insects in the home should emphasize avoidance. Screens should be used on all doors and windows. Various forms of flypaper traps with and without attractants are effective and environment friendly. One highly recommended type is clear and is placed on glass doors and windows, another uses 7-W light bulbs. Control of fleas from pets, particularly in warm and humid areas, can be extremely difficult. Veterinarians can recommend several programs, including the use of growth regulators that are fed to dogs and cats to prevent development of adult fleas and substances that are spotted onto the animal

and absorbed through the skin or others that are injected. The extensive use of anti-acetylcholinesterase pesticides is ineffective and leads to development of resistant fleas. Animals should be regularly washed and carpets and furniture regularly vacuumed to help control fleas.

Bedbug infestations should be eliminated by treatment with appropriate pesticides, preferably by a licensed professional. Reduviid bugs are primarily outdoor insects and are best controlled by eliminating their definitive hosts around houses. *T. protracta* comes from wood rat nests, but other species have varied hosts. Professional assistance is recommended.

Horseflies, deerflies, and blackflies are primarily found around water. They can be extremely difficult to avoid in these areas. The almost ubiquitous mosquito is extremely difficult to avoid. The use of repellants containing DEET (*N*,*N*′-diethyl-*m*-toluamide) can help; these should be used with caution in small children. Many other repellants are less effective. Covering up of as much exposed skin as possible and avoiding being outdoors at high-risk times, like early morning and evening, can help. Avoidance of areas of high mosquito density should be practiced. Sources of standing water should be minimized or eliminated. Mosquito netting and the use of citronella candles can also reduce mosquito density. An ultraviolet bug light can also help, particularly after dark. Both electrocuting and trap models are available. Use of yellow or orange light bulbs outdoors minimizes the attraction of insects to porches and garages.

Reliable guidance can be obtained from websites sponsored by various government agencies: for example, http://wwwn.cdc.gov/travel/yellowBookCh2-InsectsArthropods.aspx, http://www.epa.gov/pesticides/health/mosquitoes/insectrp.htm, and http://www.ces.ncsu.edu/depts/ent/notes/Urban/repellents.htm.

IMMUNOTHERAPY

Evidence for Efficacy

There is very limited controlled study evidence for the efficacy of immunotherapy in preventing life-threatening systemic reactions to insect bites. There are a significant number of anecdotal reports, most of which describe variants of large local reactions. The only challenge verified trial with an insect salivary gland–derived vaccine was reported in a noncontrolled study with *T. protracta* in 1984 (53). Immunotherapy provided protection in all five patients with no significant side effects. Immunologic changes were also observed in parallel with protection as assessed by bite challenge.

A report of treatment with deerfly whole-body vaccine, although not controlled, suggests efficacy for patients with systemic reactions (54). Immunotherapy with whole-body extracts has been tried in cases of life-threatening allergy to mosquito bites (55). Results have been mixed with some patients developing the ability to tolerate a larger number of bites and others developing major complications as described below.

It should be noted that in the United States and most other countries, there are no licensed extracts of insect saliva or salivary glands and that most whole-body extracts from biting insects are not approved for use in allergen vaccine therapy. These products should only be used under an investigational new drug (IND) application, as part of a controlled study. One study (56) demonstrated that it is possible to prepare substantially more potent vaccines from biting insects than are available in current commercial products. In some countries, special unlicensed products can be prepared for individual patients.

Most cases of severe allergy to mosquito bites are best managed by prophylactic use of antihistamines such as cetirizine (57). In controlled trials, cetirizine has been shown to reduce pruritus, significantly decrease large local reaction development, and may also prevent systemic reactions (57,58). The antihistamine, loratadine, used prophylactically reduces wheeling and pruritis from mosquito bites in children; it also reduces the size of bite lesions at 24 hours (59).

Known Risks

Immunotherapy with mosquito whole-body vaccine has been shown to cause local pain, swelling, and redness in a patient who tolerated injections at lower concentrations. Another patient in the same report developed arthralgias, fatigue, myalgias, weakness and swelling of

distal extremities, despite treatment with terfenadine, cimetidine, and prednisone (55). Life-threatening anaphylactic reactions have been observed in studies of experimental vaccines derived from mosquito cell tissue culture (60).

The use of other biting insect vaccines has not been reported to cause unusual reactions, and the experiences reported in the literature correspond to those seen with other allergens routinely used in allergen vaccine therapy.

Potential Risks

The existence of species and genus specificity for many biting insect reactions requires the use of more sophisticated diagnostic reagents than are currently commercially available. There is a significant risk of using an ineffective preparation and a potential risk of sensitization. Also, many hematophagous insects are vectors for serious diseases (parasitic, viral, rickettsial, and bacterial). Extracts prepared from salivary glands must be carefully monitored to be free of infectious agents. The use of biting insect extracts in allergen vaccine therapy is, for the most part, an experimental procedure, and proper safety procedures and regulations are necessary.

MOST IMPORTANT AND SALIENT POINTS

- There are a large variety of hematophagous insects and arachnids.
- Many different arthropods can cause bite allergy.
- Many, if not most, insect bite allergies may be species and/or genus specific.
- Insect saliva varies widely, but most species contain potent anticoagulants and digestive enzymes.
- The best diagnostic reagents are insect saliva or salivary gland extract, but none are commercially available or licensed in the United States or in Europe.
- Immunotherapy has been shown to be effective prophylaxis for severe systemic reactions for *T. protracta*, deerflies, and mosquitoes in uncontrolled studies.
- Immunotherapy with mosquito whole-body vaccine has been associated with significant side effects.
- Immunotherapy with biting insect whole body, salivary gland, and saliva extracts is still, for the most part, an experimental procedure.
- Control of many biting insects is difficult, but risk of exposure to bites can be greatly reduced.
- Reactions to mosquito bites are best managed by prophylaxis with cetirizine or loratadine.

REFERENCES

1. Hoffman DR. Allergic reactions to biting insects. In: Levine MI, Lockey RF, eds. Monograph on Insect Allergy. 4th ed. Milwaukee: American Academy of Allergy, Asthma and Immunology, 2003:161–173.
2. Van Wye JE, Hsu Y-P, Lane RS, et al. IgE antibodies in tick bite induced anaphylaxis. J Allergy Clin Immunol 1991; 88:968–970.
3. Gauci M, Loh RKS, Stone BF, et al. Allergic reactions to the Australian paralysis tick, *Ixodes holocyclus*. Diagnostic evaluation by skin test and radioimmunoassay. Clin Exp Allergy 1989; 74:279–283.
4. Arnett RH. American Insects. New York: Van Nostrand Reinhold, 1985.
5. Ribeiro JM. Blood-feeding arthropods: live syringes or invertebrate pharmacologists? Infect Agents Dis 1995; 3:143–152.
6. Tabachnik WJ. Pharmacological factors in the saliva of blood-feeding insects. Ann N Y Acad Sci 2000; 916:444–452.
7. Kerlin RL, Hughes S. Enzymes in saliva from four parasitic arthropods. Med Vet Entomol 1992; 6:121–126.
8. Theodos CM, Ribeiro JM, Titus RG. Analysis of enhancing effect of sand fly saliva on *Leishmania* infection in mice. Infect Immun 1991; 59:1592–1598.
9. Donovan MJ, Messmore AS, Scrafford DA, et al. Uninfected mosquito bites confer protection against infection with malaria parasites. Infect Immun 2007; 75:2523–2530.
10. Holt RA, Subramanian GM, Halpern A, et al. The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 2002; 298:129–149.

11. Ribeiro JMC, Arca B, Lombardo F, et al. An annotated catalogue of salivary gland transcripts in the adult female mosquito, *Aedes aegypti*. BMC Genomics 2007; 8:6.
12. Ribeiro JMC, Anderson J, Silva-Neto MAC, et al. Exploring the sialome of the blood-sucking bug, *Rhodnius prolixus*. Insect Biochem Mol Biol 2004; 34:61–79.
13. Valenzuela JG, Francischetti IM, Pham VM, et al. Exploring the sialome of the tick *Ixodes scapularis*. J Exp Biol 2002; 205:2843–2864.
14. Ribeiro JM, Nussenzveig RH, Tortorella G. Salivary vasodilators of *Aedes triseriatus* and *Anopheles gambiae* (Diptera: Culicidae) J Med Entomol 1994; 31:747–753.
15. Champagne DE, Smartt CT, Ribeiro JM, et al. The salivary gland-specific apyrase of the mosquito *Aedes aegypti* is a member of the 5′-nucleotidase family. Proc Nat Acad Sci U S A 1995; 92:694–698.
16. James AA, Blackmer K, Racioppi JV. A salivary gland-specific, maltase-like gene of the vector mosquito, *Aedes aegypti*. Gene 1989; 75:73–83.
17. Argentine JA, James AA. Characterization of a salivary gland-specific esterase in the vector mosquito, *Aedes aegypti*. Insect Biochem Mol Biol 1995; 25:621–630.
18. Stark KR, James AA. A factor Xa-directed anticoagulant from the salivary glands of the yellow fever mosquito *Aedes aegypti*. Exp Parasitol 1995; 81:321–331.
19. James AA, Blackmer K, Marinotti O, et al. Isolation and characterization of the gene expressing the major salivary gland protein of the female mosquito, *Aedes aegypti*. Mol Biochem Parasitol 1991; 44:245–253.
20. Peng Z, Beckett AN, Engler RJ, Hoffman DR, Ott NL, Simons FER. Immune responses to mosquito saliva in 14 individuals with systemic allergic reactions to mosquito bites. J. Allergy Clin. Immunol. 2004; 114:1189–94.
21. Peng Z, Li HB, Simon FER. Immunoblot analysis of IgE and IgG binding antigens in extracts of mosquitos *Aedes vexans*, *Culex tarsalis* and *Culiseta inornata*. Int Arch Allergy Immunol 1996; 110:46–51.
22. Li H, Simons FER, Peng Z. Immunoblot analysis of salivary allergens in 10 mosquito species with worldwide distribution and the human IgE responses to these allergens. J Allergy Clin Immunol 1998; 101:498–505.
23. Peng Z, Simons FE. Cross-reactivity of skin and serum specific IgE responses and allergen analysis for three mosquito species with worldwide distribution. J Allergy Clin Immunol 1997; 100:192–198.
24. Xu W, Lam H, Peng Z, et al. Mosquito allergy: expression, purification and characterization of Aed a 2, an *Aedes aegypti* salivary allergen. J Allergy Clin Immunol 1997; 99:S152 (abstr).
25. Brummer-Korvenkontio H, Kalkkinen N, Palusuo T, et al. Molecular characterization of the major 22kD *Aedes communis* mosquito saliva allergen. J Allergy Clin Immunol 1997; 99:S353 (abstr).
26. Brummer-Korvenkonito H, Palusuo T, Francois G, et al. Characterization of *Aedes communis*, *Aedes aegypti* and *Anopheles stephensi* mosquito saliva antigens by immunoblotting. Int Arch Allergy Immunol 1997; 112:169–174.
27. Cupp MS, Ribeiro JM, Cupp EW. Vasodilative activity in black fly salivary glands. Am J Trop Med Hyg 1994; 50:241–246.
28. Cupp MS, Ribeiro JMC, Champagne DE, et al. Analyses of cDNA and recombinant protein for a potent vasoactive protein in saliva of a blood-feeding fly, *Simulium vittatum*. J Exp Biol 1998; 201:1553–1561.
29. Wirtz HP. Bioamines and proteins in the saliva and salivary glands of palaearctic blackflies (Diptera: Simuliidae). Trop Med Parasitol 1990; 41:59–64.
30. Farkas J. Simuliosis. Analysis of dermatological manifestations following blackfly (Simuliidae) bites as observed in the years 1981–1983 in Bratislava (Czechoslovakia). Derm Beruf Umwelt 1984; 32: 171–173.
31. Grevelink SA, Youssef DE, Loscalzo J, et al. Salivary gland extracts from the deerfly contain a potent inhibitor of platelet aggregation. Proc Natl Acad Sci U S A 1993; 90:9155–9158.
32. Reddy VB, Kounga K, Mariano F, et al. Chrysoptin is a potent glycoprotein Iib/IIIa fibrinogen receptor antagonist present in salivary gland extracts of the deerfly. J Biol Chem 2000; 275:15861–15867.
33. Hemmer W, Focke M, Vieluf D, et al. Anaphylaxis induced by horsefly bites: identification of a 69kd IgE-binding salivary gland protein from *Chrysops* spp. (Diptera, Tabanidae) by western blot analysis. J Allergy Clin Immunol 1998; 101:134–136.
34. Hall LR, Titus RG. Sand fly vector saliva selectively modulates macrophage functions that inhibit killing of *Leishmania major* and nitric oxide production. J Immunol 1995; 155:3501–3506.
35. Belkaid Y, Valenzuela JG, Kamhawi S, et al. Delayed-type hypersensitivity to *Phlebotomus papatasi* sand fly bite: an adaptive response induced by the fly? Proc Natl Acad Sci U S A 2000; 97:6704–6709.
36. Champagne DE, Nussenzveig RH, Ribeiro JM. Purification, partial characterization, and cloning of nitric oxide-carrying heme proteins (nitrophorins) from salivary glands of the blood sucking insect *Rhodnius prolixus*. J Biol Chem 1995; 270:8691–8695.
37. Noeske-Jungblut C, Haendler B, Donner P, et al. Triabin, a highly potent exosite inhibitor of thrombin. J Biol Chem 1995; 270:28629–28634.

38. Haendler B, Becker A, Noeske-Jungblut C, et al. Expression, purification and characterisation of recombinant pallidipin, a novel platelet aggregation inhibitor from the hematophageous triatome bug *Triatoma pallidipennis*. Blood Coagul Fibrinolysis 1996; 7:183–186.
39. Pereira MH, Souza ME, Vargas AP, et al. Anticoagulant activity of *Triatoma infestans* and *Panstrongylus megistus* saliva (Hemiptera/Triatominae). Acta Trop 1996; 61:255–261.
40. Montfort WR, Weichsel A, Anderson JF. Nitrophorins and related antihemostatic lipocalins from *Rhodnius prolixus* and other blood-sucking arthropods. Biochim Biophys Acta 2000; 1482:110–118.
41. Amino R, Tanaka AS, Schenkman S. Triapsin an unusual activatable serine protease from the saliva of the hematophagous vector of Chagas' disease *Triatoma in*festans (Hemiptera: Reduviidae). Insect Biochem Mol Biol 2001; 31:465–472.
42. Chapman MD, Marshall NA, Saxon A. Identification and partial purification of species-specific allergens from *Triatoma protracta* (Heteroptera:Reduviidae). J Allergy Clin Immunol 1986; 78:436–442.
43. Paddock CD, McKerrow JH, Hansell E, et al. Identification, cloning and recombinant expression of procalin, a major triatomine allergen. J Immunol 2001; 167:2694–2699.
44. Valenzuela JG, Walker FA, Ribeiro JM. A salivary nitrophorin (nitric-oxide-carrying hemoprotein) in the bedbug *Cimex lectularius*. J Exp Biol 1995; 198:1519–1526.
45. Valenzuela JG, Guimaraes JA, Ribeiro JM. A novel inhibitor of factor X activation from the salivary glands of the bed bug *Cimex lectularius*. Exp Parasitol 1996; 83:184–190.
46. Ribeiro JM, Vaughn JA, Azad AF. Characterization of the salivary apyrase activity of three rodent flea species. Comp Biochem Physiol B 1990; 95:215–219.
47. McDermott MJ, Weber E, Hunter S, et al. Identification, cloning, and characterization of a major cat flea salivary allergen (Cte f 1). Mol Immunol 2000; 37:361–375.
48. Ribeiro JM, Endris TM, Endris R. Saliva of the soft tick, *Ornithodoros moubata*, contains anti-platelet and apyrase activities. Comp Biochem Physiol A 1991; 100:109–112.
49. Sanders ML, Scott AL, Glass GE, et al. Salivary gland changes and host antibody responses associated with feeding of male lone star ticks (Acari:Ixodidae). J Med Entomol 1996; 33:628–634.
50. Gauci M, Stone BF, Thong YH. Isolation and immunological characterization of allergens from salivary glands of the Australian paralysis tick *Ixodes holocyclus*. Int Arch Allergy Appl Immunol 1988; 87:208–212.
51. Gauci M, Loh RKS, Stone BF, et al. Evaluation of partially purified salivary gland allergens from the Australian paralysis tick, *Ixodes holocyclus* in diagnosis of allergy by RIA and skin prick test. Ann Allergy 1990; 64:297–299.
52. Marshall NA, Chapman MD, Saxon A. Species specific allergens from the salivary glands of Triatominae (Heteroptera:Reduviidae). J Allergy Clin Immunol 1986; 78:430–435.
53. Rohr AS, Marshall NA, Saxon A. Successful immunotherapy for *Triatoma protracta*-induced anaphylaxis. J Allergy Clin Immunol 1984; 73:369–375.
54. Wilbur RD, Evans R. An immunologic evaluation of deerfly hypersensitivity. J Allergy Clin Immunol 1975; 55:72.
55. McCormack DR, Salata KF, Hershey JN, et al. Mosquito bite anaphylaxis: immunotherapy with whole body extracts. Ann Allergy Asthma Immunol 1995; 74:39–44.
56. Peng ZK, Simon FER. Comparison of proteins, IgE and IgG binding antigens, and skin reactivity in commercial and laboratory-made mosquito extracts. Ann Allergy Asthma Immunol 1996; 77:371–376.
57. Reunala T, Brummer-Korvenkontio H, Karppinen A, et al. Treatment of mosquito bites with cetirizine. Clin Exp Allergy 1993; 23:72–75.
58. Karppinen A, Rantala I, Vaalasti A, et al. Effect of cetirizine on the inflammatory cells in mosquito bites. Clin Exp Allergy 1996; 26:703–709.
59. Karppinen A, Kautianen H, Reunala T, et al. Loratadine in the treatment of mosquito-bite-sensitive children. Allergy 2000; 55:668–671.
60. Scott RM, Shelton AL, Eckels KH, et al. Human hypersensitivity to a sham vaccine prepared from mosquito cell culture fluids. J Allergy Clin Immunol 1984; 74:808–811.

16 Occupational Allergens

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INTRODUCTION

Allergen immunotherapy (AIT) is the repeated administration of specific allergens to patients with IgE-mediated diseases, the purpose of which is to provide protection against the allergic symptoms and inflammatory reactions associated with the natural exposure to said allergens (1). This therapy is efficacious to treat patients with allergic rhinitis due to seasonal pollen allergens (2) (e.g., trees, grasses, and weeds) and for perennial allergic rhinitis induced by house-dust mites (3) and cat allergens (4). Similarly, AIT is effective in treating patients with aeroallergen-induced asthma attributable to sensitization with cat and grass pollens (2,5,6).

AIT is seldom considered in the initial management of workers with occupational rhinitis (OR) or occupational asthma (OA). Environmental control measures to reduce exposures to offending workplace aeroallergens are usually prioritized and emphasized and may account for the lack of published clinical studies about efficacy and safety of AIT in patients with occupational respiratory allergic diseases. However, where appropriate, AIT is considered only in workers in whom specific IgE sensitization to natural protein allergens is confirmed by skin testing to commercially available extracts or in vitro specific IgE tests. Although specific IgE is rarely demonstrated to reactive chemicals known to induce OA (7), AIT with chemical antigens (e.g., hapten-conjugated proteins) cannot be safely recommended or considered because of unknown short-term and long-terms risks of chemical-induced side effects.

The primary treatment of choice for OA or OR remains the elimination or reduction of occupational exposure to causative aeroallergens. Such interventions consist of reassignment to a different low-exposure job in the same work facility or job transfer to another workplace where exposure is eliminated or reduced sufficiently to control or prevent work-related allergic respiratory symptoms. Failure to reduce exposure to causative agents in patients with uncontrolled OA can result in disability and even fatal asthma (8,9). Therefore, pharmacotherapy and AIT should be used as ancillary treatments in selected patients but not as substitutes for effective environmental interventions.

There are specific situations in which AIT may be appropriate to treat occupational IgE-mediated respiratory disorders. AIT may be considered in symptomatic workers whose exposure to workplace allergens can be reduced but not entirely eliminated. Workers, recognizing the potential impact of a job change on their income and livelihood, may choose to continue work in a job where there is persistent exposure to an occupational allergen. In these cases, the best possible clinical outcomes may result from a combined approach, including institution of personal safety measures (e.g., respirators or masks), pharmacotherapy, and AIT, when appropriate. At the same time, these workers must be medically monitored to assure that persistent exposure does not lead to more severe illnesses or worsening of airway obstruction.

NATURAL RUBBER LATEX

Natural rubber latex (NRL) is derived from the sap of the rubber tree, *Hevea brasiliensis*, a complex mixture of at least 13 allergenic proteins that bind human IgE antibodies (Hev b allergens) (10). NRL gloves are a common source of occupational allergen exposure for health care workers and an important cause of allergic occupational disease. A FDA-approved NRL skin test reagent is not commercially available, although there are FDA-cleared immunoassays to detect NRL-specific IgE in serum (11). The primary intervention for individuals with established NRL allergy is avoidance of NRL-containing products (12).

The first multicenter, double-blind, placebo-controlled trial with NRL was performed in a French cohort for one year in 2000 (13). Seventeen patients had NRL allergy, defined by a clinical history of rhinitis and conjunctivitis, asthma (9 of 17), a positive skin prick test (SPT) with a standardized latex extract (Stallergènes SA, Antony, France), a positive latex conjunctival provocation challenge, and elevated NRL-specific IgE defined as more than or equal to class 2 level (Pharmacia, Uppsala, Sweden).

After six months of NRL subcutaneous immunotherapy (SCIT), there were significant improvements in rhinitis symptom scores ($p < 0.04$) and conjunctivitis symptom scores ($p < 0.02$) compared with placebo associated with NRL exposure. After one year of SCIT, improvement in rhinitis ($p < 0.05$) and conjunctivitis ($p = 0.05$) symptoms was maintained with added improvement in cutaneous signs ($p < 0.03$). No effect on work-associated asthma symptoms was demonstrated after one year. The mean medication scores with treatment also improved by 79%. Conjunctival challenge with aqueous latex extract was performed pre- and post-SCIT; conjunctival reactivity decreased with active versus placebo treatment ($p < 0.02$).

Safety was a concern. There was a higher rate of injection-related reactions in the treatment group as compared to placebo, with almost 50% in the treatment group experiencing immediate large local reactions at injection sites. Almost half of actively treated patients (4 of 9) had at least one anaphylactic reaction to NRL injections, characterized by angioedema, bronchospasm, pharyngeal edema, giant urticaria, and/or hypotension (13).

Another randomized, double-blind, placebo-controlled trial of NRL AIT was reported in 2003 (14). In a six-month trial with an experimental crude NRL extract, 24 patients were randomized to active NRL SCIT treatment ($n = 16$) or placebo ($n = 8$). This Spanish cohort consisted primarily of health care workers with contact urticaria ($n = 24$), rhinitis ($n = 17$), and/or asthma ($n = 15$) related to NRL exposure. Compared to placebo, patients receiving NRL SCIT had a significant decrease in SPT reactivity to NRL ($p < 0.01$), decrease in reactions to a latex rubbing test (NRL glove rubbed on wet skin for 30 seconds) ($p = 0.047$), and in reactions to a glove use test ($p = 0.046$). However, treatment had no effect on NRL-specific IgE levels, symptoms or medication scores, methacholine PC20, or the NRL glove exposure time to elicit a 15% decreased in forced expiratory volume in one second (FEV_1) by specific inhalation challenge. As in the previous study, a high number of systemic reactions were reported. Of 387 injections, there were 31 mild systemic reactions (8% of doses), with a third of these reactions classified as delayed reactions. These systemic reactions occurred in 11 actively treated patients (68.7%). The authors reported no severe systemic reactions.

Sublingual immunotherapy (SLIT) to NRL also reduced skin reactivity to NRL and reactivity on a conjunctival challenge test (14,15). Twenty-four patients with symptoms of urticaria, rhinoconjunctivitis, asthma, and/or anaphylaxis associated with NRL exposure were treated with a commercially available sublingual NRL vaccine in an open uncontrolled study (14,16). After 10 weeks of treatment, a total of 1044 doses were administered. Systemic reactions occurred with 38 doses (3.6%) and in 12 patients (46.2%). One patient developed anaphylaxis. Local oral reactions were reported with 223 doses (21.4%). Compared to baseline, there were decreased symptom scores with the latex glove use test ($p = 0.0004$) and with the latex glove rubbing test (latex glove rubbed on wet skin for 30 seconds) ($p = 0.037$).

Another nonblinded study evaluated the use of NRL SLIT in 24 patients, including 10 health care workers, with symptoms of asthma ($n = 17$), urticaria ($n = 18$), and/or rhinoconjuntivitis ($n = 10$) with NRL exposure (15). All had a positive SPT to a standardized NRL extract. Twelve underwent a four-day rush treatment protocol, and 12 others were untreated and served as controls. Two actively treated patients had local reactions in three months of treatment with NRL SLIT ($n = 12$). No systemic reactions were reported. Significant improvements in symptom scores with NRL challenge were seen in the treatment group before and after treatment with SLIT. The group treated with SLIT also had significant improvements in symptom scores compared with the untreated control group with sublingual NRL challenge ($p < 0.001$), with NRL gloved finger in mouth challenge ($p = 0.0002$), with cutaneous NRL extract challenge ($p < 0.001$), and with conjunctival NRL challenge ($p < 0.01$).

Treatment of NRL allergy with AIT seems to be effective in improving rhinitis symptom scores elicited by NRL exposure. However, proven benefit for asthma or decreased bronchial hyperactivity after NRL-specific inhalational challenge has not been confirmed. The lack of benefit from AIT may be due to inability to achieve optimal maintenance doses, secondary to the unacceptably high risk of systemic reactions associated with the SCIT. Nevertheless, the

Table 1 Summary of Immunotherapy Efficacy Studies for NRL

Allergen (route)	Study design	Number studied	Indication	Results	Level of evidence[a]	Reference
NRL (SCIT)	DBPC	17	Rhinitis, asthma, conjunctivitis	Improvement in rhinitis and conjunctivitis	A	13,41
NRL (SCIT)	DBPC	24	Contact urticaria, rhinitis, asthma	Decrease in SPT reactivity to NRL Decrease in symptoms with glove use	A	14,41
NRL (SLIT)	Unblinded, no placebo	24	Urticaria, rhinitis, asthma, anaphylaxis	Improvement of symptom scores with glove use	C	16
NRL (SLIT)	Unblinded, placebo-controlled	24	Asthma, urticaria, rhinitis, conjunctivitis	Improvement of symptom scores with NRL challenge	B	15

[a]Level of evidence
A: Evidence based on randomized controlled trial
B: Evidence based on controlled trial without randomization or other quasi-experimental study design
C: Evidence based on nonexperimental descriptive studies
Abbreviations: NRL, natural rubber latex; DBPC: double-blind, placebo-controlled; SCIT: subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SPT, skin prick test.

sublingual approach (SLIT) may be helpful if future evidence from placebo-controlled studies supports its safety and efficacy.

On the basis of the evidence, environmental control of the work environment and avoidance of NRL products remains the primary treatment. Since NRL allergy is rapidly declining because of better control of latex allergen exposure in health care facilities, there may be little need for a commercially available AIT product in the future (Table 1).

WHEAT FLOUR

Bakers and pastry makers exposed to wheat flour, containing high molecular weight protein allergens, often develop OR and bakers' asthma. The prevalence of bakers' asthma is estimated to be 9% among bakery employees (17). One double-blind, placebo-controlled study evaluated 139 patients who worked with wheat flour (17). Of the 139 subjects, 35 (25%) had a positive SPT with a crude wheat flour extract or elevated in vitro–specific IgE to wheat flour. Of these, 30 asthmatic patients were selected for the SCIT trial. Eight were treated for 10 months with wheat flour extract, 8 for 20 months with wheat flour extract, and 10 received placebo. Four in the active treatment group stopped therapy because of a change of jobs with resolution of symptoms. AIT-treated patients had a significant decrease in wheat flour extract SPT sensitivity as determined by the wheal area ($p = 0.002$) and a significant decrease in bronchial hyperresponsiveness to methacholine ($p < 0.001$). By 20 months of treatment, there was a significant decrease in wheat flour–specific IgE compared to placebo ($p < 0.005$) and improvement in symptoms associated with exposure to wheat flour compared to placebo ($p < 0.001$). No severe systemic reactions were reported, although one patient developed transient urticaria after an injection. This study indicates that SCIT, with a wheat flour vaccine, may be effective in treating bakers' asthma. However, these positive results will require confirmation in larger controlled clinical trials (Table 2).

Table 2 Summary of Immunotherapy Efficacy Studies for Wheat Flour

Allergen (route)	Study design	Number studied	Indication	Results	Level of evidence[a]	Reference
Wheat flour (SCIT)	DBPC	30	Asthma	-Decrease in SPT reactivity to wheat flour -Decrease in bronchial hyperreponsiveness on methacholine challenge -Decrease in wheat flour–specific IgE -Improvement of symptoms on exposure	A	17

[a]Level of evidence
A: Evidence based on randomized controlled trial
Abbreviations: DBPC, double-blind, placebo-controlled; SCIT, subcutaneous immunotherapy; SPT, skin prick test.

ANIMAL ALLERGY (MAMMALIAN PROTEINS)

Allergic reactions among laboratory animal workers is an important occupational health problem, with an estimated incidence of 1.32 per 100 person years and an estimated prevalence of 22% (18). Of all animal workers, veterinarians are at the greatest risk of developing occupational allergic disorders, such as atopic dermatitis, allergic rhinitis, and asthma. Among veterinarians, 38% report rhinitis or conjunctivitis and 20% asthma symptoms related to their work environment (18). Cats are the most commonly reported (58%) animals causing work-related symptoms.

Avoidance of laboratory animals and animal-housing facilities is usually recommended in symptomatic workers. However, because of career and job considerations, it is rarely feasible for affected workers to eliminate all exposure to animals. When complete avoidance is not possible, there have been reported cases when AIT with laboratory animal allergens has been attempted.

Subcutaneous AIT with rodent allergens was used in 11 patients with allergic symptoms upon exposure to laboratory animals and compared with a group of matched untreated control patients in an unblinded manner (19). Laboratory animal allergy was confirmed by intracutaneous skin testing and leukocyte histamine release to animal allergens, such as mouse, rabbit, rat, guinea pig, and hamster. All had rhinoconjunctivitis symptoms upon exposure to at least one of these animals, and most had symptoms on exposure to several different species. Nine of 11 patients reported a decrease in symptoms after SCIT. Active treatment was associated with significantly increased titers of blocking antibody determined by serum inhibition of allergen-induced histamine release with relevant laboratory animal allergens in comparison to untreated controls ($p < 0.0001$). In three of four patients in whom AIT was discontinued, blocking antibodies slowly decreased to pretreatment levels after 16 to 36 months.

Both cat-induced allergic rhinitis and asthma have been effectively treated by use of SCIT (20). In a randomized, double-blind, placebo-controlled trial, 17 patients with asthma associated with cat exposure were followed (5). All patients developed cough, wheeze, or shortness of breath with cat exposure and had a positive SPT and bronchial challenge test result to the major cat allergen, Fel d 1. Compared to placebo ($n = 8$), treatment was associated with a significant increase in PD_{20} FEV_1 on bronchial provocation with Fel d 1 ($p < 0.05$). A higher concentration of allergen was necessary to induce a 3-mm wheal in the treatment versus placebo group on skin prick titration testing ($p < 0.01$). The treatment group had increased time to ocular ($p < 0.05$) and pulmonary ($p < 0.05$) but not nasal symptoms with cat exposure compared to placebo. Ocular, nasal, and pulmonary symptom scores following cat exposure ($p \leq 0.03$) also improved with treatment. Furthermore, active treatment was associated with increased Fel d 1–specific IgG compared to placebo ($p < 0.001$) (Table 3).

Table 3 Summary of Immunotherapy Efficacy Studies for Animal Allergy (Mammalian Proteins)

Allergen (route)	Study design	Number studied	Indication	Results	Level of evidence[a]	Reference
Rodent (mouse, rabbit, rat, guinea pig, hamster) (SCIT)	Unblinded, matched, untreated controls	11	Rhinitis, conjunctivitis	-Decrease in symptoms on exposure -Increase in titers of blocking antibodies	B	19
Fel d1 (SCIT)	DBPC	17	Asthma	-Increase in PD_{20} FEV_1 with bronchial provocation -Decrease in SPT reactivity -Improvement of ocular, nasal, and pulmonary symptom scores	A	5

[a]Level of evidence
A: Evidence based on randomized controlled trial
B: Evidence based on controlled trial without randomization or other quasi-experimental study design
Abbreviations: DBPC, double-blind, placebo-controlled; SCIT, subcutaneous immunotherapy; SPT: skin prick test.

SEA SQUIRT

Major allergens from the body fluid of the sea squirt, *Styela plicata*, are the acidic glycoproteins, Gi-rep, Ei-M, and DIIIa. Asthma related to exposure to sea squirt allergens primarily occurs among Japanese oyster shucking workers. Oyster shucking workers continuously inhale the sea squirt antigens in the mist of the body fluid of sea squirts. In 1963, the reported prevalence of sea-squirt asthma was 36% (21). With industrial hygiene improvements, the reported prevalence of sea-squirt asthma has decreased to 8%, with an incidence of 10.1% (21).

In an uncontrolled study, 123 asthmatic patients received SCIT with one of three known major sea squirt allergens, Gi-rep ($n = 47$), Ei-M ($n = 62$), or DIIIa ($n = 14$) (22). The maximum dose of each AIT injection was 50 μg of antigen. After one year of treatment, 72% of those treated with Gi-rep were able to shuck oysters with minimal or no medications. Better results were obtained with Ei-M; 90% of patients reported improved symptoms. DIIIa was the least effective, with only 36% reporting improved symptoms. These results are consistent with a previous study that shows DIIIa is significantly less effective than Gi-rep or Ei-M with AIT (23). Beneficial effects of AIT were maintained over five years of treatment. A significant increase in IgG titer specific for Ei-M occurs within the first year of SCIT and correlates with the therapeutic effect, similar to previous studies (22) (Table 4).

HOUSE-DUST MITE

House-dust mite is the most common indoor allergen (24). Exposure to house-dust mite (DM) allergens occurs in a variety of occupations, including domestic cleaners and janitorial staff personnel. High levels of house-dust mite species allergens, *Dermatophagoides pteronyssinus* (Der p1) and *Dermatophagoides farinae* (Der f1), are present in house dust of rooms with wall-to-wall carpeting.

Several studies evaluated the efficacy of DM AIT, although not specifically in worker cohorts. Twenty-seven patients with a positive SPT to Der p1 and/or Der f1 and perennial

Table 4 Summary of Immunotherapy Efficacy Studies for Sea Squirt

Allergen (route)	Study design	Number studied	Indication	Results	Level of evidence[a]	Reference
Gi-rep, Ei-M, DIIIa (SCIT)	Unblinded, no placebo	123	Asthma	-Improvement of asthma symptoms -Decrease in medication use with oyster shucking	C	22

[a]Level of Evidence
C: Evidence based on nonexperimental descriptive studies
Abbreviations: SCIT, subcutaneous immunotherapy.

rhinitis and/or mild asthma received SCIT with aluminum hydroxide-adsorbed standardized dust-mite extracts (50% Der p1 and 50% Der f1) and were followed yearly for three years in a double-blind, placebo-controlled trial (25). After three years of AIT, there was a significant decrease in medication scores for asthma ($p < 0.033$) and for rhinitis ($p = 0.0007$). Subjective symptom scores for asthma were significantly improved after one year ($p = 0.016$) and continued through three years of treatment ($p = 0.0008$). Rhinitis symptom scores were significantly improved at both two and three years ($p = 0.0006$). Both SPT reactivity and conjunctival reactivity to Der p1 and/or Der f1 significantly decreased over all three years ($p < 0.0001$). Nonspecific bronchial hyperresponsiveness, as measured by methacholine challenge, also improved after three years of SCIT as compared to placebo ($p < 0.0001$). No significant systemic reactions to injections were reported.

In another double-blind, placebo-controlled study, 29 asthmatic patients with SPT positivity to Der p1 were randomized to either SCIT or placebo (26). After three years of SCIT with Der p1, there were no significant differences in the FVC, FEV_1, or FEF25–75. However, there was a significant decrease in the number of annual asthma exacerbations after one year of treatment ($p < 0.01$). Furthermore, there was a significant increase in the number of medication-free days (e.g., bronchodilators or systemic glucocorticoids) between the treatment and placebo groups ($p < 0.01$). Along with these findings, there was a significant improvement in bronchial hyperresponsiveness to methacholine. As for safety, no major systemic reactions with injections were reported.

A larger randomized, double-blind, placebo-controlled trial included 95 patients with asthma and percutaneous sensitization to Der p1 and/or Der f1 (27). SCIT was administered for three years using extracts containing Der p1 and/or Der f1 allergens; 72 patients completed the study. There was a significant decrease in percutaneous skin sensitivity to Der p1 and/or Der f1 antigens ($p < 0.05$) with AIT. Small, but significant improvements were seen in peak expiratory flow rates in the AIT group (mean increase of 1.6–5.5% of predicted from baseline). Asthma symptom scores did not significantly change, but there was an increase in the proportion of patients not requiring use of bronchodilators in the treatment group ($p < 0.01$). There was no significant change in the FEV_1 or in bronchial hyperresponsiveness to methacholine in the treatment versus the placebo group. Mild bronchospasm after injections was reported on two occasions.

One other study evaluated the effects of rush AIT in 10 Der p1 SPT-positive asthmatic patients to evaluate the underlying immune changes associated with clinical improvement with AIT (28). While treatment was not blinded, the outcomes were compared one to two days prior to the start of rush AIT and one to two days after reaching maintenance in a blinded manner. There was a significant decrease in percutaneous reactivity to Der p1 by endpoint SPT titration with AIT ($p < 0.01$). Decreased nasal reactivity to Der p1, symptom scores, and nasal obstruction was found in the AIT treated versus the placebo group ($p < 0.01$). A significant increase in anti-Der p1 IgG4 was observed in the AIT treated group ($p < 0.01$). There was also a significantly decreased lymphocyte proliferative response to Der p1 after AIT ($p < 0.05$) (Table 5).

Table 5 Summary of Immunotherapy Efficacy Studies for House-Dust Mite

Allergen (route)	Study design	Number studied	Indication	Results	Level of evidence[a]	Reference
Der p1/Der f1 (SCIT)	DBPC	27	Rhinitis, asthma	Decrease in medication scores for asthma and rhinitis Improvement of rhinitis and asthma symptom scores Decrease in SPT and conjunctival reactivity Improvement of bronchial hyperresponsiveness by methacholine challenge	A	25
Der p1 (SCIT)	DBPC	29	Asthma	Decrease in number of annual asthma exacerbations Increase in medication-free days Improvement of bronchial hyperresponsiveness by methacholine challenge	A	26
Der p1/Der f1 (SCIT)	DBPC	95	Asthma	Decrease in SPT reactivity Improvement of PEFR Decrease in use of bronchodilators	A	27
Der p1 (Rush SCIT)	Unblinded, no placebo	10	Rhinitis, asthma	Decrease in SPT reactivity Decrease in nasal reactivity and obstruction Decrease in asthma and rhinitis symptom scores	C	28

[a]Level of evidence
A: Evidence based on randomized controlled trial
C: Evidence based on nonexperimental descriptive studies
Abbreviations: DBPC, double-blind, placebo-controlled; SCIT, subcutaneous immunotherapy; SPT, skin prick test; PEFR, peak expiratory flow rate.

HYMENOPTERA VENOM

Stinging insect hypersensitivity is an occupational problem for farm workers, greenhouse workers, forest rangers, biologists, and beekeepers. Hymenoptera insects in the families Apidae (bees) and Vespidae (wasps) are primarily responsible and include the honeybee (*Apis melifera*), yellow jacket (*Vespula vulgaris*), wasp (*Polisites annularis*), white-faced hornet (*Dolichovespula maculata*), and yellow hornet (*Dolichovespula arenaria*) (29) (see chap. 14).

The prevalence of systemic reactions to Hymenoptera stings ranges from 0.8% to 3.3% in the general population (30,31). Several studies show that the rate of systemic allergic reactions is much higher in beekeepers, between 14% and 35% (32,33). A prospective evaluation of 35 Greek beekeepers was performed to estimate the incidence of sensitization among previously unsensitized workers (30). The workers were evaluated by intradermal skin testing every six months for the five years of the study. During the five-year period, 10 of 35 beekeepers (28.6%) were sensitized to honeybee venom as compared with 3 of 26 controls (8.3%). While none of the sensitized subjects reported a systemic reaction after honeybee stings, 5 of 35 beekeepers (14.3%) experienced large local reactions. Among the beekeepers, the number of stings per year correlated with the probability of sensitization to honeybee venom. In contrast, other studies show that tolerance can be induced by very

frequent stings (34,35). Beekeepers with more than 200 stings per year appear to be protected from systemic sting reactions (no reactions) as compared with beekeepers stung less than 25 times a year (45% with reactions).

SCIT with purified Hymenoptera venoms is the treatment of choice to modify future risk of anaphylactic reactions in workers sensitized to insect venoms. Full protection can be achieved in 83% to 95% of patients with a previous history of anaphylactic reactions (33). Four treatment regimens include conventional (weekly intervals for increasing doses as an outpatient), rush (inpatient induction phase over 4–7 days), ultrarush (reaching maintenance dose in 1–2 days), or cluster (a modified rush approach with a cluster of injections given at the first visit and reaching a maintenance dose within 6 weeks).

Studies demonstrate the efficacy and safety of the rush venom protocols. For example, 97 patients received rush AIT to bee venom, *Vespula* venom, or both (32). Severe sting induced allergic reactions were reported in 48 of them. Specific AIT with bee venom alone in 5, *Vespula* venom alone in 73, and with both in 19 was prescribed. The majority of these patients (90) had cutaneous reactions at the injection site. Unusual adverse events included blood pressure elevation in 11 patients, moderate hypotension in 2, and rhinitis in 1. No anaphylaxis was reported, and the protocol was deemed safe by the authors. No evidence of efficacy was presented.

Another study evaluated the safety of rush AIT with Hymenoptera venoms in 101 patients (35). Bee venom was used in 52 and yellow jacket in 49 patients. Maintenance dose was reached in all but one patient. Bee venom injections induced a higher rate of systemic reactions than did yellow jacket. The risk for systemic reactions was 0.79% per injection in the bee venom group as compared with 0.12% with yellow jacket venom. Systemic reactions were reported at all stages of the protocol and were generally mild to moderate, with only two severe reactions reported.

The efficacy and safety of the rush protocol also was evaluated prospectively in 18 patients with a history of allergic reactions to Hymenoptera stings (36). Seven patients were treated with bee venom and seven with yellow jacket venom during a seven-day protocol. After completing one year of AIT, there was a significant increase in specific IgG4 levels compared with baseline levels ($p < 0.05$). There were no changes in specific IgE levels, mean wheal diameter on SPT, or intradermal skin test reactivity after one year of AIT. Four systemic reactions were reported for bee venom (1.7%), while none were reported with yellow jacket venom. During the course of AIT, two patients had field sting events, including a beekeeper who was re-stung several times, without consequence. Another study evaluated 67 patients with confirmed Hymenoptera venom allergy who were treated with an ultrarush protocol (37). Thirty-four were treated with wasp venom, 20 with honeybee venom, and 13 with both wasp and honeybee venoms. The maintenance dose was reached in 97.5% of the ultrarush courses. Side effects showed no dose dependency and were mainly mild, with 14 (17.5%) patients experiencing allergic reactions. Furthermore, there was no significant difference in the number of systemic reactions in those receiving honeybee venom (15.2%) and wasp venom (19.1%).

While bumblebees are generally not very aggressive and allergic reactions rare, the expanding use of domesticated bumblebees for pollination of crops has resulted in an increase number of reports of occupational allergic reactions (38). Patients with allergic reactions to bumblebees are treated with honeybee venom because of the high degree of cross-reactivity with this venom. However, case reports indicate that this may not always be effective (39). Two patients, treated with honeybee venom, were later stung by a bumblebee at work and developed anaphylactic reactions. Repeat AIT was performed using bumblebee venom. An in-hospital bumblebee sting challenge resulted in a local cutaneous reaction but no systemic reaction. Another case series presented seven cases of bumblebee venom allergy among workers on a bumblebee farm, all of whom had anaphylactic reactions to bumblebee stings (40). They were treated with bumblebee venom–specific AIT. During maintenance AIT, at least 36 bumblebee stings were reported, none of which resulted in systemic reactions. Ultrarush AIT with bumblebee venom was evaluated in one case of a biologist with anaphylaxis after bumblebee stings (34). Two months after reaching maintenance, the patient had another sting at the workplace. Despite the re-sting event, the patient experienced no systemic reaction (Table 6).

Table 6 Summary of Immunotherapy Efficacy Studies for Hymenoptera Venom

Allergen (route)	Study design	Number studied	Indication	Results	Level of evidence[a]	Reference
Honeybee, *Vespula sp.* (Rush SCIT)	Unblinded, no placebo	97	Anaphylaxis	-Protocol appears safe -No episodes of anaphylaxis	C	32
Honeybee, yellow jacket (Rush SCIT)	Unblinded, no placebo	101	Anaphylaxis	-Higher rate of systemic reactions with honeybee venom vs. yellow jacket	C	35
Honeybee, yellow jacket (Rush SCIT)	Unblinded, placebo-controlled	18	Anaphylaxis	-Increase in specific IgG4 -Two field stings without reaction	B	36
Wasp, honeybee (Ultrarush SCIT)	Unblinded, no placebo	67	Anaphylaxis	-Protocol appears safe -17.5% with mild allergic reaction during treatment	B	37
Bumblebee (Rush SCIT)	Case reports	2	Anaphylaxis	-Inhospital sting challenge with local reactions only	D	39
Bumblebee (Rush SCIT)	Case series	7	Anaphylaxis	-36 field stings after AIT without systemic reactions	C	40
Bumblebee (Ultra-rush SCIT)	Case report	1	Anaphylaxis	-One field sting after AIT without systemic reaction	D	34

[a]Level of evidence
B: Evidence based on controlled trial without randomization or other quasi-experimental study design
C: Evidence based on nonexperimental descriptive studies
D: Evidence based on expert opinion
Abbreviations: DBPC, double-blind, placebo-controlled; SCIT, subcutaneous immunotherapy; SPT, skin prick test.

CONCLUSION

AIT is rarely indicated to treat OR or OA. It should be considered only to modify respiratory allergic symptoms associated with workplace exposure to natural allergens and when commercial extracts are available both to confirm sensitivity by skin test and for treatment. AIT should not be considered as an alternative to cessation or modification of exposure to the offending allergens in the work environment. Although there is only modest evidence to

support efficacy and safety in worker populations, it appears reasonable to base treatment decisions on data extrapolated from controlled studies of AIT in nonworker populations.

SALIENT POINTS

- Environmental control measures to reduce workplace exposures is the primary intervention in management of OA and OR and, therefore, AIT to offending aeroallergens is seldom initially considered.
- If appropriate, AIT should be considered in symptomatic workers whose exposure to workplace allergens can be reduced but not entirely eliminated.
- Although AIT may be considered in workers with sensitization to protein allergens encountered in the workplace, it cannot be safely recommended for chemical sensitizers because of potential toxicity.
- Although there is sparse evidence proving efficacy and safety in worker populations, rational treatment decisions for individual workers should be based on controlled clinical trials of AIT obtained in nonoccupational study populations.
- If indicated, AIT should be strongly considered in workers with OA and/or OR caused by workplace allergens for which commercial and preferably standardized allergens are readily available. Examples include house-dust mite in cleaning workers, stinging insect venom in beekeepers, and cat allergen extracts in veterinary workers.

REFERENCES

1. Allergen immunotherapy: a practice parameter. American Academy of Allergy, Asthma and Immunology. American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 2003; 90:1–40.
2. Calderon MA, Alves B, Jacobson M, et al. Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev 2007:CD001936.
3. Varney VA, Tabbah K, Mavroleon G, et al. Usefulness of specific immunotherapy in patients with severe perennial allergic rhinitis induced by house dust mite: a double-blind, randomized, placebo-controlled trial. Clin Exp Allergy 2003; 33:1076–1082.
4. Varney VA, Edwards J, Tabbah K, et al. Clinical efficacy of specific immunotherapy to cat dander: a double-blind placebo-controlled trial. Clin Exp Allergy 1997; 27:860–867.
5. Ohman JL Jr., Findlay SR, Leitermann KM. Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of in vivo and in vitro responses. J Allergy Clin Immunol 1984; 74:230–239.
6. Walker SM, Pajno GB, Lima MT, et al. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. J Allergy Clin Immunol 2001; 107:87–93.
7. Bernstein DI, Zeiss CR. Guidelines for preparation and characterization of chemical-protein conjugate antigens. Report of the Subcommittee on Preparation and Characterization of Low Molecular Weight Antigens. J Allergy Clin Immunol 1989; 84:820–822.
8. Carino M, Aliani M, Licitra C, et al. Death due to asthma at workplace in a diphenylmethane diisocyanate-sensitized subject. Respiration 1997; 64:111–113.
9. Ehrlich RI. Fatal asthma in a baker: a case report. Am J Ind Med 1994; 26:799–802.
10. Sussman GL, Beezhold DH, Kurup VP. Allergens and natural rubber proteins. J Allergy Clin Immunol 2002; 110:S33–S39.
11. Bernstein DI, Biagini RE, Karnani R, et al. In vivo sensitization to purified Hevea brasiliensis proteins in health care workers sensitized to natural rubber latex. J Allergy Clin Immunol 2003; 111:610–616.
12. NIOSH alert: preventing allergic reactions to natural rubber latex in the workplace. Hosp Technol Ser 1997; 16:10–13.
13. Leynadier F, Herman D, Vervloet D, et al. Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. J Allergy Clin Immunol 2000; 106:585–590.
14. Sastre J, Fernandez-Nieto M, Rico P, et al. Specific immunotherapy with a standardized latex extract in allergic workers: a double-blind, placebo-controlled study. J Allergy Clin Immunol 2003; 111:985–994.
15. Patriarca G, Nucera E, Pollastrini E, et al. Sublingual desensitization: a new approach to latex allergy problem. Anesth Analg 2002; 95:956–960, table of contents.
16. Cistero Bahima A, Sastre J, Enrique E, et al. Tolerance and effects on skin reactivity to latex of sublingual rush immunotherapy with a latex extract. J Investig Allergol Clin Immunol 2004; 14:17–25.

17. Armentia A, Martin-Santos JM, Quintero A, et al. Bakers' asthma: prevalence and evaluation of immunotherapy with a wheat flour extract. Ann Allergy 1990; 65:265–272.
18. Susitaival P, Kirk JH, Schenker MB. Atopic symptoms among California veterinarians. Am J Ind Med 2003; 44:166–171.
19. Wahn U, Siraganian RP. Efficacy and specificity of immunotherapy with laboratory animal allergen extracts. J Allergy Clin Immunol 1980; 65:413–421.
20. Kay AB, Larche M. Allergen immunotherapy with cat allergen peptides. Springer Semin Immunopathol 2004; 25:391–399.
21. Ohtsuka T, Tsuboi S, Katsutani T, et al. [Results of 29-year study of hoya (sea-squirt) asthma in Hatsukaichi, Hiroshima prefecture]. Arerugi 1993; 42:214–218.
22. Jyo T, Kodomari Y, Kodomari N, et al. Therapeutic effect and titers of the specific IgE and IgG antibodies in patients with sea squirt allergy (Hoya asthma) under a long-term hyposensitization with three sea squirt antigens. J Allergy Clin Immunol 1989; 83:386–393.
23. Suzuki H, Oka S, Shigeta S, et al. Isolation and characterization of an asthma-inducing sea-squirt antigen. J Biochem (Tokyo) 1984; 96:849–857.
24. Platts-Mills TA, Vervloet D, Thomas WR, et al. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol 1997; 100:S2–S24.
25. Pichler CE, Helbling A, Pichler WJ. Three years of specific immunotherapy with house-dust-mite extracts in patients with rhinitis and asthma: significant improvement of allergen-specific parameters and of nonspecific bronchial hyperreactivity. Allergy 2001; 56:301–306.
26. Pifferi M, Baldini G, Marrazzini G, et al. Benefits of immunotherapy with a standardized Dermatophagoides pteronyssinus extract in asthmatic children: a three-year prospective study. Allergy 2002; 57:785–790.
27. Maestrelli P, Zanolla L, Pozzan M, et al. Effect of specific immunotherapy added to pharmacologic treatment and allergen avoidance in asthmatic patients allergic to house dust mite. J Allergy Clin Immunol 2004; 113:643–649.
28. Lack G, Nelson HS, Amran D, et al. Rush immunotherapy results in allergen-specific alterations in lymphocyte function and interferon-gamma production in CD4+ T cells. J Allergy Clin Immunol 1997; 99:530–538.
29. Moffitt JE, Golden DB, Reisman RE, et al. Stinging insect hypersensitivity: a practice parameter update. J Allergy Clin Immunol 2004; 114:869–886.
30. Kalogeromitros D, Makris M, Gregoriou S, et al. Pattern of sensitization to honeybee venom in beekeepers: a 5-year prospective study. Allergy Asthma Proc 2006; 27:383–387.
31. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. Int Arch Allergy Immunol 2000; 123:99–106.
32. Laurent J, Smiejan JM, Bloch-Morot E, et al. Safety of Hymenoptera venom rush immunotherapy. Allergy 1997; 52:94–96.
33. Lerch E, Muller UR. Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. J Allergy Clin Immunol 1998; 101:606–612.
34. Roll A, Schmid-Grendelmeier P. Ultrarush immunotherapy in a patient with occupational allergy to bumblebee venom (Bombus terrestris). J Investig Allergol Clin Immunol 2005; 15:305–307.
35. Sturm G, Kranke B, Rudolph C, et al. Rush Hymenoptera venom immunotherapy: a safe and practical protocol for high-risk patients. J Allergy Clin Immunol 2002; 110:928–933.
36. Pasaoglu G, Sin BA, Misirligil Z. Rush hymenoptera venom immunotherapy is efficacious and safe. J Investig Allergol Clin Immunol 2006; 16:232–238.
37. Roll A, Hofbauer G, Ballmer-Weber BK, et al. Safety of specific immunotherapy using a four-hour ultra-rush induction scheme in bee and wasp allergy. J Investig Allergol Clin Immunol 2006; 16:79–85.
38. de Groot H, de Graaf-in 't Veld C, van Wijk RG. Allergy to bumblebee venom. I. Occupational anaphylaxis to bumblebee venom: diagnosis and treatment. Allergy 1995; 50:581–584.
39. Stern A, Wuthrich B, Mullner G. Successful treatment of occupational allergy to bumblebee venom after failure with honeybee venom extract. Allergy 2000; 55:88–91.
40. Kochuyt AM, Van Hoeyveld E, Stevens EA. Occupational allergy to bumble bee venom. Clin Exp Allergy 1993; 23:190–195.
41. Allergen immunotherapy: a practice parameter second edition update. J Allergy Clin Immunol 2007; Sep; 120 (3 suppl):S25–S85.

17 Standardized Allergen Vaccines in the United States

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INTRODUCTION

Allergen vaccines and other biologics were first regulated by the Hygienic Laboratory of the Public Health and Marine Hospital Service. In 1930, the Hygienic Laboratory was renamed the National Institute (singular) of Health (NIH). The NIH continued to regulate biologics (beginning in 1955, through its Division of Biologics Standards) for over 40 years. In 1972, regulatory authority over biologics was transferred to the Bureau of Biologics at the Food and Drug Administration (FDA). In 1982, the FDA merged the Bureau of Biologics and the Bureau of Drugs into a single Center for Drugs and Biologics; five years later, the entities that regulated drugs and biologics were once again separated, and the Center for Biologics Evaluation and Research (CBER) assumed responsibility for allergenics regulation (1,2).

CBER's authority to regulate allergen vaccines derives from two laws enacted by Congress, the Food, Drug, and Cosmetic Act of 1938 and the Public Health Service Act of 1944. The specific regulations that govern CBER's regulation of allergens appear in part 680 of title 21 of the Code of Federal Regulations (21 CFR 680), although other parts of 21 CFR also apply to allergen regulation. Over the past 20 years, two features of CBER's regulatory program have had a significant impact on allergen manufacturers and enhanced the safety of allergen vaccines marketed to the American public. The first is the enforcement of current good manufacturing practice (cGMP) standards (21 CFR 210, 211, and 600–680) on the manufacture of allergen products. cGMPs include requirements regarding organization and personnel, buildings and facilities, equipment, control of components and drug product containers and closures, production and process controls, holding and distribution, quality control, laboratory controls and records and reports. cGMPs have been in effect since the 1960s.

A second feature is allergen standardization. 21 CFR 680.3(e) specifies that when a potency test exists for a specific allergenic product, and when CBER has notified manufacturers that the test exists, manufacturers will be required to determine the potency of each lot of the product prior to release. Since the 1980s, 19 allergen vaccines have been standardized (Table 1). What follows focuses on these standardized products and the tests that are used to ascertain extract potency.

ALLERGEN VACCINES CURRENTLY ON THE MARKET (STANDARDIZED AND NONSTANDARDIZED)

Allergen vaccines are manufactured and sold worldwide for the diagnosis and treatment of immunoglobulin E (IgE)-mediated allergic disease. These vaccines are complex mixtures of natural biomaterials. Each extract contains proteins, carbohydrates, enzymes, and pigments of which the allergens—presumably the active ingredients—may constitute only a small proportion (3). Traditionally, allergen vaccines have been labeled either with a designation of extraction ratio (w/v) or with a protein unit designation that is determined using the Kjeldahl method (protein nitrogen units/mL). However, there is little correlation between these two designations and the biological measures of allergen potency (4,5).

The views expressed in this chapter are the personal opinions of the author and not the official opinion of the U.S. Food and Drug Administration or the Department of Health and Human Services.

Table 1 Standardized Allergen Vaccines Currently Licensed in the United States

Allergen vaccine	Current lot release tests	Labeled unitage
Dust mite (*Dermatophagoides farinae*)	Competition ELISA Protein[a]	AU/mL (equivalent to BAU/mL)
Dust mite (*Dermatophagoides pteronyssinus*)		
Cat pelt (*Felis domesticus*)	Fel d 1 (RID)	BAU/mL
Cat hair (*Felis domesticus*)	IEF	5–9.9 Fel d 1 U/mL = 5000 BAU/mL
	Protein	10–19.9 Fel d 1 U/mL = 10,000 BAU/mL
Bermuda grass (*Cynodon dactylon*)	Competition ELISA	BAU/mL
Red top grass (*Agrostis alba*)	IEF	
June (Kentucky blue) grass (*Poa pratensis*)	Protein[a]	
Perennial ryegrass (*Lolium perenne)*		
Orchard grass (*Dactylis glomerata*)		
Timothy grass (*Phleum pratense*)		
Meadow fescue grass (*Festuca elatior*)		
Sweet vernal grass (*Anthoxanthum odoratum*)		
Short ragweed (*Ambrosia artemisiifolia*)	Amb a 1 (RID)	Amb a 1 units/mL
Yellow hornet (*Vespa* spp.)	Hyaluronidase and phospholipase activity	μg protein
Wasp (*Polistes* spp.)		
Honey Bee (*Apis mellifera*)		
White faced hornet (*Vespa* spp.)		
Yellow jacket (*Vespula* spp.)		
Mixed vespid (*Vespa* + *Vespula* spp.)		

[a]Test for informational purposes only. *Abbreviations*: IEF, isoelectric focusing; RID, radial immunodiffusion.

In the absence of a concerted effort to maintain product consistency, lot-to-lot variations in allergen content may be considerable. Product consistency may be enhanced by the inherent nature of the raw materials; for example, pollen and pure mite vaccines (6) generally have greater lot-to-lot consistency than mold, house dust, and insect vaccines (7). In addition, manufacturers can increase the consistency of their products by controlled collection, storage, and processing of the raw materials; by reproducible and optimized extraction and manufacturing techniques; and by expiration dates based on real-time stability data. However, consistency can only be assured by measuring the potency of each lot of vaccines and by marketing only those lots whose potency falls within an acceptable range.

The FDA's allergen standardization regulation mandates that when an appropriate potency test exists, manufacturers must test each lot of an allergen vaccine for potency prior to sale. This regulation takes product consistency one step further by establishing a U.S. standard of potency for each standardized product. The purpose of allergen standardization is to ensure that the vaccines are well characterized in terms of allergen content and that variation between lots is minimized even among different manufacturers (8). Since standardized vaccines are compared with a single, national potency standard, patients and their physicians can switch from one manufacturer's product to another with minimized risk of causing an adverse reaction.

There are 19 standardized allergen vaccines available from manufacturers in the United States (Table 1). For each of these vaccines, there is a U.S. standard of potency to which each lot of the vaccine is compared prior to release for sale to the public. The potency measures, and the assays used to determine these measures, are specified in the approved product license applications of each manufacturer for each product. Manufacturers may use the methods described in CBER's *Methods of the Allergen Products Testing Laboratory* (9) or may seek approval to use alternative test methods that provide equally reliable measures of product potency and meet regulatory requirements.

The level of quality control for the 19 standardized allergen vaccines is the exception rather than the rule. In vitro potency tests that correlate with in vivo clinical responses have not been developed for the hundreds of nonstandardized vaccines available in U.S. product lines. Thus, for most allergen vaccines manufactured in the United States, consistency cannot be assured by potency testing.

THE BASIS OF ALLERGEN STANDARDIZATION IN THE UNITED STATES

In the United States, allergen standardization comprises two important components: the selection of a reference preparation of allergenic vaccine and the selection of the procedures to compare manufactured products with the reference extract (10–12). The use of a biological model of allergen standardization has permitted the assignment of bioequivalent allergen units for most standardized allergens (11). Once a specific unitage is assigned to a reference, then all allergen vaccines from the same source can be assigned units based on the relative potency (RP), with respect to the reference using the established quantitative in vitro potency method (13). In contrast, commercial allergens in the European Union are labeled in disparate manufacturer-specific units, and products are standardized using manufacturers' in-house references (14). While earlier European efforts to establish biological standards were similar to those in the United States (described below), recent work to develop a common European platform has focused exclusively on the validation of major allergens to facilitate studies that will establish whether the amounts of these molecules can be used as the sole potency label (15).

In theory, standardizing an allergen vaccine might involve purifying each allergen in the extract and establishing with precision the importance of these allergens. However, most allergen vaccines are complex mixtures of several relevant allergens of as yet uncertain immunodominance. In addition, an individual allergen may be less "allergenic" in a particular lot due to instability or denaturation. The choice of the best potency test depends on the allergen vaccine to be standardized. In the absence of data supporting the safety of potency designations based on single allergen content, a measure of overall allergenicity may be a better predictor of safe dosing. For two allergen vaccines (short ragweed and cat hair), data support the use of single allergen determinations (Amb a 1 and Fel d 1, respectively); for cat pelt and Hymenoptera venoms, the presence of two allergens (Fel d 1 and albumin for cat pelt; hyaluronidase and phospholipase A2 for Hymenoptera venoms) is verified for each lot; for dust mites and grass pollens, overall allergenicity is determined.

For initial overall allergenicity assessment, CBER developed a method using erythema size, following serial intradermal testing of highly allergic individuals. Intradermal testing was chosen over prick/puncture testing to achieve greater dosing accuracy; erythema size was chosen over wheal size to achieve greater accuracy in reaction measurements (16). This method is called $ID_{50}EAL$ (IntraDermal dilution for 50-mm sum of Erythema determines the bioequivalent ALlergy units) and can be used to compare the allergenicity of vaccines, regardless of source. Subsequent comparisons of vaccines from the same source material are made by a variant analysis called the parallel line bioassay. Both of these methods are described in *Methods of the Allergenic Products Testing Laboratory* (9) and are discussed briefly below.

In the $ID_{50}EAL$ method, allergenic vaccines are evaluated in subjects maximally reactive to the respective reference concentrates. Each subject is tested with serial threefold dilutions of the reference extract. After 15 minutes, the sum of the longest and midpoint orthogonal diameters of erythema (ΣE) is determined at each dilution, and the log dose producing a 50-mm ΣE response (D_{50}) is calculated (13). Vaccines that produce similar D_{50} responses can be considered bioequivalent and are assigned similar units, the bioequivalent allergy unit (BAU). Because the modal D_{50} of a series of vaccines was 14 (a 3^{-14} or 1:4.8 million dilution), vaccines with a mean D_{50} of 14 were arbitrarily assigned the value of 100,000 BAU/mL (11). Thus, the formula for the determination of potency from the D50 is

$$\text{Potency} = 3^{-(14-\text{mean D50})} \times 100{,}000 \text{ BAU/mL}$$

In a successful application of this method, the biological potencies of three commercial German cockroach allergen extracts were estimated to be between 1738 and 8570 BAU/mL (17,18).

By a similar technique and analysis, bioequivalent doses of test vaccines from the same source as the reference extract can be determined by the parallel-line bioassay (16). The inverse ratio of the doses of test extract required to produce identical D_{50} responses to a reference extract is the RP of that extract. This analysis requires that the log dose-response curves of the test extract and the reference extract be parallel; if the two dose-response lines are not parallel, then the ratio of skin test doses for identical responses—and the RP—will vary with the dose. In this situation, which strongly suggests compositional differences between the

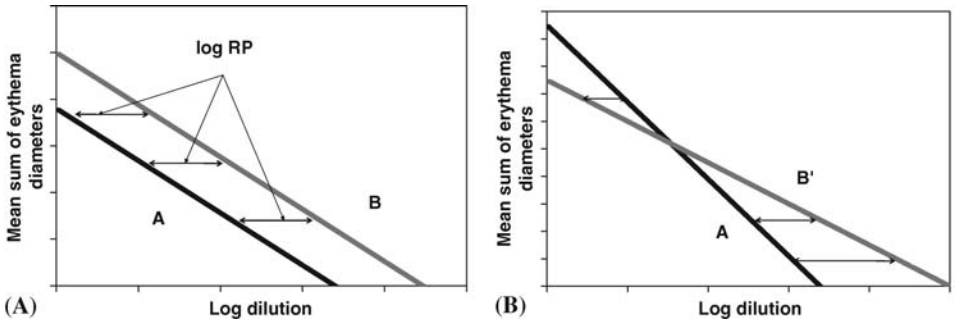

Figure 1 Hypothetical parallel line bioassay curves. In panel A, the bioassay curves are parallel, and the difference of log dilutions resulting in the same diameters is constant at all diameters. The log relative potency (log RP) of test sample B compared with reference A is represented by the difference. In panel B, the curves are not parallel, and the differences vary with the strength of the reaction. Thus, the log RP of B' compared with A cannot be calculated.

two vaccines, the distance between the two lines is different at each dose and a meaningful RP cannot be determined (10,19) (Fig. 1).

In the original 1994 protocol, the mean D_{50} for 15 highly allergic individuals was used to determine the D_{50} for the vaccine. In a reanalysis of the statistical considerations underlying such potency studies, Rabin et al. (20) applied the following formula for the number of study subjects, n, that would be required:

$$n = 2\left(\frac{\sigma}{\delta}\right)^2 (z_{1-\alpha} + z_{1-\beta/2})^2$$

where σ is the standard deviation of the measurement, δ is the acceptable difference in the D_{50}s of two equivalent products, and the z values are the critical values from the cumulative normal distribution table for a significance level α and a power of $1 - \beta$ (21). From this formula, n is a function of the squares of σ and δ. The value of n will depend on the particular allergen to be tested but, as may be seen in sample calculations represented in Table 2, n will usually be larger than 15.

Although skin testing is an essential component of the allergen standardization program, it is not intended for routine use in the testing of manufactured lots of vaccines prior to release. In vitro potency assays that accurately predict the in vivo activity of vaccines have been developed (19). Once an in vivo assay has been used to assign unitage to a reference extract, then an appropriate surrogate in vitro assay can be used to assign units to test vaccines from the same sources. These methods can be based on quantitation of the total protein content (Hymenoptera venoms), the specific allergen content within the allergen vaccines (short ragweed and cat hair), or the inhibition of the binding of IgE from pooled allergic sera to reference allergen (grasses, mites) (22). For the Hymenoptera venom allergens, the potency

Table 2 Sample size estimates

σ/δ	β	n
1.0	0.05	26
	0.10	22
	0.20	18
1.5	0.05	59
	0.10	49
	0.20	39
2.0	0.05	104
	0.10	87
	0.20	69

Estimates of sample size n from the formula $n = 2\left(\frac{\sigma}{\delta}\right)^2 (z_{1-\alpha} + z_{1-\beta/2})^2$ to demonstrate equivalence at the $\alpha = 0.05$ level by the two one-sided test formalism for a variety of β, tolerance intervals δ and standard deviations σ ($z_{0.975} = 1.96$; $z_{0.95} = 1.645$; $z_{0.90} = 1.282$).

determination is also based on the content of the known principal allergens within the extract, hyaluronidase and phospholipase, which is determined by enzyme activity (Table 1).

The potency units for short ragweed vaccines were originally assigned on the basis of their Amb a 1 content. Subsequent data suggests that 1 unit of Amb a 1 is equivalent to 1 μg of Amb a 1, and 350 Amb a 1 units/mL is equivalent to 100,000 BAU/mL. However, the original unitage has been retained. Grass pollen vaccines are labeled in BAU/mL on the basis of ID_{50}EAL testing. In some cases, the assignment of potency units to standardized allergenic vaccines in the United States has changed as better bioequivalence data have become available (13). Cat vaccines were originally standardized on the basis of their Fel d 1 content, with arbitrary unitage (AU/mL) tied to the Fel d 1 determinations. Subsequent ID_{50}EAL testing suggested that the 100,000 AU/mL cat vaccines, which contained 10–19.9 Fel d 1 units/mL, should be relabeled as 10,000 BAU/mL (23). In addition, 20% of individuals allergic to cat were found to have antibody to non–Fel d 1 proteins (24), and the identification of a cat albumin (Fel d 2) band on isoelectric focusing (IEF) was added as a requirement for cat pelt vaccines. Dust mite vaccines were originally standardized (in AU/mL) on the basis of the radioallergosorbent test (RAST) inhibition assays. Subsequent ID_{50}EAL testing indicated that the arbitrary unitage was statistically bioquivalent to BAU/mL (25); in this case, the original unitage was retained (26).

The identity of an allergen vaccine may be verified by visualizing the separated allergen proteins based on their size and isoelectric points (3). The IEF assay is an important safety test in the lot release of grass pollen and cat vaccines. The patterns produced by the crude allergen mixtures are reproducible enough to consistently indicate the presence of known allergens, to identify possible contaminants present in the vaccines, and to check lot-to-lot variation in the vaccines (27). In addition, IEF is used to verify the presence of Fel d 2 in cat pelt vaccines.

TESTS CURRENTLY APPLIED TO STANDARDIZED ALLERGENS

Several in vitro tests have been established for the testing the potency and identity of standardized allergens (Table 1). Tests for *potency* include assays for the specific allergen content, for the RP, and for the enzyme activity of allergenic vaccines. In addition, the *identity* of standardized vaccines may be tested by the qualitative assessment of allergen content.

The specific allergen content of certain allergenic vaccines can be measured by the radial immunodiffusion assay (RID). This assay is currently applied to two standardized allergenic vaccines, short ragweed and cat, in which the immunodominant allergens (Amb a 1 and Fel d 1, respectively) have been identified and defined. In this assay, monospecific antiserum is added to an agar solution, which is allowed to solidify. Wells are then cut into the agar and test allergen is placed in the wells. As the specific allergen diffuses out into the agar, a precipitin ring forms, which delineates the equivalence zone for antigen-antibody binding. The radius of the precipitin ring can then be measured. Since the antibody concentration in the agar is constant, the antigen concentration decreases with increasing distance from the well and is proportional to the log of the concentration of the applied test allergen in comparison to the reference extract.

The potency of those standardized allergen vaccines for which the immunodominant components have not been identified with certainty may be estimated using assays for IgE antigen binding that compare the overall IgE binding properties of test and reference vaccines, using pooled allergic sera. Initially, a RAST inhibition assay was used for this purpose; CBER adopted the competition ELISA as its standard assay because of its greater precision and convenience. After coating the wells of the polystyrene microtiter plate with the reference allergen and blocking the wells with bovine albumin, a mixture of the allergen vaccine to be tested and a reference serum pool is added to the wells. The greater the amount of immunoreactive allergen in the mix, the less free IgE antibody will be available from the serum pool to bind to the immobilized allergen on the plate. Once again, the concentration of the allergens in the allergen vaccine is determined by comparison to the reference allergen extract. However, since this assay does not explicitly measure a specific allergen, the allergen concentration is expressed as RP, with the reference vaccine assigned an arbitrary RP of 1. Early studies showed good an excellent correlation between RP assigned by titration skin testing and RP determined by RAST inhibition (11); subsequent studies showed the competition ELISA to be equivalent as well (28).

Hymenoptera venoms contain multiple glycoproteins enzymes, the most important of which are hyaluronidase and phospholipases A1 and A2. Venom allergen vaccines are standardized using enzymatic assays, which estimate hyaluronidase and phospholipase content on the basis of their enzymatic activity. In these assays, an agar solution is prepared with the appropriate enzymatic substrate and test samples are then added to cut wells. As the enzyme present in the sample diffuses into the agar, it digests the substrate, forming clearing zones around the wells. The radius of the clear zones is then measured and calculated as the log of the concentration of the enzyme present in the sample.

In addition to determining the potency of these allergen vaccines, manufacturers are expected to confirm the identity of certain standardized vaccines (Table 1) by IEF. This technique separates the proteins in the test extract on the basis of their isoelectric points. The profile obtained in this technique is compared with the CBER standard to confirm the stated identity of the allergen vaccine (27).

In the past, manufacturers were required to perform ninhydrin protein assays on most standardized allergen vaccines. CBER developed and adopted a modification of the more cumbersome ninhydrin technique for protein determination (29) in response to concerns about the inaccuracy of the more standard protein estimation techniques. However, release limits were not established for the total protein content of standardized allergen vaccines. Rather, the results of the ninhydrin assay were required for information only. When the results were checked as part of CBER's lot release program, CBER required that the results of the CBER assay be within 40% of the manufacturer's result.

In effect, the protein assay requirement was a quality control test; in this phase of the allergen standardization program, CBER did not have data on the protein content of the standardized allergens, or the effect of the protein content on potency assays. The requirement that manufacturers perform the ninhydrin assay on their standardized allergen vaccines was reexamined (30). As a result of these considerations, CBER no longer requires the use of the ninhydrin assay for standardized mite and grass allergen vaccines. However, as part of ongoing quality control, manufacturers should continue to perform a validated protein assay on each lot of material, and CBER continues to require this information as part of its lot release program. The choice of protein assay is left to the manufacturer. Currently approved protein assay methods for other allergens (standardized cat hair, short ragweed, and Hymenoptera venoms) are unchanged.

HOW SHOULD RELEASE LIMITS BE CHOSEN?

Fundamental to the standardization process is establishing an acceptable range of comparability or equivalence. Limits that are too broad lead to unacceptable risk to patients (anaphylaxis when the physician changes from one bottle to another, or changes to a different manufacturer), while limits that are too narrow lead to unacceptable risk for manufacturers (the rejection of a large percentage of safe and effective lots of product). In the competition ELISA, potency limits have been set according to the precision of the test; the candidate vaccines are expected to be statistically equivalent to the reference extract at a specified level of confidence with a specified test. Mite and grass pollen vaccines are currently expected to be identical to reference at the 98% confidence level, using three replicates of a validated competition ELISA; the standard deviation σ in log (RP) for a single replicate is 0.1375 (28). The 98% confidence interval is given by $10^{\pm 2.326\sigma/\sqrt{3}}$. Consequently, a lot whose RP falls in the range 0.654 to 1.530 is within the 98% confidence interval and is approved for release. This criterion also implies that, on average, 2% of lots submitted to CBER will fall outside of the release limits even if they are identical to the reference extract. Lots that are not identical to the reference would fail at predictably higher rates, while a small fraction of lots with RP outside the limits (as could be established by more exhaustive testing) will pass release testing.

An alternative approach would be to base the potency limits on acceptable ranges established in clinical studies. Three criteria would appear to be important. The first, therapeutic equivalence, addresses the efficacy of allergen vaccines for immunotherapy. Thus, an RP range will have the property of therapeutic equivalence if, for the allergen vaccine in question, lots with RPs anywhere in that range have an equal likelihood of effecting clinical

improvement in an immunotherapy trial. Likewise, diagnostic equivalence addresses the efficacy of allergen vaccines for in vivo diagnostics. Finally, safety equivalence reflects the likelihood of the safe administration of the vaccine for either diagnostic or therapeutic indications. The acceptable limits should fall within the narrowest of the equivalence ranges established by these criteria.

The aggregate consistency of manufactured lots might also be taken into account when developing testing methods and limits. For example, if typical lot-to-lot consistency is very high and well within clinical limits, then testing protocols could be adjusted to eliminate outliers while rarely failing lots with RP close to 1. On the other extreme, if the distribution of lots is broad, equivalence to the reference would be imposed. This would narrow the distribution, but at a cost: at 95% equivalence, 5% of lots with RP = 1 would fail release.

In an analysis of studies using ragweed and dust mite allergens (6), the range of therapeutic equivalence was at least 10-fold, and the ranges of diagnostic equivalence and safety equivalence were approximately fourfold. In the same study, the lot-to-lot consistency of 412 lots of grass pollen vaccines and 91 lots of dust mite vaccines were analyzed. The variability of the samples was comparable to the assay variability. Furthermore, the mean ratio (in RP) of two randomly selected lots of allergen would be 1.12 (for mites) and 1.18 (for grass pollen). The calculated 95th percentile ratios were 1.48 and 1.8, respectively. Thus, the equivalence ranges appear to be considerably broader than the current lot release limits (twofold) and the expected variations in product potency using current manufacturing and quality control practices. On the basis of these estimates, CBER broadened the internal release limits for standardized dust mite and grass pollen allergen vaccines to 0.5 to 2.0 (31).

FUTURE DIRECTIONS

The effort to standardize allergens in the United States has resulted in the development of a core group of highly used allergen vaccines that are better characterized and more consistent than their nonstandardized predecessors. Standardized allergens also facilitate accurate and informative scientific studies of the efficacy, safety, and mechanisms of allergen immunotherapy and will be essential for the study of novel immunotherapeutic products in the future. In spite of these clear advantages, most allergens marketed in the United States remain unstandardized. At a minimum, all allergen vaccines should be subject to potency testing and compared with a reference extract, whether manufacturer-specific, industry-wide, national, or international. CBER continues to work with the allergen vaccine industry to establish and maintain U.S. standards of potency for an increasing number of allergen vaccines and to improve the consistency of those products that are not standardized.

Once an allergen standardization target is selected, the marketed products that contain the allergen are examined and compared with the best products available worldwide. Biological potency is established using the ID_{50}EAL method, and a surrogate test is identified for lot release purposes. When a test for a standard of potency exists, the FDA notifies manufacturers [under 21 CFR 680.3(e)]. The regulation requires that manufacturers comply with the standard and test each lot of the specified extract prior to release for sale.

As described above, existing allergen extract potency assays estimate extract potency by one of two methods. When the specific allergen is known, it is determined using specific assays. Examples of this include Fel d 1 for cat hair extracts, Amb a 1 for short ragweed pollen extracts, and hyaluronidase and phospholipase A2 for Hymenoptera venom extracts. When the specific allergen is not yet known, overall potency is estimated using a polyclonal human IgE antiserum. The strength of the latter approach is that potency may be estimated even when the specific allergens are unknown. However, data from the CBER laboratory show that the overall potency approach may be insensitive to clinically significant changes in individual allergens (32). Thus, large fluctuations within an extract of potentially important specific allergens may go undetected if overall potency assays are used.

To address this issue, the CBER laboratory is developing a multiplex allergen extract potency assay. The purpose of this assay is to assess the overall potency of complex allergen mixtures as the integral of multiple discrete allergen assays. Early versions of this assay, using scFv antibodies selected by phage display technology (33) and used in a microbead format (34), are extremely promising.

SALIENT POINTS

- Allergen standardization in the United States is based on skin test responses in highly allergic individuals.
- Most allergen vaccines in the United States are not standardized.
- Nonstandardized allergens are labeled in units (PNU/mL or w/v) that may be unrelated to potency.
- All United States allergen vaccines, whether standardized or nonstandardized, must be manufactured in accordance with cGMPs.
- The number of individuals needed to establish the potency of a product by skin testing is related to the square of the ratio of the standard deviation (σ) of the skin test results and the acceptable difference (δ) in potency between two identically labeled products.
- The unitage adopted for standardized allergens in the United States is based on the best available scientific understanding of the specificity of responses in allergic individuals.
- The potencies of individual lots of standardized allergen vaccines are determined by specific surrogate in vitro tests that have been determined to correlate with the skin test results. In some cases, the potencies are based on specific allergen determinations.
- Release limits for lots of standardized allergens are established based on manufacturing capabilities, potency assay performance, and clinical data.

REFERENCES

1. Harden VA. A short history of the National Institutes of Health. Available at: http://history.nih.gov/exhibits/history/index.html (Accessed August 2007).
2. Food and Drug Administration. Milestones in U.S. food and drug law history. Available at: http://www.fda.gov/opacom/backgrounders/miles.html (Accessed August 2007).
3. Yunginger JW. Allergenic extracts: characterization, standardization and prospects for the future. Pediatr Clin North Am 1983; 30:795–805.
4. Baer H, Maloney CJ, Norman PS, et al. The potency and Group I antigen content of six commercially prepared grass pollen extracts. J Allergy Clin Immunol 1974; 54(3):157–164.
5. Baer H, Godfrey H, Maloney CJ, et al. The potency and antigen E content of commercially prepared ragweed extracts. J Allergy 1970; 45(6):347–354.
6. Slater JE, Pastor RW. The determination of equivalent doses of standardized allergen vaccines. J Allergy Clin Immunol 2000; 105(3):468–474.
7. Patterson ML, Slater JE. Characterization and comparison of commercially available German and American cockroach allergen extracts. Clin Exp Allergy 2002; 32:721–727.
8. Yunginger JW. Allergens: recent advances. Pediatr Clin North Am 1998; 35:981–993.
9. Food and Drug Administration. Methods of the allergenic products testing laboratory (Docket 94N-0012). Federal Register 1994; 59:60362–60363.
10. Turkeltaub PC. In-vivo Standardization. In: Middleton EJ, Reed CE, Ellis EF, eds. Allergy, Principles and Practice. St. Louis: C.V. Mosby Co., 1988:388–401.
11. Turkeltaub PC. Biological standardization of allergenic extracts. Allergol Immunopathol (Madr) 1989; 17(2):53–65.
12. Turkeltaub PC. Biological standardization. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 1997; 91:145–156.
13. Turkeltaub PC. Allergen Vaccine Unitage Based on Biological Standardization: Clinical Significance. In: Lockey R, Bukantz SC, eds. Allergens and Allergen Immunotherapy. New York: Marcel Dekker, Inc., 1999:321–340.
14. Alvarez-Cuesta E, Bousquet J, Canonica GW, et al. Standards for practical allergen-specific immunotherapy. Allergy 2006; 61(suppl 82):1–20.
15. van Ree R. The CREATE project: an introduction. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 2006;(95):71–74.
16. Turkeltaub PC, Rastogi SC, Baer H, et al. A standardized quantitative skin-test assay of allergen potency and stability: studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results. J Allergy Clin Immunol 1982; 70(5):343–352.
17. James R, Mitchell H, Gergen PJ, et al. Statistical analysis of ID50EAL data. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 2006; 95:117–127.
18. Slater JE, James R, Pongracic JA, et al. Biological potency of German cockroach allergen extracts determined in an inner city population. Clin Exp Allergy 2007; 37(7):1033–1039.

19. Turkeltaub PC. In Vivo methods of standardization. Clin Rev Allergy 1986; 4:371–387.
20. Rabin RL, Slater JE, Lachenbruch P, et al. Sample size considerations for establishing clinical bioequivalence of allergen formulations. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 2003;(94):24–33.
21. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokinet Biopharm 1987; 15(6):657–680.
22. Platts-Mills TAE, Rawle F, Chapman MD. Problems in allergen standardization. Clin Rev Allergy 1985; 3:271–290.
23. Matthews J, Turkeltaub PC. The assignment of biological allergy units (AU) to standardized cat extracts. J Allergy Clin Immunol 1992; 89:151.
24. Turkeltaub PC, Matthews J. Determination of compositional differences (CD) among standardized cat extracts by in vivo methods. J Allergy Clin Immunol 1992; 89:151.
25. Turkeltaub PC, Anderson MC, Baer H. Relative Potency (RP), compositional differences (CD), and assignment of allergy units (AU) to mite extracts (Dp and Df) assayed by parallel line skin test (PLST). J Allergy Clin Immunol 1987; 79:235.
26. Turkeltaub PC. Use of skin testing for evaluation of potency, composition, and stability of allergenic products. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A MInstitut 1994; 87:79–87.
27. Yunginger JW, Adolphson CR. Standardization of allergens. In: Rose NR, de Macario EC, Fahey JL, et al. eds. Manual of clinical laboratory immunology. Washington, DC: American Society for Microbiology, 1992:678–684.
28. Lin Y, Miller CA. Standardization of allergenic extracts: an update on CBER's standardization program. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 1997; 91:127–130.
29. Richman PG, Cissel DS. A procedure for total protein determination with special application to allergenic extract standardization. J Biol Stand 1988; 16(4):225–238.
30. Slater JE, Gam AA, Solanki MD, et al. Statistical considerations in the establishment of release criteria for allergen vaccines. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 2000; 93:47–56.
31. Food and Drug Administration. Guidance for Reviewers: Potency Limits for Standardized Dust Mite and Grass Allergen Vaccines: A Revised Protocol. Available at: http://www.fda.gov/cber/gdlns/mitegrasvac.pdf (Accessed August 2007).
32. Soldatova LN, Paupore EJ, Burk SH, et al. The stability of house dust mite allergens in glycerinated extracts. J Allergy Clin Immunol 2000; 105(3):482–488.
33. Finlay WJ, deVore NC, Dobrovolskaia EN, et al. Exploiting the avian immunoglobulin system to simplify the generation of recombinant antibodies to allergenic proteins. Clin Exp Allergy 2005; 35 (8):1040–1048.
34. deVore NC, Slater JE. An antibody-based multiplex bead assay to determine the potency and composition of allergen extracts. J Allergy Clin Immunol 2008; 121(2):557.

18 Manufacturing and Standardizing Allergen Extracts in Europe

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INTRODUCTION

History of Standardization in Europe

Specific allergy treatment, i.e., specific immunotherapy or specific allergy vaccination, has been performed for almost a century, since it was first described by Noon in 1911 (1). The discovery in 1966 of the IgE molecule (2), and the central role of IgE in allergy facilitated a better understanding of the immunological mechanisms, led to an improvement of diagnostic tools, and consolidated the concept of specific allergy diagnosis and treatment. Scientific methods were introduced to standardize allergen extracts in the seventies and eighties (3) and, in combination with gradual improvement of the clinical procedures, established specific allergy treatment as a scientifically based, reproducible, and safe treatment for allergic diseases.

The first international initiative on allergen standardization was based on the Danish Allergen Standardization 1976 program (4) and was published as the Nordic Guidelines in 1989 (5). These guidelines established the first regulatory demands for allergen extracts. The guidelines introduced the biological unit (BU), based on skin testing, for potency measures. Each manufacturer was instructed to produce an in-house reference preparation (IHRP), adjust the potency in BU, and use the IHRP for batch-to-batch control using scientifically based laboratory testing. The significance of using the major allergen content for the biological activity was recognized in the early nineties and is now established in the WHO recommendations (6) and in the European pharmacopoeia (7). This chapter describes important issues in the control of source materials and in the preparation of extracts as part of the standardization process the way it is performed in Europe. Procedures differ from those used in the United States, as does the selection of extracts for vaccination in common allergy practice (see chap. 17).

Standardization of Allergen Extracts

Allergen extracts/vaccines are used for specific diagnosis and treatment of allergic diseases and indirectly for the detection of environmental allergens. Allergen extracts are aqueous solutions of allergenic source materials, such as pollen, animal hair and dander, dust-mite bodies or cultures, insect venoms, or mold mycelia and spore particles. Since no structural feature defining an allergen has hitherto been described, the definition of an allergen is based on the functional criterion of being able to elicit an IgE response in susceptible individuals. All allergens are proteins and they are readily soluble in water. Airborne allergens are carried by particles in the micrometer range, a characteristic that is compatible with the concept that the particle carrying the allergen is inhaled, and the allergen is deposited on the mucosal surface of the lower airways, thereby stimulating the immune system. The allergen is thus defined by the immune system of the individual patient.

By this definition, any immunogenic protein (antigen) has allergenic potential, even though most allergic patients have IgE specific for a relatively limited number of "major" allergens. Analysis of a larger number of patients leads to the identification of still more

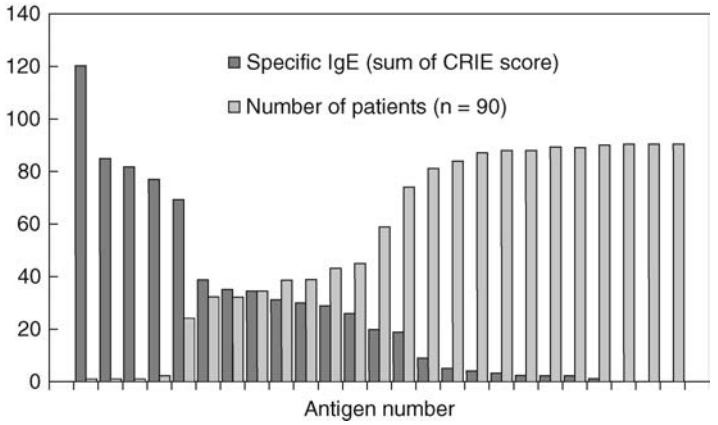

Figure 1 Complexity of patients' responses to allergen extracts. Serum samples from 90 grass-allergic patients were analyzed by crossed radio-immunoelectrophoresis (CRIE) using timothy, *Phleum pratense*, allergen extract. Labeled precipitates were assigned an arbitrary score for each patient dependent on the staining intensity of the autoradiogram. In this way, a graduated score for the specific IgE reactivity of each individual patient with each individual allergen was obtained. The scores were summed for each allergen and the antigens were arranged in ascending order and depicted on the ordinate axis. The score is depicted in the dark columns.The light column represents the cumulative number of patients having all their IgE specificities covered by the antigen in question and all other antigens to the left of the antigen in question.
Examples: A hypothetical extract containing the six most important allergens will cover all IgE specificities for 32 of the patients. Twenty-two allergens are needed to cover all IgE specificities of all 90 patients.

IgE-binding proteins (Fig. 1). Thus, the number of allergens in a given source material converge toward the total number of antigens, and any antigen has the potential to elicit an IgE response.

A major objective in the manufacture of allergen extracts, therefore, is to secure an adequate complexity reflecting the composition of water-soluble components of the allergenic source material. Another important matter of batch-to-batch control is standardization of potency, i.e., the overall IgE-binding capacity, which is a reflection of the anaphylactic potential of the preparation. The third important aspect of allergen extract manufacturing is controlling the major allergen content. The major allergens have distinct importance for the activity of allergen extracts/vaccines in diagnosis as well as treatment.

All aspects of the manufacturing procedure from selection and collection of raw materials, extract preparation and storage, to validation of assays and reagents, have impact on extract quality and are considered part of the standardization procedure.

PREPARATION OF ALLERGEN EXTRACTS

Source Materials

Inhalant allergens are present in airborne particles derived from natural allergen sources. The particles are inhaled and constitute the material to which humans are exposed. The most important allergen sources are found among the particles most frequently inhaled. Table 1 lists the most important allergen extracts in Europe and the United States.

The aim of selecting raw materials for allergen extract production is to gather materials containing the same active allergens in a manageable form. In most cases, the optimal source material is rather obvious, but in some cases, the allergen source is still debated, i.e., cat saliva/pelt/hair and dander or mouse urine/hair and dander. The source materials should be selected with attention to the need for specificity and for inclusion of all relevant allergens in sufficient amounts (8). The collection of the source materials should be performed by qualified personnel, and reasonable measures must be employed by the producer of allergen extracts to

Table 1 Most Important Allergen Extracts[a]

Europe		North America	
Temperate grasses	*Lolium perenne* *Phleum pratense* *Poa pratensis* *Festuca pratensis* *Dactylis glomerata* *Secale cereale*	House-dust mites	*Dermatophagoides pteronyssinus* *Dermatophagoides farinae*
		Temperate and subtropical grasses	*Lolium perenne* *Phleum pratense* *Poa pratensis* *Festuca pratensis* *Dactylis glomerata* *Cynodon dactylon*
House-dust mites	*Dermatophagoides pteronyssinus* *Dermatophagoides farinae*		
Trees	*Alnus glutinosa* *Betula verrucosa* *Corylus avellana*	Ragweed	*Ambrosia* spp.
		Cat	*Felis domesticus*
		Dog	*Canis familiaris*
Parietaria	*Parietaria* spp.	Lambs quarter	*Chenopodium* spp.
Olive	*Olea europea*	Mugwort	*Artemisia* spp.
Yellow jacket	*Vespula* spp.	Pigweed	*Amranthus* spp.
Mugwort	*Artemisia vulgaris*	Plaintain	*Plantago* spp.
Molds	*Alternaria* spp. *Cladosporium* spp. *Aspergillus* spp. *Penicillium* spp.	Molds	*Alternaria* spp. *Cladosporium* spp. *Aspergillus* spp. *Penicillium* spp.
Cat	*Felis domesticus*	Hymenoptera venoms	Apis mellifera
Honey bee	*Apis mellifera*		*Vespula* spp.
Dog	*Canis familiaris*		

[a]The two most important allergen sources in the world are the house-dust mites and the grass pollens. Patients often cross-react between the two important mite species, i.e., *D. pteronyssinus* and *D. farinae*, and between several species of the grasses. Commercial extracts are often based on mixtures of species within these groups. Important worldwide are also the indoor allergens from cat, dog, and molds, as well as the extracts derived from Hymenoptera venoms. In local regions other species may dominate. Examples are ragweed in large parts of the U.S., birch in Northern Europe, and *Parietaria* and olive in Southern Europe.

assure that collector qualifications and collection procedures are appropriate to verify the identity and quality of the source materials. This means that only specifically identified allergenic source materials that do not contain avoidable foreign substances should be used in the manufacture of allergen extracts. Means of identification and limits of foreign materials should meet established acceptance criteria for each source material. Where identity and purity cannot be determined by direct examination of the source materials, other appropriate methods should be applied to trace the materials from their origin. This includes complete identity labeling and certification from competent collectors. The processing and storage of source materials should be performed in a way to ensure that no unintended substances including microbial organisms are introduced into the materials. When possible, source materials should be fresh or stored in a manner that minimizes or prevents decomposition. Records should describe source materials in as much detail as possible, including the particulars of collection, pretreatment, and storage.

Pollen

The natural sources of inhalant allergens from plants are the pollen. Pollen may be obtained either by collection in nature or from cultivated fields or greenhouses. The collection may be performed by several methods, such as vacuuming or drying flower heads followed by grinding. Pollens are cleaned by dry or wet sieving and by fluid bed drying separation. Finally, pollen are dried under controlled conditions and stored in sealed containers at $-20^{\circ}C$. The maximum level of accepted contamination with pollen from other species is 1%. It should also be devoid of flower and plant debris, with a limit of 5% to 10% by weight. Pollen may show some variation in relative composition depending on cultivar, season, and location of growth.

Established criteria for collection should be validated in order to achieve a constant composition for the production of allergen extracts.

Acarids

For the production of allergen extracts of house-dust mites, the mites are grown in pure cultures. Constituents of the culture medium should be of pharmaceutical grade and devoid of contaminating substances from other allergen sources. Source materials for the extraction are either pure mite bodies (PMB) or whole mite cultures (WMC). The advantage of the WMC extract is that it contains all the material to which a mite-allergic patient is exposed under natural conditions, whereas the advantage of the PMB extract is higher homogeneity and lot-to-lot consistency and avoidance of contamination debris from the culture medium. The WMC extract includes material from mite bodies, eggs, larvae, and fecal particles as well as mite decomposition material and contaminants from the culture medium that should not be allergenic. The PMB extract contains only material extracted from mite bodies, including eggs and fecal particles. The relative concentration of group 1 and 2 allergens in the extract is dependent on the source materials, but clinical trials comparing vaccines based on WMC and PMB show both types to have clinical efficacy in specific allergy vaccination (9).

Mammals

Allergens of mammalian origin may emanate from various sources, i.e., hair, dander, serum, saliva, or urine. The allergens to which humans are exposed depend on the normal behavior of the animal. Therefore, the optimal source of allergens from mammals cannot be generalized. Whether derived from dander or deposited from body fluids, however, most allergens are present in the fur. Source materials should be collected only from animals that are declared overtly healthy by a veterinarian at the time of collection. Established procedures should minimize the risk of inclusion of zoonoses, i.e., diseases that can be transmitted from animals to humans, and the absence of viral particles in the final extract should meet established criteria. When killed animals are used, the conditions for storing should minimize postmortem decomposition until the source materials can be collected. The optimal source materials are often close-cropped hair including dander, which should be free from visible traces of blood, serum, or other extractable materials generally of low allergenic activity.

Due to the quantitative differences in the yield of the various allergens from different dog breeds, a mixture of material from a minimum of five different breeds is recommended.

Insects

The optimal source for insect allergens is dependent on the natural route of exposure, i.e., inhalation, bite, or sting. Where whole insects or insect debris are inhaled, the whole insect body is selected as allergen source. In the case of stinging insects, venom is the ideal allergen source. With biting insects, saliva would be ideal since it contains the relevant allergens.

Fungi

Raw materials are obtained by growing the fungi under controlled conditions. The harvested raw materials should consist of mycelia and spores. Due to difficulties in maintaining a constant composition of fungal cultures, an extract should be derived from at least five independent cultures of the same species. Production of the source material should be conducted under aseptic conditions to reduce the risk of contamination by microorganisms or other fungi. The inoculum should be obtained from established fungal culture banks, i.e., American Type Culture Collection (ATCC), Manassas, Virginia (http://www.lgcpromochem-atcc.com/) or Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands (http://www.cbs.knaw.nl/). The cultivation medium should be synthetic or at least devoid of antigenic constituents, i.e., proteins. Controls performed in fungal allergen extract production must include tests for suspected toxins.

Foods

Foods constitute a diversified area, and the market for standardized allergen extracts is scarce. Foods are often derived from various cultivars and subspecies grown under a broad variety of

conditions reflecting geographical regions worldwide. In addition, foods are often cooked prior to ingestion, and cooking unpredictably affects the allergenicity of the foods. Consequently, the source of allergen exposure, qualitative as well as quantitative, is highly variable (10).

Ideally, source materials for food allergen extracts should reflect local subspecies, conditions, and habits for the cultivation, harvesting, storing, and cooking of the foods. However, ingested foods are increasingly derived from distant parts of the world. The best solution to these problems may be to combine materials from as many sources as possible to reflect variation in as many parameters as possible. The quality of the source material should be suitable for human consumption and the origin and identity of the raw material should be documented.

For some plant foods, differences in tissue distribution and solubility properties of individual allergens may prevent optimal yields in a single extraction procedure (11). In such cases, an optimal extract may be derived only by combining extracts produced using different buffers and different parts of the plant, i.e., peel and pulp for fruits, as raw material.

A further problem in food allergen extract production is the presence in many foods of natural or microbial toxins, pesticides, antibiotics, preservatives, and other additives that may be concentrated in the allergen extract manufacturing process. Raw materials containing these substances in excess should be avoided as well as overtly artificial foods based on, e.g., genetically modified foods (GMO) or farmed fish. In general, the use of organic source material should be preferred.

Aqueous Allergen Extracts

Preparation of Allergen Extracts

The production process of allergen extracts imposes a number of constraints upon both selection of source materials and the physicochemical conditions used during the extraction procedure. The process must neither denature the proteins/allergens nor significantly alter the composition, including the quantitative ratio between soluble components. The extraction should be performed under conditions resembling the physiological conditions in the human airways, i.e., pH and ionic strength, and suppressing possible proteolytic degradation and microbial growth (12). The optimal extraction time is always a compromise between yield and degradation/denaturation of the specific allergens.

Low molecular weight materials (below 5000 Da) often include irritants, such as histamine, and should be removed from the final extract. This can be accomplished by dialysis, ultrafiltration, or size exclusion chromatography. Any substance excluded from the final extract should be verified nonallergenic. The production procedure should include assessment of known toxins, viral particles, microorganisms, and free histamine, and where relevant, verifying their concentration below defined thresholds.

The final extract should be stored under conditions that impede deterioration of the allergenic activity either by lyophilizing or by storing it at low temperatures ($-20^{\circ}C$ to $-80^{\circ}C$), possibly in the presence of stabilizing agents such as 50% glycerol or a nonallergenic protein, such as certified human serum albumin.

The most widely used extraction media are aqueous buffer systems of pH 6 to 9 and ionic strength 0.05 to 0.2. In general, nonaqueous solvents should be avoided due to the risk of protein denaturation.

Modified Allergen Extracts/Vaccines

Introduction

The efficacy of traditional immunotherapy, i.e., specific allergen vaccination, is related to the dose of vaccine administered, but the inherent allergenic properties of the vaccine imply a limitation due to the risk of inducing anaphylaxis. The risk of allergic side effects is minimized by administering repeated injections of increasing size over extended time periods. Physical or chemical modification of the extract can further reduce this risk. Physical modification involves adsorption of the allergens to inorganic gels, such as aluminum hydroxide or alum, for the purpose of attaining a depot effect characterized by a slow release of the allergens. Chemical modification includes cross-linking of the allergens by treatment with agents, such as formaldehyde, for the purpose of reducing allergenic reactivity. Modified allergen vaccines are used for allergy vaccination but are not used for diagnosis since they were intentionally modified to reduce interaction with IgE.

Physical Modification of Allergens

Physical modification of allergens involves adsorption of the allergen extract with insoluble complexes of inorganic salts, such as aluminum hydroxide or calcium phosphate. Aluminum hydroxide, $Al(OH)_3$, is especially useful for vaccination purposes and is used in both human and veterinary medicine (13). Its advantages are based on two characteristics of the complexes, the depot effect and the adjuvant effect. The allergens bind firmly to the inorganic complexes, giving rise to slow release of the proteins, thereby lowering the concentration of allergen in the tissue and reducing the risk of systemic side effects. Furthermore, the depot effect reduces the number of injections needed in the course of specific allergy vaccination. Although the significance of the adjuvant effect is unclear, higher levels of IgG antibodies are observed when alum-adsorbed vaccines are used in specific allergy vaccination, as compared with aqueous vaccine (14). Compared with aqueous vaccines, patients receiving depot preparations seem to experience fewer systemic side effects (15), particularly severe early reactions. The frequency of late reactions, which seem to be milder and can be managed by the patient, is reduced to a lesser extent, especially in asthmatic patients (16).

Preparation of aluminum hydroxide-adsorbed extract. Aluminum hydroxide is available as a stable viscous homogeneous gel with a high capacity for noncovalent coupling of proteins. The adsorption is performed simply by mixing the aqueous extract and the gel. After a few minutes at room temperature, the adsorption is complete. Buffer conditions need to be controlled, as the binding capacity varies with buffer composition, ionic strength, pH, and additives (17).

Standardization of the allergen extract must be completed prior to adsorption, as the insoluble complex is difficult to analyze. Therefore, it is difficult to verify the amount of protein adsorbed. In practice, a known amount of standardized allergen extract is adsorbed, and the amount of unbound protein is determined following precipitation of the complex by centrifugation. Manufacturers must specify criteria to withdraw batches above certain thresholds, as different allergens are bound to the complex with different efficiency. Thus, if a large fraction of the allergen extract is unbound, the relative composition of the vaccine may not reflect the composition of the standardized extract. In each case the binding capacity has to be empirically determined (18).

Chemically Modified Allergens

The theory behind chemical modification of allergen extracts is based on the observation that successful allergy vaccination is accompanied by an increase in allergen-specific IgG. Thus, if the allergen could be modified in such a way as to reduce allergenic reactivity, i.e., IgE binding, while preserving immunogenicity, higher doses could be administered without the risk of systemic reactions, leading to higher levels of allergen-specific IgG and improved outcome of specific allergy vaccination (19).

Formaldehyde had been used for extract development in detoxification of bacterial toxins, when Marsh and coworkers in 1970 applied formaldehyde treatment of allergens for allergy vaccination (19). The allergens are incubated with formaldehyde yielding so-called "allergoids," high molecular weight covalently coupled allergen complexes. Compounds with similar immunological properties can be produced using glutaraldehyde instead of formaldehyde. The rationale behind the reduced allergenicity of allergoids is threefold: (*i*) the large polymeric structures would contain concealed antigenic determinants (epitopes) unable to react with IgE; (*ii*) polymeric antigens would have a lower "epitope concentration" and thus reduced ability to cross-link IgE on mast cells; and (*iii*) high molecular weight polymers would diffuse more slowly through tissue.

Preparation of chemically modified allergens. Several allergens are heat labile and thus not readily applicable to the standard procedure of incubation with formaldehyde at elevated temperatures. Instead, a two-step procedure has been applied (20): The first step is incubation with 2 M formaldehyde at 10°C in aqueous buffer at pH 7.5 yielding a stabilized intermediate. After 16 days, the reaction is diluted fourfold and incubated another 16 days at 32°C. The first step at low temperature results in limited inter- and intramolecular cross-linking, thus stabilizing the allergen complex. The intermediate can be cross-linked further at elevated

temperature. Residual formaldehyde is removed by dialysis, and the allergoid is distributed stabilized by addition of 50% glycerol, lyophilized, or coupled to aluminum hydroxide.

Other Modifications

Approaches have been taken to reduce the allergenicity of allergen extracts by disruption of the tertiary structure of allergen molecules using denatured or degraded antigens or peptides, however, with reduced efficacy in allergy vaccination as compared with native allergens. Such molecules do have reduced IgE-binding activity but also substantially reduced immunizing capacity leading to insufficient stimulation of a protective immune response.

The employment of structural and molecular biology has revealed molecular details to the atomic level of several important major allergens. Biotechnology and epitope engineering may facilitate the development of safer allergen molecules in the form of mutated recombinant allergens (21), which can be standardized as chemical entities, obviating the problems of current allergen standardization (22).

Standardization of Modified Allergen Extracts

Most of the techniques used to characterize and standardize aqueous allergen extracts are not applicable to modified ones. It is, therefore, recommended that standardization be completed using the intermediate allergen preparation (IMP) prior to modification, and the reproducibility of the modification process be documented by methods specific to the procedure in question. Standardization of aqueous allergen extracts is discussed elsewhere in this chapter. A brief discussion of the methods suitable for the documentation of the modification processes in aluminum hydroxide-adsorbed and formaldehyde-treated allergen extracts follows.

Protein content in itself is not a suitable standardization parameter but may be a useful measure in terms of normalization of other activities; for example, RAST inhibition capacity per Lowry unit of protein. Determination of the reduction in primary amino groups is a good indication of the degree of modification in aldehyde-treated allergen extracts, since aldehydes react preferentially with primary amino groups. This measure can also be used for stability monitoring of the allergoid, as a reversal of the coupling will lead to an increase in the number of primary amino groups.

It is essential to verify that all protein is bound for adsorbed allergen vaccines. The acceptable level of allergen in the supernatant following centrifugation should be considerably below the initial dose used in the up-dosing schedule of allergy vaccination.

Electrophoretic techniques, such as acrylamide gel electrophoresis and isoelectric focusing (IEF) possibly combined with immunoblotting, are widely used for allergen characterization. For analysis of allergens liberated from adsorbed complexes, acrylamide gel electrophoresis is preferred. However, for "allergoids," acrylamide gel electrophoresis is not useful because of the high molecular weight. As formaldehyde preferentially reacts with primary amino groups, the isoelectric point (pI) of the allergoid is more acidic relative to the allergens. The shift in pI can be monitored by IEF. Size exclusion chromatography, preferably conducted by high-performance liquid chromatography (HPLC), is suited to control for the increase in molecular weight of allergoids relative to the allergens.

Crossed (radio-) immunoelectrophoresis cannot be used to analyze modified allergen extracts. RAST inhibition or related techniques, however, are readily applicable to both alum-adsorbed allergen extracts and allergoids for the purpose of assessing the reduction in allergenicity. These methods are also suited for stability studies.

In vivo testing in patients to standardize modified allergen vaccines is theoretically attractive; however, it is not practical. First, it would not be ethically acceptable to base production of all batches of extracts on routine in vivo assays. There are also large differences in the immune responses of individual patients necessitating large patient panels for such assays. Second, in vivo tests are expensive in terms of labor, time, and money.

Comparison of Modified Extracts

Allergen extracts contain a variety of enzymatic activities, including proteolytic activities, resulting in reduced stability of aqueous extracts when stored in solution. Both chemical and physical modification enhances the stability of allergoid preparations; however, the chemical

Figure 2 Molecular structure of the major allergen from Birch, Bet v 1. The main feature of the structure is a 25 amino acid long α-helix surrounded by a seven-stranded antiparallel β-sheet. A most unusual feature of the structure is a large internal cavity with three openings to the surface. This is the first experimentally determined structure of a clinically important inhalant major allergen (52).

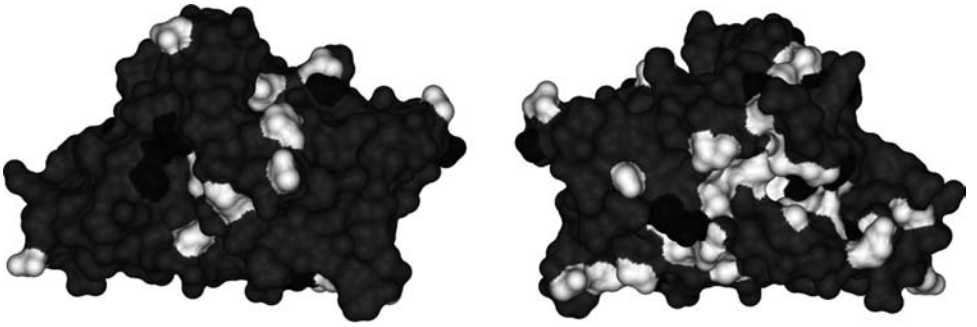

Figure 3 The molecular basis of cross-reactivity. Front and back view of the molecular structure of Bet v 1. Gray patches represent areas on the surface completely conserved among the homologous major allergens of alder, birch, and hazel. Conservative substitutions occur in dark gray areas. The conserved areas represent potential highly cross-reactive IgE epitopes on the protein surface.

modification process is slow and may permit proteolytic breakdown before completion. In addition, both physical (aluminum hydroxide) and chemical (formaldehyde) modification result in reduced allergenicity.

Acquired immune responses are driven by contact with epitopes, which are structural elements of the allergens (antigens). T-cell epitopes are linear fragments of its polypeptide chain, whereas B-cell epitopes (antibody-binding epitopes) are sections of the surface structure present only in the native conformation of the allergen (Figs. 2 and 3). Both T- and B-cell epitopes are essential for effective initiation and stimulation of immune responses; however, the repertoire of epitopes functional in any individual is highly heterogeneous (23,24).

Whereas the modification introduced by aluminum hydroxide adsorption is biologically reversible, the chemical modification of individual amino acids will irreversibly inactivate B- and T-cell epitopes. This chemical effect decreases immunogenicity explaining why higher doses of allergoid are needed to achieve clinical efficacy as compared with native allergen extract. The chemical modifications are not randomly distributed, as ε-amino groups on lysine residues are preferentially modified. Some epitopes are consequently more sensitive to modification than others, which may enhance the patient-to-patient variation when allergoids are used for allergy vaccination.

Contrary to expectation, however, allergoids are not safer in practical allergy vaccination compared with native allergens. This was documented in a report from the German Federal Agency for Sera and Vaccines which analyzed all reported adverse reactions to allergen vaccines over a 10-year period, 1991 to 2000, including 555 life-threatening, nonfatal events (25). Laboratory studies comparing commercial products available for birch pollen allergy vaccination in Europe demonstrated large variation among different allergoid preparations, but all allergoid products investigated showed reduced immunogenicity compared with intact allergen vaccine (26). This conclusion was confirmed by another study excluding differences based on the composition of the allergen extract, since the same extract was compared with or without chemical modification (27). In conclusion, allergoid products currently available on

the market in Europe do not fulfill the allergoid concept as originally formulated by Marsh and coworkers (19).

STANDARDIZATION OF ALLERGEN EXTRACTS

Allergen extracts are complex mixtures of antigenic components. They are produced by extraction of naturally occurring source materials known to vary considerably in composition depending on time and place. Without intervention, this variation would be reflected in the final products.

The purpose of standardization is to minimize the variation in composition, qualitative as well as quantitative, of the final products for the purpose of obtaining a higher level of safety, efficacy, accuracy, and simplicity for allergy diagnosis and allergen vaccination. Standardization of allergen extracts can never be absolute; standardization should be progressively improved as new methodologies and technologies are developed, and the understanding of the properties of the allergens and of the immune responses of allergic patients increases. The benefits for the clinician from improved standardization of allergen vaccines include easier differentiation between allergic and nonallergic subjects, a more precise definition of the specificity and degree of allergy, and a more reliable and reproducible outcome of specific allergy vaccination.

Standardization of allergen extracts is complicated due to their complexity, the allergen molecules, and their epitopes. Allergens are complex mixtures of isoallergens and variants, differing in amino acid sequence (Fig. 4). Some allergens are composed of two or more subunits, the association and dissociation of which will affect IgE binding. In addition, partial denaturation or degradation, which may be imposed by physical or chemical conditions in the production process, is difficult to assess and has a significant effect on the IgE-binding activities of the allergens. The B-cell epitopes that bind to IgE are largely conformational by disposition, meaning that they will be missing from the extract if the allergens are irreversibly denatured.

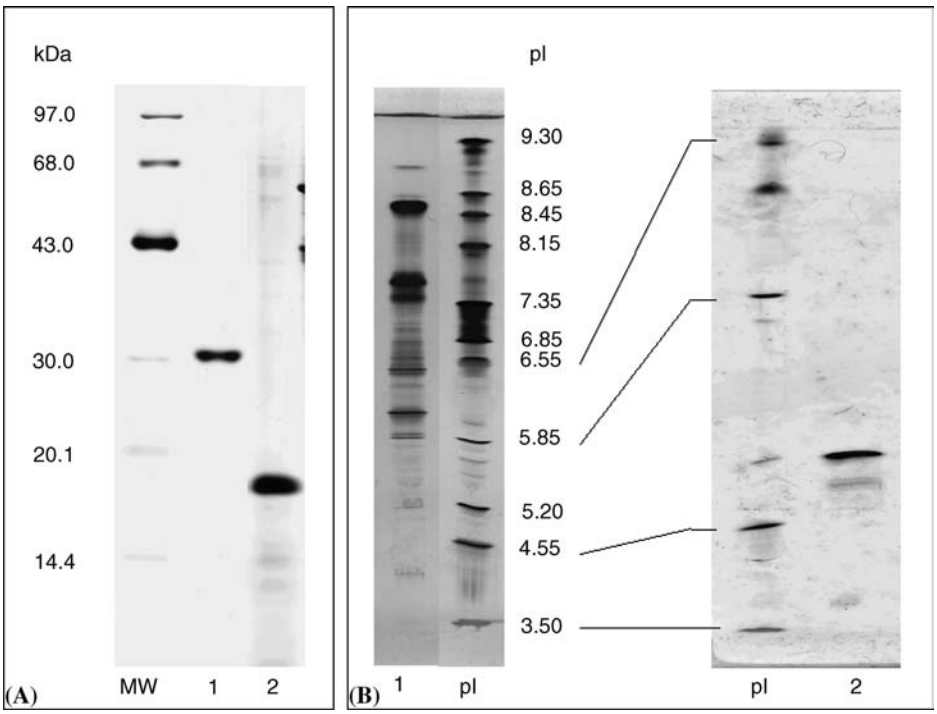

Figure 4 Isoallergenic variation. Allergens are mixtures of isoallergenic variants differing in amino acid sequence, whereas recombinant allergens are homogenous. (Panel A) A silver-stained SDS gel, lane MW: molecular weight markers, lane 1: purified natural Phl p 1, lane 2: purified recombinant Bet v 1. (Panel B) Silver-stained isoelectric focusing gels of pI markers, and the same preparations of purified allergens (lanes 1 and 2).

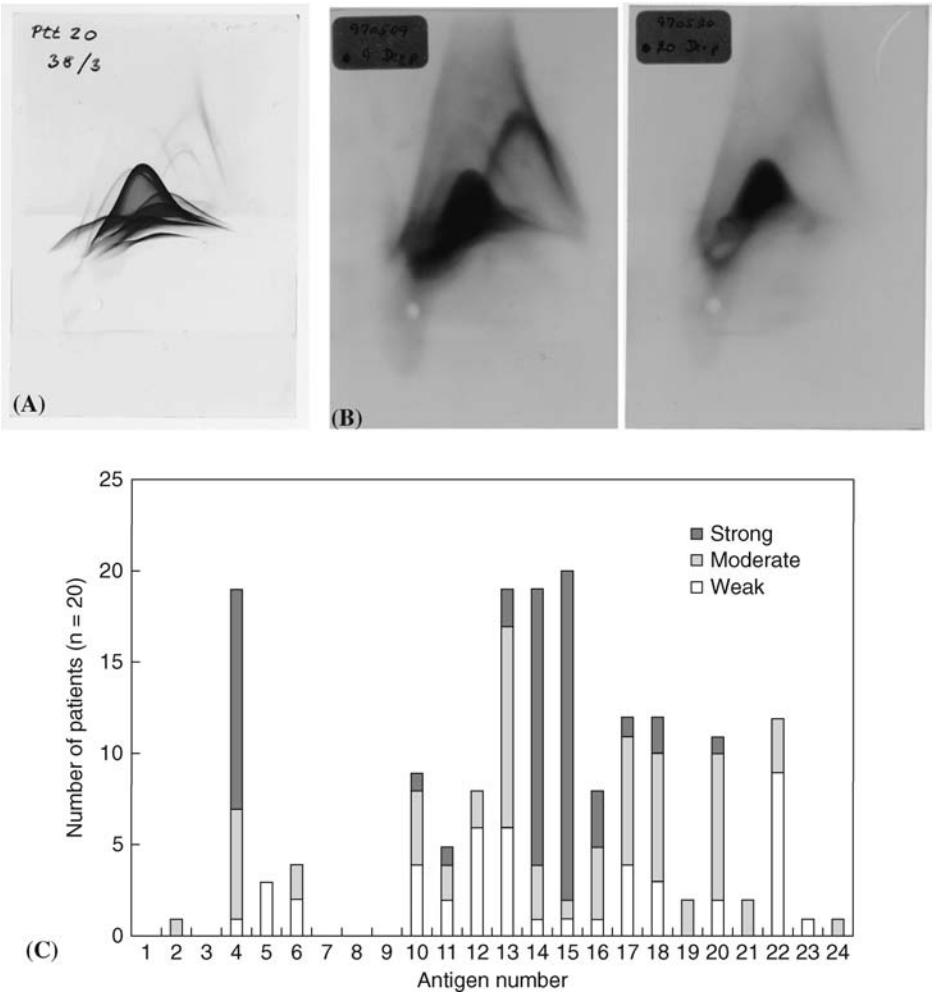

Figure 5 Complexity of allergen extracts. Crossed (radio-) immunoelectrophoresis used for the determination of important allergens. (Panel A) A crossed immunoelectrophoresis plate of a *Dermatophagoides pteronyssinus* allergen extract. Each bell-shaped precipitate represents the reaction of an antigen in the extract with the corresponding antibody present in a rabbit antiserum, raised by repeated immunization with the extract. (Panel B) An autoradiogram of similar plates after incubation with patient's serum and a radiolabeled anti-IgE antibody. Stained precipitates represent allergens. Precipitates from panel A are arbitrarily numbered, and the number of sera in a patient panel showing IgE-reactivity with each precipitate is recorded and displayed in an allergogram (Panel C). Der p 1 corresponds to antigen number 15, Der p 2 to antigen 14.

Another complicating aspect is the complexity of the immune responses of individual patients. Patients respond individually to allergen sources with respect to both specificity and potency. Allergens are proteins, and all proteins are potential allergens. A major allergen is defined as an allergen that is frequently recognized by patients' serum IgE when a large panel of patient sera is analyzed. A minor allergen binds IgE less frequently (below 50%) (28). Furthermore, patients respond individually to B- and T-cell epitopes and hence to isoallergens and variants.

A major objective of allergens' extract standardization is to ensure an adequate complexity in their composition. Knowledge of all essential allergens is a precondition for the safety of ensuring their presence in the final products (Fig. 5).

The other important aspect of standardization is the control of the total allergenic potency. The total IgE-binding activity is intimately related to the content of major allergen (29) and, for an optimal standardization procedure, control of the content of major allergen is essential.

A variety of techniques are available to assess allergen extract complexity and potency. Most techniques use antibodies as reagents, adding another level of complexity to the standardization procedure. Both human IgE and antibodies raised by immunization of animals are subject to natural variation and, in addition, may change over time.

These problems are handled by the establishment of reference and control extracts. International collaboration is necessary to ensure that manufacturers, government authorities, clinicians, and research laboratories worldwide can refer to the same preparations when comparing the results of quality control studies and potency estimates for different allergen extracts. Ideally, standards for reagents should also be established to promote and assist international collaboration.

Standards, References, and Controls

The Establishment and Use of International Standards

Guidelines for the establishment of international standards (IS) were formulated by a subcommittee under the International Union of Immunological Societies (IUIS) in 1980 to 1981. It was assumed that the collaboration and joint authority of WHO would be essential for international acceptance. In the following years, the subcommittee selected, characterized, and produced international standards from several allergenic sources. These included: *Ambrosia artemisiifolia* (short ragweed) (30), *Phleum pratense* (timothy grass) (31), *Dermatophagoides pteronyssinus* (house-dust mite) (32), *Betula verrucosa* (birch) (33), and *Canis familiaris* (dog) (34). Additional standards were planned for the *Alternaria alternata* (a mold) (35), *Cynodon dactylon* (Bermuda grass) (36) and *Lolium perenne* (rye grass) (37), *Felis domesticus* (cat), and *D. farinae* (house-dust mite). This initiative failed because of a lack of consensus and acceptance, primarily due to the differences in practical standardization between Europe and the United States (see section on "Standardization and Allergy Vaccination in Europe and in The United States").

Each of these standard reference extracts has been thoroughly investigated in collaborative studies involving laboratories and clinics worldwide. The results of the characterization and comparison of several coded extracts, which were made available by allergen manufacturers on a voluntary basis, as well as the selection of the international standards, are published and are available to all interested parties. Each international standard has been produced in 3000 to 4000 lyophilized, glass-sealed ampules, which can be obtained from the National Institute for Biological Standards and Control (NIBSC), London, United Kingdom (http://www.nibsc.ac.uk).

The content of each ampule is defined by the arbitrary assignment of 100,000 IU. This means that each ampule contains 100,000 IU of any included individual allergen and 100,000 IU of potency measured by any relevant method. Potency estimates will depend on methods and reagents, which must be stated, whereas IUs are independent of methods and reagents.

It is important to realize that the international standards are only recommended for use as calibrators (standards for measurement of relative potency). They are not recommended for use as prototypes, materials to which an extract is to be matched in all respects. None of the international standards have been tested in clinical trials of specific allergy vaccination, and no potency measures of their therapeutic effect are established.

Purified Allergens as International Standards

From November 2001 to April 2005, the European Commission funded an allergen standardization project called "Development of Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification" or in short the CREATE project (38). The two major objectives of the project were evaluation of the potential use of purified recombinant allergens as certified reference materials (CRM) and the evaluation of available ELISA assays for measurement of major allergens using the candidate CRM as a standard (38). The allergens included were major inhalant allergens in Europe, i.e., Bet v 1 from birch pollen, Phl p 1 and Phl p 5 from grass pollen, Ole e 1 from olive pollen, and Der p 1, Der p 2, Der f 1, and Der f 2 from house-dust mites. The result of the CREATE project was the establishment of the basis for the first two recombinant allergens as CRM: rBet v 1 and rPhl p 5.01 (previously called Phl p 5a). Both rBet v 1 and rPhl p 5.01 were found to be correctly folded molecules, and they fulfilled the necessary stability requirements and were similar to their natural counterparts with respect to

immunological characteristics (39). For each CRM, two ELISA assays were identified with potential for future use as reference methods. A follow-up study of CREATE has now been approved (BSP090) under the auspices of the European Directorate of the Quality of Medicines. The goals of this project are the establishment of rBet v1 and rPhl p 5.01 as European Pharmacopoeia reference preparations, calibrated in S.I. units, and validation of the candidate ELISA for the measurement of the two allergens in a true ring trial.

As soon as other GMP-produced recombinant major allergens with sufficient quality become available, it is expected that they will be included in the program.

The existence and availability of purified allergen references will enable the assignment of a major allergen content in mass units to the internal reference preparations (see below), which are in use in different laboratories of manufacturers, allergen research groups, or control authorities. Furthermore, the references can be used for standardizing major allergen content in bath-to-batch standardization.

The Establishment and Use of In-House Reference Preparations

Having established a production process including control of raw materials, batch-to-batch standardization is performed relative to an IHRP. The IHRP must be thoroughly characterized by in vitro laboratory methods to demonstrate an adequate complexity as well as an appropriate content of relevant major allergen(s). The potency of the IHRP must be determined by in vivo methods, such as skin testing, and ideally the content of major allergen(s) should be determined in absolute amounts. Furthermore, the IHRP should prove efficacious in clinical trials of specific allergy vaccination.

The IHRP serves as a blueprint of the allergy extract to be matched in all aspects by each and every following batch. Specific activities of the IHRPs should be compared with international standards. In this way, measures from different manufacturers can be compared and consistency in internal standardization achieved (4).

Strategy for Standardization

It is impossible to assess the clinical efficacy of each and every batch in the production of routine batches of allergen extracts. In practice, the batches are compared with the IHRP using a combination of different in vitro techniques to achieve a uniform composition, content of major allergen, and potency of extracts. The batch-to-batch standardization can be performed using the following three-step procedure:

1. Determination of allergen composition to ensure that all important allergens are present.
2. Quantification of specific allergens to ensure that essential allergens are present in constant ratios.
3. Quantification of the total allergenic activity to ensure that the overall potency of the extract is constant (in vivo and/or in vitro).

Methods for the Assessment of Allergen Extract Quality

The quality of an allergen extract is a measure of the complexity of the composition, including the concentration of each constituent. Having established careful control of raw materials and a robust production process, a relatively constant ratio between individual components can be achieved independently by quantifying only one or two components, i.e., the major allergens.

The complexity of the composition of allergen extracts can be assessed by several techniques. These techniques are standard biochemical and immunochemical separation techniques. Polyacrylamide gel electrophoresis with sodium dodecylsulfate (SDS-PAGE) (40) is a widely used high-resolution technique available in rapid and partly automated systems. The proteins are separated, but only after denaturation, according to size. Densitometric scanning has been reported, but this technique is not quantitative due to differences in staining intensities. It should only be used for a qualitative assessment of the allergen extract. In combination with electroblotting (41), the proteins can be immobilized on protein-binding membranes, such as nitrocellulose, and stained using a variety of dyes or labeled antibodies (immunoblotting), thereby considerably increasing the sensitivity. Some allergens, however, are irreversibly denatured by SDS treatment and may escape detection by IgE immunoblotting (42).

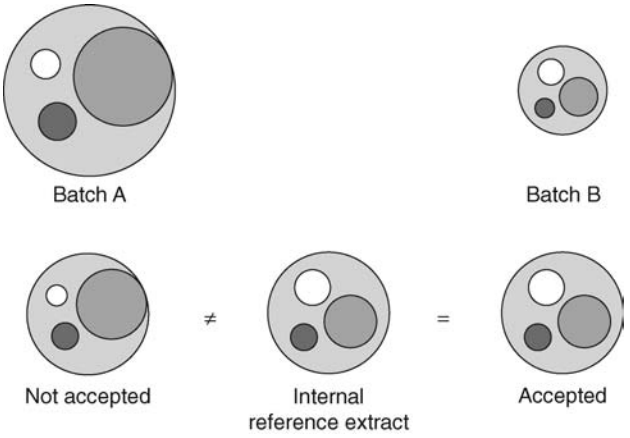

Figure 6 Standardization of allergen extracts. Complexity of allergen extracts represented by a model with three major allergens. The area of shaded circles represents the relative potency of individual components. The area of outer circles represents the total allergenic potency of the extracts. The total allergenic potency of batch A and B may be adjusted by dilution or concentration but the composition of the extracts still may vary accentuating the significance of the measurement of individual components.

IEF (43) is a qualitative electrophoretic technique that separates proteins according to charge pI. Individual allergens are difficult to identify as many proteins form several bands due to charge differences between isoallergens and variants.

Crossed immunoelectrophoresis (CIE) (44) is a technique by which individual antigens are distinguished in agarose gels in the form of bell-shaped antigen-antibody precipitates. The technique is dependent on the availability of broadly reactive polyspecific rabbit antibodies, but the method yields information on the relative concentrations of several important antigens in a single experiment. In crossed radio-immunoelectrophoresis (CRIE) (45), the plates are incubated with patient serum for the identification of allergens.

Methods based on HPLC and mass spectrometry (MS) are being evaluated for use in allergen standardization. Both methods have the potential of generating both qualitative and quantitative information on individual components in a complex mixture.

Quantification of Specific Allergens

Having determined an adequate complexity in composition, an allergen extract may still theoretically be deficient in the content of major allergen (Fig. 6). It is important to independently assess the content of major allergen(s), especially for allergen vaccines used for allergy vaccination. The maintenance dose in effective allergy vaccination contains a defined amount of major allergen (5–20 μg, regardless of vaccine), and the major allergen content is therefore a usable measure relating vaccine potency and therapeutic effect (Table 2).

The significance of controlling individual allergens in extracts is gaining more importance among government regulators and clinicians. Allergen extract manufacturers today have access to the published purification procedures of most major allergens. The purified major allergens can be used to produce antibodies for independent quantification, even in complex mixtures, such as allergen extracts. Polyspecific or monospecific polyclonal rabbit antibodies or murine monoclonal antibodies are most often used for this purpose.

Several immunoelectrophoretic techniques might be applied for the quantitative determination of individual allergens. These techniques are referred to as quantitative immunoelectrophoresis (QIE) (44), and they are convenient and reliable techniques to measure allergen concentrations relative to an in-house standard.

The area of a diffusion ring formed by the precipitated antigen in the monospecific antibody-containing gel can be correlated to the amount of antigen applied in single radial immunodiffusion (SRID), also known as the Mancini technique. The area of the precipitate, alternatively, the height of the precipitate, formed by electrophoresis of the antigen into the agarose gel containing the monospecific antibody, is proportional to the antigen concentration in rocket immunoelectrophoresis (RIE) or quantitative CIE. Both SRID and RIE are dependent on monospecific antibodies, whereas CIE is dependent on polyspecific antibodies.

The ELISA technique (46), in which the allergen is directly bound to a microtiter plate or captured using a monoclonal or polyclonal, monospecific antiserum coated to the plate, and

Table 2 Maintenance Doses in Effective Specific Allergy Vaccination[a]

Allergen source	Major allergen	Major allergen in maintenance dose	Approximate equivalent FDA potency	References
Cat				53–56
Felis domesticus	Fel d 1	14.6 μg	2,500 BAU	
House-dust mite				9,57
Dermatophagoides pteronyssinus	Der p 1	9.8 μg	740 AU	
Dermatophagoides farinae	Der f 1	13.8 μg	2,628 AU	
Ragweed				58
Ambrosia artemisiifolia	Amb a 1	10.0 μg	3,000 AU	
Grasses				59–61
Lolium perenne	Lol p 5	12.5 μg	3,948 BAU	
Phleum pratense	Phl p 5	20.2 μg	5,220 BAU	
Dactylis glomerata	Dac g 5	12.0 μg	2,956 BAU	
Festuca pratense	Fes p 5	18.6 μg	12,568 BAU	

[a]Discrepancy between diagnostic and therapeutic potency illustrated by the recommended maintenance doses of various clinical studies. For the average patient the recommended maintenance dose contains 5 to 20 μg of major allergen.

subsequently detected using monoclonal or polyclonal, monospecific antiserum, is a technique offering the possibility of multisample testing and partial automation. When optimized properly, the technique is very accurate. Monoclonal antibody-based ELISA is the most widely used technique for allergen measurement in mass units (47), and a number of validated ELISA assays for major allergens from the main allergenic sources are available. Furthermore, an initiative to establish centrally available validated ELISA assays for selected major allergens in the regulation of allergen extracts in Europe is ongoing (see section on "Purified Allergens as International Standards").

The standard ELISA format is a two-site sandwich assay. An allergen-specific mAb is coated to the microtiter plate and, upon incubating the allergen vaccine, the allergen molecules are captured and subsequently detected using a second mAb or a polyclonal antiserum. An in-house reference, calibrated against a purified allergen preparation, is used as standard. The advantages of mAb-based ELISA assays are their suitability for automation, well-defined specificity and an inexhaustible reagent supply, precise quantification in mass units of allergen, detection limits in the range of 0.1 to 5 ng/mL, and good reproducibility (intra-assay coefficient of variation in the 10% to 15% range).

A potential problem of mAb-based ELISA assays is the specificity of the mAb(s) used. Allergens are heterogeneous mixtures of isoallergens and variants, and in some cases it has been shown that specific mAb reacts to individual subsets of isoallergens (48) introducing a bias in the allergen measurement. A solution to this problem is to use a cocktail of mAb's on the solid phase of the ELISA and a polyclonal antibody as the second reagent.

Allergen Extract Potency

The potency of an allergen extract is the total allergen activity, i.e., the sum of the contribution to allergenic activity from any individual IgE molecule specific for any epitope on any molecule in the allergen extract. It follows that potency measures will always depend on the serum pool or patient panel selected as well as the methodology used. The potency of an allergen extract may be expressed mathematically as shown in equation (1):

$$a = \sum_{i=1}^{n} f_i c_i \tag{1}$$

The potency of an allergen extract can be expressed mathematically as the sum of the activities of all individual allergens, where "a" is the total allergen activity, and "c_i" and "f_i" are the concentration and activity coefficient, respectively, of molecule number "i."

Methods used for the assessment of allergen extract potency may be divided in two techniques: in vitro or in vivo. The dominating in vitro technique for the estimation of relative

allergenic potency is RAST inhibition (49) or related methods. A standardized reference extract is coupled to a solid phase, paper discs, sepharose gels, or magnetic particles. A serum pool is added, and bound IgE is detected using labeled anti-IgE. In RAST inhibition, the binding of IgE to the solid phase is inhibited by the simultaneous addition of a dilution series of the allergen extract subject to testing. The activity is determined relative to the reference extract itself; parallel inhibition curves indicate similar composition, whereas nonparallel curves indicate that the extracts differ both qualitatively and quantitatively.

The results are dependent upon the patient panel selected. The serum pool is a critical reagent and should contain sera from 20 or more different patients with clinically established allergy to the allergen source in question. A large serum pool should be made in order to ensure continuity, and care should be taken when the control serum pool is changed. Techniques based on ELISA using microtiter plastic trays as a solid phase may be applied using the same principles.

Tests of histamine release from washed human leukocytes utilize the quantification of histamine liberated from allergic patients' leukocytes upon stimulation with allergen (50). The tests are dependent on freshly drawn blood samples from a panel of allergic individuals, thus limiting the practical applicability in routine allergen extract potency determination.

Direct skin testing of human allergic subjects is the main in vivo method to assess allergen extract potency (51). It is impractical to use in vivo testing as a routine assay for production batch release; however, production batches can be compared by suitable in vitro methods to internal reference extracts, the in vivo activity of which has been already established. Patient selection criteria for in vivo assays are important since all in vivo methods will ultimately depend on the selected patient panel.

Skin testing in humans is the principle underlying the establishment of biological units of allergen extract potency. Several units are used. In Europe, the potency unit is based on the dose of allergen, which results in a wheal comparable in size to the wheal produced by a given concentration of histamine. This unit was originally called histamine equivalent potency (HEP). The Nordic Guidelines introduced the biological unit (BU) (5). One thousand BU is the equivalent of 1 HEP.

Determination of Clinical Efficacy

The potency of allergen extracts used for specific allergy vaccination should ideally be expressed in units describing clinical efficacy, since there is no relationship between therapeutic dose and skin test potency. Approaches to relate extract potency and clinical efficacy have been performed in the United States and Europe and commented on by the WHO. For several standardized vaccines, various trials have established an optimal maintenance dose. This dose corresponds to 5 to 20 μg of major allergen (Table 2), which is a useful measure for quantification.

However, determinations of clinical efficacy are extremely laborious. They can only be performed by using highly standardized vaccines, which have been described in detail with respect to composition and in vitro and in vivo potency, so that they fulfill the criteria for internal standards.

Standardization and Allergy Vaccination in Europe and in the United States

Standardization of allergy extracts in Europe is regulated by the European pharmacopoeia. The regulation is different from the United States, where regulation is enforced by the Food and Drug Administration (FDA). Whereas allergy extract consistency in Europe is maintained primarily through the use of in-house standards and international references, this goal is achieved by the FDA by mandating detailed standardization procedures and reagents for use by all manufacturers. An advantage of the European system is that it provides options for the doctor to choose from different products and for manufacturers to continuously improve quality and incorporate new methodology in analysis and control of the extracts. The advantage of the American system is that it results in a higher degree of consistency of extracts among manufacturers. Another difference between Europe and the United States is in the formulation of the extracts used for allergy vaccination. Physicians in the United States primarily use aqueous vaccines, whereas in Europe, alum-adsorbed vaccines, either chemically modified or native, are most often used (Table 3).

Table 3 Major Differences Between United States and Europe in Allergen Vaccine Standardization and Performing of Specific Allergy Vaccination

United States	Europe
Standardization of allergen vaccines	
FDA select representative extract as FDA reference (FDAR)	Manufacturer select representative extract as in-house reference preparation (IHRP) according to the European Pharmacopoeia
Biological activity (in vivo and in vitro potency/total allergen activity) relative to FDAR	Biological activity (in vivo and in vitro potency/total allergen activity) relative to IHPR
Concentration of major allergen molecules (FDA optional) relative to FDA major allergen reference	Concentration of major allergen molecules (cf. WHO recommendations) relative to IHPR
Methods and reagents selected and distributed by the FDA	Methods and reagents selected and developed by manufacturer
Performing specific allergy vaccination	
Predominantly aqueous vaccines	Predominantly aluminum hydroxide–adsorbed vaccines
Non-modified vaccine	Non-modified or chemically modified vaccines
Vaccines are mixed for multi-allergic patients	Vaccines are predominantly injected separately

CONCLUSION

In Europe each manufacturer uses individual BUs for the measure of allergen extract potency. This may cause confusion, but is enforced by differences in allergen extract composition hampering direct comparison. Furthermore, BUs in current use are based primarily on skin reactivity measurements, which may not be relevant for therapeutic efficacy. Since there is a remarkable coherence between the content of major allergen in the optimal maintenance dose comparing various allergen sources, the content of major allergen for many allergen extracts could be used as a marker relating vaccine potency to therapeutic efficacy. The provision of certified standards and assays for convenient major allergen determination will facilitate comparison of the major allergen content of different extracts. However, major allergen content in and of itself does not completely determine potency of current allergen vaccines, since other allergens, which may vary between extracts, also contribute to their biological potency. It is, therefore, necessary to assess biological potency to avoid the misunderstanding that extracts/vaccines having equal major allergen content are interchangeable.

SALIENT POINTS

- All allergens are proteins and all water-soluble proteins are potential allergens.
- Allergen extracts are complex, biological mixtures, and standardization is essential to ensure safety and efficacy of diagnosis and treatment.
- The process of extraction is highly dependent on physicochemical conditions. Extreme conditions are likely to destroy allergen epitopes and affect activity.
- Statistically patients' IgE binds to some antigens more frequently than to others, thereby defining major allergens.
- The effective maintenance dose in specific allergy vaccination for the average patient is proportional to the content of major allergen in an allergen vaccine.
- Major allergen content alone is not a sufficient measure of extract potency.
- Chemically modified allergen extracts are deficient in specific epitopes.
- The existence and use of internal as well as external standards are essential for standardization and control of allergen extracts.
- The quality of an allergen extract is dependent on the qualitative as well as quantitative composition.
- The potency of an allergen extract is determined by the combination of the concentration of one or more major allergens and the composition, qualitative as well as quantitative, of the allergen extract.

REFERENCES

1. Noon L. Prophylactic inoculation against hay fever. Lancet 1911; 177(4580):1572–1573.
2. Ishizaka K, Ishizaka T, Hornbrook MM. Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol 1966; 97(1):75–85.
3. Løwenstein H. Report on behalf of the International Union of Immunological Societies (I.U.I.S.) Allergen Standardization Subcommittee. Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 1983; 78:41–48.
4. Løwenstein H. Physico-Chemical and Immunochemical Methods for the Control of Potency and Quality of Allergenic Extracts. Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 1980; 75:122–132.
5. Nordic Council on Medicines. Registration of Allergen preparations: Nordic Guidelines. NLN Publication 1989, No 23, 1–34.
6. Bousquet J, Lockey RF, Malling HJ. eds. WHO Position Paper—Allergen immunotherapy: therapeutic vaccines for allergic diseases. Geneva: January 27–29 1997. Allergy 1998; 53(suppl 44):1–42.
7. Council of Europe. European Pharmacopoeia, 5th edition, volume 2, supplement 5.8. Directorate for the Quality of Medicines of the Council of Europe, Strasbourg, France, 2001. European Treaty Series 50.
8. Løwenstein H. Selection of reference preparation. IUIS reference preparation criteria. Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 1987; 80:75–78.
9. Wahn U, Schweter C, Lind P, et al. Prospective study on immunologic changes induced by two different *Dermatophagoides pteronyssinus* extracts prepared from whole mite culture and mite bodies. J Allergy Clin Immunol 1988; 82(3 pt 1):360–370.
10. Lemanske RF, Taylor SL. Standardized extracts, foods. Clin Rev Allergy 1987; 5(1):23–36.
11. Ahrazem O, Jimeno L, López-Torrejón G, et al. Assessing allergen levels in peach and nectarine cultivars. Ann Allergy Asthma Immunol 2007; 99(1):42–47.
12. Løwenstein H, Marsh DG. Antigens of *Ambrosia elatior* (short ragweed) pollen. I. Crossed immunoelectrophoretic analyses. J Immunol 1981; 126(3):943–948.
13. Butler NR, Voyce MA, Burland WL, et al. Advantages of aluminium hydroxide adsorbed combined diphtheria, tetanus and pertussis vaccines for the immunization of infants. Br Med J 1969; 1(5645): 663–666.
14. Norman PS, Lichtenstein LM. Comparisons of alum-precipitated and unprecipitated aqueous ragweed pollen extracts in the treatment of hay fever. J Allergy Clin Immunol 1978; 61(6):384–389.
15. Mellerup MT, Hahn GW, Poulsen LK, et al. Safety of allergen-specific immunotherapy. Relation between dosage regimen, allergen extract, disease and systemic side-effects during induction treatment. Clin Exp Allergy 2000; 30(10):1423–1429.
16. Tabar AI, Garcia BE, Rodriguez A, et al. A prospective safety-monitoring study of immunotherapy with biologically standardized extracts. Allergy 1993; 48(6):450–453.
17. al-Shakhshir RH, Regnier FE, White JL, et al. Contribution of electrostatic and hydrophobic interactions to the adsorption of proteins by aluminium-containing adjuvants. Vaccine 1995; 13(1):41–44.
18. Weeke B, Weeke E, Løwenstein H. The adsorption of serum proteins to aluminium hydroxide gel examined by means of quantitative immunoelectrophoresis. In: Axelsen NH, ed. Quantitative Immunoelectrophoresis, New Developments and Applications. Oslo, Norway: Universitetsforlaget, 1975: 149–154.
19. Marsh DG, Lichtenstein LM, Campbell DH. Studies on 'allergoids' prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. Immunology 1970; 18(5):705–722.
20. Marsh DG, Norman PS, Roebber M, et al. Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. J Allergy Clin Immunol 1981; 68(6):449–459.
21. Holm J, Gajhede M, Ferreras M, et al. Allergy vaccine engineering: epitope modulation of recombinant Bet v 1 reduces IgE binding but retains protein folding pattern for induction of protective blocking-antibody responses. J Immunol 2004; 173(8):5258–5267.
22. Løwenstein H, Larsen JN. Recombinant allergens/allergen standardization. Curr Allergy Asthma Rep 2001; 1(5):474–479.
23. Larsen JN. Isoallergens—significance in allergen exposure and response. ACI News 1995; 7:141–146.
24. van Neerven RJJ, Ebner C, Yssel H, et al. T-cell responses to allergens: epitope-specificity and clinical relevance. Immunol Today 1996; 17(11):526–532.
25. Lüderitz-Püchel U, Keller-Stanislawski B, Haustein D. Neubewertung des Risikos von Test- und Therapieallergenen. Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 2001; 44:709–718.
26. Lund L, Henmar H, Würtzen PA, et al. Comparison of allergenicity and immunogenicity of an intact allergen vaccine and commercially available allergoid products for birch pollen immunotherapy. Clin Exp Allergy 2007; 37(4):564–571.

27. Würtzen PA, Lund L, Lund G, et al. Chemical modification of birch allergen extract leads to a reduction in allergenicity as well as immunogenicity. Int Arch Allergy Immunol 2007; 144:287–295.
28. King TP, Hoffman D, Løwenstein H, et al. Allergen nomenclature. J Allergy Clin Immunol 1995; 96(1):5–14.
29. Dreborg S, Einarsson R. The major allergen content of allergenic preparations reflects their biological activity. Allergy 1992; 47(4 pt 2):418–423.
30. Helm RM, Gauerke MB, Baer H, et al. Production and testing of an international reference standard of short ragweed pollen extract. J Allergy Clin Immunol 1984; 73(6):790–800.
31. Gjesing B, Jäger L, Marsh DG, et al. The international collaborative study establishing the first international standard for timothy (*Phleum pratense*) grass pollen allergenic extract. J Allergy Clin Immunol 1985; 75(2):258–267.
32. Ford A, Seagroatt V, Platts-Mills TAE, et al. A collaborative study on the first international standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. J Allergy Clin Immunol 1985; 75(6): 676–686.
33. Arntzen FC, Wilhelmsen TW, Løwenstein H, et al. The international collaborative study on the first international standard of birch (*Betula verrucosa*) pollen extract. J Allergy Clin Immunol 1989; 83(1): 66–82.
34. Larsen JN, Ford A, Gjesing B, et al. The collaborative study of the international standard of dog, *Canis domesticus*, hair/dander extract. J Allergy Clin Immunol 1988; 82(3 pt 1):318–330.
35. Helm RM, Squillace DL, Yunginger JW. Members of the international collaborative trial. Production of a proposed international reference standard *Alternaria* extract II. Results of a collaborative trial. J Allergy Clin Immunol 1988; 81(4):651–663.
36. Baer H, Anderson MC, Helm RM, et al. The preparation and testing of the proposed international reference (IRP) Bermuda grass (*Cynodon dactylon*)-pollen extract. J Allergy Clin Immunol 1986; 78(4 pt 1):624–631.
37. Stewart GA, Turner KJ, Baldo BA, et al. Standardization of rye-grass pollen (*Lolium perenne*) extract. An immunochemical and physicochemical assessment of six candidate international reference preparations. Int Arch Allergy Appl Immunol 1988, 86 (1):9–18.
38. van Ree R. CREATE Partnership. The CREATE project: EU support for the improvement of allergen standardization in Europe. Allergy 2004; 59(6):571–574.
39. Fernandez-Rivas M, Aalbers M, Fötisch K, et al. Immune reactivity of candidate reference materials. Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 2006; 95:84–88.
40. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227(5259):680–685.
41. Kyhse-Andersen J. Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. J Biochem Biophys Methods 1984; 10 (3–4):203–209.
42. Ipsen H, Larsen JN. Detection of antigen-specific IgE antibodies in sera from allergic patients by SDS-PAGE immunoblotting and crossed radioimmunoelectrophoresis. In Bjerrum O, Heegaard NHH, eds. Handbook of Immunoblotting of Proteins, Vol II. Boca Raton: Chemical Rubber Company, 1988:159–166.
43. Brighton WD. Profiles of allergen extract components by isoelectric focussing and radioimmunoassay. Dev Biol Stand 1975; 29:362–369.
44. Løwenstein H. Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. Prog Allergy 1978; 25:1–62.
45. Weeke B, Søndergaard I, Lind P, et al. Crossed radio-immunoelectrophoresis (CRIE) for the identification of allergens and determination of the antigenic specificities of patients' IgE. In: Axelsen NH, ed. Handbook of Immunoprecipitation-in-Gel Techniques. Scand J Immunol 1983; 17(suppl 10): 265–272.
46. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. J Immunol 1972; 109 (1):129–135.
47. Carreira J, Lombardero M, Ventas P. New developments in in vitro methods. Quantification of clinically relevant allergens in mass units. Seventh International Paul-Ehrlich-Seminar, Langen, Germany, Sept 9–11, 1993.
48. Park JW, Kim KS, Jin HS, et al. Der p 2 isoallergens have different allergenicity, and quantification with 2-site ELISA using monoclonal antibodies is influenced by the isoallergens. Clin Exp Allergy 2002; 32(7):1042–1047.
49. Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. J Allergy Clin Immunol 1972; 49(1):1–9.
50. Siraganian RP. Automated histamine analysis for *in vitro* allergy testing. II. Correlation of skin test results with *in vitro* whole blood histamine release in 82 patients. J Allergy Clin Immunol 1977; 59(3): 214–222.

51. Platts—Mills TAE, Chapman MD. Allergen standardization. J Allergy Clin Immunol 1991; 87(3):621–625.
52. Gajhede M, Osmark P, Poulsen FM, et al. X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. Nat Struct Biol 1996; 3(12):1040–1045.
53. Sundin B, Lilja G, Graff-Lonnevig V, et al. Immunotherapy with partially purified and standardized animal dander extracts. I. Clinical results from a double-blind study on patients with animal dander asthma. J Allergy Clin Immunol 1986; 77(3):478–487.
54. van Metre TE, Marsh DG, Adkinson NF, et al. Immunotherapy for cat asthma. J Allergy Clin Immunol 1988; 82(6):1055–1068.
55. Hedlin G, Graff-Lonnevig V, Heilborn H, et al. Immunotherapy with cat- and dog-dander extracts V. Effects of 3 years of treatment. J Allergy Clin Immunol 1991; 87(5):955–964.
56. Hedlin G, Heilborn H, Lilja G, et al. Long-term follow-up of patients treated with a three-year course of cat or dog immunotherapy. J Allergy Clin Immunol 1995; 96(6 pt 1):879–885.
57. Haugaard L, Dahl R, Jacobsen L. A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. J Allergy Clin Immunol 1993; 91(3):709–722.
58. Creticos PS, Reed CE, Norman PS, et al. Ragweed immunotherapy in adult asthma. N Engl J Med 1996; 334(8):501–506.
59. Østerballe O. Immunotherapy in hay fever with two major allergens 19, 25 and partially purified extract of timothy grass pollen. Allergy 1980; 35(6):473–489.
60. Varney VA, Gaga M, Frew AJ, et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. Br Med J 1991; 302(6771):265–269.
61. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999; 341(7):468–475.

19 Preparing and Mixing Allergen Vaccines for Subcutaneous Immunotherapy

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COMMERCIALLY AVAILABLE ALLERGEN VACCINES

Allergen immunotherapy is appropriately performed with vaccines of inhalant allergens or the venom from stinging insects. The term vaccine refers to the solution administered to the patient. The materials used to prepare the vaccine are referred to as extracts. In the United States, allergen extracts are either standardized or nonstandardized extracts in a variety of formulations: lyophilized, adsorbed to aluminum, aqueous or 50% glycerin solutions. Potency is expressed as allergen units (AUs), bioequivalent allergen units (BAUs), content of the major allergen, weight by volume (wt/vol), or protein nitrogen units (PNU).

Standardized Extracts

The manufacturing and sale of allergen extracts in the United States is regulated by the Center for Biologics Evaluation and Research (CBER) of the Food and Drug Administration (FDA) (1). CBER has established reference extracts and reference serum pools to be used by extract manufacturers to standardize certain allergen extracts. The potency of some of the CBER standard extracts was established by titrated intradermal skin testing (2). In this method, serial threefold dilutions of the extract are tested on the back of a group of subjects highly sensitive to that inhalant. On the basis of the dilution which yields an area of erythema with the sum of orthogonal diameters of 50 mm (the D50), the extract is assigned a BAU numerical value. Allergen extract companies then compare their extract with the CBER reference using radioallergosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA) inhibition and a potency is assigned. In contrast, the extracts of cat (3) and short ragweed (4) are standardized by their content of the major allergen, expressed in arbitrary FDA units, instead of standardization with quantitative skin testing.

The inhalant allergen extracts that are currently standardized (Table 1) are house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), cat hair (which is low in cat serum albumin) and cat pelt (which contains substantial amounts of cat albumin), short ragweed (*Ambrosia elatior*), and eight grasses (Bermuda, June, meadow fescue, orchard, red top, rye, sweet vernal, and timothy).

While standardization of short ragweed, house dust mite and cat resulted in more consistently potent extracts than were previously available; the standardization in 1997 of the eight grasses resulted in a decrease, in some instances quite substantial, of the strength of the most potent extract available. This resulted from a CBER decision to reduce these grass extracts to a maximum testing potency of 10,000 BAU/mL and for treatment to 100,000 BAU/mL, which is considerably less than the potency of many of the previously available grass pollen extracts (5).

A second group of standardized extracts are the venoms of the stinging Hymenoptera (Table 1). These are standardized on the basis of a venom protein content of 100 μg/mL for all the individual species, but 300 μg/mL for the mixed vespids.

Physical Form and Diluent of Available Allergen Extracts

Standardized extracts are available in a lyophilized state (cat and Hymenoptera venoms), in 50% glycerin-saline (grasses, short ragweed, cat, house dust mites), and in aqueous solution (short ragweed). Nonstandardized extracts are available in either a 50% glycerin or an aqueous solution. The 50% glycerin contains equal parts of glycerin and normal saline. The aqueous extract consists of normal saline and 0.4% phenol. Glycerin at 50% concentration inhibits microbial growth and maintains the potency of allergenic extracts. Phenol, which is added to

Table 1 Allergen Extracts: CBER Basis for Standardization

Cat hair: 10,000 BAU/mL contains 10.0–19.9 units Fel d 1/mL and "little" cat serum albumin by isoelectric focusing
Cat pelt: 10,000 BAU/mL contains 10.0–19.9 units Fel d1/mL and substantial amounts of cat serum albumin by isoelectric focusing
Short ragweed: Designated weight by volume or protein nitrogen units, but with the Amb a 1 content in units/mL on the vial
House dust mite (*D. pteronyssinus* and *D. farinae*): 5000, 10,000, and 30,000 AU/mL determined by quantitative skin testing
Grasses: 10,000 and 100,000 BAU/mL determined by quantitative skin testing
Hymenoptera: Expressed as the venom protein content (100 μg/mL) for each individual insect species (honey bee, yellow jacket, wasp, yellow- and white-faced hornet)

Abbreviation: CBER, Center for Biologics Evaluation and Research.

aqueous extracts to inhibit bacterial and fungal growth, has an adverse effect on the potency of stored extracts. The choice between the two extracting and diluting fluids would favor glycerin were it not for the discomfort associated with injection of 50% glycerin (6).

A limited number of pollen extracts are available adsorbed to aluminum to delay their absorption from the injection site. When the initial extraction is performed with aqueous extracting fluids and the aluminum is subsequently added, the resulting vaccine is equally efficacious compared with aqueous vaccines (7) but is associated with a decreased incidence of systemic reactions (8). However, aluminum-precipitated vaccines, which have been initially extracted in pyridine, are markedly less potent than comparable aqueous vaccines (9,10).

Expressed Extract Potency

The traditional expressions of extract potency are weight by volume and protein nitrogen units. Neither provides precise information regarding the allergenic potency of the extract. However, it is likely that within broad limits the initial potency of many extracts obtained from the same commercial supplier have reproducible batch-to-batch potency (11). Thus it has been possible, as a general practice, to refill allergy treatment vaccines with new lots of the same stated potency from the same manufacturer without untoward reactions by reducing the first injection from the new vial by one-third to one-half of the previous dose.

Weight by volume is the simplest way to express the potency of allergen extracts. It is only necessary to weigh the material to be extracted and measure the volume of extracting fluid. Thus, 10 g of pollen extracted in 100 mL of extracting solution yields a final concentration of 1:10 wt/vol. One advantage of this method of expressing potency is that the extract need not be further diluted to achieve the desired level of potency.

PNUs were introduced in an attempt to more accurately express the allergen content of extracts (12). First, the protein nitrogen content is determined and then the content is converted to units (one unit equal to 0.00001 mg of protein nitrogen). The major allergens usually represent only a few percent of the total protein content of allergen extracts. Therefore, PNU offers little advantage as an expression of allergenic potency over weight by volume. The distinct disadvantage of PNU is that extracts are commercially available in specific concentrations (e.g., 20,000–40,000 PNU/mL). This requires that the extract be diluted from the strength obtained during the extraction process and therefore the most potent PNU extract available will be weaker than the most concentrated weight by volume for any given allergen.

The CBER BAU is based on quantitative intradermal skin testing with serial threefold dilutions of the extract in at least 15 highly allergic individuals (2). The endpoint is the dilution of the extract that results in an erythema with the sum of the largest and the midpoint orthogonal diameters of 50 mm. If the endpoint dilution is 9.0 to 10.9, the extract is assigned 1000 BAU/mL; if the endpoint dilution is 11.0 to 12.9, the concentration is 10,000 BAU/mL; and if it is 13.0 to 14.9, the concentration is 100,000 BAU/mL. Alternatively, the BAU potency can be determined by RAST- or ELISA-inhibition methods in comparison with the CBER reference product, whose BAU potency has been determined by quantitative intradermal skin testing. For a potency designation of 10,000 BAU/mL by the RAST-inhibition method, the relative potency in relation to the reference product must be 0.47 to 2.12 and for the ELISA-inhibition method 0.699 to1.431. The CBER standardization method is likely more reproducible when content of major allergen is used as a basis. For Amb a 1, the major allergen of ragweed,

Table 2 Immunotherapy Dose and Outcome of Asthma

Treatment group	Number evaluable	Wheezing with exertion (%)	Wheezing with upper respiratory tract infections (%)
Highest tolerated dose (maximum 1:250 wt/vol)	43	9	9
1:5000 wt/vol	39	31	10
1:10,000,000 wt/vol	49	45	55
Saline	42	64	74

Source: From Ref. 13.

determinations by gel diffusion with a range of plus or minus 25% is allowed, as opposed to plus or minus 100% for quantitative skin testing.

ADEQUATE DOSING FOR DEMONSTRATED EFFICACY

Studies with Vaccines Prepared from Nonstandardized Extracts

Johnstone and Crump (13) conducted a study of the efficacy of allergen immunotherapy employing a broad range of doses. New patients with perennial bronchial asthma referred to the pediatric allergy clinic of Strong Memorial Hospital for immunotherapy were randomly assigned to receive treatment with buffered saline, or all inhalable allergens to which the child reacted on skin testing but administered to maximum concentrations of 1:10,000,000 wt/vol, 1:5000 wt/vol, or the highest tolerated dose up to a maximum of 1:250 wt/vol. The child, parent, and evaluator were unaware of the patient's assigned group. Two hundred children were randomized and 173 were available for evaluation during the winter of the fourth year of treatment. The results suggested that clinical improvement increased with increasing dosage of antigen (Table 2).

Lowell and Franklin (14) demonstrated, in a study meeting all the requirements for adequate blinding, that immunotherapy with ragweed pollen vaccine was clinically effective. They then applied the same study design to examine the effect of two doses of ragweed pollen vaccine on seasonal rhinitis symptoms (15). Twenty-five ragweed sensitive subjects were recruited who were still symptomatic during the ragweed pollen season despite receiving allergen immunotherapy that contained ragweed pollen extract. They were paired by severity of symptoms during the ragweed pollen season. One of each pair continued to receive ragweed vaccine at the customary level (median dose 0.3 mL of a 1:50 wt/vol concentration) while the other member of the pair received a dose reduced by 95% (median dose 0.3 mL of 1:1000 wt/vol). During the ensuing ragweed season, those receiving the reduced dose experienced significantly more symptoms of allergic rhinitis.

Studies with Vaccines Prepared from Standardized Extracts

One of the major advantages of using standardized vaccines is that proven treatment regimens from controlled studies can be more effectively applied by physicians to their clinical practices. There have been a number of double-blind, controlled studies employing the standardized vaccines that are now available in the United States (Table 3). In some instances only one concentration was employed, but the clinical benefit was demonstrable within a few months to a year and clinically relevant. In other studies, more than one dose was employed, and a dose response was demonstrated. In all of these studies, the dose of allergen employed was expressed in the concentration of one of the major allergens, since this is the only method of standardization recognized internationally. To allow general application of this information to standardized extracts available in the United States, representative values for the major allergen content of specific lots of extracts standardized in BAUs are given in Table 4. It must be appreciated, however, that standardized extracts, labeled with the same BAU potency may contain different amounts of the major allergens.

House Dust Mites

The study by Ewan et al. (16) demonstrated that a maintenance dose containing 11.9-μg Der p 1 reduced symptoms and objective responses significantly after only three months, but with a

Table 3 Documented Optimal Effective Doses of Major Allergens

Allergen	First author	Optimal dose
Dermatophagoides	Ewan (16)	11.9-μg Der p 1
	Haugaard (17)	7.0-μg Der p 1
	Olsen (18)	7.0-μg Der p 1 10.0-μg Der f 1
Cat dander	Van Metre (19)	13.8-μg Fel d 1
	Alvarez-Cuesta (20)	11.3-μg Fel d 1
	Hedlin (21)	17.3-μg Fel d 1
	Varney (22)	15-μg Fel d 1
	Ewbank (23)	15-μg Fel d 1
	Nanda (24)	15-μg Fel d 1
Dog dander	Lent (25)	15-μg Can f 1
Grass	Varney (26)	18.6-μg Phl p V
	Dolz (27)	15-μg Dac q V, Lol p V, Phl p V
	Walker (28)	20-μg Phl p V
Short ragweed	Van Metre (29)	11-μg Amb a 1
	Creticos (30)	12.4-μg Amb a 1
	Creticos (31)	6-μg Amb a 1
	Furin (32)	24-μg Amb a 1

Table 4 Representative Values for Major Allergen Content of U.S. Standardized Extracts[a]

Allergen extract (n = number of extracts tested)	Expressed concentration	Major allergen	Mean content major allergen (μg/mL)	Maximum content major allergen (μg)	Minimum content major allergen (μg)
Orchard $n = 14$	100,000 BAU/mL	Dac g 5	918	2,414	294
Fescue $n = 12$	100,000 BAU/mL	Fes p 5	152	204	75
Rye $n = 14$	100,000 BAU/mL	Lol p 5	337	526	157
Kentucky $n = 23$	100,000 BAU/mL	Poa p 5	300	482	118
Timothy $n = 28$	100,000 BAU/mL	Phl p 5	680	1,336	354
Short ragweed $n = 21$	1:10 wt/vol	Amb a 1	424	763	144
Mixed ragweed $n = 10$	1:10 wt/vol	Amb a 1	174	402	56
D. pteronyssinus $n = 38$	10,000 AU/mL	Der p 1	76	241	19
D. farinae $n = 59$	10,000 AU/mL	Der f 1	56	144	14
Cat hair $n = 45$	10,000 BAU/mL	Fel d 1	43	79	24
A-P dog	1:100 wt/vol	Can f 1	140		

[a]Values provided by ALK-Abello (updated July, 2006).
Abbreviation: A-P, acetone precipitated.
Source: U.S. FDA reference extracts and extracts from ALK-Abello and other U.S. pharmaceutical firms.

high incidence of systemic reactions (approximately 15% of injections). The dose-response study by Haugaard et al. (17) demonstrated that there was marginal reduction in bronchial reactivity to mite allergen after two years of treatment with a maximum dose of 0.7-μg Der p 1, but the reduction with a dose of 7 μg was significantly greater. A higher dose (21 μg) did not result in any additional benefit but caused more than twice as many systemic reactions per injection as the 7 μg (7.1% vs. 3.3%). Therefore, the investigators concluded that a maintenance dose of 7-μg Der p 1/injection appeared to be near optimal based on benefit/risk considerations. Olsen treated 23 adult patients with asthma for one year with a maintenance dose of 7.0-μg Der p 1 or 10.0-μg Der f 1 (18). Compared with patients who received placebo, those treated with mite vaccines had significantly decreased symptoms of asthma, and required less β-adrenergic agonists and inhaled corticosteroids.

Cat Dander

Four studies have demonstrated significant improvement employing a narrow range of doses. Treatment by Van Metre et al. (19) with a maximum Fel d 1 dose of 13.8 μg/injection reduced both bronchial and skin reactions to cat dander extract. Alvarez-Cuesta et al. (20), treating with a maximum dose of 11.3-μg/injection Fel d 1 for one year, also noted decreased skin,

conjunctival, and bronchial sensitivity, as well as a 90% reduction in symptom medication scores. Hedlin et al. (21), treating with a maximum dose of 17.3-μg/injection Fel d 1 for three years, observed not only a reduced bronchial sensitivity to cat dander but also a significantly reduced response to bronchial challenge with histamine. Patients of Varney et al. (22), treated to a maintenance dose of 15-μg/injection Fel d 1, had significantly reduced symptoms on exposure in a house contaminated with cat dander.

Ewbank et al. (23) compared the immunological response shortly after achieving maintenance doses containing 0.6-μg Fed d 1, 3.0-μg Fel d 1, or 15-μg Fel d 1 by a cluster buildup. Both higher doses of vaccine produced significant decreases in prick skin test sensitivity and increases in cat-specific IgG_4, but only the vaccine containing a dose of 15-μg/injection Fel d 1 produced a significant reduction in the percent of $CD4^+/IL4^+$ peripheral blood mononuclear cells. This study was duplicated in 28 additional cat-allergic patients, and outcomes were assessed both after reaching maintenance and again after one year of maintenance injections (24). Again there was a dose-related suppression of prick skin tests. Cat-specific IgG_4 was significantly increased with the two higher doses, but only the dose containing 15 μg/injection of Fel d 1 produced sustained reduction in symptoms on nasal challenge. The conclusion of these two studies was that a maintenance dose of cat vaccine containing 15 μg of Fel d 1 was optimal and superior to one containing 3 μg of Fel d 1, while the results with a maintenance dose containing only 0.6 μg/injection was similar to placebo.

Dog Dander Vaccine

Although dog dander extracts are not standardized in the United States, the major allergen of dog, Can f 1, can be measured in dog allergen extracts. Most of the commercial dog dander extracts contain very low levels of Can f 1; however, the acetone-precipitated (A-P) dog allergen extract 1:100 wt/vol manufactured by Hollister-Steir (Spokane, Washington, U.S.) contains an average of 140-μg/mL Can f 1. A study, similar in design to those conducted with cat extract by Ewbank and Nanda, compared the response to Hollister-Steir dog vaccine containing 0.6 μg/injection, 3.0 μg/injection, or 15 μg/injection of Can f 1 with placebo (25). There was a dose-related rise in dog-specific IgG_4, suppression of the titrated prick skin test, and reduction of in vitro secretion of TNF-α from allergen-stimulated lymphocytes. The conclusion was that the greatest and most consistent response was seen with a dose of dog extract containing 15 μg of Can f 1.

Grass Pollen Vaccine

Varney et al. (26) conducted a preseasonal, double-blind trial of immunotherapy with timothy pollen vaccine in subjects with seasonal, grass pollen–allergic rhinitis. A maximum dose of 18.6-μg/injection Phl p 5 reduced symptoms and medication use by more than 50% compared with placebo. This treatment also reduced conjunctival sensitivity and the delayed skin test response to timothy grass extract. Dolz et al. (27) treated allergic subjects for three years with a vaccine containing 15 μg/injection of the major allergen of each component of a grass mixture. He observed a progressive decrease in ocular, nasal, and pulmonary symptoms over the three years of the study. Walker et al. (28) treated subjects with both seasonal allergic rhinitis and asthma with a timothy vaccine containing, at maintenance, 20 μg of Phl p 5. Immunotherapy not only diminished rhinitis but also markedly reduced chest symptoms and blocked the seasonal increase in methacholine sensitivity.

In summary, grass pollen vaccines are effective for allergic rhinitis and conjunctivitis and may improve asthma. The lowest effective dose has not been determined but a maintenance dose of 15 to 18.6 μg is effective.

Ragweed Pollen Vaccine

The most extensive experience with vaccines containing known amounts of the major allergens is with ragweed. Studies at Johns Hopkins University have included both single and multiple maintenance doses. However, the comparative dose studies have been either progressively increasing doses in the same individuals or the different doses have been administered for different number of years. There have been no studies in which groups of subjects receive different maximum doses for the same duration of treatment. Nevertheless, the data show that

clinical and objective benefits occur within months and are maintained with maximum maintenance doses of 11 μg (29) to 24.8 μg (30) of the major ragweed allergen, Amb a 1. Similar benefits were observed in a group who received a maintenance dose of 6-μg/injection Amb a 1 for three to five years (31). However, the response to 0.6 μg/injection (30) or to 2 μg/injection (32) was inconsistent and less than the response to higher doses.

Hymenoptera Venom Vaccines

Immunotherapy of insect-sensitive patients with Hymenoptera venom is effective in reducing reactions to intentional sting challenge (33). The original studies employed maximum doses of 100 μg/injection of the venom proteins, an amount exceeding the 50 μg that is usually injected by the sting of the insect. Treatment with the 100-μg dose has proven effective in the majority of sensitized subjects, therefore there have been few studies of alternative dosing.

CONSIDERATIONS IN FORMULATING AN ALLERGEN VACCINE FOR TREATMENT

The considerations in formulating an allergen vaccine for immunotherapy are (*i*) inclusion of an adequate dose of each extract in a vaccine to achieve an optimal response, (*ii*) utilization of allergenic relationships and cross-allergenicity to achieve similar effective doses for non-cross-reacting and cross-reacting allergens, (*iii*) combination of the individual extracts in vaccines to assure compatibility and stability, and (*iv*) selection of type of diluent.

Adequate Doses of Each Allergen

The optimal maintenance dose of the major allergens that are proven effective in placebo-controlled studies are listed in Table 3, and the approximate content of these major allergens in the U.S. standardized extracts are given in Table 4. From this information, it is possible to estimate the amount of standardized extract in a vaccine to be given per injection to achieve optimal dose. The amount will differ not only with different sources of the same extract but also with different extracts, as is suggested by different BAU and AU/mL values (e.g., grasses 100,000 BAU/mL, house dust mites 10,000 AU/mL).

To formulate a 10-mL maintenance treatment vaccine containing optimal concentrations of each standardized extract administered in a 0.5-mL maintenance injection, the mean effective dose, in major allergen content, for that allergen is multiplied by 20. This calculation provides the total amount of major allergen required in the 10-mL vial. There is a range of values for each vaccine depending on the major allergen content of that particular lot. An example of a vaccine mix containing optimal amount of the standardized extracts is given in Table 5.

What about the majority of allergens for which there is no information on optimal doses and no standardized extracts? Here it is necessary to work with the best clinical information available. The study by Johnstone and Crump (13) showed an inhalant allergen with a mix containing 1:250 wt/vol of each allergen was better than one with 1:5000 wt/vol of each allergen. In a similar fashion, the study by Franklin and Lowell (15) indicated that treatment with 1:50 wt/vol of ragweed was superior to treatment with 1:1000 wt/vol of ragweed.

Table 5 Representative Prescription for an Optimal Maintenance Dose Vaccine using U.S. Standardized Extracts

Extract	Concentration	Optimal dose, etc	Amount etc. (mL)	Amount expressed in U.S. CEBR units
Timothy	100,000 BAU/mL	18.6-μg Phl p 5	0.5	5,000 BAU
Short ragweed	1:10 wt/vol	12-μg Amb a 1	0.6	N.A.
D. pteronyssinus	10,000 AU/mL	3.5-μg Der p 1	0.9	900 AU
D. farinae	10,000 AU/mL	5-μg Der f 1	1.8	1,800 AU
Cat dander	10,000 BAU/mL	15-μg Fel d 1	6.2	6,200 BAU

This prescription is based on the mean documented optimal effective doses (Table 3) and the examples of the amount of major allergens contained in U.S. standardized extracts (Table 4). Major allergen content will vary among manufacturers for extracts of the same labeled potency.

Table 6 Representative Values for Major Allergen Content of Nonstandardized Extracts

Allergen	Expressed concentration	Major allergen	Major allergen concentration (μg/mL)	Reference
White birch	1:10 wt/vol	Bet v 1	390	ALK-Abello
English plantain	1:10 wt/vol	Pla l 1	30	ALK-Abello
European olive	1:20 wt/vol 50% Glycerin	Ole e 1	90	ALK-Abello
European olive	1:10 wt/vol Aqueous	Ole e 1	430	ALK-Abello
Sagebrush	1:10 wt/vol	Art v 1	1300	ALK-Abello
Dog	1:10 wt/vol 50% Glycerin	Can f 1	5–10	ALK-Abello
Alternaria	1:20 wt/vol 50% Glycerin	Alt a 1	1–5	ALK-Abello
Alternaria alternaria	1:10 and 1:20 wt/vol 50% Glycerin $n = 15$	Alt a 1	<0.01 to 6.1	34
Alternaria alternata	1:5 to 1:20 wt/vol $n = 7$	Alt a 1	0.04 to 2.04	35
Aspergillus fumigatus	1:10 and 1:20 wt/vol 50% Glycerin $n = 15$	Asp f 1	<0.01 to 64.0	34

Table 7 Representative Prescription for a Maintenance Vaccine Using Standardized and Nonstandardized Extracts

Extract	Concentration	Amount (mL)
Oak, White	1/10 wt/vol	1
Elm, American	1/10 wt/vol	1
Kochia	1/10 wt/vol	0.5[a]
Russian thistle	1/10 wt/vol	0.5[a]
Ragweed, short	1/10 wt/vol	0.5[a]
Ragweed, giant	1/10 wt/vol	0.5[a]
Timothy	100,000 BAU/mL	0.25[a]
June grass	100,000 BAU/mL	0.25[a]
Orchard grass	100,000 BAU/mL	0.25[a]
Meadow fescue	100,000 BAU/mL	0.25[a]
Diluent (to make 10 mL)	Saline with 0.03% HSA	5.0

The final concentration for each nonstandardized allergen group is approximately 1:100 wt/vol.

[a]Extracts included in reduced amounts to compensate for significant cross-allergenicity (37).The rationale for a target of 1:100 wt/vol or a 10-fold dilution from the strongest available stock extract is by analogy with clinical studies on standardized ragweed (29–32) vaccines and published studies with nonstandardized pollen vaccines (13,15).

Abbreviation: HSA, human serum albumin.

Limited data on major allergen content of nonstandardized pollen extracts suggests a range of major allergen content similar to that of standardized pollens (Table 6). This information would suggest that, at maintenance, a 1 to 10 dilution of the maximum concentration commercially available should be effective. Extracts that are less potent cannot be diluted to the same degree. Examples include cat dander and house dust mite extracts, for which substantially larger amounts of their concentrate must be added to the maintenance vaccine to provide adequate potency compared with ragweed or timothy. In the case of the very weak, nonstandardized extracts, such as dog dander (other than A-P dog) and various fungi (Table 6) and cockroach, it would be very difficult to deliver a major allergen dose in the range that has been shown to be clinically effective for other allergen extracts. The potencies of the 50% glycerin concentrate of a number of commercial extracts of German cockroach were found to be 10 to 782 BAU/mL and of American cockroach to be 10 to 250 BAU/mL (36). It is unlikely that effective doses can be attained in vaccines with many of these weak extracts. An example of a representative prescription for a maintenance vaccine containing nonstandardized vaccines is given in Table 7.

Botanical Relationships and Cross-Allergenicity

In formulating the example of a maintenance vaccine (Table 7), the calculated amount of each dust mite is reduced by half. This reflects the high degree of cross-allergenicity between these

Table 8 Patterns of Botanical Cross-Allergenicity

There is rarely significant cross-allergenicity between families.
There is generally a degree of cross-allergenicity between tribes or genera of a family, but this is variable.
There is generally a high degree of cross-allergenicity between species of the same genus.

Table 9 Patterns of Significant Cross-allergenicity Among the Tree Pollen

Birch family (39)
Birch
Alder
Hazelnut
Hornbeam
Olive family (40)
European olive
Ash
Privet
Russian olive (unrelated)
Cupressaceae (cypress) family (38)
Cedar
Cypress
Juniper
Arbor vitae
Fagaceae family (38)
Beech
Oak
Genus *Carya* (38)
Pecan
Hickory
Genus *Populus* (38)
Poplar
Aspen
Cottonwood

two species of *Dermatophagoides.* Cross-allergenicity among closely related plant pollen is also the rule (Table 8). If these relationships are not recognized, allergen vaccine mixtures may contain excessive amounts of some groups of allergens. This is likely to occur with the grasses, since most of the prevalent species in the United States fall into two non-cross-reacting botanical subfamilies (37), the first is northern pasture grasses typified by timothy and the second is Bermuda and related grasses. Other important cross-reacting groups are the individual members of the Ambrosia subtribe, the *Artemesia* genus, the Chenopod-Amaranth families of weeds, and members of certain tree groups, such as the genus *Populus* containing aspen, poplar, and cottonwood species and the junipers and cedars of the family *Cupressaceae* (38).

Patterns of Cross-Allergenicity

Trees. Among the trees there are few cases of cross-allergenicity sufficiently strong to restrict inclusion to only one representative in an allergen tree vaccine mix. These are listed in Table 9.

Grasses. Two non-cross-reacting subfamilies of grasses have been recognized. They are represented by the northern pasture grasses and Bermuda with its cross-reacting native prairie grasses (37). Each should be treated separately, using either timothy or a mixture for the northern pasture grasses and Bermuda for the other. There are also some regional grasses such as Bahia and Johnson that are in distinct subfamilies. Although they share allergens with the northern pasture grasses (Table 10), if locally important, they should be added as an additional component of the grass vaccine.

Immunotherapy with timothy and Bermuda grasses resulted in equivalent suppression of prick skin test reactivity to 10 different grasses from all three of the subfamilies listed in Table 10 (41). If more than one member of each of these subfamilies of grasses is to be included in a vaccine, the amount of each grass should be reduced to compensate for the marked cross-allergenicity.

Table 10 Botanical and Allergenic Relationships Among the Grasses

Festucoideae: Northern pasture grasses—orchard, timothy, June, red top, etc.
Eragrostoideae: Bermuda grass, grama, several western prairie grasses
Panicoideae: Bahia, Johnson

Source: From Ref. 39.

Table 11 Botanical and Allergenic Relationships Among the Weeds

Ambrosia
Ragweeds
Cocklebur
Burweed
Artemesia
Sages
Wormwood
Mugworts
Chenopods
Russian thistle
Kochia (burning bush)
Lambs quarters
Atriplex
Amaranths
Pigweed
Palmer's amaranth
Western water hemp

Source: From Ref. 38.

Weeds. There are three major groups of weeds (Table 11). Two are in the *Compositae* family, the Ambrosia, which includes the ragweeds and related species, and the Artemesia, which includes the sages, wormwoods, and mugworts. The Chenopod-Amaranth families include many of the prominent weeds of the Western United States. The major ragweeds (short, giant, western, and false) are strongly cross-reactive, whereas southern and slender ragweeds are allergenically distinct (42). The locally most important of the four major ragweeds or a mixture should be used for treatment (43). There is no clinically important cross-allergenicity of the ragweeds with other members of the Ambrosia tribe, such as cocklebur and burweed, nor is there significant cross-reactivity between ragweeds and the other clinically significant group in the *Compositae* family, the *Artemesia* (43). Within the *Artemesia,* however, there is strong cross-reactivity and one representative species should suffice for treatment (43). The Chenopod-Amaranth families, which share some allergenicity, are best viewed as three groups: the *Atriplex* and the Amaranths, both of which are strongly cross-reactive, and the Chenopods, which share some allergens. If several species of Chenopods are locally important (e.g., Russian thistle and Kochia), it is better to include them as a mix rather than using one representative species to cover the whole family. Locally important weeds such as sorrel, dock, and plantain should be treated as distinct allergens (38).

House dust mites. The house dust mites, *D. pteronyssinus* and *D. farina,* are strongly cross-reactive (44). A mix of both major species is probably best employed if both are locally important.

Components of Allergen Vaccines That May Have a Deleterious Effect on Other Extracts with Which They Are Mixed in a Vaccine

Some extracts of pollen (45,46) contain enzymes that may cause autodigestion and contribute to loss of potency of a vaccine. Extracts of a number of fungi (molds) and insects contain proteases that are capable of degrading allergenic proteins in other extracts with which they may be mixed in a vaccine (47–50) (Table 12). Major allergens in American cockroach and house dust mites are gut derived and very likely are digestive enzymes (47,51). However, protease activity is lower in American house dust mite extracts than in European, since only mite bodies are extracted and not spent culture medium containing fecal particles (Robert Esch, Greer laboratories, personal communication). Detectable trypsin-like proteolytic activity is absent from extracts derived from animal dander and pollen (47) (Table 12).

Table 12 Protease Content of Allergen Extracts

Extract	Protease content[a]	Potency of ryegrass extract
Pollen		
Sagebrush	<1	1.18
Ragweed	<1	0.70
Oak	<1	0.89
Epithelia		
Cat epithelia	<1	0.95
Dog epithelia	<1	0.90
Insects/mites		
D. pteronyssinus	14[b]	0.86
D. farinae	24[b]	0.44
P. americana	168	0.17
Fungi		
Alternaria alternata	29	0.22
Penicillium notatum	242	0.19

The extract listed in the first column was mixed with perennial ryegrass extract and stored at 4°C for one month. Potency of the ryegrass extract was compared with a reference preparation of 1.0. Potency of ryegrass was determined by IgE ELISA inhibition.
[a]μg Trypsin equivalent units/mL.
[b]U.S. house dust mite extracts are derived from washed mite bodies without spent culture medium and contain less protease activity than is typical for European extracts.
Source: From Ref. 47.

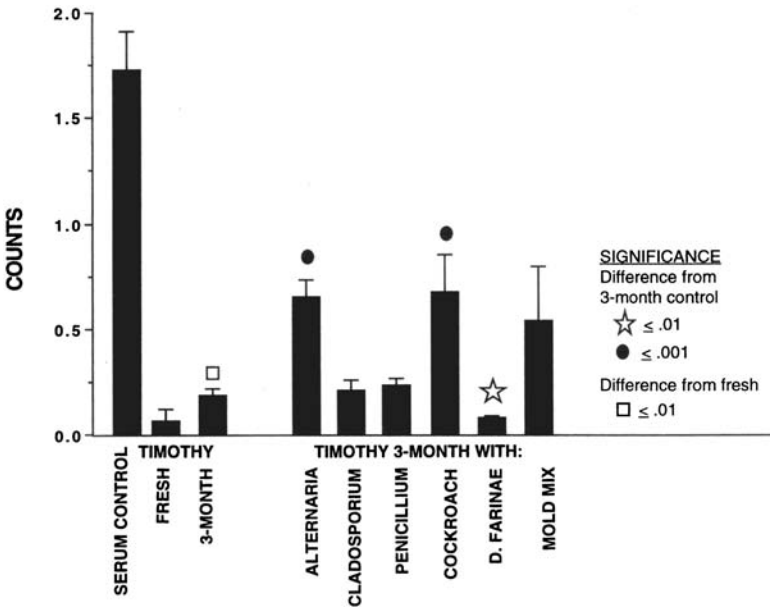

Figure 1 Stability of timothy grass alone and in mixtures. The potency of a 10-fold dilution of timothy grass stored under different conditions was compared by ELISA inhibition to that of a freshly diluted aliquot. After three months the diluted timothy extract had a significant decrease in potency compared with the fresh. In addition, those aliquots of timothy stored in combination with *Alternaria,* cockroach, and the mixture of *Alternaria, Cladosporium, Penicillium,* and cockroach showed significantly greater loss of potency than the timothy extract stored alone.

Grass pollen extracts are uniquely susceptible to these proteolytic enzymes (Fig. 1) (47,49,50,52). Birch pollen lost 70% of its allergenic potency over a period of 60 days when mixed with *Fusarium* (50). In a systematic assessment (Table 13), a number of pollen and animal dander extracts lost potency when mixed with one or more protease-containing extracts, while others were quite resistant (Fig. 2) (52). As illustrated in Table 13, the effect of mixing allergen extracts with potential protease-containing extracts is variable. *Alternaria*

Table 13 Effects of Mixing Extracts on Allergen Vaccines

Extract	Alt	Clad	PCN	CR	Mix	Mite	Overall *p*
Timothy	+	−	−	+	+	−	<0.0001
Bermuda	+	−	−	−	−	−	<0.0001
Short ragweed	−	−	−	−	−	−	0.64
Russian thistle							
1st	−	−	−	+	+	−	<0.0001
2nd	−	−	−	−	+	−	<0.01
White oak							
1st	+	−	−	−	−	−	<0.01
2nd	−	−	−	−	+	−	<0.02
Box elder							
1st	+	−	−	+	+	−	<0.0001
2nd	−	−	−	−	−	−	0.02
D. farinae							
1st	−	−	−	−	−	−	<0.01
2nd	−	ND	−	ND	ND	ND	0.49
Cat							
1st	−	+	−	−	−	−	<0.002
2nd	+	−	ND	ND	ND	ND	<0.001

The reference extracts listed on the ordinate were stored at a 10-fold dilution of the most concentrated available for three months either diluted in HSA-saline or combined with the extracts listed across the top of the Table. After three months, the residual allergenic activity of the reference allergen extract in the mixes was compared with that of the same extract stored alone. The *p* value is the overall difference among the seven conditions of storage (alone and six different combinations with other allergenic extracts). A (+) indicates significant degradation of the reference allergen extract due to mixing.

Abbreviations: Alt, *Alternaria*, Clad, *Cladosporium*, PCN, *Penicillium*, CR, cockroach, Mix, mixture of Alt, Clad, PCN, and CR. Mite, house dust mite; 1st, first of two studies with the same combinations; 2nd, second study; +, $p < 0.05$; –, $p > 0.05$; ND, not done.

Source: From Ref. 52.

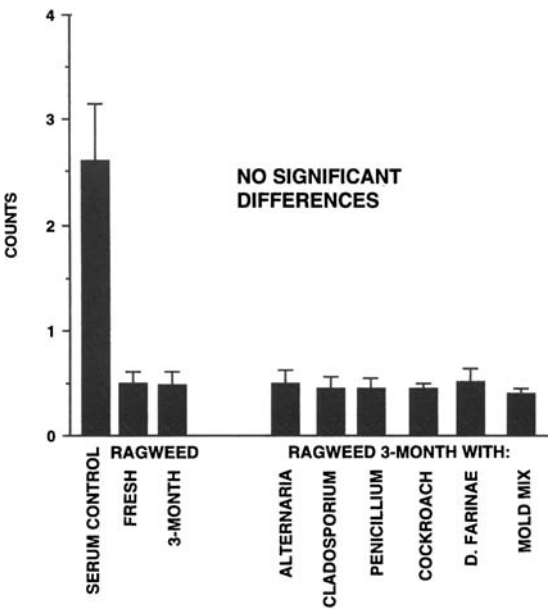

Figure 2 Stability of short ragweed alone and in mixtures. The potency of a 10-fold dilution of short ragweed stored under different conditions was compared by ELISA inhibition to that of a freshly diluted aliquot. After three months the diluted short ragweed extract and those aliquots of ragweed stored in combination with *Alternaria*, *Penicillium*, *Cladosporium*, and cockroach alone and in combination were all equal in potency to the freshly diluted aliquot of short ragweed.

significantly reduced the potency of five of eight extracts, cockroach reduced the potency of three of eight, while *Cladosporium* reduced the potency of only one extract. *Cladosporium* and cockroach reduced the potency of some extracts that were not affected by *Alternaria*. Furthermore, the effect of *Alternaria* extracts was inconsistent from lot to lot, suggesting varying quantities of protease activity were present in different lots of *Alternaria* extract.

The extracts which have deleterious effects on the potency of other extracts include *Alternaria* (52,53), *Cladosporium* (52,53), cockroach (49,52,53), *Helminthosporium* (49), *Penicillium* (47,53), *Aspergillus* (47,53), *Fusarium* (50), *Bipolaris* (53), and *Epicoccum* (53). House dust mite extracts have had no effect on other extracts (52,53) despite their content of proteases (47). This possibly results from their having low protease activity and having been tested in a diluent containing glycerin (52,53). While no single inhibitor will protect against all proteases (48), glycerin has been shown to have protective effects against some (47,53).

The degree of loss of potency due to mixing extracts in a vaccine may be marked. Allergenic activity of perennial ryegrass was reduced to 4% by mixing with *Helminthosporium* and to 11% by mixing with cockroach (49). Over 50% of timothy grass extract potency was lost within three days of being mixed with *Fusarium* (50). Some allergenic activity always remains suggesting that not all allergens in these extracts are susceptible to proteolytic digestion. On mixing with *Fusarium*, Bet v 6 and Phl p 5 were almost entirely degraded, while Bet v 1 and Phl p 1 remained relatively stable (50). However, even though there may be a significant amount of overall allergenic activity remaining, the selective reduction in certain allergens will make the vaccine less suitable for treatment and may place the patient at risk when they are treated with a freshly prepared vaccine that contains allergens no longer present in the mix that they had been receiving for immunotherapy. The results of testing of the effects of mixing extracts are not always consistent. Ragweed has been reported to be resistant (52) and susceptible (53), cat dander extract has been reported to be susceptible (52) and resistant (53), while mixing with *Fusarium* has been reported to cause loss of potency (50) and to have no effect (53). These differing results may reflect the marked variability in the composition of fungal and cockroach extracts and differing methods of assessing loss of potency.

In summary, many pollen and animal dander extracts are susceptible to accelerated loss of potency when mixed in a vaccine with protease-containing extracts. Despite the variable reports, the safest practice is to not include pollen and dander extracts in a vaccine with cockroach or fungal extracts. House dust mite extracts in even 10% glycerin appear to be neither susceptible to exogenous proteases nor to cause loss of potency due to their protease content (52,53). Degradation of fungal extracts by proteases in other fungal extracts when contained in a vaccine has not been demonstrated but has not been extensively investigated. Mixing cockroach, *Alternaria*, *Cladosporium*, and *Penicillium* extracts did not further reduce their potency in a vaccine (52). It is probable that the proteins susceptible to protease digestion had already been degraded by proteases in their own extract.

Diluents Employed in Mixing Allergen Vaccines

Because allergen extracts tend to lose potency with time, an effect that is enhanced by storing at higher temperatures and greater dilutions, a number of substances have been added to extracts both to preserve potency and prevent growth of microorganisms. The most effective preservation of extract integrity and potency is not by adding a preservative but by lyophilization (54,55). This is not routinely employed because the lyophilization process adds to the cost of the extracts.

Glycerin

Glycerin is the most effective preservative for allergen extracts (56). It is very effective at a 50% concentration. At this concentration it inhibits some, but not all, proteolytic enzymes (47,50,55). This may contribute to but does not completely explain its effectiveness as a preservative. Decreasing effectiveness as a preservative has been demonstrated with 25% and 10% concentrations of glycerin (53,56,57); however, even 10% glycerin is as effective as 0.03% human serum albumin. It is possible that the presence of glycerin accounts for the lack of proteolytic degradation of pollen extracts by the house dust mite extracts in several mixing studies (49,52,53).

Human Serum Albumin

The preservative effect of human serum albumin is likely due to reduction of adsorption of allergenic proteins to the vial surface (58) and protection of allergenic proteins from phenol denaturation (59). Human serum albumin has not been shown to have protective effects

against proteolytic enzymes (47). Similar degrees of preservative effect were found with concentrations of 0.03%, 0.1%, and 1.0% human serum albumin (56).

Concern has been expressed that patients may become sensitized by repeated injections of human serum albumin possibly altered or aggregated in commercial processing (60). However, no cases of sensitization to human serum albumin in allergen vaccines has been reported, and one study which looked for evidence of positive skin tests or IgG antibodies directed toward human serum albumin was negative (61).

Phenol

Phenol is added to multidose vials of allergen extracts to prevent growth of microorganisms. Phenol denatures proteins, including those in allergen extracts (54) and the deleterious effect of phenol increases with increasing allergen dilutions (56). Phenol degrades extracts in vaccines that are in 50% glycerin solutions (59,62). Human serum albumin is relatively more protective than glycerin against the effect of phenol on extract potency (59,62).

Others

A number of other approaches to preserve extract potency have been suggested but have not found wide acceptance. Siliconization of vials has been suggested to decrease adsorption of proteins to their surface. Testing this method revealed it to be without effect (56). Polysorbate 80 in concentrations of 0.002% to 0.2% had a slight effect in preserving potency, but it was less effective than human serum albumin (56).

Epsilon-aminocaproic acid (EACA) has been suggested as a preservative (60) since pollens are known to contain enzymatic activity that may contribute to their loss of potency and EACA is a potent enzyme inhibitor. However, EACA was found to be ineffective against a variety of fungal proteases (47). In studies on preservation of extract potency, EACA was found to be less effective than glycerin (60). EACA was found to have less protective effect than human serum albumin on potency over the short term. This observation may be due to human serum albumin reducing adsorption of allergenic proteins to the walls of the vial whereas EACA provides protection from prolonged thermal denaturation (60).

In the absence of preservatives, extracts stored in saline buffered with bicarbonate lost potency to a greater extent than those stored in phosphate buffered saline or normal saline (55,57).

Mixing Extracts to Constitute a Vaccine

Extracts which are stored combined with several other extracts retain their potency to a greater extent than the same extract, at the same dilution, stored alone (57). This preservative effect is probably related to total protein content. In this instance, the proteins in the other extracts are functioning in a manner analogous to human serum albumin when combined in a vaccine.

CONDITIONS OF STORAGE

Maintenance of potency of a therapeutic allergen vaccine is a function of the dilution, the diluent, the effectiveness of preservatives, the temperature of storage, and the presence of proteolytic enzymes. The processes that lead to loss of vaccine potency and the measures that can be used to reduce the effect are given in Table 14.

Temperature

Allergen extracts and vaccines are susceptible to loss of potency if maintained at room rather than refrigerator temperature (57,62). Loss of activity with storage at room temperatures is likely

Table 14 Mechanisms of Loss of Potency of Allergenic Extracts

Mechanism	Favored by	Avoidance by
Adsorption	High dilution, high surface to volume ratio	Human serum albumin, glycerin
Thermal denaturation	High temperature	Storage at low temperature
Enzymatic autodigestion	Enzymes in extract	Glycerin, storage at low temperature

caused by the proteases (54) while loss of potency with brief exposure to higher temperatures is probably related to heat lability of some of the allergenic proteins (63). Some extracts, such as cat (62), have been reported to be relatively resistant to this thermal lability effect. Other extracts, including white ash, elm, orchard grass, Bermuda grass (64), ragweed (63), and house dust mites (62), lose potency at high temperature (25°C–100°C). Since the loss of potency is a result of either protease susceptible or heat labile proteins, the stored extract will have an altered pattern of specificity due to the preferential persistence of the resistant proteins, resulting in an altered pattern of skin test reactivity and potential therapeutic efficacy (63).

Less extreme temperature exposure of allergen extracts and vaccines, such as exposure to room temperature for 13 hr/wk, resulted in significant loss of potency (57). The effect on allergen potency of repeated freezing and thawing has not been extensively studied but reports of reduced potency of ragweed (45) and dilute *Lolium perenne* (60) extracts are available.

Dilution

Extracts and vaccines are more susceptible to loss of potency when stored diluted rather than concentrated (52,56). The increased susceptibility of diluted extract is likely the result of lesser protein content and hence relatively greater adsorption of allergens to the container wall (54,57,58). However, addition of human serum albumin does not completely prevent this loss and all allergen extracts are not equally susceptible to this effect (52), suggesting other factors may be involved.

Occasional studies of diluted extracts report unexpectedly preserved potency for prolonged periods. Thus, intradermal skin test concentrations of timothy, birch, cat, and house dust mite preserved with human serum albumin showed preserved potency after 24 months' storage at 6°C (62). The explanation for these apparently aberrant results is unclear but may relate to the pattern of sensitivity of the population studied.

Loss of potency is related to the total protein content of the extract or vaccine. Not entirely filling a vial of extract has been reported to enhance loss of potency due to the greater surface area relative to the volume of solution from which protein is available for adsorption. This effect is diminished by including other extracts in a vaccine, thus increasing the total protein content (57). The same protective effect can be achieved by added extraneous protein, such as human serum albumin (57,58).

PATTERNS OF LOSS OF POTENCY

Assessment

A variety of methods have been employed to assess the residual potency of allergenic extracts (56). The two approaches most commonly employed, the RAST or ELISA inhibition (57) and skin testing (65), have been reported to yield similar results. In occasional studies, residual activity has been greater by skin testing than by RAST inhibition (54,55). However, a careful comparison of titrated intradermal skin testing and ELISA-inhibition yielded very similar results with multiple allergen extracts (49), suggesting that, properly done, the results with the two methods are comparable.

Individual Allergens

The stability of allergen extracts and vaccines to degradation can vary due to differing heat susceptibility of their components, different total protein content affecting the percent adsorbed to the container wall, and the content of proteolytic enzymes that may cause autodigestion (Figs. 3, 4). Stability varies with the addition of phenol as well as the presence of proteases in extracts used for the vaccine. As would be expected, studies have shown differing loss of potency for different extracts stored under similar conditions. Therefore, it is best to follow general principles that will protect the potency of the most susceptible extracts. These include (*i*) avoiding mixing fungal and insect extracts with pollen and dander extracts in formulating a vaccine (an exception appears to be house dust mite extracts, (*ii*) keeping the total protein content high by using concentrated extracts and adding human serum albumin to dilutions, and (*iii*) keeping the extracts and vaccines at refrigerator temperatures except when actually being used (for dilute vaccines left at room temperature consider using a refrigerated tray when exposed to room temperatures).

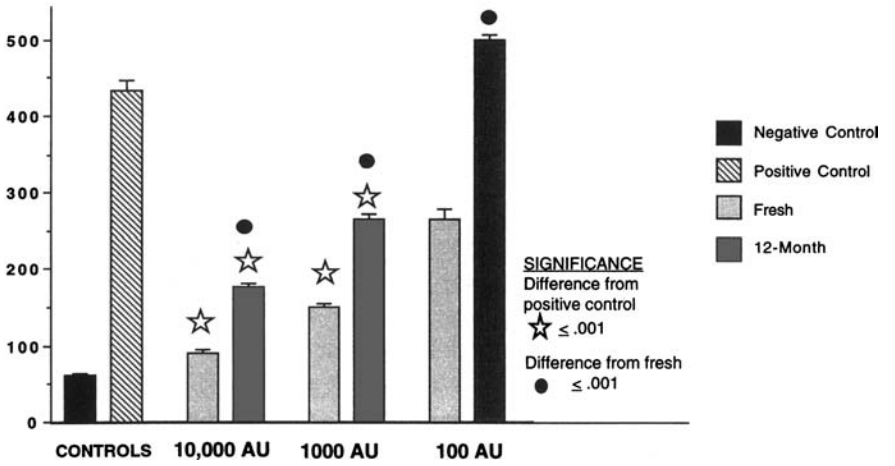

Figure 3 The potency of Bermuda grass extract stored at 4°C in concentrations of 100 AU/mL, 1000 AU/mL, and 10,000 AU/mL was compared after 12 months by ELISA inhibition to freshly diluted aliquots of the same Bermuda extract. There was, as indicated, significant loss of potency after 12 months in all dilutions. Negative control contained the Bermuda disc but no serum. Positive control contained Bermuda disc and mixed grass-allergic patients serum, but no Bermuda extract, while the tested aliquoted contained Bermuda disc, Bermuda-allergic serum and dilutions of Bermuda extract.

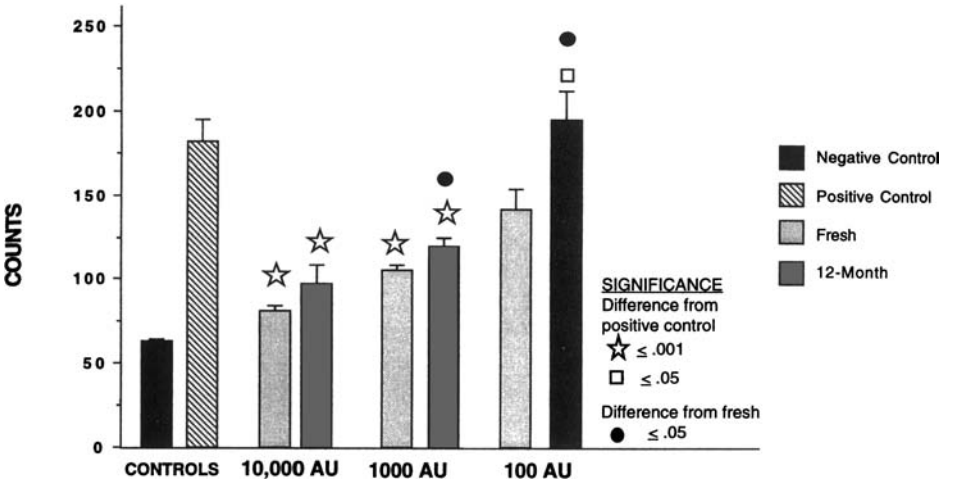

Figure 4 The potency of short ragweed extract stored at 4°C in concentrations of 100 AU/mL, 1000 AU/mL, and 10,000 AU/mL was compared after 12 months by ELISA inhibition to freshly diluted aliquots of the same short ragweed extract. There was no loss of potency after 12 months in the 10,000 AU/mL aliquot, but there was a significant loss of potency in the other two dilutions. Negative control contained the short ragweed disc but no serum. Positive control contained short ragweed disc and mixed ragweed-allergic patients serum, but no short ragweed extract, while the tested aliquots contained short ragweed disc, ragweed-allergic serum, and dilutions of short ragweed extract.

With attention to these details, some diluted vaccines will lose potency even after three months at concentrations used for maintenance immunotherapy (Figs. 1, 3), whereas others will not lose any potency at the same dilution over a year (Figs. 2, 4). Full strength extracts and vaccines are probably stable at refrigerator temperatures for their stated shelf-life. Full-strength extracts in 50% glycerin, as used for prick skin testing, are consistently stable until their expiration date (56). Diluted extracts, used for intradermal skin testing, have been found to be stable for prolonged periods by some investigators (62), but not by others (56). Some allergen extracts are susceptible to rapid loss of potency at high temperatures (25°C–100°C) (62–64), but the loss with exposure to room temperature is not rapid (57). It is unlikely that shipping

allergen extracts and vaccines through the mail would result in exposure to temperatures that would be deleterious.

SALIENT POINTS

- Standardized extracts of cat pelt and hair, house dust mites, short ragweed, eight grasses, and the venoms of four Hymenoptera are commercially available in the United States.
- Neither of the two expressions for potency used for nonstandardized extracts, weight by volume or protein nitrogen units, adequately reflects allergenic potency of a vaccine.
- There are two immunotherapy studies that relate dose to outcome employing nonstandardized vaccines. In one study employing multiple allergens, the outcome with 1:250 wt/vol vaccines was better than that with 1:5000 wt/vol. In another study, results with 1:50 wt/vol ragweed vaccine were significantly better than with 1:1000 wt/vol.
- Examples of representative values for the major allergen content of U.S. standardized vaccines are listed in Table 4. However, the major allergen contents in Table 4 are only representative and may vary significantly from one manufacturer to another.
- Placebo-controlled studies that demonstrated clinical effectiveness have been performed with the extracts that are standardized in the United States (house dust mites, cat dander, grass, and ragweed). In each instance, the effective dose of major allergen has been in the range of 7 to 20 μg (Table 3).
- The information in Tables 3 and 4 allows formulation of an allergen vaccine mixture containing concentrations of the major allergens that approximate those that have been proven to be clinically effective (Table 5).
- Vaccines should contain effective quantities of each aeroallergen. If two or more components of the allergen mixture cross-react, the amount of each should be decreased so that the sum of the cross-reacting aeroallergens is equal to the optimal effective dose for a single allergen of the mixture.
- Most fungal and cockroach extracts contain proteases that are capable of degrading allergenic proteins contained in other extracts when constituted together in a vaccine. Therefore, mixing fungal and cockroach extracts with pollen or dander extracts in a vaccine is to be avoided (Table 12).
- Degradation of allergen extracts and vaccines is increased by dilution and by the time they are maintained at room temperature.
- Glycerin is the most effective preservative but is poorly tolerated by injection at 50% solution. Ten percent and 20% glycerin solutions cause some brief discomfort, but are usually tolerated. Human serum albumin is less effective but well tolerated. Glycerin or human serum albumin should be included in all dilute vaccines.

REFERENCES

1. De Weck AL. Allergen Standardization at a Crossroads? ACI International 1997; 9:25–30.
2. Turkeltaub PC. In vivo standardization. In: Middleton E Jr., Reed CE, Ellis EF, et al. Allergy: Principles and Practice. 3rd ed. St. Louis: CV Mosby, 1988:388–401.
3. Allergy Update. Greer Laboratories. Vol 1, No 1. NC: Le Noir, May 1992.
4. Technical Bulletin. Vol. 1, No. 1. Spokane, WA: Hollister-Steir.
5. American Preventive Medical Association. Letter to the President, American Academy of Allergy and Immunology. May 26, 1994.
6. Van Metre TE Jr., Rosenberg GL, Vaswani SK, et al. Pain and dermal reaction caused by injected glycerin in immunotherapy solutions. J Allergy Clin Immunol 1996; 97:1033–1039.
7. Norman PS, Winkenwerder WL, Lichtenstein LM. Trials of alum-precipitated pollen extracts in the treatment of hay fever. J Allergy Clin Immunol 1972; 50:31–44.
8. Nelson HS. Long-term immunotherapy with aqueous and aluminum-precipitated grass extracts. Ann Allergy 1980; 45:333–337.
9. Lichtenstein LM, Norman PS, Winkenwerder WL. Antibody response following Immunotherapy in ragweed hay fever: Allpyral vs whole ragweed extract. J Allergy 1968; 41:49–57.

10. Guerin B, Hewitt B. The effect of commonly used extracting media on the allergenic composition of cat fur extract. Ann Allergy 1981; 47:166–170.
11. Sherman WB. Hypersensitivity: Mechanisms and Management. Philadelphia: WB Saunders, 1968:422.
12. Stull A, Cooke RA, Tennant J. The allergic content of pollen extracts: its determination and its deterioration. J Allergy 1933; 4:455–467.
13. Johnstone DE, Crump L. Value of hyposensitization therapy for perennial bronchial asthma in children. Pediatrics 1961; 27:39–44.
14. Lowell FC, Franklin W. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. N Engl J Med 1965; 273:675–679.
15. Franklin W, Lowell FC. Comparison of two dosages of ragweed extract in the treatment of pollenosis. JAMA 1967; 201:915–917.
16. Ewan PW, Alexander MM, Snape C, et al. Effective hyposensitization in allergic rhinitis using a potent partially purified extract of house dust mite. Clin Allergy 1988; 18:501–508.
17. Haugaard L, Dahl R, Jacobsen L. A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. J Allergy Clin Immunol 1993; 91:709–722.
18. Olsen OT, Larsen KR, Jacobsen L, et al. A 1-year, placebo-controlled double-blind house dust mite immunotherapy study in asthmatic adults. Allergy 1997; 52:853–859.
19. Van Metre TE Jr., Marsh DG, Adkinson NF Jr., et al. Immunotherapy for cat asthma. J Allergy Clin Immunol 1988; 82:1055–1068.
20. Alvarez-Cuesta E, Cuesta-Herranz J, Puyana-Ruiz J, et al. Monoclonal antibody-standardized cat extract immunotherapy: risk benefit effects from a double-blind placebo study. J Allergy Clin Immunol 1994; 93:556–566.
21. Hedlin G, Graff-Lonnevig V, Heilbron H, et al. Immunotherapy with cat- and dog-dander extracts. V. Effects of 3 years of treatment. J Allergy Clin Immunol 1991; 87:955–964.
22. Varney VA, Edward J, Tabbahk, et al. Clinical efficacy of specific immunotherapy to cat dander, a double-blind, placebo-controlled trial. Clin Exp Allergy 1997; 27:860–867.
23. Ewbank PA, Murray J, Sanders K, et al. A double-blind, placebo-controlled immunotherapy dose-response study with standardized cat extract. J Allergy Clin Immunol 2003; 111:155–161.
24. Nanda A, O'Connor M, Anand M, et al. Does dependence and time course of the immunologic response to administration of standardized cat allergen extract. J Allergy Clin Immunol 2004; 14:1339–1344.
25. Lent AM, Harbeck R, Strand M, et al. Immunologic response to administration of standardized dog allergen extract at differing doses. J Allergy Clin Immunol 2006:118:1249–1256.
26. Varney VA, Gaga M, Frew AJ, et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. Br Med J 1991; 302:530–531.
27. Dolz I, Martinez-Cocera C, Barlolome JM, et al. A double-blind, placebo-controlled study of immunotherapy with grass pollen extract Alutard SQ during a three-year period with initial rush immunotherapy. Allergy 1996; 51:489–500.
28. Walker SM, Pajno GB, Torres Lima M, et al. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. J Allergy Clin Immunol 2001:107:87–93.
29. Van Metre TE Jr., Adkinson NF, Amodio FJ, et al. A comparative study of the effectiveness of the Rinkel method and the current standard method of immunotherapy for ragweed pollen hay fever. J Allergy Clin Immunol 1979; 66:500–513.
30. Creticos PS, Marsh DG, Proud D, et al. Responses to ragweed-pollen nasal challenge before and after immunotherapy. J Allergy Clin Immunol 1989; 84:197–205.
31. Creticos PS, Adkinson NF Jr., Kagey-Sobotka A, et al. Nasal challenge with ragweed pollen in hay fever patients: effect of immunotherapy. J Clin Invest 1985; 76:2247–2253.
32. Furin MJ, Norman PS, Creticos PS, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. J Allergy Clin Immunol 1991; 88:27–32.
33. Hunt KJ, Valentine MD, Sobotka AK, et al. A controlled trial of immunotherapy in insect hypersensitivity. N Engl J Med 1978; 299:157–161.
34. Vailes L, Sridhara S, Cromwell O, et al. Quantitation of the major fungal allergens, Alt a 1 and Asp f1, in commercial allergenic products. J Allergy Clin Immunol 2001; 107:641–646.
35. Esch RE. Manufacturing and standardizing fungal allergen products. J Allergy Clin Immunol 2004; 113:210–215.
36. Patterson ML, Slater JE. Characterization and comparison of commercially available German and American cockroach allergen extracts. Clin Exp Allergy 2002; 32:721–727.
37. Martin BG, Mansfield LE, Nelson HS. Cross-allergenicity among the grasses. Ann Allergy 1985; 54:99–104.
38. Weber RW. Patterns of pollen cross-allergenicity. J Allergy Clin Immunol 2003; 112:229–239.
39. Valenta R, Breiteneder H, Pettenburger K, et al. Homology of the major birch-pollen allergen, Bet v I with the major pollen allergens of alder, hazel, and hornbeam at the nucleic acid level as determined by cross-hybridization. J Allergy Clin Immunol 1991; 87:677–682.

40. Kernerman SM, McCullough J, Green J, et al. Evidence of cross-reactivity between olive, ash, privet and Russian olive tree pollen allergens. Ann Allergy 1992; 69:493–496.
41. Leavengood DC, Renard RL, Martin B, et al. Cross allergenicity among grasses determined by tissue threshold changes. J Allergy Clin Immunol 1985; 76:789–794.
42. Leiferman KM, Gleich GJ, Jones RT. The cross-reactivity of IgE antibodies with pollen allergens II. Analyses of various species of ragweed and other fall weed pollens. J Allergy Clin Immunol 1976; 58:140–148.
43. Weber R. Cross-reactivity of pollen allergens: recommendations for immunotherapy vaccines. Curr Opin Allergy Clin Immunol 2005; 5:563–569.
44. Heymann PW, Chapman MD, Aalberse RC, et al. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp). J Allergy Clin Immunol 1989; 83:1055–1067.
45. Center JG, Shuller N, Zeleznick LD. Stability of antigen E in commercially prepared ragweed pollen extracts. J Allergy Clin Immunol 1974; 54:305–310.
46. Bousquet J, Marty JP, Coulomb Y, et al. Enzyme determination and RAST inhibition assays for orchard grass (*Dactylis glomerata*): a comparison of commercial pollen extracts. Ann Allergy 1978; 41:164–169.
47. Esch RE. Role of proteases on the stability of allergenic extracts. Arbeiten aus dem Paul-Ehrlich-Institut. Stuttgart: Gustav Fischer Verlag, 1991:171–179.
48. Wongtim S, Lehrer SB, Salvaggio JE, et al. Protease activity in cockroach and basidiomycete allergen extracts. Allergy Proc 1993; 14:263–268.
49. Kordash TR, Amend MJ, Williamson SL, et al. Effect of mixing allergenic extracts containing *Helminthosporium*, *D farinae*, and cockroach with perennial ryegrass. Ann Allergy 1993; 71:240–246.
50. Haff M, Krail M, Kastner M, et al. *Fusarium culmorum* causes strong degradation of pollen allergens in extract mixtures. J Allergy Clin Immunol 2002; 109:96–101.
51. Stewart GA, Ward LD, Simpson RJ, et al. The group III allergen from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. Immunology 1992; 75:29–35.
52. Nelson HS, Ikle D, Buchmeier A. Studies of allergen extract stability: the effects of dilution and mixing. J Allergy Clin Immunol 1996; 98:382–388.
53. Grier TJ, LeFevre DM, Duncan EA, et al. Stability of standardized grass, dust mite, cat and short ragweed allergens after mixing with mold or cockroach extracts. Ann Allergy Asthma Immunol 2007; 99(2):151–160.
54. Ayuso R, Rubio M, Herrera T, et al. Stability of *Lolium perenne* extract. Ann Allergy 1984; 53:426–431.
55. Anderson MC, Baer H. Antigenic and allergenic changes during storage of a pollen extract. J Allergy Clin Immunol 1982; 69:3–10.
56. Nelson HS. The effect of preservatives and dilution on the deterioration of Russian thistle (*Salsola pestifer*), a pollen extract. J Allergy Clin Immunol 1979; 63:417–425.
57. Nelson HS. Effect of preservatives and conditions of storage on the potency of allergy extracts. J Allergy Clin Immunol 1981; 67:64–69.
58. Norman PS, Marsh DG. Human serum albumin and Tween 80 as stabilizers of allergen solutions. J Allergy Clin Immunol 1978; 62:314–319.
59. Naerdal A, Vilsvik JS. Stabilization of a diluted aqueous mite allergen preparation by addition of human serum albumin. An intracutaneous test study. Clin Allergy 1983; 13:149–153.
60. Van Hoeyveld EM, Stevens EAM. Stabilizing effect of epsilon-aminocaproic acid on allergenic extracts. J Allergy Clin Immunol 1985; 76:543–550.
61. Brown JS, Ledoux R, Nelson HS. An investigation of possible immunologic reactions to human serum albumin used as a stabilizer in allergy extracts. J Allergy Clin Immunol 1985; 76:808–812.
62. Niemeijer NR, Kauffman HF, van Hove W, et al. Effect of dilution, temperature, and preservatives on the long-term stability of standardized inhalant allergen extracts. Ann Allergy Asthma Immunol 1996; 76:535–540.
63. Baer H, Anderson MC, Hale R, et al. The heat stability of short ragweed pollen extract and the importance of individual allergens in skin reactivity. J Allergy Clin Immunol 1980; 66:281–285.
64. Hale R, Grater WC, Haykik IB, et al. Report of the Committee on standardization of allergenic extracts: a Study of the heat stability of white oak, elm, orchard grass, and Bermuda grass. Ann allergy 1985; 55:86–87.
65. Bousquet J, Djoukadar F, Hewitt B, et al. Comparison of the stability of a mite and a pollen extract stored in normal conditions of use. Clin Allergy 1985; 15:29–35.

20 Subcutaneous Administration of Allergen Vaccines

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INTRODUCTION

Subcutaneous allergen immunotherapy (SCIT) was first described in the United States by Noon and Freeman in 1911 (1) and has been used subsequently for the treatment of allergic symptoms due to inhalant allergens. In 1918, Dr. Robert Cooke suggested a mechanism of action for allergen injections as a "desensitization or hyposensitization." Prausnitz and Kustner elucidated the more specific immunologic basis for allergic disease, demonstrating that allergic sensitivity could be transferred by the serum of a sensitive person to the skin of a nonallergic person (2). SCIT is defined as the repeated administration of specific allergens to patients with IgE-mediated conditions for the purpose of providing protection against the allergic symptoms and inflammatory reactions associated with natural exposure to these allergens (3). The technique of SCIT differs from the process of desensitization, the term applied to the rapid, progressive administration of an allergenic substance, usually a drug, to render effector cells less reactive.

In the United States, SCIT with inhalant allergens most commonly consists of once or twice weekly subcutaneous aqueous allergen vaccines injections of increasing dose. Once the highest dose (i.e., the maintenance dose) is achieved, the interval between injections is increased up to four weeks (4–6). The timing and route of administration are the same in many other countries, but modified vaccines rather than aqueous vaccines are sometimes used. Numerous variations of inhalant immunotherapy are practiced relative to route of administration, timing, frequency, duration, and dosage.

ADMINISTRATION OF INHALANT VACCINES

General Considerations

SCIT for inhalant allergy is effective for allergic asthma and allergic rhinoconjunctivitis (SCIT for Hymenoptera hypersensitivity is reviewed in chap. 24). SCIT has not been proven effective for urticaria or food allergies (7). Limited data support its use in atopic dermatitis (8). SCIT with vaccines to a variety of pollen species, including grasses, ragweed, *Parietaria* species, and mountain cedar, is effective for allergic rhinitis due to these airborne pollen-producing plants. Vaccines with house-dust mite are effective treatment for both allergic asthma and allergic rhinitis (9). Limited data support that immunotherapy provides immunologic changes (10) or clinical efficacy (11) in patients allergic to cats. Likewise, efficacy data are limited for molds such as *Alternaria* (12) and *Cladosporium* species (13). Aeroallergens vary with geographic location; lists of pollen by geographic location and month are available in several standards texts (14–16). Familiarity with the significant aeroallergens in a physician's geographic location is critical to appropriately choose allergens for cutaneous testing and SCIT. Due to the mobility of today's population, one must also have a working knowledge of different geographic regions to effectively treat some patients (16). For example, Bermuda grass is an important allergen in the southern United States but is not present in northern climates. For a patient

living in Madison, Wisconsin, this may hold little importance. However, should this person spend the summer months in Florida, Bermuda grass becomes a potential allergen. The clinical relevance of an aeroallergen depends on certain key properties: (*i*) its allergenicity, (*ii*) its aerodynamic properties, (*iii*) whether it is produced in large enough quantities to be sampled, (*iv*) whether it is sufficiently buoyant to be carried long distances, and (*v*) whether the plant releasing the pollen is widely and abundantly prevalent in the region (3).

As outlined in the Allergen Immunotherapy Practice Parameter endorsed jointly by the American Academy of Allergy, Asthma, and Immunology (AAAAI) and the American College of Allergy, Asthma, and Immunology (ACAAI), SCIT should only be administered where adverse reactions can be appropriately managed (3). This includes proper training for physicians and staff in terms of reaction recognition and treatment. The Practice Parameter recommends that patients remain in the medical facility for 30 minutes after injections. It goes on to suggest which medical equipment and pharmaceuticals should be on hand in any setting in which immunotherapy is administered. A suggested listing of available equipment and medications is: a stethoscope, a sphygmomanometer, tourniquets, syringes/needles, epinephrine at 1:1000 (wt/vol), oxygen equipment, IV fluid setup, IV antihistamines, IV glucocorticosteroids, IV dopamine (or other pressor agents), and an intubation kit or oral airway as appropriate for the treating physician's skill with these devices.

Aqueous Vaccines

In the United States, aqueous vaccines are most commonly used for SCIT. Aqueous extracts are prepared by extracting proteins from fresh source material at physiologic pH and ionic strength and at low temperatures to delay proteolytic degradation and microorganism contamination. Some are standardized by cutaneous endpoint titration and quantified by either allergy units (AUs) (as in the case of dust mites), bioequivalent allergen units (BAUs) (as for grass pollen), or major allergen content (such as for ragweed and cat) (17). However, many extracts are labeled with protein nitrogen units (PNU) or as weight/volume (wt/vol). One PNU is approximately 10 ng of total protein nitrogen, and weight/volume refers to the weight (in grams) of the source material that is extracted in a given volume (in milliliters). Neither of these is reliably associated with allergenic potency (18). In other countries, different standard units are used to express allergenic potency. In Europe, especially in the Scandinavian countries, the biologic unit (BU), which is based on comparative histamine skin testing, is used (19). Allergen standards developed by the International Union of Immunologic Societies (IUIS) are quantified in international units (IUs) (20) (see chap. 20).

The starting SCIT dose is usually 1000-fold to 10,000-fold less than the maintenance dose and can even be lower for highly sensitized patients. The maintenance dose with standardized allergy vaccines is approximately 600 AU for dust mite or 4000 bioequivalent allergen units (BAU) for grass. For non-standardized vaccines, a suggested maintenance dose is 3000 to 5000 PNU or 0.5 mL of a 1:100 (wt/vol) dilution of manufacturer's extract. For vaccines with a known major allergen concentration, a recommended maintenance dose contains between 5 and 20 μg of that major allergen (3).

Whenever possible, standardized extracts should be used to prepare vaccine treatment sets. Some commonly used allergens are standardized. As of 2007, these include cat hair, cat pelt, *Dermatophagoides pteronyssinus*, *D. farinae*, short ragweed, Bermuda grass, Kentucky bluegrass, perennial ryegrass, orchard grass, timothy grass, meadow fescue, red top, sweet vernal grass, and Hymenoptera venoms (yellow jacket, honeybee, wasp, yellow hornet, and white-faced hornet) (3). The following extracts are standardized but not commercially available: American elm, mountain cedar, paper birch, red alder, white oak, red oak, box elder, white ash, olive, cottonwood, mulberry, pecan, black walnut, sycamore, dog epithelium, German and American cockroaches, mugwort, Johnson grass, Bahia grass, and select molds (*Alternaria alternata, Cladosporium herbarum, C. cladosporioides, Penicillium chrysogenum, Aspergillus fumigatus, Epicoccum nigrum, Helminthosporium solani*).

Allergen extracts and vaccines should be maintained at recommended conditions until used for diagnostic tests or immunotherapy. Because extracts/vaccines lose their potency when stored at room temperature and with freezing and thawing, specific steps must be taken to avoid these conditions. Allergen extracts/vaccines should be kept at about 4°C. Dilutions of concentrated extracts/vaccines lose their potency more rapidly than the concentrates. Extracts

that have questionable potency, such as those exposed to freezing and thawing, should be discarded (21) (see chap. 19).

Subcutaneous Immunotherapy

Administration of inhalant vaccines is usually by subcutaneous injection. In 1949, the first controlled study of the efficacy of SCIT with house dust was published (22). Numerous subsequent studies report the efficacy of SCIT with aqueous vaccines of tree (23,24), grass (25,26), and weed pollens (27,28); fungi (12,13); and house-dust mites (29,30). Early studies were inconclusive on SCIT efficacy for cat, but some data support its use (11). One study showed that the immunologic changes with cat SCIT are similar to those found with SCIT using other allergens (10). Dog SCIT alters skin prick testing and levels of dog-specific IgG4 but does not improve symptoms as described in available publications (31). High-dose perennial SCIT is the dose schedule resulting in the best efficacy (14–16). SCIT only during the pollen season (co-seasonal therapy), or only in the few months prior to the pollen season, (preseasonal therapy), are not recommended in the United States (15). These protocols are used in other countries.

When more than one inhalant allergen is required, the vaccines may be administered either individually or incorporated into a single vial. Since most patients would rather receive as few injections as possible, combined vials are preferable for comfort and convenience. Some data on house-dust mite and fungal extracts suggest the enzymes in those extracts might degrade proteins with which they are mixed in a combined vaccine (32). There are, however, no data on the efficacy of mixed versus individual allergen vaccines to clarify the clinical relevance of the protease content of component extracts in a vaccine (see chap. 19). The Allergy Immunotherapy Practice Parameter suggests that allergens with high protease activity (dust mite, mold, and cockroach) may be mixed with each other but should be kept separate from those with low protease activity (grass pollen, tree pollen, weed pollen, and animal allergens) (3). Ragweed may be combined with any of the other allergens. Occasionally, patients who are extremely sensitive to one inhalant allergen may benefit from administration of that allergen in a separate injection in order to limit local reactions and to allow optimal dose adjustments. The dosage schedule for that allergen likely would be more conservative than for other inhalant allergens administered.

Dosage Schedules

Three basic SCIT dosage schedules are proven to be beneficial for the treatment of inhalant allergy: standard weekly immunotherapy, cluster immunotherapy, and rush immunotherapy (6). A generally accepted principle of immunotherapy is that, within certain limits, the larger the cumulative dose, the greater the efficacy (27). The optimal dose is defined as the dose of allergen vaccine inducing a clinically relevant effect in the majority of patients without causing unacceptable side effects (33). Studies by investigators at Johns Hopkins reported that ragweed vaccines administered in annual cumulative doses of approximately 50 μg antigen E or *Amb a I* are likely to be effective (34). Effective cumulative doses for other vaccines are not as well defined; however, studies of cat vaccines standardized by *Fel d I* content (35), of mite vaccines standardized by *Der p I* content (36), and of pollen vaccines standardized by BUs (37) support the hypothesis that greater cumulative doses of inhalant allergen are more likely to result in amelioration of symptoms of aeroallergen allergy.

Conventional immunotherapy regimens. There are two phases of conventional SCIT administration: the initial buildup phase, when the dose and concentration of vaccine are slowly increased, and the maintenance phase, when the patient receives an optimal immunizing dose over a period of time (7). Perennial immunotherapy commonly follows a once or twice weekly dosage schedule (Table 1). If the vaccine is labeled in AU or BAU, the initial concentration is generally 0.1 to 1 AU/mL or BAU/mL, with an initial volume of 0.05 mL. In preparation for the buildup phase of immunotherapy, serial dilutions should be produced from each maintenance concentrate vaccine. Typically, these are 10-fold dilutions, although other dilutions are occasionally used. These vials should be labeled in terms of volume per volume to indicate that these are dilutions derived from the maintenance

Table 1 Illustrative Schedule for an Inhalant Allergen Vaccine

Dilution (vol/vol)	Volume (mL)
1:1000	
	0.05
	0.10
	0.20
	0.40
1:100	
	0.05
	0.10
	0.20
	0.30
	0.40
	0.50
1:10	
	0.05
	0.07
	0.10
	0.15
	0.25
	0.35
	0.40
	0.45
	0.50
Maintenance concentrate	
	0.05
	0.07
	0.10
	0.15
	0.20
	0.25
	0.30
	0.35
	0.40
	0.45
	0.50

concentrate. For example, serial 10-fold dilutions from the maintenance concentrate would be labeled as 1:10 (vol/vol), 1:100 (vol/vol), and so on (3). Injections during the buildup phase are commonly administered once or twice weekly; after reaching the maintenance dose, the interval generally increases to biweekly (every 2 weeks), triweekly, and finally monthly. The maintenance dose is usually administered at two- to four-week intervals provided that the injections are well tolerated and symptoms improve. If a systemic reaction or anaphylaxis occurs, the physician should review the patient's immunotherapy history and adjust the dose of immunotherapy accordingly. Though recommendations vary, the practice at Northwestern University is to decrease the vaccine dose by at least half after systemic reactions. Others reduce the dose by 10-fold. Subsequently, the dose is increased as tolerated at weekly or twice-weekly intervals. The maintenance vial is replaced with a newly prepared one every 6 to 12 months, reducing the dose of the first injection from a new vial by one-third to one-half due to the potentially greater potency of a fresher vaccine. The dose is subsequently increased weekly, biweekly, or monthly to the routine maintenance dose (21).

Rush immunotherapy regimens. Numerous schedules have been published for administration of allergen immunotherapy via a rush injection schedule. Initial doses are generally similar to those of more conventional schedules as listed in Table 1. A typical rush immunotherapy schedule entails multiple injections on the first day of treatment. On the second and third days, fewer injections are administered, with the proportional increases of allergen dose also declining with each successive injection. During the first few days, injections are given at intervals of 30 minutes to 2 hours. Patients reach a maintenance dose in 3 to 7 days.

Some rush protocols increase the dosages more rapidly than outlined in Table 1, some with increases of 50% to 100% per injection. The obvious advantage of a rush schedule is that the patient can attain the maintenance dose and associated symptom relief more quickly (38). Efficacy and immunologic changes have been reported to be similar to conventional SCIT with equivalent cumulative doses. The major disadvantage, though, is that rush immunotherapy is associated with a greater risk of systemic reaction than that reported in conventional weekly immunotherapy; this increased reaction rate appears to be particularly significant in children (39). Reported rates of systemic reactions have been as high as 36.8% of patients with select allergens (40). This type of protocol has been most extensively studied in Hymenoptera venom immunotherapy, with rates of systemic reactions in approximately 7% of patients in multiple studies (40). Premedication with prednisone and an H_1 histamine receptor antagonist, with or without an H_2 histamine receptor antagonist, has been reported to reduce the risk of systemic reactions (41,42). Omalizumab, a humanized monoclonal anti-IgE antibody, has been studied as pretreatment as well. It was found to decrease the rate of systemic reactions to venom rush immunotherapy from 25.6% to 5.6% per patient (43). In addition to the more typical rush schedules, studies have investigated more extreme protocols, often termed "ultra-rush." One publication reported only 8 systemic reactions in over 2000 injections. It is important to note, though, that a modified extract not available in the United States was used (44).

Cluster immunotherapy. In cluster SCIT protocols, starting doses are similar to those of conventional SCIT (45). As with conventional protocols, weekly visits are necessary. However, at each visit more than one injection is administered, with the interval between injections varying from 30 minutes to 2 hours. Once the maintenance dose has been achieved, the interval between visits is increased. The advantage of the cluster regimen is probably most obvious for the patient who must travel several hours in order to receive injections; receiving more than one injection per visit reduces overall travel time by reducing the number of visits. Efficacy and immunologic changes are likely similar to conventional SCIT with equivalent cumulative dose. The disadvantage to cluster SCIT is that the reaction rate is probably greater than with conventional schedules (45), though reaction rates in some studies are similar to conventional SCIT schedules with dust mite (46). Several alterations to the cluster protocol may increase its safety, including premedication with antihistamines, four or less injections per visit, and twice-weekly clusters (40).

Procedures for Subcutaneous Injection

Prior to administering inhalant immunotherapy, it is imperative that the patient, the immunotherapy vial(s) of the appropriate dilution, and the dosage schedule(s) be clearly identified. Error in dosage can cause serious systemic reactions to inhalant allergen injections (47). Several systems may be used to decrease the risk of errors, including color coding of vials, confirmation checks with a second member of the health care team, and dosing confirmation with the patient. Careful documentation of the administration of SCIT is extremely important. Information that should be noted in the medical record includes the concentration given, a record of the bottle's label and its contents, the volume of vaccine scheduled and given, which arm was used for the injection, peak expiratory flow before and 20 to 30 minutes after injection as indicated for high-risk patients, a history of reactions from previous injections, treatment of any reactions that occurred, and any adjustments from the standard schedule and the reasons for the changes (7). If a patient's asthma is not controlled, treatment should be provided before an injection is administered because of the potentially dangerous synergistic effects of asthma and anaphylaxis. In one survey-based study, 15 of 17 fatal reactions occurred in the setting of poorly controlled asthma (48). Injections should be administered with a 0.5 to 1 mL syringe to insure dosage measurement accuracy. The needle gauge should be greater than 25, and the injection should be subcutaneous and not intradermal, intramuscular, or intravenous. The subcutaneous adipose tissue in the mid-portion posterior aspect of the arm, typically at the junction of the deltoid and triceps muscle, is the most common site for injections (Fig. 1). Prior to vaccine injection, the syringe plunger should be withdrawn to insure that the needle tip is not intravenous. If blood appears in the syringe, it should be withdrawn, discarded, and replaced with a new needle and syringe. After injections of inhalant allergen vaccines, the

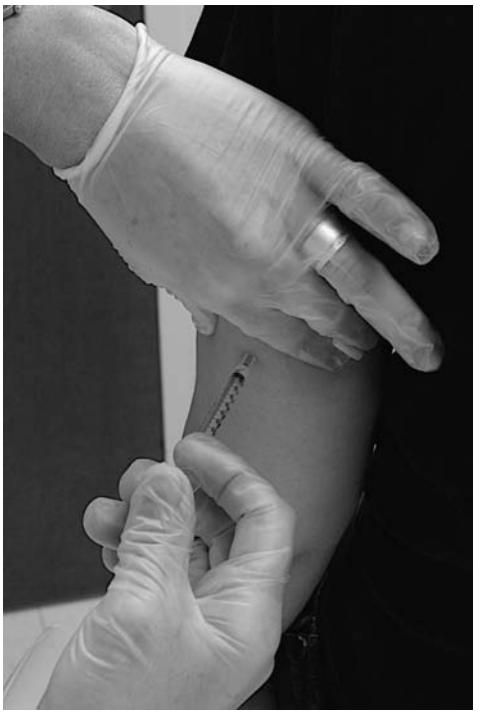

Figure 1 Proper technique for administration of an immunotherapy injection.

patient should be observed at least 30 minutes for reactions to injections and longer if he/she is considered to be at increased risk for a systemic reaction (3).

Reactions and Dosage Adjustment

Local reactions characterized by erythema and/or induration less than 2 cm and lasting less than 2 days are common and of no consequence. Large local reactions, defined as induration greater than 2 cm lasting more than 2 days, may result in the repetition or decrease of the next dose. Reactions greater than 2 cm in diameter may need to be treated with topical application of ice to reduce local blood flow, oral antihistamine therapy, and possibly topical corticosteroid therapy. These methods usually are sufficient, but rarely a large or biphasic local reaction may be so uncomfortable that a day or two of oral glucocorticosteroids may be beneficial. Oral antihistamines are often used in an effort to prevent local reactions. There appeared to be a trend favoring montelukast over loratidine for prevention of local reactions in one study (49), but the difference failed to reach statistical significance.

Most data do not correlate local reactions with risk for systemic responses but there are exceptions (50). One group analyzed the rates of systemic reactions under two different protocols: one that modified allergen vaccine doses based on local reactions and one that did not (51). Modification of dosing did not affect systemic reaction rates after analysis of nearly 8000 injection visits. These findings were confirmed by another group with a similar protocol design (52). Furthermore, the latter group found no increase in local reaction rates preceding systemic reactions. Changes in SCIT dosing schedules in response to local reactions, therefore, are unlikely to alter the risk of systemic reactions. However, they may offer some benefit at reducing subsequent local reactions and patient discomfort.

SCIT may result in systemic reactions or anaphylaxis manifested by urticaria, angioedema, generalized pruritus and erythema, laryngeal edema, headache, nausea, vomiting, bronchospasm, hypotension, shock, and even death. Most systemic reactions occur during the buildup phase or in highly allergic individuals, though anaphylaxis occurs in the maintenance phase as well. Physicians who administer allergen SCIT vaccines must be prepared to treat anaphylaxis. If a systemic reaction occurs, the subsequent SCIT dose should be reduced to one-half to one-tenth the dose that resulted in the systemic response, depending upon the severity of the symptoms. In patients who have experienced a systemic reaction, the

rate of increase in dosage may need to be reduced. After each systemic reaction, the risks and benefits of continuing immunotherapy generally should be reevaluated and discussed with the patient.

A common cause for dosage adjustment is a hiatus in therapy. Depending upon the patient's previous history of reactions and the length of the hiatus compared with the usual frequency of injections, the dosage may be repeated or reduced. If a patient has a pattern of being unable to comply with the injection schedule, particularly if the maintenance dose has not been achieved, the risks and benefits of continuing SCIT should be reevaluated.

When to Stop Immunotherapy

From our experience at Northwestern University, approximately 90% of properly selected SCIT-treated allergic patients will usually notice a reduction in symptoms and/or medications after receiving the maintenance dose for one year or more. Further, SCIT will not likely benefit and usually should be discontinued if maintenance therapy for over a year has not resulted in some improvement.

In a controlled study in which SCIT for grass pollen allergy was discontinued after three to four years of successful treatment, seasonal symptom scores and the use of rescue medication were decreased for three to four years after the discontinuation of SCIT. There was no significant clinical difference between patients who continued and those who discontinued SCIT (53). Evidence is not available to provide an absolute, optimal length of time to continue inhalant allergen vaccines. After two or three years of improvement, consideration of discontinuing SCIT is an option. In our experience, most patients whose inhalant allergen immunotherapy is discontinued at that time will continue to maintain their reduction of symptoms and/or medications. Several studies have demonstrated this, though most have been with few enrolled subjects (54). There is, however, a risk of relapse, and this should be discussed with the patient prior to stopping SCIT. In the setting of venom IT, there appear to be several factors associated with increased risk of recurrent reactivity: severity of sting reaction, systemic reaction to venom IT, allergy to honeybee, and immunotherapy duration less than five years (54) (see chap. 24).

Subcutaneous Immunotherapy in Pregnancy

Allergen SCIT is effective in the pregnant patient, and maintenance doses may be continued during pregnancy. When a woman receiving SCIT reports that she is pregnant, the dose of immunotherapy usually is not increased; rather, the patient is maintained on the dose she is receiving at the time of notification. Alternatively, some specialists slightly reduce the maintenance dose as an additional precaution. Allergen immunotherapy is usually not initiated during pregnancy because of the risks of systemic reaction and their treatment. These recommendations stem from the understanding that systemic reactions are more likely to occur during the build-up phase of immunotherapy. Possible obstetrical complications of anaphylaxis or its treatment include spontaneous abortion, premature labor, and fetal hypoxia. The initiation of immunotherapy may be considered during pregnancy for the pregnant patient with life-threatening Hymenoptera sensitivity (3).

Local Nasal Immunotherapy, Oral Immunotherapy, and Sublingual Immunotherapy

See chapters 23, 25 and 28.

Modified Extracts/Vaccines

Two major concerns exist despite the established efficacy of SCIT using conventional aqueous inhalant allergen vaccines. First, SCIT may induce systemic reactions that can be life-threatening. Second, the build-up phase of conventional SCIT generally requires 25 to 30 injections, each of which involves time and cost to the patient. Many investigators have attempted to modify inhalant allergen vaccines to reduce the risk of anaphylaxis or minimize the number of buildup injections (55). These modifications can be divided into three approaches: slowing absorption, inducing tolerance, and reducing allergenicity while retaining immunogenicity.

Alum-precipitated extracts are an example of the slow absorption approach (56). They are the only modified extracts available in the United States and are the most commonly used extracts in other countries (4). Ragweed, cat, and grass alum–precipitated vaccines have been studied and found to have equivalent efficacy when compared with aqueous vaccines. Systemic reactions are decreased, with rare prolonged local reactions the major side effect (21). To date, the FDA has not approved any modified extract other than those that are alum precipitated, and most immunotherapy outside of the United States employs alum and other modified extracts.

Experimental data demonstrate the potential to induce tolerance and enhance safety and efficacy of immunotherapy, though most of these data are from animal experiments. Possible modifications include addition of monophosphoryl lipid A, heat-killed bacteria, or CpG motifs (57). CpG motifs have been used in an effort to stimulate Toll-like receptors during immunotherapy, thereby inducing tolerance. In human studies with vaccines containing Toll-like stimulators, one group found improvement in allergic rhinitis symptom and quality-of-life scores, but failed to reach statistical significance on the primary endpoint of vascular permeability (58).

Aggregation or cross-linking of the proteins of an aqueous extract reduces the allergenicity while preserving the immunogenicity of the subsequent vaccine. Two methods of modification have accomplished this goal: polyethylene glycol-treated allergens and glutaraldehyde-treated allergens (polymerized allergen extracts). One study assessing the safety of these modifications found a rate of systemic reactions to be 1.31% per patient, or 0.12% per injection, using a protocol that required only four to six injections to reach maintenance (59). This compares favorably with published reaction rates of 0.8% per injection for aqueous vaccines used in conventional SCIT protocols (42). Direct comparison of modified to unmodified dust-mite allergen yielded one-third the number of systemic reactions in the modified group compared with the unmodified one (60). Furthermore, 10-fold higher allergen concentrations were tolerated in the modified allergen subjects. A double-blind, placebo-controlled trial of modified dust mite allergen immunotherapy showed a substantial improvement in bronchial provocation testing and symptom scores, with no systemic reactions (61). Similar findings have been published for recombinant grass pollen immunotherapy, with extracts produced via transfection of grass pollen cDNA into *Escherichia coli* (62).

Dosage Schedules

A variety of dosage schedules have been published for modified vaccines. An example of an efficacious protocol for glutaraldehyde-polymerized vaccines is shown in Table 2. Most modified vaccines require fewer than half the injections necessary with standard aqueous immunotherapy.

Techniques

Most modified vaccines are marketed for subcutaneous injection at weekly intervals. However, modified dosage schedules such as rush or cluster schedules have been published (63).

Table 2 Example of Dosage Schedule for Polymerized Ragweed Injections

Week	*Amb a I* (μg/mL)	Volume
1	25	0.10
2		0.25
3		0.50
4	250	0.10
5		0.25
6–15	250	0.50

Reactions and Dosage Adjustment
The package insert that accompanies modified vaccines generally advises physicians of the relative risk of reactions and dosage adjustment following reactions. For the most part, the reactions and dose adjustments are similar to those with unmodified aqueous vaccines.

When to Stop
For glutaraldehyde polymerized vaccines, 10 to 15 injections result in efficacy for up to six years (64). Most other modified vaccines have not been studied as to duration of efficacy after discontinuation of therapy. It should be noted that some forms of modification that reduce allergenicity can also denature the allergens. Therefore, modified vaccines, such as pyridine-extracted allergens, should only be used if clinical efficacy has been demonstrated by appropriately controlled clinical trials. In general, package inserts accompanying modified allergen vaccines include recommendations relative to a course of therapy.

SALIENT POINTS

- Allergen SCIT is defined as the repeated administration of specific allergens to patients with IgE-mediated conditions for the purpose of providing protection against the allergic symptoms and inflammatory reactions associated with natural exposure to these allergens.
- The most commonly used form of inhalant allergen immunotherapy in the United States is subcutaneous injection of aqueous vaccines (SCIT).
- While various low-dose immunotherapy regimens have been published, optimal efficacy results from a relatively high-dose immunotherapy schedule as illustrated in Table 1.
- Most systemic or anaphylactic reactions occur during the buildup phase and in highly allergic patients, especially those with poorly controlled asthma.
- Administering an incorrect dose can result in severe systemic reactions. To minimize risk, the patient, vial, dilution, and immunotherapy schedule must be individually identified prior to administration of an injection of inhalant allergen. If there was a significant reaction to the previous immunotherapy dose or if the time interval between injections is longer than designated, the dose will require adjustment.
- If a patient has been on SCIT maintenance doses for more than a year without improvement, the SCIT treatment should probably be discontinued. If improvement has occurred, the patient should be treated for two or three improved seasons or three years of more before consideration of discontinuation.
- Standardized, commercially available extracts as of 2007 include cat hair, cat pelt, *Dermatophagoides pteronyssinus, D. farinae*, short ragweed, Bermuda grass, Kentucky bluegrass, perennial ryegrass, orchard grass, timothy grass, meadow fescue, red top, sweet vernal grass, and Hymenoptera venoms (yellow jacket, honeybee, wasp, yellow hornet, and white-faced hornet).
- Allergen SCIT is effective in the pregnant patient and maintenance doses may be continued during pregnancy. Allergen immunotherapy is usually not initiated or advanced in dose or concentration during pregnancy because of risks associated with a potential systemic reaction and its treatment. Possible complications of systemic reactions include spontaneous abortion, premature labor, and fetal hypoxia.

ACKNOWLEDGMENT

Supported by the Ernest S. Bazley Grant to Northwestern Memorial Hospital and Northwestern University Feinberg School of Medicine, Chicago, Illinois, U.S.A.

REFERENCES

1. Freeman J, Noon L. Further observation on the treatment of hayfever by hypodermic inoculations of pollen vaccine. Lancet 1911; 2:814–817.
2. Reisman R., Tronolone M. Immunotherapy: a practical review and guide. Immunol Allergy Clin North Am 2000; 20(3):469–478.
3. Cox L, Li J, Nelson H, et al. Allergen immunotherapy: a practice parameter second update. J Allergy Clin Immunol 2007; 120(3 suppl):S25–S85.
4. Grammer LC. Principles of immunologic management of allergic diseases due to extrinsic antigens. In: Grammer LC, Greenberger PA, eds. Patterson's Allergic Diseases: Diagnosis and Management. Philadelphia: J. B. Lippincott, 2002:183–194.
5. Norman PS. Allergic rhinitis. In: Austen KF, Frank MM, Atkinson JP, et al., eds. Samter's Immunologic Diseases. Philadelphia: Lippincott Williams and Wilkins, 2001: 817–824.
6. Nelson HS. Immunotherapy for inhalant allergens. In: Adkinson NF, Yunginger JW, Busse WW, et al., eds. Allergy: Principles and Practice) Philadelphia, PA: C. V. Mosby, 2003:1455–1473.
7. Portnoy J. Immunotherapy for inhalant allergies—guidelines for why, when and how to use this treatment. Postgrad Med 2001; 109:89–106.
8. Nelson HS. Advances in upper airway diseases and allergen immunotherapy. J Allergy Clin Immunol 2007; 119(4):872–880 [Epub February 9, 2007].
9. Bousquet J, Michel FB. Specific immunotherapy in asthma: is it effective? J Allergy Clin Immunol 1994; 94:1–11.
10. Ewbank PA, Murray J, Sanders K, et al. A double-blind, placebo-controlled immunotherapy dose-response study with standardized cat extract. J Allergy Clin Immunol 2003; 111(1):155–161.
11. Alvarez-Cuesta E, Cuesta-Herranz J, Puyana-Ruiz J, et al. Monoclonal antibody-standardized cat extract immunotherapy: risk-benefit effects from a double-blind placebo study. J Allergy Clin Immunol 1994; 93:556–566.
12. Horst M, Hejjaoui A, Horst V, et al. Double-blind, placebo-controlled rush immunotherapy with a standardized *Alternaria* extract. J Allergy Clin Immunol 1990; 85:460–472.
13. Malling HJ, Dreborg S, Weeke B. Diagnosis and immunotherapy of mold allergy. V. Clinical efficacy and side effects of immunotherapy with Cladosporium herbarum. Allergy 1986; 41:507–519.
14. Esch RE, Bush RK. Aerobiology of outdoor allergens. In: Adkinson NF, Yunginger JW, Busse WW, et al., eds. Allergy: Principles and Practice. Philadelphia, PA: C. V. Mosby, 2003:529–555.
15. Solomon, WR. Airborne pollen prevalence in the United States. In: Grammer LC, Greenberger PA, eds. Patterson's Allergic Diseases. Philadelphia: Lippincott Williams & Wilkins, 2002:131–144.
16. Sichere SH, Eggleston PA. Environmental allergens. In: Lieberman P, Anderson JA, eds. Allergic Diseases: Diagnosis and Treatment. Totowa, NJ: Humana Press, 2000:41–51.
17. Baer H. Potency units for allergenic extracts in the USA. Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M 1987; 80:167–168.
18. Lockey RF, Slater JE, Esch RE. Preparation and standardization of allergen extracts. In: Adkinson NF, Yunginger JW, Busse WW, et al., eds. Allergy: Principles and Practice. Philadelphia, PA: C. V. Mosby, 2003:529–553.
19. Dreborg S. Precision of biologic standardization of allergenic preparations. Allergy 1992; 47:291–294.
20. Helm RM, Gauerke MB, Baer H, et al. Production and testing of an international reference standard of short ragweed pollen extract. J Allergy Clin Immunol 1984; 73:790–800.
21. Tippet J. Comprehensive care in the allergy/asthma office. Immunol Allergy Clin North Am 1999; 19(1):129–148.
22. Bruun E. Controlled examination of the specificity of specific desensitization in asthma. Acta Allergol 1949; 2:122–128.
23. Pence HL, Mitchell DQ, Greely RL, et al. Immunotherapy for mountain cedar pollinosis: a double-blind controlled study. J Allergy Clin Immunol 1976; 58:39–50.
24. Rak S, Hakanson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during the pollen season. J Allergy Clin Immunol 1990; 86:706–713.
25. Ortolani C, Pestorello E, Moss RB, et al. Grass pollen immunotherapy: a single year double blind placebo controlled study in patients with grass pollen induced asthma and rhinitis. J Allergy Clin Immunol 1984; 73:283–290.
26. Reid MJ, Moss RB, Hsu YP, et al. Seasonal asthma in northern California: allergic causes and efficacy of immunotherapy. J Allergy Clin Immunol 1986; 78:590–600.
27. Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of ragweed hayfever with antigen E: comparison with whole pollen extract and placebos. J Allergy 1968; 42:93–108.
28. Van Meter TE, Adkinson NF, Amodio FJ, et al. A comparative study of the effectiveness of the Rinkel method and of the current standard method of immunotherapy for ragweed pollen hay fever. J Allergy Clin Immunol 1980; 66:500–513.

29. McHugh SM, Lavelle B, Kemeny DM, et al. A placebo controlled trial of immunotherapy with two extracts of *Dermatophagoides pteronyssinus* in allergic rhinitis, comparing clinical outcome with antigen specific IgE, IgG, and IgG subclasses. J Allergy Clin Immunol 1990; 86:521–531.
30. Bousquet J, Hejjaoui A, Clauzel AM, et al. Specific immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. II. Prediction of efficacy of immunotherapy. J Allergy Clin Immunol 1988; 78:971–977.
31. Lent AM, Harbeck R, Strand M, et al. Immunologic response to administration of standardized dog allergen extract at differing doses. J Allergy Clin Immunol 2006; 118(6):1249–1256.
32. Nelson HS, Ikle D, Buchmeier A. Studies of allergen extract stability: the effects of dilution and mixing. J Allergy Clin Immunol 1996; 98:382–388.
33. Bousquet J, Lockey R, Malling H. WHO panel members. Allergen immunotherapy: therapeutic vaccines for allergic diseases, a WHO position paper. J Allergy Clin Immunol 1998; 102:558–562.
34. Norman PS, Winkenwerder WL, Lichtenstein LM. Trials of alum-precipitated pollen extracts in the treatment of hayfever. J Allergy Clin Immunol 1972; 50:31–44.
35. Van Metre TE, Marsh DG, Adkinson NF, et al. Immunotherapy decreases skin sensitivity to cat extract. J Allergy Clin Immunol 1989; 83:888–899.
36. Bousquet J, Calvayrac P, Guerin B, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. J Allergy Clin Immunol 1985; 76:734–744.
37. Bousquet J, Guerin B, Dotte A, et al. Comparison between rush immunotherapy with a standardized allergen and an alum adjuved pyridine extracted material in grass pollen allergy. Clin Allergy 1985; 15:179–193.
38. Bousquet J, Becker WM, Hejjaoui A, et al. Differences in clinical and immunologic reactivity of patients allergic to grass pollens and to multiple pollen species. II Efficacy of a double blind, placebo controlled specific immunotherapy with standardized extracts J Allergy Clin Immunol 1991; 88:43–53.
39. Hejjaoui JJ, Ferrando R, Dhivert H, et al. Systemic reactions occurring during immunotherapy with standardized pollen extracts. J Allergy Clin Immunol 1992; 89:925–933.
40. Cox L. Accelerated immunotherapy schedules: review of efficacy and safety. Ann Allergy Asthma Immunol 2006; 97(2):126–137.
41. Portnoy J, Bagstad K, Kanarek H, et al. Premedication reduces the incidence of systemic reactions during inhalant rush immunotherapy with mixtures of allergenic extracts. Ann Allergy 1994; 73:409–418.
42. Tankersley MS, Walker RL, Butler WK, et al. Safety and efficacy of an imported fire and rush immunotherapy protocol with and without prophylactic treatment. J Allergy Clin Immunol 2002; 109:556–562.
43. Casale TB, Busse WW, Kline JN, et al. Immune Tolerance Network Group. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2006; 117(1):134–140 [Epub December 2, 2005].
44. Casanovas M, Martin R, Jimenez C, et al. Safety of an ultra-rush immunotherapy build-up schedule with therapeutic vaccines containing depigmented and polymerized allergen extracts. Int Arch Allergy Immunol 2006; 139(2):153–158 [Epub December 22, 2005].
45. Van Metre TE, Adkinson NF, Amodio FJ, et al. A comparison of immunotherapy schedules for injection treatment of ragweed hayfever. J Allergy Clin Immunol 1982; 69:181–193.
46. Tabar AI, Echechipia S, Garcia BE, et al. Double-blind comparative study of cluster and conventional immunotherapy schedules with Dermatophagoides pteronyssinus. J Allergy Clin Immunol 2005; 116 (1):109–118.
47. Lockey RF, Benedict LM, Turkeltaub PC, et al. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1987; 79:660–677.
48. Bernstein DI, Wanner M, Borish L, et al. Immunotherapy Committee, American Academy of Allergy, Asthma and Immunology. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990 to 2001. J Allergy Clin Immunol 2004; 113(6):1129–1136.
49. Wohrl S, Gamper S, Hemmer W, et al. Premedication with montelukast reduces local reactions of allergen immunotherapy. Int Arch Allergy Immunol 2007; 144(2):137–142.
50. Roy SR, Sigmon JR, Olivier J, et al. Increased frequency of large local reactions among systemic reactors during subcutaneous allergen immunotherapy. Ann Allergy Asthma Immunol 2007; 99:82–86.
51. Kelso JM. The rate of systemic reactions to immunotherapy injections is the same whether or not the dose is reduced after a local reaction. Ann Allergy Asthma Immunol 2004; 92(2):225–227.
52. Tankersley MS, Butler KK, Butler WK, et al. Local reactions during allergen immunotherapy do not require dose adjustment. J Allergy Clin Immunol 2000; 106(5):840–843.
53. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999; 341:468–475.
54. Cox L, Cohn JR. Duration of allergen immunotherapy in respiratory allergy: when is enough, enough? Ann Allergy Asthma Immunol 2007; 98(5):416–426.
55. Grammer LC, Shaughnessy MA, Patterson R. Modified forms of allergen immunotherapy. J Allergy Clin Immunol 1986; 76:297–301.

56. Varney VA, Gaga M, Frew AJ, et al. Usefulness of immunotherapy in patients with severe hayfever uncontrolled by antiallergic drugs. Br Med J 1991; 302:265–269.
57. Francis JN, Durham SR. Adjuvants for allergen immunotherapy: experimental results and clinical perspectives. Curr Opin Allergy Clin Immunol 2004; 4(6):543–548.
58. Creticos PS, Schroeder JT, Hamilton RG, et al. Immune Tolerance Network Group. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006; 355(14): 1445–1455.
59. Casanovas M, Martin R, Jimenez C, et al. Safety of immunotherapy with therapeutic vaccines containing depigmented and polymerized allergen extracts. Clin Exp Allergy 2007; 37(3):434–440.
60. Casanovas M, Fernandez-Caldas E, Alamar R, et al. Comparative study of tolerance between unmodified and high doses of chemically modified allergen vaccines of Dermatophagoides pteronyssinus. Int Arch Allergy Immunol 2005; 137(3):211–218 [Epub Jun 9, 2005].
61. Garcia-Robaina JC, Sanchez I, de la Torre F, et al. Successful management of mite-allergic asthma with modified extracts of Dermatophagoides pteronyssinus and Dermatophagoides farinae in a double-blind, placebo-controlled study. J Allergy Clin Immunol 2006; 118(5):1026–1032 [Epub September 18, 2006].
62. Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005; 116(3):608–613.
63. Bousquet J, Hejjaoui JA, Skassa-Brociek W, et al. Double blind placebo controlled immunotherapy with mixed grass pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass pollen extract. Allergy Clin Immunol 1987; 80:591–598.
64. Grammer LC, Shaughnessy MA, Suszko IM, et al. Persistence of efficacy after a brief course of polymerized ragweed allergen: a controlled study. J Allergy Clin Immunol 1984; 73:484–489.

21 Administration of Sublingual Vaccines

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INTRODUCTION

Historical Perspective

Since inception (1), specific immunotherapy has generally been administered via subcutaneous injections [also known as subcutaneous immonutherapy (SCIT)], but other modalities of administration were proposed and investigated during the 20th century. In some approaches, the rationale was to specifically desensitize the target organs (nose or bronchi) by giving the allergen as a nasal spray (local nasal immunotherapy) or inhaled aerosol (bronchial immunotherapy). Alternatively, a systemic desensitization was targeted by administering the allergen orally or sublingually. Among these "local" or "noninjection" routes of administration, intranasal immunotherapy was extensively investigated with favorable clinical results (2). In contrast, the oral (allergen immediately swallowed) and the local bronchial routes were abandoned in the 1970s because of lack of efficacy or unacceptable side effects (3). In 1986, the British Committee for the Safety of Medicines (4) reported several deaths associated with SCIT and raised serious concerns about the safety and the risk/benefit ratio of immunotherapy. Although some cases of life-threatening events were due to avoidable human errors (wrong dose, improper prescription, incorrect administration) (5), most cases of anaphylaxis are unexplained and unpredictable (6).

The sublingual administration of allergens, which was clinically ineffective, has been used in the USA for many years (7). However, low-dose sublingual immunotherapy (SLIT) for respiratory allergy was firstly described in a controlled trial in 1986 (8) and it appeared as a promising therapeutic option, expecially for the favourable safety profile. The original rationale of SLIT was that of achieving a prompt and rapid absorption of the vaccine through the oral mucosa. It was then demonstrated that no relevant, direct absorption occurs through the sublingual mucosa, but SLIT proved effective in a number of trials (9,10) and became the most used noninjection route for immunotherapy in Europe. After a review of the literature existing in 1998, a panel of experts of the World Health Organization concluded that SLIT is a viable alternative to SCIT (11). This statement was confirmed in a position paper of the European Academy of Allergology and Clinical Immunology (12) and in the ARIA (allergic rhinitis and its impact on asthma) document (13) that extended the indications of SLIT to children. A summary of the history of SLIT is depicted in Figure 1.

General Aspects of Sublingual Vaccines

The history of SLIT is chronologically short and encompasses a period of only 20 years. The sublingual approach was initially proposed empirically, without knowledge of the biodistribution of allergens and of the possible mechanism of action. As a consequence, the practical aspects of SLIT (i.e., allergen dose, frequency of administration, build-up modality) were selected by investigators on the basis of personal experience, often translating into SLIT, the protocols used for SCIT. The result is significant variability in administration schedules, dosages, and duration of SLIT courses (Fig. 2). Nonetheless, the clinical trials in the latter 20th and early 21st century began to address this variability, and there is a trend toward more uniform SLIT protocols, similar to the previous development for SCIT. Because of regulatory issues, SLIT is not used worldwide but is employed in clinical practice in Europe and other select countries and regions, including South Africa and Latin America.

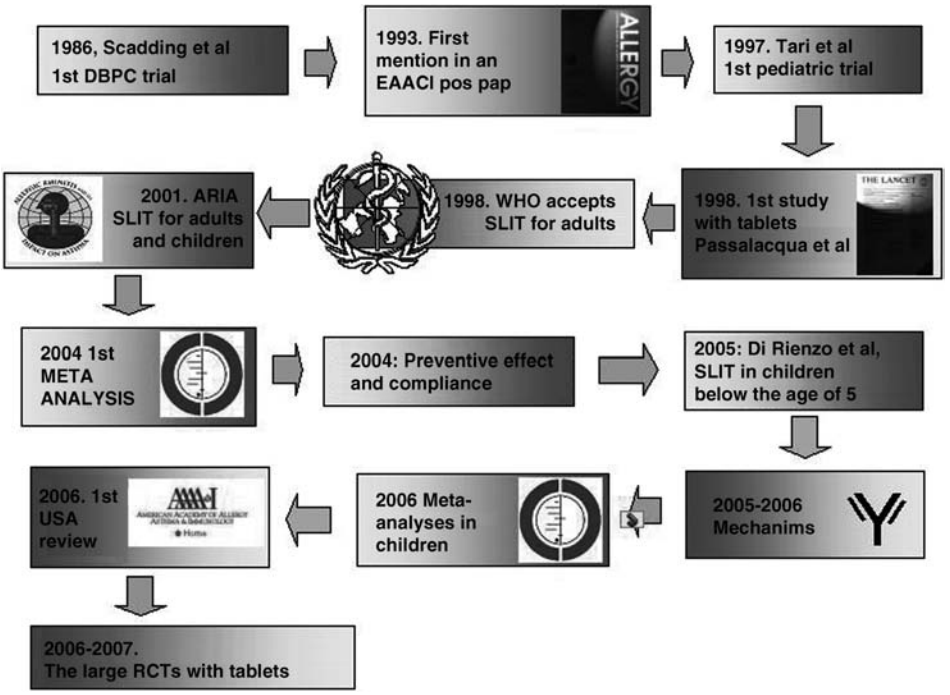

Figure 1 The main steps in the development of SLIT.

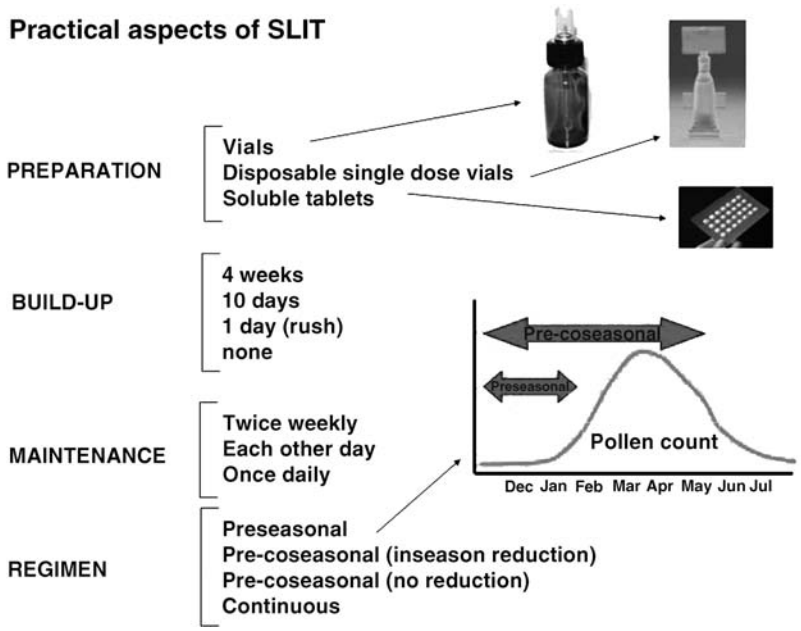

Figure 2 The variable practical aspects of SLIT.

PRACTICAL ADMINISTRATION OF SLIT

Vaccines

SLIT is currently marketed by several European manufacturers, and the administration schedules and amount of allergen(s) largely vary, depending on the producer. Almost all the SLIT vaccines commercialized in Europe are standardized, either biologically or immunologically (14). As is true for SCIT, the standardization methods are largely based on in-house

references; thus, the extracts are labeled in units that differ from one manufacturer to another. Some of the more common labeling units are allergen units (AU), index of reactivity (IR), biological units (BU), standard units (STU). The content in micrograms of the major allergens is available for many extracts, and this has allowed a quantitative approach to determine the optimal SLIT dose. In general, the maintenance dose of allergen is 5 to 300 times higher (according to the manufacturer) with SLIT compared to SCIT. The term "high-dose SLIT" is sometimes used to indicate this dose difference.

The vaccines for SLIT are available in two principle pharmaceutical forms.

1. Buffered solution to be delivered by drop-counters, predosed actuators (minipumps) or disposable single-dose vials.
2. Tablets with appropriate composition facilitating slow (1–2 minutes) dissolution in the mouth upon contact with saliva.

For the build-up or updosing phase, vials or blisters of tablets at increasing concentrations are provided by the manufacturers.

SLIT is not approved in the United States, as there are no FDA-licensed products. The American Medical Association's manual *Current Procedural Terminology 2005* defines immunotherapy as "parenteral administration of allergenic extracts as antigens at periodic intervals" (15). Currently, U.S. physicians who give SLIT might incur increased medical liability, particularly since two cases of anaphylaxis with heterogeneous mixtures of allergens have been described with SLIT (16,17).

Technique of Administration

The vaccine, in tablets or solution, is usually administered in the morning with the patient in the fasting state. The drops or tablets are kept under the tongue for one to two minutes and then swallowed (*sublingual-swallow* mode). Alternatively, in some trials, the allergen is kept under the tongue and then spat out (*sublingual-spit* mode) (18–20). In biodistribution studies (21), at least 30% of the allergen is retained on the oral mucosa even after spitting. However, because of practical considerations and clinical results, the sublingual-swallow route is the modality currently used in trials and clinical practice. The acronym SLIT, unless stated differently, indicates the sublingual-swallow modality.

Immunotherapy in Europe is prescribed for one or, at most, a few allergens. Mixtures are not used as commonly as in the United States. There is no contraindication to administering multiple allergens with SLIT, except for the possibility of one allergen extract enzymatically digesting another extract with loss of potency/stability. A study of SLIT treatment with a mixture of two unrelated allergens (grass and birch) showed more efficacy than the use of the single allergen alone (22). The administration of numerous extracts mixed together may increase the risk of adverse events. In support of this point, two of three cases of anaphylaxis described with SLIT used heterogeneous mixtures of more than three different allergens (16,17).

Buildup and Maintenance

SLIT traditionally involves a build-up phase (with gradually increasing doses) and a maintenance phase with the maximum dose. This approach is similar to SCIT but more accelerated. The build-up phase is usually four to six weeks. The vaccine is prepared in separate vials (or in separate blisters of tablets) at increasing concentrations. The treated subject starts with the lowest concentration and gradually increases, using the different dosage preparations, until the maintenance dose is reached. An example of build-up regimens is reported in Tables 1 and 2. European manufacturers provide for each extract a suggested maintenance dose on the basis of clinical trials. Of note, the relative safety of SLIT does not strictly limit the maximum dose, and this contributes to the variability of the maintenance dosages used in clinical trials. This is evident in a dose-ranging, safety study of SLIT completed in 2006 (23). In this study, one of the groups received 1,000,000 standardized quality units per day, corresponding to 200 μg of Phl p 5 allergen and 40-fold greater than the lowest dose (23). Dose-dependent side effects occurred, but even with the greatest amount of allergen, the untoward effects were mostly local (oral itching/swelling) and mild or moderate in severity.

Table 1 Example of a Traditional SLIT Schedule with Tablets[a]

	1st BLISTER tab 25 AU	2nd BLISTER tab 100 AU	3rd BLISTER tab 300 AU	4th BLISTER tab 1.000 AU
Wk 1	1 tab e.o.d.			
Wk 2	1 tab e.o.d.			
Wk 3		1 tab e.o.d.		
Wk 4		1 tab e.o.d.		
Wk 5			1 tab e.o.d.	
Wk 6			2 tab e.o.d.	
Maintenance				1 tab twice weekly

[a]e.o.d., every other day.

Table 2 Example of a Traditional SLIT Schedule with Drops

	1st VIAL 0.1 IR/mL	2nd VIAL 1 IR/mL	3rd VIAL 10 IR/mL	4th VIAL 100 IR/mL
Day 1	1 drop			
Day 2	2 drops			
Day 3	3 drops			
Day 4	4 drops			
Day 5	6 drops			
Day 6	8 drops			
Day 7	10 drops			
Day 8–14		The same as Vial 1		
Day 15–21			The same as Vial 2	
Day 22–28				The same as Vial 3, up to 20 drops
Maintenance				20 drops thrice weekly

The treatment at the various doses up to 1,000,000 standardized quality units did not result in any serious, systemic, or significant (leading to withdrawal) adverse events. Similar results were obtained in another study, investigating the safety of SLIT in patients with grass-induced asthma (24). A consequence of these observations is that with SLIT the concept of "maximum tolerated dose" is less applicable than with SCIT.

The suggested maintenance dose interval varies among manufacturers and investigators. The most used regimen is once daily (25), but alternate day (26) and once weekly administration have been utilized (27).

SLIT can be administered either preseasonally (start prior to the season and stop at the beginning of the season), pre-coseasonally (start prior to the season and stop at the end of the season) or continuously. Preseasonal and pre-coseasonal schedules are commonly used for pollen allergy. In this case, the treatment is commenced about two months before the expected pollen season. In the pre-coseasonal regimen, no dose reduction during the pollen season is usually applied. On the other hand, for nearly perennial or perennial allergens, a continuous treatment (all year round) is preferred.

Duration of SLIT

There is no controlled study on the optimal duration of a SLIT treatment, thus the recommendations are derived from the SCIT experience. A typical SLIT course is usually three to five years and discontinued if there is no benefit after two years of treatment (11,13). A 15-year follow-up of subjects treated with SLIT for three, four, or five years suggests that a four-year course represents the best compromise between clinical efficacy and long-term effect (28).

PRESCRIPTION OF SLIT

SLIT appears efficacious for IgE-mediated respiratory allergy. SLIT is specific for the allergen and not the disease. The major issue to be determined before initiating SLIT, or any allergen immunotherapy, is the causal importance of a specific allergen or allergens.

The allergy diagnosis is made by clinical history, physical examination, skin testing, and/or serum specific IgE assays. Since the European preference is the administration of one extract, or at most a few extracts, in a vaccine, the selection of allergens responsible for allergic symptoms may be facilitated with conjunctival or nasal provocation tests in some cases (29,30). SLIT is generally an individually prepared product in Europe that is sent directly to the patient or to the doctor by the manufacturer. The preparation follows recommendations of the treating physician. This is similar to prescribing a pharmaceutical therapy. The introduction of standardized tablets, which have been mutually recognized by European pharmaceutical agencies, will make SLIT even more similar to usual pharmaceuticals. SLIT is self-managed by the patient at home, thus detailed instructions, schedule of administration, and possible side-effect discussions are mandatory. The manufacturers usually provide written instructions with SLIT, but it is common practice for most allergists to see the patient before he/she starts the treatment, to provide additional information and verify understanding. A contact physician for telephone reporting of adverse events should be provided.

As per guidelines, SLIT is indicated in patients with rhinitis or asthma or both, especially those who refuse injections or previously experienced severe adverse reactions to SCIT. Although there is no formal evidence of increased risk, SLIT is usually not recommended for patients with severe/uncontrolled asthma. The clinical efficacy of SLIT in mild, intermittent rhinitis is less defined. Ideally, SLIT should be used in an integrated treatment plan, including avoidance measures and appropriate pharmaceutical therapy.

Finally, although SLIT is safe and easy to use, SLIT should be prescribed by a specialist after a detailed diagnostic workup. In support of this point, a controlled study in which patients were selected for SLIT by general practitioners showed only a marginal benefit (31).

ADHERENCE AND COSTS

Adherence

SLIT is self-administered and usually managed at home; thus, concerns about compliance (32), monitoring, or adherence are potential problems. Adherence with SCIT is documented, since it is given in the presence of a physician. Some studies have attempted to quantify the adherence to SLIT therapy by means of unscheduled telephone interviews. This could be done as the treatments were prepared as tablets or single-dose vials, allowing a count of the remaining doses and a calculation of adherence. In one study, involving 126 adult patients receiving SLIT in tablets, the compliance was reported as greater than 90% over a one-year period (33). In another observational study of 442 patients, the compliance measured at three and six months was greater than 75% in 86% of the patients (34). Similar results were documented in a population of 71 children (35). Compliance data were available for all children at three months, and for 56 children at six months. At three months, 85% of subjects had a compliance rate greater than 75% (69% greater than 90%) and at six months, 84% had a compliance rate greater than 75% (66% greater than 90%). Finally, in a large, randomized controlled trial with grass vaccine in tablets (25), adherence was 94% to 98%. In conclusion, published trial data show adherence to SLIT is very high.

Costs

The cumulative dose of allergen given via sublingual route is greater than SCIT, and therefore, the cost of the vaccine is greater. The cost of the vaccine is offset by the reduced need for medical and nursing time, so the global cost of SLIT may be less than SCIT. A cost-benefit analysis, using a validated pharmacoeconomic model (36) shows that SLIT for pollinosis in patients with rhinitis and/or asthma leads to significant savings in terms of both direct and indirect costs. Similar analyses, based on mathematical models, were conducted with SLIT in tablets for grass allergy in two multinational studies in northern (37) and southern Europe (38). In both cases, the treatment with tablet SLIT was economically superior to symptomatic treatment alone, after adjusting for the comparable cost differences among national health care services. SLIT resulted in a significant gain of quality-adjusted life years (QALY) and in a significant cost saving.

NEW DIRECTIONS FOR SLIT ADMINISTRATION

The favorable safety profile of SLIT is reported in adults (9,39) and in children younger than five years (40,41). The safety of SLIT has led some investigators to try ultra-rush (reaching maintenance within 2 hours of initiating SLIT) or no-updosing (initiation of SLIT with maintenance vaccine concentration). Both of these approaches may be feasible (24,25,42–46). Such protocols are simpler and more convenient to manage. Two large, randomized trials (24,25) were performed with the no-updosing regimen, and safety was as favorable as studies performed with the traditional updosing approach. A third randomized trial compared the safety of the traditional updosing regimen with no-updosing (46) in 135 patients and showed no difference in the rate and type of adverse events between the two groups of patients.

SLIT can be administered in different forms (drops, single-dose vials, predosed dispensers, tablets). Soluble tablets were first introduced in Italy during the 1980s (47) and proved to be a convenient and easy way to manage modality for treatment. Large trials have used soluble tablets in the later 20th and early 21st centuries. Tablets have the advantage of simplicity, thus minimizing possible dosing errors. The rate of dissolution in the oral cavity can be adjusted by modifying the formulation. Some allergen tablets can be stored at room temperature, improving the convenience of the treatment. On the other hand, soluble tablets cannot be divided, and therefore adjusting or reducing the dose may be a problem. Nonetheless, most likely soluble tablets will be the preferred method of SLIT administration in the future.

POTENTIAL CHALLENGES

The efficacy of SLIT has been demonstrated by clinical trials and meta-analyses (10,48–51). Nevertheless, answers to several questions are needed. These include the following:

1. The optimal dose of allergen is probably the most important variable to be defined. Clinical trials were conducted with variable doses of allergens, usually labeled in arbitrary units and often not reported with the content of allergens in micrograms. Positive and negative clinical results occur with doses ranging from 5 to 300 times greater than the doses used in an analogous SCIT course. Thus, it is currently not possible to specify the optimal SLIT dose. A 2006 study with grass allergen SLIT in tablets compared the effects of three different doses labeled as 2500, 25,000, and 75,000 SQ-T (25). These doses correspond to 0.5, 5, and 15 μg of Phl p 5, respectively. The results show dose-dependent efficacy of SLIT, with the greatest dose (15 μg of Phl p 5) being the most efficacious. This is the first rigorous dose-ranging trial performed with SLIT. Such studies are complex, expensive, and time-consuming but necessary before SLIT can be confidently used in clinical care.
2. Long term benefits from SLIT, such as persistent symptom relief after discontinuations or prevention of disease progression, are not fully established. The optimal SLIT duration for maximum, if any, long-term benefit is not known. Persistent symptom improvement has been suggested by the results of a single, open, nonrandomized trial (52). Additional data with more rigorous designs are needed. There is only one randomized, nonblinded trial, suggesting a reduced occurrence of asthma after completion of a course of SLIT (53).
3. The mechanisms of action of SLIT are not yet fully understood. Some data would suggest that SLIT may act via the Treg cells (54), but other studies suggest that the mechanisms of action differ from SCIT (55,56). A more detailed knowledge of the molecular events underlying clinical efficacy are required to improve the treatment (57).
4. Combination therapy with multiple allergens has not been sufficiently evaluated from both an efficacy and safety perspective. Adverse reactions to SLIT, including anaphylaxis, may occur, particularly with multiple allergen mixtures or non-standardized products. Since SLIT is generally administered at home without professional supervision, the risk of serious reactions needs optimal clarification to properly inform the treated patient and protect the treating physician.

CONCLUSIONS

Specific immunotherapy is a cornerstone in the management of respiratory allergy since it is allergen specific, immunomodulating, and affects disease progression. SLIT, introduced about 20 years ago, is potentially a significant advance, offering an excellent safety and acceptance profile. The efficacy trials and experience with SLIT have lead to confusion concerning the wide range of doses and variable frequency and regimens of administration. SLIT is commercialized in Europe as an individualized prepared product offered by several manufacturers, each with their own standardization procedure and suggested maintenance dose, similar to variability associated with SCIT. There is a trend to more consistent dosing and administration of SLIT. Moreover, in the more recent trials, the definition of an optimal dose is better identified, and there is a general trend toward more standardization of the administration. The potential of using little or no updosing with the convenience of once-daily maintenance is very promising, and such products have been approved by the European drug agency. SLIT is self-administered and managed by the patient at home, thus detailed instructions and careful follow-up of treated patients are required. The prescription of SLIT should be made by a specialist with detailed knowledge of the strengths, limitations, and adverse effects of SLIT compared with alternative therapies, including SCIT. Effective application of SLIT requires an accurate diagnosis of allergic disease and an evaluation of the expected benefit/cost ratio compared with other alternatives.

SALIENT POINTS

- Low-dose SLIT was first introduced in 1986, and it is now accepted as a viable alternative to the injection route (SCIT) and is routinely used in many European countries.
- The clinical efficacy of SLIT has been demonstrated in many clinical trials and meta-analyses.
- SLIT can be administered as either drops or tablets, preseasonally or continuously.
- Regimens and maintenance doses are highly variable among manufacturers.
- The usual SLIT course involves a build-up phase followed by a maintenance phase. The excellent safety profile has resulted in regimens without an induction or updosing phase. This approach is desirable for simplicity.
- The SLIT prescription requires a fund of knowledge similar to SCIT. Thus, the decision to initiate SLIT should be made by a trained specialist after thorough evaluation, proper diagnosis, and consideration of other therapeutic options.
- The enduring and potential disease modifying effects of SLIT need to be confirmed.
- The mechanisms of action of SLIT, although partially similar to SCIT, need to be clarified, and additional studies will be necessary.

REFERENCES

1. Noon L. Prophylactic inoculation against hay fever. Lancet 1911; i:1572–1573.
2. Passalacqua G, Albano M, Riccio A. Local nasal immunotherapy: experimental evidences and general considerations. Allergy 1997; 52(suppl 33):10–16.
3. Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. J Allergy Clin Immunol 2003; 111:437–448.
4. Committee on the safety of medicines. CSM update. Desensitizing vaccines. Br Med J 1986; 293:948.
5. Bernstein DI, Wanner M, Borish L, et al. Immunotherapy Committee, American Academy of Allergy, Asthma and Immunology. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990–2001. J Allergy Clin Immunol 2004; 113:1129–1136.
6. Lockey RF, Benedict LM, Turkeltaub PC, et al. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1987;79:660–677.
7. American Academy of Allergy Asthma and Immunology. Position statements. Controversial techniques: provocation testing (sublingual). J Allergy Clin Immunol 1981; 67:337–338.
8. Scadding K, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to dust mite. Clin Allergy 1986; 16:483–491.

9. Cox LS, Linnemann DL, Nolte H, et al. Sublingual immunotherapy: a comprehensive review. J Allergy Clin Immunol. 2006; 117:1021–1035.
10. Passalacqua G, Durham SR. ARIA update: allergen specific immunotherapy. J Allergy Clin Immunol 2007; 119:881–891.
11. Bousquet J, Lockey R, Malling HJ. World Health Organization Position Paper. Allergen immunotherapy: therapeutical vaccines for allergic diseases. Allergy 1998; 53(suppl 44):1–42.
12. Malling HJ, Abreu-Nogueira J, Alvarez-Cuesta E, et al. EAACI Position Paper: local immunotherapy. Allergy 1998; 53:933–944.
13. Bousquet J, Van Cauwenberge P. Allergic Rhinits and its impact on asthma. J Allergy Clin Immunol 2001; 108(suppl 5):S147–S334.
14. Becker WM, Vogel L, Vieths S. Standardization of allergen extracts for immunotherapy: where do we stand? Curr Opin Allergy Clin Immunol 2006; 6:470–475.
15. American Medical Association. American Medical Association, Current Procedural Terminology (CPT) 2005. Standard ed. Chicago: American Medical Association, 2005.
16. Dunsky E, Goldstein MF, Dvorin MJ, et al. Anaphylaxis due to sublingual immunotherapy. Allergy 2006; 61:1235.
17. Eifan AO, Keles S, Bahceciler NN, et al. Anaphylaxis to multiple pollen allergen sublingual immunotherapy. Allergy 2007; 62:567–568.
18. Pajno GB, Morabito L, Barberio G, et al. S. Clinical and immunological effects of longterm sublingual immunotherapy in asthmatic children sensitized to mite: a double blind study. Allergy 2000; 55:842–849.
19. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double blind study. Allergol Immunopathol 1990; 18: 277–284.
20. Hirsch T, Sahn M, Leupold W. Double blind placebo controlled study of sublingual immunotherapy with house dust mite extracts in children. Pediatr Allergy Immunol 1997; 8:21–27.
21. Passalacqua G, Altrinetti V, Villa G, et al. Sublingual swallow or spit? Allergy 2001; 56:578.
22. Marogna M, Spadolini I, Massolo A, et al. Effects of sublingual immunotherapy for multiple or single allergens in polysensitized patients. Ann Allergy Asthma Immunol 2007; 98:274–280.
23. Kleine-Tebbe J, Ribel M, Herold DA. Safety of a SQ-standardised grass allergen tablet for sublingual immunotherapy: a randomized, placebo-controlled trial. Allergy 2006; 61:181–184.
24. Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. Allergy 2006; 61:185–190.
25. Durham SR, Yang WH, Pedersen MR, et al. Sublingual immunotherapy with once-daily grass-allergen tablets: a randomised controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117:802–809.
26. Bordignon V, Parmiani S. Variation of the skin end-point in patients treated with sublingual specific immunotherapy. J Investig Allergol Clin Immunol 2003; 13:170–176.
27. Lombardi C, Gargioni S, Venturi S, et al. Controlled study of preseasonal immunotherapy with grass pollen extract in tablets: effect on bronchial hyperreactivity. J Investig Allergol Clin Immunol 2001; 11:41–45.
28. Marogna M, Spadolini I, Massolo A, et al. Long lasting effect according to the duration of SLIT: a 15-year prospective study [abstr]. XXVI EAACI Meeting, 2–13 June, 2007; Goteborg, Sweden; 2007:276.
29. Melillo G, Bonini S, Cocco G, et al. EAACI provocation tests with allergens. Report prepared by the European Academy of Allergology and Clinical Immunology Subcommittee on provocation tests with allergens. Allergy 1997; 52(suppl 35):5–35.
30. Gosepath J, Amedee RG, Mann WJ. Nasal provocation testing as an international standard for evaluation of allergic and nonallergic rhinitis. Laryngoscope 2005; 115(3):512–516.
31. Roder E, Berger MY, Hop WC, et al. Sublingual immunotherapy with grass pollen is not effective in symptomatic youngsters in primary care. J Allergy Clin Immunol 2007; 119:892–898.
32. Osterberg L, Blashke T. Adherence to medication. N Engl J Med 2005; 353:487–497.
33. Lombardi C, Gani F, Landi M, et al. Quantitative assessment of the adherence to sublingual immunotherapy. J Allergy Clin Immunol 2004; 113:1219–1220.
34. Passalacqua G, Musarra A, Pecora S, et al. Quantitative assessment of the compliance with a once daily sublingual immunotherapy in real life. J Allergy Clin Immunol 2006; 117:946–948.
35. Passalacqua G, Musarra A, Pecora S, et al. Quantitative assessment of the compliance with once daily sublingual immunotherapy in children. Pediatr Allergy Immunol 2007; 18(1):58–62.
36. Berto P, Passalacqua G, Ortolani C, et al. Economic evaluation of sublingual immunotherapy versus symptomatic treatment in adults with pollen-induced respiratory allergy: the SPAI study. Ann Allergy Asthma Immunol 2006; 97:615–621.
37. Bachert C, Vestenbaek U, Christensen J, et al. Cost-effectiveness of grass allergen tablet (GRAZAX) for the prevention of seasonal grass pollen induced rhinoconjunctivitis - a Northern European perspective. Clin Exp Allergy 2007; 37:772–779.
38. Canonica GW, Poulsen PB, Vestenbaek U. Cost-effectiveness of grass allergen tablet (GRAZAX) for the prevention of grass pollen induced rhinoconjunctivitis in Southern Europe. Respir Med 2007; 101(9): 1885–1894.

39. Gidaro GB, Frati F, Sensi L, et al. The safety of sublingual-swallow immunotherapy: an analysis of published studies. Clin Exp Allergy 2005; 35:565–571.
40. Di Rienzo V, Musarra A, Sambugaro R, et al. Post marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. Clin Exp Allergy 2005; 35:560–564.
41. Fiocchi A, Pajno G, La Grutta S, et al. Safety of SLI T in children aged 3 to 7 years. Ann Allergy Asthma Immunol 2005; 95:254–258.
42. Rossi R, Monasterolo RE. A pilot study of feasibility of ultra-rush (20-25 minutes) sublingual-swallow immunotherapy in 679 patients (699 sessions) with allergic rhinitis and/or asthma. Int J Immunopathol Pharmacol 2005; 18:277–285.
43. Tripodi S, Di Rienzo Businco A, Benincori N, et al. Safety and Tolerability of Ultra-Rush Induction, Less than One Hour, of Sublingual Immunotherapy in Children. Int Arch Allergy Immunol 2005; 139:149–152.
44. Grosclaude M, Bouillot P, Alt R, et al. Safety of various dosage regimens during induction of sublingual immunotherapy. Int Arch Allergy Immunol 2002; 129:248–253.
45. Guerra L, Compalati E, Rogkakou A, et al. Randomized open comparison of the safety of SLIT in a no updosing and traditional updosing schedule in patients with parietaria allergy. Allergol Immunopathol 2006; 34:82–83.
46. Rodriguez F, Boquete M, Ibanez MD, et al. Once daily sublingual immunotherapy without updosing: a new treatment schedule. Int Arch Allergy Immunol 2006; 140:321–326.
47. Passalacqua G, Albano M, Fregonese L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite induced rhinoconjunctivitis. Lancet 1998; 351:629–632.
48. Wilson DR, Torres L, Durham SR. Sublingual immunotherapy for allergic rhinitis. Allergy 2005; 60:3–8.
49. Penagos M, Passalacqua G, Compalati E, et al. Meta-analysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. Chest 2008; 133:599–609.
50. Penagos M, Compalati E, Tarantini F, et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in children. Meta analysis of randomized controlled trials. Ann Allergy Asthma Immunol 2006; 97:141–148.
51. Calamita Z, Saconato H, Bronhara Pelà A, et al. Efficacy of Sublingual immunotherapy in asthma. Systematic review of randomized clinical trials. Allergy 2006; 61:1162–1172.
52. Di Rienzo V, Marcucci F, Puccinelli P, et al. Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a ten year prospective study. Clin Exp Allergy 2003; 33: 206–210.
53. Novembre E, Galli E, Landi F, et al. Coseasonal sublingual immunotherapy reduces the development of asthma in children with allergic rhinoconjunctivitis. J Allergy Clin Immunol 2004; 114:851–857.
54. Cosmi L, Santarlasci V, Angeli R, et al. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. Clin Exp Allergy 2006; 36:261–272.
55. Rolinck-Werninghaus C, Kopp M, Liebke C, et al. Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. Int Arch Allergy Immunol 2005; 136:134–141.
56. Dehlink E, Eiwegger T, Gerstmayr M, et al. Absence of systemic immunologic changes during dose build-up phase and early maintenance period in effective specific sublingual immunotherapy in children. Clin Exp Allergy 2006; 36:32–39.
57. Moingeon P, Batard T, Fadel R, et al. Immune mechanisms of allergen-specific sublingual immunotherapy. Allergy 2006; 61:151–165.

22 Subcutaneous Immunotherapy for Allergic Rhinoconjunctivitis, Allergic Asthma, and Prevention of Allergic Diseases

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INTRODUCTION

Allergic rhinitis, the most common allergic disease, affects about 20% of the adult population (1). Allergic asthma varies in prevalence in different countries, but in the Western world it usually affects more than 5% of the population (2). These diseases, which predominantly affect children and young adults, impair both physical and cognitive functions and quality of life (3). The anatomy and physiology of nasal and bronchial mucosa are very similar, and most patients with allergic asthma also have rhinitis (4). The coincident upper and lower airway dysfunction suggests a continuum of disease between rhinitis and asthma. This is the basis for the concept that the upper and the lower airways be considered a single entity, influenced by a common and evolving inflammatory process, which may be sustained and amplified by interrelated mechanisms (5).

Allergic rhinitis constitutes a major risk factor for development of allergic asthma with approximately 20% of patients with allergic rhinitis subsequently developing asthma (3,6,7). Patients with upper airway disease and associated bronchial hyperreactivity (BHR) are more likely to develop asthma (8,9).

OBJECTIVES OF ALLERGEN-SPECIFIC IMMUNOTHERAPY IN ALLERGIC DISEASES

Allergic rhinitis, especially in seasonal pollen allergy, is a disease in which allergy is the dominant or single eliciting factor, and consequently allergen immunotherapy is more likely to be highly effective. Asthma is a multifactorial and more complex disease in which allergic factors and nonallergic triggers interact and result in bronchial obstruction and inflammation (10). The inhalation of allergens leads to a complex activation of various cell types and the release of proinflammatory mediators. On the basis of allergen sensitization, different situations exist in indoor (house dust mites and animal dander) and outdoor allergy (mostly pollens). Although very few pollen grains can reach the lower airways, these allergens, carried on fragmented pollen or other particles (usually less than 10 μm in diameter), frequently induce asthma via an IgE-mediated mechanism (10,11). Pollen-induced allergic reactions, prolonged over several days, almost always lead to nonspecific BHR. This BHR is usually transient in patients allergic only to pollens, lasting from a few weeks to a few months after the end of the pollen season (12). House dust mites and other indoor allergens induce a sustained inflammation of the bronchi leading to variable BHR. Symptoms are caused by a combination of allergen-induced inflammation and specific and nonspecific BHR (13). The persistent allergen exposure results in a chronic, persistent inflammatory state. Some patients with chronic asthma develop airway remodeling or irreversible structural changes (14). Inflammation and airway remodeling may be involved in the "accelerated decline" of the pulmonary function characterized by a poorly reversible bronchial obstruction appearing after decades of chronic asthma (15). Loss of lung function is associated with permanent bronchial wall

alterations revealed by CT scans (16). The role of allergy in airway remodeling is not definitely defined.

These points suggest that (*i*) allergen-specific immunotherapy may be more rapidly effective in patients allergic to pollens than in those sensitized to indoor allergens; (*ii*) after a long course of the disease, patients with persistent asthma may have permanent airway abnormalities that cannot be reversed by allergen immunotherapy; (*iii*) grass pollen or other predominately seasonal pollen allergy may be an ideal model to study the effects of immunotherapy in patients with normal bronchi; and (*iv*) immunotherapy in mite allergy may be used to examine the effects of treatment in patients with a variable degree of bronchial inflammation and remodeling (17).

The major objectives of the immunomodulation of allergen immunotherapy are to reduce the allergic triggers eliciting symptoms in the short term and to decrease airway inflammation and BHR in the long run. This treatment ideally is prior to significant bronchial damage. Allergen immunotherapy appears to be the only treatment that modifies the course of the disease, either by preventing the development of new sensitivities or by altering the natural history of allergic airway diseases.

TREATMENT STRATEGY

The management of allergic airway diseases is based on combining three essential interventions, allergen avoidance, pharmacological treatment, and allergen-specific immunotherapy, with education of the patient on the nature of the disease. Education should include identification of triggers and relievers, appropriate variation of drug treatment, and recognition of disease involvement of the lower airways when appropriate (3). Nonspecific interventions are not restricted to the causative allergen but rather to the avoidance of nonspecific triggers. Specific interventions include avoidance of the causative allergen and application of allergen-specific immunotherapy.

The relative advantage of each of these three interventions, allergen avoidance, pharmacotherapy, and allergen immunotherapy, is variable, but the combination of interventions should improve the clinical outcome. Allergen avoidance always is the first-line attempt and may reduce the need for additional intervention even when not completely effective (18). A meta-analysis does not confirm the efficacy of single measures (19). A multifaceted intervention in selected subjects may be effective, but more data are needed. Drug treatment is often the next step to reduce symptom severity. However, for patients with a constant need for preventive (local steroids) pharmacotherapy, early initiation of allergen immunotherapy is advantageous, while the severity of the disease is modest and when the possibility for prevention of asthma is greatest (20,21). Allergen immunotherapy interferes with the pathophysiological mechanisms of allergic inflammation, with a potential for a prolonged effect compared with symptomatic pharmacological treatment (20,21). Although drugs are highly effective and with limited side effects, they represent a symptomatic treatment, while immunotherapy represents the only treatment that might alter the natural course of the disease (22). Using an appropriate allergen vaccine for correct indications, immunotherapy will significantly reduce the severity of the allergic disease, reduce the need for antiallergic drugs, and consequently improve the quality of life for allergic patients (23,24).

A significant proportion of subjects with persistent rhinitis have inflammation in the lower airways during allergen exposure (25). The symptoms from the lower airways often do not disturb the patients and the inflammation is therefore inconsistently or insufficiently treated. Allergen immunotherapy, as a solitary treatment, may ameliorate inflammatory reactions independent of the shock organ, that is, the treatment potentially will help rhinitis and simultaneously improve asthma or BHR. Consequently, considering the allergen-IgE-mediated disease as a multiorgan disease, it is important to consider immunotherapy based on the allergen sensitization rather than the disease or symptoms (21). The advantage of combining allergen avoidance, immunotherapy, and drug treatment requires further investigation, focusing on patient compliance, long-term preventative aspects, and cost-effectiveness/side effects.

The advantages of introducing allergen-specific, disease-modifying interventions (allergen avoidance and immunotherapy) in allergic disease management should be considered

when developing a treatment strategy. It is advantageous to apply a specific treatment to interfere with the activation of the immunological mechanisms rather than to only treat symptoms. Furthermore, the exclusive use of drug treatment may affect the identification of specific allergen sensitization in the future by partially masking the association of allergen exposure and symptoms. Allergen identification is essential for prescribing specific treatment. Moreover, the lack of knowledge concerning the specific allergens reduces the possibility of avoiding allergens.

New insights into allergic inflammation provide a platform for further investigations of the advantages and drawbacks of different intervention strategies and for the development of new, effective and safe treatment principles. Conventional immunotherapy using native allergen extracts may in the future be substituted by a more refined and precisely targeted, allergen-specific immunological intervention based on knowledge of immunological mechanisms. One example of this approach is the use of recombinant allergens for immunotherapy.

ESTIMATING CLINICAL EFFICACY OF SUBCUTANEOUS IMMUNOTHERAPY

Allergic patients suffer from the clinical manifestations of the disease, i.e., rhinitis, conjunctivitis, and asthma, and clinical efficacy should reflect a significant reduction in disease severity (26). Consequently, only randomized, placebo-controlled clinical trials can be used to evaluate efficacy. The primary end point, if possible, should be a single end point, globally assessing the patient and the comorbidities. In the case of allergic rhinitis induced by pollens, the total symptom score including all nasal symptoms (nasal obstruction, rhinorrhea, sneezing, and pruritus) with one or more ocular symptoms is preferable. The use of rescue medication reflects symptom severity. Therefore, a primary end point may include both symptom severity and intake of rescue medications. Different approaches to combine the two scoring systems have been proposed, but there is no standardized method. For pollen allergy, the pollen count is important and the clinical efficacy of allergen immunotherapy should preferably be recorded during the entire pollen season. However, the primary outcome analysis can be made for the peak of the pollen season, represented for instance by the weeks including 50% of the total pollen load. House dust mites and animal dander can induce both intermittent and persistent symptoms, thus, the patients with persistent rhinitis and/or asthma should be carefully selected for study (27). For evaluating efficacy in asthma, bronchial symptoms (wheezing, shortness of breath, cough) can be used as a primary outcome, but a less subjective measure, such as FEV_1 or peak flow, is usually included as a co-primary end point. As secondary outcomes, the control of the disease and quality of life appear to be important parameters. Other outcomes, such as nonspecific BHR, may complement the study. Changes in immunological parameters and response to airway or mucosal challenge may be of interest in elucidating mechanisms but cannot replace the clinical evaluation.

The magnitude of clinically significant efficacy, clinically relevant reduction in disease severity, may be debated. By including a large number of participants, statistically significant but clinically irrelevant differences may be observed. The magnitude of efficacy should be relevant, i.e., the reduction in symptom scores and drug consumption should, from a clinical point and patient perspective, significantly reduce the morbidity of the disease. A review of 68 placebo-controlled, double-blind (PCDB) subcutaneous immunotherapy (SCIT) studies providing symptom/medication scores (26) suggests that a mean reduction in disease severity of $>30\%$ above the placebo effect is clinically relevant. Depending on the risks of intervention, costs, patient preferences, and inconvenience, this figure is subject to change.

When evaluating the clinical efficacy of interventions, the study design is critical to providing conclusive answers. The primary and, when appropriate, secondary outcome measures should be clearly defined. The sample size should be large enough to have a high probability (power) of statistically detecting a clinically important difference. Patients should be randomized to study groups to avoid bias in group characteristics, and the study should be a PCDB trial (27).

The majority of studies evaluating clinical efficacy focus on short-term efficacy, i.e., efficacy obtained during active treatment, often after a rather short period of treatment for one-half to one year. This degree of efficacy is important, but a major argument for instituting

allergen immunotherapy is to obtain long-term disease modifying capacity and to prevent disease progression.

CONTROLLED STUDIES TO DEMONSTRATE EFFICACY

Published studies indicate that SCIT is an effective treatment of allergic airway diseases, under the conditions of a careful selection of patients and the use of quality allergen extracts for treatment vaccines (26). In 1998, the author reviewed all double-blind, placebo-controlled immunotherapy studies of rhinitis or asthma. The evaluation criteria stipulated that the studies were peer reviewed, printed in English, included symptom-medication scores, and published since 1980 (26). The reason for not including older studies is that this arbitrary limit coincides with a general use of vaccines constituted from adequately standardized allergen extracts. Clinical efficacy was estimated by symptom-medication scores or by measuring the area under the curve of symptom-medication scores during the study period for both the active group and the placebo group (Fig. 1). The magnitude of improvement induced by active treatment was calculated as the percentage reduction in disease severity (symptom-medication scores) compared with placebo treatment. In 43 studies fulfilling the strict inclusion criteria, a mean clinical improvement of 45% greater than placebo was observed in 1120 actively treated subjects. Prior to the analysis, an improvement of at least 30% (mean) was arbitrarily chosen as the lower limit for clinical efficacy based on statistical significance and for balancing a clinically relevant efficacy with the inconvenience and risks of injection immunotherapy. Thus, the studies showed a clinically significant mean improvement.

An update of the original review (MedLine search terminated June, 2007) identified 59 double-blind, placebo-controlled studies, giving 65 comparable groups as some studies include more than one actively treated groups, evaluating the clinical efficacy of SCIT in rhinitis and 21 (25 comparable groups) in asthma (Malling H-J, personal communication). The update confirms the conclusion of the initial review (26) but adds birch and other pollens to the list of effective allergen vaccines. To evaluate efficacy an approach relying on statistical change was used. Studies are graded as "unequivocal efficacy" in the situation that both symptom scores and drug scores show a statistical significant difference between active treatment and placebo. In studies where either symptom scores or drug scores show a statistical significance, the grading "possible efficacy" is used (these studies probably are effective, but the documentation is not as convincing). Studies without statistical significance between treatment groups are graded as "no efficacy." For rhinitis, 8% (5 out of 65 comparable groups) were graded no efficacy, 6% (4 of 65) possible efficacy, and 86% (55 of 65) efficacy. For asthma the figures are no efficacy in 16% (4 of 25), possible efficacy in 20% (5 of 25), and efficacy in 64% (16 of 25). The mean clinical effect in these studies is a reduction in rhinitis severity of 40% and asthma severity of 45%, comparable with the 45% originally described. Including studies published since the original review (26), the additional number of actively treated patients

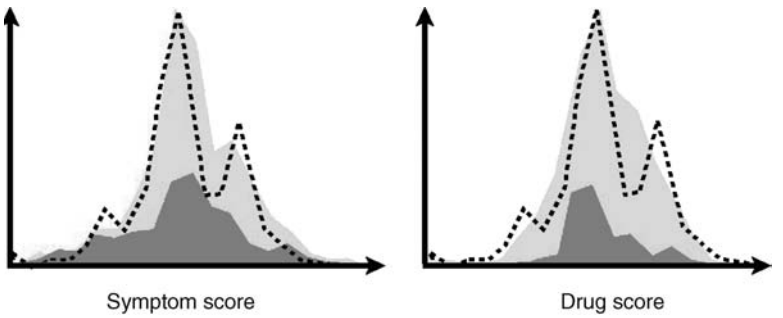

Figure 1 Magnitude of clinical efficacy estimated by measuring the area under the curve of immunotherapy treated (*darkly shaded areas*) as percentage of placebo treated (*lightly shaded areas*). The median symptom score and medication score of the immunotherapy group is 39% and 28% of the placebo group, respectively, indicating a mean reduction in symptoms of 61% and of 72% in medication scores. The dotted line denotes airborne grass pollen counts. *Source*: Data adapted from Ref. 52.

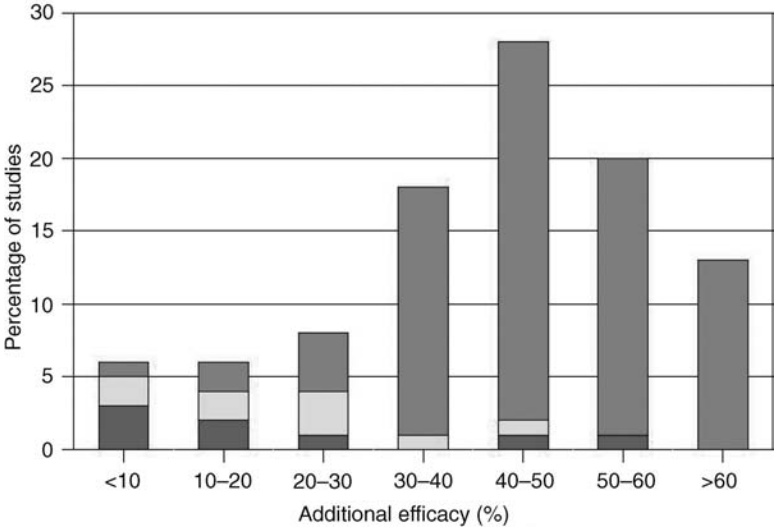

Figure 2 (*See color insert.*) Graded magnitude of clinical efficacy of placebo-controlled, double-blind rhinitis immunotherapy studies published in 1980–2007, including 2253 actively treated patients (1776 rhinitis patients and 477 asthma patients). The additional efficacy compared to placebo is given in intervals of 10%. The 80 clinical studies result in 90 groups comparable with placebo as some studies include two actively treated groups. Furthermore, the statistical significance is related to the magnitude of efficacy: green indicates significant difference from placebo for both symptom scores and drug scores (clear cut clinical efficacy), yellow indicates significance for either symptom scores or drug scores (possible clinical efficacy), and red indicates no significant difference for either symptom scores or drug scores (no efficacy).

brings the total number of patients supporting the evidence of efficacy to about 2250. Figure 2 shows the relation between the magnitude of efficacy (in 10% intervals) and the grading system based on the statistical analysis. If the original choice of 30% improvement more than placebo is chosen as a clinically significant threshold to justify the inconvenience of SCIT (26), then almost 80% of published studies in 2007 document a benefit with SCIT. This magnitude of efficacy is equivalent to or better than intranasal corticosteroids and considerably better than antihistamines or leukotriene inhibitors (3,28).

The allergens in the studies reviewed include seasonal pollens in 74%, dust mite in 19%, and animal danders and molds in less than 5% (Fig. 3). Pollen allergens, especially grass and ragweed, have the best documentation for efficacy. The efficacy for dust mite immunotherapy is convincing, but careful selection of candidates is crucial. The number of studies using animal danders and molds is insufficient for confident conclusions.

Pollen Allergens

The best documentation for efficacy of SCIT is in pollen allergy. The majority of patients suffer from rhinitis that may be complicated by asthma with high pollen exposure. Efficacy seems greater for asthma developing in subjects with allergic rhinitis than in subjects with asthma without rhinitis. This is probably due to the shorter duration of the allergic asthma symptoms and limited airway remodeling (29). Studies documenting clinical efficacy have been carried out with allergen vaccines of grass, ragweed, birch, mountain cedar, *Parietaria*, cypress, and other pollens. The dose dependency for clinical efficacy, based on natural exposure to grass pollens, is documented in one large-scale study (24).

An interesting non-placebo-controlled, but double-blind, comparative study evaluated the clinical efficacy of preseasonal birch pollen immunotherapy versus nasal glucocorticosteroid administered as budesonide, 400 μg daily (30). Symptom scores in the two groups were identical during the first four weeks of the six-week season, and only during the final two weeks did nasal glucocorticosteroid-treated subjects show fewer symptoms than immunotherapy-treated subjects. No difference in medication scores was observed indicating that a short course of preseasonal immunotherapy is almost as effective as this relatively high-dose

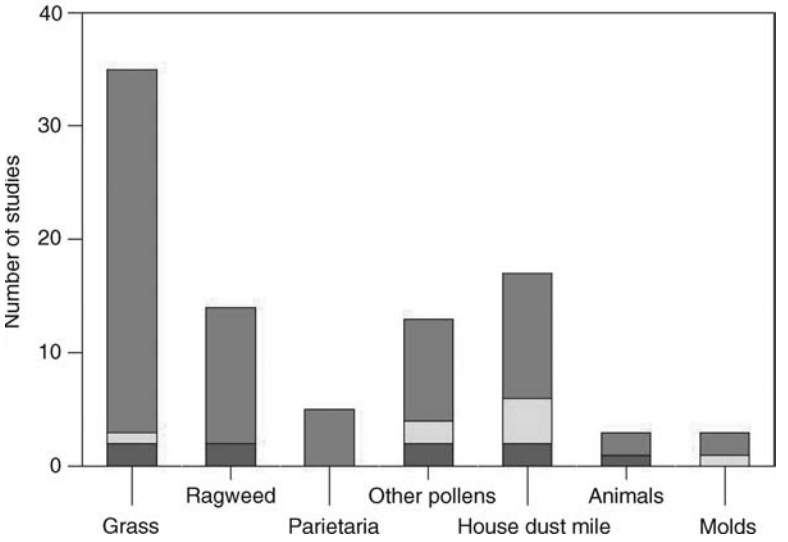

Figure 3 (*See color insert.*) Allergen vaccines used in placebo-controlled, double-blind rhinitis immunotherapy studies published in 1980–2007. Clinical efficacy is graded based on statistical significance (*green* indicated significance for both symptoms scores and drug scores (clear cut clinical efficacy), yellow indicates significance for either symptom scores or drug scores (possible clinical efficacy); and red indicates no significant difference for either symptom scores or drug scores (no efficacy).

intranasal glucocorticosteroid. An additional effect of immunotherapy, reinforcing that it represents a systemic treatment with effect on both the upper and the lower airways, was that seasonal peak expiratory flow values decreased significantly only in the nasal glucocorticosteroid-treated subjects. Furthermore, only immunotherapy prevented the seasonal BHR and eosinophil activation in asthmatic subjects.

House Dust Mite Allergens

Inhaled house dust mite allergen may induce both rhinitis and asthma in the same patient. Most studies evaluating the clinical effect of house dust mite immunotherapy use asthma as the indication. On the basis of clinical symptoms and medication scores, clinical efficacy is documented for house dust mite immunotherapy using vaccines from both *Dermatophagoides pteronyssinus* and *D. farinae*. House dust immunotherapy reduces bronchospasm following bronchial challenges with house dust mite extracts and increases the threshold dose to elicit immediate bronchial obstruction. Finally, the late-phase reaction following bronchial challenge with house dust mite is inhibited in many studies after SCIT (31). The decrease in the late-phase reaction is important as it indicates a decrease in the inflammatory reaction in the airway mucosa. However, some of the results with house dust SCIT are not impressive and, especially in adults, are sometimes negative. Subjects with house dust mite asthma often have perennial symptoms with occasional exacerbations, and the relative contribution of house dust mite sensitization to clinical symptoms may be difficult to determine. The variable role of allergen sensitization in determining symptoms with perennial allergic disease may be the reason for negative studies with SCIT. The glucocorticosteroid-sparing effect of SCIT was shown in a study demonstrating no improvement in asthma symptoms (32). Patients requiring at least the equivalent of 500-μg inhaled fluticasone propionate daily were included; and in patients with moderate persistent asthma, the reduction in inhaled corticosteroids was statistically significant for those treated with SCIT compared with placebo after year 2 and year 3. The median dose reductions after three years were 90% for SCIT and 42% for placebo ($p = 0.04$).

The importance of the allergen dose required to induce a significant clinical effect has been investigated with house dust mite allergen (33). Using a maximal dose of 7 μg of Der p 1, subjects experienced significant improvement in $PD_{20}FEV_1$ (provocative dose sufficient to decrease FEV_1 by 20%), but the maximal effect was observed with a dose of 21 μg of Der p 1. Pretreatment asthma severity is critically related to the outcome of immunotherapy. Subjects

with severe asthma, FEV_1 under 70% of predicted values after optimal pharmacological treatment, demonstrated less improvement with immunotherapy than those with milder asthma (34).

Other Allergens

A number of studies demonstrate significant improvement in bronchial sensitivity, clinical symptoms, and medication needs in subjects with cat-allergic asthma following cat immunotherapy (35–37). Immunotherapy with dog is not validated, with no studies documenting clinical efficacy (38).

Immunotherapy studies with several allergens or potential allergens have not shown consistent benefit. These include most molds (39), *Candida albicans*, *Trichophyton*, and bacterial vaccines. This may be the result of poor quality of the extracts for many of these antigens. However, SCIT with standardized *Cladosporium* and *Alternaria* vaccines is highly effective in rhinitis and/or asthma in two studies (40,41), although less effective in a third (42). DBPC studies of SCIT with bacterial vaccines for treatment of rhinitis and/or asthma do not show efficacy (43). There is no study of immunotherapy with *C. albicans* or *Trichophyton*, and the quality of the extracts is generally inadequate.

Multiple Allergens

Few studies have investigated SCIT with multiple allergens. A controlled trial of SCIT, using a mixture of allergens, for treatment of mild to severe asthma in a population of optimally treated allergic children shows no significant difference compared with placebo (44). There are several methodological factors that may have led to the negative results. Among them, the study was carried out using mixtures of allergens including mold extracts, some potentially important allergens were not used, the dose of individual allergens was below the optimal dose, and the population of asthmatic children was not optimally selected (21). A major difference between American and European allergists/immunologists is the use of allergen mixtures in SCIT. Mixing of unrelated allergens is not recommended in Europe (45), whereas this is common practice in the United States (46). Mixing of allergens has not been documented to be effective in clinical studies, but existing data are insufficient to judge if mixed SCIT is effective (47,48). Other than mixing allergens in a vaccine that result in mutual degradation or diluting the dose of each allergen below the optimal dose, there is no rationale to explain why mixed vaccines would not be effective with SCIT.

Meta-Analysis

Using the Cochrane methodology, meta-analysis documents that SCIT for respiratory allergy is effective for both seasonal rhinitis (49) and asthma (47). The meta-analysis for rhinitis only includes pollen allergens. The rhinitis analysis identifies 51 studies fulfilling the inclusion criteria, but the data on symptom scores is based on only 15 trials. The overall reductions in symptom scores and medication scores are statistically significant, with a standard mean difference (SMD) of -0.73 (95% confidence interval, -0.97 to -0.50) for symptoms and -0.57 (95% confidence interval, -0.82 to -0.33) for medication scores in 13 trials.

Interpretation of the clinical importance of the effect size is difficult. The authors' interpretation is that "SCIT in suitable selected patients with seasonal allergic rhinitis results in a significant reduction in symptom scores and medication use. SCIT has a known and relatively low risk of severe adverse events."

The updated asthma analysis includes 75 randomized, controlled trials (47). There are 36 trials of SCIT with house dust mite, 20 with pollen extracts, 10 with animal dander, two with *Cladosporium*, one with latex, and six trials with a mixture of multiple allergens. Concealment of allocation was assessed as clearly adequate in only 15 of these trials. Significant heterogeneity among these 75 trials was evident in a number of comparisons. Overall, there was a significant improvement in asthma symptoms, SMD -0.72 (95% confidence interval, -0.99 to -0.33), and medication use, SMD -0.80 (95% confidence interval, -1.13 to -0.48). SCIT reduced allergen-specific BHR with some reduction in nonspecific BHR. There was no consistent effect on pulmonary function with SCIT. The reviewer's conclusions are that "immunotherapy reduces asthma symptoms and use of asthma medications and improves bronchial hyperreactivity. One trial found that the size of the benefit is possible comparable to inhaled steroids. The possibility of adverse effects (such as anaphylaxis) must be considered."

On the basis of meta-analysis, the documentation for efficacy of SCIT is evidence based with the strength of A for both rhinitis and asthma (50).

LONG-TERM EFFICACY AND ALTERATION OF THE NATURAL COURSE OF ALLERGIC DISEASE

A fundamental objective in assessing the value and applicability of SCIT is to document its long-term efficacy and preventative capacity. Without long-term reduction in disease severity and disease modification after terminating treatment, immunotherapy may not be a viable alternative to pharmacological treatment due to cost and risk (20). Some studies indicate that the treatment may have a long-lasting effect. The PCDB study published by Durham et al (51) is a continuation of a previous study documenting efficacy SCIT (52). Patients with severe grass pollen rhinitis, uncontrolled by standard pharmacotherapy including topical glucocorticosteroids, were treated with preseasonal grass-SCIT and placebo. After less than one year of grass-SCIT, subjects experienced improved symptoms and a 60% reduction in the need for rescue medications. After three years of active treatment, subjects were rerandomized to continue with SCIT or to receive placebo. At the termination of the study, the symptom-medication scores between subjects continuing and discontinuing immunotherapy did not differ. Treated subjects, compared with a matched control group not receiving immunotherapy, had a >75% reduction in disease severity. An open study compared 13 patients, six years after having terminated a three-year course of grass pollen SCIT, with 10 patients of the control group who were not treated with SCIT (53). Clinical symptoms and the use of rescue drugs in the previously SCIT-treated subjects were significantly lower than in the control group. The patients were reevaluated after 12 years, and the beneficial effect of SCIT persisted (54). A retrospective, questionnaire-based evaluation compared SCIT-treated asthmatic subjects with dust mite or grass allergy with a matched control group approximately nine years after cessation of SCIT (55). The 48 SCIT-treated patients were three times less likely to experience asthmatic symptoms as the control group. However, asthma pharmacological therapy did not statistically differ between the two groups (55).

SAFETY

Injections of allergens into an IgE-sensitized patient always include a risk of inducing anaphylaxis. On the basis of the controlled studies included in the efficacy evaluation (Malling H-J, personal communication), 68% (44 out of 65) of studies in rhinitis and 72% (18 of 25) of studies in asthma reported systemic side effects. Anaphylactic shock, the clinically most important side effect, was observed in 6% (4 of 65) of rhinitis studies and 20% (5 of 25) of asthma studies. These data support the view that asthma is a separate risk factor for serious systemic side effects during SCIT (56) and also confirm the importance of the administration of SCIT as described in international recommendations (45,46).

INDICATION AND CONTRAINDICATIONS

When defining the indication for SCIT for inhalant allergies, it is advantageous to recognize that the allergen-IgE reaction results in a multiorgan disease, with symptoms in many patients occurring in the eyes, nose, and lungs (21). Some patients have symptoms predominantly from one organ, which does not indicate an absence of inflammation in other parts of the airways (25). Consequently, all symptoms should be considered when selecting the most appropriate treatment of the allergic disease (21). Before instituting immunotherapy, the following must be considered: (*i*) the severity and duration of symptoms, (*ii*) the requirement for and the effect of pharmaceutical therapy, (*iii*) the risk incurred by the treatment and the risk of the disease, (*iv*) psychological factors, and (*v*) the patient's attitude related to alleviating symptoms (drugs) versus interfering with the pathophysiological background of the disease (specific treatment).

The indication for allergen immunotherapy in allergic airway diseases relates to both the severity of the disease and the duration of the symptoms (20,21). Mild rhinitis symptoms that

respond adequately to oral or topical antihistamines are not generally an indication for beginning SCIT. However, SCIT is a consideration if there is a need for repeated courses of topical glucocorticosteroids, both for a short or a long season, or symptoms lasting several months, even if these symptoms are rather mild and respond to pharmacological treatment (20).

In asthma, the number of drug doses needed to reduce symptoms, the frequency of daily administrations, and the number of organs needing treatment has an important influence on the rationale for initiating SCIT. SCIT offers the possibility of reducing the requirement for drugs and minimizing symptoms. It is a mistake to institute SCIT only in patients who do not respond to drug treatment or who develop side effects during drug treatment (20). Even though some guidelines recommend giving allergen SCIT only when all other forms of treatment fail, SCIT may be considered in patients with mild disease. Optimal results of SCIT are obtained in patients with mild disease, i.e., requiring a rather modest pharmacological treatment (34). The advantages of SCIT include minimizing medications, symptoms, and disease progression. The latter has not been achieved with pharmacological therapy.

Children probably respond better to immunotherapy than adults (20). Although this observation may relate to the chronological age of the patient, it is more likely related to the duration of the allergic disease. Attempts to interfere with the natural course of the disease should be introduced at a time when the patient has the capacity to respond positively, i.e., before the disease becomes a chronic, irreversible condition (57).

Risk-benefit assessments of both the allergic disease and treatment options are important for evaluating the indication for SCIT (20,21). The disabling nature of allergic rhinitis, which diminishes performance capacity in schoolwork, job, and social contacts, is of great importance for the affected subject's quality of life (58). Of additional concern is that a number of allergic rhinitis patients develop asthma during the course of their disease (59,60). Asthma is more severe than rhinitis in relation to acute attacks, hospital admissions, and possibly in days of missed work or school. Furthermore, asthma may develop into chronic, irreversible pulmonary impairment. Therefore, inadequate treatment may be associated with increased risk of remodeling and permanently decreased lung function (61,62). However, no treatment, including SCIT, has been proven to affect the risk of developing irreversible loss of lung function.

The hazards of SCIT are related to the risk of inducing anaphylaxis. The rate of severe, systemic reactions in patients with rhinitis treated with high potency vaccines is variable but is approximately 0.5% to 5% of the injections (63,64). Reactions are more likely during the induction phase but may occur at any time during the course of treatment (20). In asthma, the risk of systemic reactions is greater, primarily due to the increased possibility of bronchial obstruction. Consequently, it is advisable to monitor lung function before injections and to ensure optimal asthma pharmacotherapy during SCIT (20,21). Systemic reactions or anaphylaxis is a general limitation for the use of SCIT. Therefore, the decision to initiate SCIT and its administration should be directed by a specialist knowledgeable about these issues and prepared to treat anaphylaxis (20,21,45).

Psychological factors potentially affecting the decision to use SCIT include compliance with pharmacotherapy, concern of possible medication side effects, and the patient's perception of having a chronic disease with a continual need for medications. Studies of drug compliance or adherence in asthmatic patients show that only approximately half or less of the prescribed drugs are used (65,66). Drug compliance may be greater in rhinitis due to the usually shorter duration of symptoms in seasonal allergic rhinitis and recognition of drug efficacy due to more rapid or more evident symptom response (67). However, perennial, persistent, or chronic rhinitis is often the reason patients seek specialty care. These subjects are less likely to perceive symptom response with medications, making the likelihood of adherence lower due to the chronic nature of the therapy. It is important in treating allergic diseases to be aware of the patient's perception of disease severity, the psychological motivation associated with seeking care, the benefit or lack of benefit of prior pharmacotherapy, and the scientific understanding of the rationale for treating allergic inflammation.

Several aspects of the treatment of allergic rhinitis require careful consideration: (*i*) about 20% of subjects with rhinitis experience symptoms from the lower airways—often neither considered nor treated as asthma; (*ii*) patients with presenting symptoms only from the upper airways have a significant risk of developing asthma; (*iii*) patients with less intensive

Table 1 Considerations for Initiating Immunotherapy in Allergic Airway Diseases

1. IgE-mediated disease demonstrated by the presence of clinically significant positive skin tests and/or serum-specific IgE to relevant allergens
2. Specific allergen sensitivity responsible for clinical symptoms and the severity of disease
   - Confirmed by appearance of symptoms after natural exposure to allergens identified by allergy testing
   - Rarely confirmed by challenge with relevant allergens
3. Nonspecific triggers may play an additional, but only minimal, role in the induction of symptoms
4. Severity and duration of symptoms
   - Involvement of lower airways in patients with rhinitis
   - Increasing symptoms induced by succeeding seasons or perennial exposure
5. Inadequate response of symptoms to initial therapy
   - Response to allergen avoidance/reduction
   - Response to pharmacotherapy
6. Availability of standardized or high-quality allergen extracts ideal for preparation of vaccines for immunotherapy
7. Ideally clinical efficacy and safety of immunotherapy with selected allergens confirmed by randomized placebo-controlled, double-blind studies
8. Psychological and social considerations
   - Cost of pharmacotherapy and immunotherapy
   - Patient comfort level with pharmacotherapy side effects or potential side effects
   - Impaired quality of life despite adequate pharmacotherapy
   - Concern about prevention of long-term disease progression
9. No relative contraindications

Source: Adapted from Refs. 20, 21.

Table 2 General Indications for Immunotherapy in Allergic Airway Diseases

- Patients with symptoms induced predominantly by allergen exposure that cannot be avoided
- Patients with a prolonged season or with increasing symptoms induced by succeeding pollen seasons
- Rhinitis associated with symptoms from the lower airways during peak allergen exposure
- Patients with insufficient symptom control with pharmacotherapy
- Patients who do not wish to be on constant or long-term pharmacotherapy
- Patients with undesirable side effects with pharmacotherapy

symptoms need less symptomatic treatment, but intervention affecting the natural course of the disease is more successful. There are no definite rules for the institution of SCIT in allergic rhinitis or asthma. The initiation of SCIT is based on a careful balancing of advantages and disadvantages, taking into consideration the patient's attitude to both the symptoms and possible treatments of the disease (20). Analyzed in this way, SCIT is not considered to be the ultimate treatment but, instead, is a supplement to pharmacotherapy in the early phase of the disease (20,21). The considerations for initiating immunotherapy in allergic rhinitis and asthma are illustrated in Table 1 and the indications in Table 2 (3,20,21,45). The complexity of these decisions support the practice standard of knowledgeable specialists being involved in the decision rather than remote testing services or vaccine vendors.

Contraindications

In contrast to the multiple factors defining the indications for SCIT, the contraindications are more straightforward (20,21). Relative contraindications involve serious immunopathological or immunodeficiency diseases including some forms of malignancy or cancer treatment (in the European guidelines any malignancy is an absolute contraindication), significant cardiovascular diseases (due to the risk of hypotension and the potential administration of epinephrine), treatment with β-blockers (reduces the effectiveness of epinephrine in the treatment of

anaphylaxis), severe asthma uncontrolled by pharmacotherapy and irreversible airway obstruction defined as FEV_1 consistently <70% of predicted value in spite of adequate drug treatment, severe psychological disorders, and low probability of compliance. Other relative contraindications include initiating SCIT during pregnancy due to the risk of anaphylaxis for the mother and possible associated detrimental effect on the fetus. Well-tolerated and effective SCIT may be continued if pregnancy occurs after a woman has received immunotherapy for several months. Age is a relative contraindication. In children less than five years of age, SCIT with inhalant allergens is less commonly utilized because of less evidence or proof of the role of inhalation allergens in the total manifestations of disease, because of the fact that some infants improve with growth, and because of the difficulty in recognizing systemic reactions in very young children. In patients >50 years of age, allergy may not be as important in the development of symptoms and there are limited data demonstrating efficacy. However, in appropriate adults older than 50 years of age, SCIT is a reasonable consideration (20,21).

SCIT should be prescribed by specialists and administered by physicians familiar with this special treatment and capable of treating anaphylaxis (21,45). The recommended equipment for settings where SCIT is administered includes epinephrine for injection; equipment for administering intravenous fluids and oxygen, including an oral airway; equipment for monitoring blood pressure; and pharmacological agents, including glucocorticosteroids, antihistamines, and vasopressors for injection (20,21). When SCIT is administered remote from emergency care access, additional rescue equipment may be appropriate. However, the most important recommendation is the prompt recognition of systemic reactions or anaphylaxis and the immediate administration of epinephrine.

DURATION OF IMMUNOTHERAPY

The international guidelines recommend perennial treatment because it attains a higher cumulative allergen dose while reducing the side effects that are a problem predominantly during the induction phase (20,21). Injection of a significant maximum allergen dose, in the range 5 to 20 μg of the major allergen, is associated with symptom-reduction efficacy and possibly disease modification with improvement persisting after discontinuation. Because there may be poorer patient compliance with perennial treatment, several short-term immunotherapy studies have been published (68,69). The problem with this design is that the preseasonal, 7-injection regimen results in low-dose immunotherapy because of a low cumulative allergen dose, and consequently, the level of efficacy obtained is only during the allergen season immediately following the preseasonal treatment. The immunological changes do not accomplish a fundamental change in the allergic phenotype. This form of treatment is comparable to symptomatic drug treatments that suppress the clinical symptoms while being administered but do not modify the long-term disease. From a cost-effectiveness perspective, SCIT, without documented long-term efficacy and preventive capacity, is less attractive, especially when considering the potential risk of inducing anaphylaxis. A perennial maintenance regimen using depot-allergen vaccines, administered six times per year (an interval of 8 weeks between injections) reduces the total number of injections, provides considerably higher doses of allergen, and minimizes risks compared with aqueous vaccines (56).

DISCONTINUATION OF IMMUNOTHERAPY

SCIT is usually administered for a defined time, typically three to five years. Efficacy is normally manifest after one year of treatment. If no improvement is observed after two years of treatment, the probability of obtaining efficacy is low and SCIT generally should be terminated (20,45). Short-term treatment has no protracted effect after SCIT is discontinued. The recommendation for a minimum of three years of maintenance SCIT is based on the observation that discontinuing SCIT after less than three years results in a higher relapse rate (70,71). Given the relatively slow onset of action and the knowledge of the likely immune mechanisms, there is a tendency toward extending the treatment period to five years, as is now commonly used for venom allergy. This is not based on scientific data obtained but is proposed in an attempt to

reduce the risk of reoccurrence of symptoms. In patients who improve with SCIT and deteriorate following discontinuation, the course of SCIT should probably be prolonged. In nonresponders, i.e., patients showing marginal or no efficacy after one year of treatment or 18 months in subjects with a major seasonal component, the indication for SCIT should be carefully reassessed with the view of determining whether allergy due to the identified allergens is responsible for the persisting symptoms (20). Patients not benefiting from SCIT usually should be discontinued after no more than two years of treatment at the optimal dose or consideration given to alternative allergens contributing to symptoms. Likewise, in patients experiencing severe anaphylaxis with SCIT, considerations should be given to alternative treatments. Nonadherence or limitations imposed by travel or scheduling would also be indications for discontinuation (45,72).

DISEASE PREVENTION WITH ALLERGEN IMMUNOTHERAPY

The capacity of SCIT to reduce the risk of developing new sensitizations was suggested by two large-scale studies in patients sensitized to one allergen only (73,74). The Purello-D'Ambrosio et al. (73) study followed for three years after SCIT, in an open retrospective design, 7182 originally monosensitized (to a variety of allergens) children treated with SCIT for four years (total of 7 years of observation). The control group consisted of 1214 comparable patients followed for seven years. The development of sensitization to new allergens at the five-year follow-up (two years after stopping SCIT) was 68% in the control group versus 24% in the SCIT group. Corresponding figures at the seven-year follow-up (4 years after stopping SCIT) were 78% and 27%, respectively. The difference between the control group and SCIT-treated group is statistically and clinically convincing. The Pajno et al. (74) study followed 75 SCIT-treated children, monosensitized to house dust mites, and 63 comparable controls treated pharmacologically for six years. The results show that 74% in the SCIT-treated group versus 33% in the control group remained monosensitized (specific IgE to only one allergen).

Likewise, SCIT as a prevention for progression of rhinitis into asthma has been suggested in studies. A multicenter trial, the Preventive Allergy Treatment Study, investigated the capacity of SCIT of allergic rhinitis to prevent the disease progressing into asthma (75). Children with allergy to birch and grass pollens, without clinical evidence of lower airway BHR, were randomized to receive either SCIT or optimal pharmacotherapy. After three years of treatment, the number of patients developing clinical asthma was statistically reduced in the SCIT group (24%) versus the pharmacotherapy group (44%). These data demonstrate the clinically important risk of developing lower airway symptoms in subjects with allergic rhinitis and demonstrate risk reduction with SCIT. Two years after terminating SCIT (5-year follow-up) (76) and seven years after discontinuing SCIT (10-year follow-up) (77), the beneficial effect of SCIT persisted (Fig. 4). Asthma prevention not only occurred with seasonal (pollen-induced)

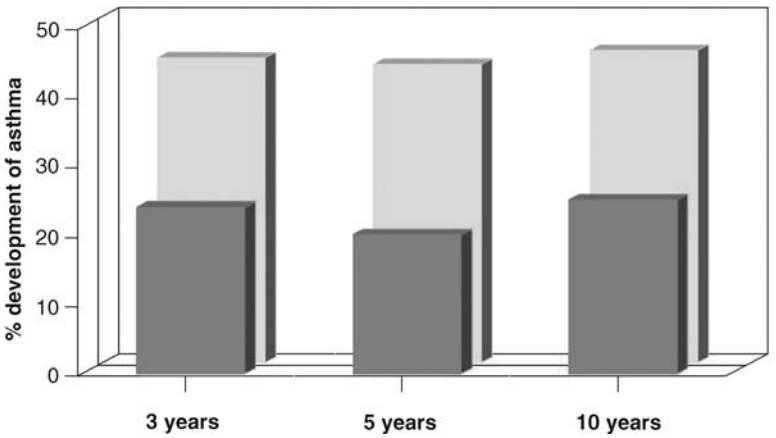

Figure 4 Frequency of the development of asthma in SCIT-treated children (*dark grey*) and controls (*light grey*) at discontinuation of immunotherapy (3 years), two years after termination (5 years total) and seven years after termination (10 years total). *Source*: From Ref. 77.

asthma but also with perennial asthma. Furthermore, the study showed that careful evaluation for asthma uncovered 20% of unidentified mild seasonal asthma in the 205 children recruited for rhinitis and more than one-third of the subjects with rhinitis had seasonal BHR measured by methacholine challenge (75). BHR to methacholine decreased significantly in SCIT-treated patients. However, only 2 out of 40 patients with asthma at inclusion were free of asthma after three years, indicating that SCIT has a greater capacity for asthma prevention than remission.

SALIENT POINTS

- The benefits of allergen immunotherapy for allergic rhinitis and asthma are well documented and based on PCDB studies with an appropriate number of carefully selected patients and the use of potent, well-characterized allergen vaccines at sufficient dosage.
- In clinical practice, immunotherapy ideally should be performed with allergen vaccines, which in clinical trials have demonstrated clinical efficacy and safety.
- The magnitude of clinical efficacy as assessed by symptom reduction is equivalent to or better than results obtained with optimal pharmacotherapy.
- The advantage of immunotherapy compared with pharmacotherapy is the capacity to reduce symptoms and simultaneously reduce the need for medications and potentially improve the natural course of the disease. The result is improved clinical outcomes with SCIT and reduction of progression into more severe disease.
- Allergen-specific immunotherapy is the only treatment that may alter the natural course of the disease with documented long-term efficacy and modulation of disease progression after termination of the treatment.
- Immunotherapy should be introduced at a time when the patient has the capacity to respond positively, i.e., before the disease becomes chronic or irreversible. Immunotherapy does not replace pharmacotherapy but complements or supplements if used in the early phase of allergic disease.
- Candidates for immunotherapy are subjects with rhinitis and/or asthma, symptoms induced by allergens and limited evidence of irreversible, structural airway changes. Age may affect response with younger subjects generally responding better than older individuals.
- Perennial SCIT is the only treatment in severe allergic rhinitis documented to reduce the development of asthma.
- Immunotherapy ideally should be continued for at least three, and probably five years, to achieve long-term, persistent clinical efficacy.

REFERENCES

1. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. Eur Respir J 2004; 24:758–764.
2. Masoli M, Fabian D, Holt S, et al. Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004; 59:469–478.
3. Bousquet J, Van Cauvenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001; 108:S147–S334.
4. Vignola AM, Chanez P, Godard P, et al. Relationships between rhinitis and asthma. Allergy 1998; 53(9):833–839.
5. Braunstahl GJ, Kleinjan A, Overbeek SE, et al. Segmental bronchial provocation induced nasal inflammation in allergic rhinitis patients. Am J Respir Crit Care Med 2000; 161:2051–2057.
6. Linna O, Kokkonen J, Lukin M. A 10-year prognosis for childhood allergic rhinitis. Acta Paediatr 1992; 81:100–102.
7. Settipane RJ, Hagy GW, Settipane GA. Long-term risk factors for developing asthma and allergic rhinitis: a 23-year follow-up study of college students. Allergy Proc 1994; 15:21–25.
8. Madonini E, Briatico Vangosa G, et al. Seasonal increase of bronchial reactivity in allergic rhinitis. J Allergy Clin Immunol 1987; 79:358–363.
9. Braman SS, Barrows AA, DeCotiis BA, et al. Airway hyperresponsiveness in allergic rhinitis. A risk factor for asthma. Chest 1987; 91:671–674.

10. Anto JM, Sunyer J. Thunderstorms: a risk factor for asthma attacks (editorial, comment). Thorax 1997; 52:669–670.
11. Knox RB. Grass pollen, thunderstorms and asthma. Clin Exp Allergy 1993; 23:354–359.
12. Sotomayor H, Badier M, Vervloet D, et al. Seasonal increase of carbachol airway responsiveness in patients allergic to grass pollen: reversal by corticosteroids. Am Rev Respir Dis 1984; 130:56–58.
13. Platts-Mills TA. The role of allergens in the induction of asthma. Curr Allergy Asthma Rep 2002; 2:175–180.
14. Vignola AM, Gagliardo R, Siena A, et al. Airway remodeling in the pathogenesis of asthma. Curr Allergy Asthma Rep 2001; 1:108–115.
15. Ten Hacken NH, Postma DS, Timens W. Airway remodeling and long-term decline in lung function in asthma. Curr Opin Pulm Med 2003; 9:9–14.
16. Viggnola AM, Paganin F, Capieu L, et al. Airway remodelling assessed by sputum and high-resolution computed tomography in asthma and COPD. Eur Respir J 2004; 24:910–917.
17. Bousquet J, Hejjaoui A, Michel FB. Specific immunotherapy in asthma. J Allergy Clin Immunol 1990; 86:292–305.
18. Halken S, Host A, Niklassen U, et al. Effect of mattress and pillow encasings on children with asthma and house dust mite allergy. J Allergy Clin Immunol 2003; 111:169–176.
19. Sheikh A, Hurwitz B, Shehata Y. House dust mite avoidance measures for perennial allergic rhinitis. Cochrane Database Syst Rev 2007; (1):CD001563 (review).
20. Malling H-J, Weeke B. EAACI Immunotherapy position papers. Allergy 1993; 48(suppl 14):9–35.
21. Bousquet J, Lockey RF, Malling H-J, eds. WHO Position Paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998; 53(suppl 44):1–42.
22. Jacobsen L. Preventive allergy treatment as part of allergy disease management. J Investig Allergol Clin Immunol 1997; 7:367–368.
23. Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005; 116:608–613.
24. Frew AJ, Powell RJ, Corrigan CJ, et al. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117:319–325.
25. Ciprandi G, Buscaglia S, Pesce G, et al. Minimal persistent inflammation is present at mucosal level in patients with asymptomatic rhinitis and mite allergy. J Allergy Clin Immunol 1995; 96:971–979.
26. Malling H-J. Immunotherapy as an effective tool in allergy treatment. Allergy 1998; 53:461–472.
27. Canonica GW, Baena-Cagnani CE, Bousquet J, et al. Recommendations for standardization of clinical trials with allergen specific immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. Allergy 2007; 62:317–324.
28. Wilson AM, ÓByrne PM, Parameswaran K. Leukotriene receptor antagonists for allergic rhinitis: a systematic review and meta-analysis. Am J Med 2004; 116:338–344.
29. Osterballe O. Immunotherapy in hay fever with two major allergens 19, 25 and partially purified extract of timothy grass pollen: a controlled double blind study. In vivo variables, season 1. Allergy 1980; 35:473–489.
30. Rak S, Heinrich C, Jacobsen L, et al. A double-blinded, comparative study of the effects of short preseasonal specific immunotherapy and topical steroids in patients with allergic rhinoconjunctivitis and asthma. J Allergy Clin Immunol 2001; 108:921–928.
31. Bousquet J, Calvayrac P, Guerin B, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract: 1. In vivo and in vitro parameters after a short course of treatment. J Allergy Clin Immunol 1985; 76:734–744.
32. Blumberga G, Groes L, Haugaard L, et al. Steroid-sparing effect of subcutaneous SQ-standardised specific immunotherapy in moderate and severe house dust mite allergic asthmatics. Allergy 2006; 61:843–848.
33. Haugaard L, Dahl R, Jacobsen L. A controlled dose response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. J Allergy Clin Immunol 1993; 91:709–722.
34. Bousquet J, Hejjaoui A, Clauzel AM, et al. Specific immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. II. Prediction of efficacy of immunotherapy. J Allergy Clin Immunol 1988; 82:971–977.
35. Haugaard L, Dahl R. Immunotherapy in patients allergic to cat and dog dander. I. Clinical results. Allergy 1992; 47:249–254.
36. Alvarez-Cuesta E, Cuesta-Herranz J, Puyana-Ruiz J, et al. Monoclonal antibody-standardized cat extract immunotherapy: risk-benefit effects from a double-blind placebo study. J Allergy Clin Immuno1 1994; 93:556–566.
37. Varney V, Edwards J, Tabbah K, et al. Clinical efficacy of specific immunotherapy to cat dander: a double-blind placebo-controlled trial. Clin Exp Allergy 1997; 27:860–867.

38. Valovirta E, Koivikko A, Vanto T, et al. Immunotherapy in allergy to dog: a double-blind clinical study. Ann Allergy 1984; 53:85–88.
39. Salvaggio J, Aukrust L. Postgraduate course presentations: mold-induced asthma. J Allergy Clin Immunol 1981; 68:327–346.
40. Malling HJ, Dreborg S, Weeke B. Diagnosis and immunotherapy of mould allergy. V. Clinical efficacy and side effects of immunotherapy with *Cladosporium herbarum*. Allergy 1986; 41:507–519.
41. Horst M, Hejjaoui A, Horst V, et al. Double-blind, placebo-controlled rush immunotherapy with a standardized *Alternaria* extract. J Allergy Clin Immunol 1990; 85:460–472.
42. Dreborg S, Agrell B, Foucard T, et al. A double-blind, multicenter immunotherapy trial in children, using a purified and standardized *Cladosporium herbarum* preparation. I. Clinical results. Allergy 1986; 41:131–140.
43. Malling H-J. Bacterial vaccines: anything but placebo. Allergy 2000; 55:214–218.
44. Adkinson N Jr., Eggleston PA, Eney D, et al. A controlled trial of immunotherapy for asthma in allergic children. N Engl J Med 1997; 336:324–331.
45. Alvarez-Cuesta E, Bousquet J, Canonica GW, et al. Standards for practical allergen-specific immunotherapy. Allergy 2006; 61(suppl 82):1–20.
46. Li JT, Lockey RF, Bernstein IL, et al. Allergen immunotherapy: a practice parameter. Ann Allergy Asthma Immunol 2003; 90:1–40.
47. Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. Cochrane Database Syst Rev 2003; (4):CD001186 (review).
48. Malling H-J. Quality and clinical efficacy of allergen mixtures. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 2006; 95:253–257.
49. Calderon MA, Alves B, Jacobson M, et al. Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev 2007; (1):CD001936. DOI: 10.1002/14651858.CD001936.pub2.
50. Shekelle PG, Woolf SH, Eccles M, et al. Clinical guidelines: developing guidelines. Br Med J 1999; 318:593–596.
51. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999; 341:468–475.
52. Varney VA, Gaga M, Frew AJ, et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. Br Med J 1991; 302:265–269.
53. Eng PA, Reinhold M, Gnehm HPE. Long-term efficacy of preseasonal grass pollen immunotherapy in children. Allergy 2002; 57:306–312.
54. Eng PA, Borer-Reinhold M, Heijnen IA, et al. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. Allergy 2006; 61:198–201.
55. Cools M, Van Bever HP, Weyler JJ, et al. Long-term effects of specific immunotherapy, administered during childhood, in asthmatic patients allergic to either house-dust mite or to both house-dust mite and grass pollen. Allergy 2000; 55:69–73.
56. Malling H-J. Minimising the risks of allergen-specific injection immunotherapy. Drug Saf 2000; 23:323–332.
57. Bousquet J, Chanez P, Lacoste JY, et al. Asthma: a disease remodeling the airways. Allergy 1992; 47:3–11.
58. Juniper EF, Guyatt GH, Griffith LE, et al. Interpretation of rhinoconjunctivitis quality of life questionnaire data. J Allergy Clin Immunol 1996; 98:843–845.
59. Strachan DP, Butland BK, Anderson HR. Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. Br Med J 1996; 312:1195–1199.
60. Sly RM. Managed care—the key to quality of management of asthma. Ann Allergy 1996; 76:161–163.
61. Bousquet J, Chanez P, Lacoste JY, et al. Asthma: a disease remodeling the airways. Allergy 1992; 47:3–11.
62. Peat JK, Woolcook AJ, Cullen K. Rate of decline of lung function in subjects with asthma. Eur J Respir Dis 1987; 70:171–179.
63. Tabar AI, Garcia BE, Rodriguez A, et al. A prospective safety-monitoring study of immunotherapy with biologically standardized extracts. Allergy 1993; 48:450–453.
64. Rugose FV, Passalacqua G, Gambardella R, et al. Nonfatal systemic reactions to subcutaneous immunotherapy: a 10-year experience. Invest Allergol Clin Immunol 1997; 7:151–154.
65. Alessandro F, Vincenzo ZG, Marco S, et al. Compliance with pharmacologic prophylaxis and therapy in bronchial asthma. Ann Allergy 1994; 73:135–140.
66. Yeung M, O'Connor SA, Parry DT, et al. Compliance with prescribed drug therapy in asthma. Respir Med 1994; 88:31–35.
67. Bronsky WA, Dockhorn RJ, Meltzer EO, et al. Fluticasone propionate aqueous nasal spray compared with terfenadine tablets in the treatment of seasonal allergic rhinitis. J Allergy Clin Immunol 1996; 97:915–921.
68. Balda BR, Wolf H, Baumgarten C, et al. Tree-pollen allergy is efficiently treated by short-term immunotherapy (STI) with seven preseasonal injections of molecular standardized allergens. Allergy 1998; 53:740–748.

69. Klimek L, Wolf H, Mewes T, et al. The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophil cationic protein and tryptase in nasal secretions. J Allergy Clin Immunol 1999; 103:47–53.
70. Des Roches A, Paradis L, Knani J, et al. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. V. Duration of efficacy of immunotherapy after its cessation. Allergy 1996; 51: 430–433.
71. Hedlin G, Heilborn H, Lilja G, et al. Long-term follow-up of patients treated with a three-year course of cat or dog immunotherapy. J Allergy Clin Immunol 1995; 96:879–885.
72. Cohn JR, Pizzi A. Determinants of patient compliance with allergen immunotherapy. J Allergy Clin Immunol 1993; 91:734–737.
73. Purello-D'Ambrosio F, Gangemi S, Merendino RA, et al. Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study. Clin Exp Allergy 2001; 31:1295–1302.
74. Pajno GB, Barberio G, de Luca Fr, et al. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 2001; 31:1392–1397.
75. Möller C, Dreborg S, Ferdousi HA, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 2002; 109:251–256.
76. Niggemann B, Jacobsen L, Dreborg S, et al. Five year follow-up on the PAT-study: specific immunotherapy and long-term prevention of asthma in children. Allergy 2006; 61:855–859.
77. Jacobsen L, Niggemann B, Dreborg S et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy 2007; 62:943–948.

23 Sublingual Immunotherapy for Allergic Rhinoconjunctivitis, Allergic Asthma, and Prevention of Allergic Diseases

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INTRODUCTION

Sublingual immunotherapy (SLIT) is an efficacious and safe alternative to subcutaneous immunotherapy (SCIT). During the last three decades, SLIT has been widely used in many countries, primarily in central and southern Europe, where more than 50% of the current population of patients receiving specific immunotherapy are on SLIT (1).

The sublingual route has emerged as an effective alternative to SCIT. The clinical indications are broadly similar, and where both treatments are available, patient preference becomes an important determinant of choice. Selection of patients for SLIT should always be in the hands of physicians trained and experienced in allergy and immunotherapy. The favorable safety profile makes this treatment suitable for ambulatory use and, therefore, more accessible to a broader range of patients (2–4).

Efficacy of SLIT has been mainly assessed by evaluation of patients' symptom scores and medication scores before and after treatment compared with placebo. There is no general agreement as to what degree of improvement, either individually or in combination, might indicate clinically relevant efficacy (2).

This review examines all 46 double-blind, placebo-controlled randomized trials (DB PC RCT) published in peer-reviewed journals up to August 1, 2007. Cochrane and Pubmed databases were searched for studies that assessed the efficacy of SLIT in adults and children with allergic rhinitis and asthma. The review focuses only on those outcomes provided in quantitative terms.

HISTORICAL BACKGROUND

The first clinical trials on mucosal specific immunotherapy were reported by Black (5) and Feinberg (6) almost 70 years ago. However, it was not until 1986 that the first DB PC crossover randomized trial using low doses of dust-mite allergen for SLIT was published (7). The authors found that SLIT was effective in reducing symptoms in 72% of cases compared with placebo. Since then, the potential for SLIT in the treatment of respiratory allergies has been investigated by several different researchers, mostly from European centers. In 1998, the World Health Organization reported that there are sufficient efficacy and safety data available to conclude that SLIT is an acceptable method of specific immunotherapy administration (1). A subsequent publication by the European Academy of Allergy and Immunology, as well as the Allergic Rhinitis and Its Impact on Asthma (ARIA) document, endorsed the use of SLIT in adults and children (8,9) (Fig. 1).

Interest in immunotherapy has grown rapidly, on the basis of clinical evidence that supports its long-term benefits. For example, clinical efficacy remains evident up to at least three years following cessation of treatment (10). The risks of developing asthma in children with seasonal allergic rhinitis are reduced two- to threefold. Immunotherapy also prevents both the number and degree of sensitization to novel allergens following successful treatment of monosensitized children, even during a long-term follow-up (11–13). The efficacy of immunotherapy is reflected in numerous well-designed clinical studies that follow international standardized recommendations.

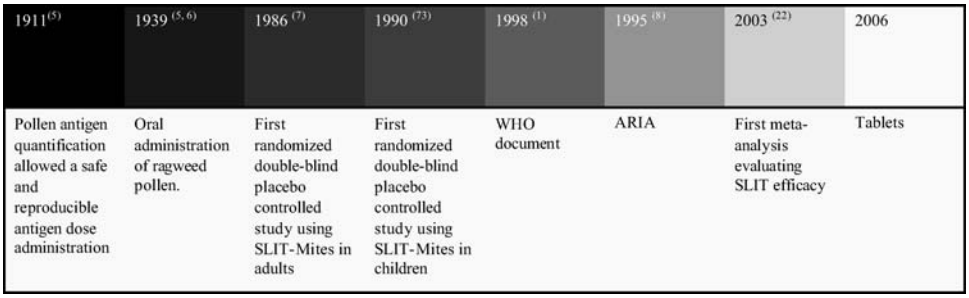

Figure 1 Chronology of sublingual immunotherapy.

SUBLINGUAL IMMUNOTHERAPY IN CLINICAL PRACTICE

SLIT is a self-administered treatment, which can be taken at home. The vaccine is administered orally and held under the tongue for one to two minutes prior to being swallowed or spat out. Sublingual protocols used for vaccination vary considerably, although these may contain up to 50- or even 500-fold more allergens than those doses used for SCIT (4,14–16). Different formulations of allergen are used, including oral drops, dissolving tablets, or a combination of the two.

Treatment duration and frequency of dosing may vary widely, from daily to weekly administrations, depending on the geographical location (study center), where treatment is given and the type of preparation administered. Duration of treatment can vary from two months to five years, according to the protocol and allergen used. SLIT has been administered preseasonally only, preseasonal continuing through the season, and continuously throughout the whole year, without regard to season. In one study, SLIT for grass pollen allergy had a better effect in reducing symptom and medication scores during the pollen season when preseasonal treatment was started at least 16 weeks before the pollen season (17).

EVIDENCE-BASED MEDICINE APPROACH TO SLIT

Evidence-based medicine (EBM) has gained in popularity. On the basis of EBM, conclusions from meta-analyses of DB PC RCT represent the most solid sources of evidence to assess the efficacy of a therapeutic intervention. Meta-analysis is a statistical procedure that incorporates the results of pooled independent studies. This allows a more objective appraisal of the evidence than a traditional narrative. A meta-analysis also provides a quantitative estimate of treatment effect sizes and may explain and quantify any heterogeneity among individual studies. Analysis is performed by the method of the weighted or standardized mean differences (SMD) and by use of fixed or random effect models (REMs). This depends on the study design, distribution of effect sizes, and outcome characteristics (18–21).

At present, four systematic reviews and meta-analyses assessing the efficacy of SLIT are published (22–25). The first systematic review and meta-analysis regarding the effects of SLIT on allergic rhinitis was published by Wilson et al. in 2003 (22). This review was conducted following Cochrane group methodology. The efficacy of SLIT was compared with placebo-assessing symptoms and medication requirements. Twenty-two trials involving 959 participants (484 SLIT and 475 placebo) were included. There was a significant reduction in both symptoms [SMD −0.42; 95% confidence interval (95% CI), −0.69 to −0.15; $p = 0.002$] and rescue medication requirements (SMD −0.43; 95% CI, −0.63 to −0.23; $p = 0.00003$) following immunotherapy (Fig. 2). A subgroup analysis failed to identify any preference for particular allergens administered. In those studies involving only children ($n = 5$), there was no significant reduction in symptoms (SMD −0.31; 95% CI, −1.32 to 0.7; $p = 0.5$) and medication scores (SMD 0.02; 95% CI, −0.34 to 0.37; $p = 0.9$), although this could reflect the relative paucity of pediatric studies when this review was performed (22).

This systematic review and meta-analysis has been updated (26). Thirty-nine DB PC RCT studies were included; of them, 19 were newly identified and 20 were from the previous

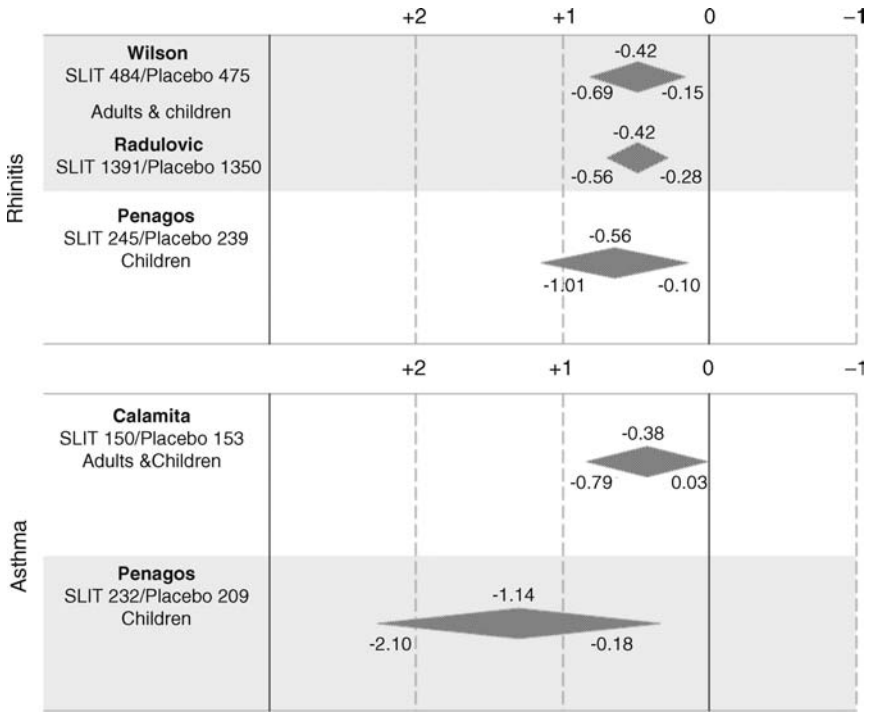

Figure 2 Meta-analyses summary.

review (1393 received SLIT and 1353 placebo). It confirmed previous findings of a significant reduction, with narrower CIs than in the original review for both symptoms (SMD −0.43; 95% CI, −0.57 to −0.28; $p < 0.00001$) and medication requirements (SMD −0.41; 95% CI, −0.55 to −0.28; $p < 0.00001$).

The predetermined subgroup analyses performed in this review show that SLIT is highly effective in both seasonal and perennial allergic rhinitis and that treatment is effective, whatever the duration, although the effect size is greatest with the treatment lasting more than 12 months for both symptoms (SMD −0.70; 95% CI, −1.19 to −0.21; $p = 0.005$) and medication scores (SMD −0.44; 95% CI, −0.84 to −0.04; $p = 0.03$). Similarly to the previous review, the authors were unable to identify a significant reduction in both symptoms and medication scores in children (26).

The efficacy of SLIT in the treatment of allergic rhinitis in children was evaluated in a meta-analysis of 10 DB PC RCTs published between 1990 and 2004 (23). Four hundred eighty-four participants (245 SLIT and 239 placebo) had a final clinical evaluation and were assessed. Overall, there was a significant reduction in both symptoms (SMD −0.56; 95% CI, −1.01 to −0.10; $p = 0.02$) and medication use (SMD −0.76; 95% CI, −1.46 to −0.06; $p = 0.03$) after immunotherapy compared with placebo (Fig. 2). Subanalysis of treatment duration shows that SLIT for more than 18 months versus shorter courses is more effective. The authors conclude that SLIT with standardized vaccines is effective in pediatric patients with allergic rhinitis when compared with placebo (23). The observed discrepancy between the effect sizes reported in the pediatric subanalysis by Radulovic (26) and those reported by the Penagos meta-analysis (23) is perhaps due to the use of different studies and different data extraction methods.

Another systematic review evaluated the effect of SLIT for asthma (24). Nine studies, including 303 participants (150 SLIT and 153 placebo), assessed asthmatic symptoms, and six studies of 254 participants (132 SLIT and 122 placebo) evaluated specific asthma medication. There was a tendency toward the improvement of asthma symptom scores (SMD −0.38; 95% CI, −0.79 to 0.03; $p = 0.07$) and reduction of asthma medication use (SMD −0.91; 95% CI, −1.94 to 0.12; $p = 0.08$] in favor of SLIT, but without statistical significance. The 95% CI includes the zero, thus indicating a nonsignificant reduction in asthmatic symptoms (Fig. 2). We found some methodological issues, which make interpretation difficult. First, there are no restrictive inclusion criteria for trial selection; this review includes open-labeled and double-blind

studies. Second, the outcomes analyzed are not clearly defined. Authors analyzed different allergic symptom scores in a collective manner. Finally, statistical analysis includes qualitative assessments instead of a quantitative approach for some outcomes (24).

A meta-analysis on the efficacy of SLIT in allergic asthma in pediatric patients, aged 3 to 18 years, also has been published (25). Nine DB PC RCTs, all published from 1990 to May 2006, fulfil the selection criteria. Four hundred and forty-one patients (232 SLIT and 209 placebo) had a final assessment and are included in the analysis. Overall, there is a significant reduction in both symptoms (SMD −1.14, 95% CI, −2.10 to −0.18; $p = 0.02$) and medication use (SMD −1.63, 95% CI, −2.83 to −0.44; $p = 0.007$) following SLIT compared with placebo (Fig. 2). The authors conclude that SLIT with standardized vaccines reduces both symptom scores and rescue medication use in children with allergic asthma compared with placebo (25). Nonetheless, in this meta-analysis, the heterogeneity is very significant (>94%), with a wide 95% CI for SMD in both outcomes. This indicates the diverse methodology used throughout studies. More studies are required to give a robust conclusion.

In a systematic review, as expected, studies brought together will differ in their outcomes for rhinoconjunctivitis symptom and medication use. In all four meta-analyses on SLIT, a significant interstudy heterogeneity is found. This may be due to the fact that diverse allergens were used and different age groups included in the analyses. Methodological diversity is also evident in the study sample sizes. Finally, variability in the treatment effects evaluated in the different trials was observed. This is known as "statistical heterogeneity," and it is a consequence of clinical and/or methodological diversity among the studies (21).

All authors analyzed their data with the REM to estimate the treatment effects. Even though REM explicitly accounts for the heterogeneity of studies through a statistical parameter representing the interstudy variation, further evaluations should be conducted to determine the impact of this variability on the global effect size (20,21).

In all four meta-analyses, a significant interstudy heterogeneity is found. This is most likely due to widely differing scoring systems for rhinoconjuctivitis symptoms and medication use, among studies. In the light of the heterogeneity, one should be cautious in the interpretation, particularly when the studies included are small. This highlights the need for more definitive studies, including dose-response studies involving large numbers of well-defined patients (21).

SLIT FOR ADULTS

Quantitative data have been identified from 31 DB PC RCT studies in adults assessing the efficacy of SLIT (Table 1) (27–57). Age ranged from 18 to 60 years. All studies include subjects with symptomatic sensitization to only one aeroallergen, but some individuals were polysensitized to other aeroallergens.

The allergens studied include seasonal pollens, such as grasses (12 studies), trees (5 studies), both grasses and trees (1 study), and weeds (5 studies) and, perennial allergens, such as house-dust mites (HDM) (5 studies) and cats (2 studies). Twenty studies used drops, seven studies tablets, and three studies both drops and tablets.

All studies assessed the clinical efficacy of SLIT in patients with moderate to severe allergic rhinoconjunctivitis; 14 of these also included patients with mild to moderate allergic asthma. Only one study investigated the efficacy of SLIT in asthmatics (29). The main clinical outcome was reduction of rhinoconjunctivitis symptoms and medication scores. In most studies, it was possible to retrieve the cumulative dose in micrograms. A wide dose range was used, which makes it difficult to perform a global analysis of the effects of the dose on the outcomes.

In Tables 1 and 2, the effect size (δ) of each of the studies is demonstrated. As defined by Cohen, this is the difference in the means between two groups divided by the standard deviation. The most usual interpretation of δ is to consider: $0.0 < \delta < 0.2$, trivial effect size; $0.2 < \delta < 0.5$, small effect size; $0.5 < \delta < 0.8$, moderate effect size, and $\delta > 0.8$, strong effect size (58).

The clinical efficacy and safety of a new fast-dissolving, once-daily oral lyophilisate tablet for sublingual administration has been investigated in adults with seasonal allergic rhinitis due to grass pollen. The tablet contains 75,000 SQ-T standardized allergen extract of grass pollen,

Table 1 Efficacy of Sublingual Immunotherapy in Adults: Evidence Review

Author, yr	Diagnosis	SLIT	Placebo	Allergen	Units	Presentation	Duration (mo)	Cumulative dose	Effect size	Dropout rate (%)
Andre et al., 2003	R, A, C	48	51	Amb a 1	IR	Tablets	6.5	1300–30,500	R: −0.45 (−0.84, −0.05)	10
Ariano et al., 2001	R, A	10	10	*Cupressus arizonica*	U RAST	Drops	8	250,000 U RAST	R: −2.06 (−3.19, −0.93)	0
Bousquet et al., 1999	A	23	27	*Dermatophagoides pteronyssinus* and *Dermatophagoides. farinae*	IR	Drops	25	Der p 1–4.2 mg Der f 1–7.3 mg	A: −0.04 (−0.60, 0.51)	41
Bowen et al., 2004	R, A, C	37	39	Amb a 1	IR	Drops	3.5	18.26 mg	R: −0.43 (−0.88, 0.03)	31
Casanovas et al., 1994	R, A, C	9	6	*Olea europaea*	HEP (w/v)	Drops	4	n/s	R: −1.00 (−2.11, 0.12)	0
Clavel et al., 1998	R, C, A	62	58	Mixed grasses (Orchard, meadow, ryegrass, sweet vernal, and timothy)	IR	Drops	6	2.6 mg/Phl p 5s	R: −0.42 (−0.82, −0.02)	12
Dahl et al., 2006a	A, R. C	61	32	*Phleum pratense*	SQ-T	Tablet	5	2.0 mg	R: −0.63 (−1.07, −0.19)	18
Dahl et al., 2006b	R, C. A	282	286	*Phleum pratense*	SQ-T	Tablet	8	2.7 mg	R: −0.52 (−0.69, −0.35)	10
Didier et al., 2007	R, C	133	146	Mixed grasses (Orchard, meadow, ryegrass, sweet vernal, and timothy)	IR	Tablet	6	4.125 mg	R: −0.43 (−0.67, −0.20)	11
Di Rienzo, 2006	R, C	18	14	*Juniperus ashei*	IR	Drops	4	12.6 mcg	R: −0.98 (−1.72, −0.23)	6
Drachenberg et al., 2001	R, C	37	12	Birch and grass/rye	AU	Drops	5	Phl p 1 1914 μg	R: −0.26 (−0.92, 0.39)	28
Durham et al., 2006	R, C, A	131	129	*Phleum pratense*	SQ-T	Tablets	9	Bet v 1 1535 μg 15 μg Phl p 5/day	R: −0.23 (−0.47, 0.02)	8
Feliziani et al., 1995	R, C, A	18	16	Grass (*Dactylis glomerata, Festuca pratensis, Lolium perenne, Phleum pratense,* and *Poa pratensis*)	BU	Drops	3	19.2 μg major grass allergen/mo	R: −1.00 (−1.72, −0.28)	0
Guez et al., 2000	R, C	36	36	*Dermatophagoides pteronyssinus* and *Dermatophagoides.farinae*	IR	Drops	24	Der p 1 = 2.2 mg; Der f l = 1.7 mg	R: −0.41 (−0.88, 0.06)	46

(*Continued*)

Table 1 Efficacy of Sublingual Immunotherapy in Adults: Evidence Review (*Continued*)

Author, yr	Diagnosis	SLIT	Placebo	Allergen	Units	Presentation	Duration (mo)	Cumulative dose	Effect size	Dropout rate (%)
Hordijk et al., 1998	R, C	35	36	Mixed grasses (Orchard, meadow, ryegrass, sweet vernal, and timothy)	BU	Drops	3	760,000 BU	R: −0.57 (−1.04, −0.09)	20
Lima et al., 2002	R, C	28	28	*Phleum pratense*	μg	Drops	12–18	16.3 mg	R: 0.01 (−0.51, 0.54)	12
Nelson et al., 1993	R, C	20	21	Fel d 1	AU	Drops	3.5	4,500,000 AU or 450–900 Fel d I Units	R: −0.56 (−1.18, 0.07)	7
Palma-Carlos AG	R, A, C	13	7	Grass pollen	AU	Tablets	24	Manteinance 2000 AU/week	NA	39
Passalacqua et al., 1998	R, C	10	9	*Dermatophagoides pteronyssinus* and *Dermatophagoides. farinae*	AU	Tablets	24	4000 AU/wk	R: −1.27 (−2.28, −0.26)	5
Passalacqua et al., 1999	R, C, A	15	15	*Parietaria judaica*	BU	Drops	6	16 μg	R: −0.02 (−0.73, 0.70)	10
Passalacqua et al., 2006	R, C	28	28	*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*	AU	Tablets	24	2000 AU/wk	R: −1.65 (−2.26, −1.04)	18
Pradalier et al., 1999	R, C, A	63	63	Mixed grasses (Orchard, meadow, ryegrass, sweet vernal, and timothy)	IR	Drops and Tablets	4.5	0.935 mg Phl p 5.	R: −0.18 (−0.53, 0.17)	6
Purello-D'Ambrosio et al., 1999	R, C, A	14	16	*Parietaria judaica*	BU/mL	Drops	9	12.77 μg (Par j 1)	R: −0.62 (−1.36, 0.12)	0
Sabbah et al., 1994	R, C	29	29	Mixed grasses (Orchard, meadow, ryegrass, sweet vernal, and timothy)	IR	Drops	4	4500 IR	NA	0

Sanchez-Palacios 2001	R, A	20	20	Fel d 1	HEP	Drops	12	3.6 mg	NA	0
Smith et al., 2004	R, C	45	51	Mixed grasses (Orchard, meadow, ryegrass, sweet vernal, and timothy)	IR	Drops and tablets	24	6264 μg Lolp1	R: 0.12 (−0.28, 0.52)	27
Tonnel et al., 2004	R, C	10	12	*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*	IR	Drops and tablets	24	3654 μg Dacg5 1.28 mg Der p 1; 1.47 mg Der f 1	R: −0.61 (−1.47, 0.25)	44
Troise et al., 1995	R, C	15	16	*Parietaria judaica*	BU	Drops	10	6.3 μg Parj1	R: −0.22 (−0.92, 0.49)	0
Vervloet et al., 2007	R, C	19	19	*Juniperus ashei*	IR	Drops	4	27.36 mg	R: 0.13 (−0.51, 0.76)	8
Voltolini et al., 2001	R, C	15	15	Alder, birch, hazel	BU	Drops	12	445 μg Betv1	R: 0.37 (−0.35, 1.10)	10
Worm, 2006	R, C	41	48	*Holcus lanatus, Dactylis glomerata, Lolium perenne, Phleum pratense, Poa pratensis, and Festuca elatior*	μg	Drops	20	24 mg	NA	52

Abbreviations: R, rhinitis; C, conjunctivitis; A, asthma.

Table 2 Efficacy of Sublingual Immunotherapy in Children: Evidence Review

Author, yr	Diagnosis	SLIT	Placebo	Allergen	Units	Presentation	Duration (mo)	Cumulative dose	Effect size	Dropout rate (%)
Bahcecilier, 2001	R, A	7	7	Dpt and Df extract 50/50	IR	Drops	6	Dpt 0.56 mg; Df 0.98 mg	R: 0.31 (−0.71, 1.34) A: 0.33 (−0.73, 1.39)	0
Bufe et al., 2004	R, A	63	63	Grass pollen extracts	AU	Drops	36	9.6 mg	R: −0.06 (−0.40, 0.28)	18
Caffarelli, 2000	R, A, C	24	20	*Holcus lanatus, Phleum pratense, and Poa pratensis*	AU	Tablets	3	37 250 AU	R: −0.98 (−1.69, −0.26) A: −3.51 (−4.50, −2.53)	8
Hirsch et al., 1997	R, A	14	15	Mites extract	μg	Drops	12	570 μg	R: 0.51 (−0.36, 1.39) A: −0.53 (−1.40, 0.35)	5
Ippoliti et al., 2003	R, A, C	18	15	*Dermatophagoides pteronyssinus*	BU	Drops	6	12 mg	R: −0.61 (−1.32, 0.09) A: −2.57 (−3.14, −1.99)	0
La Rosa et al., 1999	R	16	17	*Parietaria judaica*	IR	Drops	24	52.5 mg	R: −0.24 (−0.93, 0.44)	19
Lue et al., 2006	A	10	10	*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*	IR	Drops	6	1.7 mg Dpt 3.0 mg Df	A: −1.15 (−2.11, −0.19)	0
Niu et al., 2006	R, A	49	48	Dpt and Df extract 50/50	IR	Drops	6	1.7 mg Dpt 3.0 mg Df	A: −1.26 (−1.70, −0.82)	12
Pajno et al., 2000	A	12	9	Der p 1, Der p 2	BU	Drops	24	250 μg Der p 1 125 μg Der p 2	A: −1.24 (−2.20, −0.28)	13
Röder E et al., 2007	R	82	72	*Lolium perenne, Phleum. pratense, Dactylis glomeratein, Holcus, and Anthoxantum odoratum*	BU	Drops	24	4.5 mg Lol p 5	R: −0.17 (−0.49, 0.15)	25

Rolinck-Werninghaus et al., 2004	R, A	39	38	Mixed grasses (*Dactylis glomerata, Festuca pratensis, Lolium perenne, Phleum pratense, and Poa pratensis*)	STU	Drops	32	188 μg	R: 0.05 (−0.40, 0.49) A: 0.01 (−0.62, 0.64)	17
Tari et al., 1990	R, A	30	28	Mites extract	STU	Drops	18	365 STU	R: −2.24 (−2.91, −1.58) A: −2.53 (−3.23, −1.83)	12
Valovirta et al., 2006	R, A, C	27	29	*Brachydesmiella verrucosa, Corylus avellana*, and *Alnus glutinosa*	SQ-U	Drops	19	Low dose: 67.5 μg High dose: 562.87 μg	R: -0.49 (−1.03, 0.04) A: −0.31 (−0.84, 0.22)	14
Vourdas et al., 1998	R, A, C	34	32	*Olea europaea*	IR	Drops	24	Ole e 1 (8.1 mg)	R: −0.17 (−0.65, 0.32) A: 0.99 (0.46, 1.52)	3
Wüthrich et al., 2003	R, C	10	12	Mixed grasses (*Dactylis glomerata, Festuca pratensis, Lolium perenne, Phleum pratense,* and *Poa pratensis*)	μg	Drops	24	139.88 μg	R: −0.11 (−0.95, 0.73)	21

Abbreviations: R, rhinitis; C, conjunctivitis; A, asthma.

Phleum pratense (equivalent to 2800 BAU or 15 mcg of major allergen protein), and it is recommended as safe for home administration with no updosing phase. Over 2400 subjects have been included in the tablet evaluation. The mean rhinoconjunctivitis symptom scores and medication scores were reduced by 30% and 38%, (median results 34% and 53%), respectively, in the SLIT group as compared with placebo in the first year (38) and, by 36% and 46%, (median results 44% and 73%), respectively, in the second year (59). These reductions are highly statistically significant, ($p < 0.0001$). Furthermore, the subjects in the SLIT group report improved quality of life as assessed using Juniper's Rhinoconjunctivitis Quality-of-Life Questionnaire (60).

Another DB PC RCT study evaluated the efficacy and safety of a different sublingual tablet in adults (57). Six hundred and twenty-eight participants with grass pollen rhinoconjunctivitis were randomized into three groups, receiving 100-, 300-, and 500-IR of five-grasses pollen immunotherapy. Treatment started four months before the pollen season and was given until the end of the pollen season. Significant improvements in symptom and medication scores were found in both 300- and 500-IR groups compared with placebo. Furthermore, a very good safety profile was identified (57,61).

SLIT FOR CHILDREN

Over the last two decades, the interest on efficacy of SLIT in children has increased. Due to its good safety profile and convenience, SLIT seems to be an efficacious route to administer specific immunotherapy to children. At present, 15 DB PC RCT studies evaluating the efficacy of SLIT in children compared with placebo provide quantitative data (62–76) (Table 2). Age range varies from 3 to 18 years. The youngest age reported in DB PC RCT assessing efficacy of SLIT compared with placebo is three years (25). Moreover, in a safety survey of SLIT, the investigators included subjects as young as 23 months (77), with no severe adverse effects.

The allergens studied include seasonal pollens such as grasses (6 studies), trees (2 studies), and weeds (1 study) and perennial allergens such as dust mites (6 studies). All studies used drops, except one (64), which investigated the effect of sublingual tablets.

Thirteen studies assessed the clinical efficacy of SLIT in patients with moderate to severe allergic rhinitis. Ten of these also included patients with mild to moderate allergic asthma. Two studies (68,70) exclusively evaluate the effect of SLIT in mild to moderate allergic asthma. The main clinical outcome is reduction of rhinitis symptoms and medication scores. The effect size of each study is summarized in Table 2, where the data have been analyzed according to Cohen's method. Even though most of the studies conducted in asthmatics report a reduction in clinical scores, a statistical significant effect of SLIT was observed in seven studies.

The efficacy of preseasonal monomeric allergoid SLIT tablets was investigated in a DB PC study in children with asthma, rhinitis, and/or rhinoconjunctivitis due to grass pollen (64). Twenty-four patients received SLIT for three months before the pollen season and 24 matched patients received identically appearing placebo. Patients in the SLIT group showed a significant reduction in the symptom-medication score compared with the placebo group during the pollen season. The authors conclude that SLIT is effective for the treatment of asthma due to grass pollen in children (64).

A study in a primary care setting evaluated the efficacy of SLIT in children aged 6 to 18 years with hay fever. Participants were randomly assigned to receive grass pollen mix ($n = 91$) or placebo ($n = 77$) for two years. The authors concluded that both symptom and medication scores do not differ between those subjects allocated to SLIT or placebo. However, this study has limitations. Patients with mild disease are included, at least 70% of patients are polysensitized, a retrospective score is considered as a substitute for baseline evaluation, and study withdrawals are more than 20%; for these reasons, results have to be interpreted with care (71).

SLIT VS. SCIT

The efficacy of SLIT and SCIT in patients with respiratory allergy has been compared by various authors (78–84). Allergic rhinoconjunctivis was evaluated in all studies; in addition, three studies included mild to moderate allergic asthma (80,81,83). Different allergens have

been used, such as grass-mixed pollens, birch pollen, HDM, and multiple allergen extracts. The design of the studies also varies. Some are open (78,79,83), while others are randomized (81,84) or randomized placebo-controlled studies (80,82). All studies report no significant differences between SCIT and SLIT for improvement in symptom scores and medication scores. Thus, out of nine studies, seven do not involve a double-blind randomized protocol such that there are clear limitations in their interpretation. Two studies (80,82) are designed as DB PC RCT double-dummy studies. The first is small and does not include a placebo arm, and in view of the inevitable large placebo effect in immunotherapy trials (35), it is unclear whether the lack of difference between SCIT and SLIT implies any benefit for either treated group. The Khinchi study, although optimally designed and showing a convincing difference for both SLIT and SCIT compared with placebo, is, nonetheless, inadequately powered to detect a difference between SLIT and SCIT. On reviewing the data in the Khinchi study (82), there is a clear trend in favor of SCIT over SLIT, even though significance was not achieved.

At present, the meta-analysis data *suggest* that SCIT may be more effective than SLIT, although in view of heterogeneity among trials and overlap of the CIs, no firm conclusions can be drawn. More head-to-head comparisons of SLIT versus SCIT are needed, since, with the exception of the Kinchi study (82), the lack of a proper study design and the limited statistical power of the studies does not allow for a firm conclusion (2).

LONG-TERM EFFECT OF SLIT

Five studies evaluated the long-lasting effect of SLIT (70,85–88). In a DB PC RCT study, 24 children (8–15 years) with mild to moderate asthma, with single sensitization to mite allergen, were evaluated. After a one-year observation phase, patients were randomly allocated to receive SLIT (sublingual-spit immunotherapy) as drops with a standardized extract of *Dermatophagoides pteronyssinus*. After two years of SLIT, there was a significant reduction in asthma symptom scores (SMD −1.24 95% CI, −2.20 to −0.28; $p = 0.0001$) and medication use (SMD −8.10; 95% CI, −10.95 to −5.25; $p = 0.0001$) in the active compared with placebo group (70).

A DB RCT, not placebo controlled, of one or three years of SLIT and three years of observation, was conducted in 137 patients (10–51 years) with perennial rhinitis due to HDM (88). Efficacy of SLIT was evaluated with symptom scores, skin prick tests, and the nasal allergen challenge score. The authors found a greater improvement in the three years of SLIT group compared with the two years of treatment when they compared results over six years. However, the lack of a placebo group during discontinuation of treatment makes the validity of these results difficult to interpret (88).

A 13-year retrospective study evaluated the long-lasting effect of SLIT and also assessed if this effect could be related to the duration of treatment (87). Patients had HDM-allergic rhinitis and also had a retrospective positive methacholine challenge performed at baseline. However, the study design means that the results are difficult to interpret. Long-term well-designed DB PC RCTs in large populations are needed to assess the long-term effects of SLIT after discontinuing treatment.

PROGRESSION OF ATOPY

The progression from allergic rhinitis to allergic asthma was specifically evaluated in an open study conducted by Novembre et al. in 113 children aged 5 to 14 years (89). These children had hay fever limited to grass pollen. This was an open study in which patients were randomized to receive preseasonal and coseasonal SLIT for three years or to observation only with standard symptomatic therapy. At recruitment, none of the children reported more than three episodes of seasonal asthma per year. The SLIT-treated group used less medication in the second and third years of therapy, and their symptom scores tended to be lower. From the second year of SLIT, subjective evaluation of overall allergy symptoms was favorable in the actively treated children. Development of asthma after three years was 3.8 times more frequent in the control subjects.

In an open, controlled, two parallel group study, 511 patients, with allergic rhinitis with or without intermittent asthma, were randomized to drugs only or drugs and SLIT for three years (90). At the end of the treatment period, one or more new sensitizations to allergens as determined by skin tests versus those existing at the time of randomization appeared in 16 of 271 (5.9%) patients in the SLIT group and 64 of 170 (38%) patients in the control group ($p = 0.01$).

The long-lasting effects of SLIT in children (mean age 8.5 years) with asthma due to HDM was evaluated in a 10-year prospective open study by Di Rienzo et al. (85). The authors found that the mean peak expiratory flow result was significantly higher in the active than in the control group. They concluded that SLIT is effective and maintains the clinical efficacy for four to five years after discontinuation. Although these studies are encouraging, at present, there is insufficient evidence to draw firm conclusions on the long-term effects of SLIT on the development of new sensitizations.

DOSE EFFICACY

Evaluation of efficacy by dose response has been evaluated in three large multicenter DB PC RCT studies (38,57,74). The literature was reviewed to determine whether the allergen SLIT dose is related to clinical outcomes. Studies that reported allergen dose in mass units and symptom and/or medication scores were reviewed. Of the 46 RCT DB PCT identified, clinical scores were available in 42 and the allergen dose in mass units in 35 studies. Thirty-one studies were included in this analysis. Twelve studies used grasses, nine HDM, five tree pollens, and five weeds. The median administration time for SLIT was 10 months (range 3–36 months) (Fig. 3). Cumulative dose was variable, ranging from a monthly dose of 0.6 to 6840 μg. Allergen dose was not significantly correlated with lower clinical scores.

We are currently conducting a meta-regression analysis to corroborate these findings Nonetheless, the data imply that large definitive trials will be needed to determine dose-response relationships for SLIT. There are currently two reported. Durham et al. evaluated the

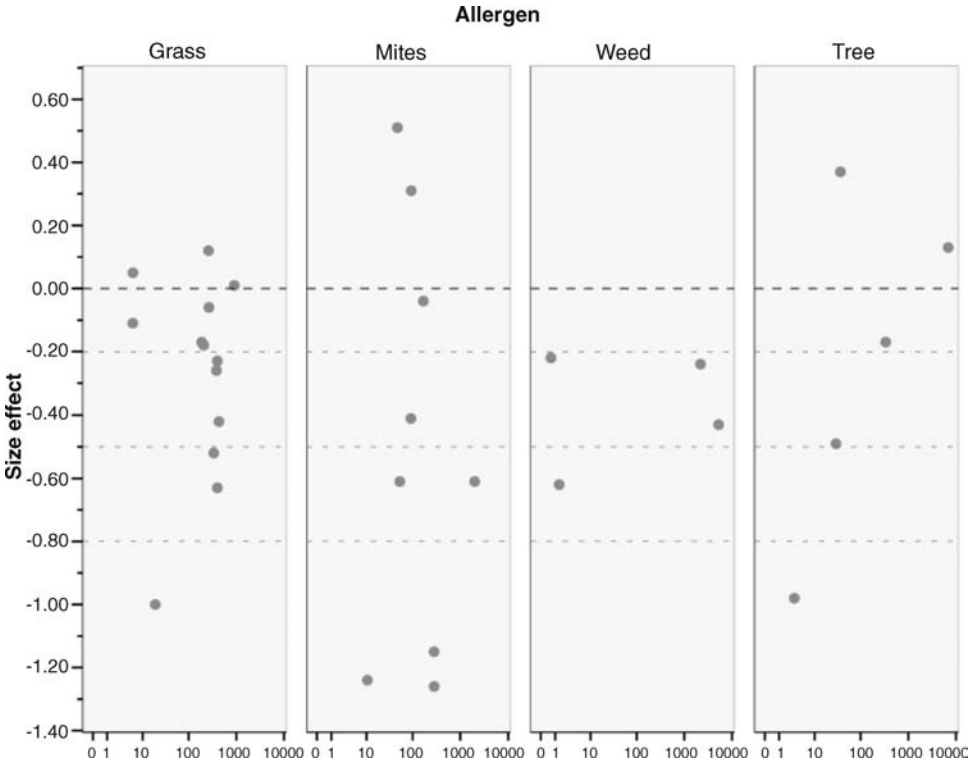

Figure 3 Effect of the allergen dose on clinical efficacy.

Table 3 Efficacy of Sublingual Immunotherapy: In Vivo Studies

Author, yr	Immediate skin reactivity	Nasal provocation test	Immediate conjunctival reactivity
Ariano et al., 2001		2.00 (0.88, 3.12)	
Bahceciler et al., 2001	−1.22 (−2.36, −0.09)		
Bufe et al., 2004	0.05 (−0.29, 0.39)		
Drachenberg et al., 2001	0.65 (−0.02, 1.31)		
Lue et al., 2006	0.34 (−0.55, 1.22)		
Marcucci et al., 2003		0.36 (−0.45, 1.17)	
Rolinck-Werninghaus et al., 2004	0.51 (0.05, 0.96)	−0.31 (−0.76, 0.14)	0.17 (−0.28, 0.62)
Sanchez-Palacios, 2001	0.15 (−0.47, 0.77)		
Troise et al., 1995		0.66 (−0.06, 1.39)	
Vourdas et al., 1998	−0.46 (−0.94, 0.03)		
Wessner et al., 2003		0.36 (−0.35, 1.06)	
Wuthrich, 2003		−0.45 (−1.30, 0.40)	

effect of three doses of a grass pollen tablet (2500, 25,000, and 75,000 SQ-T units of *P. pratense*) in 855 adults. Treatment was administered daily for a mean duration of 18 weeks. The authors found a significant improvement in medication and symptom scores only in the highest-dose treatment group, 75,000 SQ-T (equivalent to 15 mg of Phl p 5) compared with the placebo group (38). Similarly, in another study, 628 adults with grass pollen rhinoconjunctivitis were randomized in three groups, receiving 100-, 300-, and 500-IR of a five-grass pollen SLIT tablet. Treatment started four months before the grass pollen season and was given until the end of the pollen season. Significant improvements in symptom and medication scores were found in both 300- and 500-IR groups compared with placebo (57). These two studies confirm a dose-response relationship and the need for high doses of grass pollen SLIT using preseasonal protocols of up to four months. Whether more prolonged courses of SLIT, for example, one to three years, will require lower doses than for preseasonal protocols, remains to be determined.

IN VIVO TESTS

Twenty-nine studies carried out in vivo tests to evaluate the efficacy of SLIT compared with placebo. Only 12 studies provided quantitative data suitable for statistical analysis (Table 3). Seven studies reported results of immediate skin reactivity by means of skin prick tests (37,50,62,63,68,72,75). A significant increase of skin reactivity was found by Bufe (63) and Rolinck-Werninghaus (72) in children treated with SLIT. Those children who clinically improved by SLIT did not show a significant reduction of sensitivity in skin prick tests.

Nasal provocation tests were carried out in six studies (28,53,72,76,91,92). Only in Ariano's study (28) did the challenge tests result in a significant increase in the allergen dose required to develop a positive test. Immediate conjunctival reactivity was evaluated by Rolinck-Werninghaus (72). Even though an increase in allergen dose was found, the difference is not statistically significant. A significant heterogeneity exists in all studies, because most of the trials are designed to assess clinical efficacy as the primary end point.

CONCLUSION

Evidence-based medicine demonstrates that SLIT is effective in reducing symptoms and there is need for rescue medication in patients with allergic rhinoconjunctivitis due to grass pollen. This clinical effect is associated with an excellent safety profile and makes SLIT suitable for home administration. SLIT offers efficacy, safety, and convenience, and therefore provides the ideal characteristics for allergen immunotherapy to be given to a wider population of allergic people. More studies are needed to confirm the optimal dose and schedule to be used and to clarify indications for SLIT in children. Evaluation of the influence of SLIT on long-term efficacy and progression of atopic diseases requires further well-designed clinical studies in large cohorts.

SALIENT POINTS

- EBM shows that SLIT is efficacious in improving symptom and medication scores.
- SLIT has a very good safety profile.
  - Minor local side effects (itching and swelling of the oral mucosa) were reported almost universally, but were rarely of significance.
- The good clinical effect of SLIT associated with its excellent safety profile makes it suitable for home administration.
- Long-term efficacy and progression of atopy of SLIT needs to be confirmed with further well-designed clinical studies in large cohorts.
- More studies are needed to confirm the optimal dose and schedule to be used.
- SLIT can be considered as an alternative to SCIT.

REFERENCES

1. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO Position Paper. J Allergy Clin Immunol 1998; 102:558–562.
2. Durham SR. Tradition and innovation: finding the right balance. J Allergy Clin Immunol 2007; 119:792–795.
3. Canonica GW, Passalacqua G. Non-injection routes for immunotherapy. J Allergy Clin Immunol 2003; 111:437–448.
4. Cox LS, Linnemann DL, Nolte H, et al. Sublingual immunotherapy: a comprehensive review. J Allergy Clin Immunol 2006; 17:1021–1035.
5. Black JH. The oral administration of ragweed pollen. J Allergy 1939; 10:156–158.
6. Feinberg SM, Foran FL, Lichtenstein M. Oral pollen therapy in ragweed pollinosis. J Am Med Assoc 1940; 115:23–39.
7. Scadding GK, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to house-dust mite. Clin Allergy 1986; 16:483–491.
8. Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop Group, World Health Organization. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001; 108:S147–S334.
9. Brozek JL, Baena-Cagnani C, Bonini S, et al. Methodology for development of the Allergic Rhinitis and its Impact on Asthma Guideline 2008 update. Allergy 2008; 63(1):38–46.
10. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999; 341:468–475.
11. Moller C, Dreborg S, Ferdousi HA, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 2002; 109: 251–256.
12. Niggemann B, Jacobsen L, Dreborg S, et al. PAT Investigator Group: Five-year follow-up on the PAT study: specific immunotherapy and long-term prevention of asthma in children. Allergy 2006; 61(7): 855–859.
13. Jacobsen L, Niggemann B, Dreborg S, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy 2007; 62:943–948.
14. Passalacqua G, Guerra L, Pasquali M, et al. Efficacy and safety of sublingual immunotherapy. Ann Allergy Asthma Immunol 2004; 93:3–12.
15. Bousquet J, Demoly P. Specific immunotherapy—an optimistic future. Allergy 2006; 61:1155–1158.
16. Leatherman BD, Owen S, Parker M, et al. Sublingual immunotherapy: past, present, paradigm for the future? A review of the literature. Otolaryngol Head Neck Surg 2007; 136:S1–S20.
17. Calderon MA, Birk AO, Andersen JS, et al. Prolonged preseasonal treatment phase with Grazax sublingual immunotherapy increases clinical efficacy. Allergy 2007; 62:958–961.
18. Akobeng AK. Principles of evidence based medicine. Arch Dis Child 2005; 90:837–840.
19. Sackett DL. Clinical epidemiology. What, who, and whither. J Clin Epidemiol 2002; 55:1161–1166.
20. Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. BMJ 1997; 315:1533–1537.
21. Higgins JPT, Green S, eds. Formulating the problem. Cochrane Handbook for Systematic Reviews of Interventions 4.2.6 (updated September 2006); Section 6b.2. In: The Cochrane Library, Issue 4. Chichester, UK: John Wiley, 2006.
22. Wilson DR, Torres-Lima M, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005; 60:4–12.
23. Penagos M, Compalati E, Tarantini F, et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: a meta-analysis of randomized, placebo-controlled, double-blind trials. Ann Allergy Asthma Immunol 2006; 97:141–148.

24. Calamita Z, Saconato H, Pela AB, et al. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. Allergy 2006; 61: 1162–1172.
25. Penagos M, Passalacqua G, Compalati E, et al. Meta-analysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. Chest 2007; [Epub ahead of print]: DOI:10.1378/chest.06-1425.
26. Radulovic S, Calderon M, Wilson S, et al. Sublingual immunotherapy for allergic rhinitis: an updated Cochrane systematic review and meta-analysis. Allergy 2007; 62(s83):167–551.
27. Andre C, Perrin-Fayolle M, Grosclaude M, et al. A double-blind placebo-controlled evaluation of sublingual immunotherapy with a standardized ragweed extract in patients with seasonal rhinitis. Evidence for a dose-response relationship. Int Arch Allergy Immunol 2003; 131:111–118.
28. Ariano R, Spadolini I, Panzani RC. Efficacy of sublingual specific immunotherapy in Cupressaceae allergy using an extract of Cupressus arizonica. A double blind study. Allergol Immunopathol (Madr) 2001; 29:238–244.
29. Bousquet J, Scheinmann P, Guinnepain MT, et al. Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. Allergy 1999; 54:249–260.
30. Bowen T, Greenbaum J, Charbonneau Y, et al. Canadian trial of sublingual swallow immunotherapy for ragweed rhinoconjunctivitis. Ann Allergy Asthma Immunol 2004; 93:425–430.
31. Casanovas M, Guerra F, Moreno C, et al. Double-blind, placebo-controlled clinical trial of preseasonal treatment with allergenic extracts of Olea europaea pollen administered sublingually. J Investig Allergol Clin Immunol 1994; 4:305–314.
32. Clavel R, Bousquet J, André C. Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. Allergy 1998; 53:493–498.
33. Purello-D'Ambrosio F, Gangemi S, Isola S, et al. Sublingual immunotherapy: a double-blind, placebo-controlled trial with Parietaria judaica extract standardized in mass units in patients with rhinoconjunctivitis, asthma, or both. Allergy 1999; 54:968–973.
34. Dahl R, Kapp A, Colombo G, et al. Efficacy and safety of sublingual immunotherapy with grass allergen tablet for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 118:434–440.
35. Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. Allergy 2006; 61:185–190.
36. Di Rienzo V, Pagani A, Parmiani S, et al. Postmarketing surveillance study on the safety of sublingual immunotherapy in children. Allergy 1999; 54:1110–1113.
37. Drachenberg KJ, Pfeiffer P, Urban E. Sublingual immunotherapy—results from a multi-centre, randomised, double-blind, placebo-controlled study with a standardised birch and grass/rye pollen extract. Allergologie 2001; 24:525–534.
38. Durham SR, Yang WH, Pedersen MR, et al. Sublingual once-daily grass-pollen immunotherapy: a randomised controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117: 802–809.
39. Feliziani V, Lattuada G, Parmiani S, et al. Safety and efficacy of sublingual rush immunotherapy with grass allergen extracts. A double blind study. Allergol Immunopathol (Madr) 1995; 23(5):224–230.
40. Guez S, Vatrinet C, Fadel R, et al. House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis: a double-blind, placebo-controlled study. Allergy 2000; 55:369–375.
41. Hordijk GJ, Antvelink JB, Luwema RA. Sublingual immunotherapy with a standardised grass pollen extract; a double-blind placebo-controlled study. Allergol Immunopathol (Madr) 1998; 26:234–240.
42. Lima MT, Wilson D, Pitkin L, et al. Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. Clin Exp Allergy 2002; 32:507–514.
43. Nelson HS, Oppenheimer J, Vatsia GA, et al. A double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized cat extract. J Allergy Clin Immunol 1993; 92:229–236.
44. Palma-Carlos AG, Santos AS, Branco-Ferreira M, et al. Clinical efficacy and safety of preseasonal sublingual immunotherapy with grass pollen carbamylated allergoid in rhinitic patients. A double-blind, placebo-controlled study. Allergol Immunopathol (Madr) 2006; 34:194–198.
45. Passalacqua G, Albano M, Fregonese L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. Lancet 1998; 351:629–632.
46. Passalacqua G, Albano M, Riccio A, et al. Clinical and immunologic effects of a rush sublingual immunotherapy to Parietaria species: a double-blind, placebo-controlled trial. J Allergy Clin Immunol 1999; 104:964–968.
47. Passalacqua G, Pasquali M, Ariano R, et al. Randomized double-blind controlled study with sublingual carbamylated allergoid immunotherapy in mild rhinitis due to mites. Allergy 2006; 61:849–854.
48. Pradalier A, Basset D, Claudel A, et al. Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. Allergy 1999; 54:819–528.

49. Sabbah A, Hassoun S, Le SJ, et al. A double-blind, placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. Allergy 1994; 49(5):309–313.
50. Sánchez Palacios A, Schamann F, García JA. Sublingual immunotherapy with cat epithelial extract. Personal experience. Allergol Immunopathol (Madr) 2001; 29:60–65.
51. Smith H, White P, Annila I, et al. Randomized controlled trial of highdose sublingual immunotherapy to treat seasonal allergic rhinitis. J Allergy Clin Immunol 2004; 114:831–837.
52. Tonnel AB, Scherpereel A, Douay B, et al. Allergic rhinitis due to house dust mites: evaluation of the efficacy of specific sublingual immunotherapy. Allergy 2004; 59:491–497.
53. Troise C, Voltolini S, Canessa A, et al. Sublingual immunotherapy in Parietaria pollen-induced rhinitis: a double-blind study. J Investig Allergol Clin Immunol 1995; 5:25–30.
54. Vervloet D, Birnbaum J, Laurent P, et al. Safety and efficacy of Juniperus ashei sublingual-swallow ultra-rush pollen immunotherapy in cypress rhinoconjunctivitis. A double-blind, placebo-controlled study. Int Arch Allergy Immunol 2007; 142:239–246.
55. Voltolini S, Modena P, Minale P, et al. Sublingual immunotherapy in tree pollen allergy. Double-blind, placebo-controlled study with a biologically standardised extract of three pollens (alder, birch and hazel) administered by a rush schedule. Allergol Immunopathol (Madr) 2001; 29:103–110.
56. Worm M. Efficacy and tolerability of high dose sublingual immunotherapy in patients with rhinoconjunctivitis. Allerg Immunol (Paris) 2006; 38:355–360.
57. Didier A, Malling HJ, Worm M, et al. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol 2007; 120(6):1338–1345.
58. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed. New Jersey: Lawrence Erlbaum, 1988.
59. Emminger W, Dahl R, Kapp A, et al. Progressive effect of grass tablet immunotherapy during long-term treatment in patients with rhinoconjunctivitis. Allergy 2007; 62(S83): 240 (abstr).
60. Rak S, Yang WH, Pedersen MR, et al. Once-daily sublingual allergen-specific immunotherapy improves quality of life in patients with grass pollen-induced allergic rhinoconjunctivitis: a double-blind, randomised study. Qual Life Res 2007; 16(2):191–201; [Epub 2006, Oct 11].
61. Malling H, Mélac M, de Beaumont O, et al. Efficacy of sublingual immunotherapy (SLIT) tablets for grass pollen rhinoconjunctivitis in patients with or without mild asthma, mono- or polysensitised. Allergy 2007; 62(supp 83):167–551.
62. Bahceciler NN, Isik U, Barlan IB, et al. Efficacy of sublingual immunotherapy in children with asthma and rhinitis: a double-blind, placebo-controlled study. Pediatric Pulmonology 2001; 32:49–55.
63. Bufe A, Ziegler-Kirbach E, Stoeckmann E, et al. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. Allergy 2004; 59:498–504.
64. Caffarelli C, Sensi LG, Marcucci F, et al. Preseasonal local allergoid immunotherapy to grass pollen in children: a double-blind, placebo-controlled, randomized trial. Allergy 2000; 55:1142–1147.
65. Hirsch Th, Sähn M, Leupold W. Double-blind placebo-controlled study of sublingual immunotherapy with house dust mite extract (D.pt.) in children. Pediatr Allergy Immunol 1997; 8:21–27.
66. Ippoliti F, De Sanctis W, Volterrani A, et al. Immunomodulation during sublingual therapy in allergic children. Pediatr Allergy Immunol 2003; 14:216–221.
67. La Rosa M, Ranno C, Andre C, et al. Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized Parietaria judaica in children with allergic rhinoconjuntivitis. J Allergy Clin Immunol 1999; 104:425–432.
68. Lue KH, Lin YH, Sun HL, et al. Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. Pediatr Allergy Immunol 2006; 17:408–415.
69. Niu CK, Chen WY, Huang JL, et al. Efficacy of sublingual immunotherapy with high-dose mite extracts in asthma: A multi-center, double-blind, randomized, and placebo-controlled study in Taiwan. Respir Med 2006; 100(8):1374–1383. (Epub 2006, Jan 5.).
70. Pajno GB, Morabito L, Barberio G, et al. Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. Allergy 2000; 55:842–849.
71. Röder E, Berger MY, Hop WC, et al. Sublingual immunotherapy with grass pollen is not effective in symptomatic youngsters in primary care. J Allergy Clin Immunol 2007; 119:892–898.
72. Rolinck-Werninghaus C, Wolf H, Liebke C, et al. A prospective, randomized, double-blind, placebo-controlled multi-centre study on the efficacy and safety of sublingual immunotherapy (SLIT) in children with seasonal allergic rhinoconjunctivitis to grass pollen. Allergy 2004; 59:1285–1293.
73. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double-blind study. Allergol Immunopathol (Madr) 1990; 18(5):277–284.
74. Valovirta E, Jacobsen L, Ljorring C, et al. Clinical efficacy and safety of sublingual immunotherapy with tree pollen extract in children. Allergy 2006; 61:1177–1183.

75. Vourdas D, Syrigou E, Potamianou P, et al. Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due a olive pollen sensitization. Allergy 1998; 53:662–672.
76. Wüthrich B, Bucher Ch, Jörg W, et al. Double-blind placebo-controlled study with sublingual immunotherapy in children with seasonal allergic rhinitis to grass pollen. J Invest Allergol Clin Immunol 2003; 13:145–148.
77. Agostinis F, Tellarini L, Canonica GW, et al. Safety of sublingual immunotherapy with a monomeric allergoid in very young children. Allergy 2005; 60:133.
78. Piazza I, Bizzaro N. Humoral response to subcutaneous, oral, and nasal immunotherapy for allergic rhinitis due to Dermatophagoides pteronyssinus. Ann Allergy 1993; 71:461–469.
79. Bernardis P, Agnoletto M, Puccinelli P, et al. Injective versus sublingual immunotherapy in Alternaria tenuis allergic patients. J Investig Allergol Clin Immunol 1996; 6:55–62.
80. Quirino T, Iemoli E, Siciliani E, et al. Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study. Clin Exp Allergy 1996; 26:1253–1261.
81. Mungan D, Misirligil Z, Gurbuz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma-a placebo controlled study. Ann Allergy Asthma Immunol 1999; 82:485–490.
82. Khinchi MS, Poulsen LK, Carat F, et al. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. Allergy 2004; 59:45–53.
83. Herrscher RF. Evaluation of three allergen specific immunotherapy methods: standard injection, rush injection and sublingual. In: Proceedings of the annual meeting of American College of Allergy, Asthma, and Immunology; 2005 Nov 5–7; Anaheim: ACAAI; 2005. Abstract nr 54.
84. Mauroa M, Russelloa M, Incorvaiab C, et al. Comparison of efficacy, safety and immunologic effects of subcutaneous and sublingual immunotherapy in birch pollinosis: a randomized study. Eur Ann Allergy Clin Immunol 2007; 39:119–122.
85. Di Rienzo V, Marcucci F, Puccinelli P, et al. Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study. Clin Exp Allergy 2003; 33(2):206–210.
86. Cingi C, Aynaci S, Cakli H, et al. Efficacy of long-term sublingual-oral immunotherapy in allergic rhinitis. Acta Otorhinolaryngol Ital 2005; 25:214–219.
87. Marogna M, Bruno M, Massolo A, et al. Long-lasting effects of sublingual immunotherapy for house dust mites in allergic rhinitis with bronchial hyperreactivity: a long-term (13-year) retrospective study in real life. Int Arch Allergy Immunol 2007; 142:70–78.
88. Tahamiler R, Saritzali G, Canakcioglu S. Long-term efficacy of sublingual immunotherapy in patients with perennial rhinitis. Laryngoscope 2007; 117:965–969.
89. Novembre E, Galli E, Landi F, et al. Coseasonal sublingual immunotherapy reduces the development of asthma in children with allergic rhinoconjunctivitis. J Allergy Clin Immunol 2004; 114:851–857.
90. Marogna M, Spadolini I, Massolo A, et al. Randomized controlled open study of sublingual immunotherapy for respiratory allergy in real-life: clinical efficacy and more. Allergy 2004; 59:1205–1210.
91. Wessner D, Rakoski J, Ring J. Efficacy of sublingual immunotherapy in grass pollen allergy. Chem Immunol Allergy 2003; 82:53–61.
92. Marcucci F, Sensi L, Frati F, et al. Effects on inflammation parameters of a double-blind, placebo controlled one-year course of SLIT in children monosensitized to mites. Allergy 2003; 58:657–662.

24 Immunotherapy for Hymenoptera Venom Hypersensitivity

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INTRODUCTION

Insect stings, especially by Hymenoptera of the families *Apidae* (the honeybee and the bumblebee), *Vespidae* (with the species *Vespula*, *Dolichovespula*, *Vespa* and *Polistes*), and in some regions also *Formicidae* (the ants), are one of the major causes for severe, generalized, immunoglobulin E (IgE)-mediated hypersensitivity reactions that may be fatal. According to the registered data of the Swiss Statistical Department, 132 individuals died from Hymenoptera stings in Switzerland between 1962 and 2004, an average of three per year (Fig. 1). Extrapolation of the Swiss data (population 7.5 million) to the European Union (population 485 million), indicates about 200 yearly fatalities from Hymenoptera stings in the European Union. Government statistics in the United States show at least 40 deaths each year from insect stings, although it is likely that many others are not reported.

The first attempts at immunotherapy for Hymenoptera sting allergic patients were made at the end of the 1920s. Insect venom or venom sac vaccines were first used. The high frequency of side effects with these vaccines and the report of the successful treatment of a beekeeper with whole body vaccine of bees led to their worldwide use. The results of immunotherapy with these preparations were favorable in uncontrolled studies (1). It was only in the late sixties and seventies of the last century that venoms were shown to be superior to whole body extracts for diagnosis and controlled studies documented the superiority of venoms over whole body vaccines for immunotherapy of Hymenoptera sting allergic individuals (2,3). Venoms obtained by electrostimulation or by venom sac extraction were commercially introduced in 1979 and have since been used worldwide successfully for immunotherapy of patients allergic to stings by *Apidae* and *Vespidae*. Venom preparations are as yet not commercially available for *Formicidae*. From the section titled "Indications" to the section "Special Aspects of Ant Hypersensitivity," various aspects of immunotherapy for Hymenoptera sting hypersensitivity are dealt with. The last section considers new approaches to venom immunotherapy (VIT).

INDICATIONS

History

The indications for VIT include only two factors: a history of systemic allergic reaction to a sting and positive diagnostic tests (4,5). The history is especially important because diagnostic tests with venoms are positive in 10% to 20% of asymptomatic individuals. There is an absolute need to correlate the history with the test results (6,7).

Systemic reactions to stings consist of any one or more of the signs and symptoms of anaphylaxis or may be limited to cutaneous manifestations, which is more common in children

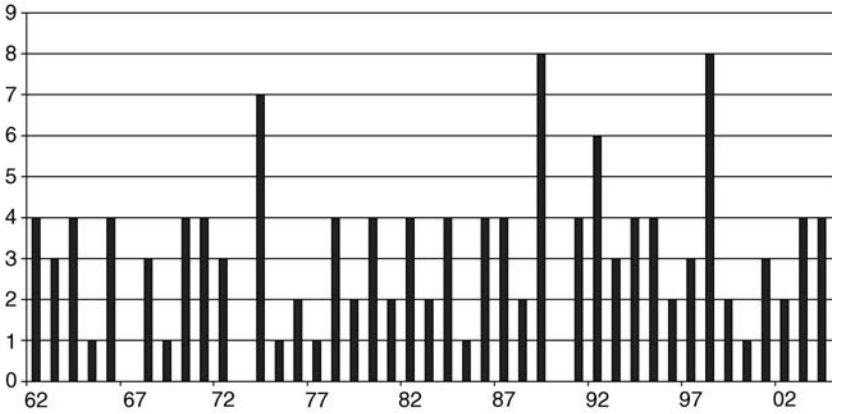

Figure 1 132 fatalities from Hymenoptera sting reactions in Switzerland from 1962 to 2004.

(60%) than in adults (15%) (8,9). Respiratory symptoms occur with equal frequency in about 40% of children and adults. Cardiovascular signs and symptoms are common in adults (30%) but infrequent in children (10%). It is sometimes difficult to be certain whether symptoms are truly anaphylactic because they may result from anxiety, pain, or toxic effects from the stings, especially when many stings occur. It is most helpful when objective signs of anaphylaxis are noted, e.g., generalized urticaria, angioedema, documented hypotension, wheezing, reduced airflow, or oxygen desaturation. The severity of the reaction is one of the most important factors determining the need for VIT, the duration of treatment, and the risk of adverse reactions to injections (10). Although identification of the stinging insect by patients and physicians is unreliable, the identity of the culprit insect is important because honeybee allergy is associated with greater risks and less reliable treatment efficacy (11). In the absence of a history of a sting-induced allergic reaction, sensitization by an asymptomatic sting has been reported to be associated with a 17% chance of a systemic reaction to a future sting, but is not considered an indication for VIT, especially since asymptomatic sensitivity is transient in many cases (6). For this reason, venom allergy testing and treatment is not recommended when requested by some individuals, out of fear alone, such as a family member of someone who had a fatal reaction to a sting.

Contraindications for VIT are the same as for immunotherapy for inhalant allergy, but are relative in nature because of the life-saving potential of therapy. The relative contraindications include severe immunodeficiency, autoimmune and neoplastic diseases, and chronic infections. Treatment with β-blockers may increase the severity of allergic reactions when patients are restung. Elderly patients with cardiovascular disease are at increased risk of developing severe or even fatal sting reactions. Many are on a β-blocker and have a significantly increased survival rate on this treatment. Therefore, VIT on β-blockers may be indicated even in such patients (12).

VIT should not be started during pregnancy, but well-tolerated maintenance VIT may be continued during pregnancy (4). Sting reactions such as Henoch-Schoenlein syndrome, vacuities, acute disseminated encephalomyelitis, or interstitial nephritis should not be treated by VIT, while patients who develop cerebrovascular or myocardial infarction during anaphylaxis and have positive tests are candidates for such therapy (13,14).

Diagnostic Testing: Skin Tests and In Vitro Serum IgE

The decision to begin VIT requires confirmation of allergic sensitivity to venom allergens by positive venom skin tests or detection of venom-specific IgE antibodies in the serum (Table 1).

The standard skin test uses the intradermal test technique with commercially available Hymenoptera venom preparations. For Hymenoptera venom testing, prick tests at 0.1 to 1 μg/mL (6,15) may be used initially for patients with a history of very severe reactions. Aqueous venom preparations are used for intradermal tests beginning with concentrations of 0.001 to 0.01 μg/mL and increasing, if necessary, to 1.0 μg/mL to find the minimum concentration giving a positive reaction. Honeybee venom is somewhat more irritating and can

Table 1 Clinical Recommendations Based on History of Sting Reactions and Results of Venom Skin Test or RAST

Reaction to previous sting	Skin test or RAST	Risk of systemic reaction	Clinical advice
No reaction	Positive	10–15%	Avoidance
Large local	Positive	5–10%	Avoidance
Cutaneous systemic	Positive—Child	10%	No VIT
	Adult	15–20%	VIT
Anaphylaxis	Positive	50–75[a]%	VIT
	Negative	2–5%	Repeat skin test/RAST

[a]According to controlled studies.

Table 2 Results of Evaluation of 309 Patients with a Positive History of Systemic Reaction

Skin test positive	68%
ST negative with RAST positive	14%
ST negative and RAST negative with	
sting challenge negative	17%
sting challenge positive	1%

induce weak positive reactions in nonallergic individuals. Yellow jacket (*Vespula*) venom causes false-positive reactions primarily at the 10 μg/mL concentration in 10% of nonallergic subjects (15).

Most patients with a convincing history of insect allergy have positive venom tests but some have negative skin tests (16) (Table 2). Negative skin tests can be due to loss of sensitivity and can also occur in up to 50% during the refractory period of four to six weeks following a sting reaction. They are also caused by antihistamines, certain neuroleptic, and antidepressant drugs. When there is a history of severe anaphylaxis and venom skin tests are negative, in vitro tests for venom-specific IgE antibodies should be obtained, and the patient should continue avoidance precautions. In many cases like this, the in vitro test may be positive. Some cases of apparent sting anaphylaxis are thought to be non–IgE mediated, which would account for the negative in vivo and in vitro test results.

The detection in vitro of allergen-specific IgE antibodies is useful. A high level of venom-specific IgE is usually diagnostic but must be correlated with the history. A low level of venom-IgE is more difficult to interpret. Even a very low level of venom-IgE can be associated with near-fatal anaphylaxis. The venom skin test and in vitro test results may not correlate as the in vitro test results are negative in approximately 20% of skin test positive subjects (16–19). Skin tests are preferred clinically for their greater sensitivity. The converse is also true: approximately 10% of skin test negative patients have a positive in vitro test. Therefore, European allergists recommend skin testing and estimation of venom-specific IgE in all individuals with a history of systemic allergic reactions. If both tests are negative in the presence of a strong history, cellular tests like CAST (cellular antigen stimulation test) or BAT (basophil activation test), although more expensive, may be helpful (20,21). A positive result with these tests may also point to a non–IgE mediated mast cell activation mechanism.

Neither the degree of skin test sensitivity nor the titer of specific IgE correlate reliably with the degree of clinical sting reaction. Patients who have had only large local reactions may have very high levels of sensitivity of both skin and in vitro tests but have a very low risk of anaphylaxis, whereas some patients who have had an abrupt and near-fatal anaphylactic reaction have only weak skin test or serologic positivity. In fact, almost 25% of patients presenting for evaluation of systemic allergic reactions to stings are skin test positive only at the 1.0 μg/mL concentration, demonstrating the importance of testing with the full diagnostic range of venoms. Again, these points emphasize the importance of the history in making the correct diagnosis, assessing prognosis, and instituting appropriate treatment.

Another diagnostic option is a supervised live sting challenge. The history and test results help identify patients at high risk, but even patients with previous severe reactions only

have a 50% to 75% chance of reacting to a future sting. The sting challenge helps select those patients who will most likely have another systemic reaction to a field sting (22). However, even a negative sting challenge does not rule out future reactions, because 20% of patients who did not react to one challenge sting do react to a repeat challenge sting (23). Others have considered the diagnostic sting challenge to be unethical and recommend it only as a test to evaluate the efficacy of immunotherapy (24).

Some patients with positive venom skin tests were at low risk of anaphylaxis because their systemic reactions were mild. Subsequent stings usually cause no systemic reaction or a reaction that tends to be equal or less severe than previous reactions. However, there are patients who have reactions of increasing severity to subsequent stings. As currently practiced in North America, adults with sting-induced generalized urticaria and angioedema, as well as patients of all ages with more severe reactions, including pharyngeal symptoms, dyspnea, dizziness, or hypotension are advised to undergo VIT. The majority of children ($\leq$15 years) have reactions confined to the skin (generalized erythema, itching, and urticaria and angioedema) but no involvement of the pharynx or respiratory or circulatory systems. In 15 to 20 year follow-up studies, stings caused no systemic reactions in 87%; 7% had another mild cutaneous and 6% a moderate systemic reaction, but none a severe systemic reaction (8,9). Therefore VIT is not generally recommended in this situation. However, it is sometimes given to highly exposed children with repeated reactions to improve their quality of life. The same recommendation for VIT is also used in Europe in adults with reactions limited to the skin because prospective studies indicate a low risk (15–20%) to develop a generalized reaction at reexposure (4).

Some patients with positive venom tests are at a relatively low risk of anaphylaxis because they never had anaphylaxis to a previous sting. Those children and adults with a large local, but no systemic reaction, seem to have a 4% to 10% chance of a subsequent systemic reaction (8,25). Therapy is not usually recommended for test positive patients with a history of a large local reaction, but may be helpful in heavily exposed individuals like farmers or gardeners. There is limited evidence that VIT prevents these reactions (26).

Patients with systemic mastocytosis (27,28) are at a high risk of developing severe or even fatal cardiovascular sting reactions. Two studies (29,30) indicate that severe cardiovascular reactions are associated with an elevated baseline serum tryptase (>11.4 ng/mL) in up to 25% of individuals, indicating an increased whole body mast cell load as a risk factor for life-threatening sting reactions. A routine baseline serum tryptase is, therefore, recommended in all patients with systemic sting reactions.

Selection of Venoms

The selection of venom vaccines for immunotherapy is dependent on the venom skin test reaction and presence of serum specific IgE antibodies. North American allergists/immunologists recommend that all venoms resulting in positive tests be included for VIT because preventing future sting reactions is not possible without specific therapy. Some investigators recommend treatment only with the venom of the suspected insect culprit (31). When vespids are involved, the most common practice is to treat with *Vespula* venom alone or, in North America, with the mixed vespid venom preparation. It contains equal parts of yellow jacket (*Vespula* spp), yellow hornet (*Dolichovespula arenaria*) and white-faced hornet (*Dolichovespula maculata*) venoms (32), which are not available in Europe. Although *Dolichovespula* are by no means rare, they are responsible for only a small percentage of vespid stings. These insects do not forage on human food and therefore, almost exclusively sting in the proximity of their nests. The same is true for the European hornet, *Vespa crabro*. Moreover, European *Dolichovespula*, in contrast to American *D. maculata*, can be distinguished from *Vespula* only by those trained to do so, with a magnifying glass. Finally, in vitro studies demonstrate ample crossreactivity among venoms of *Vespula, Dolichovespula,* and *Vespa*. Therefore, vespid allergic patients in Europe are treated by *Vespula* venom alone, which is effective in more than 95% (1,4) of treated patients.

The skin test is also positive to wasp (*Polistes*) venoms in at least 50% of vespid allergic patients. When positive, it is usually included in therapy as a separate injection, at least in areas where *Polistes* is important, as in the Gulf states of the United States and the Mediterranean countries in Europe. Therapy with *Vespula* or mixed vespid venoms protects against *Polistes* stings, but this has only been established for patients whose *Polistes*-specific

IgE antibodies completely cross-react with *Vespula* venom as assessed by radioallergosorbent test (RAST) inhibition (32).

Dual positivity of diagnostic tests with *Vespula* and honeybee venom in up to 50% of patients is observed, especially in countries where bee venom allergy is more frequent than vespid venom allergy (1). The history sometimes helps to identify the culprit insect, since vespids do not usually sting in spring and do not, in contrast to the honeybee, usually leave the stinger in the skin. However, in the United States, *V. maculifrons* leaves the stinger in 30% to 50% of cases. The limited cross-reactivity on peptide basis between protein allergens of *Vespula* and honeybee venoms is largely confined to hyaluronidase. Double positivity is often due to so-called CCDs (crossreacting carbohydrate determinants), which are probably of no clinical relevance. When tests are definitely positive with the two venoms, both should be included for VIT, unless complete cross-reactivity can be demonstrated by RAST inhibition with whole venoms and CCDs, like horseradish peroxidase or bromelain (33,34).

EFFICACY, SAFETY, AND MONITORING OF VIT

The recommended maintenance dose is 100 μg of venom, both in children and adults. This dose was originally suggested because it was believed to be equivalent to two stings. This is true of honeybee, but may be closer to 10 *Vespula* stings. Doses lower than 100 μg are not reliably effective in adults (35). VIT with honeybee venom gives full protection in 80% to 90% of cases, whereas therapy with *Vespula* venoms is 95% to 98% effective (11,24). When treatment with 100 μg is not fully effective, patients may be protected by using a higher dose (36).

VIT is safer than originally thought. Systemic reactions were expected to be more frequent or severe because of the underlying anaphylactic syndrome, but that has not been confirmed. The incidence of adverse reactions to venom is similar to that reported for inhalant allergen immunotherapy (37). For unexplained reasons, systemic allergic reactions are considerably more frequent during immunotherapy with honeybee venom (11). Systemic symptoms occur in 5% to 15% of patients on vespid venoms and 20% to 40% on honeybee venom, most often during the first weeks of treatment, regardless of the regimen used. Most reactions are mild. In the unusual case of recurrent systemic reactions to injections, therapy may be streamlined to a single venom and given in divided doses, 30 minutes apart. Large local reactions, which may be larger (8–10 cm) than are generally accepted during inhalant allergen immunotherapy, occur in up to 50% of patients, especially during updosing in the dose ranges of 20 to 50 μg. Large local reactions, however, are not predictive of systemic reactions to subsequent injections and do not prevent attaining the maintenance target dose of VIT.

Annual visits with the allergist serve to review the treatment plan and to assure that the patient does not have a new medication or medical condition which influences therapy. There is no need for annual skin tests or blood tests, although repeating the skin tests every two to three years is recommended to identify patients who possibly can stop treatment because their skin tests become negative. The venom-specific IgE level and skin test sensitivity usually increase in the first months of therapy, return to baseline after 12 months, and then decline steadily during maintenance treatment. This decline continues even after therapy is stopped or after a sting (38) (Fig. 2). Even after three to five years of treatment, these tests turn negative only in a minority of patients. Less than 20% of patients are skin test negative after five years, but 50% to 60% become negative after 7 to 10 years (39). Specific IgE may decrease more rapidly than skin sensitivity but also may persist at very low levels even when venom skin tests become negative (40).

Venom-specific IgG antibodies, especially IgG4, are high in beekepers, and passive immunotherapy with beekeeper gamma globulin protects bee venom–allergic individuals (41). Assays for venom-specific IgG correlate with clinical protection but cannot accurately predict the outcome of stings in every individual. The test may be used to confirm protective levels after initiating therapy and then to verify that the venom-IgG level is adequately maintained at the longer intervals used for maintenance treatment. In one study, the IgG level was considered protective with serum concentrations of >3 μg/mL during the first four years of maintenance therapy, but protection was independent of the IgG after four years of treatment, probably because of other mechanisms of action (42). Profound changes in the T-cell reactivity to allergen stimulation of venom allergic patients with a shift from a TH2- to a TH1- or

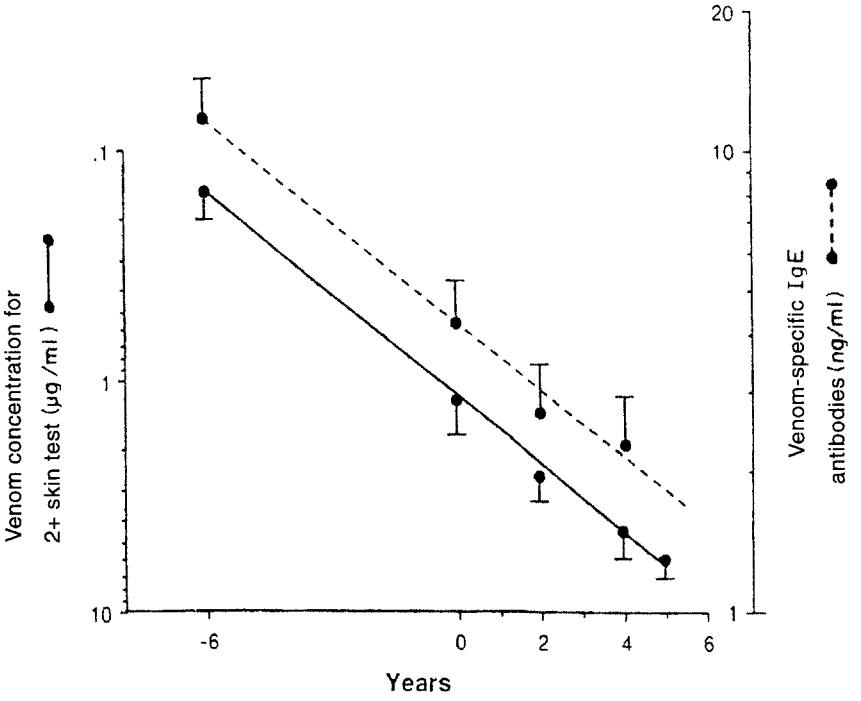

Figure 2 Mean venom skin test sensitivity (concentration in µg/mL for 2+ reaction) and venom-specific IgE antibody level (in ng/mL) shown before venom immunotherapy (time, −6 years), after a mean of six years of treatment (time 0), and 2, 4, and 5 years after stopping therapy. *Source*: From Ref. 38.

TH0- pattern and induction of IL-10–producing T regulatory cells are described during VIT (43). Data on the relation of these alterations to the efficacy of the treatment, as indicated by a tolerated sting, are not available.

IMMUNOTHERAPY PROTOCOLS

The starting dose of VIT is between 0.001 and 0.1 µg. The recommended maintenance dose is 100 µg of venom protein, corresponding to one to two bee stings and probably many more *Vespula* stings (4,5,44). Higher maintenance doses (200 µg or more) are recommended for beekeepers (45), who may be stung by several insects at the same time and in treatment failures or incomplete treatment success (4,36). The success rate increases with higher maintenance doses.

A number of protocols are used for the buildup phase, some of which are summarized in Table 3 (conventional, cluster, rush, or ultrarush protocols) (4,46). Many allergists in Europe use aluminium hydroxide–adsorbed venom for conventional protocols, while others use aqueous preparations for the buildup with accelerated protocols and then change to aluminium hydroxide–adsorbed venoms, which have a comparable efficacy to aqueous venoms. They are usually somewhat better tolerated for maintenance immunotherapy (47). In the United States, only aqueous preparations are available. Rush and ultrarush protocols have the advantage of inducing a more rapid protection and are therefore recommended in highly exposed individuals during the Hymenoptera flying season. Moreover, the number of visits during the buildup phase is greatly reduced. However, the incidence of side effects is somewhat higher in these rapid buildup protocols, especially in bee venom allergic individuals (11), and in rush protocols with high cumulative daily doses (46).

Once the maintenance dose is reached, the interval between injections is extended to four weeks in the first year and to six weeks from the second year of immunotherapy, provided that

Table 3 Treatment Protocols for VIT (after 4)

Protocol	Dose in μg venom					
Day	Hour	Ultrarush	Rush	Cluster	Conventional	Aluminium hydroxide adsorbed
1	0	0.1	0.01	0.001	0.01	0.02
	0.5	1	0.1	0.01	0.1	
	1	10	1	0.1		
	1.5	20				
	2.5	30	2			
		40				
2	0		4			
	1		8			
			10			
			20			
3	0		40			
	1		60			
	2		80			
4	0		100			
8	0		100	1	1	0.04
	1			5	2	
				10		
15	0	50	100	20	4	0.08
	1	50		30	8	
22	0	100		50	10	0.2
	1			50	20	
29			100	100	40	0.4
36				100	60	0.8
43		100	100		80	2
50					100	4
57					100	6
64				100		8
71		100	100		100	10
78						20
85					100	40
92				100		60
99		100	100			80
106				100		100

Further injections of the maintenance dose of 100 mg every four weeks during the first year, every six weeks during further years of VIT. *Abbreviation*: VIT, venom immunotherapy

the treatment is tolerated. An even longer interval is not recommended for honeybee allergic patients since beekeepers with less than 10 stings a year, are those who develop systemic allergic reactions most frequently (45). While the buildup phase of VIT should usually be performed by an allergist, injections can be continued by a general practitioner once the maintenance dose is achieved. Some allergists premedicate with antihistamines during the buildup phase because several controlled studies demonstrate reduced side effects (48).

DURATION OF VIT

After introduction in 1979, VIT was—and by some still is today—continued for life or at least until both skin tests and serum venom–specific IgE become negative. However, even after prolonged VIT, only a small proportion of patients develop negative diagnostic tests and compliance with continuation of VIT for many years often decreases (1,4).

For this reason, a number of studies address the protection rate after stopping VIT. In a first series, the reaction to a sting challenge (CH) one to three years after stopping VIT of at least three years duration was analyzed (Table 4) (49–54). All studies report continued

Table 4 Prospective Studies with Sting Provocation Test After Stopping VIT

Author	No. of patients	Insect	Sting challenge (CH)	
			After years	No. with GR (%)
Urbanek (49)	29	Honeybee	1	1 (3)
	14		2	2 (14)
Golden (50)	29	m *Vespula*	1	0
Müller (51)	86	Honeybee	1	15 (17)
Haugaard (52)	25	*Vespula*	2	0
Keating (53)	51	m *Vespula*	1	2 (4)
Van Halteren (54)	75	*Vespula*	1–3	6 (8)

Abbreviations: CH, sting challenge; GR, generalized allergic reaction; m Vespula, mostly Vespula.

Table 5 Long-Term Protection After Discontinuation of VIT

Author	No. of patients	Insect	Observation years after stop	Re-exposure	No. with GR (%)
Reisman (55)	113	mV	1–>5	FS	10 (9)
Golden (38)	74	mV	5	CH	7 (9.5)
Golden (40)	26	mV	3–7	FS	5 (19)
Lerch (44)	120	B	3–7	FS/CH	19 (15.8)
	80	V	3–7	FS/CH	6 (7.5)

Abbreviations: GR, generalized allergic reaction; mV, mostly *Vespula*; B, honey bee; FS, field sting; CH, sting challenge; no, number.

protection in the vast majority (83–100%) of patients. Results are somewhat more favorable in *Vespula* than in bee venom–allergic individuals and in children rather than adults.

Long-term protection for up to seven years after discontinuing VIT (Table 5) is analyzed in four studies (38–40,55,56). Reisman (55) observed relapses following a field sting up to more than five years after stopping VIT in 10 of 113 (9%) mostly *Vespula* venom allergic patients. Golden (38) followed 74 predominantly *Vespula* venom allergic patients for five years after stopping VIT of at least five years duration with a challenge every year (29 patients), every second year (25 patients) or only after two years (20 patients). Seven (9.5%) developed at least one generalized mild allergic reaction (GR). The same group (39) observed a GR to a field sting in 5 of 26 (19%) patients out of 125 who were followed up to seven years after VIT, and some of these reactions were severe. Lerch (56) reported on 358 patients up to seven years after stopping successful VIT. Two-hundred patients were reexposed by either a field sting or a challenge and 25 (12.5%) developed a general reaction: In 14 this was only cutaneous, in eight, there were also respiratory, in three cardiovascular symptoms. In 8 of the 11 patients with respiratory or cardiovascular symptoms, these occurred only after the second sting after stopping VIT. These studies of a prolonged observation after stopping VIT found relapses somewhat more frequently than the earlier shorter follow-up studies. Still, the great majority, 80% or more, remained protected when re-stung up to seven years after VIT was discontinued.

A number of risk factors for the recurrence of GR following Hymenoptera stings can be identified.

Age: Children generally have a more favorable prognosis than adults (8,9), both without VIT and also after discontinuing VIT. Urbanek (49) saw relapses in only 3% of BV allergic children, whereas Müller (51) observed 17% in 86 mostly adult patients after BV-immunotherapy. Lerch (56) recorded 8.3% relapses in 24 children as compared to 13.1% in 176 adults who were reexposed up to seven years after stopping VIT.

Insect: Analysis of the results presented in Table 5 as well as the recurrence rates after VIT reported by Lerch (56) of 7.5% for *Vespula* venom and 15.8% for bee venom treated patients indicate a higher risk of relapse in bee venom than in *Vespula* venom allergic patients. The reason for this difference is not entirely clear but has been discussed elsewhere (4,11,24).

Severity of pretreatment reaction: In four prospective studies involving 386 patients, relapses were observed in 5 (4.1%) of 123 with mild but 38 (14.5%) of 263 with severe pretreatment GR (53,55–57) ($X^2 = 9.128$, $p < 0.01$). In addition there is also a higher risk that a recurring reaction after stopping VIT in these patients will be more severe than in those with milder pretreatment reactions.

Safety and efficacy of VIT: Patients who develop generalized allergic side effects to VIT injections have a relapse risk of 38%, whereas those who do not, have a relapse risk of 7% (51). Similarly, incomplete protection with a re-sting during VIT is associated with an increased risk of relapse (38).

Duration of VIT: The risk of a relapse seems to be reduced with more prolonged VIT. Only 4.8% of 82 patients with a VIT duration of ≥ 50 months, as opposed to 17.8% of 118 with a VIT duration of 33 to 49 months, developed GRs when restung after discontinuation ($X^2 = 7.382$; $p < 0.01$) (56).

Elevated basal serum tryptase, mastocytosis: Insect venom allergy in patients with urticaria pigmentosa is most often associated with severe shock reactions (27). Two female patients with urticaria pigmentosa and *Vespula* venom sensitivity died from a re-sting 1.3 and 9 years after stopping VIT (28). One further fatal sting reactions was observed in a patient with systemic mastocytosis: The patient had been successfully treated by VIT for severe anaphylaxis following bee stings, but died when he was stung by a yellow jacket (58). Up to one quarter of patients with severe shock reactions following Hymenoptera stings have an elevated baseline serum tryptase level indicating the presence of an increased whole body mast cell load (29,30). Patients with mastocytosis and/or elevated baseline serum tryptase are at an increased risk to develop very severe or even fatal sting reactions. Since VIT is effective in such patients (27), it should be continued for life.

Repeated reexposure after stopping VIT: About half of the relapses occur after the first, the other half after later re-stings (56). The risk of a severe reaction increases significantly in the presence of repeated re-stings. Golden et al. (40) also described an increasing frequency of generalized reactions four years after stopping VIT compared with the first one to two years. Such was also the case in patients who developed systemic reactions after 7 to 13 years off VIT despite a nonreaction to a previous sting during the first few years after discontinuing VIT. On the basis of these observations, sting challenge after stopping VIT is not recommended.

High sensitivity according to diagnostic tests: An association of re-sting reactions has been observed with a persisting high sensitivity with intradermal skin testing (40). Others (51,56) are unable to confirm this. Specific serum IgE and IgG antibodies, per se, have no predictive value with regard to the re-sting risk after stopping therapy. Diagnostic tests currently utilized are of limited predictive value with regard to long-term protection after VIT. Only the combination of a negative intracutaneous skin test at 1 μg/mL and the absence of venom specific serum IgE-antibodies is associated with a strongly diminished risk of relapse (51,56). Gender and a history of atopic disease do not seem to influence the risk of a relapse after stopping VIT.

Most patients with Hymenoptera venom sensitivity remain protected, following discontinuation of VIT of at least three to five years duration. An even longer treatment duration has to be considered in the high-risk situations discussed above. Because of the small but relevant risk of re-sting reactions, epinephrine for self-administration should be considered for patients stopping VIT.

SPECIAL ASPECTS OF ANT HYPERSENSITIVITY

Classification

There are nearly 10,000 species of ants (order Hymenoptera, family *Formicidae*) recognized worldwide. Some of these sting victims, as do other Hymenoptera, and human reactions span the spectrum from a self-limited local reaction to life-threatening anaphylaxis. In the United States, only members of the genera *Solenopsis* (*S.*) (Fig. 3), the imported fire ant (IFA), and

Figure 3 Imported fire ant (*Solenopsis invicta*).

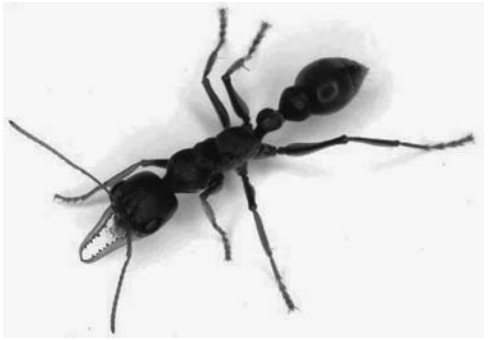

Figure 4 Jack jumper ant (*Myrmecia pilosula*).

Pogonomyrmex, the harvester ant, induce such reactions (59). In Australia, 88 species of the genus *Myrmecia* (bull ants) are of clinical relevance (60). The most well known, the jack jumper ant (*Myrmecia pilosula*) (Fig. 4), is responsible for one quarter of all anaphylaxis treated in some Australian areas. Different species of stinging ants that cause anaphylaxis have also been found in other parts of the world. Details of the taxonomy of stinging ants and their worldwide significance in Hymenoptera venom allergy are outlined in chap. 14.

Reactions

The IFA attaches to the skin by means of a powerful mandible and stings, releasing venom that produces a characteristic "fire-like" pain. If not removed, the IFA will continue to rotate in a pivotal fashion, repeatedly injecting small amounts of venom provoking a sharp pain. An initial local reaction begins as a 25- to 50-mm erythematous flare. This is followed a few minutes later by a larger wheal and, within the next 24 hours, an umbilicated pustule forms that usually remains for 3 to 10 days later rupturing and leaving a residual macule, nodule, or scar.

Stings of the IFA commonly produce large local reactions that are similar to those induced by stings of the other flying Hymenoptera. Following an initial wheal-and-flare, a large local reaction may develop several hours later. This includes erythema and edema that extends more than 10 cm from the initial sting site. This reaction is thought to occur in up to 30% to 50% of IFA stings.

Systemic allergic reactions can manifest all the symptoms of anaphylaxis including generalized erythema, urticaria, angioedema, nausea, vomiting, diarrhea, laryngeal edema, asthma, as well as shock and death. Anaphylaxis to the IFA sting is thought to occur in up to 1% of stings (61). In 1989, a survey of 29,300 physicians reported a total of 32 deaths thought to be secondary to anaphylaxis induced by ant stings (62). Although the species of ant was not identified in most cases, *Solenopsis* and *Pogonomyrmex* species were implicated in these deaths. Postmortem case reports of deaths following IFA stings describe findings of acute pulmonary changes and cerebral vascular congestion compatible with shock due to anaphylaxis. Neurological sequelae due to the IFA are rare but include mononeuropathy, focal motor, and grand mal epileptic seizures (63,64).

Allergens of the IFA

The venom of the IFA is unique when compared with other Hymenoptera. The venom of the IFA, unlike other Hymenoptera venoms, has an extremely low protein content in the aqueous fraction, less than 0.1%, with a prominence of toxic alkaloids. The aqueous component contains the allergenic proteins. Alkaloids comprise 95% and the aqueous 5% of the venom. The alkaloids are responsible for the hemolytic, bactericidal and cytotoxic properties that result in formation of a sterile pustule. This alkaloid portion, however, is nonallergenic (65). The venom of the harvester ant, *Pogonomyrmex*, more closely resembles that of the flying Hymenoptera and consists of 73% protein. *Solenopsis* and *Pogonomyrmex* proteins do not cross-react (65). IFA whole body extract (WBE), unlike the WBE of other members of Hymenoptera, contains the clinically important allergens responsible for hypersensitivity (65). Both IFA WBE and venom produce positive skin tests in sensitized individuals. Skin testing with IFA venom is more sensitive and specific than IFA WBE. The venom is also thought to be 10 times more potent and better tolerated for skin testing, and RAST testing with IFA venom more sensitive than WBE. IFA venom, however, is not commercially produced currently leaving IFA WBE as the only available option for testing and immunotherapy. Similarly, WBE and not venoms are available for *Pogonomyrmex* species.

Diagnosis

There is a high rate of false-positive results when skin testing is performed on patients in endemic regions. Therefore, only patients who have experienced a systemic allergic reaction following an ant sting should undergo skin testing with IFA WBE. Skin testing should be done at least thirty days after the systemic reaction. A prick-puncture test with IFA WBE is performed first, and if there is no response, it is followed by serial intradermal testing beginning with a 1:1,000,000 weight/volume (w/v) dilution. A great majority of patients who are sensitive react before reaching a 1:500 w/v dilution (66). In vitro tests for IFA IgE should be obtained in history positive skin test negative patients.

Immunotherapy

With other Hymenoptera (honeybee, wasp, hornet and yellow jacket), children, who have had only a cutaneous systemic reaction (generalized erythema, urticaria, and/or pruritus), are not candidates for immunotherapy (8,9). However, with IFA, there are no data to indicate that children, who have had only a cutaneous reaction to an IFA sting, will not respond to subsequent stings with a more serious systemic reaction. Therefore, patients of all age groups, with positive skin or in vitro tests, should receive IFA immunotherapy, regardless of the severity of their systemic reaction to IFA.

IFA immunotherapy is begun with 0.05 mL of the highest dilution of WBE that produces a positive skin test (usually 1:10,000 or 1:100,000 w/v). The dose is increased with each injection, either weekly or biweekly. Once a maximum tolerated dose or 0.5 mL of a 1:10 w/v is achieved, the interval between injections is extended to every four to six weeks.

A two-day rush protocol for IFA immunotherapy has been studied in a small population of patients and shown to be safe and efficacious (67). Of 58 patients, only three patients (5.2%) experienced a mild systemic reaction during the two-day rush protocol, a final dose of 0.3 mL of 1:100 w/v. When 56 patients (total of 112 stings) had sting challenges on day 22, all (98.2%) tolerated the sting except one. He complained of lightheadness, which resolved without intervention. Prophylactic pretreatment with H1 and H2 antihistamines and glucocorticosteroids did not reduce the systemic reaction rate associated with rush immunotherapy, which was underpowered with only three reactions (67).

IFA immunotherapy can be discontinued when the individual becomes negative on repeat skin testing. Otherwise, the decision to discontinue such therapy after five years is determined by the physician in consultation with the patient, since no data exist when IFA immunotherapy can be discontinued when skin tests remain positive.

Only whole ant body extracts are used for ant immunotherapy. However, a double-blind placebo-controlled crossover study on VIT in patients allergic to the jack jumper ant, *Myremcia pilosula* was reported from Tasmania, Australia (68). Of 29 patients on placebo, 21 (72%) developed a systemic allergic skin reaction, while only one of the 35 (3%) on ant venom developed a mild urticarial reaction when purposely stung. When the placebo group switched to VIT, all 26 patients except one (mild urticaria) tolerated a sting challenge. Ant venom

immunotherapy to the jack jumper ant, *Myremcia pilosula*, could offer some benefit to prevent life-threatening sting anaphylaxis in southeastern Australia. Such impressive evidence of the efficacy of VIT to the jack jumper ant, *Myremcia pilosula*, supports more research investment in this area. Currently, ant venom products are not commercially available for immunotherapy.

NEW APPROACHES TO IMMUNOTHERAPY

Insect venom allergy is often considered as a model to study IgE-mediated reactions. Diagnostic tests (skin tests and RAST) are reliable and specific VIT is safe and effective. However, the specificity of skin tests with insect venom extracts and tests for venom specific serum IgE antibodies are far from perfect. Up to 20% of individuals with no history of systemic sting reactions have positive tests and only 30% to 75% (2,3,24,68) of those with positive tests and a history of a systemic sting reaction, react to a subsequent sting by the incriminated insect. In addition, following a sting challenge about 95% of patients allergic to vespid stings are completely protected as compared to only 80% to 90% (11,24) of those allergic to honeybee venom to a sting provocation test during VIT. Systemic allergic side effects to honeybee-venom immunotherapy injections may occur in 20 to 40% of patients and 5% to 10% to vespid venom immunotherapy. Thus, there is room for considerable improvement to diagnose and treat Hymenoptera allergy.

RECOMBINANT VENOM ALLERGENS

A number of honeybee, vespid, and ant allergens in recombinant form are available today (Table 6) (69,70). The IgE binding capacity of these recombinant allergens correlates closely with that of their respective natural purified preparations. Some disparities, however, revealed by RAST-inhibition and western blot studies indicate that all natural preparations are contaminated with trace amounts of other venom allergens. Recombinant allergens, therefore, should be superior to highly purified natural preparations when the true clinical relevance of the individual allergen is determined.

The use of recombinant cocktails is also promising for diagnosis. Venom-specific IgE antibodies were measured in 85 bee venom allergic patients with positive and 20 nonallergic controls with negative skin tests. None of the negative controls reacted to a recombinant cocktail containing phospholipase A2, hyaluronidase, and melittin as compared to 15% who reacted to the whole bee venom, indicating superior specificity of the recombinant cocktail. On the other hand, 87% of the patients were positive with the recombinant cocktail versus 95% with the whole bee venom (69). The somewhat lower sensitivity of the cocktail could probably be improved by the addition of further relevant bee venom allergens such as acid phosphatase and protease in the recombinant forms. The sensitization pattern of an individual patient can be determined once all relevant allergens of a venom are available in recombinant form. A

Table 6 Recombinant Hymenoptera Venom Allergens [76–77]

Species	Allergen	MW kDa
Apis mellifera	Api m 1 phospholipase A2	16–20
	Api m 2 hyaluronidase	43
	Api m 3 acid phosphatase	49
Vespula vulgaris	Ves v 1 phospholipase A1	35
	Ves v 2 hyaluronidase	45
	Ves v 5 antigen 5	25
Dolichovespula maculata	Dol m 1 phospholipase A1	35
	Dol m 2 hyaluronidase	45
	Dol m 5 antigen 5	25
Polistes annularis	Pol a 5 antigen 5	25
Solenopsis invicta	Sol i 2	30
	Sol i 3 antigen 5	25

patient-tailored cocktail containing all the allergens to which the patient has IgE antibodies could then be prepared for immunotherapy. The mostly conformational B-cell epitopes have been shown to be reduced in unrefolded or point-mutated recombinant allergens. Cocktails of such preparations will have a strongly reduced reactivity to IgE antibodies fixed on effector cells, therefore, will induce much less mediator release and will be theoretically safer. Their capacity to interact with T cells, thus inducing protective immunologic effects, will be preserved. So far no clinical trials on immunotherapy with recombinant venom allergens have been performed.

In another study, a recombinant chimeric protein consisting of overlapping fragments of the bee venom allergens, phospholipase A2, hyaluronidase, and melittin, was engineered and shown to have strongly reduced allergenicity but preserved T-cell epitopes (71).

T-Cell Epitope Peptides

Peptide immunotherapy has been studied experimentally both in cat and bee venom allergy (72). Its goal is to provide effective immunotherapy without sideeffects, by destroying IgE reactive conformational B-cell epitopes, but preserving the linear T-cell epitopes. Peptides can be prepared synthetically or expressed as recombinant fragments. In one study, three short linear peptides of 11–18 amino acids of phospholipase A2 (the major bee venom allergen) were identified that were unable to bind to the respective specific IgE-antibodies in sera from bee venom allergic patients. However, they induced strong proliferation of their T lymphocytes in vitro. Immunotherapy with an equimolar mixture of these major synthesized T-cell epitope peptides was performed in five bee venom allergic patients in a preliminary trial. No side effects were observed. A honeybee sting challenge after 10 weeks of peptide immunotherapy resulted in complete protection of three and partial protection of two patients (73). In vitro studies on lymphocyte cultures of the patients suggested the induction of phospholipase A2 specific tolerance by this form of peptide immunotherapy.

In another study longer synthetic peptides consisting of 45 to 60 residues and covering the whole molecule of phosphlipase A2 were used in a phase I clinical trial and shown to induce an increase in IL-10, IFNγ and allergen specific IgG4, but caused some mild adverse reactions (74). In patients treated with a mixture of 4 to 18 residues peptides with high affinity to commonly expressed MHC (major histocompatibility complex) class II molecules, no side effects and significantly reduced late phase reactions were observed (72).

DNA-Vaccination

This technique consists of the injection of DNA-plasmids that encode the allergen and induce a TH1 response. Successful DNA-vaccination of phospholipase A2 sensitized mice with the corresponding DNA-sequence has been reported (75). Protection against anaphylaxis was complete when vaccination was done before sensitization but only 65% of the mice survived when it was performed after intraperitoneal sensitization.

SALIENT POINTS

- VIT is highly effective for Hymenoptera venom allergy.
- Indication for VIT is based on a history of systemic allergic reactions to Hymenoptera stings and positive diagnostic tests.
- Rush and ultrarush protocols for immunotherapy provide more rapid protection than conventional protocols but may be associated with more side effects.
- Most patients remain protected for many years after stopping VIT of three to five years.
- Immunotherapy with whole body vaccines of the fire ant (*Solenopsis invicta*) and probably other ants appears to be effective, in contrast to other Hymenoptera, where only venoms induce protection.
- The most promising new approaches to VIT include treatment with modified recombinant allergens, T-cell epitope peptides and DNA vaccination.

REFERENCES

1. Müller UR. Insect sting allergy. Clinical picture, diagnosis and treatment. Stuttgart/New York: Gustav Fischer, 1990.
2. Hunt KJ, Valentine MD, Sobotka AK, et al. A controlled trial of immunotherapy in insect hypersensitivity. N Engl J Med 1978; 299:157–161.
3. Müller U, Thurnheer U, Patrizzi R, et al. Immunotherapy in bee sting hypersensitivity: Bee venom versus wholebody extract. Allergy 1979; 34:369–378.
4. Bonifazi F, Jutel M, Bilo B, et al. Prevention and treatment of hymenoptera venom allergy: Guidelines for clinical practice. Allergy 2005; 60:1459–1470.
5. Moffitt JE, Golden DBK, Reisman RE, et al. Stinging insect hypersensitivity: A practice parameter update. J Allergy Clin Immunol 2004; 114:869–886.
6. Golden DBK, Marsh DG, Freidhoff LR, et al. Natural history of Hymenoptera venom hypersensitivity in adults. J Allergy Clin Immunol 1997; 100:760–766.
7. Antonicelli L, Bilo B, Bonifazi B. Epidemiology of Hymenoptera allergy. Curr Opin Allergy Clin Immunol 2002; 2:341–346.
8. Valentine MD, Schubert KC, Kagey Sobotka A., Graft DF, Kwiterowitch KA, Szklo M, Lichtenstein LM. The value of immunotherapy with venom in children with allergy to insect stings. N Engl J Med 1990; 323:1601–1613.
9. Golden DBK, Kagey-Sobotka A, Norman PS, et al. Outcomes of Allergy to insect stings in children, with and without venom immunotherapy. New Engl J Med 2004; 351:668–674.
10. Reisman RE. Natural history of insect sting allergy: Relationship of severity of symptoms of initial sting anaphylaxis to re-sting reactions. J Allergy Clin Immunol 1992; 90:335–339.
11. Müller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. J Allergy Clin Immunol 1992; 89:529–535.
12. Müller U, Haeberli G. Use of β-blockers during immunotherapy for hymenoptera venom allergy. J Allergy Clin Immunol 2005; 115:606–610.
13. Reisman RE. Unusual reactions to insect stings. Curr Opin Allergy Clin Immunol 2005; 5:355–358.
14. Müller U. Cardiovascular disease and anaphylaxis. Curr Opin Allergy Clin Immunol 2007; 7:337–341.
15. Bilo B, Rueff F, Mosbech H, et al. Diagnosis of hymenoptera venom allergy. Allergy 2005; 60:1339–1349.
16. Schwartz HJ, Lockey RF, Sheffer AL, et al. A multicenter study on skin test reactivity of human volunteers to venom as compared with whole body Hymenoptera antigens. J Allergy Clin Immunol 1981; 67:81–85.
17. Golden DBK, Kagey-Sobotka A, Hamilton RG, et al. Insect allergy with negative venom skin tests. J Allergy Clin Immunol 2001; 107:897–901.
18. Hunt KJ, Valentine MD, Sobotka AK, et al. Diagnosis of allergy to stinging insects by skin testing with Hymenoptera venoms. Ann Intern Med 1976; 85:56–59.
19. Day J, Buckeridge D, Welsh A. Risk assessment in determining systemic reactivity to honeybee stings in sting-threatened individuals. J Allergy Clin Immunol 1994; 93:691–705.
20. Hamilton RG, Adkinson NF. In vitro assays fort he diagnosis of IgE-mediated disorders. J Allergy Clin Immunol 2004; 114:213–225.
21. Ebo DG, Sainte-Laudy J, Bridts CH, et al. Flow-assisted allergy diagnosis: current applications and future perspectives. Allergy 2006; 61:1028–1039.
22. van der Linden PG, Hack CE, Struyvenberg A, et al. Insect-sting challenge in 324 subjects with a previous anaphylactic reaction: Current criteria for insect-venom hypersensitivity do not predict the occurrence and the severity of anaphylaxis. J Allergy Clin Immunol 1994; 94:151–159.
23. Franken HH, Dubois AEJ, Minkema HJ, et al. Lack of reproducibility of a single negative sting challenge response in the assessment of anaphylactic risk in patients with suspected yellow jacket hypersensitivity. J Allergy Clin Immunol 1994; 93:431–436.
24. Rueff F, Przybilla B, Muller U, et al. The sting challenge test in Hymenoptera venom allergy. Allergy 1996; 51:216–225.
25. Graft DF, Schubeth KC, Kagey-Sobotka A, et al. A prospective study of the natural history of large local reactions following Hymenoptera stings. J Pediatr 1984; 104:664–668.
26. Kelly D, Golden DBK, Hamilton RG, et al. Venom immunotherapy reduces large local reactions to insect stings. J Allergy Clin Immunol 2007; 119:S79.
27. Fricker M, Helbling L, Schwartz L, et al. Hymenoptera sting anaphylaxis and urticaria pigmentosa: clinical findings and results of venom immunotherapy in ten patients. J Allergy Clin Immunol 1997; 100:11–15.
28. Oude-Elberink J, deMonchy J, Kors J, et al. Fatal anaphylaxis after a yellow jacket sting despite venom immunotherapy in two patients with mastocytosis. J Allergy Clin Immunol 1997; 99:153–154.
29. Ludolph-Hauser D, Rueff F, Fries C, et al. Constitutively raised serum concentration of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. Lancet 2001; 357:361–362.

30. Haeberli G, Brönnimann M, Hunziker T, et al. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003; 33:1216–1220.
31. Reisman RE. Venom hypersensitivity. J Allergy Clin Immunol 1994; 94:651–658.
32. Hamilton RH, Wisenauer JA, Golden DBK, et al. Selection of Hymenoptera venoms for immunotherapy based on patients' IgE antibody cross-reactivity. J Allergy Clin Immunol 1993; 92:651–659.
33. Hemmer W, Focke M, Kolarich D, et al. Identification by immunoblot of venom glycoproteins displaying IgE-binding N-glycans as cross-reactive allergens in honeybee and yello-jacket venom. Clin Exp Allergy 2004; 34:460–469.
34. Jappe U, Raulf-Heimsoth M, Hoffmann M, et al. In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. Allergy 2006; 61:1220–1229.
35. Golden DBK, Kagey-Sobotka A, Valentine MD, et al. Dose dependence of Hymenoptera venom immunotherapy. J Allergy Clin Immunol 1981; 67:370–374.
36. Rueff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving conventional Hymenoptera venom immunotherapy are protected by increased venom doses. J Allergy Clin Immunol 2001; 108:1027–1032.
37. Lockey RF, Turkeltaub PC, Olive ES, et al. The Hymenoptera venom study III: Safety of venom immunotherapy. J Allergy Clin Immunol 1990; 86:775–780.
38. Golden DBK, Kwiterovich KA, Kagey-Sobotka A, et al. Discontinuing venom immunotherapy: Outcome after five years. J Allergy Clin Immunol 1996; 97:579–587.
39. Golden DBK, Kwiterovich KA, Addison BA, et al. Discontinuing venom immunotherapy: Extended observations. J Allergy Clin Immunol 1998; 101:298–305.
40. Golden DBK, Kagey-Sobotka A, Lichtenstein LM. Survey of patients after discontinuing venom immunotherapy. J Allergy Clin Immunol 2000; 105:385–390.
41. Müller UR, Morris T, Bischof M, et al. Combined active and passive immunotherapy in honeybee-sting allergy. J Allergy Clin Immunol 1986; 78:115–122.
42. Golden DBK, Lawrence ID, Kagey-Sobotka A, et al. Clinical correlation of the venom-specific IgG antibody level during maintenance venom immunotherapy. J Allergy Clin Immunol 1992; 90:386–393.
43. Jutel M, Akdis M, Blaser K, et al. Mechanisms of allergenspecific immunotherapy – T cell tolerance and more. Allergy 2006; 61:796–807.
44. Hoffman DR, Jacobson RS, Allergens in Hymenoptera venoms XII. How much venom is in a sting? Ann Allergy 1984; 52:276–278.
45. Müller U. Bee venom allergy in beekeepers and their families. Curr Opin Allergy Clin Immunol 2005; 5:343–347.
46. Birnbaum J, Charpin D, Vervloet D. Rapid Hymenoptera venom immunotherapy: comparative safety of three protocols. Clin Exp Allergy 1993; 23: 226–230.
47. Rueff F, Wolf H, Schnitzel J, et al. Specific immunotherapy with honey bee venom: a comparative study using aqueous and aluminium hydroxide adsorbed preparations. Allergy 2004; 59:589–595.
48. Müller U, Hari Y, Berchtold E. Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. J Allergy Clin Immunol 2001; 197:81–86.
49. Urbanek R, Forster J, Kuhn W, et al. Discontinuation of bee venom immunotherapy in children and adolescents. J Pediatrics 1985; 107:367–371.
50. Golden DBK, Addison BI, Gadde J, et al. Prospective observations on stopping prolonged venom immunotherapy. J Allergy Clin Immunol 1989; 84:162–167.
51. Müller U, Berchtold E, Helbling A. Honeybee venom allergy: Results of a sting challenge 1 year after stopping successful venom immunotherapy in 86 patients. J Allergy Clin Immunol 1991; 87:702–709.
52. Haugaard L, Nörregaard OF, Dahl R. In-hospital sting challenge in insect venom-allergic patients after stopping venom immunotherapy. J Allergy Clin Immunol 1991; 87:699–702.
53. Keating MU, Kagey-Sobotka A, Hamilton RG, et al. Clinical and immunologic follow-up of patients who stop venom immunotherapy. J Allergy Clin Immunol 1991; 88:339–348.
54. van Halteren HK, van der Linden PWG, Burgers JA, et al. Discontinuation of yellow jacket venom immunotherapy: Follow-up of 75 patients by means of deliberate sting challenge. J Allergy Clin Immunol 1997; 100:767–770.
55. Reisman RE. Duration of venom immunotherapy: Relationship to the severity of symptoms of initial insect sting anaphylaxis. J Allergy Clin Immunol 1993; 92:831–836.
56. Lerch E, Müller UR. Long-term protection after stopping venom immunotherapy: Results of re-stings in 200 patients. J Allergy Clin Immunol 1998; 101:606–612.
57. Golden D, Johnson K, Addison BI, et al. Clinical and immunologic observations in patients who stop venom immunotherapy. J Allergy Clin Immunol 1986; 77:435–442.
58. Reimers A, Müller U. Fatal outcome of a Vespula sting in a patient with mastocytosis after specific immunotherapy with honey bee venom. J World Allergy Org 2005; (suppl 1):69–70.

59. Lockey RF. Systemic reactions to stinging ants. J Allergy Clin Immunol 1974; 54:132–146.
60. Brown SG, Wu QX, Kelsall GR, et al. Fatal anaphylaxis following jack jumper ant sting in southern Tasmania. Med J Aust 2001; 175:644–647.
61. deShazo RD, Butcher BT, Banks WA. Reactions to the stings of the imported fire ant. N Engl J Med 1990; 323:462–466.
62. Rhoades RB, Stafford CT, James FK Jr. Survey of fatal anaphylactic reactions to imported fire ant stings. Report of the Fire Ant Subcommittee of the American Academy of Allergy and Immunology. J Allergy Clin Immunol 1989; 84:159–162.
63. Candiotti KA, Lamas AM. Adverse neurologic reactions to the sting of the imported fire ant. Int Arch Allergy Immunol 1993; 102:417–420.
64. Fox RW, Lockey RF, Bukantz SC. Neurologic sequelae following the imported fire ant sting. J Allergy Clin Immunol 1982; 70:120–124.
65. Hoffman DR. Fire ant venom allergy. Allergy 1995; 50:535–544.
66. Moffitt J, Barker J, Stafford C. Management of imported fire ant allergy: results of a survey. Ann Allergy Asthma Immunol 1997; 79:125–130.
67. Tankersley MS, Walker RL, Butler WK, et al. Safety and efficacy of an imported fire ant rush immunotherapy protocol with and without prophylactic treatment. J Allergy Clin Immunol 2002; 109:556–62.
68. Brown S, Wiese M, Blackman K, et al. Ant venom immunotherapy: a double-blind placebo-controlled, crossover trial. Lancet 2003; 361:1001–1006.
69. Müller U. Recombinant Hymenoptera venom allergens. Allergy 2002; 57:570–576.
70. Grunwald T, Bockisch B, Spillner E, et al. Molecular clining and expression in insect cells of honey bee venom allergen acid phosphatase (Api m3). J Allergy Clin Immunol 2006; 117:848–854.
71. Karamloo F, Schmid-Grendelmeier P, Kussebi F, et al. Prevention of allergy by a recombinant multi-allergen vaccine with reduced IgE binding and preserved T cell epitopes. Eur J Immunol 2005; 35:3268–3276.
72. Larché M. Update on the current status of peptide immunotherapy. J Allergy Clin Immunol 2007; 119:906–909.
73. Müller U, Akdis C, Fricker M, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. J Allergy Clin Immunol 1998; 101; 747–754.
74. Fellrath J, Kettner A, Dufour N, et al. Allergen specific T cell tolerance induction with allergen derived long synthetic peptides: results of a phase 1 trial. J Allergy Clin Immunol 2003; 111:854–861.
75. Jilek S, Barbey C, Spertini F, et al. Antigen-independent suppression of the allergic immune response to bee venom phospholipase A2 by DNA vaccination in CBA/J mice. J Immunol 2001; 166:3612–3621.

25 Experimental Forms of Allergen Immunotherapy with Modified Allergens and Adjuvants

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INTRODUCTION

Allergen-specific immunotherapy (SIT) is an effective form of treatment for allergic diseases caused by inhalant allergens (e.g., pollen, mites, animal dander) and insect venom. Despite being initially described at the turn of the 20th century, SIT remains the only "curative" approach to atopic allergic disease. In addition to the ability to modulate existing disease, SIT is capable of modifying the natural course by preventing the worsening of symptoms (from rhinitis to asthma) and sensitization against new allergens (1,2). The treatment consists of administering increasing doses of natural allergen preparations on a regular basis, and the beneficial effects for the patient depend on the amount of allergen used. Typically, between 5 and 20 μg of the major allergen is required in each monthly maintenance injection to achieve optimal clinical efficacy. Because treatment involves injecting allergens into a sensitized individual, the occurrence of typical allergic symptoms, both local and systemic reactions, is not unexpected, with risks increasing with the concentration of allergen administered per treatment. Several immunological pathways seem to be involved in the clinical improvement achieved by the common SIT schedules (3,4). These mechanisms include (*i*) increase in allergen-specific IgG antibodies, in particular IgG4, which is believed to exert its effect by neutralizing allergen and blocking IgE-facilitated allergen presentation to T cells (5); (*ii*) generation of IgE modulating CD8+ T cells (6); (*iii*) reduction in the number of mast cells and eosinophils and the release of mediators by these cells (7,8); and (*iv*) modulation of allergen-specific T cells by shifting the response from a Th2 to Th1 cytokine pattern. The latter results in a decrease of IL-4 and IL-5 production accompanied by an increase of IFN-γ (immune deviation) (9,10). Moreover, the induction of an anergic state in peripheral T cells (immunologic tolerance) has been reported. Tolerance may be mediated by IL-10 and has been characterized by suppressed proliferative and cytokine responses against major allergens (11).

Although this type of therapy is widely established, three major problems are still associated with SIT. First, SIT is performed with allergen extracts containing mixtures of allergens, nonallergenic proteins, and/or potentially toxic substances, including bacterial endotoxin and other macromolecules that are difficult to standardize. Indeed, new IgE reactivities to allergenic components may develop during SIT administration of complex, allergen extracts (12). Second, severe IgE-mediated side effects can occur during the treatment with anaphylaxis to fully active allergens. Third, therapeutically effective doses often cannot be achieved because of adverse events or poor standardization of extracts in the vaccines.

RECOMBINANT AND ENGINEERED ALLERGENS

Many of the problems associated with SIT could be overcome, in principle, by the use of genetically engineered allergens (13,14). Cocktails of pure and standardized recombinant allergens can be formulated to replace natural extracts, and the selected recombinant allergens can be engineered to reduce the risk of IgE-mediated side effects. Genetic engineering of allergens for SIT should aim at the production of modified molecules with reduced IgE-binding epitopes (hypoallergens) while preserving structural motifs necessary for T-cell recognition (T-cell epitopes) and for induction of IgG antibodies reactive with the natural allergen (blocking antibodies). The uptake of allergens by antigen-presenting cells (APCs) is mediated and facilitated by the interaction of the allergen with specific IgE (15,16) and leads to enhanced production of Th2 cytokines and IgE production. Engineered allergens lacking IgE binding are designed to avoid these pathways and preferentially target APCs that use phagocytosis or pinocytosis for antigen uptake (e.g., monocytes, macrophages, and dendritic cells). This in turn induces a balanced Th0 or Th1-like cytokine production by T cells and low IgE and high IgG production by B cells. The presence of intact T-cell epitopes in hypoallergens enables targeting of T cells, allowing administration of higher doses to induce tolerance of allergen-specific T cells and alteration of cytokine production towards a Th1-like pattern. In this way, recombinant allergens could replace natural extracts in vaccines and increase the efficacy and safety of SIT.

Low IgE-Binding Natural Isoforms

Complementary deoxyribonucleic acid (cDNA) cloning of allergens demonstrates that many major allergens are encoded by gene families. Within individual allergen sequences, polymorphisms occur, such as those found in ragweed Amb a 1 (17), hazel Cor a 1 (18), birch Bet v 1 (19), group 1 (20) and group 5 grass allergens (21), apple Mal d 1 (22), celery Api g 1 (23), Parietaria Par j 1 (24), olive Ole e 1 (25), group 2 dust-mite allergens (26), latex Hev b 7 (27), and cow dander Bos d 2 (28). Because of sequence variations, isoallergens may have different antigenic and/or allergenic activities. Differences in T-cell reactivity of isoforms have previously been reported for Cor a 1 (29), Bet v 1 (30), Phl p 5 (31), and for Der p 2 (32).

Investigation of the IgE-binding activity of isoallergens led to the identification of naturally occurring Bet v 1 hypoallergens (33). Isoforms Bet v 1d (Bet v 1.0401), Bet v 1g (Bet v 1.0701), and Bet v 1l (Bet v 1.1001) are highly antigenic in T-cell proliferation assays and poorly allergenic in vitro and in vivo. The crystal structure of the hypoallergenic isoform Bet v 1l (Bet v 1.1001) does (34) not differ significantly from the high IgE-binding isoform Bet v 1a (Bet v 1.0101). Thus, the low IgE-binding activity of certain isoforms is not due to problems in the recombinant production leading to unfolded proteins. The hypoallergenic nature of these isoforms was confirmed in a study using recombinant Bet v 1.0401 and Bet v 1.1001 (35). Compared with Bet v 1.0101, Bet v 1.0401 and Bet v 1.1001 isoforms were unable to induce measurable IgE levels in allergic individuals naturally exposed to birch pollen, whereas significantly higher levels of IgG4 antibodies were directed mostly to Bet v 1.0401 and Bet v 1.1001. Structural comparisons showed that most of the amino acid differences among the Bet v 1.0101, Bet v 1.0401, and Bet v 1.1001 isoforms were located on the surface of the proteins. Thus, naturally occurring hypoallergens could be an excellent source of vaccine candidates. Unfortunately, this approach could not be generally applied because naturally occurring hypoallergens have not been identified for other allergen families. Instead, genetic engineering has been widely used to generate low IgE-binding variants.

Engineered Allergens

Genetic engineering involves the targeted modification of a protein to alter its function or properties in a predictable manner. This usually requires the complete understanding of the relationship between structure and function/properties for precise and effective manipulation. Alteration of the gene includes changing specific base pairs (mutated gene), introduction of a new piece of DNA into the existing DNA molecule (chimeric or hybrid gene, head-to-tail and tail-to-head fusions), deletions (truncated gene), and fragmentation (gene fragment) (Fig. 1). With the exception of the DNA shuffling approach (described later), which bypasses the need to identify amino acid residues or motifs that are important to structure and function, engineering of allergens usually requires knowledge of B- and T-cell epitopes and, in some

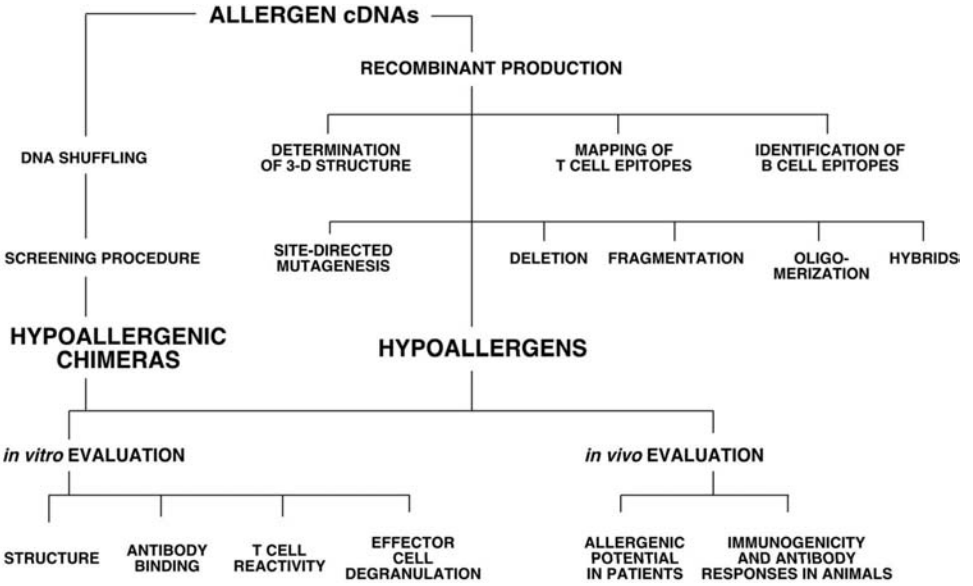

Figure 1 Task tree for genetic engineering and evaluation of hypoallergens for specific immunotherapy.

cases, knowledge of the three-dimensional structure of the allergen. No matter how allergen genes have been altered, putative hypoallergens must be subjected to a series of in vitro and in vivo evaluation procedures before being considered for therapeutic purposes (Fig. 1). To date, cDNA sequences of approximately 388 allergens (73 from pollen, 43 from mites, 15 from mammals, 76 from molds, 61 from insects, 95 from foods, 20 from other sources, e.g., latex) have been deposited in the allergen databank (http://www.allergen.org). In addition, the three-dimensional structures of approximately 40 allergens are defined and are accessible at the Protein Data Bank (PDB; http://www.rcsb.org/pdb/). Thus, the starting material and relevant information for producing new vaccines for important allergens are available.

Site-Directed Mutants

After identification of crucial amino acid residues or motifs involved in IgE recognition, dominant epitopes can be targeted using site-directed mutagenesis. The observation that Bet v 1 and other closely related tree pollen allergens consist of a mixture of closely related isoforms displaying striking differences in their ability to bind IgE (30) constituted the basis for engineering a Bet v 1 hypoallergen (36). The patterns of amino acid substitutions in tree pollen isoallergens and their IgE-binding activities were analyzed using a computer algorithm developed to predict functional residues in protein sequences (37). Using in vitro site-directed mutagenesis, the amino acid residues occurring in positions 10, 57, 112, 113, and 125 of Bet v 1a were substituted with those present in the same positions of low IgE-binding isoforms. Thus, a Bet v 1 mutant carrying point mutations was produced. This mutant protein displayed extremely low IgE-binding activity for all patients tested. In vivo (skin prick) tests indicated that the potency of the Bet v 1 mutant to induce typical wheal and flare skin reactions in allergic individuals was dramatically reduced (100–1000-fold) compared with Bet v 1a. T cell clones (TCC) established from the peripheral blood of birch pollen–allergic patients and reactive with Bet v 1a were also activated by the mutant. Other point mutations directed at molecular surfaces of Bet v 1 are described. Four- and nine-point mutants of Bet v 1 retained the pattern of the alpha-carbon backbone folding but showed significantly lower IgE-binding activity, suggesting a mutation-induced distortion of IgE-binding, B-cell epitopes (38).

IgE recognition of polcalcins, a group of EF-hand calcium-binding allergens found in pollen (39), and parvalbumin, a cross-reactive fish allergen (40), is influenced by bound calcium. This led to the idea of disruption of EF-hand calcium-binding domains for engineering hypoallergenic mutants. In this way, calcium-binding deficient mutants of Bet v 4 (41) and Bra r 1 (turnip) (42) with reduced IgE-binding activities were generated. However,

data concerning T-cell recognition of calcium-binding allergens and their engineered counterparts are not available.

The introduction of point mutations in highly conserved sequence domains of Lol p 5 (rye grass) was based on investigations of B- and T- cell epitopes and sequence comparison of group 5 allergens from different grasses. Hypoallergenic forms of Lol p 5 were produced that contained all relevant T-cell epitopes (43). Likewise, hypoallergenic variants of latex Hev b 6.02 (44) and Hev b 5 (45), egg Gal d 1 (22), apple Mal d 1 (46), and peanut Ara h 1, Ara h 2, and Ara h 3 (47,48) were also generated by site-directed mutagenesis. Modified peanut allergens retained their ability to stimulate T-cell proliferation in peripheral blood mononuclear cells (PBMCs) from allergic patients. In addition, peanut hypoallergens have been tested in a murine model of peanut anaphylaxis (48) and shown to be effective.

Conformational Variants

Akdis et al. (49) showed that bee venom phospholipase A2 (PLA, Api m 1) preparations lacking native conformation and antibody-binding activity were exclusively presented by monocytes and induced a Th1-biased cytokine profile leading to IgG4 production by B cells. In contrast, folded PLA with full antibody-binding activity was processed and presented by B cells, stimulated Th2-like cytokines, and induced IgE antibodies. Thus, the three-dimensional structure of an antigen and its recognition by different APCs are likely crucial aspects in the development of distinct T-cell cytokine patterns. These findings give further support to the use of hypoallergens in allergen immunotherapy.

The importance of the correct native fold and its stability for antibody binding was further demonstrated for Api m 1. Introduction of multiple mutations and deletions in exposed areas of Api m 1, but outside of previously identified T-cell epitope regions, led to a progressive loss of specific IgE and IgG reactivity (50). Although this Api m 1 mutant shared a similar content of secondary structure with the wild-type protein, the surface mutations dramatically affected the thermal stability of the protein. Hypoallergenic variants of the major allergen of Par j 1 (Parietaria) with altered folding were produced and characterized (51,52). Par j 1 is a member of the nonspecific lipid transfer proteins (nsLTPs) with a characteristic α-α-α-α-β structure, which is stabilized by four disulfide bonds. Targeting these disulfide bonds by site-directed mutagenesis resulted in molecules with altered conformation and decreased IgE-binding activity that retain their ability to stimulate T-cell proliferation. Disruption of native conformation by targeting disulfide bonds could be a generally applicable approach for engineering allergenic nsLTPs, including food-derived members. Disulfide bonds stabilizing the antigenic structure of major allergens of dust mites have also been targeted by site-directed mutagenesis. Hypoallergenic variants of Der p 2 (53), Der f 2 (54), and Lep d 2 (55) were produced and evaluated for subsequent IgE-mediated reactions and cellular responses. However, caution is warranted when targeting the conformation of allergens. Conformation changes may reduce the solubility of the final product, and denatured or unfolded proteins tend to form aggregates. Protein preparations consisting of aggregates or insoluble molecules would not be suitable candidates for therapeutic products. Thus, to avoid such problems, hypoallergenic variants of the major cat allergen Fel d 1 were generated by a rational approach using structural information and knowledge of B- and T-cell epitopes. The three-dimensional structure was systemically altered by duplication of selected T-cell epitopes (DTE) and disruption of disulphide bonds (56). When applied to the cat allergen Fel d 1, DTE accounted for reduction in IgE-binding capacity, whereas mutagenesis improved protein refolding and solubility. This novel concept may be a valuable approach for the design of new hypoallergenic derivatives of well-characterized allergens.

Deletion Mutants

Knowledge of IgE-reactive regions of allergens can be used to engineer hypoallergenic variants by deleting the corresponding DNA segment in the gene. This approach was successfully used for the timothy grass pollen allergen Phl p 5b (57). Epitope mapping was performed using overlapping recombinant fragments, and at least four continuous IgE-binding epitopes were identified. Deletions eliminating identified T-cell epitopes were then performed within these IgE-binding regions. Some of these deletion mutants showed reduced IgE-binding properties, no histamine-releasing activity, reduced skin reactivity, and no significant changes in T-cell

reactivity. A similar approach was used to engineer hypoallergens of the American cockroach, Per a 1 (58). Based on the results obtained by proteolytic fingerprinting, a deletion mutant of rye grass, Lol p 1, was produced that displayed decreased IgE-binding activity and did not trigger histamine release up to a concentration of 10 mg/mL (59). The mutant was not tested in T-cell proliferation assays or for skin reactivity.

Allergen Oligomers

Vrtala et al. (60) constructed a Bet v 1 oligomeric form consisting of three copies of full-length Bet v 1 cDNA linked by short oligonucleotide (ODN) spacers in one open reading frame. Recombinant Bet v 1 monomer and trimer produced in *Escherichia coli* showed comparable in vitro IgE-binding activity and strongly stimulated Bet v 1-specific TCC and PBMCs from birch pollen–allergic patients. Interestingly, the Bet v 1 trimer released minimal amounts of histamine from patients' basophils and exhibited reduced skin reactivity compared with the monomer. When injected in mice and rabbits, the trimer induced IgG antibodies that inhibited human IgE binding to Bet v 1 monomer. Several explanations were proposed to explain why the IgE-binding Bet v 1 trimer showed reduced anaphylactic potential: (*i*) lower affinity for IgE binding, (*ii*) reorientation of IgE epitopes preventing efficient cross-linking of FcεRI-bound IgE antibodies, and (*iii*) microaggregation, steric hindrance, and/or unfavorable charge interactions causing concealment of IgE epitopes required for efficient cross-linking.

Following this line, monomers, dimmers, and trimers of wild-type and mutant Dau c 1, the major carrot allergen, were constructed and characterized. Mice were used to assess the contribution of mutations and oligomerization to IgE-binding activity and to the induction of blocking IgG antibodies (61). The results demonstrated that destruction of the native conformation by introduction of multiple mutations, rather than oligomerization of the wild-type allergen, is the appropriate strategy for the development of novel reagents for standard allergen-SIT, at least in mice.

Chimeras: Allergen Hybrids and Shuffled Molecules

King et al. (62) reported an approach to genetically modify allergens in which hybrids were prepared consisting of a small portion of the "guest" allergen of interest and a large portion of a homologous but weakly cross-reacting host protein. In this scenario, the homologous host protein serves as a scaffold to maintain the native structure of the guest allergen of interest to preserve conformation-dependent B-cell epitopes, but at a reduced density. The homologous allergens from yellow jacket venom Ves v 5 and from paper wasp Pol a 5 (59% sequence identity) show very limited cross-reactivity of antibodies from sensitized patients. Hybrids of these two molecules containing 10 to 49 residues of Ves v 5 showed a 100- to 3000-fold reduction in allergenicity in the histamine release assay with basophils from yellow jacket–sensitized patients.

Hybrids consisting of head-to-tail fusions of Phl p 5 and Phl p 1 and of Phl p 6 and Phl p 2 allergens were engineered by polymerase chain reaction (PCR) with the aim of producing combination vaccines for grass pollen immunotherapy (63). These hybrids were not hypoallergens and contained most of the IgE and T-cell epitopes of natural grass pollen extract. However, these hybrids showed higher immunogenic activity than the individual allergens when injected in mice, and thus may be useful for vaccine development.

A similar approach was reported for the major bee venom allergens. Kussebi et al. (64) fused the two major allergens of bee venom, Api m 1 and Api m 2. The hybrid molecule lost conformational B-cell epitopes but retained the important T-cell epitopes. The same could be demonstrated for hybrids of the major allergens from Parietaria, Par j 1 and Par j 2 (65).

The strategy of tail-to-head reassembly of hypoallergenic fragments within one molecule was demonstrated for timothy grass profilin, Phl p 12 (66). Non-IgE-reactive fragments of Phl p 12 were identified by synthetic peptide chemistry and restructured (rs) as a new molecule. Phl p 12-rs showed decreased IgE-binding activity but preserved T-cell reactivity in allergic patients. IgG antibodies induced by immunization of mice and rabbits with Phl p 12-rs cross-reacted with pollen Phl p 12 and other wild-type profilins. In addition, Phl p 12-rs inhibited profilin-induced basophil degranulation.

As discussed above, directed mutagenesis has been used to generate hypoallergens. Such a rational design method requires an understanding of the relationship between structure and

function for the target allergen. The approach has the limitation that substitutions of amino acids or segments must be appropriate for the specific position in which they are found in the protein. The effects of substitutions, even chemically conserved ones, are practically impossible to predict with the current knowledge-based system. DNA shuffling, or molecular breeding, is a novel approach that mimics natural evolution and can be performed without prior knowledge of structural or functional characteristics of the target molecules (67). It allows the generation of large and complex libraries of novel chimeric genes, from which variants with desired properties can be selected using appropriate screening methods. The features of the DNA family shuffling method would allow the generation of allergen chimeras having T-cell epitopes derived from several family members, reduced anaphylactic potential, and improved immunogenicity (68). This approach was used to generate molecules that are suitable for SIT, not only for birch pollen but also cross-reactive tree pollens like alder, hazel, hornbeam, and oak (69). The data demonstrated that it is possible to randomly recombine in vitro T- and B-cell epitopes of a family of related allergens and to select chimeric proteins–matching criteria presently thought to be relevant for improving SIT. The low IgE-binding activity and reduced capacity to induce release of inflammatory mediators of the shuffled chimeras combined with the high potency to react with T lymphocytes is expected to minimize IgE-mediated side effects without compromising SIT efficacy. A similar approach was successfully used to evolve two isoforms of group 2 house-dust mite allergens, Lep d 2 and Gly d 2 (70).

Allergen DNA Vaccines

An attractive alternative for immunotherapy using allergen proteins is the use of allergen genes in genetic immunization approaches. Intramuscular or intradermal injection of plasmid DNA encoding clinically relevant allergens can induce long-lasting immune responses with a Th1 bias and promote the formation of interferon (IFN)-γ-producing CD4+ T cells (71–73). After subcutaneous administration of plasmid DNA encoding an allergen, transcripts have been simultaneously detected in several tissues. Furthermore, immunization of mice with an allergen-cDNA cloned in a plasmid vehicle resulted in an allergen-specific IgG2a and Th1-like response, with no detectable IgE response. This is in contrast to immunization with allergen, which induced an IgE antibody response. However, despite the potential of this approach, no studies have been performed in humans to date, largely because of concerns over the introduction of "active" genetic material into man. In the mouse model, allergen-gene DNA vaccines were shown to efficiently protect from IgE expression, experimental airway inflammation, and airway hyperreactivity (AHR) by induction of Th1-biased immune reactions (74).

However, before clinical application of allergy DNA vaccines, two major concerns should be addressed: (*i*) anaphylactic side effects as the result of cross-linking preexisting IgE molecules by the translated native gene product and (*ii*) weak immunogenicity of parenterally injected conventional DNA vaccines in humans. The first problem was solved with DNA vaccines coding for hypoallergenic derivatives, such as fragmented allergen genes (75) or hypoallergens developed by mutation or gene shuffling (76). Moreover, a routine method was established for the production of hypoallergenic vaccines with any given allergy gene of interest by forced ubiquitination (77). Replicase-based DNA vaccines are immunogenic at 10,000-fold lower concentrations than conventional DNA vaccines (78), thus elegantly addressing the immunogenicity problem. Moreover, even at such ultralow doses, this vaccine type displayed highly effective protection from allergic reactions in a mouse model. Taken together, these latest DNA vaccine generations fulfill all necessary requirements for clinical trials: (*i*) they can be produced with high purity, (*ii*) they are allergen specific, (*iii*) they are safe with respect to anaphylactic side effects, (*iv*) they do not trigger new "therapy-induced" IgE antibodies, and (*v*) they likely can be used at nanogram doses in humans.

ALLERGEN FRAGMENTS

Large Allergen Fragments

IgE epitopes can be formed by linear sequences of amino acids (continuous epitopes) or by nonadjacent sequence elements brought together by folding (discontinuous or conformation-dependent epitopes). IgE recognition of continuous epitopes may also depend on their

conformation, which might only occur in the context of the folded allergen molecule. Thus, the disruption of the three-dimensional structure by fragmentation could be a useful approach to reduce the anaphylactic potential of allergens. The three-dimensional structure of Bet v 1 was disrupted by expressing two fragments of the cDNA, corresponding to amino acids 1-74 and 75-160, in *E. coli* (79). The fragments exhibited random coil conformation and almost no allergenicity. Together, the fragments harbored all relevant T-cell epitopes. Skin reactivity and histamine release were greatly reduced when compared with the native intact Bet v 1 allergen (80). Moreover, immunization of mice and rabbits with Bet v 1 fragments induced IgG antibodies that inhibited binding of IgE from patients allergic to wild-type Bet v 1 (81). Bet v 1 fragments were used to vaccinate 124 birch pollen–allergic patients in a double-blind, placebo-controlled study (82). Active treatment induced protective IgG antibodies that inhibited allergen-induced release of inflammatory mediators. The authors also observed a reduction of cutaneous sensitivity and an improvement of symptoms in actively treated patients. In addition, increases of allergen-specific IgE induced by seasonal birch pollen exposure were significantly reduced in vaccinated patients. This was the first clinical trial using a modified recombinant allergen preparation.

On the basis of IgE epitope mapping data of Phl p 6 from grass pollen, three allergen fragments have been produced, comprising amino acids 1-33, 1-57, and 1-110 (83). All fragments lacked a structural fold, which was accompanied by a strong reduction of IgE reactivity and allergenic activity. However, immunizing mice with fragments, adsorbed to an adjuvant allowed for human use, induced Phl p 6–specific IgG antibodies with only one of the fragments (31–110).

Nonanaphylactic fragments of the major house-dust mite allergen Der f 2 were produced by C- and N-terminal deletions. These fragments were mixed after separate refolding of the denatured fragments (84). Fragments of the calcium-binding allergens Bet v 4 (birch) (85) and Aln g 4 (alder) (86), and an N-terminal fragment of Lol p 1 from rye grass (59), also showed decreased IgE-binding activities. However, these fragments have yet to be studied with respect to T-cell reactivity and immunogenicity.

T-Cell Peptide Epitopes

Peptide immunotherapy is an approach that targets CD4+ T cells by using allergen-derived peptides containing short linear T-cell epitopes to induce tolerance. This approach is similar in many ways to the use of allergen fragments but employs smaller sequences specifically selected for activity as T-cell epitopes. The principle has been established in animal models, and the reduced ability of short peptide epitopes to cross-link surface-bound IgE may provide an attractive alternative to SIT in humans.

In vitro experiments have demonstrated that different concentrations of peptides can induce activation or hyporesponsiveness, depending on dose. Lamb and colleagues (87) employed high doses (50 μg) of peptide to render human T cells nonresponsive in vitro. Influenza virus hemagglutinin-specific human Th0 TCC were exposed to supraoptimal doses of peptide in the absence of APCs. Subsequent whole antigen challenge was characterized by antigen-specific T-cell hyporesponsiveness (anergy), which could be prevented or reversed by the addition of IL-2 (88). Further studies using supraoptimal doses of peptides in human CD4+ TCC reactive to house-dust mite reproduced clonal anergy and showed that this state was accompanied by downregulation of IL-2 and IL-4 and maintenance of IFN-γ secretion (89).

The principle that T-cell peptide epitopes may be employed to induce antigen-specific hyporesponsiveness has been extensively investigated in rodent models of autoimmune disease and allergic sensitization. Translation of the approach to human subjects was first attempted in individuals allergic to cats.

Allervax®CAT

Approximately 95% of individuals with a clinical history of cat allergy are sensitive to one protein, Fel d 1 (*Felis domesticus*), found in cat dander and saliva. The allergen is transported on clothing and is ubiquitous in the environment, present not only in the homes of cat owners but also in public places (90). Cloning, sequencing, epitope mapping, and preclinical studies resulted in the selection of two 27-amino acid sequences, IPC-1 and IPC-2, for evaluation in clinical trials (91).

The efficacy of Allervax®CAT (IPC-1/IPC-2) was evaluated by Norman and colleagues in a double-blind, placebo-controlled trial (92). Four weekly subcutaneous injections of placebo or peptides at doses of 7.5, 75, or 750 μg were administered. Modest improvements in symptom and medication scores were observed in the highest dose group, six weeks after treatment. Clinical outcomes included nasal and lung symptoms during a 60-minute exposure in a "cat room." Treatment was associated with a significant number of early and late adverse events, including chest tightness, nasal congestion, and flushing. These occurred a few minutes to several hours after administration of the peptides. In an associated study, a decrease in IL-4 production by IPC-1/IPC-2-specific T-cell lines from subjects in the high-dose group was demonstrated. However, proliferative responses to either peptide or whole allergen remained unchanged (93).

Immunotherapy with Allervax®CAT was also evaluated in cat-allergic asthmatic subjects, using inhaled allergen challenge (94). The investigators measured allergen PD_{20} [inhaled dose sufficient to reduce forced expiratory volume in 1 second (FEV_1) by 20%] before and after a variable cumulative dose of peptides. Six weeks after ending therapy, posttreatment PD_{20} was not significantly different between the treated and placebo groups. However, in the middle- and high-dose groups, there was a significant increase in allergen tolerance between baseline and posttreatment days. In addition, IL-4 release was significantly reduced in the high-dose group. No change was observed in IFN-γ production.

In a randomized, double-blind, parallel-group study (95), 40 cat-allergic subjects received subcutaneous injections of 250 μg of the same peptide preparation as used in the two studies described previously. Subjects received consecutively, four weekly injections. No change was seen in either early- or late-phase skin reactivity to whole cat extract up to 24 weeks after the last injection. No significant change in cat-antigen-specific cytokine production was observed. Frequent adverse events, including symptoms of asthma, rhinitis, and pruritus, were reported.

An additional study was a multicenter, randomized, double-blind, placebo-controlled study of 133 cat-allergic patients. Maguire and coworkers demonstrated modest improvements in some clinical outcome measures with peptide SIT (96). Subjects received subcutaneous injections of either 75 or 750 μg of peptide or placebo twice weekly for two weeks in two treatment phases, four months apart (a total of 8 injections). Pulmonary function improved in the 750-μg group. A large number of adverse events were recorded, including the requirement for systemic epinephrine in three peptide-treated patients. The majority of adverse events were associated with respiratory symptoms, occurred a few hours after injection, and declined with successive doses.

HLA-based Peptide Vaccines

In contrast to defined T-cell epitopes, other studies have focused on studies involving a large number of peptides, hence expanded recognition by several human leukocyte antigen (HLA) molecules, which are referred to as HLA-based peptide vaccines. Haselden and colleagues (97) administered intradermally a mixture of three short (16 to 17 residue-long peptides, compared to 27 residue-long peptides in Allervax®CAT) peptides from the cat allergen Fel d 1 to cat-allergic asthmatics. In a subset of individuals, an isolated late asthmatic reaction (LAR) was observed, characterized by a decline in FEV_1 two to four hours after peptide administration. Induction of bronchoconstriction was demonstrated to be IgE-independent and HLA-restricted, implying a T-cell-mediated reaction. Subjects receiving Fel d 1 peptides and experiencing isolated LAR subsequently displayed markedly reduced reactivity to injected peptides. In later studies, 12 overlapping peptides (16/17 residues each) were synthesized. This group of peptides spanned the majority of chains 1 and 2 of Fel d 1. Encompassing the majority of the molecules increased the number of HLA haplotypes able to bind and present peptide (98). Using this preparation of peptides, the magnitude, as well as the frequency, of isolated LARs in cat-allergic asthmatic subjects was dose dependent, with 50% of individuals developing a LAR when challenged with a single dose of 5 μg of peptide. A second injection of peptides was associated with a marked reduction or absence in LARs, with a return to baseline values of bronchial responsiveness over a period of up to 40 weeks. Thus, peptide-induced hyporesponsiveness was long lived.

A subsequent open study employing an updosing protocol with peptides (0.1, 1.0, 5, 10, 25 ug) injected at two-weeks' intervals demonstrated the ability to induce T-cell hyporesponsiveness in the absence of LARs. These results indicate that incremental dosing protocols,

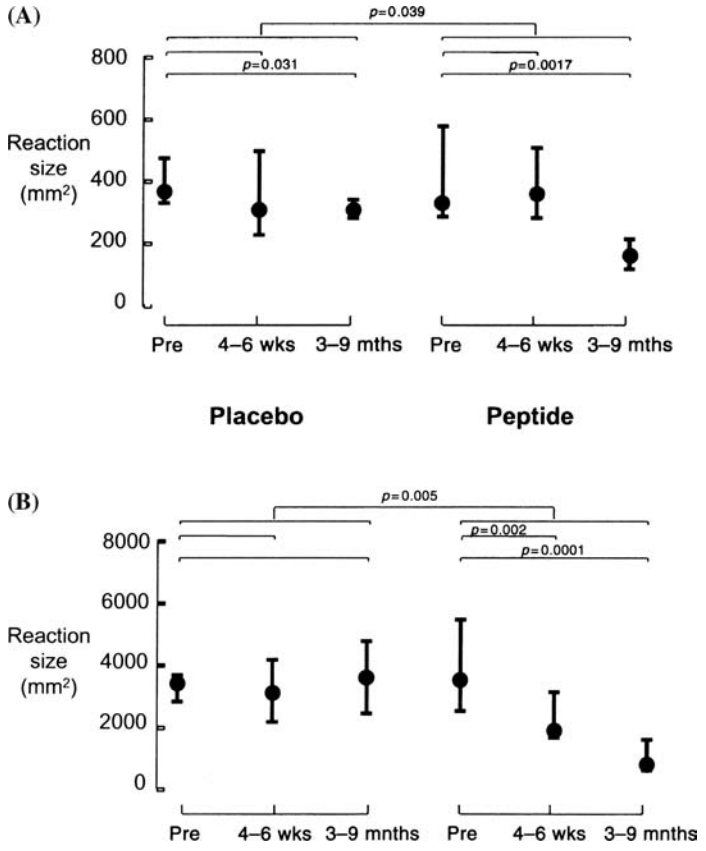

Figure 2 Peptide immunotherapy reduced both the early-phase cutaneous reaction to allergen challenge (**A**) and the late-phase cutaneous response to allergen challenge (**B**). A randomized, double-blind, placebo-controlled study of peptide immunotherapy with a mixture of 12 synthetic overlapping peptides derived from the primary sequence of the major cat allergen, Fel d 1, was performed in cat-allergic volunteers with asthma. A total of 90 μg of each peptide was administered. Measurements of early-phase (15 minutes) and late-phase (6 hours) skin reactions to intradermal challenge with purified Fel d 1 protein were recorded before therapy, 4 to 6 weeks after therapy and 3 to 9 months after therapy. Statistically significant reductions in the magnitude of both early and late reactions were observed in the peptide-treated group.

starting at a dose that is too low to induce LARs, may allow peptide immunotherapy to be used safely in the treatment of asthma without undesirable bronchoconstriction (99).

Initial observations show that a single peptide injection reduced cutaneous late phase reactions to intradermal challenge with whole cat dander extract (98). To confirm these observations, a small, double-blind, placebo-controlled study was performed. Subjects received either placebo or peptide via an incremental dosing protocol up to a total dose of 90 μg (100). Following peptide treatment, a significant reduction in both the early and late cutaneous reactions to allergen challenge was observed, indicating peptide-induced modulation of both the early-phase and the late-phase reactions. Changes in late cutaneous reactions were observed within weeks of completing treatment and persisted for several months (Fig. 2). Changes in early cutaneous reactions were only apparent at long-term follow-up (3–9 months). No significant differences were demonstrated in bronchial responsiveness to either methacholine or whole cat dander. However, the study was not powered to detect such outcomes. The important clinical observation was that peptide-treated subjects were better able to tolerate subsequent exposure to cats.

In vitro T-cell responses measured before and after peptide treatment demonstrated a significant decrease in peptide- and whole-allergen-induced proliferation of peripheral PBMCs and reduced production of both Th2 (IL-4, IL-13) and Th1 cytokines and IFN-γ in cultures. Furthermore, peptide treatment was associated not only with decreases in whole cat dander–induced proinflammatory cytokines by PBMCs but also with increased IL-10 production. Thus, induction of T-cell hyporesponsiveness in man may be associated with the induction and/or expansion of a population of regulatory T cells.

ADJUVANTS

Approaches that have been exploited to enhance the immunogenicity of vaccine antigens include strategies based on adjuvants, epitopes, and particulate antigens. An emerging area in the vaccinology of allergic diseases involves the use of various adjuvants to direct and redirect protective immune responses. Substantial progress has been made in some of these approaches, leading to clinical trials and licensure. However, safety concerns and economic considerations have limited their commercialization. The experimental research on improving immunotherapy has involved a combination of standardized allergens or purified/cloned allergens with an appropriate adjuvant. The latter may consist of a live or killed microorganism as a vaccine vector or molecular immunomodulators, such as those carrying cytosine-phosphoguanosine (CpG) sequences or plasmids (pDNA) encoding protective cytokines (101).

Microbial Adjuvants

To date, a large repertoire of live vaccine vectors, either avirulent or nonpathogenic organisms, capable of expressing important immunomodulatory molecules, has been investigated. These include vectors with small genome sizes, such as vaccinia virus, avipoxviruses, adenoviruses, polioviruses, salmonella, and vectors with large genomes, such as the herpes viruses and the mycobacterium bacillus calmette-guerin (BCG) (102–104). cDNAs encoding major allergens are available, which can be cloned into these nonpathogenic organisms to generate effective reagents for prophylaxis and treatment of allergies. Such organisms are expected to provide a more effective and longer-lasting adjuvant effect compared with the pDNA immunostimulants (105), presumably because of their persistence within cells of the body.

BCG

BCG is considered an excellent vaccine vector because it offers the following unique advantages (106,107): (*i*) BCG lends itself to the development of a *multiantigen vaccine*, as would be required for downregulation of specific allergies. (*ii*) Live attenuated BCG has been used for immunization of more than 2.5 billion people worldwide since 1948, with a low incidence of serious complications (case fatality rates of $0.19/10^6$). (*iii*) BCG has been shown to be a potent adjuvant in experimental animals and man, particularly in relation to induction of Th1 cells. (*iv*) BCG is heat stable and inexpensive to produce. (*v*) BCG can be administered by the oral route. (vi) BCG can be given at birth or at any time afterwards and is unaffected by maternal antibodies. (vii) BCG given as single inoculum sensitizes to tuberculoproteins for 5 to 50 years. (*viii*) Various in vitro studies of human and murine systems show that BCG-reactive CD4+ T cells are potent producers of IFN-γ (108), the principal mediator of antituberculous resistance. (*ix*) The evidence that BCG can be a resident within the phagosome of the long-lived macrophage for years suggests that a BCG-based vaccine may induce a potentially persistent or long-lasting allergen-specific immune response (109).

Controversial evidence in support of a role for BCG vaccination in the determination of allergic phenotype has come from the observation that Japanese children displaying a positive delayed-type hypersensitivity skin test reaction following prior BCG immunization exhibited lower prevalence of atopic allergic disease (110).

Previously, Erb et al. demonstrated that infection of mice with BCG suppressed allergen-induced airway eosinophilia (111). In order to establish if live BCG would provide an adjuvant effect similar to that of complete Freund's adjuvant, mice were vaccinated with BCG and subsequently immunized with recombinant allergen in alum. This protocol induced a decrease in total IgE and specific IgE by 5- and 10-fold, respectively. Concomitantly, IgG2a increased by 10-fold (109). Furthermore, the production of both specific and total IFN-γ was increased when cells from mice, vaccinated in vivo with BCG followed by recombinant Kentucky bluegrass allergen in alum, were stimulated in vitro with the same allergen. The result was an increase in the ratio of IFN-γ to IL-4. These findings demonstrate that live BCG as an adjuvant is capable of inducing a protective Th1 type of response.

In an effort to provide specificity to the effects of BCG, many investigators have developed recombinant BCG (rBCG) producing foreign antigens within the macrophage. Oral immunization with rBCG induces both cellular and humoral responses against foreign antigen (112). Experiments have examined the effects of rBCG vaccination on allergic responses in a

murine model (109). A BCG-*E. coli* shuttle vector was developed with the promoter and signal sequence and one of the major antigens of *Mycobacterium bovis* (A-antigen), and the vector was tested using *E. coli* β-galactosidase as the model antigen and allergen (109). This vector enabled the expression of the *E. coli* β-galactosidase (GAL) gene in BCG, which was detected in its protein extract by immunoblotting analysis. Vaccination of mice with a single dose of 10^6 rBCG generated a β-galactosidase-specific antibody response. The splenocytes of vaccinated mice, compared with controls, produced significantly higher levels of IFN-γ ($p < 0{\cdot}01$) and IL-2 ($p < 0{\cdot}05$) and lower levels of IL-5 ($p < 0{\cdot}01$). Mice vaccinated with rBCG had significantly less ($p < 0{\cdot}01$) serum IgE compared with controls. These results demonstrate that rBCG-secreting antigens or allergens may be used for the induction of a Th1-like response and the downregulation of IgE antibody response.

Cohon et al. examined the effects of BCG as an adjuvant in dust-mite allergen immunotherapy in asthmatics (113). After 12 to 16 weeks of SIT with *Dermatophagoides pteronyssinus* extract, clinical outcomes and immunologic parameters improved in asthmatic children with mite allergy. However, simultaneous BCG vaccination at the initiation of SIT provided no additional benefit, suggesting that immunomodulatory responses that might have been induced by BCG were not sufficient to translate into further clinical improvement of patients with established allergic disease. Thus, the role of BCG as an immune-redirecting adjuvant for allergy and asthma remains to be established in further studies.

Mycobacterium Vaccae

Mycobacterium vaccae has been examined for its therapeutic effect for allergy and asthma. Wang and Rook showed that *M. vaccae* injected twice after mice were sensitized with ovalbumin resulted in a reduction in IgE and allergen-specific IL-5 synthesis, suggesting a potential clinical application of this organism in the immunotherapy of allergic diseases (108). Like BCG, *M. vaccae* also evokes a strong IFN-γ response and has been suggested to be effective in enhancing antiallergic response in clinical trials. In a murine model of asthma, *M. vaccae* inhibited airway inflammation via regulating Th1/Th2 balance, suggesting that it may be beneficial in the treatment of asthma (114,115). Furthermore, studies with newborn mice show that immunization with *M. vaccae* prevents some of the chronic airway changes in asthma. Treatment of mice with SRP299 (a killed *M. vaccae* suspension) gave rise to allergen-specific $CD4{+}CD45RB_{(Lo)}$-regulatory T cells, that conferred protection against airway inflammation (116). This specific inhibition was mediated through IL-10 and transforming growth factor-beta (TGF-β), as antibodies against IL-10 and TGF-β completely reversed the inhibitory effect. Thus, regulatory T cells generated by mycobacterial treatment may have a role in restoring the balance of the immune system to prevent and treat allergic diseases.

A double-blind, randomized study investigated whether heat-killed *M. vaccae* (SRL172), a potent downregulator of Th2 cytokines, could reduce allergen-induced airway responses in patients with atopic asthma (117). A total of 24 male asthmatics participated in this study. Bronchial allergen challenge was performed along with measurement of early asthmatic responses and LARs, two weeks before and three weeks after a single intradermal injection of SRL172 or placebo. SRL vaccination prior to allergen challenge improved lung function following bronchial challenge compared with animals without SRL vaccination, but the magnitude of the effect did not reach statistical significance. SRL172 caused a trend for a reduction in serum IgE and IL-5 synthesis in vitro three weeks posttreatment ($p = 0.07$). These results suggest that multiple dosing of *M. vaccae* may be required to achieve significant benefit in asthma.

Heat-killed *Listeria monocytogenes* (HKL) potently stimulates IFN-γ production in CD4 T lymphocytes and, when used as an adjuvant for immunotherapy, reduces IgE production and reverses established allergen-induced AHR in a murine model of asthma (118). In another study, Frick et al. (119) showed that HKL plus allergen treatment markedly improved established food allergic responses in dogs, suggesting that such an immunotherapeutic strategy in humans might improve the condition of individuals with food allergy and anaphylaxis.

These studies have identified the potential of live BCG or other microorganisms, either by themselves or in the form recombinant organisms expressing target allergens, as adjuvants for the potentiation of treatments for allergic diseases. However, these preliminary studies need to be extended in clinical trials to determine if such compounds offer real advantages to the management of human allergic diseases.

Adjuvant Effects of Toll-like Receptor Agonists

Several formulations have been developed on the basis of the concept that linking or activating specific toll-like receptors (TLRs) can modulate and bias the immune response to a preferred profile (120). Although experimental evidence indicates that lipopolysaccharide (LPS) has a kinase-like behavior on T-cell profiles, TLR-4, which binds LPS, seems to consistently support the development of a Th1 response. Thus, Pollinex Quattro™, a cocktail of several allergens that use monophosphoryl lipid A (MPL) as adjuvant has been developed. MPL is a detoxified derivative of the LPS moiety of *Salmonella minnesota* R595. It binds to and specifically activates TLR4 and promotes the development of a Th1 response (121). MPL also acts through TLR2, which may enhance the function of CD4+ CD25+ regulatory T-cell function in a MyD88-dependent fashion (122–124).

This formulation is currently marketed in Europe as an ultrashort course of immunotherapy in patients suffering from allergic rhinitis during the pollen season. Besides the use of MPL, the grass/rye-derived allergens are treated with glutaraldehyde adsorbed onto an L-tyrosine depot, reducing the potential for adverse side effects (125). Clinical studies demonstrate that Pollinex Quattro reduces allergen-specific T-cell proliferation (126), rhinoconjuctivitis symptoms, and medication use (127–129). In addition, Pollinex Quattro blunted the elevation of IgE after allergen exposure while increasing allergen-specific IgG titers, particularly in the IgG4 subclass (128–130).

CRX-675 (aminoacyl-glucosamine 4 phosphatide), a soluble TLR4 agonist, is another promising adjuvant showing improvement in nasal symptom scores in patients suffering from allergic rhinitis. This formulation has no documented adverse side effects (131). Unfortunately, there are no indications thus far about the future marketing of this compound.

Potentially, new compounds could result from the determination of the immunomodulatory molecules in *L. monocytogenes* based on the adjuvant studies, both in an experimental, allergic-asthma mouse model and in food allergic dogs (119). Thus, a number of promising research strategies may help achieve the goal of modulating the T-cell profile through the proper stimulation of specific TLRs.

An additional, extensively studied TLR agonist is CpG-ODN. In contrast to eukaryotic DNA, the genetic material of prokaryotes is rich in sequences containing unmethylated CpG dinucleotide motifs. CpG DNA is most often coadministered with antigen in the form of synthetic oligodeoxynucleotides (CpG-ODN), which are made with a nuclease-resistant phosphorothioate backbone. Synthetic ODNs containing CpG motifs activate both innate and acquired immune responses through a signaling pathway involving TLR9. Depending on the sequence, length, number, and positions of CpG motifs in an ODN, distinct immunostimulatory profiles can be observed. These immunostimulatory profiles can be further modified and fine-tuned by appropriate chemical modifications, leading to preclinical and clinical development of CpG-ODN treatments in cancer, allergy, asthma, and infectious diseases. CpG-ODN sequences tend to induce Th1-like cytokine responses (132). The adjuvant may be injected with natural allergens or may be genetically linked with allergen cDNA. CpG-ODN, administered in conjunction with antigen, downregulates established Th2 responses. This immunomodulation is neither murine strain dependent nor model dependent. Although the effects of CpG-ODNs are associated with the induction of the Th1 cytokines IFN-γ and IL-12, neither cytokine is essential for the effect (133).

Experimental evidence indicates that CpG improves allergic airway inflammation by both inhibiting Th2 activation and preventing IgE-dependent release of Th2 cytokines (134). Specific immunostimulatory sequences containing CpG motifs have been evaluated in allergen immunotherapy (135,136). A randomized, double-blind, placebo-controlled phase 2 trial of a vaccine consisting of Amb a 1 conjugated to CpG-ODNs administered to patients allergic to ragweed shows a significant improvement in allergic rhinitis. This formulation also induces a transient increase in Amb a 1–specific IgG antibodies but suppresses the seasonal increase in Amb a 1–specific IgE antibodies. A reduction in the number of IL-4-positive basophils was also observed in formulation-treated patients. Interestingly, this treatment has long-lasting immunomodulatory effects confirmed by observations in subsequent ragweed seasons (137). A new formulation targeting allergies to house-dust mite has entered phase II clinical trials, and these results will help to determine the applicability of CpG adjuvants in a broader context (138). Treatment of rhinitis in mice sensitized to *Dermatophagoides farinae*

supports the potential use of CpG-ODNs in vaccines for the treatment of house-dust mite allergies (139). The effects observed include a reduction in IL-5 and an increase in IFN-γ in nasal lavage fluid.

Immunostimulatory ODN sequences from different sources have also been explored. ODN-BL07S from a probiotic strain, *Bifidobacterium longum*, inhibits allergen-specific IgE production and significantly reduced the levels of Th2 cytokines in an experimental mouse model. This also is accompanied with an increase in the levels of IgG2a (140).

In a mouse model sensitized to the major bee venom allergen phospholipase A2, the testing of several formulations using different TLR agonists as adjuvants shows the potential of polyriboinosinic polyribocytidylic (polyI:C) acid, a TLR3 agonist, as an adjuvant for prophylactic vaccination. This adjuvant skews the antibody response toward an IgG profile. In addition, formulations using adjuvants such as CpG, polyI:C, or peptidoglycan bias the T-cell response to a Th1 profile. In a therapeutic scenario, formulations using CpG, polyI:C, or 3M003 show excellent immunomodulatory properties (141).

Compounds such as CRX-527 (GSK), E6020 (Esai), and "Compound 1" (Biomira) are expected to be evaluated in the allergy field (104). Potentially, new compounds could result from determination of the molecules responsible for immunomodulation in *L. monocytogenes* (119). All the above indicate the promise of research to develop methods of modulating the T-cell profile through the proper stimulation of specific TLRs.

Microparticle or Nanoparticle Polymers

To improve the administration of allergy vaccines, the use of different chemistries to develop new assemblages, such as microparticles and nanoparticles, has also been explored in experimental allergic mouse models. Biodegradable polylactide-co-glycolide (PLGA) was used to prepare microparticles as a way to carry and deliver CpG-ODNs along with PLA2. This formulation was administered to mice sensitized to PLA2, resulting in an increased Th1 response and antibody profile biased to IgG2a (132). PLGA microparticles were also developed to deliver DNA vaccination against PLA2, resulting in a Th2-to-Th1 immune deviation, an increase in IL-10 production, and a reduction in IgE production (133). Use of a PLGA-based formulation could also potentially reduce both the treatment course and the amount of allergen and CpG administered in each dose, without preventing the development of strong Th1 responses (142). PLGA microparticles were also tested as carriers for allergen extracts, such as Ole e 1, in BALB/c mice previously sensitized to this allergen. This formulation also elicited a specific Th1-like response (143).

Chitosan, a biodegradable polymer, has been described as a vaccine adjuvant (144–146). Hall et al. (147) investigated whether intranasal administration of an allergen-derived peptide, either alone or adsorbed to chitosan, could prevent the induction of Th2-mediated pulmonary inflammation after sensitization and challenge of the airways with allergen. The results demonstrate that mice given peptide adsorbed to chitosan have significant reductions in airway eosinophilia, which correlate with reduced levels of IL-4 and IL-5 in the bronchoalveolar lavage fluid. There was decreased recruitment of activated CD4+ T cells into the airways after allergen challenge, which correlated with a loss of Der p 1–specific, T-cell cytokine responses in the periphery and localized production of IL-10 by antigen-specific T cells in bronchial lymph nodes. In another study, using a standardized mouse model of allergic asthma, Hellermann et al. showed that chitosan nanoparticles could be used to encapsulate allergen, which could then be delivered orally or as a nasal solution to reduce the sensitivity of the animals. The allergenicity of soy protein was greatly decreased by the attachment of chitosan through the Maillard reaction (148). Chew et al. (149) explored the use of chitosan-DNA nanoparticles for oral immunization to induce immune responses specific to both the left and right domains of Der p 1. Plasmid constructs encoding Der p 1[pDer p 1; (1-222) and pDer p 1 (114-222)] were complexed with chitosan and delivered orally, followed by an intramuscular injection of pDer p 1 (1-222) 13 weeks later. Such an approach has successfully primed Th1-skewed immune responses against both domains of Der p 1. This strategy can be further optimized for more efficacious gene vaccination for full length Der p 1. These studies indicate that chitosan may not only act as a gene carrier but also as an immunostimulant in SIT.

Allergens Conjugated/Linked to Human Ig Receptors

An interesting approach is to modify allergens by conjugating them to molecules that play modulatory roles in the immune system. A chimeric fusion protein comprising human Fcγ linked to Fel d 1 was developed. This molecule binds to FcγRII in a fashion equivalent to that of human IgG and thereby coaggregates FcγRIIb with FcεRI-bound IgE. The result is inhibition of mediator release, such as histamine, in a dose-dependent fashion and inhibition of IgE-mediated Syk and extracellular signal-regulated kinase (ERK) phosphorylation in basophils from cat-allergic subjects. These results were also corroborated in cat allergen–sensitized mice (134).

In an alternate approach, Fel d 1 was linked to a single-chain fragment of the variable region (sFv) of the humanized anti-CD64 mAb H22 (H22-Fel d1). This fusion protein was administered to (*i*) patients allergic to cat allergen and (*ii*) control subjects without Fel d 1–specific IgE or IgG. It was found to induce cytokine-secreting subtypes consistent with Th0 and Th1 cells, mainly in the subset population of allergic individuals, and it did not induce a proallergic CD4+ T-cell repertoire. Although H22-Fel d 1 upregulates both an IL-5- and an IL-10-secreting T-cell population, IL-10 seems to dominate and control IL-5+ CD4+ T cells (140).

Cytokines as Adjuvants

Advances in gene transfer technology now make it possible to deliver cytokine genes to the target organ (Fig. 3). This approach will allow the evaluation of cytokines as adjuvants with antigen in vaccine or immunotherapy formulations. IL-12 and IFN-γ have been considered as potentially important adjuvants for the induction of Th1 cell-mediated, protective immunity (141). The functional effects of IL-12 in many systems are likely to be mediated through secondary production of IFN-γ. However, the Th1-inducing effect of IL-12 in vivo may not be accompanied by a long-lasting suppression of Th2 development, and IL-12 is toxic in some

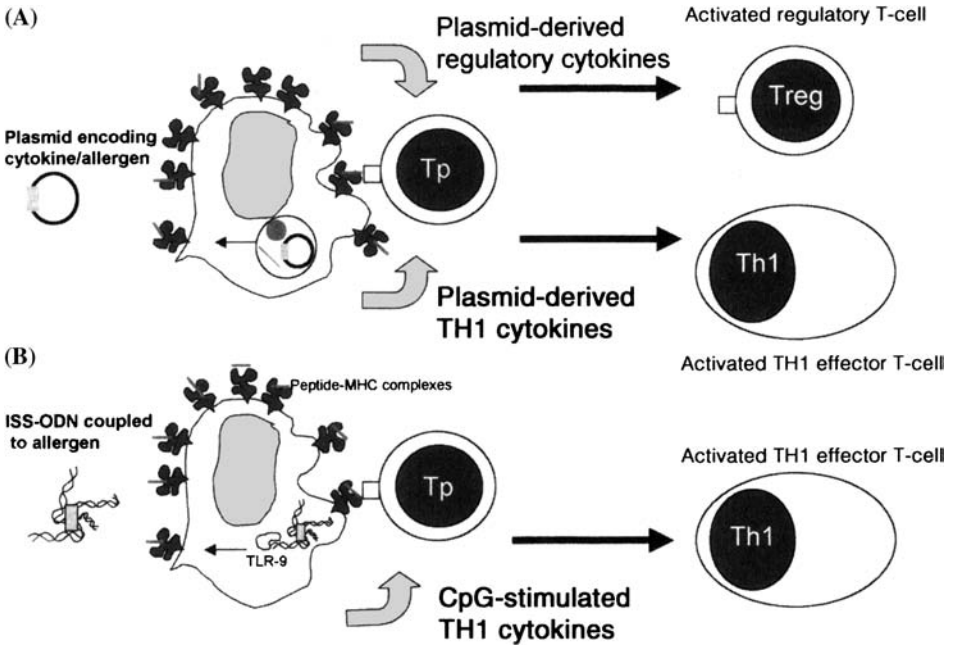

Figure 3 DNA-based approaches for the treatment of allergic diseases. (**A**) Plasmid DNA vectors encoding allergens and cytokine genes may be used to generate allergen-specific responses in an environment rich in proinflammatory, Th1 cytokines, such as IL-12 and IFN-γ, or regulatory cytokines, such as IL-10 and TGF-β. Presentation of allergen-derived peptides to Tp leads to the generation of robust Th1 or T-regulatory allergen-specific responses. (**B**) Allergens covalently coupled to ISS-ODN may be used to deliver allergen molecules to APCs together with TLR-9 activation leading to the production of Th1-stimulating cytokines such as IL-12. Presentation of allergen-derived peptides to Tp leads to the generation of robust Th1 allergen-specific responses. *Abbreviations*: DNA, deoxyribonucleic acid; ISS-ODN, immunostimulatory oligodeoxynucleotides; TLR-9, toll-like receptor-9; Tp, precursor T cells.

systems. IFN-tau, a type I IFN that lacks the toxicity associated with other type I IFNs, inhibits IgE production in a murine model of allergy and in an IgE-producing human myeloma cell line.

Administration of recombinant allergen (ovalbumin) with an IL-12 (subunit p40) fusion protein vaccine downregulates ovalbumin-specific IgE responses in vivo (104). In another model, IL-12 was delivered intranasally during allergen immunotherapy (150). In a typical protocol, DBA/2 mice were immunized intraperitoneally with GAL and then immunized parenterally with grass allergen with or without IL-12. Treatment of sensitized mice with a combination of allergen plus IL-12 shows the highest IFN-γ production and decreases the Th2-like response in GAL-stimulated splenocyte culture. IL-12 also inhibits GAL-induced IgE production and enhances GAL-specific IgG2a in GAL-presensitized mice. Intranasal delivery of IL-12 attenuates airway hyperresponsiveness and BAL eosinophilia in sensitized mice. Analysis of IL-12 receptor expression suggests a shift in the expression profile of IL-12Rβ1 and β2 in the lung tissue, consistent with the observed shift in the cytokine profile from a Th2- to a Th1-like response. These results suggest that intranasal delivery of IL-12 inhibits allergic airway inflammation in asthma via an IFN-γ-dependent pathway, involving regulation of the expression of the IL-12 receptor.

The immunomodulatory role of plasmid DNA-expressing cytokines IFN-γ (pIFN-γ) and/or IL-12 (pIL-12) as adjuvants was assessed in a murine model of Kentucky blue grass (KBG) allergy (Mohapatra, unpublished observations). Mice vaccinated with cytokine plasmid adjuvants had relatively less total serum IgE and higher levels of grass allergen–specific IgG2a when compared with the control mice injected with the empty vector plasmid. The lowest IgE and the highest IgG2a levels were found in mice vaccinated with combined pIFN-γ and pIL-12 as adjuvant. The IgG1 titers of all mice remained unchanged. The most profound decrease in airway hyperresponsiveness and pulmonary inflammation was observed in mice receiving both pIFN-γ and pIL-12 as adjuvants. These studies provide evidence that a combination of pIFN-γ and pIL-12 provides a more effective adjuvant to the grass allergen vaccine than either one of these plasmids alone, and the combination may enhance the effectiveness of allergen immunotherapy in humans.

CONCLUSION

Rapid progress is being made in the development of novel immunotherapeutics for the treatment of allergic diseases. Recombinant DNA technology has enabled the cloning and sequencing of genes encoding several hundred important aeroallergens. Knowledge of nucleotide and amino acids sequences enables the standardized production of recombinant molecules, fragments, and peptides to high degrees of purity for development as vaccine candidates. Furthermore, detailed evaluation of structural features of allergens and their isoforms has led to strategies aimed at decreasing the allergenicity of proteins while retaining the ability to induce protective immunity.

Parallel development of adjuvant technology, including the use of bacteria or their products to activate the innate immune response and also the use of Th1-stimulating plasmid-encoded cytokines, offers the potential to redirect allergic responses safely and effectively. Many of these approaches are currently being evaluated in clinical trials and hold considerable promise for future therapeutic intervention in allergic diseases.

SALIENT POINTS

- SIT has several advantages; however, current methods are not without the potential for systemic reactions.
- Recombinant allergens can be formulated to replace natural extracts.
- Allergens can be engineered to create low IgE-binding isoforms.
- Allergen DNA vaccines offer a novel form of allergen immunotherapy for the future.
- Allergen fragments, representing B- and/or T-cell epitopes, can be used for allergen immunotherapy with less side effects.

- Microbial adjuvants enhance the potency of allergen vaccines and shift the immune response away from the allergic response.
- TLR agonists and antagonists may play a role in creating adjuvant effects.
- Microparticle or nanoparticle polymers act as adjuvants for allergen vaccines and enhance vaccine efficacy in allergic animal models.
- Cytokines or other immune molecules may function as immune response modifiers for allergen vaccines.

REFERENCES

1. Bousquet J, Lockey R, Malling HJ, et al. Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American academy of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 1998; 81:401–405.
2. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999; 341:468–475.
3. Akdis CA, Blaser K. Immunologic mechanisms of allergen-specific immunotherapy. Adv Exp Med Biol 2001; 495:247–259.
4. Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. J Allergy Clin Immunol 1998; 102:157–164.
5. RJ van Neerven, Wikborg T, Lund G, et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. J Immunol 1999; 163:2944–2952.
6. Rocklin RE, Sheffer AL, Greineder DK, et al. Generation of antigen-specific suppressor cells during allergy desensitization. N Engl J Med 1980; 302:1213–1219.
7. Durham SR, Varney VA, Gaga M, et al. Grass pollen immunotherapy decreases the number of mast cells in the skin. Clin Exp Allergy 1999; 29:1490–1496.
8. Otsuka H, Mezawa A, Ohnishi M, et al. Changes in nasal metachromatic cells during allergen immunotherapy. Clin Exp Allergy 1991; 21:115–119.
9. Ebner C, Siemann U, Bohle B, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. Clin Exp Allergy 1997; 27:1007–1015.
10. Secrist H, Chelen CJ, Wen Y, et al. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. J Exp Med 1993; 178:2123–2130.
11. Akdis CA, Blesken T, Akdis M, et al. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998; 102:98–106.
12. Moverare R, Elfman L, Vesterinen E, et al. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. Allergy 2002; 57:423–430.
13. Chapman MD, Smith AM, Vailes LD, et al. Recombinant allergens for diagnosis and therapy of allergic disease. J Allergy Clin Immunol 2000; 106:409–418.
14. Valenta R. The future of antigen-specific immunotherapy of allergy. Nat Rev Immunol 2002; 2:446–453.
15. Maurer D, Ebner C, Reininger B, et al. The high affinity IgE receptor (Fc epsilon RI) mediates IgE-dependent allergen presentation. J Immunol 1995; 154:6285–6290.
16. van der Heijden FL, Joost van Neerven RJ, van Katwijk M, et al. Serum-IgE-facilitated allergen presentation in atopic disease. J Immunol 1993; 150:3643–3650.
17. Griffith IJ, Pollock J, Klapper DG, et al. Sequence polymorphism of Amb a I and Amb a II, the major allergens in Ambrosia artemisiifolia (short ragweed). Int Arch Allergy Appl Immunol 1991; 96:296–304.
18. Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, et al. Four recombinant isoforms of Cor a I, the major allergen of hazel pollen, show different IgE-binding properties. Eur J Biochem 1993; 212:355–362.
19. Swoboda I, Jilek A, Ferreira F, et al. Isoforms of Bet v 1, the major birch pollen allergen, analyzed by liquid chromatography, mass spectrometry, and cDNA cloning. J Biol Chem 1995; 270:2607–2613.
20. Chang ZN, Peng HJ, Lee WC, et al. Sequence polymorphism of the group 1 allergen of Bermuda grass pollen. Clin Exp Allergy 1999; 29:488–496.
21. Muller WD, Karamfilov T, Kahlert H, et al. Mapping of T-cell epitopes of Phl p 5: evidence for crossreacting and non-crossreacting T-cell epitopes within Phl p 5 isoallergens. Clin Exp Allergy 1998; 28:1538–1548.
22. Son DY, Scheurer S, Hoffmann A, et al. Pollen-related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen. Eur J Nutr 1999; 38:201–215.

23. Hoffmann-Sommergruber K, Ferris R, Pec M, et al. Characterization of api g 1.0201, a new member of the Api g 1 family of celery allergens. Int Arch Allergy Immunol 2000; 122:115–123.
24. Duro G, Colombo P, Assunta Costa M, et al. Isolation and characterization of two cDNA clones coding for isoforms of the Parietaria judaica major allergen Par j 1.0101. Int Arch Allergy Immunol 1997; 112:348–355.
25. Gonzalez EM, Villalba M, Lombardero M, et al. Influence of the 3D-conformation, glycan component and microheterogeneity on the epitope structure of Ole e 1, the major olive allergen. Use of recombinant isoforms and specific monoclonal antibodies as immunological tools. Mol Immunol 2002; 39:93–101.
26. Smith AM, Benjamin DC, Derewenda U, et al. Sequence polymorphisms and antibody binding to the group 2 dust mite allergens. Int Arch Allergy Immunol 2001; 124:61–63.
27. Sowka S, Hafner C, Radauer C, et al. Molecular and immunologic characterization of new isoforms of the Hevea brasiliensis latex allergen hev b 7: evidence of no cross-reactivity between hev b 7 isoforms and potato patatin and proteins from avocado and banana. J Allergy Clin Immunol 1999; 104:1302–1310.
28. Rautiainen J, Auriola S, Konttinen A, et al. Two new variants of the lipocalin allergen Bos d 2. J Chromatogr B Biomed Sci Appl 2001; 763:91–98.
29. Schenk S, Hoffmann-Sommergruber K, Breiteneder H, et al. Four recombinant isoforms of Cor a 1, the major allergen of hazel pollen, show different reactivities with allergen-specific T-lymphocyte clones. Eur J Biochem 1994; 224:717–722.
30. Ferreira F, Hirtenlehner K, Jilek A, et al. Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. J Exp Med 1996; 183:599–609.
31. Wurtzen PA, Bufe A, Wissenbach M, et al. Identification of isoform-specific T-cell epitopes in the major timothy grass pollen allergen, Phl p 5. Clin Exp Allergy 1999; 29:1614–1625.
32. Hales BJ, Hazell LA, Smith W, et al. Genetic variation of Der p 2 allergens: effects on T cell responses and immunoglobulin E binding. Clin Exp Allergy 2002; 32:1461–1467.
33. Arquint O, Helbling A, Crameri R, et al. Reduced in vivo allergenicity of Bet v 1d isoform, a natural component of birch pollen. J Allergy Clin Immunol 1999; 104:1239–1243.
34. Z Markovic-Housley, Degano M, Lamba D, et al. Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. J Mol Biol 2003; 325:123–133.
35. Wagner S, Radauer C, Bublin M, et al. Naturally occurring hypoallergenic Bet v 1 isoforms fail to induce IgE responses in individuals with birch pollen allergy. J Allergy Clin Immunol 2008; 121:246–252.
36. Ferreira F, Ebner C, Kramer B, et al. Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. FASEB J 1998; 12:231–242.
37. Casari G, Sander C, Valencia A. A method to predict functional residues in proteins. Nat Struct Biol 1995; 2:171–178.
38. Holm J, Gajhede M, Ferreras M, et al. Allergy vaccine engineering: epitope modulation of recombinant Bet v 1 reduces IgE binding but retains protein folding pattern for induction of protective blocking-antibody responses. J Immunol 2004; 173:5258–5267.
39. Valenta R, Hayek B, Seiberler S, et al. Calcium-binding allergens: from plants to man. Int Arch Allergy Immunol 1998; 117:160–166.
40. Swoboda I, A Bugajska-Schretter, Verdino P, et al. Recombinant carp parvalbumin, the major cross-reactive fish allergen: a tool for diagnosis and therapy of fish allergy. J Immunol 2002; 168:4576–4584.
41. Engel E, Richter K, Obermeyer G, et al. Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. J Biol Chem 1997; 272:28630–28637.
42. Okada T, Swoboda I, Bhalla PL, et al. Engineering of hypoallergenic mutants of the Brassica pollen allergen, Bra r 1, for immunotherapy. FEBS Lett 1998; 434:255–260.
43. Swoboda I, N De Weerd, Bhalla PL, et al. Mutants of the major ryegrass pollen allergen, Lol p 5, with reduced IgE-binding capacity: candidates for grass pollen-specific immunotherapy. Eur J Immunol 2002; 32:270–280.
44. Karisola P, Kotovuori A, Poikonen S, et al. Isolated hevein-like domains, but not 31-kd endochitinases, are responsible for IgE-mediated in vitro and in vivo reactions in latex-fruit syndrome. J Allergy Clin Immunol 2005; 115:598–605.
45. Beezhold DH, Hickey VL, Sussman GL. Mutational analysis of the IgE epitopes in the latex allergen Hev b 5. J Allergy Clin Immunol 2001; 107:1069–1076.
46. Mine Y, Sasaki E, Zhang JW. Reduction of antigenicity and allergenicity of genetically modified egg white allergen, ovomucoid third domain. Biochem Biophys Res Commun 2003; 302:133–137.
47. Bannon GA, Cockrell G, Connaughton C, et al. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. Int Arch Allergy Immunol 2001; 124:70–72.
48. Li XM, Srivastava K, Huleatt JW, et al. Engineered recombinant peanut protein and heat-killed Listeria monocytogenes coadministration protects against peanut-induced anaphylaxis in a murine model. J Immunol 2003; 170:3289–295.

49. Akdis CA, Blesken T, Wymann D, et al. Differential regulation of human T cell cytokine patterns and IgE and IgG4 responses by conformational antigen variants. Eur J Immunol 1998; 28:914–925.
50. Buhot C, Chenal A, Sanson A, et al. Alteration of the tertiary structure of the major bee venom allergen Api m 1 by multiple mutations is concomitant with low IgE reactivity. Protein Sci 2004; 13:2970–2978.
51. Bonura A, Amoroso S, Locorotondo G, et al. Hypoallergenic variants of the Parietaria judaica major allergen Par j 1: a member of the non-specific lipid transfer protein plant family. Int Arch Allergy Immunol 2001; 126:32–40.
52. Colombo P. Hypoallergenic variants of the Parietaria judaica major allergen Par j 1: in vitro and in vivo evaluation. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 2003:198–203; discussion 204.
53. Smith AM, Chapman MD. Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen Der p 2. Mol Immunol 1996; 33:399–405.
54. Takai T, Yokota T, Yasue M, et al. Engineering of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy. Nat Biotechnol 1997; 15:754–758.
55. Kronqvist M, Johansson E, Whitley P, et al. A hypoallergenic derivative of the major allergen of the dust mite Lepidoglyphus destructor, Lep d 2.6Cys, induces less IgE reactivity and cellular response in the skin than recombinant Lep d 2. Int Arch Allergy Immunol 2001; 126:41–49.
56. Saarne T, Kaiser L, Gronlund H, et al. Rational design of hypoallergens applied to the major cat allergen Fel d 1. Clin Exp Allergy 2005; 35:657–663.
57. Schramm G, Kahlert H, Suck R, et al. "Allergen engineering": variants of the timothy grass pollen allergen Phl p 5b with reduced IgE-binding capacity but conserved T cell reactivity. J Immunol 1999; 162:2406–2414.
58. Wu CH, Lee MF, Yang JS, et al. IgE-binding epitopes of the American cockroach Per a 1 allergen. Mol Immunol 2002; 39:459–464.
59. Tamborini E, Faccini S, Lidholm J, et al. Biochemical and immunological characterization of recombinant allergen Lol p 1. Eur J Biochem 1997; 249:886–894.
60. Vrtala S, Hirtenlehner K, Susani M, et al. Genetic engineering of recombinant hypoallergenic oligomers of the major birch pollen allergen, Bet v 1: candidates for specific immunotherapy. Int Arch Allergy Immunol 1999; 118:218–219.
61. Reese G, BK Ballmer-Weber, Wangorsch A, et al. Allergenicity and antigenicity of wild-type and mutant, monomeric, and dimeric carrot major allergen Dau c 1: destruction of conformation, not oligomerization, is the roadmap to save allergen vaccines. J Allergy Clin Immunol 2007; 119:944–951.
62. King TP, Jim SY, Monsalve RI, et al. Recombinant allergens with reduced allergenicity but retaining immunogenicity of the natural allergens: hybrids of yellow jacket and paper wasp venom allergen antigen 5s. J Immunol 2001; 166:6057–6065.
63. Linhart B, B Jahn-Schmid, Verdino P, et al. Combination vaccines for the treatment of grass pollen allergy consisting of genetically engineered hybrid molecules with increased immunogenicity. FASEB J 2002; 16:1301–1303.
64. Kussebi F, Karamloo F, Rhyner C, et al. A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy. J Allergy Clin Immunol 2005; 115:323–329.
65. R Gonzalez-Rioja, Ibarrola I, Arilla MC, et al. Genetically engineered hybrid proteins from Parietaria judaica pollen for allergen-specific immunotherapy. J Allergy Clin Immunol 2007; 120:602–609.
66. Westritschnig K, Linhart B, M Focke-Tejkl, et al. A Hypoallergenic Vaccine Obtained by Tail-to-Head Restructuring of Timothy Grass Pollen Profilin, Phl p 12, for the Treatment of Cross-Sensitization to Profilin. J Immunol 2007; 179:7624–7634.
67. Crameri A, Raillard SA, Bermudez E, et al. DNA shuffling of a family of genes from diverse species accelerates directed evolution. Nature 1998; 391:288–291.
68. Punnonen J. Molecular breeding of allergy vaccines and antiallergic cytokines. Int Arch Allergy Immunol 2000; 121:173–182.
69. Wallner M, Stocklinger A, Thalhamer T, et al. Allergy multivaccines created by DNA shuffling of tree pollen allergens. J Allergy Clin Immunol 2007; 120:374–380.
70. Gafvelin G, Parmley S, T Neimert-Andersson, et al. Hypoallergens for allergen-specific immunotherapy by directed molecular evolution of mite group 2 allergens. J Biol Chem 2007; 282:3778–3787.
71. Hartl A, Kiesslich J, Weiss R, et al. Immune responses after immunization with plasmid DNA encoding Bet v 1, the major allergen of birch pollen. J Allergy Clin Immunol 1999; 103:107–113.
72. Hsu CH, Chua KY, Tao MH, et al. Inhibition of specific IgE response in vivo by allergen-gene transfer. Int Immunol 1996; 8:1405–1411.
73. Raz E, Tighe H, Sato Y, et al. Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. Proc Natl Acad Sci U S A 1996; 93:5141–5145.

74. Weiss R, Hammerl P, Hartl A, et al. Design of protective and therapeutic DNA vaccines for the treatment of allergic diseases. Curr Drug Targets Inflamm Allergy 2005; 4:585–597.
75. Hochreiter R, Ferreira F, Thalhamer J, et al. TH1-promoting DNA immunization against allergens modulates the ratio of IgG1/IgG2a but does not affect the anaphylactic potential of IgG1 antibodies: no evidence for the synthesis of nonanaphylactic IgG1. J Allergy Clin Immunol 2003; 112:579–584.
76. Ferreira F, Wallner M, Thalhamer J. Customized antigens for desensitizing allergic patients. Adv Immunol 2004; 84:79–129.
77. Bauer R, Scheiblhofer S, Kern K, et al. Generation of hypoallergenic DNA vaccines by forced ubiquitination: preventive and therapeutic effects in a mouse model of allergy. J Allergy Clin Immunol 2006; 118:269–276.
78. Gabler M, Scheiblhofer S, Kern K, et al. Immunization with a low-dose replicon DNA vaccine encoding Phl p 5 effectively prevents allergic sensitization. J Allergy Clin Immunol 2006; 118:734–741.
79. Vrtala S, Hirtenlehner K, Vangelista L, et al. Conversion of the major birch pollen allergen, Bet v 1, into two nonanaphylactic T cell epitope-containing fragments: candidates for a novel form of specific immunotherapy. J Clin Invest 1997; 99:1673–1681.
80. M van Hage-Hamsten, Kronqvist M, Zetterstrom O, et al. Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1: results obtained with a mix of two recombinant Bet v 1 fragments and recombinant Bet v 1 trimer in a Swedish population before the birch pollen season. J Allergy Clin Immunol 1999; 104:969–977.
81. Vrtala S, Akdis CA, Budak F, et al. T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. J Immunol 2000; 165:6653–6659.
82. Niederberger V, Horak F, Vrtala S, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. Proc Natl Acad Sci U S A 2004; 101(suppl 2):14677–14682.
83. Vrtala S, Focke M, Kopec J, et al. Genetic engineering of the major timothy grass pollen allergen, Phl p 6, to reduce allergenic activity and preserve immunogenicity. J Immunol 2007; 179:1730–1739.
84. Takai T, Mori A, Yuuki T, et al. Non-anaphylactic combination of partially deleted fragments of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy. Mol Immunol 1999; 36:1055–1065.
85. Twardosz A, Hayek B, Seiberler S, et al. Molecular characterization, expression in Escherichia coli, and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. Biochem Biophys Res Commun 1997; 239:197–204.
86. Hayek B, Vangelista L, Pastore A, et al. Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, Aln g 4: structural basis for calcium-modulated IgE recognition. J Immunol 1998; 161:7031–7039.
87. Lamb JR, Skidmore BJ, Green N, et al. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. J Exp Med 1983; 157:1434–1447.
88. Essery G, Feldmann M, Lamb JR. Interleukin-2 can prevent and reverse antigen-induced unresponsiveness in cloned human T lymphocytes. Immunology 1988; 64:413–417.
89. RE O'Hehir, Yssel H, Verma S, et al. Clonal analysis of differential lymphokine production in peptide and superantigen induced T cell anergy. Int Immunol 1991; 3:819–826.
90. Custovic A, Green R, Taggart SC, et al. Domestic allergens in public places. II: Dog (Can f1) and cockroach (Bla g 2) allergens in dust and mite, cat, dog and cockroach allergens in the air in public buildings. Clin Exp Allergy 1996; 26:1246–1252.
91. Counsell CM, Bond JF, Ohman JL Jr., et al. Definition of the human T-cell epitopes of Fel d 1, the major allergen of the domestic cat. J Allergy Clin Immunol 1996; 98:884–894.
92. Norman PS, Ohman JL Jr., Long AA, et al. Treatment of cat allergy with T-cell reactive peptides. Am J Respir Crit Care Med 1996; 154:1623–1628.
93. Marcotte GV, Braun CM, Norman PS, et al. Effects of peptide therapy on ex vivo T-cell responses. J Allergy Clin Immunol 1998; 101:506–513.
94. Pene J, Desroches A, Paradis L, et al. Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. J Allergy Clin Immunol 1998; 102:571–578.
95. Simons FE, Imada M, Li Y, et al. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. Int Immunol 1996; 8:1937–1945.
96. Maguire P, Nicodemus C, Robinson D, et al. The safety and efficacy of ALLERVAX CAT in cat allergic patients. Clin Immunol 1999; 93:222–231.
97. Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. J Exp Med 1999; 189:1885–1894.
98. Oldfield WL, Kay AB, Larche M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. J Immunol 2001; 167:1734–1739.
99. Alexander C, Oldfield WLG, Shirley KE, et al. A dosing protocol of allergen-derived T cell peptide epitopes for the treatment of allergic disease. J Allergy Clin Immunol 2001; 107:S217–S217.

100. Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. Lancet 2002; 360:47–53.
101. Hedley ML. Gene therapy of chronic inflammatory disease. Adv Drug Deliv Rev 2000; 44:195–207.
102. Broder CC, Earl PL. Recombinant vaccinia viruses. Design, generation, and isolation. Mol Biotechnol 1999; 13:223–245.
103. Bumann D, Hueck C, Aebischer T, et al. Recombinant live Salmonella spp. for human vaccination against heterologous pathogens. FEMS Immunol Med Microbiol 2000; 27:357–364.
104. FW van Ginkel, Nguyen HH, JR McGhee. Vaccines for mucosal immunity to combat emerging infectious diseases. Emerg Infect Dis 2000; 6:123–132.
105. Yew NS, Zhao H, Wu IH, et al. Reduced inflammatory response to plasmid DNA vectors by elimination and inhibition of immunostimulatory CpG motifs. Mol Ther 2000; 1:255–262.
106. Husson RN, James BE, Young RA. Gene replacement and expression of foreign DNA in mycobacteria. J Bacteriol 1990; 172:519–524.
107. Stover CK, VF de la Cruz, Fuerst TR, et al. New use of BCG for recombinant vaccines. Nature 1991; 351:456–460.
108. Wang CC, Rook GA. Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed Mycobacterium vaccae. Immunology 1998; 93:307–313.
109. Kumar M, Behera AK, Matsuse H, et al. A recombinant BCG vaccine generates a Th1-like response and inhibits IgE synthesis in BALB/c mice. Immunology 1999; 97:515–521.
110. Shirakawa T, Enomoto T, Shimazu S, et al. The inverse association between tuberculin responses and atopic disorder. Science 1997; 275:77–79.
111. Erb KJ, Holloway JW, Sobeck A, et al. Infection of mice with Mycobacterium bovis-Bacillus Calmette-Guerin (BCG) suppresses allergen-induced airway eosinophilia. J Exp Med 1998; 187: 561–569.
112. Lagranderie M, Murray A, Gicquel B, et al. Oral immunization with recombinant BCG induces cellular and humoral immune responses against the foreign antigen. Vaccine 1993; 11:1283–1290.
113. Cohon A, Arruda LK, Martins MA, et al. Evaluation of BCG administration as an adjuvant to specific immunotherapy in asthmatic children with mite allergy. J Allergy Clin Immunol 2007; 120:210–213.
114. Hopfenspirger MT, Parr SK, Hopp RJ, et al. Mycobacterial antigens attenuate late phase response, airway hyperresponsiveness, and bronchoalveolar lavage eosinophilia in a mouse model of bronchial asthma. Int Immunopharmacol 2001; 1:1743–1751.
115. Ozdemir C, Akkoc T, Bahceciler NN, et al. Impact of Mycobacterium vaccae immunization on lung histopathology in a murine model of chronic asthma. Clin Exp Allergy 2003; 33:266–270.
116. C Zuany-Amorim, Sawicka E, Manlius C, et al. Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells. Nat Med 2002; 8:625–629.
117. Camporota L, Corkhill A, Long H, et al. The effects of Mycobacterium vaccae on allergen-induced airway responses in atopic asthma. Eur Respir J 2003; 21:287–293.
118. Yeung VP, Gieni RS, Umetsu DT, et al. Heat-killed Listeria monocytogenes as an adjuvant converts established murine Th2-dominated immune responses into Th1-dominated responses. J Immunol 1998; 161:4146–4152.
119. Frick OL, Teuber SS, Buchanan BB, et al. Allergen immunotherapy with heat-killed Listeria monocytogenes alleviates peanut and food-induced anaphylaxis in dogs. Allergy 2005; 60:243–250.
120. Takahashi N, Kitazawa H, Iwabuchi N, et al. Immunostimulatory oligodeoxynucleotide from Bifidobacterium longum suppresses Th2 immune responses in a murine model. Clin Exp Immunol 2006; 145:130–138.
121. Puggioni F, Durham SR, Francis JN. Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favour of Th1 responses. Allergy 2005; 60:678–684.
122. Liu H, M Komai-Koma, Xu D, et al. Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells. Proc Natl Acad Sci U S A 2006; 103:7048–7053.
123. Sutmuller RP, MH den Brok, Kramer M, et al. Toll-like receptor 2 controls expansion and function of regulatory T cells. J Clin Invest 2006; 116:485–494.
124. A Zanin-Zhorov, Cahalon L, Tal G, et al. Heat shock protein 60 enhances CD4+ CD25+ regulatory T cell function via innate TLR2 signaling. J Clin Invest 2006; 116:2022–2032.
125. Johansen P, Senti G, JM Martinez Gomez, et al. Toll-like receptor ligands as adjuvants in allergen-specific immunotherapy. Clin Exp Allergy 2005; 35:1591–1598.
126. V von Baehr, Hermes A, R von Baehr, et al. Allergoid-specific T-cell reaction as a measure of the immunological response to specific immunotherapy (SIT) with a Th1-adjuvanted allergy vaccine. J Investig Allergol Clin Immunol 2005; 15:234–241.
127. Drachenberg KJ, Heinzkill M, Urban E, et al. Efficacy and tolerability of short-term specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A (MPL) for children and adolescents. Allergol Immunopathol (Madr) 2003; 31:270–277.

128. PL McCormack, Wagstaff AJ. Ultra-short-course seasonal allergy vaccine (Pollinex Quattro). Drugs 2006; 66:931–938.
129. Patel P, Salapatek AM. Pollinex Quattro: a novel and well-tolerated, ultra short-course allergy vaccine. Expert Rev Vaccines 2006; 5:617–629.
130. Mothes N, Heinzkill M, Drachenberg KJ, et al. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. Clin Exp Allergy 2003; 33:1198–1208.
131. Tomai M, Miller R, Lipson K, et al. Immune response modifiers: Imiquimod and future drugs for modulating the immune response. Drug Discovery Today 2006; 3:343–352.
132. Martinez Gomez JM, Fischer S, Csaba N, et al. A protective allergy vaccine based on CpG- and protamine-containing PLGA microparticles. Pharm Res 2007; 24:1927–1935.
133. Jilek S, Walter E, Merkle HP, et al. Modulation of allergic responses in mice by using biodegradable poly(lactide-co-glycolide) microspheres. J Allergy Clin Immunol 2004; 114:943–950.
134. Zhu D, Kepley CL, Zhang K, et al. A chimeric human-cat fusion protein blocks cat-induced allergy. Nat Med 2005; 11:446–449.
135. Creticos PS, Balcer SL, Schroeder JT, et al. Initial immunotherapy trial to explore the safety, tolerability and immunogenicity of subcutaneous injections of an Amb a 1 immunostimulatory oligonucleotide conjugate [Aic] in ragweed allergic adults. J Allergy Clin Immunol 2001; 107:S216–S216.
136. Creticos PS, Eiden JJ, Balcer SL, et al. Immunostimulatory oligonucleotides conjugated to Amb a 1: Safety, skin test reactivity, and basophil histamine release. J Allergy Clin Immunol 2000; 105:S70–S70.
137. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006; 355:1445–1455.
138. Kanzler H, Barrat FJ, Hessel EM, et al. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. Nat Med 2007; 13:552–559.
139. Mo JH, Park SW, Rhee CS, et al. Suppression of allergic response by CpG motif oligodeoxynucleotide-house-dust mite conjugate in animal model of allergic rhinitis. Am J Rhinol 2006; 20:212–218.
140. Hulse KE, Reefer AJ, Engelhard VH, et al. Targeting Fel d 1 to FcgammaRI induces a novel variation of the T(H)2 response in subjects with cat allergy. J Allergy Clin Immunol 2008; 121:756–762.
141. Bliss J, V Van Cleave, Murray K, et al. IL-12, as an adjuvant, promotes a T helper 1 cell, but does not suppress a T helper 2 cell recall response. J Immunol 1996; 156:887–894.
142. Diwan M, Elamanchili P, Cao M, et al. Dose sparing of CpG oligodeoxynucleotide vaccine adjuvants by nanoparticle delivery. Curr Drug Deliv 2004; 1:405–412.
143. Batanero E, Barral P, Villalba M, et al. Encapsulation of Ole e 1 in biodegradable microparticles induces Th1 response in mice: a potential vaccine for allergy. J Control Release 2003; 92:395–398.
144. Kumar M, Behera AK, Lockey RF, et al. Intranasal gene transfer by chitosan-DNA nanospheres protects BALB/c mice against acute respiratory syncytial virus infection. Hum Gene Ther 2002; 13:1415–1425.
145. Kumar M, Kong X, Behera AK, et al. Chitosan IFN-gamma-pDNA Nanoparticle (CIN) Therapy for Allergic Asthma. Genet Vaccines Ther 2003; 1:3.
146. Mohapatra SS. Mucosal gene expression vaccine: a novel vaccine strategy for respiratory syncytial virus. Pediatr Infect Dis J 2003; 22:S100–S103; discussion S103-4.
147. Hall G, Lund L, Lamb JR, et al. Kinetics and mode of peptide delivery via the respiratory mucosa determine the outcome of activation versus TH2 immunity in allergic inflammation of the airways. J Allergy Clin Immunol 2002; 110:883–890.
148. Usui M, Tamura H, Nakamura K, et al. Enhanced bactericidal action and masking of allergen structure of soy protein by attachment of chitosan through maillard-type protein-polysaccharide conjugation. Nahrung 2004; 48:69–72.
149. Chew JL, Wolfowicz CB, Mao HQ, et al. Chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen, Der p 1 for oral vaccination in mice. Vaccine 2003; 21:2720–2729.
150. Matsuse H, Kong X, Hu J, et al. Intranasal IL-12 produces discreet pulmonary and systemic effects on allergic inflammation and airway reactivity. Int Immunopharmacol 2003; 3:457–468.

26 Anti-IgE Therapy

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INTRODUCTION

Despite the advanced and increasing understanding of the pathophysiology of asthma and other allergic diseases, current treatment remains nonspecific and targets late events within the allergic cascade [e.g., antihistamines, β2 agonists, topical and systemic corticosteroids (SCS), and chromones] (1). Although the majority of asthmatic patients are sufficiently treated with standard medication, a subpopulation of asthmatics remains who still suffer from the disease despite optimal drug treatment according to guidelines or encounter severe side effects from medication with high-dose inhaled and/or oral steroids. New treatment options are therefore required that target the pathophysiological cascade of allergen-mediated airway disease earlier and in a more *specific* way (2,3). Because of the pivotal role of IgE for the development and severity of allergic diseases, antagonizing or inhibiting IgE by anti-IgE antibodies provides a novel and promising approach to treat allergic asthma (4,5).

THE RATIONALE FOR TREATING ALLERGIC ASTHMA WITH ANTI-IgE

Role of IgE in Allergic Asthma

Binding of IgE to high-affinity (FcεRI) receptors on effector cells, such as mast cells and basophils, and subsequent exposure to allergen initiates a cascade resulting in release of proinflammatory mediators that contribute to the acute and chronic symptoms of allergic airway diseases (6).

The IgE molecule is comprised of fragments known as Fab (antigen-binding fragment) and Fc (crystallizable fragment) (Fig. 1). The Fab region binds to specific components (or epitopes) of the allergen, whereas the Fc region binds to FcεRI receptors present on mast cells and circulating basophils. FcεRI receptors consist of four polypeptide chains, $\alpha\beta\gamma_2$. The α chain binds to five amino acids (330–335) of the cε3 domain of the Fc segment of IgE to orientate the IgE molecule so that it lies on its side with the allergen-binding site facing outward (Fig. 2) (6).

Binding of allergen to two or more membrane-bound IgE molecules results in receptor clustering, and through interactions involving the α and β chains, sets into motion intracellular biochemical events that trigger mast-cell or basophil activation (6). This activation releases a variety of preformed and newly generated proinflammatory mediators and cytokines, including histamine, interleukins, leukotrienes, and prostaglandins (type-I hypersensitivity reaction), and the release of IL-4, IL-13 (increasing IgE synthesis), and IL-5 (increasing eosinophil accumulation) that contribute to the chronic inflammatory response (Fig. 3). Eosinophilia is a well-recognized feature of inflammation in asthma and reflects asthma severity and the risk of exacerbations (7,8).

IgE upregulates the expression of FcεRI on mast cells and basophils (9). This results in mast-cell stimulation and mediator release at lower concentrations of allergen, and/or in the release of increased amounts of mediators and cytokines for a given level of stimulus (10). IgE also binds to FcεRI receptors on dendritic cells and enhances allergen uptake and presentation to T cells (11), leading to increased T-cell proliferation and eosinophil differentiation and activation.

The risk of developing asthma increases with increasing levels of serum IgE (12). However, asthma severity is poorly associated with total IgE levels (13). The association between specific IgE and asthma severity is currently under investigation. FcεRI receptors are upregulated on eosinophils, mast cells, macrophages, and dendritic cells in patients with rhinitis (14) and allergic asthma (15). In addition, a significant correlation has been observed between serum IgE levels and FcεRI expression on precursor dendritic cells (pDCs) from

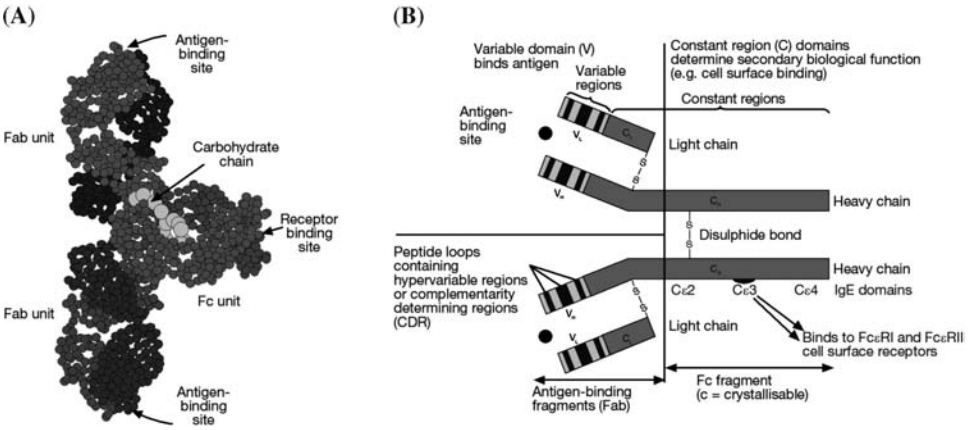

Figure 1 Molecular structure and schematic diagram of IgE.

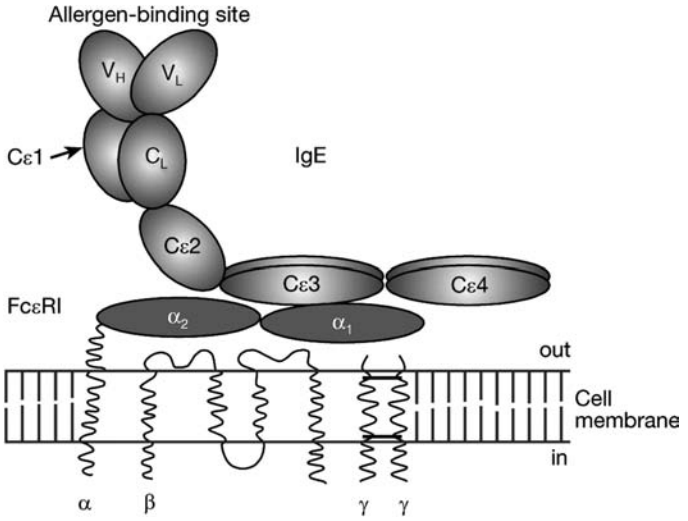

Figure 2 Binding of IgE to the high-affinity (FcεRI) receptor.

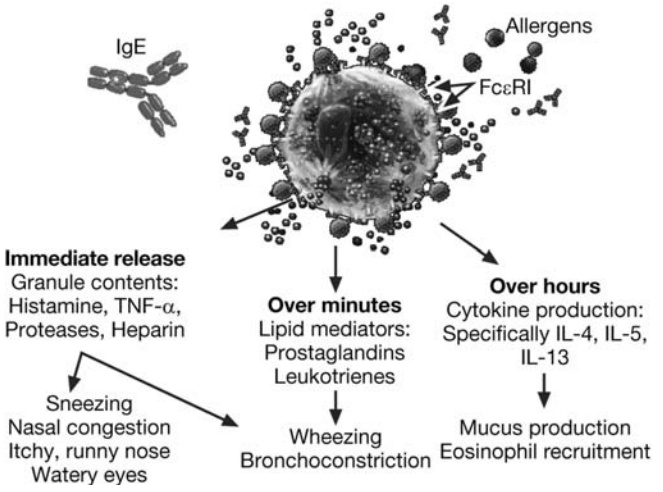

Figure 3 IgE-dependent release of inflammatory mediators.

subjects with allergic asthma (16). A possible relationship between FcεRI expression and fatal asthma has also been reported (17). This latter study examined postmortem human lung tissue and found that cases of fatal asthma were associated with higher levels of FcεRI+ cells within the lamina propria compared with subjects who died of other causes or with biopsy tissue from patients with mild asthma.

Targeting of IgE

Omalizumab (Xolair®) is a recombinant, humanized monoclonal antibody directly targeting IgE. Omalizumab reduces free IgE by approximately 95% by binding to the cε3 region on free IgE (Fig. 2), thereby blocking the binding of IgE to its specific high-affinity receptor. Importantly, anti-IgE treatment reduces the expression of FcεRI receptors on basophils as shown in subjects with perennial allergic rhinitis (PAR) and with positive skin test to dust mite (18,19). Basophil receptor density measurements were taken prior to treatment and three months after treatment initiation. With omalizumab treatment, basophil FcεRI density decreased by approximately 97% ($p = 0.0022$) (18). Reflecting the reduction in receptor density, the responsiveness of the basophils to challenge with dust mite antigen (*Dermatophagoides farinae*) decreased by approximately 90% ($p = 0.0022$) (18). During the period following discontinuation of omalizumab treatment, basophil FcεRI receptors were upregulated, concurrent with a gradual rise of serum-free IgE levels (to 16% of pretreatment levels over an 8-week period) (19). However, a more rapid rise in antigen-triggered histamine release responses by the cells implies a reduction of the threshold for basophil activation. Thus, the effects of omalizumab treatment on mast cells and basophils are achieved through a combination of (*i*) reduced free IgE levels and (*ii*) IgE receptor downregulation leading to the inhibition of IgE-mediated proinflammatory mediator release. This dual effect of omalizumab is important, since otherwise complete removal of free IgE would be necessary to elicit functional consequences on mast cells and basophils (18,19).

Omalizumab significantly reduces submucosal IgE+ and FcεRI+ cells in patients with allergic asthma. The effect of omalizumab on the number of cells expressing IgE or FcεRI was assessed in a placebo-controlled study in 45 patients with corticosteroid-naïve, mild-to-moderate asthma (20). Treatment with omalizumab for 16 weeks significantly reduced IgE+ cells in the bronchial submucosa compared with baseline or placebo (both $p < 0.001$). Similarly, treatment with omalizumab for 16 weeks significantly reduced FcεRI+ cells in the bronchial submucosa compared with baseline or placebo (both $p < 0.001$). As mentioned previously, a close correlation has also been observed between serum IgE levels and FcεRI expression on pDCs from subjects with allergic asthma (16). The effect of omalizumab on FcεRI receptor expression on pDC subtypes pDC1 and pDC2 (these subtypes promote the T cell to shift toward the Th1 and Th2 phenotype, respectively) has been assessed in a randomized, double-blind, placebo-controlled study in patients with seasonal allergic rhinitis (SAR) (21). Omalizumab significantly reduced FcεRI expression ($p \leq 0.002$) from day 7 onward for both pDC1 and pDC2 cells, indicating that omalizumab causes a rapid decrease in FcεRIα surface expression by DCs. By downregulating FcεRI expression on DCs, omalizumab may inhibit antigen processing and presentation to T cells.

The depletion of IgE in airway tissue is associated with a marked reduction in airway eosinophils (20). Omalizumab significantly reduces eosinophil numbers in airway tissue and induced sputum in patients with mild or moderate allergic asthma (20). Omalizumab treatment reduced the percentage of inflammatory cells in the sputum that were identified as eosinophils (4.8% at baseline, 0.6% posttreatment, $p < 0.001$; $p = 0.05$ vs. placebo). The reduction in sputum eosinophil counts with omalizumab treatment was mirrored by a significant decrease in the numbers of eosinophils in both the epithelial and submucosal compartments.

The effects of omalizumab at a cellular level are reflected in clinical observations on patients with asthma. Omalizumab inhibited the early-phase and late-phase asthmatic responses to inhaled allergens as demonstrated in bronchial challenge tests (22,23). Assessed as the mean maximal fall in forced expiratory volume in 1 second (FEV_1), omalizumab reduced the early asthmatic response by 85% ($p = 0.01$) and the late asthmatic response by 65% ($p = 0.047$) compared with placebo in 18 patients with mild allergic asthma (22).

As omalizumab binds to the cε3 site (third domain of the constant region) on free IgE, the same site that binds to the FcεRI receptor (Fig. 2), omalizumab cannot interact with IgE molecules bound to FcεRI receptors on basophils and mast cells and does not activate effector

cells by receptor cross-linking (23). Omalizumab is therefore expected not to cause anaphylaxis in clinical use.

ANTI-IgE FOR TREATMENT OF ALLERGIC DISEASES

Drug Profile

The main *characteristics* of anti-IgE are therefore (24) that it

1. inhibits the binding of IgE to the high-affinity receptor, FcεRI;
2. recognizes and binds free but not complexed IgE, and not IgM, IgG, and IgA;
3. forms complexes with free IgE in serum;
4. is not specific regarding the allergen specificity of the target IgE antibody, meaning that it binds to any IgE molecule;
5. does not bind to IgE already bound to mast cells or basophils, meaning that it does not cause degranulation ("non-anaphylactic antibody");
6. blocks mast cell or basophil degranulation following passive sensitization in vitro or challenge with allergen in vivo;
7. decreases FcεRI expression on immune effector cells.

Clinical Pharmacology

The binding of anti-IgE to IgE results in the formation of small (~1000 kDa), nonprecipitating and non-complement-activating immune complexes (25) that are no longer able to bind IgE receptors. Anti-IgE concentrations were highest in the serum compartment and no specific organ deposition was observed. The immune complexes were eliminated by urinary excretion.

The decrease of IgE serum levels follows a dose-dependent response within five minutes after intravenous administration and within 24 hours after subcutaneous injections as seen in studies with single- and multidose studies in adults with and without allergic disease (26,27). The decrease of IgE lasted, depending on the antibody dose, for about four to six weeks after a single injection with anti-IgE (28). Further, anti-IgE was well tolerated in all of these trials.

The most effective route of administration was studied in a large phase II study in adults with SAR (29). Treatment via SC versus IV route at different dosages (0.15-mg/kg body weight SC, 0.15 mg/kg IV, or 0.5 mg/kg IV, 7 injections within 84 days) was compared. Pharmacodynamics between the SC and IV routes of administration did not differ. Anti-IgE decreased serum IgE levels in a dose- and baseline IgE–dependent fashion. However, not all routes of administration were similarly effective. Fahy et al. (30) studied the effects after aerosolized application of anti-IgE in patients with mild allergic asthma and did not find significant suppression of serum IgE levels and nor effects on the early asthmatic response to allergen, despite detectable levels of anti-IgE.

Since the reduction of free IgE below 25 IU/mL is a prerequisite for efficacy and for practical and safety reasons, 375 mg of omalizumab every two weeks is considered to be the maximum applicable SC dose at present. Higher dosages of omalizumab have not been studied so there is no advice concerning safety and efficacy. Therefore, a treatment of patients with total IgE levels above 400 to 1300 IU/mL, depending on the body weight, is not recommended.

Safety and Tolerability

The safety and tolerability profile of omalizumab was evaluated in an analysis of data from completed phase I, II, and III studies involving more than 7500 adult and adolescent patients with asthma, rhinitis, or related conditions (31). More than 5300 of these patients received omalizumab, including 3700 with moderate-to-severe persistent asthma. In all controlled studies, adverse events (AEs) were reported in 74.8% of patients receiving omalizumab and in 75.2% of control group patients. Serious AEs were reported by 4.2% of patients receiving omalizumab and by 3.8% of control group patients. AEs were generally of mild-to-moderate severity and of short duration. Severe AEs occurred more frequently in the control group than in the omalizumab group (omalizumab 10.8%, control 12.6%). In placebo-controlled-allergic asthma studies, the overall incidence of suspected drug-related AEs was similar in the omalizumab and placebo groups (omalizumab 9.2%, placebo 7.6%). Postlaunch data in the

United States with approximately 40,000 patients are consistent with safety results obtained from omalizumab clinical trials. The most significant, serious AE is anaphylaxis or anaphylaxis-like reactions, which occur in 0.1% to 0.2% of treated subjects. Despite the infrequent occurrence of this event, the FDA recommended in 2007 a boxed warning on the label devoted to anaphylaxis. A numerical increase in malignancy in omalizumab-treated subjects is being investigated in an ongoing, long-term trial.

Anti-IgE for Treatment of Asthma

Treatment of asthma has substantially improved over the last decades. Inhaled corticosteroids (ICS) are the standard anti-inflammatory medication and normally effectively control symptoms of mild-to-moderate asthma. Some patients, however, remain symptomatic even with persistent ICS treatment, or even require systemic steroids to control the disease. In this patient group, the efficacy and safety profile of omalizumab has been extensively investigated in a series of clinical studies. Of particular interest is the INvestigatioN of Omalizumab in seVere Asthma TrEatment (INNOVATE) study (32) since it assessed, in a 28-week randomized, placebo-controlled trial, the efficacy of omalizumab in patients with inadequately controlled, severe persistent allergic asthma despite high-dose ICS plus long-acting β2 agonist (LABA). Efficacy was further analyzed in pooled data (33) from seven clinical trials with omalizumab carried out in patients with moderate-to-severe or severe asthma (32,34–41). Five of these seven studies were randomized, double-blind, placebo-controlled trials in which patients received omalizumab or placebo. Two studies were randomized and controlled but open label.

The INNOVATE Study

Patients included in the INNOVATE study (32) represented the asthma population with the most severe disease and the highest unmet medical need. All patients were receiving high-dose ICS [>1000 μg/day beclomethasone diproprionate (BDP) equivalent] plus a LABA with requirements for additional controller medication in approximately 60%, including maintenance, oral corticosteroids in 22% of patients. Sixty-seven percent of the patients were considered to be at high risk of asthma-related death; quality of life (QoL) was profoundly restricted by an average of 31 missed school/work days due to asthma in the year prior to the treatment protocol. The primary endpoint was the incidence and frequency of exacerbations with one secondary outcome parameter of improvement in QoL.

Adding omalizumab to high-dose ICS plus a LABA significantly reduced the rate of clinically significant exacerbations, defined as asthma worsening requiring treatment with SCS, by 26% versus add-on placebo (adjusted post hoc for an observed imbalance in exacerbation history (0.68 vs. 0.91, $p = 0.042$)) (Fig. 4) (unadjusted result: 19% reduction (0.74 vs. 0.92, $p = 0.153$)). Even more importantly, omalizumab significantly reduced the severe exacerbation rate by 50% versus placebo (0.24 vs. 0.48, $p = 0.002$) (Fig. 4), translating into

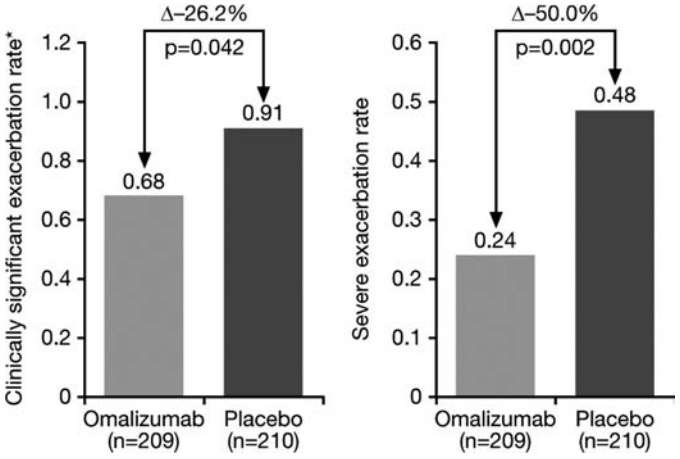

Figure 4 Effect of omalizumab on clinically significant and severe exacerbations. (*) Indicates adjustment due to a pre-study imbalance in exacerbation rate; –19.4% ($p = 0.153$) reduction unadjusted. *Source*: Adapted from Ref. 32.

reduced emergency visit rate by 44% versus placebo (0.24 vs. 0.43, $p = 0.038$). The number needed to treat (NNT) with omalizumab in addition to Global Initiative for Asthma (GINA) step 4 therapy to prevent one event above placebo per year was 2.2 for clinically significant exacerbations, 2.2 for severe exacerbations, and 2.8 for total emergency visits (32).

Clinically meaningful, add-on omalizumab treatment also provided significant QoL improvements for patients with changes in Asthma Quality of Life Questionnaire (AQLQ) baseline scores of 0.91 in the omalizumab group compared with 0.46 in the placebo group ($p < 0.001$). Improvements were achieved in significantly more patients taking omalizumab compared with placebo (60.8% vs. 47.8%, $p = 0.008$), measured across the domains of activity limitations, asthma symptoms, emotional function, and environmental exposure.

Pooled Analysis

Efficacy of treatment with omalizumab was further assessed in an analysis of pooled data from seven clinical trials (including INNOVATE) (33) comprising a total of 4308 patients, with 2511 patients receiving omalizumab and 93% of patients with severe persistent asthma (GINA 4). Five of these studies were multicenter, randomized, double-blind, parallel-group, placebo-controlled studies and included large patient groups with moderate-to-severe asthma and requirements for daily ICS therapy. The protocol for these five studies was similar, consisting of four phases with (*i*) a run-in period of 4 weeks to stabilize disease while on therapy with BDP, (*ii*) a stable phase with no change in corticosteroid therapy for 16 weeks, (*iii*) a corticosteroid-withdrawal phase with tapering of corticosteroid dosages by 25% every 2 weeks for 8 weeks, and (*iv*) an extension period with stable, lowest tolerated dose of BDP and continuation of placebo or anti-IgE therapy for 5 months beyond the first 28 weeks. Omalizumab was administered SC at a dose of 0.016 mg/kg body weight/IU total serum IgE, once every four weeks or every two weeks, depending on dose, starting after the run-in period.

Adding omalizumab to current asthma therapy in the larger pooled population from all seven studies consistently supported the findings seen in the INNOVATE study: add-on omalizumab significantly reduced asthma exacerbation rates by 38.3% versus placebo (0.91 vs. 1.47, $p < 0.0001$) (Table 1), and total emergency visits were significantly less frequent (by 47% vs. control, 0.332 vs. 0.623, $p < 0.0001$). Accordingly, hospital admission rates were reduced by 51% ($p = 0.041$), emergency room visits by 60% ($p = 0.013$), and unscheduled doctor visits by 43% ($p = 0.0003$).

QoL was measured in six clinical trials, with 1221 patients receiving omalizumab and 1032 receiving placebo or control medication across the six studies (42). Omalizumab consistently improved QoL, resulting in significantly greater improvements in AQLQ overall score compared with placebo/control (AQLQ overall score: 1.01 vs. 0.61, $p < 0.001$). The proportion of patients recording a clinically meaningful improvement in AQLQ overall score of $\geq$0.5 point was significantly higher in the omalizumab group compared with placebo/control (66.3% omalizumab vs. 52.4% placebo/control, $p < 0.001$).

Table 1 Effect of Omalizumab Vs. Placebo on Asthma Exacerbation Rates

Study	Difference in annual exacerbation rate	Reduction vs. control/placebo (%)	Significance (*p* value)
INNOVATE study (32)	0.49	26.6	0.042[a]
ETOPA study (34)	1.49	60.4	<0.001
SOLAR study (38)	0.29	37.5	0.027
Busse study (37)	0.40	40.3	<0.001
Solèr study (34)	0.70	57.6	<0.001
Holgate study (39)	0.42	26.5	0.165
ALTO study (41)	0.18	15.3	0.077
Pooled (33)	0.56	38.3	<0.0001

Pooled analysis.

[a]Adjusted for an observed relevant imbalance in pre-study history of clinically significant asthma exacerbations between the omalizumab (0.68/yr) versus placebo (0.91/yr) group.

Source: From Ref. 30.

Anti-IgE for Treatment of Allergic Rhinitis

Beside studies on asthmatic patients, safety and efficacy of treatment with anti-IgE was evaluated in clinical trials on patients with seasonal and PAR.

Anti-IgE for Treatment of SAR

The first large-scale (240 adults), double-blind, placebo-controlled, multicenter phase II trial was performed to assess optimal dosing regimens and test the efficacy of anti-IgE for the treatment of ragweed-induced SAR (29). Omalizumab decreased serum IgE levels in a dose- and baseline IgE–dependent fashion, and specific IgE levels correlated significantly with symptom scores. Only patients with IgE levels below detection level (<25 IU/mL) on treatment with omalizumab experienced a marked reduction of symptoms. However, the average symptom score before and during the peak of the season was small (before season 0.6, during season 0.91 in placebo, and 0.7–0.8 in anti-IgE groups, n.s.), making the study less capable of proving a difference with treatment even if one existed. The use of rescue medication was similar between the groups, suggesting that omalizumab had relatively little effect. Furthermore, neither overall specific skin test reactivity was altered over the course of the study nor did the scores of QoL estimations differ. The one exception of a change in specific IgE was the high-dose anti-IgE group, which showed a marginally significant increase in average, endpoint titration allergen concentration.

The results of the previous study (29) warranted further evaluations of the efficacy of anti-IgE therapy in SAR, utilizing higher anti-IgE doses in a second randomized, double-blind, placebo-controlled, multicenter phase III study involving 251 adults (17–66 years), with moderate-to-severe SAR to birch pollen (history $\geq$2 years, positive specific skin test, baseline serum IgE 30–700 IU/mL) (43). Twenty-one percent of actively treated patients reported complete control of symptoms (vs. 2% of placebo treated), 59% reported some improvement (vs. 35%), and only 2% experienced worsening (vs. 13%) (43). Average daily nasal symptom severity scores did not change during the course of the study in the anti-IgE group (0.71 at baseline vs. 0.70 total average during study), but increased in the placebo group during the pollen season (0.78 vs. 0.98, $p < 0.001$). Similarly, ocular symptom severity scores decreased in anti-IgE-treated patients (0.47–0.43), but increased in the placebo group (0.43–0.54), resulting in a significant difference between the groups ($p = 0.031$). Importantly, the average use of rescue medication was significantly lower in the anti-IgE group compared with the placebo group (0.59 vs. 1.37 tablets/day, $p < 0.001$), and the proportion of days on which no SAR medication was required was almost twice as high (49% vs. 28%, $p < 0.001$). Statistically significant differences in favor of anti-IgE were similarly observed for estimation of QoL. The rather limited effect of anti-IgE treatment may be explained by an unexpected early pollen season during that specific year of the trial in Scandinavia, which resulted in the beginning of anti-IgE treatment less than one week in advance of the first pollen exposure in more than 50% of all patients. Conclusively, this study confirmed that anti-IgE was safe and effective in controlling birch pollen–induced SAR compared with placebo, reducing use of rescue medication and improving QoL.

Anti-IgE for Treatment of PAR

An additional study investigated the efficacy of anti-IgE on mean daily nasal severity scores in 289 adults and adolescents with moderate-to-severe symptomatic PAR in a 16-week, double-blind subcutaneous treatment [at least 0.016 mg/kg/IgE (IU/mL) per 4 weeks] (44). The mean daily nasal severity score was significantly lower in anti-IgE-treated patients than with placebo ($p < 0.001$) and was paralleled by a reduction in use of rescue antihistamine ($p \leq 0.005$ overall) and improved QoL of patients.

Anti-IgE for Treatment of SAR in Combination with SCIT

Subcutaneous allergen immunotherapy (SCIT) is the only curative treatment for SAR and allergic asthma, and beneficial effects can be observed even years after discontinuation (45,46). Especially in children, administration of SCIT may prevent the development of lower airway disease or asthma in subjects with upper airway disease or rhinitis. Application of SCIT, however, is limited by the number of allergens that can be administered per injection, the lack

of standardized vaccines for all important allergens, and the limited ability to determine the optimal dose (47). In addition to these limitations, SCIT carries the rare risk of serious side effects, such as anaphylactic reactions (5.4 severe events per million injections) and even death (1 per 2.5 million injections). Since anti-IgE reduces the serum concentration of IgE and thereby reduces IgE-mediated reactions, it was hypothesized that concomitant treatment of anti-IgE and SCIT may improve the risk-benefit ratio of SCIT in polysensitized patients during the consecutive pollen seasons and thereby may prove clinically superior to treatment with SCIT alone.

Anti-IgE Plus SCIT for Treatment of SAR in Children and Adolescents

A randomized, double-blind, parallel-group, multicenter phase III study in children with SAR investigated whether combined therapy with SCIT and anti-IgE is superior to single treatment alone (48). Two hundred and twenty-one patients (6–17 years) with moderate-to-severe SAR and sensitization to birch *and* grass pollen were started on SCIT-birch (2 groups) *or* SCIT-grass (2 groups) for 14 weeks prior to start of the birch pollen season. After SCIT titration (12 weeks), placebo *or* anti-IgE was added for 24 weeks to one of the two groups of the respective SCIT arms. When analyzed separately by season, the two groups receiving unrelated SCIT (i.e., birch-SCIT for grass season and grass-SCIT for birch season, respectively) were considered as placebo controls. Anti-IgE was administered SC at two- or four-week intervals at a dose equivalent to a minimum of 0.016 mg/kg/IU IgE/mL serum in four weeks. Primary endpoint was symptom load, the sum of mean daily symptom severity score plus mean daily rescue medication use.

Combination therapy of anti-IgE and SCIT reduced symptom load (sum of mean daily symptom severity score plus mean daily rescue medication use) over the entire pollen seasons (birch and grass) by 48% compared with SCIT alone ($p < 0.001$). Reduction of symptom load due to combination therapy was highly significant in allergen seasons, birch and grass, when compared with the SCIT alone (Fig. 5).

Differentiating the two components adding to symptom load revealed that rescue medication reduction was a major contributing factor to the observed improvements. Over both pollen seasons, addition of omalizumab resulted in reduction of the median rescue medication score of about 80% compared with the two SCIT groups. Oral Corticosteroids (OCS) as the last rescue medication to be used was reported by 15 patients receiving SCIT + placebo compared with five patients on SCIT + anti-IgE. Symptom severity, the second component of symptom load, was significantly reduced by the combination of anti-IgE with SCIT in the separate groups. Moreover, experimental support for the clinical benefit was provided by the relationship of serum IgE and in vitro mediator release. The decline of serum IgE levels accomplished by anti-IgE treatment correlated with the decreased leukotriene release after in vitro stimulation of mast cells with allergen (49).

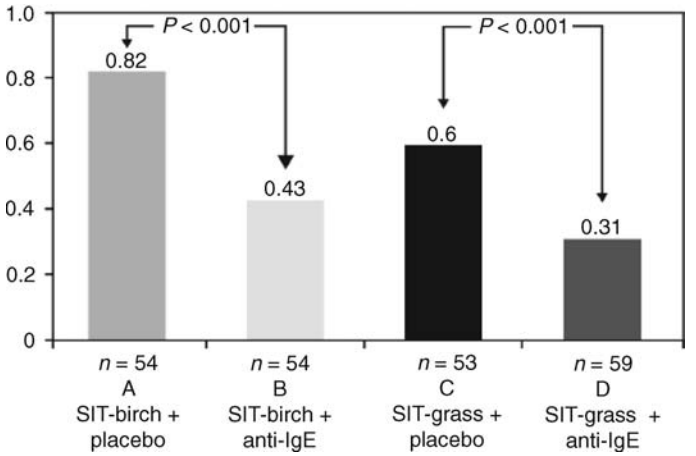

Figure 5 Treatment with allergen-specific immunotherapy with birch (SIT-birch) or grass (SIT-grass) allergen combined with omalizumab reduces symptom load (sum of symptom score + rescue medication score) during entire pollen season (birch and grass). *Source*: Adapted from Ref. 48.

Anti-IgE Plus Rush-SCIT for Treatment of SAR in Adults

Rush immunotherapy (rush SCIT) is a method of accelerated desensitization with aeroallergens and offers an attractive alternative since it potentially provides better compliance, faster response, and lower costs compared with conventional SCIT. However, rush SCIT is accompanied with a higher risk of systemic anaphylactic reactions due to IgE-mediated type-I hypersensitivity reactions (50). In a 2006 trial, the outcomes in safety and efficacy of the combination of rush SCIT and anti-IgE for SAR were analyzed (51). Patients with ragweed-induced SAR were included in a three-center, double-blind, placebo-controlled phase III trial. The objective was to examine whether omalizumab, given 9 weeks before and 12 weeks together with rush-SCIT, increases safety and efficacy compared with SCIT alone. A total of 159 patients between the ages of 18 and 50 years, with a minimum two-year history of ragweed SAR and no recent immunotherapy, were randomized into four treatment groups: (*i*) rush SCIT + anti-IgE, (*ii*) placebo-rush SCIT + anti-IgE, (*iii*) rush SCIT + placebo anti-IgE, and (*iv*) placebo-rush SCIT + placebo anti-IgE.

The number, scope, and severity of AEs associated with rush SCIT were highest in those patients receiving rush SCIT with placebo anti-IgE, a fivefold increase in the risk to develop AE compared with placebo SCIT ($p = 0.001$). Only small differences in AEs were noted between study groups receiving omalizumab plus rush SCIT (33.3%), omalizumab plus placebo-rush SCIT (29.7%), or placebo omalizumab plus placebo-rush SCIT (18.9%). AE increased in a dose response in correlation with the total amount of allergen administered. The percentages of patients with severe adverse events (SAE) during rush SCIT were 2.6 for omalizumab plus rush SCIT, 0 for omalizumab only, and 15.0 for rush SCIT only, compared with 5.0 for placebo-only group. Patients receiving omalizumab in combination with rush-SCIT showed a significant improvement in severity scores during the ragweed season compared with patients on rush-SCIT alone (0.69 vs. 0.86, $p = 0.044$).

In summary, treatment with omalizumab prior to and during rush SCIT offers substantial protection from serious IgE-mediated allergic reactions due to SCIT. The data of the discussed studies show that omalizumab treatment results in safer and more effective use of SCIT, even with accelerated treatment programs.

Anti-IgE for Treatment of Food Allergy

The potent anti-allergic effect of anti-IgE therapy has also been demonstrated in adult patients with food allergy (52). In this study, the threshold amount of peanut leading to allergic symptoms after oral intake was significantly increased after injection of anti-IgE antibody, showing the significant consequences of a treatment directly targeting IgE-mediated immediate type of hypersensitivity reactions.

CONCLUSION

Current pharmacotherapy of bronchial asthma decreases airway inflammation and provides symptomatic relief of bronchoconstriction, but does not specifically target the underlying disease. It is sufficient for the majority of adult and pediatric patients, but not for a subpopulation of patients still suffering severe symptoms or restrictions of QoL with traditional medical regimens. For this group of patients, additional strategies for treatment are needed. The enhanced understanding of the mechanisms underlying the development of allergic diseases, such as asthma, has prompted the development of several novel strategies toward an immunological approach of therapy. These tend not to act as nonspecific anti-inflammatory drugs reducing the ongoing allergic response, but rather to reduce the development of allergic diseases by a specific approach in the allergic inflammatory response. The first line of these immune-modulating regimens have now passed initial evaluations in clinical trials, and the results are not always in favor of the new compounds (53,54). The conclusion one may draw from these first studies is that the clinical situation of asthma in humans is clearly more complex than the experimental situation in animal models (55,56). Simply targeting one single cellular or molecular mediator or pathway may not be adequate to effectively interfere with the complex and redundant inflammatory processes involved in the development of human asthma (57).

The only novel approach for therapy of asthma that went beyond trial status and was accepted for clinical treatment of severe (Europe) or moderate-to-severe (United States) allergic asthma is omalizumab, Xolair. Targeting IgE aims at the common and most distinct phenotype evident in all patients suffering from allergic diseases: increased IgE serum levels. Although atopy may be a coincident rather than a causal factor in the pathogenesis of asthma, there is a significant body of evidence demonstrating a pivotal role of IgE in the development of bronchial asthma and allergic airway disease. Even in patients with apparently nonallergic asthma and normal IgE serum levels, production of IgE in the airways has been found (58). On the other hand, experimental data from murine models demonstrates that development of airway inflammation and hyperreactivity to nonspecific stimuli may occur independent of B cells (59), IgE production (59,60), or IgE-mediated mast-cell activation (61). In these models of allergen-mediated inflammatory response of the airways without B-cell activation, treatment with anti-IgE antibodies is not likely to affect the development of airway inflammation or Airway Hyperresponsiveness (AHR) (62). The human equivalent could be asthma without a significant allergic component, and anti-IgE antibody therapy would likely be less effective even with airway inflammation, eosinophilia, and possibly increased total IgE.

The pooled analysis of seven large-scaled clinical *phase III* trials with omalizumab in patients with bronchial asthma showed that it is effective for patients with symptomatic, moderate-to-severe allergic asthma (33). Similarly, a meta-analysis of eight studies listed in the Cochrane Airways Group Asthma trials register and contributing a total of 2037 mild-to-severe allergic asthmatic participants underscored the efficacy of omalizumab for treatment of allergic asthma (63,64) (the latter being the more recent update). Herein, significant increases in the number of participants who were able to reduce or completely withdraw their daily steroid intake were observed (Table 2). Importantly, this benefit was also shown for patients with severe asthma, suggesting effects by anti-IgE beyond mere reduction of steroid requirements. Finally, omalizumab is safe and well tolerated and improved QoL in all studies observing this parameter (42). Although some of the improvements in symptom scores, exacerbation rates, and lung function data during the course of the trials were also seen in the placebo-treated group, presumably demonstrating the benefits of continuous physician monitoring, the differences between omalizumab and placebo treatment group reached significance for almost all important endpoints in favor of anti-IgE.

A major limitation for a broader use of anti-IgE is the high cost-benefit ratio that by far exceeds that of present standard asthma medication (ICS plus LABA), which provide good asthma control for the majority of patients. Therefore, omalizumab was launched for treatment of adults and children with corticosteroid-dependent or corticosteroid-resistant asthma who require high doses of oral or inhaled steroids and who still have poor asthma control. In such patients, analysis of the direct and indirect costs related to frequent exacerbations and hospitalization may validate the higher costs of anti-IgE therapy. Finally, future trials ideally should better delineate predictors to better define "responders" versus "nonresponders" for anti-IgE therapy. This will ultimately help identify the target group of asthmatic patients and, subsequently, candidates among patients suffering from other allergic diseases who will offer the lowest cost-benefit ratio for anti-IgE therapy.

Table 2 Efficacy of Omalizumab Vs. Placebo for Treatment of Patients with Allergic Asthma with ICS Therapy

Outcomes	No. of trials	No. of participants	Steroid phase	Odds ratio (95% CI)
No. of participants with exacerbation	4	1634	Steroid reduction	0.47 0.37–0.60
No. of participants with at least one exacerbation	4	1651	Stable steroid intake	0.49 0.38–0.64
Complete steroid withdrawal	4	1634	Steroid reduction	2.50 2.00–3.13
$\geq$50% Reduction in steroid usage	4	1634	Steroid reduction	2.50 2.02–3.10

Results of the Cochrane analysis.
Abbreviation: ICS, inhaled corticosteroids.
Source: From Ref. 65.

SALIENT POINTS

- Increased production of IgE is a cardinal feature of allergic diseases and asthma.
- IgE-mediated activation of mast cells is a key element of the early asthmatic reaction and significantly contributes to airway inflammation and airway hyperreactivity.
- Targeting of IgE is an effective anti-allergic approach for treatment of asthma and other allergic diseases since it directly addresses a key element in the pathophysiology.
- Anti-IgE (omalizumab, Xolair) is the first approved "biological" therapy available for treatment of severe allergic asthma.
- Several phase III and phase IV omalizumab clinical trials on subjects with moderate-to-severe asthma show significant reduction of exacerbation rates and corticosteroid therapy and improvement in QoL versus placebo.
- Clinical trials with omalizumab for treatment of other allergic diseases, such as seasonal or PAR or food allergy, are promising and warrant further investigation.

REFERENCES

1. Creer T, Wigal J, Kotses H, et al. A Life Activities Questionnaire for Childhood Asthma. J Asthma 1993; 30:467–473.
2. Frew A. Immunotherapy of allergic disease. J Allergy Clin Immunol 2003; 111:S712–S719.
3. Bousquet J, Lockey R, Malling H. The WHO panel members. J Allergy Clin Immunol 1998; 102:558–562.
4. Abramson M, Puy R, Weiner J. Allergen immunotherapy for asthma (Cochrane Review). In: The Cochrane Library. Issue 3. Oxford: Update Software, 2003.
5. Alton E, Norris A. Chloride transport and the actions of nedocromil sodium and cromolyn sodium in asthma. J Allergy Clin Immunol 1996; 98:S102–S106.
6. Holgate ST. Asthma and allergy—disorders of civilization. QJM 1998; 91:171–184.
7. Louis R, Lau LC, Bron AO, et al. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000; 161:9–16.
8. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002; 360:1715–1721.
9. MacGlashan D Jr., Lichtenstein LM, McKenzie-White J, et al. Upregulation of FcεRI on human basophils by IgE antibody is mediated by interaction of IgE with FcεRI. J Allergy Clin Immunol 1999; 104:492–498.
10. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. J Allergy Clin Immunol 2000; 105:847–859.
11. Maurer D, Ebner C, Reininger B, et al. The high affinity IgE receptor (FcεRI) mediates IgE-dependent allergen presentation. J Immunol 1995; 154:6285–6290.
12. Burrows B, Martinez FD, Halonen M, et al. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320:271–277.
13. Bousquet J, Rabe K, Humbert M, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. Respir Med 2007; 101(7):1483–1492.
14. Rajakulasingam K, Durham SR, O' Brien F, et al. Enhanced expression of high-affinity IgE receptor (FcεRI) α chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. J Allergy Clin Immunol 1997; 100:78–86.
15. Rajakulasingam K, Till S, Ying S, et al. Increased expression of high affinity IgE (FcεRI) receptor-α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. Am J Respir Crit Care Med 1998; 158:233–240.
16. Foster B, Metcalfe DD, Prussin C. Human dendritic cell 1 and dendritic cell 2 subsets express FcεRI: correlation with serum IgE and allergic asthma. J Allergy Clin Immunol 2003; 112:1132–1138.
17. Fregonese L, Patel A, van Schadewijk A, et al. Expression of the high-affinity IgE receptor (FcεRI) is increased in fatal asthma. Am J Respir Crit Care Med 2004; 169:A297.
18. MacGlashan DW Jr., Bochner BS, Adelman DC, et al. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 1997; 158:1438–1445.
19. Saini SS, MacGlashan DW, Sterbinsky SA, et al. Down-regulation of human basophil IgE and FcεRIα surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. J Immunol 1999; 162:5624–5630.
20. Djukanović R, Wilson SJ, Kraft M, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. Am J Respir Crit Care Med 2004; 170:583–593.
21. Prussin C, Griffith DT, Boesel KM, et al. Omalizumab treatment downregulates dendritic cell FcεRI expression. J Allergy Clin Immunol 2003; 112:1147–1154.

22. Fahy JV, Fleming HE, Wong HH, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med 1997; 155:1828–1834.
23. Boulet LP, Chapman KR, Cote J, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. Am J Respir Crit Care Med 1997; 155:1835–1840.
24. Presta LG, Lahr SJ, Shields RL, et al. Humanization of an antibody directed against IgE. Immunology 1993; 151:2623–2632.
25. Fox J, Hotaling T, Struble C, et al. Tissue distribution and complex formation with IgE of an anti-IgE antibody after intravenous administration in cynomolgus monkeys. J Pharmacol Exp Ther 1996; 279:1000–1008.
26. Prussin C, Griffith D, Boesel K, et al. Omalizumab treatment downregulates dendritic cell FcepsilonRI expression. J Allergy Clin Immunol 2003; 112:1147–1154.
27. Fick RB. Anti-IgE as novel therapy for the treatment of asthma. Curr Opin Pulm Med 1999; 5:76–80.
28. Jardieu P, Fick RB. IgE inhibition as a therapy for allergic disease. Int Arch Allergy Immunol 1999; 118:112–115.
29. Casale T, Bernstein L, Busse W, et al. Use of an anti-lgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. J Allergy Clin Immunol 1997; 100:110–121.
30. Fahy J, Cockroft D, Boulet L, et al. Effect of aerosolized anti-lgE (E25) on airway responses to inhaled allergen in asthmatic subjects. Am J Respir Crit Care Med 1999; 160:1023–1027.
31. Corren J, Casale T, Lanier BQ, et al. Omalizumab is well tolerated in adolescent/adult patients ($\geq$12 years) with moderate-to-severe persistent asthma. J Allergy Clin Immunol 2005; 115:S75.
32. Humbert M, Beasley R, Ayres J, et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. Allergy 2005; 60:309–316.
33. Bousquet J, Cabrera P, Berkman N, et al. The effect of treatment with omalizumab, an anti-IgE antibody, on asthma exacerbations and emergency medical visits in patients with severe persistent asthma. Allergy 2005; 60:302–308.
34. Solèr M, Matz J, Townley R, et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. Eur Respir J 2001; 18:254–261. Erratum in: Eur Respir J 2001; 18:739–740.
35. Lanier BQ, Corren J, Lumry W, et al. Omalizumab is effective in the long-term control of severe allergic asthma. Ann Allergy Asthma Immunol 2003; 91:154–159.
36. Buhl R, Solèr M, Matz J, et al. Omalizumab provides long-term control in patients with moderate-to-severe allergic asthma. Eur Respir J 2002; 20:73–78.
37. Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol 2001; 108:184–190.
38. Vignola AM, Humbert M, Bousquet J, et al. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with concomitant allergic asthma and persistent allergic rhinitis (SOLAR). Allergy 2004; 59:709–717.
39. Holgate ST, Chuchalin AG, Hébert J, et al. Efficacy and tolerability of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. Clin Exp Allergy 2004; 34:632–638.
40. Ayres JG, Higgins B, Chilvers ER, et al. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with poorly controlled (moderate-to-severe) allergic asthma. Allergy 2004; 59:701–708.
41. Genentech ALTO study. Available at: http://clinicalstudyresults.gene.com/q2143g.pdf.
42. Chipps B, Buhl R, Beeh K-M, et al. Improvement in quality of life with omalizumab in patients with severe allergic asthma. Curr Med Res Opin 2006; 22:2201–2208.
43. Ädelroth E, Rak MS, Haahtela T, et al. Recombinant humanized mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2000; 106:253–259.
44. Chervinsky P, Casale T, Townley R, et al. Omalizumab, an anti-IgE antibody, in the treatment of adults and adolescents with perennial allergic rhinitis. Ann Allergy Asthma Immunol 2003; 91: 160–167.
45. Durham S, Walker S, Varga E-M, et al. Long-term clinical efficacy of grass pollen immunotherapy. N Engl J Med 1999; 341:468–475.
46. Walker SM, Varney VA, Gaga M, et al. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. Allergy 1995; 50:405–413.
47. Practice parameters for allergen immunotherapy. Joint Task Force on Practice Parameters, representing the American Academy of Allergy, Asthma and Immunology, the American College of Allergy, Asthma and Immunology, and the Joint Council of Allergy, Asthma and Immunology. J Allergy Clin Immunol 1996; 98:1001–1011.
48. Kuehr J, Brauburger J, Zielen S, et al. Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis. J Allergy Clin Immunol 2002; 109:274–80.

49. Kopp MV, Brauburger J, Riedinger F, et al. The effect of anti-IgE treatment on in vitro leukotriene release in children with seasonal allergic rhinitis. J Allergy Clin Immunol 2002; 110(5):728–735.
50. Lockey RF, Nicoara-Kasti GL, Theodoropoulos DS, et al. Systemic reactions and fatalities associated with allergen immunotherapy. Ann Allergy Asthma Immunol 2001; 87(1 suppl 1):47–55.
51. Casale TB, Busse WW, Kline JN, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2006; 117(1):134–140.
52. Leung DY, Sampson HA, Yunginger JW, et al. Avon Longitudinal Study of Parents and Children Study Team: Effect of Anti-lgE therapy in patients with peanut allergy. N Engl J Med 2003; 348:986–993.
53. Leckie M, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000; 356:2144–2148.
54. Bryan S, O'Connor B, Matti S, et al. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000; 356:2149–2153.
55. Bluemchen K, Kallinich T, Hamelmann E. Interleukin-5: a novel target for asthma therapy. Expert Opin Biol Ther 2001; 1:433–453.
56. Barnes P. Cytokine-directed therapies for asthma. J Allergy Clin Immunol 2001; 108:S72–S76.
57. Hamelmann E, Gelfand E. IL-5-induced airway eosinophilia-the key to asthma? Immunol Rev 2001; 179:182–191.
58. Humbert M, Grant J, Taborda-Barata L, et al. High-affinity IgE receptor (FcεRI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. Am J Respir Crit Care Med 1996; 153:1931–1937.
59. Hamelmann E, Takeda K, Schwarze J, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness requires interleukin-5 but not immunoglobulin E or B lymphocytes. Am J Respir Cell Mol Biol 1999; 21:480–489.
60. Oettgen H, Martin T, Wynshaw B, et al. Active anaphylaxis in IgE-deficient mice. Nature 1994; 370:367–370.
61. Takeda K, Hamelmann E, Joetham A, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. J Exp Med 1997; 186:449–454.
62. Hamelmann E, Cieslewicz G, Schwarze J, et al. Anti-interleukin 5 but not anti-IgE prevents airway inflammation and airway hyperresponsiveness. Am J Respir Crit Care Med 1999; 160:934–941.
63. Walker S, Monteil M, Phelan K, et al. Anti-IgE for chronic asthma. Cochrane Database Syst Rev 2003; 3:CD003559.
64. Walker S, Monteil M, Phelan K, et al. Anti-IgE for chronic asthma in adults and children. Cochrane Database Syst Rev 2004; Art. No. CD003559.pub2.
65. Corren J, Casale T, Deniz Y, et al. Omalizumab, a recombinant humanized anti-IgE antibody, reduces asthma-related emergency room visits and hospitalizations in patients with allergic asthma. J Allergy Clin Immunol 2003; 111:87–90.

27 Immunotherapy for Food and Latex Allergy

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INTRODUCTION

Food allergies affect 2% to 4% of adults and 6% to 8% of young children in westernized societies and account for approximately 30,000 emergency room visits with an estimated 150 deaths per year in the United States (1,2). Peanut, tree nuts, cow's milk, egg, soybean, wheat, and seafood are responsible for over 90% of food allergies in children. In adults, peanut, tree nuts, and seafood are the primary food allergens causing severe reactions, and fruits and vegetables trigger milder symptoms, primarily oral pruritus (3–5). Childhood food allergy to cow's milk, egg, soybean, and wheat typically resolve, whereas peanut, tree nut, and seafood allergy tend to be lifelong (6,7). Epidemiological data indicate that the prevalence of peanut allergy doubled in the past 5 to 10 years (3,8), now affecting about 1% of young children. Peanut is the leading trigger of food allergy fatalities (9). Considering the above factors, food allergy is an important target for research on curative treatments.

Natural rubber latex allergy more commonly occurs among exposed health care workers (HCW) and patients undergoing procedures that include latex. Latex also shares allergens with certain foods (10).

This chapter reviews developments for immunotherapeutic treatments for immunoglobulin E (IgE)-mediated food and latex allergy. A variety of immunological strategies are discussed.

IMMUNOTHERAPY WITH NATURAL FOOD/RELATED PROTEINS

Subcutaneous Peanut Immunotherapy

The feasibility of subcutaneous immunotherapy (SCIT) with peanut was evaluated in a small controlled study (11). Three treated subjects displayed a 67% to 100% decrease in symptoms, as evaluated by double-blind, placebo-controlled food challenges, (DBPCFC) and had a two- to five-log reduction in end point prick skin test (PST) reactivity to peanut. One placebo-treated subject completed the study and had no change in DBPCFC symptom scores or PST sensitivity to peanut. A follow-up study of 12 subjects (6 treated) used treatment with a maintenance dose of 0.5 mL of 1:100 wt/vol peanut vaccine. This dose was achieved by a rush injection protocol (12). All treated subjects experienced increased tolerance to peanut and decreased sensitivity on titrated peanut PST, whereas controls experienced no changes. However, systemic reactions with urticaria and angioedema occurred a mean of 4.8 times in the six treated patients. Systemic anaphylaxis with respiratory involvement occurred a mean of 7.7 times during the 12-month study, with an average of 9.8 epinephrine injections required per study subject. Only three subjects achieved the intended maintenance dose because of adverse events. This pivotal study demonstrates that injected food allergen could be successfully used to induce tolerance, but clinical application is limited by practical safety concerns.

BIRCH POLLEN IMMUNOTHERAPY FOR POLLEN-FOOD ALLERGY SYNDROME

Individuals who become sensitized to airborne pollen proteins may develop oral-pharyngeal pruritus from ingestion of raw foods with homologous proteins. This pollen-food allergy syndrome (PFAS) is exemplified by sensitization to birch pollen major allergen (Bet v 1), resulting in reactions from the homologous apple protein, Mal d 1 (13). Pollen immunotherapy (IT) is an established treatment for allergic rhinitis, and this therapy theoretically should be

beneficial for PFAS. A published study investigated an open trial of birch pollen SCIT in 49 adults with birch pollinosis and oral symptoms provoked by apple (14). Forty-one subjects (84%), compared with no controls, reported a significant reduction (50–95%) or a complete resolution of apple oral allergy symptoms ($p < 0.001$). Birch IT also induced a marked reduction in skin reactivity against fresh apple in 43 subjects (88%). In a follow-up study, the duration of the effect of birch IT was evaluated in 30 birch pollen-allergic subjects who experienced resolution of apple oral allergy and loss of skin test reactivity to fresh apple (15). Symptoms and skin test reactivity were compared at the end of the 12-month IT course and 30 months after IT was discontinued. Over 50% of subjects still tolerated apple at the 30-month follow-up visit, although the majority showed evidence of resensitization by PST. Subsequent clinical trials, in which oral allergy to apple was diagnosed with DBPCFC, confirmed a beneficial effect of birch IT in some subjects (16,17). Similar observations were reported from an observational study of 16 adult subjects suffering from PFAS (hazelnut, walnut, lettuce, peach, and cherry) and plane tree pollinosis who were treated with plane tree pollen IT (18). The mean amount of food to provoke objective symptoms increased from 2.19 to 13.74 g ($p < 0.05$), and 6 of 11 subjects tolerated the highest amount (25 g) of the challenge food following treatment.

In a study using sublingual immunotherapy (SLIT), rather than subcutaneous injection, subjects with birch pollinosis and apple PFAS were treated with birch pollen (maintenance dose equaling 4.5 μg Bet v 1 daily, a relatively low dose for SLIT). Nine of 15 had improved nasal provocation scores to birch pollen after 12 months of therapy; however, their apple-induced oral allergy symptoms were not significantly reduced (19). Successful birch pollen SLIT induced Bet v 1-reactive but not Mal d 1-reactive IgE and IgG4 antibodies and reduced Bet v 1-specific but not Mal d 1-specific T-cell proliferation.

Asero postulated that for some subjects with PFAS, IT doses higher that typically needed to produce improvement in birch pollen rhinitis may be necessary to improve birch-PFAS. He also pointed out that most significant effects on PFAS were observed in the studies that included adults monosensitized to birch tree pollen and not to other pollens (20). An alternative explanation is that the T-cell immune response to birch cross-reactive food allergens, such as apple Mal d 1, hazelnut Cor a 1, and carrot Dau c 1, is at least in part Bet v 1-independent (19,21). If so, vaccines based on modified, recombinant food allergens may represent a superior approach to PFAS.

Oral Immunotherapy for Food Allergy

Oral IT to food is generating increasing interest as a potential approach to treatment of food allergy (22,23). The rationale for using the oral route is to involve cells and immune pathways involved in induction of oral tolerance. Animal studies suggest that high-dose feeding of an antigen results in anergy or deletion of antigen-specific T lymphocytes, and continuous low-dose ingestion may induce protective suppressive responses from regulatory T cells. In contrast, intermittent feedings or nonoral exposures may induce sensitization and allergy (24). A distinction should be made between approaches that induce "desensitization," where the allergen is ingested without symptoms during treatment but maintenance requires daily, uninterrupted ingestion, compared with true "tolerance," where the food may be ingested without allergy symptoms despite periods of abstinence.

Evidence in support of oral desensitization is limited primarily to nonrandomized clinical trials and case reports. Available data do not distinguish the effects of oral desensitization versus the natural resolution of food allergy (25,26). In some subjects who ultimately tolerate a maintenance dose, even for a significant period of time, allergic symptoms redevelop on eating if the food is not ingested on a regular basis, highlighting a concern that true tolerance is not achieved (22,27). In some subjects, a full maintenance dose (e.g., 100 mL of cow's milk) cannot be achieved because of allergic symptoms, though the patient may benefit from an increased threshold of reactivity, e.g., a patient who previously experienced allergic reactions because of trace amounts of food contamination is protected from such inadvertent exposures as long as he/she continues to ingest the daily dose of the food in question (26). Additional unresolved issues regard safety. For example, food-dependent exercise-induced anaphylaxis (FDEIA) was noted in children undergoing oral desensitization (28,29). It is unclear whether FDEIA is a concern for all subjects or only for those who did not achieve the status of persistent tolerance. Results of studies of oral IT for food allergy are summarized in Table 1 (22,25–27,30–39).

Table 1 Summary of Current Experience with Oral Food Desensitization for IgE-Mediated Food Allergy

Study	Foods	Subjects	Starting dose	Time to maintenance	Success rate[a]	Comments
Schofield, 1908	Egg	$N = 1$	1/10,000 of an egg	6 mo	1/1	Negative oral food challenge to egg after 6 mo; the subject continued to ingest an egg daily
Patriarca, 1984 (30); open clinical trial	CM (8) Egg (8) Fish (2) Orange (1)	$N = 19$ Age: 5–55 yr	10 drops of CM in 10 mL of water; 4 drops/day; 10 drops of beaten egg in 100 mL of water, 4 drops/day; 10 mL of mixed fish commercial extract (eel, sardine, codfish, anchovy) in 90 mL of water, 4 drops/day; Unspecified	100 mL of undiluted CM/day in 104 days; 120 drops of pure beaten egg/day in 90 days; 200 g of cooked fish/day in 120 days; 3 mo	5/8 6/8 2/2 1/1	Side effects in 11/19 patients: urticaria, pruritus, emesis, angioedema, abdominal pain, rhinitis, dyspnea; patients followed for 3–12 mo; subject with orange allergy reported resolution of allergy to plums and peaches. No insight into mechanism of desensitization
Shenassa, 1985 (31)	Peanut	$N = 6$	0.05 mL of the strongest dilution of peanut extract 1:10 wt/vol that caused no erythema on PST	Not specified	4/6	4 subjects reported to tolerate peanut daily, 2 subjects did not complete the desensitization protocol
Patriarca, 1998 (32); clinical trial	CM (6) Egg (5) Fish (2) Apple (1)	$N = 14$ Age: 4–14 yr	Modification of previously published protocol (30)	Modification of previously published protocol (30)	12/14	All of the children who achieved maintenance continued to tolerate the foods at least 2–3 times/wk for 3–6 yr. 10/14 patients experienced side effect during treatment
Bauer, 1999 (33)	CM	$N = 1$ Age: 12 yr	1 mL/day of 0.01% milk diluted in water	Rush protocol: dose doubled every other hour; final dose 200 mL of undiluted milk achieved in 5 days	1/1	Patient tolerated CM daily for at least 6-mo follow-up
Nucera, 2000 (34)	CM	$N = 1$ Age: 6 yr	10 drops of milk in 10 mL of water; 4 drops/day	100 mL of undiluted CM/day in 104 days	1/1	Child able to ingest CM and dairy products after 6 mo. After 7 mo: PST to BLG, ALA, CS became negative; IgE to milk proteins decreased significantly, whereas milk-IgG and IgA increased. PBMC stimulated with BLG produced significantly less IL-4 at 18 mo than at baseline

(*Continued*)

Table 1 Summary of Current Experience with Oral Food Desensitization for IgE-Mediated Food Allergy (*Continued*)

Study	Foods	Subjects	Starting dose	Time to maintenance	Success rate[a]	Comments
Rueff, 2001 (35)	Celery	$N = 1$ Age: 49 yr	0.1 mL of a commercial natural celery juice 5 times a day	5 mL 5 times daily for 3 mo	1/1	At 3 mo patient tolerated 10 g of raw celery but developed flushing to 20 g; continued to ingest 25 mL of raw celery juice for 3 yr
Patriarca, 2003 (25); clinical trial	CM (29) Egg (15) Fish (11) Orange (2); and other[b]	$N = 59$ Age: 3–55 yr	Modification of previously published protocol (110)	Modification of previously published protocol (110)	45/54 (83.3%)	51% of patients experienced urticaria, emesis, diarrhea, or abdominal pain; in 9 patients (16.7%), protocol was stopped because of side effects; no differences between children and adults; PST became negative after 18 mo in 78%; food-IgE decreased and food-IgG4 increased after 18 mo
Meglio, 2004 (26)	CM	$N = 21$ Age: 5–10 yr	1 drop of CM diluted 1:25 in water	200 mL undiluted CM/day over 180 days	15/21 (71.4%)	3/21 reacted to minimal dose of diluted CM; 3/21 tolerated only 40–80 CM/day; 15/21 tolerated 200 mL CM/day for 6 mo; side effect rate 13/21; PST to BLG and CS significantly decreased at 6 mo ($p < 0.001$), CN-IgE levels not significantly different
Rolinck-Werninghaus, 2005 (22)	CM (1) Egg (2)	$N = 3$ Age: 4–12 yr	0.0006 mL of CM per day 0.01 mg egg per day at home	100 mL CM/day over 37 wk, 2.5 g egg/day (1/2 egg) over 41–52 wk	3/3	All patients had acute symptoms to food reexposure following discontinuation of food for 2–14 days. The only study to rechallenge patients to food following a period of avoidance, while on maintenance
Patriarca, 2006 (36)	Peanut	$N = 1$ Age: 38 yr	5.6 mg peanut/day on day 1	40 g peanut/day over 7 days in the hospital	1/1	PST to peanut became negative at 6 mo, no significant changes in peanut-IgE (baseline 2.1, 6 mo–1.5) or peanut-IgG

Buchanan, 2006 (37)	Peanut	$N = 7$ Mean age: 4.4 yr	Not specified	Rush phase and dose escalation in the hospital, maintenance at home	7/7 at 6 mo	During rush phase, 4/7 required oral antihistamine. At 6 mo, there was mean 2.3-fold increase in peanut-IgG and mean change in peanut-IgE of 0.9 fold.
Mansfield, 2006 (38)	Peanut	$N = 1$ Age: 6 yr (with history of anaphylaxis to peanut)	Half a peanut kernel 3 times daily	Initial dose and dose increases in the allergy office over 8 wk, maintenance 2 whole peanuts twice daily	1/1	Accidental exposures (contact and ingestion) tolerated without symptoms. Peanut-IgE (kIU/L) was >100 at baseline, 74 at 6 mo and 42 at 12 mo
Buchanan, 2007 (32)	Egg	$N = 7$ Mean age: 4 yr (subjects with history of egg-induced anaphylaxis were excluded)	0.1 mg of powdered egg white followed by doubling doses every 30 min until the highest tolerated dose was determined	Modified rush and build-up phase in the hospital, maintenance dosing once a day at home. Increases by 25 mg every 2 wk until 150 mg, then by 50 mg until reaching maintenance of 300 mg	4/7	4 subjects tolerated egg challenge at the end of 24 mo. 2 of them reacted to a subsequent egg challenge done 3 mo after treatment was stopped. Egg-specific IgG increased significantly from baseline to 24 mo ($p = 0.002$). 5 subjects showed an overall decrease in egg-specific IgE
Morisset, 2007 (39) randomized clinical trial	CM (57) Egg (84)	$N = 141$ mean age: CM: 2.2 yr Egg: 3.5 yr	CM: 1 mL/day Egg: 1 g of hard boiled egg yolk	CM: home buildup over 6 wk, up to a dose of 250 mL/day; Egg: home buildup over 4 wk to 4 g of yolk and 4 g of egg white once a day, every other day	CM: 89% Egg: 69%	Only children tolerating at least 60 mL of CM or 965 mg of raw egg white on a baseline food challenge were included. PST sizes and specific IgE levels were significantly decreased in children that developed tolerance to CM or egg

[a]Success rate defined as regular ingestion of the tested food for at least six months.
[b]One of each apple, peach, lettuce, orange, beans, and corn.
References are shown in parenthesis.
Abbreviations: CM, cow's milk; PST, prick skin test; BLG, beta-lactoglobulin; ALA, alpha-lactalbumin; CS, casein; PBMC, peripheral blood mononuclear cells.

SLIT with Food

Another approach to induce reduced hypersensitivity or tolerance is SLIT with food. An initial case report described modified SLIT with fresh kiwi pulp extract in a 29-year-old woman with history of kiwi anaphylaxis (40). The extract or kiwi cube was kept under the tongue for one minute before swallowing (e.g., combined SLIT and oral therapy). There was a diminished IgE-reactivity to the major kiwi allergen, Act c 1 (30 kD), in western blots with kiwi extract. Five years into kiwi-modified SLIT, this subject tolerated without problems resumption of modified SLIT after a treatment interruption of four months (41).

Subsequently, a randomized, double-blind, placebo-controlled trial of SLIT for treatment of hazelnut allergy was conducted with a commercial hazelnut extract (42). Adult subjects with hazelnut allergy (54.5% with history of oral allergy symptoms), confirmed by DBPCFC, were randomly assigned to two groups, hazelnut IT ($n = 12$) or placebo ($n = 11$). Subjects kept the IT solution in the mouth for at least three minutes and then expectorated. All subjects receiving hazelnut IT reached the planned maximum dose with a four-day rush protocol, followed by a daily maintenance dose (containing 188.2 μg of Cor a 1 and 121.9 μg of Cor a 8, major hazelnut allergens). Systemic reactions were observed in 0.2% of the total doses administered and limited to the rush build-up phase and were treated successfully with oral antihistamines. Local reactions, mainly in the form of immediate oral itching, were observed in 7.4% (109 reactions/1466 doses). Four patients in the active group reported abdominal pain several hours after the ingestion on one occasion each and only during the build-up phase. All local reactions during the maintenance phase were limited to oral itching and only occurred in one patient. After five months of SLIT, the mean threshold dose of ingested hazelnut resulting in allergic symptoms increased from 2.3 to 11.6 g ($p = 0.02$; active group) versus 3.5 to 4.1 g (NS; placebo). Almost 50% of subjects who underwent active treatment tolerated the highest dose (20 g) of hazelnut during follow-up DBPCFC, compared with 9% in the placebo group. Levels of serum hazelnut-specific IgG_4 antibody and total serum IL-10 increased only in the active group, but there were no differences in hazelnut-specific IgE antibody levels pre- and post-IT.

Another study evaluated SLIT in eight children with cow's milk allergy (43). A day after an initial positive milk food challenge, children started SLIT with 0.1 mL of cow's milk for the first two weeks, increasing by 0.1 mL every 15 days until 1 mL/day was given. Cow's milk was kept in the mouth for two minutes and then discharged. One patient withdrew from the study because of oral symptoms, thus seven subjects completed the protocol. After six months of treatment, the provocative dose of milk increased from a mean of 39 mL at baseline to 143 mL ($p < 0.01$).

These preliminary data on oral IT and SLIT are encouraging, especially with regard to safety. However, additional studies must address multiple factors, including optimal dose, ideal duration of IT, degree of protection, efficacy for different ages, severity and type of food allergy responsive to treatment, and need for patient protection during home administration. The latter includes defining the manifestations of anaphylaxis, documentation of the patient's ability to recognize these manifestations, and demonstrating the proper use of epinephrine autoinjectors. Additionally, mechanistic studies are needed to understand the immunological changes induced by oral IT and SLIT with foods.

TREATMENT WITH RECOMBINANT FOOD PROTEINS

An increasing number of food allergens are being characterized (44). Many recombinant food proteins have been evaluated for binding specific IgE antibody from allergic subjects' sera and by skin prick testing (PST) (44–47). Availability of recombinant allergens should allow for customizing and standardizing improved diagnostic tests and therapies. In addition, having animal models of food allergy to test these and other treatment strategies is crucial. For example, Li et al. developed a well-characterized murine model of IgE-mediated cow's milk and peanut hypersensitivity that uses the oral route for sensitization. These sensitized mice experience anaphylaxis from oral food challenge (48,49) In a peanut allergy model, C3H/HeJ mice were sensitized orally with peanut using cholera toxin as an adjuvant. After sensitization, the animals were challenged with peanut orally three and five weeks later (49). The symptoms were similar to those seen in human subjects (Fig. 1). Ara h 1- and Ara h 2-specific IgE antibodies were detected in the sera of mice with peanut allergy. Furthermore, these Ara h 2-specific IgE

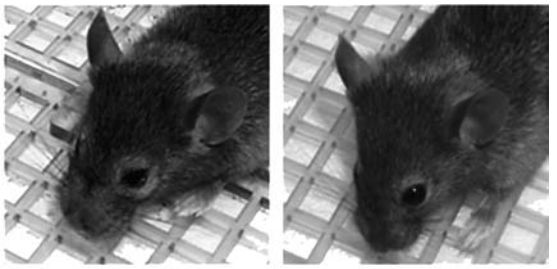

Figure 1 Murine Model of Peanut Anaphylaxis. (**A**) Peanut allergic-mouse with snout, eye and paws edema, and pilar erecti following oral peanut challenge (score 3). (**B**). Naive (non-allergic mouse), asymptomatic on feeding with peanut (score 0). C3H/HeJ mice were sensitized intragastrically (i.g.) with PN and cholera toxin on days 0, 7, and 14. At week 3, mice were challenged with 10-mg crude peanut extract i.g. Symptoms were scored using a 0- to 5-point scale, with 0 for no signs of reaction and 5 for death. *Source*: From Ref. 66.

antibodies bound the same Ara h 2 isoforms and major allergenic epitopes as antibodies of human subjects with peanut allergy.

IT with injections of plasmid DNA encoding recombinant peanut proteins was also evaluated in murine models. Intramuscular immunization of naïve AKR/J (H-2^K), BALB/c, and C3H/HeJ (H-2^K) mice with plasmid DNA encoding Ara h 2, prior to intraperitoneal peanut sensitization, had a protective effect in AKR/J and BALB/c mice. These strains were asymptomatic during subsequent intraperitoneal injections of peanut protein at three and five weeks following sensitization. However, peanut challenge induced anaphylaxis in the majority of the sensitized C3H/HeJ mice (60% mortality rate) (50). In another study, oral chitosan (nanoparticle composed of chitin) embedded with Ara h 2 had a protective effect in preventing sensitization in AKR mice (51). These data suggest that the prophylactic effects of plasmid DNA-based IT may be strain dependent.

Synthetic immunostimulatory oligodeoxynucleotides containing unmethylated CpG motifs (ISS)-conjugated to allergen is another approach that could reduce risks of food allergen IT. Risk reduction is possibly due to enhancing Th1 and reducing Th2 responses. This strategy was explored by Li et al. who immunized C3H/HeJ mice intradermally with ISS-linked Ara h 2 (peanut) or ISS-linked Amb a 1 (ragweed) as a control (52). Four weeks following immunization, mice were sensitized intragastrically with peanut and later challenged with Ara h 2. ISS-Ara h 2-treated mice did not develop symptoms and had significantly lower plasma histamine levels following oral challenge compared with controls. Nguyen et al. also found that intradermal immunization with a mixture of ISS and β-galactosidase (β-gal), but not with ISS alone or β-gal alone, provides protection against fatal anaphylaxis induced by intraperitoneal β-gal sensitization and challenge (53). These results suggest that antigen-ISS immunization may have a prophylactic effect against allergy; however, the ability to reverse established food allergy is unknown.

Immunotherapy with Recombinant Engineered Food Proteins

Another approach to avoid IgE activation during IT is to engineer proteins with reduced IgE binding (54). This goal can be accomplished with point mutations introduced by site-directed mutagenesis in the known IgE-binding epitopes of major food allergens. This strategy has been successfully used for generation of hypoallergenic mutants of peanut, fish, and apple allergens (46,55–59). Polymerization is another technique to modify food allergens to reduce IgE binding but preserve immunogenicity. This has been applied to apple and carrot but probably is less effective than allergen modification or site-directed mutation (60,61).

RNA isolated from peanut was used to construct an expression library in *Escherichia coli* for western blot screening with serum IgE from subjects with peanut allergy. Clones with intense IgE binding were selected. Ninety-four percent of tested sera bound to both wild-type and recombinant Ara h 1. Subsequently, two additional major peanut allergens were cloned and characterized: Ara h 2 and Ara h 3 (55,56). The IgE-binding epitopes of each of these three major peanut allergens was determined and site-directed mutagenesis of the allergen cDNA used to produce engineered recombinant allergens with reduced allergenicity (57). In vitro studies of the engineered proteins confirmed a reduced IgE binding with retention of T-cell stimulation (58). Similar modifications of recombinant major fish allergen (parvalbumin from carp, rCyp c 1) reduced IgE binding by 95% but did not affect IgG binding (59). The hypoallergenic engineered recombinant proteins are therefore promising candidates for safe IT for food allergy.

In vivo efficacy of the engineered recombinant peanut proteins was tested in the murine model of peanut anaphylaxis (62). Mice were sensitized to whole peanut and then desensitized by intranasal administration of engineered recombinant Ara h 2 (3 doses/wk for 4 weeks). Desensitization with the engineered recombinant Ara h 2 protein suppressed synthesis of Ara h 2-IgE and resulted in significantly decreased severity of anaphylactic reactions following oral peanut challenge compared with a control group.

Modified food allergens may be combined with bacterial adjuvants to further reduce specific IgE production (63,64). Heat-killed *Listeria monocytogenes* (HKLM) combined with engineered peanut allergens (mAra a 1-3) have been applied to peanut-allergic mice (65). Peanut-allergic C3H/HeJ mice were treated for 10 weeks following sensitization with a mixture of the recombinant, modified major peanut allergens, and HKLM [modified (m)Ara h 1-3 + HKLM] administered subcutaneously. All mice in the sham-treated group exhibited anaphylactic symptoms, whereas only 31% of mice in the mAra h 1-3 plus HKLM group developed mild anaphylaxis. This protective effect was more potent than in the mAra h 1-3 protein alone-treated group. Though the approach of injecting heat-killed bacteria with modified proteins was effective, safety concerns about using potentially pathogenic bacteria in humans will likely limit clinical application. To potentially overcome this obstacle, a nonpathogenic strain of *E. coli* was used as an adjuvant in subsequent studies. In addition, in view of potential complications from the subcutaneous route of administration in humans, animal studies have focused on modified food allergen vaccines administered per rectum. Since nonpathogenic *E. coli* bacteria reside in the colon, it was assumed that rectal delivery would provide superior safety regarding possible infectious complications as well as limit severe adverse reactions (66). Peanut-allergic C3H/HeJ mice received 0.9 (low dose), 9 (medium dose), or 90 (high dose) μg of heat-killed *E. coli* expressing modified proteins Ara h 1-3 (HKE-MP123) per rectum, HKE-containing vector (HKE-V) alone, or vehicle alone (sham) weekly for three weeks. Mice were challenged with peanut two weeks later. Second and third peanut challenges were performed at four-week intervals. After the first peanut challenge, all three HKE-MP123 and HKE-V-treated groups exhibited reduced severity of anaphylactic reactions ($p < 0.01$, 0.01, 0.05, and 0.05, respectively) compared with the sham-treated group. Only the medium- and high-dose HKE-MP123-treated mice remained protected for up to 10 weeks after treatment (Fig. 2). Peanut-specific-IgE levels were significantly lower in all HKE-MP123-treated groups ($p < 0.001$), being most reduced in the high-dose HKE-MP123-treated group at the time of each challenge. In vitro IL-4, IL-13, IL-5, and IL-10 production by peanut-stimulated splenocytes of high-dose HKE-MP123-treated mice were significantly decreased ($p < 0.01$, 0.001, 0.001, and 0.001, respectively), and IFN-γ and TGF-β production were significantly increased ($p < 0.001$ and 0.01, respectively) compared with sham-treated mice at the time of the last challenge. The comparison of experience with native, recombinant, and engineered recombinant allergen IT for food allergy is presented in Table 2.

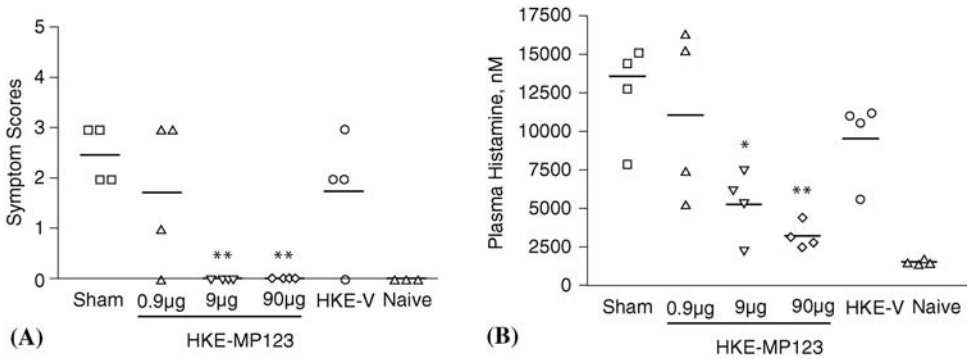

Figure 2 Persistent protection against peanut-induced anaphylactic reactions by HKE-MP 123. Mice were challenged at 10 weeks after the last HKE-MP 123 treatment. (**A**). Anaphylactic symptom scores were determined 30 minutes after challenge. Each point indicates an individual mouse. Bars indicate the median of four mice in each group. (**B**). Histamine plasma levels were measured in blood samples obtained 30 to 40 minutes after the challenge. Data are means ± SEM for each group of four mice. *$p < 0.05$ and **$p < 0.01$ versus sham. *Abbreviation*: SEM, standard errors of measurement. *Source*: From Ref. 66.

Table 2 The Comparison of Native, Recombinant, and Engineered Recombinant Allergen IT for Food Allergy

Therapy	Mechanism of Action	Effects	Comments
Conventional peanut IT	Modified T-cell responses, upregulation of suppressor cells in allergen IT	Increased oral peanut tolerance	Subcutaneous injections of gradually increasing doses of allergen, unacceptably high rate of serious adverse events
Birch pollen IT for oral allergy to apple	Marked reduction in skin test to raw apple; IT effect inversely correlated with baseline skin test, but not with serum apple or birch-IgE	Significant reduction or total resolution of oral allergy to raw Golden Delicious apple in a subset of patients receiving IT for at least 12 mo	Clinical effect lasting for up to 30 mo after discontinuation in $>$50% of patients
Oral desensitization	Presumed oral tolerance induction; decreased skin test reactivity, increased serum food-IgG/IgG4, no change in food-IgE, increased food-specific CD4+CD23 high T cells, increased IL-10 on food antigen stimulation	Tolerance to regular servings of food in most subjects (70–80%) maintained as long as food ingested on regular basis for up to 6 mo; in a subset increased threshold dose for clinical reactions	Up to 50% experience systemic side effects; some patients require uninterrupted ingestion of food to maintain desensitized tolerant state; rigorous clinical trials necessary to determine safety and efficacy
Sublingual IT with hazelnut extract	Presumed oral tolerance induction; increased serum hazelnut-IgG4 and total IL-10 level, no change in hazelnut-IgE	Increased oral hazelnut tolerance	Systemic reactions in only 0.2% of doses during build-up phase, treated with oral antihistamines; rigorous clinical trials necessary to determine safety and efficacy
Plasmid DNA-based IT	Induces prolonged humoral and cellular responses due to CpG motifs in the DNA backbone	Protection against peanut anaphylaxis in sensitized AKR/J mice, but induction of anaphylaxis in C3H/HeJ ($H\text{-}2^K$) mice; no effect on peanut-IgE antibody levels	Serious concerns regarding safety in view of strain-dependent effects in mice, concern for excessive Th 1 stimulation and autoimmunity
Immunostimulatory sequences (ISS-ODN)	Potent stimulation of Th1 via activation of antigen-presenting cells, natural killer cells, and B cells; increased Th1 cytokines	Protection against peanut sensitization in mice	Not shown to reverse established peanut allergy, concern for excessive Th1 stimulation, and potential for autoimmunity
Engineered recombinant peanut IT	Binding to mast cells eliminated, T-cell responses comparable to native peanut allergens	Protection against peanut anaphylaxis in mice	Improved safety profile compared with conventional IT, requires identification of IgE-binding sites
Heat-killed bacteria mixed with or expressing engineered recombinant peanut proteins	Potentiation of Th 1 and T-regulatory cytokine responses	Protection against peanut anaphylaxis in mice, lasting up to 10 wk after treatment	Concern for toxicity of bacterial adjuvants, excessive Th 1 stimulation, and potential for autoimmunity; heat-killed *Escherichia coli* expressing modified peanut allergens administered rectally, viewed as the safest approach for future human studies

Abbreviation: IT, Immunotherapy.

ALLERGEN NONSPECIFIC APPROACHES TO FOOD ALLERGY

In addition to research focusing on IT with native and engineered recombinant food proteins, alternative approaches to food allergy therapy have been investigated. These include monoclonal anti-IgE therapy, Chinese herbs, probiotics, and cytokine therapy. These alternative approaches are reviewed in references (67–70).

Table 3 Latex Allergens

		Allergen relevance		IgE cross-reactivity	
Allergen	Name/function	HCW	Spina bifida	Molecule	Cross-reactive allergen source
Hev b 1[a]	Rubber elongation factor	Minor	**Major**	Hev b 3 (72)	Latex
Hev b 2	β-1,3-glucananse	Minor	Minor	Plant β-1,3-glucanases (74,75)	Bell pepper, olive
Hev b 3[a]		Minor	**Major**	Hev b 1 (72)	Latex
Hev b 4	Cyanogenic glucosidase	Minor[b]	Minor[c]		
Hev b 5[d]		**Major**	Minor[c]		
Hev b 6.01[d]	Prohevein	**Major**	Minor[c]	Class I chitinases (80)	Avocado, banana
Hev b 6.02[d]	Hevein	**Major**	Minor[c]	Class I chitinases (80)	Avocado, banana
Hev b 6.03[a]	C-fragment	Minor	Minor		
Hev b 7	Patatin-like	Minor	Minor		
Hev b 8	Profilin	Minor	Minor	Profilins (74)	Bell pepper, celery tuber, Birch and Timothy pollen
Hev b 9	Enolase	Minor	Minor	Mold enolases (81)	*Cladosporium herbarum, Alternaria alternata*
Hev b 10	Manganase superoxide Dismutase	Minor	Minor	Mold manganese dismutase (82)	*Aspergillus fumigatus*
Hev b 11	Class I chitinase	Minor			
Hev b 12	Lipid transfer protein	Minor			
Hev b 13[d]	Early nodule-specific protein	**Major**	Minor	Patatin (98)	White potato

[a]CD4 epitopes were mapped for these allergens.
[b]Major allergens for latex-allergic adults (not health care workers).
[c]Major allergen for latex-allergic children (without spina bifida).
[d]CD4 epitopes were mapped and hypoallergenic recombinant-mutated molecules were generated.
References are shown in parenthesis.

LATEX IMMUNOTHERAPY

Clinical manifestations of IgE-mediated latex allergy include eczema, contact urticaria, rhinoconjunctivitis, asthma, food allergy (due to cross-reactive proteins), angioedema, and anaphylaxis. The older studies reported prevalence of latex sensitization of HCWat approximately 10% (range 0–40%), with an estimated prevalence rate of 1% in the general population. However, recent data from the Third National Health and Nutrition Examination Survey (1988–1991) showed that after adjusting for other factors associated with latex sensitization, there was no significant association between longest-held jobs in health care and latex sensitization (odds ratio = 1.49, 95% confidence interval, 0.92, 2.4) (71). Children with spina bifida and frequent surgical procedures or, in some cases, frequent fecal disimpaction or the repeated introduction of a latex catheter into the bladder have a prevalence of latex sensitization as high as 37% (72).

Thirteen latex allergens have been identified (Table 3); in addition, several other allergen candidates were identified by IgE immunoblotting of two-dimensional gel electrophoresis of latex extracts (73–82). Clinically relevant latex allergens include Hev b 1 and Hev b 3 for spina bifida subjects and Hev b 5, Hev b 6.02, and Hev b 13 for latex-allergic HCWs and children without spina bifida (72,83). The different reactivity patterns between subject groups presumably reflect the different latex allergen content in latex products to which individuals are exposed, as well as different routes of exposures and other factors (83,84). Treatment of latex allergy requires avoidance, which is simplified by many latex-free products. The reduction of exposure to latex in health care has decreased the problem. Nevertheless, efforts have been made to standardize a latex extract to offer specific IT.

SPECIFIC LATEX IMMUNOTHERAPY VIA THE SUBCUTANEOUS ROUTE (SCIT)

Initial case reports described successful use of oral and SCIT with nonstandardized latex extracts, as well as latex glove skin exposure desensitization predominantly in adult HCWs (85–87). A multicenter, randomized, double-blind, placebo-controlled trial evaluated the efficacy and safety of latex SCIT in 20 HCWs with symptoms of occupational rhinitis or asthma due to latex (88). Treatment began with a two-day course of rush IT followed by monthly maintenance therapy for one year. The latex vaccine potency was expressed as index of reactivity (IR, arbitrary units); the maximum maintenance dose was 100 IR, approximately equivalent to 20 μg/mL of latex protein. Subjects in the active treatment group had a significantly lower total rhinitis score after 6 ($p < 0.04$) and 12 months ($p < 0.05$), conjunctivitis score after six months ($p < 0.02$), and cutaneous score after 12 months ($p < 0.03$) compared with the placebo group. Almost half the subjects receiving active treatment had local reactions, starting with the first injection, and four of the nine subjects in this group experienced moderate to severe systemic reactions.

In 2003, Sastre et al. published a randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of SCIT with a standardized natural latex rubber extract in a population of 24 occupationally sensitized subjects with cutaneous and respiratory symptoms following latex exposure (89). To diagnose respiratory allergy, an inhalation challenge was performed and contact urticaria was diagnosed with glove-use and rubbing tests. Treatment consisted of a build-up phase of weekly injections over three months, followed by every other week maintenance doses for six months. Sixteen subjects received active treatment and eight received placebo. The vaccine contained a maximum of 20 μg/mL of total protein (ALK-Abelló). The average maximum-tolerated dose was 11.1 ± 9.3 μg of latex protein. Following six months of treatment, subjects in the active latex SCIT group had significantly decreased skin reactivity to the latex extract. Concentration of latex extract 12 times higher than at baseline was required to induce the same wheal area by prick testing in the treated subjects. In the placebo group, a significant improvement occurred in the rubbing and glove-use tests and bronchial challenge, but there was no change in cutaneous reactivity. There were no significant differences between active and placebo groups in symptom scores, use of medication, self-assessment, or methacholine test before and after treatment. The lack of efficacy on these parameters could be explained by the fact that all subjects were also allergic to pollens and part of the study was carried out during the pollen season. In a follow-up study, development of

new IgE sensitizations in three subjects and increased intensity of IgE immunoblot binding to latex proteins in four subjects were detected after six months of latex SCIT (90). Following latex SCIT, there was a significant increase in IgE binding in a band of approximately 22 kDa ($p = 0.032$) that may correspond to Hev b 6.01. A significant ($p = 0.012$) negative correlation ($r = -0.72$) was observed between the maximal-tolerated dose and the serum concentration of specific IgE to Hev b 6 at baseline, suggesting that Hev b 6.01 is one of the relevant latex allergen in HCWs.

In another study, 23 adult subjects with latex allergy (most of them HCWs) were randomized to receive SCIT with a standardized latex extract ($n = 11$) or placebo ($n = 12$) (91). Diagnosis of latex allergy was based on a two-year or more history of immediate allergic reactions to latex. These reactions included rhinoconjunctivitis (in 23), cutaneous manifestations (in 22), and asthma (in 20). Subjects in the active group received a two-day rush protocol, followed by a 12-month phase of maintenance treatment at the maximum-tolerated dose (target dose of 0.5 mL of 10 IR/mL). Eight subjects completed the trial in the active group and only three reached the planned maintenance dose of 5 IR, equivalent to about 1 μg of latex proteins. There were no significant changes in symptom and medication scores between baseline and following 12 months of latex IT in both subject groups. Conjunctival provocation test showed threefold increase of the threshold dose in the active group compared with no change in the placebo group, but the difference was not significant. There were no significant changes in percutaneous skin test reactivity to latex or in latex-specific IgE antibody levels. Systemic reactions occurred in 102 of 335 injections (30.4%) and affected nine subjects (81.8%) in the active group. In contrast, systemic reactions occurred in 9 of 354 doses (2.5%) and in two subjects in the placebo group (16.7%). There were 10 severe systemic reactions, including five reactions in the same subject. This study underscores the high rate of adverse reactions during latex SCIT, with many of the most severe reactions occurring during the maintenance phase. The study also demonstrates the difficulty in advancing to the planned maintenance dose that was effective in a previous trial (88).

LATEX SUBLINGUAL IMMUNOTHERAPY

Patriarca et al. conducted a randomized nonblinded clinical trial of SLIT in 24 subjects with cutaneous and respiratory symptoms to latex (92). The treatment vaccine contained a maximum dose of 500-μg/mL latex protein (ALK-Abelló). Twelve subjects underwent a four-day sublingual rush protocol for desensitization to latex, performed by putting increasing doses of latex vaccine under the tongue for three minutes and subsequently spitting them out. This was repeated every 20 minutes. The maintenance phase was five drops of the 500-μg/mL sublingual solution, three times a week for three months. Twelve subjects were controls. Following three months of maintenance SLIT, all subjects in active treatment were able to wear latex gloves for six hours and had an improvement in nonblinded respiratory, conjunctival, and mucocutaneous symptoms. Furthermore, a nonblinded conjunctival challenge test became negative in 50% of the subjects, compared with 100% positive challenges at baseline. Subjects in the control group had no improvement. There were no side effects reported during the rush phase, whereas there were two mild reactions in two subjects during the maintenance phase.

Cisteró et al. assessed the safety and efficacy of rush, nonblinded latex SLIT on latex-skin reactivity in 26 adults with cutaneous and respiratory symptoms, including three subjects with anaphylaxis due to latex (93). The vaccine contained a maximum dose of 500-μg/mL latex protein; the drops were kept under the tongue for three minutes and were subsequently swallowed (sublingual-swallow technique). All subjects underwent rush SLIT in the hospital (4 days) with a standardized latex vaccine followed by a maintenance treatment consisting of five drops of the maximum concentration (100 μg of latex protein per dose) three times a week for nine weeks. All subjects reached the maintenance dose. Of the 1044 administered doses, 257 (24.6%) produced adverse reactions, of which 21.4% were local in nature. The occurrence of adverse reactions was identical during the build-up and maintenance phases (25%). Systemic reactions were observed in 3.6% of the doses (38/1044) and required treatment in about half of them (55%). The glove-use test improved significantly after 5 days and 10 weeks of treatment ($p = 0.003$, $p = 0.0004$ respectively), whereas the rubbing test improved significantly only after 10 weeks of treatment ($p = 0.04$). No change was detected for cutaneous reactivity on PST.

In 2007, a double-blind placebo-controlled study of rush SLIT in subjects with latex-induced urticaria ($n = 30$) or asthma ($n = 10$) was reported (94). Results were available for 35 subjects, 18 treated with latex SLIT, and 17 with placebo. Treatment consisted of five concentrations of ALK-Abelló latex: 5×10^{-8}, 5×10^{-5}, 5×10^{-2}, 5, and 500 μg/ mL administered according to the sublingual-swallow technique. The induction phase lasted four days and the maintenance dose was then taken at home three times per week for 12 months. The daily symptom scores were significantly improved at 12 months in the active group, with a mean decrease of 74.1% ($p < 0.05$), and were significantly different ($p < 0.05$) than the improvement in the placebo group, mean decrease in symptom score 44.8%. Glove provocation test score was not different at baseline but significantly different at 12 months, with the active group having lower mean score than the placebo group, 1.8 versus 2.9 ($p < 0.05$). There were no significant differences between the groups in PST to latex, serum latex-IgE antibody levels, and bronchial provocation tests at 12 months. Latex SLIT was well tolerated, with limited adverse reactions in three subjects in the active group (17%) compared with one subject in the placebo group (6%). The mild symptoms occurred during the induction phase in the active group and included mouth itching and burning (2 subjects) and lip swelling (1 subject). This report highlighted a better safety profile of latex SLIT in comparison with SCIT with latex. Nevertheless, latex SLIT is also capable of inducing serious anaphylactic reactions, as reported in a single subject undergoing rush SLIT (95).

Bernardini et al. reported a randomized, double-blind, placebo-controlled 12-month trial of latex SLIT in 26 children, aged 4 to 15 years, with nonanaphylactic latex allergy (96). Eighteen children had surgical procedures in the past and 14 had latex-fruit syndrome. Twelve children were randomized to SLIT with a commercial latex extract (ALK-Abelló) and eight to placebo; an additional six children with latex allergy were enrolled as untreated controls. No side effects related to treatments were observed. The build-up phase was conducted over four days according to a rush protocol. During the first week of the maintenance phase, subjects received doses in the hospital, followed by home administration for the rest of the study duration. The drops were kept under the tongue for three minutes and subsequently swallowed; maintenance dose was two drops containing 40 μg of latex protein once a day for 12 months. A significant improvement of glove-use and rubbing test symptom score in SLIT subjects was observed at three months ($p = 0.01$). This improvement became more significant following 12 months of SLIT treatment ($p = 0.0005$). In comparison with placebo, significant improvements were observed starting at nine months ($p = 0.015$) and also at 12 months ($p = 0.005$). The number of foods triggering oral allergy symptoms increased in placebo and control subjects but not in active-treated patients ($p = 0.05$). No significant changes were observed among the groups regarding percutaneous skin test reactivity to latex, glove prick-prick test, and serum latex-specific IgE antibody levels. In this population of children with non-anaphylactic allergy to latex, latex SLIT appeared safe and resulted in symptomatic improvement, but objective immunological changes were not detected.

Together, current evidence with human studies of latex IT supports the concept of immunomodulation in the treatment of IgE-mediated latex allergy. SCIT or SLIT with natural latex extracts needs to be evaluated further regarding safety, optimal dosing, duration of therapy, and persistence of protective effect.

LATEX IMMUNOTHERAPY WITH HYPOALLERGENIC ALLERGENS

Studies have evaluated hypoallergenic (mutated) recombinant latex allergens for IT. In general, the strategies focus either on disruption of IgE-binding epitopes or generation of the peptides that retain CD4 T-cell reactivity (78,79,97–99). Known CD4 T-cell epitopes for major latex allergens, including Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Hev b 6.02, and Hev b 6.03 may serve as a basis for future peptide-based hypoallergenic vaccines (73,76,77,100).

SUMMARY

Food and latex allergies are active targets for developing ITs. Recombinant peanut major allergens engineered to decrease IgE binding and delivered within *E. coli* show great promise

in a murine model of peanut anaphylaxis. Human clinical trials are planned. Oral desensitization and SLIT with food extracts represent another approach being actively explored. Novel therapies must be carefully evaluated with respect to safety and long-lasting benefit on oral food tolerance before being applied in clinical practice. Currently, SCIT and SLIT with natural latex extract is considered an investigational therapy because of efficacy and safety concerns. Future approaches based on hypoallergenic mutated recombinant latex allergens are promising, but are at a very early stage of development.

SALIENT POINTS

- At present, immunotherapeutic approaches for food and latex allergy are primarily investigational.
- SCIT with native food allergens has essentially been abandoned for safety concerns.
- Oral IT with native food proteins has been reported to increase tolerance and provide protection from accidental ingestions. However, efficacy, appropriate IT schedules, dosing, duration, the immunological mechanisms, and the permanence of the protective effect remain to be determined.
- Sublingual IT with native food extracts (hazelnut, cow's milk) demonstrates increases in tolerated dose; more studies are needed to address the same concerns raised for oral desensitization.
- Murine models of food allergy are being used to evaluate additional approaches to food IT, including recombinant food proteins, plasmid DNA encoding recombinant food proteins, and immunostimulatory sequence-conjugated food allergens.
- Vaccine containing mutated (hypoallergenic) recombinant major peanut allergens Ara h 1, 2, and 3 expressed in nonpathogenic *E. coli* was administered rectally in peanut-allergic mice and produced significant protection from oral ingestion of peanut and decreases in peanut-specific IgE; these effects were long lasting. Human vaccine is under the Food and Drug Administration (FDA) review in preparation for phase I clinical trials.
- Birch pollen SCIT produces improvement of oral allergy to raw apple in a subset of subjects. Doses higher than typically used for birch pollen IT may be necessary to treat apple allergy.
- IT with recombinant plant food allergens may represent a superior approach to treatment of PFAS.
- Subcutaneous and sublingual native latex IT was effective in reducing symptoms on latex exposure in a subset of latex-allergic HCWs and children.
- Mutated recombinant latex allergens are being evaluated as a potential approach for latex IT.

REFERENCES

1. Sicherer SH, Sampson HA. 9. Food allergy. J Allergy Clin Immunol 2006; 117(2 suppl Mini-Primer): S470–S475.
2. Yocum MW, Butterfield JH, Klein JS, et al. Epidemiology of anaphylaxis in Olmested County: a population-based study. J Allergy Clin Immunol 1999; 104:452–456.
3. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. J Allergy Clin Immunol 2003; 112(6):1203–1207.
4. Munoz-Furlong A, Sampson HA, Sicherer SH. Prevalence of self-reported seafood allergy in the US. J Allergy Clin Immunol 2004; 113:S100.
5. Sicherer SH. Clinical implications of cross-reactive food allergens. J Allergy Clin Immunol 2001; 108 (6):881–890.
6. Wood RA. The natural history of food allergy. Pediatrics 2003; 111(6 pt 3):1631–1637.
7. Fleischer DM, Conover-Walker MK, Matsui EC, et al. The natural history of tree nut allergy. J Allergy Clin Immunol 2005; 116(5):1087–1093.

8. Grundy J, Matthews S, Bateman B, et al. Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. J Allergy Clin Immunol 2002; 110(5):784–789.
9. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001; 107(1):191–193.
10. Taylor JS, Erkek E. Latex allergy: diagnosis and management. Dermatol Ther 2004; 17(4):289–301.
11. Oppenheimer JJ, Nelson HS, Bock SA, et al. Treatment of peanut allergy with rush immunotherapy. J Allergy Clin Immunol 1992; 90(2):256–262.
12. Nelson HS, Lahr J, Rule R, et al. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. J Allergy Clin Immunol 1997; 99(6 pt 1):744–751.
13. Valenta R, Kraft D. Type I allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. J Allergy Clin Immunol 1996; 97:895.
14. Asero R. Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients. Clin Exp Allergy 1998; 28:1368–1373.
15. Asero R. How long does the effect of birch pollen injection SIT on apple allergy last? Allergy 2003; 58 (5):435–438.
16. Bucher X, Pichler WJ, Dahinden CA, et al. Effect of tree pollen specific, subcutaneous immunotherapy on the oral allergy syndrome to apple and hazelnut. Allergy 2004; 59(12):1272–1276.
17. Bolhaar ST, Tiemessen MM, Zuidmeer L, et al. Efficacy of birch-pollen immunotherapy on cross-reactive food allergy confirmed by skin tests and double-blind food challenges. Clin Exp Allergy 2004; 34(5):761–769.
18. Alonso R, Enrique E, Pineda F, et al. An observational study on outgrowing food allergy during non-birch pollen-specific, subcutaneous immunotherapy. Int Arch Allergy Immunol 2007; 143(3): 185–189.
19. Kinaciyan T, Jahn-Schmid B, Radakovics A, et al. Successful sublingual immunotherapy with birch pollen has limited effects on concomitant food allergy to apple and the immune response to the Bet v 1 homolog Mal d 1. J Allergy Clin Immunol 2007; 119(4):937–943.
20. Asero R. Effects of birch pollen SIT on apple allergy: a matter of dosage? Allergy 2004; 59(12):1269–1271.
21. Bohle B, Radakovics A, Luttkopf D, et al. Characterization of the T cell response to the major hazelnut allergen, Cor a 1.04: evidence for a relevant T cell epitope not cross-reactive with homologous pollen allergens. Clin Exp Allergy 2005; 35(10):1392–1399.
22. Rolinck-Werninghaus C, Staden U, Mehl A, et al. Specific oral tolerance induction with food in children: transient or persistent effect on food allergy? Allergy 2005; 60(10):1320–1322.
23. Niggemann B, Staden U, Rolinck-Werninghaus C, et al. Specific oral tolerance induction in food allergy. Allergy 2006; 61(7):808–811.
24. Ko J, Mayer L. Oral tolerance: lessons on treatment of food allergy. Eur J Gastroenterol Hepatol 2005; 17(12):1299–1303.
25. Patriarca G, Nucera E, Roncallo C, et al. Oral desensitizing treatment in food allergy: clinical and immunological results. Aliment Pharmacol Ther 2003; 17(3):459–465.
26. Meglio P, Bartone E, Plantamura M, et al. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy 2004; 59(9):980–987.
27. Buchanan AD, Green TD, Jones SM, et al. Egg oral immunotherapy in nonanaphylactic children with egg allergy. J Allergy Clin Immunol 2007; 119(1):199–205.
28. Calvani M, Sopo SM. Exercise-induced anaphylaxis caused by wheat during specific oral tolerance induction. Ann Allergy Asthma Immunol 2007; 98(1):98–99.
29. Caminiti L, Passalacqua G, Vita D, et al. Food-exercise-induced anaphylaxis in a boy successfully desensitized to cow milk. Allergy 2007; 62(3):335–336.
30. Patriarca C, Romano A, Venuti A, et al. Oral specific hyposensitization in the management of patients allergic to food. Allergol Immunopathol (Madr) 1984; 12(4):275–281.
31. Shenassa MM, Perelmutter L, Gerarrd JW. Desensitization to peanut. J Allergy Clin Immunol 1985; 75:177S.
32. Patriarca G, Schiavino D, Nucera E, et al. Food allergy in children: results of a standardized protocol for oral desensitization 1. Hepatogastroenterology 1998; 45(19):52–58.
33. Bauer A, Ekanayake MS, Wigger-Alberti W, et al. Oral rush desensitization to milk. Allergy 1999; 54 (8):894–895.
34. Nucera E, Schiavino D, D'Ambrosio C, et al. Immunological aspects of oral desensitization in food allergy. Dig Dis Sci 2000; 45(3):637–641.
35. Rueff F, Eberlein-Konig B, Przybilla B. Oral hyposensitization with celery juice. Allergy 2001; 56 (1):82–83.
36. Patriarca G, Nucera E, Pollastrini E, et al. Oral rush desensitization in peanut allergy: a case report. Dig Dis Sci 2006; 51(3):471–473.
37. Buchanan AD, Scurlock AM, Jone SM, et al. Oral desensitization and induction of tolerance in peanut-allergic children. J Allergy Clin Immunol 2006; 117(2):S327.

38. Mansfield L. Successful oral desensitization for systemic peanut allergy. Ann Allergy Asthma Immunol 2006; 97(2):266–267.
39. Morisset M, Moneret-Vautrin DA, Guenard L, et al. Oral desensitization in children with milk and egg allergies obtains recovery in a significant proportion of cases. A randomized study in 60 children with cow's milk allergy and 90 children with egg allergy. Allerg Immunol (Paris) 2007; 39(1):12–19.
40. Mempel M, Rakoski J, Ring J, et al. Severe anaphylaxis to kiwi fruit: Immunologic changes related to successful sublingual allergen immunotherapy. J Allergy Clin Immunol 2003; 111(6):1406–1409.
41. Kerzl R, Simonowa A, Ring J, et al. Life-threatening anaphylaxis to kiwi fruit: protective sublingual allergen immunotherapy effect persists even after discontinuation. J Allergy Clin Immunol 2007; 119 (2):507–508.
42. Enrique E, Pineda F, Malek T, et al. Sublingual immunotherapy for hazelnut food allergy: a randomized, double-blind, placebo-controlled study with a standardized hazelnut extract. J Allergy Clin Immunol 2005; 116(5):1073–1079.
43. De Boissieu D, Dupont C. Sublingual immunotherapy for cow's milk protein allergy: a preliminary report. Allergy 2006; 61(10):1238–1239.
44. Valenta R, Vrtala S, Laffer S, et al. Recombinant allergens. Allergy 1998; 53(6):552–561.
45. Reuter A, Lidholm J, Andersson K, et al. A critical assessment of allergen component-based in vitro diagnosis in cherry allergy across Europe. Clin Exp Allergy 2006; 36(6):815–823.
46. Bolhaar ST, Zuidmeer L, Ma Y, et al. A mutant of the major apple allergen, Mal d 1, demonstrating hypo-allergenicity in the target organ by double-blind placebo-controlled food challenge. Clin Exp Allergy 2005; 35(12):1638–1644.
47. Astier C, Morisset M, Roitel O, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol 2006; 118(1):250–256.
48. Li XM, Schofield BH, Huang CK, et al. A murine model of IgE-mediated cow's milk hypersensitivity. J Allergy Clin Immunol 1999; 103(2 pt 1):206–214.
49. Li XM, Serebrisky D, Lee SY, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. J Allergy Clin Immunol 2000; 106(1 pt 1):150–158.
50. Li X, Huang CK, Schofield BH, et al. Strain-dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. J Immunol 1999; 162(5):3045–3052.
51. Roy K, Mao HQ, Huang SK, et al. Oral gene delivery with chitosan–DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat Med 1999; 5(4):387–391.
52. Srivastava K, Li XM, Bannon GA, et al. Investigation of the use of ISS-linked Ara h2 for the treatment of peanut-induced allergy. J Allergy Clin Immunol 2001; 107:S233 (abstract).
53. Nguyen MD, Cinman N, Yen J, et al. DNA-based vaccination for the treatment of food allergy. Allergy 2001; 56(suppl 67):127–130.
54. Burks AW, Cockrell G, Stanley JS, et al. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. J Clin Invest 1995; 96(4):1715–1721.
55. Stanley JS, King N, Burks AW, et al. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. Arch Biochem Biophys 1997; 342(2): 244–253.
56. Rabjohn P, Helm EM, Stanley JS, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. J Clin Invest 1999; 103(4):535–542.
57. Bannon GA, Cockrell G, Connaughton C, et al. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. Int Arch Allergy Immunol 2001; 124(1–3):70–72.
58. King N, Helm R, Stanley JS, et al. Allergenic characteristics of a modified peanut allergen. Mol Nutr Food Res 2005; 49(10):963–971.
59. Swoboda I, Bugajska-Schretter A, Linhart B, et al. A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. J Immunol 2007; 178(10):6290–6296.
60. Vrtala S, Hirtenlehner K, Susani M, et al. Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. FASEB J 2001; 15(11):2045–2047.
61. Reese G, Ballmer-Weber BK, Wangorsch A, et al. Allergenicity and antigenicity of wild-type and mutant, monomeric, and dimeric carrot major allergen Dau c 1: destruction of conformation, not oligomerization, is the roadmap to save allergen vaccines. J Allergy Clin Immunol 2007; 119(4):944–951.
62. Srivastava KD, Li XM, King N, et al. Immunotherapy with modified peanut allergens in a murine model of peanut allergy. J Allergy Clin Immunol 2002; 109:S287.
63. Yeung VP, Gieni RS, Umetsu DT, et al. Heat-killed *Listeria monocytogene* as an adjuvant converts established murine TH2-dominated immune responses into TH1-dominated responses. J Immunol 1998; 161:4146–4152.
64. Frick OL, Bachanan BB, Delval G, et al. Allergen immunotherapy with heat-killed *Listeria monocytogenes* as adjuvant prevents allergeic reactions in highly sensitized dogs. J Allergy Clin Immunol 2001; 107:S232 (abstract).

65. Li XM, Srivastava K, Huleatt JW, et al. Engineered recombinant peanut protein and heat-killed Listeria monocytogenes coadministration protects against peanut-induced anaphylaxis in a murine model. J Immunol 2003; 170(6):3289–3295.
66. Li XM, Srivastava K, Grishin A, et al. Persistent protective effect of heat-killed Escherichia coli producing "engineered," recombinant peanut proteins in a murine model of peanut allergy. J Allergy Clin Immunol 2003; 112(1):159–167.
67. Leung DY, Sampson HA, Yunginger JW, et al. Effect of anti-IgE therapy in patients with peanut allergy. N Engl J Med 2003; 348(11):986–993.
68. Li XM. Traditional Chinese herbal remedies for asthma and food allergy. J Allergy Clin Immunol 2007; 120:25–31.
69. Hsieh KY, Hsu CI, Lin JY, et al. Oral administration of an edible-mushroom-derived protein inhibits the development of food-allergic reactions in mice. Clin Exp Allergy 2003; 33(11):1595–1602.
70. Lee SY, Huang CK, Zhang TF, et al. Oral administration of IL-12 suppresses anaphylactic reactions in a murine model of peanut hypersensitivity. Clin Immunol 2001; 101(2):220–228.
71. Garabrant DH, Roth HD, Parsad R, et al. Latex sensitization in health care workers and in the US general population. Am J Epidemiol 2001; 153(6):515–522.
72. Slater JE. Latex allergens. Clin Allergy Immunol 2004; 18:369–386.
73. Banerjee B, Kanitpong K, Fink JN, et al. Unique and shared IgE epitopes of Hev b 1 and Hev b 3 in latex allergy. Mol Immunol 2000; 37(12–13):789–798.
74. Raulf-Heimsoth M, Chen Z, Rihs HP, et al. Analysis of T-cell reactive regions and HLA-DR4 binding motifs on the latex allergen Hev b 1 (rubber elongation factor). Clin Exp Allergy 1998; 28(3):339–348.
75. Palomares O, Villalba M, Quiralte J, et al. 1,3-beta-glucanases as candidates in latex-pollen-vegetable food cross-reactivity. Clin Exp Allergy 2005; 35(3):345–351.
76. Bohle B, Wagner B, Vollmann U, et al. Characterization of T cell responses to Hev b 3, an allergen associated with latex allergy in spina bifida patients. J Immunol 2000; 164(8):4393–4398.
77. de Silva HD, Sutherland MF, Suphioglu C, et al. Human T-cell epitopes of the latex allergen Hev b 5 in health care workers. J Allergy Clin Immunol 2000; 105(5):1017–1024.
78. Drew AC, Eusebius NP, Kenins L, et al. Hypoallergenic variants of the major latex allergen Hev b 6.01 retaining human T lymphocyte reactivity. J Immunol 2004; 173(9):5872–5879.
79. Karisola P, Mikkola J, Kalkkinen N, et al. Construction of hevein (Hev b 6.02) with reduced allergenicity for immunotherapy of latex allergy by comutation of six amino acid residues on the conformational IgE epitopes. J Immunol 2004; 172(4):2621–2628.
80. Karisola P, Kotovuori A, Poikonen S, et al. Isolated hevein-like domains, but not 31-kd endochitinases, are responsible for IgE-mediated in vitro and in vivo reactions in latex-fruit syndrome. J Allergy Clin Immunol 2005; 115(3):598–605.
81. Wagner S, Breiteneder H, Simon-Nobbe B, et al. Hev b 9, an enolase and a new cross-reactive allergen from hevea latex and molds. Purification, characterization, cloning and expression. Eur J Biochem 2000; 267(24):7006–7014.
82. Wagner S, Sowka S, Mayer C, et al. Identification of a Hevea brasiliensis latex manganese superoxide dismutase (Hev b 10) as a cross-reactive allergen. Int Arch Allergy Immunol 2001; 125(2):120–127.
83. Yeang HY, Cheong KF, Sunderasan E, et al. The 14.6 kd rubber elongation factor (Hev b 1) and 24 kd (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. J Allergy Clin Immunol 1996; 98(3):628–639.
84. Yeang HY, Arif SA, Raulf-Heimsoth M, et al. Hev b 5 and Hev b 13 as allergen markers to estimate the allergenic potency of latex gloves. J Allergy Clin Immunol 2004; 114(3):593–598.
85. Pereira C, Rico P, Lourenco M, et al. Specific immunotherapy for occupational latex allergy. Allergy 1999; 54(3):291–293.
86. Nucera E, Schiavino D, Pollastrini E, et al. Desensitization to latex by percutaneous route. J Investig Allergol Clin Immunol 2002; 12(2):134–135.
87. Pereira C, Pedro E, Tavares B, et al. Specific immunotherapy for severe latex allergy. Allerg Immunol (Paris) 2003; 35(6):217–225.
88. Leynadier F, Herman D, Vervloet D, et al. Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. J Allergy Clin Immunol 2000; 106(3):585–590.
89. Sastre J, Fernandez-Nieto M, Rico P, et al. Specific immunotherapy with a standardized latex extract in allergic workers: a double-blind, placebo-controlled study. J Allergy Clin Immunol 2003; 111(5): 985–994.
90. Sastre J, Raulf-Heimsoth M, Rihs HP, et al. IgE reactivity to latex allergens among sensitized healthcare workers before and after immunotherapy with latex. Allergy 2006; 61(2):206–210.
91. Tabar AI, Anda M, Bonifazi F, et al. Specific immunotherapy with standardized latex extract versus placebo in latex-allergic patients. Int Arch Allergy Immunol 2006; 141(4):369–376.
92. Patriarca G, Nucera E, Pollastrini E, et al. Sublingual desensitization: a new approach to latex allergy problem. Anesth Analg 2002; 95(4):956–960.

93. Cistero BA, Sastre J, Enrique E, et al. Tolerance and effects on skin reactivity to latex of sublingual rush immunotherapy with a latex extract. J Investig Allergol Clin Immunol 2004; 14(1):17–25.
94. Nettis E, Colanardi MC, Soccio AL, et al. Double-blind, placebo-controlled study of sublingual immunotherapy in patients with latex-induced urticaria: a 12-month study. Br J Dermatol 2007; 156 (4):674–681.
95. Antico A, Pagani M, Crema A. Anaphylaxis by latex sublingual immunotherapy. Allergy 2006; 61 (10):1236–1237.
96. Bernardini R, Campodonico P, Burastero S, et al. Sublingual immunotherapy with a latex extract in paediatric patients: a double-blind, placebo-controlled study. Curr Med Res Opin 2006; 22 (8):1515–1522.
97. Rolland JM, Drew AC, O'hehir RE. Advances in development of hypoallergenic latex immunotherapy. Curr Opin Allergy Clin Immunol 2005; 5(6):544–551.
98. Arif SA, Hamilton RG, Yusof F, et al. Isolation and characterization of the early nodule-specific protein homologue (Hev b 13), an allergenic lipolytic esterase from Hevea brasiliensis latex. J Biol Chem 2004; 279(23):23933–23941.
99. Yip L, Hickey V, Wagner B, et al. Skin prick test reactivity to recombinant latex allergens. Int Arch Allergy Immunol 2000; 121(4):292–299.
100. de Silva HD, Gardner LM, Drew AC, et al. The hevein domain of the major latex-glove allergen Hev b 6.01 contains dominant T cell reactive sites. Clin Exp Allergy 2004; 34(4):611–618.

28 Unproven and Controversial Forms of Immunotherapy

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INTRODUCTION

Specific allergen immunotherapy is the accepted practice among allergy specialists throughout the world for the treatment of selected patients with respiratory atopic allergy or Hymenoptera venom anaphylaxis. The majority of placebo-controlled clinical trials document its efficacy and, therefore, support its use.

This treatment evolved from the preseasonal grass pollen injections recommended empirically by Noon and Freeman in 1910. Efforts to improve allergen immunotherapy are the result of the process being time consuming, necessitating cost, and having potentially serious adverse effects, including death. Such efforts include changes in the structure of the administered allergen, the use of immunological adjuvants, and different routes of administration. The precise mechanism by which allergen immunotherapy renders the allergic patient clinically tolerant to ambient allergen exposure remains elusive.

The purpose of this chapter is to describe certain unconventional or "alternative" methods of allergen immunotherapy (Table 1) that have been tried but cannot be recommended because they are (*i*) unproven, (*ii*) unscientific in concept, and/or (*iii*) characterized by an unfavorable risk/benefit ratio.

IMMUNOTHERAPY BASED ON SERIAL ENDPOINT TITRATION

Serial endpoint titration refers to a method that uses semiquantitative skin testing to establish the dose of allergen for initiating and optimizing injection treatment of allergic disease. Rinkel, whose name is typically associated with this method of immunotherapy, developed this technique, which others modified. The "Rinkel" method was recommended originally for treatment of respiratory diseases caused by the common inhalant allergens (pollens, molds, dust). It was subsequently recommended for the treatment of food allergy (1–4). It is currently favored by a subset of otolarygologists in the United States, who include allergy practice in their specialty.

Rinkel's method of testing uses serial fivefold decreasing dilutions (i.e., increasing concentrations) of allergen. Allergen extracts are injected intradermally in a volume of 0.01 mL until an "endpoint is achieved." The wheal diameter is recorded 10 minutes after injection (5,6). The "endpoint" of the test (i.e., a "positive" test) for each allergen is the lowest concentration that results in a 2-mm or greater increase in wheal diameter compared with the prior fivefold dilution. As many as nine serial intradermal injections are generally administered. The initial test dose is very low, generally 1:1,953,125 of the concentrated allergen.

Certain features of this testing protocol must be considered in assessing its relationship to treatment. The presence or absence of erythema accompanying the wheal is ignored (7), which could lead to false-positive results. The use of a latent period of only 10 minutes for an IgE-mediated allergic skin test reaction could result in a false-negative interpretation. In some cases this testing method does not produce the expected progressive increase in wheal diameters. These variations are referred to as bizarre, hour-glass, plateau, and flash responses (7). Proponents of the Rinkel method attribute these variations to extraneous factors such as concurrent infection, airborne allergen exposure, or incidental food allergy. There is no proof of such associations.

Table 1 Unconventional Forms of Immunotherapy

Serial endpoint titration
Neutralization or symptom-relieving therapy
Enzyme-potentiated desensitization
Autogenous urine injections

The endpoint, as it is defined above, is considered to be a safe dose to initiate immunotherapy for that particular allergen and patient. This procedure is a safe method for preventing a systemic reaction to the first subcutaneous treatment dose, although the dose is almost always too conservative (i.e., the allergen solution is excessively dilute). The low, initial dose unnecessarily prolongs the course of treatment (8–11).

In addition to establishing the initial dose of immunotherapy, an "optimal" dose is calculated as certain arbitrary multiples of the endpoint, usually between 25 and 50 times the quantity of allergen producing the endpoint. Practitioners of this procedure may vary these multiples empirically, depending on the allergen.

The optimal dose, as determined in this way, is claimed to be the dose at which symptoms will be controlled during immunotherapy. Clinical trials, however, have shown that such a calculated optimal immunotherapy dose is almost always too low, so that treatment based on the endpoint procedure leads to therapy that is ultimately no more effective than placebo (12).

Proponents of the Rinkel method recommend retesting during the course of immunotherapy to establish a new endpoint if the patient fails to improve as expected. There are no clinical studies to validate this recommendation.

PROVOCATION-NEUTRALIZATION

"Neutralization" (also called "symptom-relieving" or "tolerance") therapy is also based on a testing procedure related to the method of treatment. The testing procedure is known as provocation-neutralization, which evolved from serial endpoint titration described in the previous section. It is based on the concept that an extremely small quantity of allergen can cause the immediate appearance of a symptomatic allergic reaction; subsequently, the prompt disappearance (neutralization) of ongoing allergic symptoms may occur. In actual practice, the symptoms that are provoked and cleared in this way are subjective, nonspecific, and not consistent with those symptoms widely recognized in allergic disease (13–18). Objective physical signs are ignored.

Provocation-neutralization testing is performed in a manner similar to skin endpoint titration, using increasing or decreasing fivefold serial dilutions of the allergen (4). Many practitioners of this procedure use skin test extracts that include not only the usual inhalant and food allergens, but also solutions of environmental chemicals, drugs, hormones, and many other items that are unlikely to cause atopic disease.

Testing is performed by exposing the patient to the allergen via the intracutaneous, subcutaneous, or sublingual route. Injections are given in the arm. There is no rational explanation for selecting the route of administration. Intracutaneous testing volumes are 0.01, 0.02, or 0.05 mL. The patient keeps a written record of all "sensations" (i.e., any symptom) experienced over a 10-minute period following each injection or sublingual drop. There is no standardized protocol for grading the subjective response, so any symptom or sensation reported by the patient constitutes a positive test result. If the patient reports no symptoms, higher doses are administered in a serial fashion until symptoms are reported. Once a test result is considered positive, further testing proceeds by progressively lower concentrations until a dose is reached at which the patient reports no sensations. This is considered to be the neutralizing dose, which is then used for subsequent treatment.

Each allergen or other test substance must be given separately in a serial fashion, so complete testing can require many days, weeks, or months. No negative controls are included, and there is no provision to account for spontaneous symptoms.

There are variations on this basic protocol. Wheal diameter may be used in addition to subjective symptoms in determining a positive response, but there are no published criteria for wheal sizes indicating a positive or negative result (14). Some practitioners of provocation-neutralization use the absence of symptoms as a positive test (14,16). In this scheme, a negative test is followed by serially lowering the subsequent doses; after a positive test, the dosages are increased until a negative (neutralizing) dose is achieved.

The sublingual route for provocation-neutralization is used especially—although not exclusively—to diagnose food allergy.

The neutralizing doses of one or more tested substances are then self-administered by the patient as treatment. Where more than one substance is required for treatment, they can be used separately or in combination. Treatment can be administered intracutaneously, subcutaneously, or sublingually. The choice is arbitrary, because there are no established protocols and no published clinical trials. The patient is advised to administer the neutralizing solution either after symptoms appear or before anticipated exposure to a substance that the patient believes is the cause of the illness. Treatment can also be given on a regular maintenance schedule, usually daily or twice weekly.

Historically, this procedure evolved from the serial intradermal endpoint technique, and certain theories have been offered to justify the results. It has been claimed that allergen is present in the injected wheal and released into the systemic circulation from which it elicits symptoms (13). However, the minute quantity of allergen and the nonallergic nature of induced symptoms make this theory unlikely. Another hypothesis states that allergen introduced into the skin or under the tongue induces antibody formation with the development of circulating immune complexes, but the kinetics and time required for these events make this an untenable scenario. Other theories postulate antigen stimulation, suppression of lymphocyte function, and/or induction of immunological tolerance. Sublingual "desensitization" of lymphocytes has also been postulated as a consequence of antigen absorption from the sublingual route that bypasses its gastrointestinal metabolism. There have been no published results of experiments to test any of these theories.

Neutralizing therapy has been recommended for treating a wide variety of conditions, including atopic asthma, rheumatic diseases, premenstrual syndrome, viral infections, headache, musculoskeletal complaints, attention deficit disorder in children, and others. Neutralizing antigens include extracts of known allergens, environmental chemicals, hormones, viral vaccines, foods, histamine, serotonin, saline, and even distilled water.

Published clinical trials of neutralization therapy are few in number (17–25). One preliminary report of a double-blind, placebo-controlled crossover study of subcutaneous injections of foods administered daily to eight patients revealed improvement with both placebo and active vaccines, but the results from the latter were said to be superior (21). Another report claimed both subjective and objective improvement in 20 patients with perennial rhinitis treated with sublingual dust vaccine. These results are of questionable significance, since the duration of the study period was only two weeks, and five of the subjects were, in fact, not allergic to the house-dust mite, as determined by the investigators reporting the study (25).

ENZYME-POTENTIATED DESENSITIZATION

In 1973, McEwen reported that the enzyme beta-glucuronidase acts as an adjuvant or promoter of an immune response when added to the antigen immediately before injection (26). Since then, a small number of allergists have recommended a procedure known as enzyme-potentiated desensitization (EPD) as an improvement over conventional immunotherapy, claiming that it requires many fewer injections compared with conventional immunotherapy and has 80% effectiveness.

A very low dose of allergen (1–2.5 Noon units), which is approximately the amount delivered into the skin in a standard prick test, is mixed with a partially purified enzyme, beta-glucuronidase, in a dose (100 Fishman units, <40 μg) equivalent to the amount of enzyme normally present in 4 mL of human blood. The mixture is immediately injected intradermally in a volume of approximately 0.125 mL. This is considered sufficient immunization as a single dose, preseasonally, to produce a therapeutic effect for an entire pollen season. For perennial

allergy, the intradermal injections are given every two to six months. Both inhalant and food allergens have been used in this fashion. A single intradermal injection may contain as many as 150 allergens, typically including inhalants, foods, and certain food additives.

Proponents of this form of treatment have claimed success in treating not only allergic rhinitis, asthma, and eczema, but also sinusitis, nasal polyposis, urticaria, migraine headaches, ulcerative colitis, irritable bowel syndrome, chronic fatigue syndrome, "immune dysfunction," attention-deficit hyperactivity disorder, anxiety, rheumatoid arthritis, grand mal and petit mal seizures, and anaphylaxis from food allergy.

None of the published research findings in patients treated by EPD substantiate this theory. The effectiveness of this method and the presumed pharmacological property of beta-glucuronidase on the immune system are based on anecdotal evidence or clinical trials of generally inferior quality. Several published double-blind reports claim symptomatic improvements in adults or children with allergic rhinitis or asthma along with conflicting results of immunological changes (27–34). These studies suffer from a number of limitations, including small sample sizes, brief follow-up periods, and/or lack of objective measures of disease activity. The most rigorously performed double-blind study, which included a sample size of 183 subjects and objective outcome measures, showed no benefit to EPD in comparison with placebo for children with allergic rhinitis (34).

The proponents of EPD hypothesize that the enzyme recruits and activates a new population of CD8 lymphocytes that suppress or downregulate the response to the injected antigens, thereby suppressing the immune response. The claim that this method of treatment requires infrequent injections of allergen is based on the supposition that specific "suppressor" CD8 T cells persist for up to two years. When prescribed for perennial allergies, the first few injections are given every two months, after which the frequency may decrease to as little as once or twice yearly. For treatment of seasonal pollen allergy, a single dose is given not more than four months before the expected arrival of the season. Boosting doses are given as required. The effectiveness for house-dust allergy is said to be evident almost immediately, for hay fever after three to four weeks and for food allergy after six to nine months.

Advocates of this treatment frequently require their patients to follow certain rules to avoid treatment failure. The patients must not be exposed to allergens for which they are being treated for a period of 24 hours before and 48 hours after the injection. They must consume a special "EPD diet" of lamb, sweet potatoes, carrots, celery, lettuce, sago, tapioca, rhubarb, sea salt, and bottled water for 24 hours before and 48 hours after the injection. They are prescribed specific vitamins and minerals. The injection is given only during the first two weeks of the menstrual cycle, and a number of specified medications must be avoided. It is not to be used during pregnancy or within five days of an upper respiratory infection. The patient must not use scented products or ointments on the skin near the injection site. Exposure to heat, stress, environmental chemicals, smoke, air-conditioning, newsprint, and photocopiers must be avoided. Efficacy is also believed to be enhanced by taking zinc, folic acid, vitamin A, pyridoxine, and magnesium orally or intravenously for several days before the injection. Delayed reactions, described as a temporary return of the allergic symptoms for which the patient is being treated, are considered a favorable sign that the treatment will be effective.

AUTOGENOUS URINE IMMUNOTHERAPY

In the early 1930s, several medical publications appeared claiming that a specific substance, called "proteose," is present in the urine during the course of allergic disease (35,36). Urinary proteose refers to a mixture of partially to completely hydrolyzed protein from the glomerular filtrate. It is therefore postulated to contain allergen peptide fragments and, in particular, those peptides that are "specific" or most allergenic for each individual allergic person. This substance was believed to be a source of allergen for therapy superior to the usual allergen vaccines used in immunotherapy.

Several chemical extraction procedures were recommended for obtaining proteose from the urine of allergic patients. The extract was suspended in a buffered solution and then used for intradermal testing and for subcutaneous therapeutic injections. This practice seemed to thrive briefly in the mid-1900s, subsided after several years, and then resurfaced in the latter half of the 20th century.

The published reports consist of uncontrolled, anecdotal histories of apparently successful treatment of a variety of allergic conditions, including asthma, rhinitis, anaphylaxis, urticaria, angioedema, and serum sickness (37–39). None of these studies used proper controls and therefore cannot be used to show efficacy.

There has been no investigation of long-term safety. This is a critical issue, since small quantities of glomerular basement membrane antigens are found in normal urine. It is not unreasonable to assume that alteration by chemical treatment during the extraction process could lead to the production of altered renal proteins that might prove to be antigenic for the induction of autoantibodies, which potentially might result in autoimmune nephropathy.

SCOPE OF THE PROBLEM

Some of the unproven treatment methods discussed above, such as autogenous urine immunotherapy, are rarely used today. Others, however, persist (40). In particular, neutralization therapy using either the injection or sublingual route and EPD form an important part of the practice of those who subscribe to theories whereby certain people are believed to react to ordinary or even exceedingly minute exposures to common environmental items that can be detected by odor, such as perfumes, organic solvents, and other ubiquitous chemicals. The clinical manifestations of this condition are numerous but entirely subjective (41). Extracts of chemicals and foods are typically included in the neutralizing or "enzyme-potentiating" treatment. This "condition" has never been shown to be caused by chemicals or to involve a physical sensitivity (42), and evidence abounds that psychological factors are important in these beliefs (41).

COSTS TO THE HEALTH-CARE SYSTEM

There is no reliable method to assess or even estimate the cost of these unproven immunotherapy methods in either absolute amounts or as a percentage of the total health-care expenditure. Since they are controversial and not considered standard forms of medical practice, they are not listed or codified in the *Common Procedural Terminology* publication (43). Nonetheless, it is likely that the costs are substantial, considering that, in the United States, out-of-pocket expenditures for alternative therapies such as these are estimated to be approximately $27 billion, and rhinosinusitis and asthma are two of the most common conditions for which alternative therapies are sought (44). It is also likely that in most instances payment for these services in the United States is made by the patient directly to the practitioner and not by third-party payers, limiting the available data as to the total expenditure for unproven immunotherapy.

SALIENT POINTS

- The same controversial treatment is often claimed to be efficacious for a variety of unrelated illnesses.
- Theories in support of controversial allergy procedures frequently change.
- Controversial allergy treatments are often linked to unproven forms of allergy diagnostic testing.
- Clinicians should be familiar with unproven and controversial treatments and their pitfalls to properly advise their patients.
- Unproven treatments flourish in part because of the placebo effect inherent in every form of treatment.

REFERENCES

1. Rinkel HJ. The management of clinical allergy: Part II. Etiologic factors and skin titration. Arch Otolaryngol 1963; 77:42.

2. Rinkel HJ. The management of clinical allergy: Part III. Inhalation allergy therapy. Arch Otolaryngol 1963; 77:205.
3. Rinkel HJ. The management of clinical allergy: Part IV. Food and mold allergy. Arch Otolaryngol 1963; 77:302.
4. Rinkel HJ, Lee CH, Crown DW Jr., et al. The diagnosis of food allergy. Arch Otolaryngol 1964; 79:71.
5. Williams RI. Skin titration: testing and treatment. Otolaryngol Clin North Am 1971; 3:507.
6. Richardson AS. Titration; evaluation of an office system of allergy diagnosis and treatment: its use in otolaryngology. Ann Otol Rhinol Laryngol 1961; 70:344.
7. Willoughby JW. Serial dilution titration skin tests in inhalant allergy; a clinical quantitative assessment of biologic skin reactivity to allergenic extracts. Otolaryngol Clin North Am 1974; 7:579.
8. Hirsch S, Kalbfleisch JH, Golbert TM, et al. Rinkel method; a controlled study. Second report. J Allergy Clin Immunol 1980; 65:192.
9. Hirsch S, Kalbfleisch JH, Golbert TM, et al. Rinkel injection therapy; a multicenter controlled study. J Allergy Clin Immuno1 1981; 68:133.
10. Van Metre TE, Adkinson NF, Amodio FJ, et al. A comparative study of the effectiveness of the Rinkel method and the current standard method of immunotherapy for ragweed pollen hay fever. J Allergy Clin Immunol 1980; 66:500.
11. Van Metre TE, Adkinson NF, Lichtenstein LM, et al. A controlled study of the effectiveness of the Rinkel method of immunotherapy for ragweed pollen hay fever. J Allergy Clin Immunol 1980; 65:288.
12. Van Metre TE. Critique of controversial and unproven procedures for diagnosis and therapy of allergy disorders. Pediatr Clin North Am 1983; 30:807.
13. Morris DL. Use of sublingual antigen in diagnosis and treatment of food allergy. Ann Allergy 1971; 27:289.
14. Lee CH, Williams RT, Binkley EL. Provocative testing and treatment for foods. Arch Otolaryngol 1969; 90:87.
15. Breneman JC, Crook WC, Deamer W, et al. Report of the food allergy committee on the sublingual method of provocation testing for food allergy. Ann Allergy 1973; 31:382.
16. Willoughby JW. Provocative food test technique. Ann Allergy 1965; 23:543.
17. Lee CH, Williams RT, Binkley EL. Provocative inhalation testing and treatment. Arch Otolaryngol 1969; 90:173.
18. Kailin EW, Collier R. "Relieving" therapy for antigen exposure. JAMA 1971; 217:78.
19. Dickey LD, Pfeiffer G. Sublingual therapy in allergy. Trans Am Soc Ophthalmol Otolaryngol Allergy 1964; 5:37.
20. Warren CM. Inhalant allergy: diagnosis and treatment by provocation intracutaneous method. Med Dig 1978; 33.
21. Miller JB. A double-blind study of food extract injection therapy: a preliminary report. Ann Allergy 1977; 38:185.
22. Morris DL. Use of sublingual antigen in diagnosis and treatment of food allergy. Ann Allergy 1969; 27:289.
23. Morris DL. Treatment of respiratory disease with ultra-small doses of antigen. Ann Allergy 1970; 28:494.
24. Morris DL. Treatment of atopic dermatitis with tolerogenic doses of antigen. Acta Dermatovenerol 1980; 92(suppl):97.
25. Scadding GK, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to house dust mite. Clin Allergy 1986; 16:483.
26. McEwen LM, Nicholson M, Kitchen I, et al. Enzyme potentiated desensitization. III. Control by sugars and diols of the immunological effect of glucuronidase in mice and patients with hay fever. Ann Allergy 1973; 31:543.
27. Fell P, Brostoff J. A single dose desensitization for summer hay fever. Results of a double blind study-1988. Eur J Clin Pharmacol 1990; 38:77.
28. Cantani A, Ragno V, Monteleone MA, et al. Enzyme-potentiated desensitization in children with asthma and mite allergy: a double-blind study. J Investig Allergol Clin Immunol 1996; 6:270.
29. Astarita C, Scala G, Sproviero S, et al. Effects of enzyme-potentiated desensitization in the treatment of pollinosis: a double-blind placebo-controlled trial. J Investig Allergol Clin Immunol 1996; 6:248.
30. Di Stanisloa C, Di Berardino L, Bianchi I, et al. A double-blind, placebo controlled study of preventive immunotherapy with E.P.D., in the treatment of seasonal allergic disease. Allerg Immunol (Paris) 1997; 29:39–42.
31. Caramia G, Franceschini F, Cimarelli ZA, et al. The efficacy of E.P.D., a new immunotherapy, in the treatment of allergic diseases in children. Allerg Immunol (Paris) 1996; 28:308–310.
32. Di Stanislao C, Angelini F, Gagliardi MC, et al. Beta glucuronidase short-term immunotherapy. Allergy 2003; 58:459.

33. Galli E, Bassi MS, Mora E, et al. A double-blind randomized placebo-controlled trial with short-term β-glucuronidase therapy in children with chronic rhinoconjunctivitis and/or asthma due to dust mite allergy. J Investig Allergol Clin Immunol 2006; 16:345–350.
34. Radcliffe MJ, Lewith GT, Turner RG, et al. Enzyme potentiated desensitization in treatment of seasonal allergic rhinitis: double blind randomized controlled study. BMJ 2003; 327:251–254.
35. Steel RS. The specificity of urinary proteose. Med J Aust 1932; 2:800.
36. Oriel OH, Barber HW. Proteose in urine excreted in anaphylactic and allergic conditions. Lancet 1930; 2:1304.
37. Whitehead RW, Darley W, Dickman PA. Therapeutic use of urinary proteose. Colorado Med 1934; 56.
38. Liberman I, Bigland AD. Autogenous urinary proteose in asthma and other allergic conditions. Br Med J 1937; 1:62.
39. Plesch J. Urine therapy. Med Press 1947; 218:128.
40. Beyer K, Tueber SS. Food allergy diagnostics: scientific and unproven procedures. Curr Opin Allergy Clin Immunol 2005; 5:261–266.
41. Terr AI. Unconventional theories and unproved methods in allergy. In: Adkinson NF Jr., et al. Middleton's Allergy: Principles & Practice. 6th ed. Philadelphia: Mosby, 2003:1712–1716.
42. UNEP, ILO, WHO, et al. Conclusions and recommendations of a workshop on multiple chemical sensitivities (MCS). Regul Toxicol Pharmacol 1996; 24:S188–S189.
43. Medicode, Inc. International Classification of Diseases, 9th Revision, Clinical Modification. 5th ed. Salt Lake City, UT: Medicode Publications, 1997.
44. Institute of Medicine of the National Academies. Complementary and Alternative Medicine in the United States. Washington, DC: The National Academy of Sciences, 2005.

29 Adverse Effects and Fatalities Associated with Subcutaneous Allergen Immunotherapy

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INTRODUCTION

Most local and systemic reactions, which develop during subcutaneous allergen immunotherapy (SCIT), occur within 20 to 30 minutes of injection but can occur later than 30 minutes. Subcutaneous nodules at the site of injection are more common with aluminum-adsorbed vaccines, may persist but usually disappear, and do not necessitate an adjustment in the immunotherapy (IT) dose. Patients who develop nodules that persist should be injected with aqueous preparations.

Van Arsdel's and Sherman's (1) comprehensive review in 1957 analyzed retrospectively the incidence of constitutional reactions in a population of 8706 patients who had received a total of 1,250,000 allergen injections during the 20 years between 1935 and 1955. Their patients experienced a total of 1774 constitutional reactions, about 1 in 700 of the 1,250,000 injections given. The reactions occurred in 663 patients, an incidence of 1.9% versus the 3.5% reported in 1916 by Cooke and Vanderveer (2). Of the 663 reacting patients, 635 were pollen sensitive, representing about 15% of the 4215 pollen-sensitive patients, contrasted with a 0.6% incidence in the remaining 4491 patients. Most of the studies on adverse reactions to allergen IT have been concerned with reactions resulting from the injection itself.

Reports of adverse effects from prick-puncture skin tests prompted an analysis of data derived from the Second National Health and Nutrition Examination Survey (NHANES II) (3). This study revealed that the risk of prick-puncture allergy skin testing was low when carried out with eight extracts licensed by the U.S. Food and Drug Administration (FDA) on a randomly selected population.

The incidence of adverse reactions during IT reported in retrospective and prospective studies has varied considerably, depending on several factors, including the type of allergen vaccine preparation, the patients selected, route of administration, and treatment schedule used, with or without pretreatment and/or preventive procedures (4–12). All of these studies carried out between 1980 and 1989 established the safety of IT when performed on selected patients by experienced physicians who exercised caution and provided adequate monitoring and appropriate treatment, when anaphylaxis does occur. The nonfatal adverse reaction rate ran from less than 1% of patients on IT to 36.2% on rush IT, without pretherapy.

These data have been obtained in studies using the subcutaneous route of injection of allergen vaccines obtained by aqueous extraction of allergens. An "Immunotherapy Coalition" consisting of the American Academy of Allergy Asthma and Immunology (AAAAI), the American College of Allergy Asthma and Immunology (ACAAI), and allergy extract manufacturers supported the benefits of IT and pretreatment to reduce adverse effects associated with the therapy (13–24).

Other allergen molecules and techniques of IT have also been explored to decrease the potential for adverse reactions and to increase efficacy (25–33). These have included oral administration (34,35), nasal administration (36,37), and sublingual-swallow immunotherapy (SLIT) (38–42). A review of the available literature by the World Health Organization (WHO) in 1998 concluded that oral IT was ineffective, whereas SLIT is a viable alternative to the subcutaneous injection route (see chap. 30). These conclusions were also made in a position paper of the European Academy of Allergy and Clinical Immunology. This is reviewed in the paper by Passalacqua et al. (42). Cox et al. have reviewed the literature regarding SLIT from the AAAAI and ACAAI Task Force and report evidence of clinical efficacy and a favorable safety profile (43). SLIT is also well tolerated in children as young as two years (44).

However, in the United States, such therapy is not routinely used because it has not been approved by the U.S. FDA, and the effective dose, treatment schedules, and overall duration of treatment are not yet determined (43).

The traditionally protein-based IT has a limited scope of efficacy. A number of reagents, however, termed "DNA-based immunotherapeutics," have been effective in prevention and reversal of TH-2-mediated hypersensitivity states in mouse models of allergic disease (45,46). The four basic DNA-based IT modalities used include immunization with gene vaccines, allergen mixed with immunostimulating oligodeoxynucleotides (ISS-ODN), and physical allergen-ISS-ODN conjugates as well as immunomodulation with ISS-ODN alone. As the Horner et al. review concludes, "If these reagents prove as effective in humans, as they have proven to be in rodents and nonhuman primates, then DNA-based immunotherapeutics are likely to revolutionize the standard of care for the treatment of allergic disease." Creticos et al. randomized, double-blind, placebo-controlled phase 2 trial of a vaccine consisting of ragweed-pollen antigen, conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA (AIC), resulted in long-term clinical efficacy in the treatment of ragweed allergic rhinitis. In this study, 25 adults allergic to ragweed received six weekly injections of the AIC or placebo vaccine. The AIC group had significantly better rhinitis scores on the visual-analog scale and nasal symptom diary scores than the placebo group during the first and second ragweed seasons (47). However, for the time being, SCIT remains the standard of care throughout the United States and most other parts of the world.

FREQUENCY OF IgE SYSTEMIC REACTIONS

The risk of death after the injection of a foreign substance has been known since Lamson's report in 1929 (48). No other cited studies had reported fatalities; however in 1942, Vance and Strassman (49) reported seven cases of sudden death following injection of foreign protein, and James and Austin (50) published an analysis of six instances of fatal anaphylaxis in humans, following parenteral administration of antigen (penicillin, guinea pig hemoglobin, bee venom, and ragweed vaccine), citing several single case reports by Sheppe (51) and Blanton and Sutphin (52), as well as the seven cases of Vance and Strassman by Rosenthal (53), following penicillin injection.

Rands, a general practitioner in the United Kingdom, published a report of a single fatality of a 19-year-old female due to nonresponsive bronchoconstriction developing within five minutes following the injection of her usual maintenance dose of Pollinex (a commercial pollen vaccine manufactured in Toronto, Canada) given five weeks after the same dose had been administered without effect (54). This was followed by a report of 26 fatalities due to IT and the recommendations of the Committee on Safety of Medicine in the United Kingdom (55), which established preconditions for IT, which resulted in the virtual temporary abandonment of SCIT in the United Kingdom.

A major objective of this chapter is to compare the retrospective reviews of fatalities occurring during skin testing and IT made by the Committee on Allergen Standardization of the AAAAI and by the Paul Ehrlich Institute, the German Federal Agency for Sera and Vaccines (56). These studies analyzed the factors contributing to fatalities occurring during skin tests or IT, with a view to diminishing and, hopefully, eliminating them.

NON-IgE-MEDIATED ADVERSE REACTIONS

It is appropriate to review reports of some aspects of adverse reactions to IT that are controversial. The possible role of precipitins, as responsible for adverse reactions, was addressed by Busse et al. in a study correlating *alternaria* IgG precipitins and adverse reactions (57). Their prospective study revealed that 5 of 23 *alternaria*-sensitive persons had IgG precipitins before IT and another six developed precipitins during therapy. Only 1 of the 23 experienced a reaction to *alternaria* four to six hours after an injection of *alternaria* vaccine. They conclude that precipitins to *alternaria* are common and do not seem to be the basis for late reactions and their presence is not a contraindication to IT. A contrasting report by Kaad and Ostergaard suggests that IT of asthmatic children with mold vaccines might be hazardous

by provoking immune complex reactions (58). Of 38 children with bronchial asthma who were immunized with mold vaccines, seven (19%) were withdrawn from SCIT because of "serious" side effects that were considered clinically consistent with an immune complex reaction. These seven children exhibited a two- to fourfold increase in circulating precipitating antibodies to the injected vaccines. Of the remaining 31 patients also treated with mold vaccines, 14, who were without side effects, did not develop precipitating antibodies. The sera of these patients were not examined for immune complexes, and the authors quote the contradictory findings of the Kemler and Stein (59,60) groups as well as apparently supportive reports by Cano et al., EI-Hefny et al., Kuukiala et al., Moore and Fink, and Stendardi et al. (61–65).

Relevant to these studies is the report by Clausen and Yanari that immune complex–mediated disease is not a factor in patients on maintenance venom IT (66). They evaluated the problem in 30 adults and 15 pediatric patients receiving regular monthly doses of venom (100 μg of antigen), all for between 9 and 12 months. A serum sickness–like presentation had been reported as a sequela of Hymenoptera stings, but the possible role of immune complexes had not been addressed.

No patients developed clinical manifestations suggestive of immune complex pathology; all urinalyses were negative for gross and microscopic hematuria, no sera showed an elevation of Clq, and only 4 of the 45 patients had significantly elevated Raji cell assays. Prospective reevaluation showed the presence of immune complexes before venom administration with no change in acute phase reactants or Raji cell titers 12 hours later. The authors conclude that monthly administration of Hymenoptera venom does not appear to be associated with immune complex disease by either clinical or immunological parameters. A further relevant article was contributed by Umetsu et al. who described an 8-year-old male child with rhinitis and asthma, who developed serum sickness triggered by anaphylaxis-complicating SCIT with multiple inhalant allergens (ragweed, grass, and tree pollens; mold spores; and dust) (67). This child developed puffy eyelids one hour following a half dose of his vaccine and progressed thereafter to an impressive serum sickness syndrome characterized by severe generalized raised annular urticaria, severe asthma, angioedema, severe arthralgias, fever, and episodes of confusion and disorientation. The authors hypothesized that the enhanced vascular permeability that accompanied the anaphylaxis allowed immune complexes that may have persisted in the circulation, to deposit in the blood vessels of the patient. The immune complexes may or may not have been related to the IT itself; tests for these complexes, however, were negative. Clemmensen and Knudsen reported a patient with eczema who apparently developed contact sensitivity to aluminum while receiving IT for hay fever with an aluminum-precipitated allergen (68). Standard patch testing was positive to the aluminum discs used for testing and negative in 53 controls; the eczema disappeared when therapy was discontinued.

An association between brachial plexus neuropathy and allergen IT was reported by Wolpow (69). Two patients were described who developed acute, self-limiting, unilateral brachial plexus neuropathy in association with subcutaneous injections of dust and molds. Previous reports of this neurological illness had "in many cases followed injection of foreign substances, but usually of animal rather than vegetable origin."

Schatz et al. call attention to what they termed "nonorganic adverse reactions" to allergen IT (70). They described 10 patients who presented adverse reactions to SCIT, which mimicked immunologically mediated reactions but were believed to be nonorganic in etiology—with a high incidence of coexisting or contributory psychiatric problems.

LONG-TERM SEQUELAE

The possibility that chronic injection of foreign proteins might induce long-term sequelae has been addressed by both experimental animal studies and anecdotal reports in humans. Rabbits hyperimmunized with various vaccines make cryoprecipitating proteins, monoclonal antibody, rheumatoid factor, and anti-DNA antibodies (71–73), and such hyperimmunized animals may develop amyloidosis and myeloma (74,75). There have been anecdotal reports of multiple myeloma and Waldenström's macroglobulinemia in patients on long-term SCIT (76) and a report of a striking incidence of positive rheumatoid factors in atopic children on such therapy (77).

Levinson et al. undertook to determine if long-term allergen IT caused late sequelae, particularly those reflecting abnormal immunological responses (78). Their study, the first systematic investigation of potential adverse effects of long-term IT, examined 41 patients between 18 and 50 years who had received regular IT with three or more allergen vaccines for five or more years at the Walter Reed Army Medical Center Allergy Clinic. Twenty-one age- and gender-matched atopic individuals served as controls prior to initiating such therapy. The treated individuals showed no increased autoimmune, collagen, vascular, or lymphoproliferative disease. Furthermore, long-term allergen IT had no adverse effects on immunological reactivity as assessed by a number of immunological parameters—with a particularly noteworthy absence of immune complexes in the serum of patients undergoing long-term IT. It is true for at least this study of 41 patients, mostly Caucasian females, of an average age of 30, treated for allergic rhinitis and asthma for five or more years in the U.S. Army Allergy Clinic.

Phanuphak and Kohler (79) described in 6 of 20 consecutive patients the onset of polyarteritis nodosa, of vasculitic symptoms that coincided with SCIT for presumptive atopic (lgE-mediated) respiratory disease. Compared with 14 other patients with polyarteritis nodosa, the six on IT had significantly greater skin involvement and peripheral blood eosinophils. There was evidence for circulatory complexes with decreased hemolytic complement, increased cryoglobulins, or increased Clq binding in both groups but no allergen-precipitating antibodies.

A possible association between pemphigus vulgaris and allergen injections with cat pelt vaccine was raised by McCombs et al. (80). Although intriguing, it seems irrelevant since such therapy today is performed with purified cat allergens.

The conflicting results of studies in patients receiving long-term IT might be explained by a nonuniformity of detection methods used. This prompted a group of Australian investigators to examine a population of older patients with documented prolonged IT extending over many years. They examined 35 older patients (mean age 62, range 53–85 years) who received injections of allergen vaccines for between 2 and 30 years (mean of 13 years) and compared them with an age-matched control group (mean age 64.7, range 42–87 years). Treated patients had significantly higher IgG and lower total IgE than controls, but no increased incidence of paraproteins or evidence of immune complex disease such as urinary abnormalities, increased Clq binding levels, cryoglobulins, or rheumatoid (sic) fever (81).

FATALITIES

Fatalities, carefully documented, constitute a less controversial measure of adverse effects related to either skin testing or allergen IT. Since Lamson's first description of death from anaphylaxis associated with SCIT (48), six fatalities have been reported related to or associated with SCIT (49,50,54,82–84). More than 70 deaths (between 1895 and 1964) have been reported after skin testing, the majority of these associated with antigens such as horse serum–derived tetanus or diphtheria antitoxins and pneumococcus antiserum, none of which are currently in use. Nine of these 70 deaths from skin testing were associated with allergens similar to allergens used today. No articles on fatalities associated with IT or skin testing have been published in the United States between 1980 and 1987.

A project defining risk factors for fatalities from skin testing and IT was instituted as a retrospective study by the Committee on Allergen Standardization of the AAAAI in 1983. For this project, Lockey and his coworkers composed a 64-item questionnaire designed to obtain data on fatalities from skin testing and IT. The questionnaire was mailed to the then 3400 members and fellows of the AAAAI and its analysis was published in *The Journal of Allergy and Clinical Immunology* in April, 1987 (85). Although 46 fatalities had been reported from 1945 to 1984, only 30 (6 fatalities from skin testing and 24 from IT) had sufficient data for analysis. Tables 1 and 2 summarize the data on fatalities associated with skin testing and IT, respectively.

Although all ages were affected (range 7–70), the mean ages of the fatalities following skin testing or IT were 30 and 34 years, respectively. There was no gender predilection. Errors of administration appeared to be responsible for three fatalities and questionable for an additional three. Ten patients had died after skin tests or IT during a seasonal exacerbation of the patients' allergic disease, four in patients who had been symptomatic at the time of

Table 1 Case Reports from the Literature, *Fatalities from Skin Test*

Author/year	Age/medical disease	Injected vaccine	Onset of symptoms	Initial symptoms	Cause of death
Baagoe, 1928 (86)	Unknown	Egg white, 0.1 cc	Sudden	Dyspnea	Unknown
Lamson, 1924 (48)	5 mo, eczema	Ovomucoid, 0.05 cc	2 min	Cyanosis	Respiratory arrest
Lamson, 1929 (48)	34 yr, asthma	Buckwheat 1:500, ID	2–3 min	Lacrimation Cyanosis Respiratory difficulty	Anaphylactic shock
Vance and Strassman, 1942 (49)	4 yr	Silkworm, sheep's wool, kapok extracts, IC	5 min	Shock/ unconscious	Anaphylactic shock[a]
Wiseman and McCarthy-Brough, 1945 (87)	78 yr, asthma	17 environmental allergens, 0.01 cc, IC	5 min	Cough Asthma	Anaphylactic arrest
Swineford, 1946 (88)	49 yr, asthma	56 food extracts, 8–9 inhalant extracts, IC	3 min	"Not feeling well" Dyspnea	Cardiovascular collapse
Blanton and Sutphin, 1949 (89)	57 yr, asthma	56 skin tests, scratch, and IC	Sudden	Air hunger	Anaphylactic shock[a]
Harris and Shure, 1950 (90)	25 yr, asthma	Environmental substances, ID	1 min	Dyspnea	Respiratory arrest[a]
Dogliotti, 1968 (91)	35 yr	Penicillin scratch	4–5 min	Flush Abdominal pain	Anaphylactic shock
Lockey et al., 1987 (85)	6 subjects, 10–50 yr	Variable	3–20 min	Variable	Asthma, anaphylactic shock Others
Reid et al., 1996 (92)	1 subject	Unknown	Unknown	Unknown	Unknown
Bernstein et al., 2004 (93)	1 young subject, asthma, food allergy	90 food scratch tests	Unknown	Bronchospasm	Respiratory arrest

[a]Autopsy confirmed anaphylactic shock.
Source: Adapted from Ref. 85.

injection, two of whom had been receiving β-adrenergic blockers. Of the 24 fatalities associated with IT, 4 had experienced previous reactions, 11 had a high degree of sensitivity, and 4 had been injected with newly prepared vaccines. Fifteen of the total of thirty fatalities had received a pollen vaccine as part of the fatal injection. Five of the six fatalities associated with skin testing had occurred without prior prick-puncture testing. Signs and symptoms of systemic reactions were not reliable predictors of death. The onset of systemic reactions was 30 minutes or less after injection in 23 of 30 patients, more than 30 minutes after injection in two, and had not been reported on five. The cause of death in 14 of 16 patients with asthma was respiratory. Epinephrine had been administered to 18 patients, not given to 3, and was either not recorded or unknown in 9 patients.

A later supplemental survey conducted by Reid et al. on deaths in the United States from SCIT between 1985 and 1989 was reported at the 1990 AAAAI meeting in Baltimore, Maryland (95). There were no deaths from skin testing reported; however, 16 deaths were reported from IT. These deaths were reported in the *Journal of Allergy and Clinical Immunology* and included one additional death for a total of 17 (96). The mean age was 36 years (range 10–77), and there were 5 males and 11 females (gender of one subject not reported) versus 11 males and 13 females in the earlier study. Eighty-seven percent of the subjects had asthma, 1 was on β-blocker therapy, and 10 of 17 were "highly sensitive" by skin testing or the radio-allergosorbent test (RAST). Fourteen of seventeen were on aqueous vaccines, 10 of 17 on increasing doses, and 9 of 17 received epinephrine. The results obtained in this study are very similar to those reported previously.

Reid and Gurka presented an abstract at the 1996 Academy of Allergy, Asthma and Immunology meeting about the continuation of the above-mentioned study that covered

Table 2 Case Reports from the Literature, *Fatalities from Immunotherapy*

Author	Gender	Age (yr)	Medical disease	Allergen	Status of IT	Onset of symptoms	Symptoms	Cause of death
Lamson, 1929 (48)	M	34	Asthma	Bermuda grass pollen, 0.05 cc, 1:100	(because of "nervousness" with preceding reaction)	<3 min	Flushing, athetoid movements Dyspnea	Anaphylactic shock[a]
Waldbott, 1932 (94)	F	40		Ragweed vaccine, 1400 units	Unk	<3 min	Dyspnea U	Anaphylactic shock
Vaughn and Black 1939 (83)	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
Vance and Strassman, 1942 (49)	M	35	Asthma	Ragweed vaccine	Unk	1 hr	Unk	Anaphylactic shock[a]
James and Austen, 1964 (50)	M	56		Hay fever desensitization	Unk (11 of 21)	<45 min	Dyspnea	Anaphylactic shock[a]
Rands, 1980 (54)	F	19	Asthma	Pollinex, Migen[b]	Maintenance	3–10 min	Rushing syndrome, tachycardia	Anaphylactic shock[a]
Pollard, 1980 (84)	M	24	Asthma	Bencard Product[c]	Unk (15th injection)	25 min	Unk	Status asthmaticus
CSM Update, 1986 (55)	F, 13 M, 12 Unk, 1	11–57 ×, 31	Asthma Hayfever Unk	Varied, mite	Normal course, 16[d] Maintenance, 4 Unk, 6	<10 min, 14 <30 min, 4 <90 min, 2 Unk, 6	Asthma and anaphylaxis	Asthma and anaphylaxis
Lockey et al., 1987 (85)	F, 13 M, 11	7–70 ×, 34	Unk[e]	Varied pollens Molds	Maintenance, 7 Increasing, 9 Decreasing, 1 1st injection, 1 NA, 6	<20 min, 15 20–30 min, 3 >30 min, 2 Unk, 4	Pruritus, 3 A, 0 UAO/LAO and/or asthma, 12 Shock, 4 Coma, 9 Hypotension, 6 Myocardial infarction, 1 NA or unk, 6	UAO/LAO and/or asthma, 13 Cardiovascular, 6 Anaphylactic shock, 13 Others, 4 NA or unk, 3

Reid et al., 1990 (95)	F, 11 M, 5 Unk, 1	10–77 ×, 36	Asthma, 13 AR, 7 CV, 3 DM, 1 HtD, 1 Unk, 1	Aqueous, 14 Unk, 2	Maintenance, 3 Increasing, 9 Decreasing, 1 Unk, 3	<20 min, 8 20–30 min, 3 >30 min, 1[f] Unk, 4	U and A, 1 LAO, 5 UAO and LAO, 4 UAO, 1 Shock 4 Unk, 5	Resp, 8 Shock, 0 Both, 4 Other, 1 Unk, 3
Reid et al., 1996 (92)	Unk	12–73	Asthma, 19	Unk	Unk	Unk	Unk	Unk
Lüderitz-Puchel et al., 1996 (56)	Unk, 40 28 analyzed	Unk	Aqueous, 6 Semidepot, 22	Unk	Unk	Unk	Shock, 7 Unk, 21	
Bernstein et al., 2004 (93)	F, 6 M, 11	5–81 ×, 39	Asthma, 15 AR, 2	Mix 14, mite 3	Maintenance 10 Increasing 7	< 30 min, 10 > 30 min, 3 Unk, 4	LAO 8 UAO 2, UAO and LAO 6 Shock 13 Pruritus 3 A 3	Upper airway edema, 5 Asthma, 6 Shock, 6

[a]Autopsy confirmed.
[b]Not in article, listed only as Pollinex, pollen vaccine; Migen, house dust-mite vaccine.
[c]Bencard Allergy Unit, Brentford, Middlesex, United Kingdom.
[d]? meaning of normal course.
[e]Not requested.
[f]Not witnessed.
Abbreviations: AR, allergic rhinitis; CV, cardiovasular, DM, diabetes mellitus; HtD, heart disease, NA, not available; Unk, unknown.
LAO, lower airway obstruction; UAO, upper airway obstruction; U, Urticaria; A, angioedema.
Source: Adapted from Ref. 85.

events from January 1990 to June 1995 (92). They reported 28 deaths during this time, with an average of five deaths per year with incomplete data for 19 of the 28 reports. One of the 28 deaths was associated with intradermal skin testing and 27 with IT. Four of the IT deaths occurred following home injections or injections given with no physician present, 3 were associated with an incorrect dose, 19 occurred in individuals with atopic asthma, and 5 deaths occurred despite postreaction intervention. The age range at the time of death was 12 to 73 years and there was no gender predilection. Data in this study are similar to previous reports of fatalities.

The Committee on the Safety of Medicine of the United Kingdom reported in 1986 that 26 deaths from anaphylaxis due to allergen IT had occurred in the United Kingdom since 1957. All died from IT-induced bronchospasm and/or anaphylaxis, 11 of these since 1980 and 5 during the preceding 18 months. In most of the cases, adequate facilities for cardiorespiratory resuscitation were not available. Asthma was the indication for treatment in 16 of the 26, allergic rhinitis in 1, and the indicator was unknown in 9. In two patients, the ultimately fatal systemic reaction allegedly began more than 30 minutes after injection, resulting in a recommendation that patients remain in a medical facility for two hour after their injection (55). Currently, 26 centers throughout the United Kingdom are equipped to perform SCIT in specialist hospital settings. About 500 courses of SCIT are initiated annually, and the minimum waiting period is now 60 minutes (Durham SR, personal communication, 2007; Andersen P, personal communication, 2007).

In Sweden, introduction of potent mite, mold, and animal dander vaccines was accompanied by some anaphylactic deaths that prompted the regulatory agency to restrict the use of these vaccines to physicians and clinics specializing in this issue (97).

The Paul Ehrlich Institute in Germany reported 40 fatalities between 1977 and 1994, with complete data available for 20 reports in Germany and 8 reports elsewhere in Europe. For 23 of the 28 reports analyzed, it was not possible to rule out error on the part of the physician and/or inadequate information given to the patient as factors contributory to the fatal outcomes (56). Three cases with permanent hypoxic brain damage as a result of anaphylactic shock were also reported. Semidepot preparations, which are not used in the United States, were involved in most of the adverse and fatal reactions. Mite allergen vaccines were used in 18 of the cases reported.

There are 41 fatalities identified from SCIT in Bernstein et al. AAAAI physician member survey of fatal reactions and near-fatal reactions (NFRs) from 1990 to 2001 (92). The estimated fatality rate is 1 per 2.5 million injections, (average of 3.4 deaths/yr), similar to the results in the previous AAAAI physician member surveys (85,92,96). One skin test fatality was confirmed in a young woman, with allergic rhinitis, moderate persistent asthma, and food allergy, who had a fatal anaphylactic reaction after application of prick-puncture tests to 90 food antigens (93).

ANAPHYLAXIS ASSOCIATED WITH SKIN TESTING AND SCIT

The study by Van Arsdel and Sherman (1) supports the general safety of IT for the control of IgE-mediated allergic diseases in that over 1 million allergen vaccine injections given to 8700 patients from 1935 to 1955 had been administered without a fatality. Hepner et al. prospective study on IT, reported in 1987, that 25 out of 2989 patients, over a seven-month period, experienced systemic reactions and there were no fatalities (6). On the basis of annual studies from a panel of 2000 physicians in the United States, the National Disease and Therapeutic Index indicated that in each of the five previous years, 7 to 10 million allergen injections had been given (98). Since so many injections are administered yearly, the risk of a fatal reaction is low. Lockey et al. reported that 45 (1.4%) of 3236 patients who had a clinical history of Hymenoptera hypersensitivity and were skin tested had systemic hypersensitivity reactions during skin testing, and eight of these (0.25% of the subjects tested) were severe (99). Of 1410 patients placed on IT, 171 experienced 327 systemic reactions, of which 28 reactions (9%) were severe but not fatal (100). These studies illustrate that IT with a standardized vaccine, used as indicated, in individuals with an allergic disease that may be life threatening, induces a low incidence of adverse reactions, most of which are mild to moderate.

The systemic reaction rate to skin testing is significantly lower than the rate of reactions to SCIT, but it is not negligible. Lin et al. reported only two patients with systemic allergic

reactions to skin testing in 10,400 patients tested (101). The overall risk of inducing anaphylaxis by skin testing was 0.02%; other studies have produced similar results (101–103). The rate of systemic reactions to skin testing is likely under reported. Thompson et al. reported a systemic reaction rate of 6% of patients receiving skin testing (104). At the 2007 World Allergy Congress meeting, Bagg et al. reported a 3.5% systemic reaction rate of 1456 patients receiving skin testing in a retrospective review over one year (105). All patients were given epinephrine for any systemic symptoms that occurred during skin testing. All patients readily responded to early intervention with epinephrine (105). It is important to recognize the risk of systemic reactions from skin testing as well as the treatment for these reactions to prevent progression. The early administration of epinephrine appears to prevent more serious and late-phase reactions.

The incidence of unconfirmed NFRs from 1990 to 2001 was 23 per year (5.4 events per million injections) in the latest mentioned AAAAI survey of fatal and NFRs from SCIT (106). There were 115 systemic reactions (5.2% of patients and 0.06% of injections) from 1981 to 1990 (107) and 26 systemic reactions (1.08% of patients and 0.01% of injections) from 1991 to 2000 (108) in another retrospective analysis of nonfatal systemic reactions to SCIT.

A review by Stewart and Lockey (109) in 1992, which examined the incidence of systemic reactions to IT, concluded that the percentage of subjects experiencing a systemic reaction from IT is small but will probably increase as the IT schedule is accelerated and when or if high-dose regimens are required in highly sensitive subjects. In addition, maintenance IT is associated with fewer systemic reactions than the buildup period of rush and accelerated schedules of IT.

Premedication with a combination of methylprednisolone, ketotifen (a mast-cell stabilizer not available in the United States), and long-acting theophylline may decrease the incidence of systemic reactions associated with rush protocols. Concern was voiced over masking a mild reaction by using premedication, which might therefore be followed by a later, more serious, reaction or delay the onset of a reaction beyond the waiting period. However, premedication with antihistamine reduced the frequency of severe systemic reactions caused by conventional IT and increased the proportion of patients who achieved the target maintenance dose in one randomized controlled study (110). In other studies premedication with antihistamines significantly reduced the incidence of systemic reactions during rush IT or specific cluster IT (111–113). There was no evidence that antihistamines masked the early warning signs or delayed the onset of systemic reactions. In the Casale et al. study, pretreatment with omalizumab resulted in a fivefold decrease in risk of anaphylaxis caused by a ragweed rush protocol (114). Further studies involving larger groups of patients and different dosage regimens are necessary to define the future role of antihistamines and other pharmacological pretreatment in IT. Finally, a minimum 30-minute waiting period, as recommended by the AAAAI and the ACAAI *Allergen Immunotherapy: A Practice Parameter Second Update*, was deemed appropriate with a longer waiting period for high-risk patients (115).

RISK FACTORS FOR SKIN TESTING AND IMMUNOTHERAPY

It is essential that strict attention be paid to risk factors for systemic reactions and that techniques of management are initiated both before and after skin testing or IT to minimize these risks. Several guidelines have been suggested which emphasize thorough training of all personnel involved in these procedures as well as the prompt treatment of systemic reactions (85,97). These have encouraged the development and use of standardized vaccines and emphasize certain risk factors, including the following:

1. Patients, particularly asthmatics, suffering a seasonal exacerbation of their symptoms
2. Patients who demonstrate exquisite sensitivity to particular allergen(s) (116).
3. Patients on β-blockers (116,117)
4. Patients with asthma, especially when their asthma is unstable (118)
5. Patients in whom rush IT is used (both venoms and inhalant allergens) (109,111,113,116)
6. Patients in whom high doses of potent standardized allergen vaccines are used
7. Injections from new vials (116)
8. Dosing errors (116)

PRECAUTIONS FOR SKIN TESTING AND IMMUNOTHERAPY

The following guidelines are suggested:

1. Always begin with a percutaneous procedure for skin testing (i.e., prick-puncture).
2. When possible, do not use β-adrenergic blocking agents concomitantly during skin testing or IT.
3. Keep patients under observation for at least 30 minutes or even longer for those at greatest risk since most fatal systemic reactions begin within that time (115).
4. When SCIT is prescribed, give the patient written and/or verbal guidelines outlining methods of IT and the importance of adherence to these guidelines to prevent an adverse reaction (see chap. 32).
5. Inform patients receiving IT of its potential risk and obtain informed consent.
6. Administer IT in an office or clinical setting with a physician present and with optimal care available for the treatment of a systemic reaction.
7. Monitor patients to assure they are waiting a proper time in the facility where they receive their IT.
8. Provide adequate instructions to another physician who may give the injections elsewhere from vials of vaccine taken from the prescribing physician's office or clinic (see chap. 32).

The safety of allergen IT has been reviewed in detail by Norman and Van Metre (119).

EQUIPMENT RECOMMENDED FOR SETTINGS WHERE ALLERGEN IMMUNOTHERAPY IS ADMINISTERED

The following equipment are recommended by the Joint Task Force on Practice Parameters (115):

1. Stethoscope and sphygmomanometer.
2. Tourniquets, syringes, hypodermic needles, large-bore needles (14 gauge).
3. Aqueous epinephrine HCL 1:1000 w/v.
4. Equipment to administer oxygen by mask.
5. Equipment to administer intravenous fluids.
6. Antihistamines for injection (second-line agents for anaphylaxis, but H1 and H2 antihistamines work better together than either one alone).
7. Corticosteroids for intravenous injection.
8. Vasopressors.
9. Equipment to maintain an airway appropriate for the supervising physician's expertise and skill.

The prompt recognition of systemic reactions and the immediate use of epinephrine are the mainstays of therapy (120–122).

SALIENT POINTS

- Physicians who administer allergen IT should have the appropriate equipment and personnel to treat a systemic reaction.
- No allergen vaccine can be considered completely safe for a given patient allergic to that vaccine.
- The risk of a fatal reaction can be reduced and even eliminated by the careful selection and monitoring of allergic patients on IT, by using improved biologically standardized vaccines and by skilled and timely treatment of systemic reactions.
- The wait period of 30 minutes is adequate for most patients, but should be extended for high-risk patients.
- Patients at highest risk for allergen IT are patients with asthma, especially unstable asthma.

- Other high-risk patients include those with a seasonal exacerbation and exquisite sensitivity, those regularly treated with β-blockers, and those receiving rush or accelerated schedule immunotherapy.

REFERENCES

1. Van Arsdel PP, Sherman WB. Risk of inducing constitutional reactions in allergic patients. J Allergy 1957; 28:251–261.
2. Cooke RA, Veer AV. Human sensitization. J Immunol 1916; 1:201–305.
3. Turkeltaub PC, Gergen PJ. The risk of adverse reactions from percutaneous prick-puncture allergen skin testing, venipuncture, and body measurements: data from the second National Health and Nutrition Examination Survey 1976-80 (NHANES II). J Allergy Clin Immunol 1989; 84:886–890.
4. Greenberg MA, Kaufman CR, Gonzalez GE, et al. Late and immediate systemic-allergic reactions to inhalant allergen immunotherapy. J Allergy Clin Immunol 1986; 77:865–870.
5. Hejjaoui A, Dhivert H, Michel FB, et al. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. IV. Systemic reactions according to the immunotherapy schedule. J Allergy Clin Immunol 1990; 85:473–479.
6. Hepner M, Ownby D, MacKechnie H, et al. The safety of immunotherapy—a prospective study. J Allergy Clin Immunol 1987; 79:(133).
7. Levine MI. Systemic reactions to immunotherapy. J Allergy Clin Immunol 1979; 63:209 (abstr).
8. Nelson BL, Dupont LA, Reid MJ. Prospective survey of local and systemic reactions to immunotherapy with pollen extracts. Ann Allergy 1986; 56:331–334.
9. Osterballe O. Side effects during immunotherapy with purified grass pollen extracts. Allergy 1982; 37:553–562.
10. Rawlins MD, Wood SM, Mann RD. Hazards with desensitising vaccines. Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 1988; 82:147–151.
11. Rieckenberg MR, Khan RH, Day JH. Physician reported patient response to immunotherapy: a retrospective study of factors affecting the response. Ann Allergy 1990; 64:364–367.
12. Vervloet D, Khairallah E, Arnaud A, et al. A prospective national study of the safety of immunotherapy. Clin Allergy 1980; 10:59–64.
13. Allergen immunotherapy: a practice parameter. American Academy of Allergy, Asthma and Immunology. American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 2003; 90:1–40.
14. Akcakaya N, Hassanzadeh A, Camcioglu Y, et al. Local and systemic reactions during immunotherapy with adsorbed extracts of house dust mite in children. Ann Allergy Asthma Immunol 2000; 85:317–321.
15. Brockow K, Kiehn M, Riethmuller C, et al. Efficacy of antihistamine pretreatment in the prevention of adverse reactions to Hymenoptera immunotherapy: a prospective, randomized, placebo-controlled trial. J Allergy Clin Immunol 1997; 100:458–463.
16. Karaayvaz M, Erel F, Caliskaner Z, et al. Systemic reactions due to allergen immunotherapy. J Investig Allergol Clin Immunol 1999; 9:39–44.
17. Lockey RF, Nicoara-Kasti GL, Theodoropoulos DS, et al. Systemic reactions and fatalities associated with allergen immunotherapy. Ann Allergy Asthma Immunol 2001; 87:47–55.
18. Machin IS, Robaina JCG, Bonnet C, et al. Immunotherapy units: a follow-up study. J Investig Allergol Clin Immunol 2001; 11:167–171.
19. Malling HJ. Minimising the risks of allergen-specific injection immunotherapy. Drug Saf 2000; 23:323–332.
20. Mellerup MT, Hahn GW, Poulsen LK, et al. Safety of allergen-specific immunotherapy. Relation between dosage regimen, allergen extract, disease and systemic side-effects during induction treatment. Clin Exp Allergy 2000; 30:1423–1429.
21. Nettis E, Giordano D, Pannofino A, et al. Safety of inhalant allergen immunotherapy with mass units-standardized extracts. Clin Exp Allergy 2002; 32:1745–1749.
22. Tankersley MS, Butler KK, Butler WK, et al. Local reactions during allergen immunotherapy do not require dose adjustment. J Allergy Clin Immunol 2000; 106:840–843.
23. Winther L, Malling HJ, Mosbech H. Allergen-specific immunotherapy in birch- and grass-pollen-allergic rhinitis. II. Side-effects. Allergy 2000; 55:827–835.
24. Wuthrich B, Gumowski PL, Fah J, et al. Safety and efficacy of specific immunotherapy with standardized allergenic extracts adsorbed on aluminium hydroxide. J Investig Allergol Clin Immunol 2001; 11:149–156.
25. Bhalla PL. Genetic engineering of pollen allergens for hayfever immunotherapy. Expert Rev Vaccines 2003; 2:75–84.
26. Chen D, Maa YF, Haynes JR. Needle-free epidermal powder immunization. Expert Rev Vaccines 2002; 1:265–276.

27. Di Gioacchino M, Cavallucci E, Di Sciascio MB, et al. Allergen immunotherapy: an effective immune-modifier. Int J Immunopathol Pharmacol 1999; 12:1–5.
28. Holt PG, Rowe J, Loh R, et al. Developmental factors associated with risk for atopic disease: implications for vaccine strategies in early childhood. Vaccine 2003; 21:3432–3435.
29. Koh YY, Kim CK. The development of asthma in patients with allergic rhinitis. Curr Opin Allergy Clin Immunol 2003; 3:159–164.
30. Reyes Moreno A, Castrejon Vazquez MI, Miranda Feria AJ. [Failure of allergen-based immunotherapy in adults with allergic asthma]. Rev Alerg Mex 2003; 50:8–12.
31. Spiegelberg HL, Takabayashi K, Beck L, et al. DNA-based vaccines for allergic disease. Expert Rev Vaccines 2002; 1:169–177.
32. Tarzi M, Larche M. Peptide immunotherapy for allergic disease. Expert Opin Biol Ther 2003; 3:617–626.
33. Tella R, Bartra J, San Miguel M, et al. Effects of specific immunotherapy on the development of new sensitisations in monosensitised patients. Allergol Immunopathol (Madr) 2003; 31:221–225.
34. Criado Molina A, Guerra Pasadas F, Daza Munoz JC, et al. [Immunotherapy with an oral Alternaria extract in childhood asthma. Clinical safety and efficacy and effects on in vivo and in vitro parameters]. Allergol Immunopathol (Madr) 2002; 30:319–330.
35. Litwin A, Flanagan M, Entis G, et al. Oral immunotherapy with short ragweed extract in a novel encapsulated preparation: a double-blind study. J Allergy Clin Immunol 1997; 100:30–38.
36. Liu YH, Kao MC, Lai YL, et al. Efficacy of local nasal immunotherapy for Dp2-induced airway inflammation in mice: Using Dp2 peptide and fungal immunomodulatory peptide. J Allergy Clin Immunol 2003; 112:301–310.
37. Passali D, Bellussi L, Passali GC, et al. Nasal immunotherapy is effective in the treatment of rhinitis due to mite allergy. A double-blind, placebo-controlled study with rhinological evaluation. Int J Immunopathol Pharmacol 2002; 15:141–147.
38. Andre C, Perrin-Fayolle M, Grosclaude M, et al. A double-blind placebo-controlled evaluation of sublingual immunotherapy with a standardized ragweed extract in patients with seasonal rhinitis. Evidence for a dose-response relationship. Int Arch Allergy Immunol 2003; 131:111–118.
39. Cirla AM, Cirla PE, Parmiani S, et al. A pre-seasonal birch/hazel sublingual immunotherapy can improve the outcome of grass pollen injective treatment in bisensitized individuals. A case-referent, two-year controlled study. Allergol Immunopathol (Madr) 2003; 31:31–43.
40. Di Rienzo V, Marcucci F, Puccinelli P, et al. Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study. Clin Exp Allergy 2003; 33:206–210.
41. Grosclaude M, Bouillot P, Alt R, et al. Safety of various dosage regimens during induction of sublingual immunotherapy. A preliminary study. Int Arch Allergy Immunol 2002; 129:248–253.
42. Passalacqua G, Baena-Cagnani CE, Berardi M, et al. Oral and sublingual immunotherapy in paediatric patients. Curr Opin Allergy Clin Immunol 2003; 3:139–145.
43. Cox LS, Linnemann DL, Nolte H, et al. Sublingual immunotherapy: a comprehensive review. J Allergy Clin Immunol 2006; 117:1021–1035.
44. Cox L. Sublingual immunotherapy in pediatric allergic rhinitis and asthma: efficacy, safety, and practical considerations. Curr Allergy Asthma Rep 2007; 7:410–420.
45. Horner AA, Takabaysahi K, Zubeldia JM, et al. Immunostimulatory DNA-based therapeutics for experimental and clinical allergy. Allergy 2002; 57(suppl 72):24–29.
46. Horner AA, Van Uden JH, Zubeldia JM, et al. DNA-based immunotherapeutics for the treatment of allergic disease. Immunol Rev 2001; 179:102–118.
47. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006; 355:1445–1455.
48. Lamson R. Death associated with injection of foreign substances. JAMA 1924; 82.
49. Vance BM, Strassman, G. Sudden death following injection of foreign protein. Arch Pathol 1942; 34:849–865.
50. James LP Jr., Austen KF. Fatal systemic anaphylaxis in man. N Engl J Med 1964; 270:597–603.
51. Sheppe WM. Fatal anaphylaxis in man. J Lab Clin Med 1930; 16:372–375.
52. Blanton WV, Sutphin AK. Death during skin testing. Am J Med Sci 1949; 217:169–173.
53. Rosenthal A. Fatal anaphylactic reactions to penicillin. NY State J Med 1954; 54:1485–1487.
54. Rands DA. Anaphylactic reaction to desensitisation for allergic rhinitis and asthma. Br Med J 1980; 281:854.
55. CSM Update: Desensitizing vaccines. Br Med J 1986; 293:948.
56. Lüderitz-Puchel U. Haustein D. Incidents following hyposensitization. Münch med Wschr 1996; 138:1–7.
57. Busse WW, Storms WW, Flaherty DK, et al. *Alternaria* IgG precipitins and adverse reactions. J Allergy Clin Immunol 1976; 57:367–372.
58. Kaad PH, Ostergaard PA. The hazard of mould hyposensitization in children with asthma. Clin Allergy 1982; 12:317–320.

59. Kemler BJ, Franklin WD, Alpert E, et al. Failure to detect circulating immune complexes in allergic patients on injection therapy. Clin Allergy 1979; 9:473–478.
60. Stein MR, Brown GL, Lima JE, et al. A laboratory evaluation of immune complexes in patients on inhalant immunotherapy. J Allergy Clin Immunol 1978; 62:211–216.
61. Cano PO, Chow M, Jerry LM, et al Circulating immune complexes in patients with atopic allergy. Clin Allergy 1977; 7:167–171.
62. El-Hefny A, Ekladious EM, El-Sharkawy S, et al. Extrinsic allergic bronchiolo-alveolitis in children. Clin Allergy 1980; 10:651–658.
63. Kuuliala O, Palosuo T, Aho K, et al. Haemagglutinating antibodies to cat dander in relation to exposure and respiratory allergy. Clin Allergy 1979; 9:391–395.
64. Moore VL, Fink JN. Immunologic studies in hypersensitivity pneumonitis—quantitative precipitins and complement-fixing antibodies in symptomatic and asymptomatic pigeon breeders. J Lab Clin Med 1975; 85:540–545.
65. Stendardi L, Delespesse G, Debisschop MJ. Circulating immune complexes in bronchial asthma. Clin Allergy 1980; 10:405–411.
66. Clausen RW, Yanari SS. Immune complex-mediated disease not a factor in patients on maintenance venom immunotherapy. J Allergy Clin Immunol 1983; 72:199–203.
67. Umetsu DT, Hahn JS, Perez-Atayde AR, et al. Serum sickness triggered by anaphylaxis: a complication of immunotherapy. J Allergy Clin Immunol 1985; 76:713–718.
68. Clemmensen O, Knudsen HE. Contact sensitivity to aluminium in a patient hyposensitized with aluminium precipitated grass pollen. Contact Dermatitis 1980; 6:305–308.
69. Wolpow ER. Brachial plexus neuropathy. Association with desensitizing antiallergy injections. JAMA 1975; 234:620–621.
70. Schatz M, Patterson R, DeSwarte R. Nonorganic adverse reactions to aeroallergen immunotherapy. J Allergy Clin Immunol 1976; 58:198–203.
71. Bokisch VA, Bernstein D, Krause RM. Occurrence of 19S and 7S anti-IgGs during hyper-immunization of rabbits with streptococci. J Exp Med 1972; 136:799–815.
72. Christian CL, Desimone AR, Abruzzo JL. Anti-DNA antibodies in hyperimmunized rabbits. J Exp Med 1965; 121:309–321.
73. Eichmann K, Braun DG, Krause RM. Influence of genetic factors on the magnitude and the heterogeneity of the immune response in the rabbit. J Exp Med 1971; 134:48–65.
74. Potter M. Myeloma proteins (M-components) with antibody-like activity. N Engl J Med 1971; 284:831–838.
75. Riesen W, Rudikoff S, Oriol R, et al. An IgM Waldenstrom with specificity against phosphorylcholine. Biochemistry 1975; 14:1052–1057.
76. Penny R, Hughes S. Repeated stimulation of the reticuloendothelial system and the development of plasma-cell dyscrasias. Lancet 1970; 1:77–78.
77. Raphael SA, Nell PA, Hymchuk, Lischner HW. Positive late antiglobulin test in atopic pediatric subjects receiving hyposensitization therapy. J Allergy Clin Immunol 1976; 57:231.
78. Levinson AI, Summers RJ, Lawley TJ, et al. Evaluation of the adverse effects of long-term hyposensitization. J Allergy Clin Immunol 1978; 62:109–114.
79. Phanuphak P, Kohler PF. Onset of polyarteritis nodosa during allergic hyposensitization treatment. Am J Med 1980; 68:479–485.
80. McCombs CC, Michalski JP, Jerome DC. Case 26-1980: pemphigus vulgaris after hyposensitization injections. N Engl J Med 1980; 303:1179.
81. Katelaris CH, Walls RS. A study of possible ill effects from prolonged immunotherapy in treatment of allergic diseases. Ann Allergy 1984; 53:257–261.
82. Waldbott GL. The prevention of anaphylactic shock. JAMA 1932; 98:446–449.
83. Vaughan VA, Black JR. Practice of Allergy. St. Louis: C. V. Mosby, 1939.
84. Pollard RCH. Anaphylactic reaction to desensitization. Br Med J 1980; 281:481.
85. Lockey RF, Benedict LM, Turkeltaub PC, et al. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1987; 79:660–677.
86. Baagoe K. Case of allergic death due to intracutaneous injection of 10 cc of white of hen's egg. Ugeskr Laeger 1928; 90:550.
87. Wiseman J, McCarthy-Brough MP. Skin sensitivity in the aged-fatality following intradermal tests. J Allergy 1945; 16:250.
88. Swineford O. Anaphylactic shock from skin testing. J Allergy 1946; 17:24.
89. Blanton WB, Sutphin AK. Death during skin testing. Am J Med Sci 1949; 217:169–173.
90. Harris MC, Shure N. Sudden death due to allergy tests. J Allergy 1950; 21:208–216.
91. Dogliotti M. An instance of fatal reaction to the penicillin scratch-test. Dermatologica 1968; 136:489–496.
92. Reid M, Gurka G. Deaths associated with skin testing and immunotherapy. J Allergy Clin Immunol 1996; 97:231.

93. Bernstein DI, Wanner M, Borish L, et al. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990–2001. J Allergy Clin Immunol 2004:1129–1136.
94. Waldbatt GL. The prevention of anaphylactic shock. JAMA 1932; 98:446.
95. Reid MJ, Lockey RF, Turkeltaub PC, et al. Fatalities (F) from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1990; 85.
96. Reid MJ, Lockey RF, Turkeltaub PC, et al. Survey of fatalities from skin testing and immunotherapy 1985–1989. J Allergy Clin Immunol 1993; 92:6–15.
97. Norman PS. Fatal misadventures. J Allergy Clin Immunol 1987; 79:572–573.
98. National Disease and Therapeutic Index. Plymouth Meeting, PA: IMS America Ltd., 1978–1988.
99. Lockey RF, Turkeltaub PC, Olive CA, et al. The Hymenoptera venom study II: Skin test results and safety of venom skin testing. J Allergy Clin Immunol 1989; 84:967–974.
100. Lockey RF, Turkeltaub PC, Olive ES, et al. The Hymenoptera venom study III: Safety of venom immunotherapy. J Allergy Clin Immunol 1990; 86:775–780.
101. Lin MS, Tanner E, Lynn J, et al. Nonfatal systemic allergic reactions induced by skin testing and immunotherapy. Ann Allergy 1993; 71:557–562.
102. Liccardi G, Salzillo A, Spadaro G, et al. Anaphylaxis caused by skin prick testing with aeroallergens: case report and evaluation of the risk in Italian allergy services. J Allergy Clin Immunol 2003; 111: 1410–1412.
103. Valyasevi MA, Maddox DE, Li JT. Systemic reactions to allergy skin tests. Ann Allergy Asthma Immunol 1999; 83:132–136.
104. Thompson M, Shearer D, Lockey R, et al. Systemic reactions to percutaneous (P) and intradermal (ID) skin tests (ST). J Allergy Clin Immunol 1998; 101:S30.
105. Bagg A, Chacko T, Glaum M, et al. Systemic reactions to percutaneous and intradermal skin tests. World Allergy Congress. Bangkok, Thailand, December, 2007.
106. Amin HS, Liss GM, Bernstein DI. Evaluation of near-fatal reactions to allergen immunotherapy injections. J Allergy Clin Immunol 2006; 117:169–175.
107. Ragusa FV, Passalacqua G, Gambardella R, et al. Nonfatal systemic reactions to subcutaneous immunotherapy: a 10-year experience. J Investig Allergol Clin Immunol 1997; 7:151–154.
108. Ragusa VF, Massolo A. Non-fatal systemic reactions to subcutaneous immunotherapy: a 20-year experience comparison of two 10-year periods. Allerg Immunol (Paris) 2004; 36:52–55.
109. Stewart E, Lockey RF. Systemic reactions from allergen immunotherapy. J Allergy Clin Immunol 1992; 90:567–578.
110. Ohashi Y, Nakai Y, Murata K. Effect of pretreatment with fexofenadine on the safety of immunotherapy in patients with allergic rhinitis. Ann Allergy Asthma Immunol 2006; 96:600–605.
111. Berchtold E, Maibach R, Muller U. Reduction of side effects from rush-immunotherapy with honey bee venom by pretreatment with terfenadine. Clin Exp Allergy 1992; 22:59–65.
112. Nielsen L, Johnsen CR, Mosbech H, et al. Antihistamine premedication in specific cluster immunotherapy: a double-blind, placebo-controlled study. J Allergy Clin Immunol 1996; 97:1207–1213.
113. Portnoy J, Bagstad K, Kanarek H, et al. Premedication reduces the incidence of systemic reactions during inhalant rush immunotherapy with mixtures of allergenic extracts. Ann Allergy 1994; 73: 409–418.
114. Casale TB, Busse WW, Kline JN, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. J Allergy Clin Immunol 2006; 117: 134–140.
115. Portnoy J, Bagstad K, Kanarek H, et al. Allergen immunotherapy: a practice parameter second update. J Allergy Clin Immunol 2007; 120:S25–S85.
116. Casale TB, Busse WW, Kline JN, et al. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Geneva: January 27–29, 1997. Allergy 1998; 53:1–42.
117. Kaplan AP, Anderson JA, Valentine MD, et al. Beta-adrenergic blockers, immunotherapy, and skin testing. American Academy of Allergy and Immunology. J Allergy Clin Immunol 1989; 84:129–130.
118. Bousquet J, Hejjaoui A, Dhivert H, et al. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. Systemic reactions during the rush protocol in patients suffering from asthma. J Allergy Clin Immunol 1989; 83:797–802.
119. Norman PS, Van Metre TE Jr. The safety of allergenic immunotherapy. J Allergy Clin Immunol 1990; 85:522–525.
120. Bousquet J, Hejjaoui A, Dhivert H, et al. Personnel and equipment to treat systemic reactions caused by immunotherapy with allergenic extracts. American Academy of Allergy and Immunology. J Allergy Clin Immunol 1986; 77:271–273.
121. Bousquet J, Lockey R, Malling HJ, et al. Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American academy of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 1998; 81:401–405.
122. Kemp SF, Lockey RF. Anaphylaxis: a review of causes and mechanisms. J Allergy Clin Immunol 2002; 110:341–348.

30 Adverse Effects Associated with Sublingual Immunotherapy

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HISTORICAL PERSPECTIVE

Allergen-specific immunotherapy is a biological response modifier that affects the response to allergen at the earliest steps of allergic diseases. For this reason, immunotherapy is currently a cornerstone in the management of allergic respiratory diseases and in Hymenoptera venom allergy. Since the beginning of the 20th century, allergenic vaccines were administered subcutaneously (SCIT, subcutaneous immunotherapy). The favorable clinical results rapidly resulted in the widespread use of SCIT, with this being the only form of allergen immunotherapy for many decades. Some clinicians occasionally tried routes other than subcutaneous (1–3). Since SCIT is well established in clinical practice, alternatives to SCIT generally were of limited interest (4).

In 1986, the British Committee for the Safety of Medicines officially reported 26 fatalities associated with SCIT (5). As a consequence, the risk-benefit ratio of immunotherapy was more critically questioned. The result was revision of the indications and implementation of additional safety measures, making the treatment impractical in some countries. The safety concerns with SCIT and resulting inconvenience stimulated the search for safer IT modalities, and the noninjection routes gained new interest. Although some of the adverse events (AEs) with SCIT can be avoided, others occur without explanation (6,7), contributing to the growth in interest of noninjection routes deemed to be safer. The first controlled trial with sublingual immunotherapy (SLIT) was published in 1986 (8). The earliest studies with SLIT were conflicting but an increasing number of positive studies were subsequently published. SLIT was mentioned in immunotherapy guidelines 10 years after the first SLIT trial (9), and in some subsequent guidelines (10,11). SLIT was categorized as a reasonable alternative to SCIT. Currently, SLIT is routinely used in several European countries, where standardized extracts for the relevant allergens are available. There is no FDA-approved SLIT allergen extract/vaccine in the United States and therefore SLIT is not an approved treatment.

THE SAFETY OF IMMUNOTHERAPY: GENERAL ASPECTS

A detailed description of the AEs possibly, probably, or certainly related to a treatment is provided by carefully reporting the experience in clinical trials or collecting reports from experience after approval. SCIT may cause a wide variety of AEs, ranging from a local wheal and flare reaction to systemic side effects such as asthma, generalized urticaria, anaphylaxis and, very rarely, death. The majority of the AEs are immediate (i.e., within 30 minutes) and therefore are presumed to be due to specific IgE, but delayed reactions also occur. AEs due to SCIT have been classified according to a system introduced in Europe in 1993 and updated in 2006 (12): local (large if >10 cm), mild systemic, moderate systemic, and severe systemic (usually requiring epinephrine). With the injection route, the rate of systemic reactions depends on the administration schedule, the type of allergen, and the survey method. The occurrence of systemic AEs with SCIT is approximately 0.05% to 0.6% of doses administered (13). Near fatal and fatal events are very rare with appropriate administration (14), but the risk of severe AEs and fatalities (approximately 1 per 2.5 million injections) persists even with the best clinical practice. Reporting of AEs for SCIT is sometimes incomplete. For example, no

information is provided about AEs in approximately 20% of the published clinical trials (15). In the remaining 80% of studies, AEs occur on average in 14% of patients, with limited details provided. A survey in 2004 reported that human error remains a major cause of incorrect dosing with SCIT, but human error may not be the cause of most serious SCIT reactions (7,8,16).

AE rates are reported in the majority of the SLIT studies since this treatment was developed to minimize AEs and the prevalence and incidence of AEs were more closely monitored. An AE classification system similar to SCIT is often used: local reactions in the mouth or digestive tract, mild systemic, moderate systemic, and severe systemic. Many of the AEs with SLIT involve the digestive system at various levels: itching, swelling, burning of the lips, oral mucosa or tongue; nausea; diarrhea; and abdominal pain. Some studies report that up to 50% of treated subjects experience oral or gastrointestinal reactions. The mechanisms of AEs with SLIT are not known, but the amount of allergen and possibly the number of allergens administered play an important role. When the allergen vaccine is immediately swallowed (oral route), there are few side effects involving the mouth, whereas abdominal pain and nausea are frequent. But when the dose is held under the tongue and expectorated, oral reactions are more common and abdominal complaints are less frequent. SLIT is self managed at home by the patient and the severity of AEs is usually mild; therefore, the frequency of SLIT AEs is probably underestimated.

DATA FROM THE RANDOMIZED DOUBLE-BLIND CLINICAL TRIALS

There are 49 randomized double-blind, controlled clinical trials with SLIT (4,17,18). No life-threatening AE or fatality has been described in clinical trials, but three severe AEs have been reported in clinical experience (19–21). Ten of 46 published trials report no AE.

The most frequently described AE in children and adults is the immediate onset of oral/sublingual itching after dosing (ranging from 0–40%), followed in frequency by abdominal pain, nausea, and/or diarrhea. These AEs are usually, but not always, described as mild and self-limited and do not require dose adjustment or medical treatment. Systemic AEs (e.g., asthma, rhinitis, urticaria, angioedema) or severe abdominal complaints are reported in less than 3% of patients (22–26). SLIT was used in 14 pediatric studies, including children from 3 to 17 years of age. All of those studies report an acceptable safety profile and four studies report no side effects. Local oral side effects are the most frequent in children as well. The occurrence of nausea, abdominal pain, and oral symptoms was 20% in the active group and 2% in controls when high-dose SLIT was employed (23). Three cases of moderately severe urticaria were described by Tari et al. (22). Bufe et al. (27), Hirsch et al. (28), and Pajno et al. (29) reported one moderate asthma exacerbation in the active groups. A published randomized SLIT trial with cat vaccine reported no AEs (30).

The largest published SLIT trial involved more than 600 patients (31), with three doses of allergen. AEs were reported by 54% of patients. Almost all of these events were mild/moderate and self-limited, but one episode of uvular edema was judged as a serious AE. Only 2% of the participants withdrew for AEs probably or possibly related to SLIT. A randomized controlled trial in 2006 was designed specifically to assess safety as well as efficacy of SLIT in subjects with asthma (32). The occurrence of asthma AEs was not different with SLIT compared with placebo in the 114 subjects studied. Mild, local discomfort occurred in 53% of the SLIT subjects and in none of the placebo-treated individuals; no severe AE occurred. Another controlled dose finding study of safety (33) was conducted in 48 grass-allergic patients outside the pollen season. They received SLIT for 28-day periods at progressively increasing doses, up to 200 μg Phl p 5 or approximately 20 to 40 times the amount given with maintenance SCIT. The overall incidence of all AEs was 74%, all of mild or moderate intensity. The most frequently reported events were irritation of the throat and oral itching.

SLIT has been utilized also in diseases other than respiratory allergy. Enrique et al. (34) reported successful treatment of hazelnut-induced allergy with SLIT. Local AEs were reported with 7% and systemic AEs with 0.2% of the doses, and no AE was severe or required SLIT discontinuation. However, in a randomized controlled study with dust mite–SLIT for atopic dermatitis, treatment lead to intolerable worsening of the skin lesions in 2 out of 24 children and SLIT was discontinued (35).

DATA FROM SYSTEMATIC REVIEWS OF THE LITERATURE

André et al. (36) examined the safety of SLIT in eight trials performed with vaccines from a single manufacturer. These studies involved 690 subjects (347 active + 343 placebo), with 218 children aged 5 to 16 years (103 active + 115 placebo). The majority of AEs were local (82%) and the systemic AEs did not differ in active and placebo groups. The only differences were the oral and gastrointestinal AEs that were more frequent in SLIT patients, although mild in severity. The rates of AEs and dropouts were similar in adults and children.

A 2006 review (17) of the available studies on SLIT (including nonrandomized and observational ones) reported the following major findings. In 41 studies with information on the total number of AEs, 1047 AEs in a total of 386,149 doses were identified (2.7 reactions per 1000 doses). There were 529 AEs per 4378 patients (12%) in 49 studies with information on the number of patients treated. In the trials that specified the severity of reaction, the occurrence of severe AEs was 0.56 per 1000 doses. Overall, 14 serious AEs probably related to the treatment were reported.

DATA FROM THE POSTMARKETING SURVEYS

The data on the safety derived from postmarketing surveys are of relevance. First, AEs occur more often in a more diverse population of patients. Second, these data reflect patients' perception of AEs in an uncontrolled setting since SLIT is self-administered at home (Table 1).

Di Rienzo et al. (37) followed 268 children between 2 and 15 years of age for 3 years. The overall rate of systemic AEs was 3% of the patients and 1/12,000 doses, with no discontinuation. Only one (urticaria) of the AEs was judged of moderate severity and required oral antihistamine therapy. Another survey was performed in adults (38), with 198 patients observed for about three years. Side effects occurred in 7.5% of patients and 0.52/1000 doses administered. Four urticaria and two gastrointestinal AEs were of moderate intensity.

Table 1 Summary of the Postmarketing Surveys

First author (ref)	*N* patients	Age range	Follow-up	Allergens	Cumulative dose/yr	AE % patients	AE/1000 doses
Di Rienzo (37)	268	2–15 yr	3 yr	54% mites 25% grass 6% Parietaria 16% mixed	200 μg Der 1 39 μg group 5 24 μg Par j 1	3	0.083
Lombardi (38)	198	Adults	3 yr	35% mites 38% grass 23% Parietaria 4% others	104,000 AU for all allergens	7.5	0.5
Pajno (39)	354	5–15 yr	3–4 yr	42% mites 20% Parietaria 11% grass 7% olive 20% mixed	120 μg Der 1 30 μg Par j 1 72 μg group 5 432 μg Ole e1	6	0.15
Fiocchi (40)	65	3–7 yr	1 yr	64% mites 17% grass 12% trees 7% Parietaria	36,000 IR for all allergens	15	Not stated
Drachenberg (41)	159	6–60 yr	<1 yr	16% mites 37% grass 12% trees 35% other/mix	Not stated	6.3	Not stated
Agostinis (42)	36	3–5 yr	2 yr	mites 19 grasses 17	90,000 AU for both allergens	5	0.07
Di Rienzo (43)	128	3–5 yr	2 yr	62% mites 22% grass 12% Parietaria 4% others	174 μg Der 1 39 μg group 5 24 μg Par j 1	5.6	0.2

AEs were controlled by a temporary dose adjustment, and in no case was the SLIT discontinued. In another survey of 354 asthmatic children of 5 to 12 years of age (39), both the sublingual-swallow and the sublingual-spit modalities were considered. The occurrence of AE was 6% of treated patients and 0.15/1000 doses. Nonspecific reactions, such as headache and fatigue, were observed in 4% of patients. Fiocchi et al. (40), with a high-dose (up to 40,000 IR) SLIT regimen, reported at least one AE in 11 of 65 children, aged from 3 to 10 years. All the events were mild (oral/intestinal) except one severe AE with vomiting (39). Drachenberg et al. (41) reported eight local and four systemic AEs (3 rhinitis, 1 exanthema) in 6.3% of 159 patients.

SCIT is generally not recommended in children younger than five years because the systemic side effects are more difficult to recognize in small children. There are two postmarketing surveys of SLIT performed in children between three and five years of age. The first, in 36 subjects, reported AE in 5% of patients and a rate of 0.071/1000 doses. These events were gastrointestinal and mild (42). The other survey (43) involved 128 children and reported AEs in 5.6% of patients (0.2/1000 doses). Two AEs were local and mild and the remaining (nausea, diarrhea) were moderate.

Finally, in 2006 and 2007, three cases of anaphylaxis were first reported with SLIT (19–22). In one case, the diagnosis of anaphylaxis was not made by a health professional, no epinephrine was needed, and the SLIT used was a mixture of six different allergens, some of which were nonstandardized (19). In another case, anaphylaxis with a standardized latex extract occurred at the maximum recommended dose with an ultrarush procedure (20). In the third case, a mixture of dust mites plus five grasses plus four cereals was used. The symptoms were severe oral and gastrointestinal complaints, but criteria for severe anaphylaxis were not met and epinephrine was not necessary (21).

ADVERSE EVENTS, ALLERGEN DOSE, AND INDUCTION REGIMEN

The relationship between allergen dose and AEs with SLIT is not clearly evident from the controlled trials published during the last 20 years. This may be due to a relatively small sample size in many of the studies. The limited evidence of a dose relationship is particularly true for systemic AEs due to their limited frequency. A review of the published studies (44) evaluated the occurrence of AEs according to the dose, expressed as the ratio between SLIT and the equivalent SCIT. This review concluded that local or oral side effects are more frequent with "low" doses of allergen (less than 50 times the corresponding SCIT dose) than with higher doses. Refuting this point are the results of two randomized controlled studies of several hundred adult subjects (32,33). These trials demonstrate that local AEs are dose dependent. Gastrointestinal complaints (nausea, stomachache, and vomiting) also occur more frequently with higher doses of vaccine.

The inconsistent relationship of dose and AE with SLIT has resulted in the proposal that a slow updosing phase, or buildup, may not be necessary. One trial of differing induction regimens demonstrated that AEs were not related to the duration of the buildup (45). Subsequent reports with ultrarush (less than 2 hours) updosing show that the rapid increase of SLIT doses does not increase AEs (46,47). Preliminary experiences with the no-updosing regimen (initiating SLIT with the maintenance dosage) support the feasibility of this regimen (48). A randomized trial compared the safety of the traditional updosing regimen with the no-updosing (49) regimen in 135 patients and found no difference in AEs rate between the two groups. Four large randomized trials with grass extracts were performed with the no-updosing regimen (31–33,50), and their results in term of safety were as favorable as those of the studies with the traditional buildup protocol.

CLINICAL IMPLICATIONS

The overall safety of SLIT is confirmed by clinical trials, postmarketing surveillance, and 20 years of clinical use. The following statements summarize the published SLIT literature in the opinion of the authors (Table 2).

Table 2 SLIT: Side Effect

Local	
Oral itching/swelling	Relatively frequent; usually self-resolve after the first doses without treatment; if persist reduce the dose and treat
Stomachache	
Nausea/vomiting	
Systemic	
Urticaria/angioedema	Rare; give symptomatic treatment and reduce the dose; if persist, stop SLIT
Rhinitis	
Asthma	
Anaphylaxis	Exceptional; treat properly and stop SLIT

The occurrence and severity of AE do not differ between children (even younger than 5 years of age) and adults.

No fatality with SLIT has been reported, indicating a very low risk.

Severe uncontrolled asthma is not a relative contraindication for SLIT as with SCIT.

Anaphylaxis rarely occurs and, in two of the three cases, a mixture of more than four allergens was utilized. This suggests that SLIT may be safer with administration of a limited number of relevant allergens, the typical practice in Europe. One case of anaphylaxis occurred with an ultrarush SLIT protocol for latex allergy, suggesting that latex SLIT may have unique risk. If anaphylaxis occurs, SLIT usually should be discontinued since SLIT is administered at home without immediate access to treatment.

The severity of the large majority of the AEs reported is mild. Most AEs involve the mouth (burning or itching) or the gastrointestinal tract (abdominal pain, nausea) and usually resolve within a few days without intervention.

In case of persisting or moderately severe local AEs, a temporary dose reduction and concomitant antihistamine therapy are considered. If an AE persists and remains troublesome, SLIT should be discontinued.

Systemic AEs, such as urticaria, rhinitis, or asthma, require treatment and temporary dose reduction and likely should result in discontinuation of SLIT.

No-updosing regimens, though convenient, likely increase the occurrence of AEs.

SALIENT POINTS

- SLIT is marketed and used in current clinical practice in many European countries. Detailed data on its safety are available in clinical trials and postmarketing surveys.
- The literature consistently shows that the side effects of SLIT are mostly local (oral itching/burning, stomachache) and mild and very rarely lead to treatment discontinuation.
- Systemic AEs (rhinitis, asthma, urticaria) have been occasionally reported.
- The rate of AEs is not increased in very young children (below the age of 5 years).
- Only three cases of severe systemic reactions (one ascertained anaphylaxis) have been reported, with ultrarush latex SLIT and with mixtures of multiple allergens. These circumstances may require special caution.
- The use of no-induction (no up-dosing) regimens is well tolerated and does not seem to increase the risk.

REFERENCES

1. Herxeimer H. Bronchial hypersensitization and hyposensitization in man. Int Arch Allergy Appl Immunol 1951; 40:40–57.
2. Taylor G, Shivalkar PR. Local nasal desensitization in allergic rhinitis. Clin Allergy 1972; 2:125–126.
3. Taudorf E, Weeke B. Orally administered grass pollen. Allergy 1983; 38:561–564.

4. Canonica GW, Passalacqua G. Non injection routes for immunotherapy. J Allergy Clin Immunol 2003; 111:437–448.
5. Committee on the Safety of Medicines. CSM update. Desensitizing vaccines. Br Med J 1986; 293:948.
6. Lockey RF, Benedict LM, Turkeltaub PC, et al. Fatalities from immunotherapy and skin testing. J Allergy Clin Immunol 1987; 79:660–677.
7. Reid MJ, Lockey RF, Turkeltaub PC, et al. Survey of fatalities from skin testing and immunotherapy. J Allergy Clin Immunol 1993; 92:6–15.
8. Scadding K, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to dust mite. Clin Allergy 1986; 16:483–491.
9. Bousquet J, Lockey R, Malling HJ, eds. World Health Organization Position Paper. Allergen immunotherapy: therapeutical vaccines for allergic diseases. Allergy 1998; 53(suppl).
10. Malling HJ, ed. EAACI Position Paper on local immunotherapy. Allergy 1998; 53:933–944.
11. Bousquet J, Van Cauwenberge P, eds. Allergic Rhinits and its impact on asthma. J Allergy Clin Immunol 2001; 108(5 suppl):S146–S150.
12. Alvarez-Cuesta E, Bousquet J, Canonica GW, et al. Standards for practical allergen-specific immunotherapy. Allergy 2006; 61(suppl 82):1–20.
13. Stewart GE, Lockey RF. Systemic reactions from allergen immunotherapy. J Allergy Clin Immunol 1992; 90:567–578.
14. Lockey RF, Nikoara-Kasti GL, Theodoropoulos DS, et al. Systemic reactions and fatalities associated with allergen immunotherapy. Ann Allergy Asthma Immunol 2001; 87(1 suppl):S46–S55.
15. Malling HJ. Immunotherapy as an effective tool in allergy treatment. Allergy 1998; 53:461–472.
16. Aaronson DW, Gandhi TK. Incorrect allergy injections: allergists' experiences and recommendations for prevention. J Allergy Clin Immunol 2004; 113:1117–1121.
17. Cox L, Linneman D, Nolte H, et al. Sublingual immunotherapy: a comprehensive review. J Allergy Clin Immunol 2006; 117:1021–1035.
18. Passalacqua G, Durham SR. ARIA update: allergen immunotherapy. J Allergy Clin Immunol 2007; 119:881–991.
19. Dunsky E, Goldstein MF, Dvorin MJ, et al. Anaphylaxis due to sublingual immunotherapy. Allergy 2006; 61:1235.
20. Antico A, Pagani M, Crema A. Anaphylaxis by latex sublingual immunotherapy. Allergy 2006; 61:1236.
21. Eifan AO, Keles S, Bahceciler NN, et al. Anaphylaxis to multiple pollen allergen sublingual immunotherapy. Allergy 2007; 62:567–568.
22. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double blind study. Allergol Immunopathol 1990; 18:277–284.
23. La Rosa M, Ranno C, Andre' C, et al. Double blind placebo controlled evaluation of sublingual swallow immunotherapy with standardized parietaria judaica extract in children with allergic rhinoconjunctivitis. J Allergy Clin Immunol 1999; 104:425–432.
24. Guez S, Vatrinet C, Fadel R, et al. House dust mite sublingual swallow immunotherapy in perennial rhinitis: a double blind placebo controlled study. Allergy 2000; 55:369–375.
25. Lima MT, Wilson D, Pitkin L, et al. Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. Clin Exp Allergy 2002; 32:507–514.
26. Tonnel AB, Scherpereel A, Douay B, et al. Allergic rhinitis due to house dust mites: evaluation of the efficacy of specific sublingual immunotherapy. Allergy 2004; 59(5):491–497.
27. Bufe A, Ziegler-Kirbach E, Stoeckmann E, et al. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. Allergy 2004; 59:498–504.
28. Hirsch T, Sahn M, Leupold W. Double blind placebo controlled study of sublingual immunotherapy with house dust mite extracts in children. Pediatr Allergy Immunol 1997; 8:21–27.
29. Pajno G, Vita D, Caminiti D, et al. Impact of sublingual immunotherapy on seasonal asthma and skin reactivity in children allergic to Parietaria pollen treated with inhaled fluticasone propionate. Clin Exp Allergy 2003; 33(12):1641–1647.
30. Alvarez-Cuesta E, Berges-Gimeno P, Mancebo EG, et al. Sublingual immunotherapy with a standardized cat dander extract: evaluation of efficacy in a double blind placebo controlled study. Allergy 2007; 62(7):810–817.
31. Durham SR, Yang WH, Pedersen MR, et al. Sublingual immunotherapy with once-daily grass-allergen tablets: a randomised controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117(4):802–809.
32. Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. Allergy 2006; 61:185–190.
33. Kleine-Tebbe J, Ribel M, Herold DA. Safety of a SQ-standardised grass allergen tablet for sublingual immunotherapy: a randomized, placebo-controlled trial. Allergy 2006; 61(2):181–184.

34. Enrique E, Pineda F, Malek T, et al. Sublingual immunotherapy for hazelnut food allergy: a randomized, double-blind, placebo-controlled study with a standardized hazelnut extract. J Allergy Clin Immunol 2005; 116:1073–1079.
35. Pajno GB, Caminiti L, Vita D, et al. Sublingual immunotherapy in mite-sensitized children with atopic dermatitis: a randomized, double-blind, placebo-controlled study. J Allergy Clin Immunol 2007; 120 (1):164–170.
36. André C, Vatrinet C, Galvain S, et al. Safety of sublingual swallow immunotherapy in children and adults. Int Arch Allergy Immunol 2000; 121:229–234.
37. Di Rienzo V, Pagani A, Parmiani S, et al. Post-marketing surveillance study on the safety of sublingual immunotherapy in children. Allergy 1999; 54:1110–1113.
38. Lombardi C, Gargioni S, Melchiorre A, et al. Safety of sublingual immunotherapy in adults: a post marketing surveillance study. Allergy 2001; 56:889–892.
39. Pajno GB, Peroni DG, Vita D, et al. Safety of Sublingual immunotherapy in children with asthma. Paediatr Drugs 2003; 5:777–781.
40. Fiocchi A, Pajno G, La Grutta S, et al. Safety of SLIT in children aged 3 to 7 years. Ann Allergy Asthma Immunol 2005; 95:254–258.
41. Drachenberg KJ, Urban E, Proll S, et al. Sublingual specific immunotherapy for adults and children: a post marketing survey. Allergol Immunopathol 2004; 32:76–81.
42. Agostinis F, Tellarini L, Falagiani P, et al. Safety of SLIT in very young children. Allergy 2005; 60:133.
43. Di Rienzo V, Minelli M, Musarra A, et al. Post-marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. Clin Exp Allergy 2005; 35:560–564.
44. Gidaro GB, Frati F, Sensi L, et al. The safety of sublingual-swallow immunotherapy: an analysis of published studies. Clin Exp Allergy 2005; 35(5):565–571.
45. Grosclaude M, Bouillot P, Alt R, et al. Safety of various dosage regimens during induction of sublingual immunotherapy. Int Arch Allergy Immunol 2002; 129:248–253.
46. Rossi R, Monasterolo RE. A pilot study of feasibility of ultra-rush (20-25 minutes) sublingual-swallow immunotherapy in 679 patients (699 sessions) with allergic rhinitis and/or asthma. Int J Immunopathol Pharmacol 2005 18(2):277–285.
47. Tripodi S, Di Rienzo Businco A, Benincori N, et al. Safety and tolerability of ultra-rush induction, less than one hour, of sublingual immunotherapy in children. Int Arch Allergy Immunol 2005; 139(2): 149–152.
48. Guerra L, Compalati E, Rogkakou A, et al. Randomized open comparison of the safety of SLIT in a no updosing and traditional updosing schedule in patients with parietaria allergy. Allergol Immunopathol 2006; 34:82–83.
49. Rodriguez F, Boquete M, Ibanez MD, et al. Once daily sublingual immunotherapy without updosing—a new treatment schedule. Int Arch Allergy Immunol 2006; 140:321–326.
50. Didier A, Malling HJ, Worm M, et al. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol 2007; 120(6):1338–1345.

31 Prevention and Treatment of Anaphylaxis

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ANAPHYLAXIS AND ALLERGEN IMMUNOTHERAPY

Definitions

Anaphylaxis is not a reportable disease and both its morbidity and mortality are probably underestimated. A variety of statistics on the epidemiology of anaphylaxis are published (Table 1) (1–10). There is no universally accepted clinical definition of anaphylaxis (11,12). The traditional nomenclature for anaphylaxis reserves the term *anaphylactic* for IgE-dependent reactions and the term *anaphylactoid* for IgE-independent events, which are clinically indistinguishable. The World Allergy Organization, which is an international umbrella organization whose members represent 74 national and regional professional societies dedicated to allergy and clinical immunology, recommends the replacement of this terminology with *immunologic* (IgE mediated and non-IgE mediated, e.g., IgG and immune complex complement mediated) and *nonimmunologic* anaphylaxis and the term "anaphylaxis" refers to both (11). Anaphylaxis was defined by the National Institute of Allergy and Infectious Diseases and Food Allergy and Anaphylaxis Network (Chantilly, Virginia, U.S.). It is considered likely to be present if any one of three criteria is satisfied within minutes to hours: (*i*) acute onset of illness with involvement of skin, mucosal surface, or both, *and* at least one of the following: respiratory compromise, hypotension, or end organ dysfunction; (*ii*) two or more of the following occur rapidly after exposure to a *likely* allergen: involvement of skin or mucosal surface, respiratory compromise, hypotension, or persistent gastrointestinal symptoms; (*iii*) hypotension develops after exposure to a *known* allergen for that patient: age-specific low blood pressure or decline of systolic blood pressure of greater than 30% compared to baseline (12). In clinical practice, however, waiting until the development of multiorgan symptoms is risky since the ultimate severity of an anaphylactic reaction is difficult to predict from the outset.

Signs and symptoms of anaphylaxis vary, but cutaneous features (urticaria, angioedema, erythema) are the most common overall (13). Reactions may be immediate and uniphasic, or they may be delayed in onset, biphasic (recurrent), or protracted (discussed below). Respiratory compromise and cardiovascular collapse cause most fatalities (13,14). An analysis of 202 anaphylaxis fatalities occurring in the United Kingdom from 1992 to 2001 ascertained that the interval between initial onset of food anaphylaxis symptoms and fatal cardiopulmonary arrest averaged 25 to 35 minutes, which was longer than for insect stings (10–15 minutes) or for drugs (mean, 5 minutes in hospital; 10–20 minutes prehospital) (14).

Subcutaneous Allergen Immunotherapy and Anaphylaxis

Three studies suggest that fatalities from subcutaneous allergen immunotherapy (SCIT) occur at a rate of approximately 1/2,500,000 injections (8,15,16). However, the physicians in these surveys included only members of the American Academy of Allergy Asthma and Immunology. Therefore, the prevalence of fatalities resulting from SCIT in the hands of physicians who are not specially trained to practice allergy and immunology remains unknown. These three studies evaluated 104 fatalities from immunotherapy and skin testing between 1945 and 2001. Most of the fatalities occurred from reactions that took place within 30 minutes of the injection. Subjects with asthma or with high levels of allergen-specific IgE were more likely to die from anaphylaxis. Subjects who were symptomatic, especially with asthma, at the time of the injection or who were in their "allergy season" were also at increased risk. Some errors in vial selection, especially when advancing to the next vial, and mistakes in dosage or administration also were important in these deaths.

Table 1 Epidemiology of Anaphylaxis

Statistic	Value	Reference
Incidence	30/100,000 person-yr	1
Risk/person in United States	1–3% per person	1, 2
	1.24–16.74%	3
Risk in hospitalized patients	1/2700	4
Hospital fatalities	$154/10^6$ patients	5
Mortality rate	1%	2
Food anaphylaxis fatalities	150/yr	6
Peanuts or tree nuts	94% fatalities in registry	7
Fatal reactions to β-lactam antibiotics	400–800/yr	4
Fatalities from allergen immunotherapy	1/2,500,000 injections	8
Systemic reactions to Hymenoptera stings	0.4–4%	9
Prevalence of idiopathic anaphylaxis	34,000 subjects	10

Near-fatal reactions (NFRs) to SCIT also have been examined retrospectively. Of 646 survey allergist-immunologist respondents, 273 reported NFR. The investigators defined an NFR as respiratory compromise, hypotension, or both, requiring emergency epinephrine. Hypotension was reported in 80% and respiratory failure occurred in 10% of NFRs, exclusively in asthmatic subjects. Epinephrine was delayed or not administered in 6% of these cases (17).

Lüderitz-Püchel et al. (18) analyzed 28 of 48 SCIT-related fatalities reported to the Paul Ehrlich Institute from Germany and other countries from 1977 to 1994. Three additional subjects experienced hypoxic brain injury following anaphylaxis. In 23 of the 28 fatalities investigated, the authors could not exclude medical errors and/or inadequate information provided to the subjects as contributory factors to fatal anaphylaxis. Errors include mistakes in dosage and administration of allergen vaccines and failure to adhere to the recommended 30-minute period of observation following injection.

Stewart and Lockey (19) reviewed 38 studies for systemic reactions associated with SCIT. The percentage of subjects experiencing one or more systemic reactions ranged from 0.8% to 46.7% for conventional dose schedules (mean 12.92%, SD 10.8%). Systemic reactions per injection during conventional build up schedules occurred in 0.05% to 3.2% of subjects (mean 0.5%, SD 0.87%). All but one study reported rates per injection of 0.6% or less. The 23 studies in which rush or accelerated schedules were used were also reviewed. The percentage of subjects experiencing one or more systemic reactions during the maintenance phase of rush or accelerated SCIT ranged from 0% to 21.1% (mean 4.77%, SD 6.47%). However, the number of systemic reactions was higher during buildup when the interval between injections was reduced ("rush" or "semirush" protocols). Subjects experiencing one or more systemic reaction ranged from 0% to 66.7% with these accelerated schedules (mean 21.33%, SD 17.86%). These accelerated protocols were associated with rates of systemic reactions per injection ranging from 0% to 6.4% (mean 2.36%, SD 1.7%).

MECHANISMS OF ANAPHYLAXIS

Chemical Mediators of Anaphylaxis

The chemical mediators that cause anaphylaxis are preformed and released from granules (histamine, tryptase, glycosidases, and granulocyte chemotactic factors) or are generated from membrane lipids (prostaglandin D_2, leukotrienes, and platelet-activating factor) by the activated mast cell and basophil (13). The development and severity of anaphylaxis also depend on the responsiveness of cells targeted by these mediators. IL-4 and IL-13 are cytokines important in the initial generation of antibody and inflammatory cell responses to anaphylaxis. No comparable studies have been conducted in humans, but anaphylactic effects in the mouse depend on IL-4Rα-dependent IL-4/IL-13 activation of the transcription factor, signal transducer and activator of transcription 6 (STAT-6) (20).

Eosinophils may be proinflammatory (e.g., release of cytotoxic granule–associated proteins) or anti-inflammatory (e.g., metabolism of vasoactive mediators) (21,22). A guinea pig anaphylaxis model suggests that eosinophils already present in chronically inflamed airways

may participate in the immediate phase response to allergen exposure as well as the role traditionally expected in the late-phase allergic response (23). Potential implications for human anaphylaxis have not been determined.

Histamine is only one of the mast cell mediators released in anaphylaxis, but its systemic effects have been studied more than other mediators. In one study, investigators infused histamine into normal volunteers at doses ranging from 0.05 to 1.0 μg/kg/min over 30 minutes to determine the plasma levels required to elicit symptoms of anaphylaxis (24). A mean plasma level of 1.61 ± 0.30 ng/mL induced a 30% increase in heart rate; a level of 2.39 ± 0.52 ng/mL induced flushing and headache; and a level of 2.45 ± 0.13 ng/mL induced a 30% increase in pulse pressure. Pretreatment with an H_2-antagonist (cimetidine) did not alter these reactions. However, pretreatment with the H_1 antagonist, hydroxyzine hydrochloride, increased the level of histamine necessary to increase the heart rate by 30%. Combining the H_1 and H_2 antihistamines significantly raised the level at which histamine elicited all responses. On the basis of these results, the authors concluded that flushing, hypotension, and headache associated with histamine infusion are mediated by both H_1 and H_2 receptors, whereas tachycardia, pruritus, rhinorrhea, and bronchospasm are associated only with H_1 receptors.

H_3 receptors have been implicated in a canine model of anaphylaxis (25). These inhibitory presynaptic receptors modulate endogenous release of norepinephrine from sympathetic fibers that innervate the cardiovascular system. Pretreatment of study animals with thioperamide maleate, an H_3 receptor antagonist, is associated with a higher heart rate and greater left ventricular systolic function compared with a nontreatment group or the other treatment arms involving receptor blockade for H_1, H_2, cyclooxygenase, and leukotriene pathways (25). Potential implications for human subjects have not been studied.

Tryptase is concentrated selectively in the secretory granules of human mast cells and released when these cells degranulate. It can activate complement, coagulation pathways, and the kallikrein-kinin contact system with the potential clinical consequences of hypotension, angioedema, clotting, and clot lysis (disseminated intravascular coagulation) (22). Release of β-tryptase (mature tryptase) stored in mast cell secretory granules is more specific for activation than α-protryptase, which is an inactive monomer that is secreted constitutively at rest. Tryptase levels generally correlate with the clinical severity of anaphylaxis (26). However, a dichotomy may exist in the magnitude of tryptase elevations for those individuals experiencing anaphylaxis after parenteral exposure (e.g., injection, insect sting) versus oral exposure (e.g., food ingestion). In an analysis of anaphylaxis fatalities, the parenterally exposed subjects had higher serum levels of tryptase and lower levels of antigen-specific IgE, whereas those who succumbed after oral exposure had low tryptase levels and comparatively higher levels of antigen-specific IgE (27). This difference may be related to the mast cell phenotype first encountered by the culprit antigen. Tryptase- and chymase-containing mast cells (MC_{TC}) are threefold more common in connective tissue than tryptase-containing mast cells (MC_T). The latter predominate in the mucosa of the lung and small intestine (27).

Levels of total tryptase peak 60 to 90 minutes after the onset of anaphylaxis and can persist as long as five hours after the onset of symptoms (22). The estimated positive predictive value of tryptase elevations in 259 subjects with anesthesia-associated anaphylaxis is 92.6%, and the estimated negative predictive value of normal tryptase levels is 54.3% (28). Serial tryptase measurements might improve diagnostic sensitivity, but further investigation is needed (12).

Elevations of histamine and tryptase may not correlate clinically. In an emergency department study evaluating subjects who presented with acute allergic reactions, elevated histamine was observed in 42 of 97 subjects, but only 20 exhibited increased tryptase levels (29). Serum histamine levels also correlate with the severity and persistence of cardiopulmonary manifestations, but not with urticaria (29,30).

Nitric oxide (NO), a potent autacoid vasodilator formerly known as endothelium-derived relaxing factor, appears to be involved in the complex interaction of regulatory and counterregulatory mediators in mast cell activation, including anaphylaxis (31,32). L-Arginine is converted to NO as histamine binds to H_1 receptors during phospholipase C–dependent calcium mobilization. Physiologically, NO participates in the homeostatic control of vascular tone and regional blood pressure. Experiments with NO inhibitors in mice, rabbits, and dogs demonstrate that NO promotes bronchodilation, coronary artery vasodilation, and decreases histamine release. However, its net effects in anaphylaxis appear to be detrimental vascular smooth muscle relaxation and enhanced vascular permeability (33).

Metabolites of arachidonic acid include products of lipoxygenase and cyclooxygenase pathways. Of note, leukotriene B_4 is a chemotactic agent and thus can solicit other cells to participate in anaphylaxis. These cells theoretically contribute to biphasic (recurrent) anaphylaxis and to protracted reactions. Other effects of arachidonic acid metabolites may reflect mast cell degranulation, since elevations occur in tryptase and histamine. These effects include bronchospasm, hypotension, and skin erythema (22).

Inflammatory mediators also activate the contact system. These consist of kininogenase, kallikrein, and tryptase. Release of these mediators may induce formation of bradykinin as well as the activation of factor XII. This, in turn, may produce clotting, clot lysis, and subsequent activation of complement. In contrast, some mediators may have a salubrious effect that limits anaphylaxis. For example, chymase may activate angiotensin II, which can modulate hypotension. Heparin inhibits clotting, kallikrein, and plasmin, opposes complement formation, and modulates tryptase activity (22).

There are other inflammatory pathways that participate in anaphylactic episodes. These may be extremely important in the prolongation and amplification of anaphylaxis. Much of the supporting evidence derives from data obtained during experimental insect sting challenges. During severe episodes of anaphylaxis, there is concomitant activation of complement, coagulation pathways, and the contact (kallikrein-kinin) system. Decreases in C4 and C3, as well as the formation of C3a, have been observed in anaphylaxis. Demonstrable evidence for coagulation pathway activation during severe anaphylaxis includes decreases in factor V, factor VIII, and fibrinogen, and fatal disseminated intravascular coagulation in some instances (14,30). Of 202 anaphylaxis fatalities analyzed retrospectively over a 10-year period in the United Kingdom, seven (8%) were attributable to disseminated intravascular coagulation (14). Successful treatment with tranexamic acid is reported (34). Contact system recruitment is indicated by decreased high molecular weight kininogen and the formation of kallikrein–C1 inhibitor and factor XIIa–C1 inhibitor complexes. Kallikrein activation not only results in the formation of bradykinin but also activates factor XII. Factor XII alone can lead to clotting and clot lysis via plasmin formation, and plasmin can also activate complement (22).

Effects on the Cardiovascular System

Anaphylaxis can be associated with myocardial ischemia, conduction defects, atrial and ventricular arrhythmias, and T-wave abnormalities (35). Whether such changes are related to direct mediator effects on the myocardium, exacerbation of preexisting myocardial insufficiency by the hemodynamic stress of anaphylaxis, or from either exogenous or endogenous epinephrine is unclear (30,35–37).

Histamine exerts its pathophysiologic effects during anaphylaxis via both H_1 and H_2 receptors. H_1 receptors mediate coronary artery vasoconstriction and increase vascular permeability, whereas H_2 receptors increase atrial and ventricular inotropy, atrial chronotropy, and coronary artery vasodilation. The interaction of H_1 and H_2 receptor stimulation appears to mediate decreased diastolic pressure and increased pulse pressure (38). Animal studies suggest a possible modulatory role for H_3 receptors (25). Platelet-activating factor (PAF) also decreases coronary blood flow, delays atrioventricular conduction, and has negative inotropic effects on the heart (35).

Raper and Fisher (36) described two previously healthy subjects who developed profound myocardial depression during anaphylaxis. Echocardiography, nuclear imaging, and hemodynamic measurements confirmed the presence of myocardial dysfunction. Intraaortic balloon counterpulsation supplemented anaphylaxis treatment to provide hemodynamic support. Balloon counterpulsation was required for up to 72 hours because of persistent myocardial depression, even though other clinical signs of anaphylaxis had resolved. Both subjects recovered with no subsequent evidence of myocardial dysfunction. Thus, the heart may be a primary target for anaphylaxis, even in subjects with no preexisting cardiovascular disease.

Increased vascular permeability during anaphylaxis can shift up to 35% of intravascular volume to the extravascular space within 10 minutes (39). Intrinsic compensatory responses to anaphylaxis, e.g., endogenous catecholamines, angiotensin II, and endothelins, also influence the extent of clinical manifestations and, when adequate, may be lifesaving independent of medical intervention. These compensatory responses, however, produce variable effects on peripheral vascular resistance. Some subjects experience abnormal elevation of the peripheral

vascular resistance (maximal vasoconstriction), yet shock persists due to reduced intravascular volume, while others have decreased systemic vascular resistance despite elevated levels of catecholamines (13). These differences have important clinical implications since the latter scenario may respond favorably to therapeutic doses of vasoconstrictor agents, while the former is vasoconstrictor unresponsive and requires large-volume fluid resuscitation.

In a retrospective review of prehospital anaphylactic fatalities in the United Kingdom, the postural history was known for ten individuals (40). Four of the ten fatalities were associated with the assumption of an upright or sitting posture. Postmortem findings were consistent with pulseless electrical activity (PEA) and an "empty heart" attributed to reduced venous return from vasodilation and concomitant volume redistribution.

Since IgE attached to mast cells can trigger mast cell degranulation and mast cells accumulate at sites of coronary atherosclerotic plaques, some investigators suggest that anaphylaxis promotes plaque rupture, thus risking myocardial ischemia (41,42). Stimulation of the H_1 histamine receptor may also produce coronary artery vasospasm (42–44). Calcitonin gene–related peptide (CGRP) released during anaphylaxis may help to counteract coronary artery vasoconstriction during anaphylaxis (45,46). CGRP, a sensory neurotransmitter widely distributed in cardiovascular tissues, relaxes vascular smooth muscle and has cardioprotective effects in animal models of anaphylaxis (47).

While tachycardia is the rule, bradycardia may occur during anaphylaxis, so bradycardia may not be as useful to separate anaphylaxis from a vasodepressor reaction as previously thought. Relative bradycardia, initial tachycardia followed by a reduction in heart rate despite worsening hypotension, is reported in experimentally induced insect sting anaphylaxis (30,48,49).

Two distinct phases of physiologic response occur in mammals subjected to hypovolemia. The initial response is a baroreceptor-mediated sympathoexcitatory phase comprised of an overall increase in cardiac sympathetic drive and concomitant withdrawal of resting vagal drive, which together produce tachycardia and peripheral vasoconstriction (50). When the effective blood volume falls by 20% to 30%, a second phase follows characterized by withdrawal of the vasoconstrictor drive, relative or absolute bradycardia, increased vasopressin, further catecholamine release as the adrenal axis becomes more active, and hypotension (50,51). Hypotension in this hypovolemic scenario is independent of the bradycardia since it persists after atropine reverses bradycardia.

Conduction defects and sympatholytic medications may also produce bradycardia (13). Excessive venous pooling with decreased venous return, also present in vasodepressor reactions, may activate tension-sensitive sensory receptors in the inferoposterior portions of the left ventricle, resulting in a cardioinhibitory (Bezold-Jarisch) reflex that stimulates the vagus nerve and causes bradycardia (22).

Hemodynamic collapse may occur immediately with no cutaneous or respiratory symptoms (52,53). Of 27 subjects with anaphylaxis in Scandinavia who received prehospital treatment, there were two fatalities, 23 hospitalizations, and two subjects were discharged home after emergency department evaluation. Of subjects with cardiovascular collapse and/or respiratory failure, 70% had cutaneous symptoms, whereas 30% had gastrointestinal manifestations and 85% neurologic deficits (52).

AGENTS OF ANAPHYLAXIS

No evaluation can conclusively prove causation of anaphylaxis without directly challenging the subject with the suspected agent. Direct challenge is generally contraindicated due to safety concerns in subjects who have experienced potentially life-threatening anaphylaxis. Cause and effect may often be demonstrated historically in subjects who experience recurrent, objective findings of anaphylaxis on inadvertent reexposure to the offending agent. Specific diagnostic testing, where appropriate, may confirm the presence of specific IgE and/or the degranulation of mast cells and basophils.

Virtually any agent capable of activating mast cells or basophils may potentially precipitate anaphylaxis. Table 2 lists common causes of anaphylaxis classified by pathophysiologic mechanism. Idiopathic anaphylaxis may be one of the most common since this diagnosis accounts for approximately one-third of cases in most retrospective studies of

Table 2 Representative Agents That Cause Anaphylaxis

IgE dependent
Foods
Medications
Insect venoms
IgE independent
Nonspecific degranulation of mast cells and basophils
Opioids
Muscle relaxants
Idiopathic
Exercise
Cold, heat
Disturbance of arachidonic acid metabolism
Nonsteroidal anti-inflammatory drugs
Complement activation/activation of contact system
Radiocontrast media
ACE inhibitors
Protamine (possibly)

Source: Modified from Ref. 13.

anaphylaxis (1,22,54). Of 601 subjects evaluated over two decades in a university-affiliated practice, 356 (59%) were thought to have idiopathic anaphylaxis (55). This series excluded anaphylaxis due to SCIT and insect stings. Idiopathic anaphylaxis remains a diagnosis of exclusion.

The most common identifiable causes of anaphylaxis are foods, medications, insect stings, and allergen immunotherapy injections (Figs. 1 and 2) (1,19,22,54). Anaphylaxis to peanuts and/or tree nuts causes the greatest concern because of its life-threatening severity, especially in subjects with asthma, and the propensity for lifelong allergic sensitivity to these foods. Of added importance, investigators report that the majority (52%) of peanut-allergic children experience life-threatening symptoms with subsequent reactions, even if atopic dermatitis previously is the only adverse clinical manifestation (56). A quantitative radioallergosorbent test (RAST) for food-specific IgE can be diagnostic. For example, subjects with peanut-specific IgE levels of at least 15 kU/L as measured by one reference laboratory have at least a 95% chance of allergic manifestations, possibly anaphylaxis, if they eat peanuts (57). Thus, a diagnostic food challenge is not indicated in such individuals and might be hazardous.

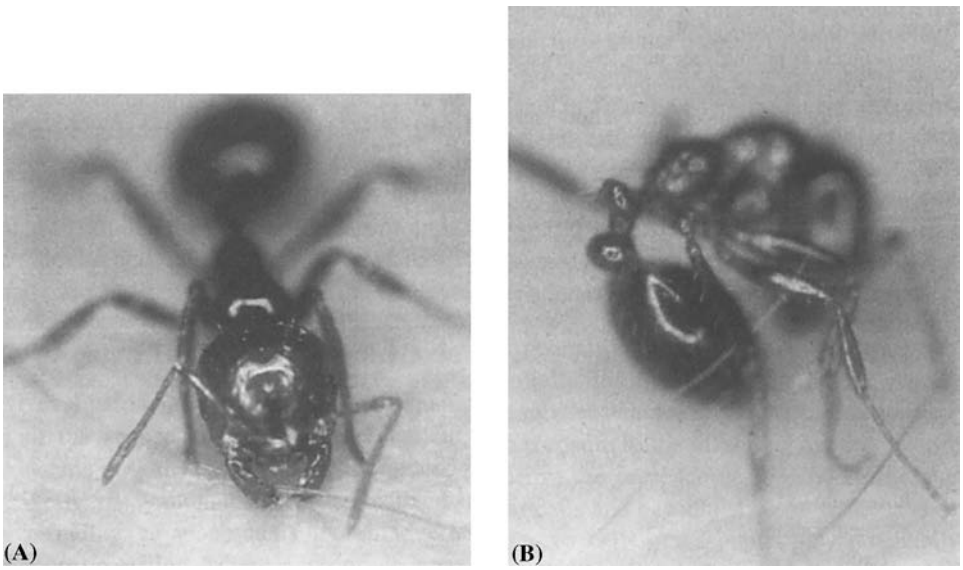

Figure 1 Imported fire ant attaches itself with its mandibles (**A**) and then stings about this anchor point in a circular pattern (**B**).

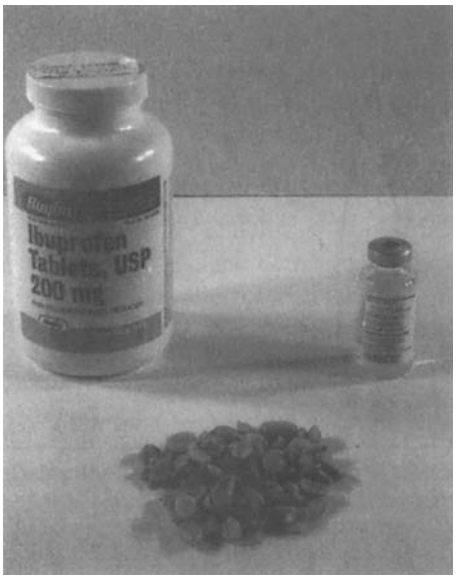

Figure 2 Nonsteroidal anti-inflammatory drugs, penicillin, and peanuts are examples of agents that cause anaphylaxis.

MANIFESTIONS AND DIFFERENTIAL DIAGNOSIS

Clinical Manifestations of Anaphylaxis

The broad spectrum of possible clinical presentations may complicate the diagnosis of anaphylaxis. Special attention should be directed toward assessment of both upper and lower airways, respiratory and pulse rates, blood pressure, tissue perfusion, and appearance of the skin. Measurement of peak expiratory flow rate and pulse oximetry may also be useful, where appropriate.

Anaphylaxis is associated with the following signs and symptoms, alone or in combination: diffuse erythema, pruritus, and urticaria; angioedema; bronchospasm; laryngeal edema; hyperperistalsis; uterine cramps; hypotension; or cardiac arrhythmias. Urticaria and angioedema are the most common manifestations (55,58,59). Anaphylaxis, however, occurs as part of a clinical continuum. It can begin with relatively minor symptoms such as itchy palms, eyes, nose, or skin and rapidly progress to a life-threatening respiratory and cardiovascular reaction. Cutaneous findings may be delayed or absent in rapidly progressive anaphylaxis. The next most common manifestations of anaphylaxis are respiratory symptoms, followed by dizziness, syncope, and gastrointestinal symptoms. The more rapid anaphylaxis occurs after exposure to an offending stimulus, the more likely the reaction is to be severe and potentially life-threatening (15,60).

Differential Diagnosis for Anaphylaxis

Several systemic disorders share clinical features with anaphylaxis, with the vasodepressor (vasovagal) reaction probably the condition most commonly confused with anaphylaxis. In vasodepressor reactions, however, urticaria is absent, the heart rate is typically bradycardic, bronchospasm or dyspnea is usually absent, the blood pressure may be normal or depressed, and the skin is usually cool and pale. Tachycardia is the rule in anaphylaxis, but it may be absent in subjects with hypovolemia (after initial tachycardia), conduction defects, increased vagal tone due to a cardioinhibitory (Bezold-Jarisch) reflex, or in those who take sympatholytic medications. Myocardial dysfunction may cause sudden hemodynamic collapse with or without an arrhythmia. A pulmonary embolism may produce tachycardia, dyspnea, tachypnea, and chest discomfort that can be pleuritic. Systemic mastocytosis, a disease characterized by mast cell proliferation in multiple organs, usually features urticaria pigmentosa (brownish macules that transform into wheals on stroking) and recurrent episodes of pruritus, flushing, tachycardia, abdominal pain, diarrhea, syncope, or headache. Pancreatic polypeptide syndromes may produce flushing and diarrhea. Other diagnostic considerations for children, in particular, include foreign body aspiration, acute poisoning, and a seizure disorder.

Signs and symptoms frequently observed in anaphylaxis may occur by themselves in other disorders. Subjects with hereditary angioedema, for example, experience episodes of nonpruritic, typically painless edema of the extremities with or without laryngeal edema and abdominal discomfort due to visceral involvement. Factitious anaphylaxis is characterized by repeated, self-induced episodes of anaphylaxis. Anaphylaxis alternatively may be surreptitiously inflicted upon a susceptible subject, an example of Munchausen syndrome by proxy. Undifferentiated somatoform idiopathic anaphylaxis likewise is a psychiatric disorder in which subjects report symptoms identical to those encountered in idiopathic anaphylaxis, but objective findings are absent, and the subjects meet established diagnostic criteria for this disorder (61).

VARIATIONS ON THE THEME

Exercise-Induced Anaphylaxis

Anaphylaxis associated with exercise occurs as two syndromes of physical allergy: exercise-induced anaphylaxis (EIA) and cholinergic urticaria (58). EIA occurs with prolonged strenuous exercise, frequently in conditioned athletes, such as marathon runners, and is usually accompanied by a short prodrome of cutaneous warmth and generalized pruritus. It may occur only after ingestion of certain foods, such as lettuce or celery, or a medication, such as aspirin, prior to exercise. Clinical manifestations may progress to generalized erythema and urticaria, nausea and diarrhea, upper or lower airway obstruction, hypotension, and possibly syncope as exercise continues (58). Prophylaxis with antihistamines, a corticosteroid, or cromolyn sodium does not consistently prevent EIA. Episodes occur sporadically, which distinguishes EIA from other forms of physical urticaria in which exercise provocation invariably produces symptoms (58). Some individuals may demonstrate symptoms during a controlled exercise challenge but the test is often negative, despite a classic clinical history (58). Subjects with EIA should be taught how to administer epinephrine and preferably should exercise with a partner educated about EIA and how to treat it.

Exercise avoidance remains the best treatment since the natural history of the syndrome is not fully understood. Shadick et al. (62) surveyed 365 subjects with EIA over an average of 10.6 years (62). Of survey respondents, 47% had fewer episodes and 46% had stabilized since diagnosis. Forty-one percent reported no episodes in the year preceding the survey. Successful respondents apparently had moderated their exercise programs and avoided provocative factors (62).

Cholinergic Urticaria

Cholinergic urticaria, also called "heat urticaria," is caused by increased core body temperature due to fever, stress, environmental factors, or exercise. Skin lesions frequently appear as 2- to 4-mm pruritic wheals ("microhives") surrounded by marked erythema. Systemic manifestations, similar to those described for EIA, may also occur but are unusual. Subjects with this syndrome might develop wheals at the site where methacholine is injected or generalized urticaria when the body is warmed, as with a plastic occlusive suit (58).

Idiopathic Anaphylaxis

Idiopathic anaphylaxis is a syndrome of repeated anaphylactic episodes for which no cause can be determined (63). It may occur in children as well as adults (59,61) and fatalities are rare (61). Within one year, almost all subjects enter a period of prolonged remission or have infrequent and less severe episodes (59). Failure to respond to prednisone should prompt consideration of another diagnosis (59). (See sect. on "Special Management Problems" for management of idiopathic anaphylaxis.)

Serial histories and diagnostic tests for foods, spices, and vegetable gums have occasionally identified the culprit agent in subjects previously presumed to have idiopathic anaphylaxis (22). The usefulness of prick skin testing for reactions to food allergens was tested in 102 subjects presumed to have idiopathic anaphylaxis (64). One-third had positive tests to one or more foods from a battery of 79 food allergens. Five subjects experienced anaphylaxis after eating a food implicated by a positive skin test. Two subjects stopped having reactions after they eliminated the implicated food from their diet, but they refused subsequent

confirmatory oral food challenge. The 10 allergens that provoked anaphylaxis in these seven subjects were aniseed, cashew, celery, flaxseed, hops, mustard, mushroom, shrimp, sunflower, and walnut. The authors conclude that skin testing with selected foods may be useful to identify food allergens that cause anaphylaxis, since 7% of subjects in the reference group previously presumed to have idiopathic anaphylaxis had food-induced anaphylaxis.

Anaphylaxis Attributed to Endogenous Progesterone

A syndrome of recurrent anaphylaxis apparently triggered or exacerbated by progesterone has been described in five female subjects (65,66). Four of these subjects reported attacks that exacerbated during pregnancy, lessened during lactation, and increased when lactation ceased. Three of the five subjects experienced remission when treated with a luteinizing hormone-releasing hormone (LHRH) analogue, which apparently antagonized LHRH and inhibited progesterone. Immediate skin test reactions to intradermal injection of 40 to 2000 μg of medroxyprogesterone were present in responders to LHRH analogue therapy. Systemic reactions characterized by urticaria and hypotension developed in two subjects after 100 μg of LHRH was administered intravenously during the luteal phase of their menstrual cycles. The authors postulated that progesterone, in some undefined way, facilitates mast cell mediator release. The three subjects whose anaphylactic symptoms were dramatically reduced by LHRH analogue therapy subsequently underwent oophorectomy with long-lasting reduction in their symptoms. One subject, however, continued to require combined H_1 and H_2 antihistamine therapy to control attacks.

Recurrent and Persistent Anaphylaxis

The reported incidence of biphasic (recurrent) anaphylaxis varies from less than 1% to a maximum of 23%. Additionally, the reported time of onset of the late phase may vary from 1 to 72 hours. Potential risk factors include severity of the initial phase, delayed or suboptimal doses of epinephrine during initial treatment, laryngeal edema or hypotension during the initial phase, delayed symptomatic onset after antigen exposure (often a food or insect sting), or prior history of biphasic anaphylaxis. It is unclear whether systemic corticosteroids administered in the initial phase can prevent or lessen late-phase reactions (67,68).

Persistent anaphylaxis, anaphylaxis which may last from 5 to 32 hours, occurred in 7 of 25 subjects (28%) in the Stark and Sullivan (69) report with two fatalities. Of 13 subjects analyzed in a report on fatal or near-fatal anaphylaxis to foods, 3 (23%) similarly experienced persistent anaphylaxis (70). Retrospective data from other investigators, however, suggest that persistent anaphylaxis is uncommon.

Neither biphasic nor persistent anaphylaxis can be predicted from the severity of the initial phase of an anaphylactic reaction since they have occurred after what were perceived initially to be mild episodes. Since life-threatening manifestations of anaphylaxis may recur, it may be necessary to monitor selected subjects up to 24 hours after their apparent recovery from the initial phase.

MANAGEMENT OF ANAPHYLAXIS

General

Practice parameters (71) and consensus emergency management guidelines (72,73) concerning anaphylaxis and its management are available. However, physicians and other healthcare professionals may not follow them. In a standardized clinical anaphylaxis scenario, as defined by U.K. Resuscitation Council guidelines, 5% of senior house officers would use the proper dose and/or route of administration for epinephrine as outlined in the published guidelines (74). Other reports examining treatment patterns for anaphylaxis in the emergency departments of civilian (75) and military hospitals (76) indicate that epinephrine is administered during anaphylaxis to 16% and 50% of patients, respectively, according to consensus guidelines.

Clinicians who perform procedures and administer medications should have the appropriate medications and equipment available to treat anaphylaxis. The following equipment and supplies are recommended (71,77,78): (*i*) stethoscope and sphygmomanometer, (*ii*) tourniquets, tuberculin syringes, and large-bore needles (e.g., 14-gauge needles),

Table 3 Management of Acute Anaphylaxis

I. Immediate intervention
   a. Assessment of airway, breathing, circulation, and adequacy of mentation
   b. Administer epinephrine *intramuscularly* every 5–15 min, in appropriate doses, as necessary, depending on the presenting signs and symptoms of anaphylaxis, to control signs and symptoms and prevent progression to more severe symptoms such as respiratory distress, hypotension, shock, and unconsiousness
   c. Place patient in recumbent position and elevate lower extremities

II. Possibly appropriate subsequent measures depending on response to epinephrine
   a. Establish and maintain airway
   b. Administer oxygen
   c. Establish venous access
   d. Normal saline IV for fluid replacement

III. Specific measures to consider after epinephrine injections, where appropriate
   a. Consider epinephrine infusion
   b. Consider H_1 and H_2 antihistamines
   c. Consider nebulized β_2 agonist (e.g., albuterol) for bronchospasm resistant to epinephrine
   d. Consider systemic corticosteroids
   e. Consider vasopressor (e.g., dopamine)
   f. Consider glucagon for patient taking β-blocker
   g. Consider atropine for symptomatic bradycardia
   h. Consider transportation to an emergency department or an intensive care facility
   i. For cardiopulmonary arrest during anaphylaxis, high-dose epinephrine and prolonged resuscitation efforts are encouraged, if necessary. (See reference for specific details.)

Source: Adapted from Ref. 71.

(*iii*) injectable aqueous epinephrine 1:1000, (*iv*) equipment for administering oxygen, (*v*) oral airway, (*vi*) equipment for administering intravenous fluids, (*vii*) injectable H_1 and H_2 antihistamines (e.g., diphenhydramine and ranitidine), (*viii*) corticosteroids for intravenous injection, and (*ix*) a vasopressor (e.g., dopamine). Depending on the clinical setting, glucagon, an automated electronic defibrillator (AED), and a one-way valve face mask with an oxygen inlet port [e.g., Pocket-Mask® (Laerdal Medical Corporation, Gatesville, Texas, U.S.) or similar device] might be desirable. The emergency kit should be up-to-date and complete. Everyone directly involved in patient care should be able to locate necessary supplies, rapidly assemble fluids for intravenous administration, and be aware of treatment guidelines for anaphylaxis.

A sequential approach to management is outlined in Table 3. The judicious use of epinephrine, maintenance of adequate oxygenation, and maintaining an effective circulatory volume are the most important considerations. Assessment and maintenance of airway, breathing, circulation, and mentation are essential initial management steps. Altered mentation may reflect underlying hypoxia. Measurement of pulse oximetry and peak expiratory flow rate, where appropriate, may be useful to guide therapy. Patients should be monitored continuously to facilitate prompt detection of new clinical findings or treatment complications. A patient should be transferred to an emergency facility depending on the clinical severity of the reaction, response to treatment, and the likelihood that other complications will occur.

Anaphylactic shock, a form of distributive shock, may shift significant fluid volume from the central to the peripheral vascular compartment, potentially resulting in inadequate venous return to the heart (39,40). Thus, the recumbent position is strongly recommended, essential in the hypotensive subject, and provides autoinfusion (or preservation) of approximately 1 to 2 L of fluid into the central vascular compartment (79).

Systemic absorption of an agent must be minimized by stopping intravenous infusion of offending medications or other biologic agents. Severe laryngeal edema may develop within 30 to 180 minutes (72). Therefore, an endotracheal tube should be inserted as soon as possible if laryngeal edema does not promptly reverse following parenteral administration of

epinephrine. H_1 and H_2 antagonists, a corticosteroid, and volume expanders can be infused once intravenous access is established.

Epinephrine

How to Use Epinephrine

Epinephrine is the treatment of choice for anaphylaxis (12,71–73,80,81). It should be given as soon as any signs or symptoms of anaphylaxis appear. Data are limited concerning the frequency with which two or more doses (reports range from 16–36%) of epinephrine are utilized to treat anaphylaxis (82–84). Fatalities from anaphylaxis can result from delayed administration or inadequate doses of epinephrine and from severe respiratory and/or cardiovascular complications (14,71). Even with ideal treatment, patients still die from anaphylaxis (7,14,70,85–87). It is important to note that *there are no absolute contraindications for epinephrine administration to treat anaphylaxis* (71). All subsequent therapeutic interventions depend on the initial response to this medication and toxicity from or inadequate response to epinephrine indicates that additional therapeutic modalities are necessary (71).

Intramuscular epinephrine every 5 to 15 minutes, or as often as necessary, should be given to control symptoms and sustain blood pressure (71–73). Comparisons of intramuscular to subcutaneous injections have not been done during anaphylaxis. However, absorption is complete and more rapid and plasma levels higher in asymptomatic adults and children given epinephrine intramuscularly in the anterolateral thigh (88,89). Obesity or other conditions that accentuate the subcutaneous fat pad may prevent or complicate intramuscular access (90).

The pharmacology of epinephrine is reviewed in detail by Westfall and Westfall (91). The α-adrenergic, vasoconstrictive effects at recommended dosages given intramuscularly reverse peripheral vasodilation and alleviate hypotension and reduce generalized cutaneous erythema and urticaria as well as angioedema. Local injection of epinephrine may reduce further absorption of antigen from a sting or injection site, but this has not been studied systematically. The β-adrenergic properties of epinephrine cause bronchodilation, increase myocardial output and contractility, and suppress further mediator release from mast cells and basophils (81,92). Epinephrine, administered in low concentrations (e.g., 0.1 μg/kg), can paradoxically produce vasodilation, hypotension, and increase the release of inflammatory mediators (91,93).

Epinephrine administration enhances coronary blood flow. Two mechanisms are probably responsible: an increased duration of myocardial diastole compared to systole and a vasodilator effect due to increased contractility. These actions usually offset the vasoconstrictor effects of epinephrine on the coronary arteries (91,94).

Common pharmacologic effects of epinephrine that occur at recommended doses via any route of administration include agitation, anxiety, tremulousness, headache, dizziness, pallor, or palpitations (91). Rarely, and usually associated with excessive doses, epinephrine administration might contribute to or cause myocardial ischemia or infarction (95–100), pulmonary edema (101,102), prolonged QTc interval (103), ventricular arrhythmias, accelerated or malignant states of hypertension, and intracranial hemorrhage in adults and children alike (92,104). Nonetheless, some patients have survived massive doses of epinephrine with no evidence of myocardial ischemia or residual complications (105,106).

Intravenous Epinephrine

Because of the risk for potentially lethal arrhythmias, epinephrine 1:10,000 or 1:100,000 vol/vol can be administered intravenously when treatment for anaphylaxis is failing, during cardiac arrest, or to unresponsive or hypotensive patients who fail to respond to intravenous volume replacement and multiple IM doses of epinephrine (71). One group of investigators suggests that the early use of intravenous epinephrine is safe, effective, and well tolerated when the rate of administration is titrated to the clinical response, but this has not been evaluated systematically by a cohort study comparing this modality to epinephrine injections (49). Continuous hemodynamic monitoring is essential.

High-dose intravenous epinephrine, i.e., the rapid progression to a high dose, should be used for subjects in cardiopulmonary arrest. Administer 1 to 3 mg (1:10,000 vol/vol dilution) IV slowly over three minutes, 3 to 5 mg IV over three minutes, and then 4 to 10 μg/min infusion is recommended (72). The recommended initial resuscitation dosage in children is

0.01 mg/kg (0.1 mL/kg of a 1:10,000 vol/vol solution), repeated every three to five minutes. Higher subsequent dosages (0.1–0.2 mg/kg; 0.1 mL/kg of a 1:1,000 vol/vol solution) may be considered for asystole or PEA. These arrhythmias are often observed during cardiopulmonary arrest that occurs in anaphylaxis. Administration of atropine in accordance with asystole/PEA algorithms may also be appropriate. Additionally, prolonged resuscitation efforts are encouraged, as necessary, since efforts are more likely to be successful in anaphylaxis, where the subject is often young with a healthy cardiovascular system (72).

No Evidence Supports Epinephrine Administration by Other Routes

No data support the use of epinephrine in anaphylaxis by a nonparenteral route. However, alternative routes of administration have been anecdotally successful. These include, for example, inhaled epinephrine in the presence of laryngeal edema or sublingual injection if an intravenous route cannot be obtained. Intraosseous (IO) administration at doses equivalent to intravenous dosing have been recommended when intravenous access is not available and in instances where the clinician has received training and has maintained proficiency in the technique. IO cannulation provides access to a noncollapsible venous plexus, which is attainable in all age groups and several studies have documented its safety and efficacy (107). Access often can be obtained in 30 to 60 seconds. Any drug or fluid that is administered IV can be given IO. The technique requires a rigid needle, preferably a specially designed IO or bone marrow needle from an IO access kit (108). Endotracheally administered dosages of epinephrine previously proposed for use when intravenous access is not available in intubated patients experiencing cardiac arrest are no longer recommended. Furthermore, recent animal studies suggest that the lower drug concentrations achieved when epinephrine is administered endotracheally may produce transient, detrimental β-adrenergic effects, such as hypotension and impaired coronary artery perfusion (107).

How to Use Ancillary Medications

H_1 and H_2 Antihistamines

The standard treatment of anaphylaxis should usually include H_1 antihistamines and corticosteroids. However, antihistamines have a much slower onset of action than epinephrine, they exert minimal effect on blood pressure, and they should not be administered as treatment alone (71). Even at maximum dosages, the antihistamines cannot abort anaphylaxis if histamine already occupies its receptor. They do, however, attenuate cutaneous symptoms, such as urticaria or generalized pruritus, and they may prevent recurrence. Diphenhydramine, 25 to 50 mg for adults and 12.5 to 25 mg for children, may be administered intravenously once the cardiovascular and respiratory conditions are stabilized by epinephrine and/or fluids. Intravenous administration ensures effective dosing will not be impaired by hemodynamic compromise, which adversely affects gastrointestinal or intramuscular absorption, but maximal effect may not be observed for one hour (109). However, oral or intramuscular administration of antihistamines may suffice for milder anaphylaxis.

The role for H_2 antihistamines, such as cimetidine and ranitidine, is more controversial (22). H_2 receptors mediate coronary vasodilation and some case reports suggest that intravenously administered H_2 antagonists may help to relieve persistent hypotension during anaphylaxis. Because cimetidine may inhibit the metabolism of β-adrenergic antagonists in vitro and theophylline metabolism in vivo, ranitidine 50 mg (1 mg/kg) in adults and 12.5 to 50 mg in children, infused over 10 to 15 minutes, is recommended (71). When bolus intravenous administration is desired, ranitidine may be diluted in 5% dextrose to a total volume of 20 mL and injected over five minutes. Cimetidine, 4 mg/kg in adults, should be administered slowly since rapid intravenous administration may produce hypotension (71). There are no established dosages for cimetidine in children with anaphylaxis. Since H_2 blockade without concomitant H_1 blockade could increase available histamine and H_1 receptor stimulation, H_2 antagonists should not be administered prior to H_1 antagonists. As above, oral administration of H_2 antihistamines may suffice for milder anaphylaxis.

Corticosteroids

Systemic corticosteroids may not produce appreciable effects for several hours, but they may prevent persistent or biphasic reactions. Subjects with asthma or other conditions recently

treated with a corticosteroid may be at increased risk for severe or fatal anaphylaxis and may receive additional benefit if corticosteroids are administered to them during anaphylaxis. We recommend corticosteroid treatment for all subjects with anaphylaxis. Corticosteroids should be given intravenously early in the treatment of anaphylaxis at a dosage equivalent to 1.0 mg/kg of methylprednisolone every six hours. Oral administration of prednisone, 0.5 mg/kg, may suffice for milder attacks.

Oxygen and β_2 Agonists

Oxygen should be administered and pulse oximetry monitored during anaphylaxis for those who require multiple doses of epinephrine, have protracted anaphylaxis, or have preexisting hypoxemia or myocardial dysfunction. Inhaled β_2 agonists, e.g., albuterol 0.5 mL or 2.5 mg of a 5% solution, may be administered for bronchospasm refractory to epinephrine.

Persistent Hypotension—Appropriate Roles of Volume Replacement and Glucagon

Usual doses of epinephrine administered during anaphylaxis to subjects taking β-adrenergic antagonists may not produce the desired clinical response and may instead cause predominantly α-adrenergic effects. In such situations, isotonic volume expansion, in some circumstances, up to 7 L of crystalloid, is necessary and glucagon administration is recommended (22,110). Glucagon directly activates adenyl cyclase and completely bypasses the β-adrenergic receptor and therefore may reverse refractory hypotension and bronchospasm associated with anaphylaxis (110). The recommended dosage for glucagon is 1 to 5 mg [20–30 μg/kg (maximum 1 mg) in children] administered intravenously over five minutes and followed by an infusion, 5 to 15 μg/min, titrated to clinical response. Protection of the airway is particularly important in severely drowsy or obtunded subjects since glucagon can cause emesis with the attendant risk for aspiration.

Some investigators report elevated endogenous levels of norepinephrine, epinephrine, and angiotensin II in individuals who experience hypotension during insect sting–induced anaphylaxis (48). This may explain why more epinephrine fails to help some subjects with anaphylaxis. The subject whose hypotension persists despite epinephrine should receive intravenous crystalloid solutions and volume expanders, such as hydroxyethyl starch (Hespan®). Large volumes are often required. A volume of 1 to 2 L of normal saline is administered to adults at a rate of 5 to 10 mL/kg in the first five minutes. Children should receive up to 30 mL/kg in the first hour. Adults receiving colloid solution should receive 500 mL rapidly followed by slow infusion (22).

Clinicians who are adequately trained and proficient at obtaining IO access for either adults or children may consider it if attempts at IV access is unsuccessful. IO cannulation provides access to a noncollapsible venous plexus, which is attainable in all age groups, and several studies have documented its safety and efficacy. Fluids administered IO for volume replacement should be infused under pressure using an infusion pump, pressure bag, or manual pressure to overcome venous resistance (107). Less than 1% of patients have complications after an IO infusion (111).

Many sites may be used for IO infusions. For young children, the proximal tibia just distal to the growth plate is most commonly used. For older children and adults, appropriate IO insertion sites include the medial or lateral malleolus, the distal tibia just proximal to the medial malleolus, the distal femur, the anterior-superior iliac spine, the distal radius or ulna, and the sternum (111).

Vasopressors, such as dopamine, should be given if epinephrine ± antihistamines and volume expansion fail to alleviate hypotension. Dopamine, 400 mg in 500 mL of 5% dextrose, should be administered at 2 to 20 μg/kg/min and titrated to maintain systolic blood pressure. Central venous access is helpful to facilitate administration of fluids and to continue to assess intravascular volume status. As mentioned above, oxygen should be administered to subjects with protracted anaphylaxis since subjects with prolonged hypoxemia and/or hypotension may experience myocardial dysfunction, possibly resulting in refractory hypotension and/or end-organ damage. A critical care specialist may need to be consulted for a subject with intractable hypotension.

SPECIAL MANAGEMENT PROBLEMS

Management of Persistent Airway Obstruction

Persistent Upper Airway Obstruction

Severe laryngeal edema may occur so quickly during anaphylaxis that endotracheal intubation becomes impossible. Therefore, an endotracheal tube should be quickly inserted if laryngeal edema is not reversed promptly with epinephrine. An endotracheal tube measuring at least 7.5 mm in diameter is preferred in adults since larger sizes reduce resistance to air flow. Aerosolized epinephrine, along with supplemental oxygen and extension of the neck, may be helpful for difficult endotracheal intubation. If intubation fails, a cricothyrotomy is next since it is more easily accomplished than is an emergency tracheostomy. To do so, the subject's neck is hyperextended, and the area of the cricothyroid membrane is palpated below the thyroid cartilage and above the cricoid cartilage. A small incision is made, the membrane is punctured, and the opening is enlarged with a blunt instrument such as a scalpel handle. Finally, a small-diameter (4–5 mm) endotracheal tube is inserted. Alternatively, high-flow oxygen delivery through an 11-gauge needle or polyethylene catheter may suffice for the short term if an endotracheal tube is not available. Potential complications of cricothyrotomy include vocal cord injury, bleeding, and subcutaneous emphysema (72).

Persistent Lower Airway Obstruction

Epinephrine reduces bronchospasm associated with anaphylaxis, but ventilation and oxygenation may remain a problem despite an adequate airway. This persistent airway obstruction should be treated as is status asthmaticus. Arterial blood gas determinations and continuous pulse oximetry help guide therapy. Subjects usually respond to inhaled β agonists, such as albuterol (0.5 mL or 2.5 mg of a 5% solution) delivered with oxygen nebulization.

Since adequate oxygenation also depends on ventilation, it may be necessary to establish and maintain an airway and/or provide ventilatory assistance. Again, one of the quickest, easiest, and most effective ways to support ventilation involves a one-way valve face mask with oxygen inlet port (e.g., Pocket-Mask or similar device). Oxygen saturations comparable to endotracheal intubation have been demonstrated in patients who require artificial ventilation via the mouth-to-mask technique with oxygen attached to the inlet port. Subjects with adequate, spontaneous respirations may breathe through the mask.

Ambubags of less than 700 mL are not recommended in adults unless an endotracheal tube is in place since ventilated volume will not overcome the 150 to 200 mL of anatomic dead space and thus provide effective tidal volumes. Recommended tidal volume during artificial ventilation is 6 to 7 mL/kg over 1.5 to 2 seconds. Ambubags may be used in children if the reservoir volume of the device is sufficient. Avoid overinflation. Endotracheal intubation or cricothyroidotomy may be considered where appropriate, depending on the skills of the physician and other health care professionals.

The rate of administered oxygen depends on the clinical response and the device used. A nasal cannula delivers 25% to 40% oxygen with a 4- to 6-L/min flow. A simple plastic face mask delivers 50% to 60% oxygen with an 8- to 12-L/min flow. By comparison, the one-way valve face mask with oxygen inlet valve permits ventilation with up to 50% oxygen at a flow rate of 10 L/min and approaches 90% to 100% if the rescuer periodically occludes the opening of the mask with his/her tongue during mouth-to-mask ventilation.

Mechanical ventilation itself may present a danger for subjects requiring ventilator support during anaphylaxis. Frequent complications of mechanical ventilation include pulmonary barotrauma and hemodynamic compromise, which may result if extremely high inspiratory pressures are necessary to overcome airway obstruction. Mechanical ventilation may have serious consequences for subjects with persistent hypotension despite adequate ventilation. High inspiratory pressure and an inadequate internal diameter of the endotracheal tube also may decrease venous return and increase right ventricular afterload, which leads to inadequate oxygen delivery, arrhythmias, and possible cardiac arrest.

Problems Posed by β-Adrenergic Antagonists During Anaphylaxis

β-Adrenergic antagonists (β-blockers) are used to treat cardiovascular disease, arrhythmias, hypertension, migraine headaches, anxiety, glaucoma, and thyrotoxicosis. Numerous cases of

unusually severe or refractory anaphylaxis are reported in patients taking topical or oral β-adrenergic blockers (71). Subjects taking β-adrenergic antagonists may be more likely to experience severe anaphylaxis characterized by paradoxical bradycardia, severe hypotension, and bronchospasm. These agents may also impede epinephrine treatment. Use of selective β_1-antagonists does not reduce the risk for anaphylaxis since both β_1 and β_2 antagonists may inhibit the β-adrenergic receptor (71).

Management of Anaphylaxis in Pregnancy

Anaphylaxis rarely occurs during pregnancy and, therefore, data are insufficient to make recommendations for treatment. With a few modifications, however, consensus treatment modalities used are identical to those used for anaphylaxis occurring in nonpregnant subjects (112). The uteroplacental arteries are very responsive to α-adrenergic stimulation, and great care is necessary when epinephrine or other agents with α-adrenergic effects are utilized. Epinephrine and diphenhydramine both increase the risk of fetal malformations. However, at least in the case of epinephrine, the lack of an equally effective substitute combined with the tentative nature of the data concerning epinephrine teratogenic risk strongly suggest epinephrine must be used during pregnancy for this potentially life-threatening emergency. Ensuring adequate oxygenation and intravascular volume are especially important during pregnancy (112).

Management of Idiopathic Anaphylaxis

Treatment for subjects with idiopathic anaphylaxis depends on its frequency. The treatment of the acute episode is the same as for any other form of anaphylaxis. Various protocols are published for use to prevent recurrent episodes. They recommend the administration of H_1 and H_2 antagonists, β agonists, leukotriene modifiers, and corticosteroids. All are successful in individual cases. The decision to begin preventive therapy should be individualized (71).

Self-Treatment by Subject

All subjects at risk for anaphylaxis should carry and know how to self-administer epinephrine (Figs. 3 and 4). Data from Vander Leek et al. suggest that epinephrine should be prescribed for any child or adult with confirmed peanut allergy, regardless of the severity of the initial reaction (56). In one report, more than 80% of subjects who died from food anaphylaxis were not given appropriate information to avoid inadvertent food-induced reactions or self-administer epinephrine to treat such reactions (7). Pumphrey determined that epinephrine was administered in 62% of fatal anaphylactic reactions in the United Kingdom, only 14% prior to cardiac arrest (113). In a follow-up analysis of 48 cases of fatal food anaphylaxis from 1999 to 2006, Pumphrey and Gowland (86) reported 19 (40%) received epinephrine autoinjectors, but over half of the fatalities occurred in patients whose previous clinical reactions were so mild that, in the opinion of the investigators, it was unlikely that a physician would have prescribed an epinephrine syringe for self-administration.

Demonstration of proper self-administration technique using a placebo trainer is recommended since two studies report that many subjects receive improper or no instructions

Figure 3 EpiPen® epinephrine autoinjector. Single devices are also available. *Source*: Photo courtesy of Dey Laboratories, Napa, California, U.S. www.anaphylaxis.com

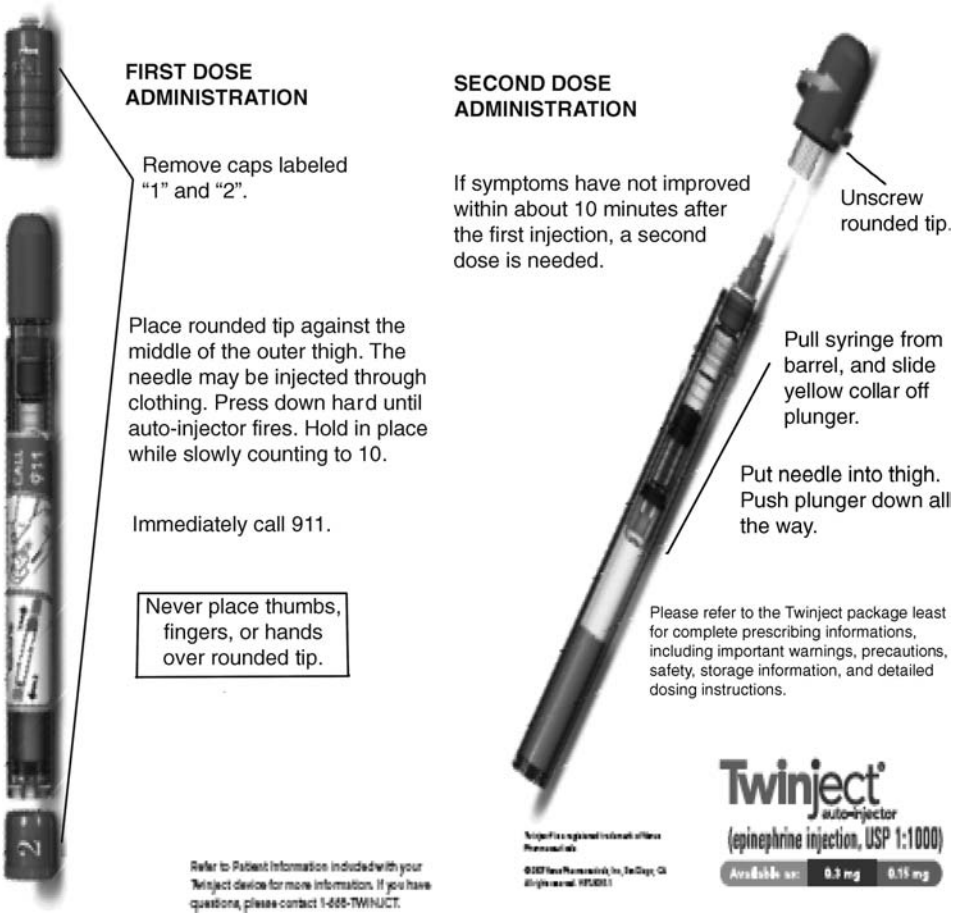

Figure 4 Twinject® epinephrine device. *Source*: Photo courtesy of Verus Pharmaceuticals, San Diego, California, U.S.

as to how to self-administer epinephrine (114,115). An EpiPen® (Dey Laboratories, Napa, California, U.S.) autoinjector for adults is available with a single 0.3 mg (1:1000 vol/vol) dose. Similarly, an EpiPen Jr.®, with a 0.15 mg (1:2000 vol/vol) dose, is available for children weighing less than 30 kg. The Twinject® (Verus Pharmaceuticals, San Diego, California, U.S.) is a pen-sized device containing two doses of epinephrine available either as a 0.15- or a 0.3-mg formulation. The first of the two doses in both cases is delivered by autoinjector while the second is injected manually.

Adherence with an action plan to keep epinephrine available at all times and to inject it during anaphylaxis is another concern. Kemp et al. (116) determined in a follow-up survey of patients that 32 (47%) of 68 did not have their epinephrine syringe with them when they again experienced anaphylaxis from a previously identified allergen. In contrast, 31 (91%) of 34 patients with idiopathic anaphylaxis had epinephrine available at a subsequent episode. Implementation of an educational protocol with emphasis on carrying epinephrine increased the frequency of adherence from 53% to 92% over the ensuing 10 years (55). Other studies indicate that 50% to 75% of patients prescribed epinephrine will carry it with them; 30% to 40% of them demonstrate proper administration technique (80). Some carry the epinephrine kit but choose not to use it (83,117) or prefer to seek emergency medical assistance with anaphylaxis (113).

Observation After Anaphylaxis

A reasonable evidence-based recommendation appears to be that observation periods after complete resolution of uniphasic anaphylaxis should be individualized, particularly since

Table 4 Preventive Measures for Subjects with Anaphylaxis

I. General measures
- Obtain thorough history to diagnose life-threatening food or drug allergy
- Identify cause of anaphylaxis and those individuals at risk for future attacks
- Provide instruction on proper reading of food and medication labels, where appropriate
- Avoidance of exposure to antigens and cross-reactive substances
- Optimal management of asthma and coronary artery disease
- Implement a waiting period of 20–30 min after injections of drugs or other biologic agents
- Consider a waiting period of 2 hr if a patient receives an oral medication in the office he/she has never previously taken

II. Specific measures for high-risk patients
- Individuals at high risk for anaphylaxis should carry self-injectable syringes of epinephrine at all times and receive instruction in proper use with placebo trainer
- MedicAlert® (MedicAlert Foundation, Turlock, California, U.S.) or similar warning bracelets or chains
- Substitute other agents for β-adrenergic antagonists, tricyclic antidepressants, monoamine oxidase inhibitors, and certain tricyclic antidepressants whenever possible. Angiotensin-converting enzyme inhibitors may also be a potential problem
- Slow, supervised administration of agents suspected of causing anaphylaxis, orally, when possible
- Where appropriate, utilize specific preventative strategies, including pharmacologic prophylaxis, short-term challenge and desensitization, and long-term desensitization

Source: Modified from Ref. 120.

there are no reliable predictors of biphasic anaphylaxis. An observation period, based on the severity and response to treatment, is appropriate. Initial phases of anaphylaxis characterized by hypotension, respiratory failure or hypoxemia, repeated doses of epinephrine, poorly controlled asthma, or prior history of biphasic anaphylaxis are reasonable indications for a prolonged observation period of 24 hours or even longer. At discharge, all patients should be provided an epinephrine autoinjector and receive proper instruction on how to self-administer it in case of a subsequent episode. Patients should also have ready and prompt access to emergency medical services for transportation to the closest emergency department for treatment. Further prospective studies on biphasic anaphylaxis are needed (67,68,71,73).

PREVENTION OF ANAPHYLAXIS

Certain Anaphylactic Reactions Are Preventable

Some anaphylactic reactions are so severe that treatment is unsuccessful and death occurs. This underscores the critical importance of education, avoidance, and prevention. Table 4 outlines basic principles for the prevention of future anaphylaxis. An allergist-immunologist can provide comprehensive professional advice on these matters.

Agents that cause anaphylaxis must be identified, whenever possible, and subjects should be instructed how to minimize future exposure. β-Adrenergic antagonists should be discontinued where feasible. There is some preliminary evidence that angiotensin-converting enzyme (ACE) inhibitors may also potentiate anaphylaxis by preventing compensatory angiotensin II mobilization during anaphylaxis (22). More clinical data are necessary to recommend that they not be used when a patient is susceptible to anaphylaxis. Monoamine oxidase inhibitors and some tricyclic antidepressants render epinephrine usage more hazardous by interfering with its degradation.

Meals may potentially have unsavory surprises for highly allergic individuals. A case report illustrates that anaphylaxis may occur in latex-allergic subjects whose food handlers wear latex gloves. Baked goods commonly contain peanuts and nuts, and accidental ingestion of these foods is common. Approximately 35% to 50% of subjects allergic to peanuts will have an inadvertent peanut ingestion within three to four years (118). Pumphrey (113) observed that commercial catering causes 76% of food-related anaphylactic reactions in the United Kingdom. Education is of paramount importance, and the Food Allergy and Anaphylaxis Network (telephone, 800-929-4040; www.foodallergy.org) is a helpful nonprofit resource for many food-allergic individuals.

The potential for anaphylaxis may be determined by skin tests in some circumstances (e.g., allergy to β-lactam antibiotics). However, the immunochemistry of most drugs and biologic agents is not well defined, and reliable in vivo or in vitro testing for most agents is unavailable (119).

Situations may arise for which it is medically necessary to administer an agent to an individual in whom it has previously caused an anaphylactic episode. Numerous protocols enable prevention or reduction of the severity of anaphylaxis. All protocols should only be conducted in clinical settings where anaphylaxis, if it occurs, can be properly managed. Examples of these protocols are antihistamine and corticosteroid prophylaxis to prevent or reduce the severity of IgE-independent reactions (e.g., radiocontrast media); administration of gradual incremental doses of medication over several hours (e.g., short-term penicillin desensitization); or the highly effective, long-term desensitization with venom immunotherapy for stinging insect anaphylaxis (see chap. 24).

Potential Future Options to Reduce Risk of Anaphylaxis

Potential future therapeutic options may feature modified allergens for SCIT to reduce allergenicity. Options being explored include novel vaccine delivery systems, DNA-based vaccination, conjugation of immunostimulatory DNA motifs to specific allergens, plasmid vectors containing DNA, vaccines with highly purified and defined allergens, and "non-anaphylactic" allergens/allergen fragments/peptides for active immunotherapy. Others include IgE-binding haptens of major allergens for passive saturation of effector cells and induction of blocking antibodies, allergen-specific antibodies and antibody fragments for passive immunotherapy in the allergic effector organs, and immunotherapy with humanized anti-IgE monoclonal antibodies or IgE mimotopes. Clinical trials are evaluating optimal dosing of oral peanut desensitization to reduce the risk of peanut anaphylaxis.

SALIENT POINTS

- Fatal anaphylaxis from SCIT occurs at a rate of approximately 1/2,500,000 injections.
- Anaphylaxis associated with SCIT occurs more frequently with accelerated dosage schedules than with traditional, more leisurely schedules.
- β-Adrenergic antagonists may increase the risk for refractory anaphylaxis.
- Mast cell tryptase levels correlate with the severity of anaphylaxis in many instances. However, a normal tryptase level does not necessarily exclude anaphylaxis.
- Some subjects with anaphylaxis have atypical findings such as bradycardia, vasomotor collapse without urticaria, or isolated gastrointestinal symptoms.
- Myocardial dysfunction and arrhythmias may be prominent features of anaphylaxis.
- Peanuts cause the greatest concern in food-associated anaphylaxis because of (*i*) the life-threatening severity of anaphylaxis to the peanut, especially in subjects with concomitant asthma, and (*ii*) the propensity for subjects to remain allergic to peanuts for life.
- Exercise avoidance remains the best treatment for EIA since medical prophylaxis is not very effective.
- Epinephrine is the most important therapeutic agent used to treat anaphylaxis. Epinephrine must be used in appropriate doses.
- Glucagon is indicated for subjects on β-blockers not responding to epinephrine given for anaphylaxis.
- Intravenous epinephrine is indicated when severe and life-threatening anaphylaxis is not responding with optimal therapy.

REFERENCES

1. Yocum MW, Butterfield JH, Klein JS, et al. Epidemiology of anaphylaxis in Olmsted County: a population-based study. J Allergy Clin Immunol 1999; 104:452–456.
2. Valentine M, Frank M, Friedland L, et al. Allergic emergencies. In: Drause RM, ed. Asthma and Other Allergic Diseases. NIAID Task Force Report. Bethesda, MD: National Institutes of Health, 1979:467–507.

3. Neugat AI, Ghatak AT, Miller RL. Anaphylaxis in the United States: an investigation into its epidemiology. Arch Intern Med 2001; 161:15–21.
4. Porter J, Jick H. Drug-induced anaphylaxis, convulsion, deafness, and extrapyramidal symptoms. Lancet 1977; 1:587–588.
5. International Collaborative Study of Severe Anaphylaxis. An epidemiologic study of severe anaphylactic and anaphylactoid reactions among hospital patients: methods and overall risks. Epidemiology 1998; 9:141–146.
6. Burks W, Bannon GA, Sicherer S, et al. Peanut-induced anaphylactic reactions. Int Arch Allergy Immunol 1999; 119:165–172.
7. Bock SA, Muñoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001; 107:191–193.
8. Bernstein DI, Wanner M, Borish L, et al. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990-2001. J Allergy Clin Immunol 2004; 113:1129–1136.
9. Graft DF, Schuberth KC, Kagey-Sobotka A, et al. A prospective study of the natural history of large local reactions after Hymenoptera stings in children. J Pediatr 1984; 104:664–668.
10. Patterson R, Hogan MB, Yarnold PR, et al. Idiopathic anaphylaxis: an attempt to estimate the incidence in the United States. Arch Intern Med 1995; 155:869–871.
11. Johansson SGO, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004; 113:832–836.
12. Sampson HA, Muñoz-Furlong A, Campbell RL, et al. Second symposium on the definition and management of anaphylaxis: summary report—second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol 2006; 117:391–397.
13. Kemp SF, Lockey RF. Anaphylaxis: a review of causes and mechanisms. J Allergy Clin Immunol 2002; 110:341–348.
14. Pumphrey R. Anaphylaxis: can we tell who is at risk of a fatal reaction? Curr Opin Allergy Clin Immunol 2004; 4:285–290.
15. Lockey RF, Benedict LM, Turkeltaub PC, et al. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1987; 79:660–677.
16. Reid MJ, Lockey RF, Turkeltaub PC, et al. Survey of fatalities from skin testing and immunotherapy 1985–1989. J Allergy Clin Immunol 1993; 92:6–15.
17. Amin HS, Liss GM, Bernstein DI. Evaluation of near-fatal reactions to allergen immunotherapy injections J Allergy Clin Immunol 2006; 117:169–175.
18. Lüderitz-Püchel U, May S, Haustein D. Zwischenfälle nach hyposensibilisierung (English translation: Incidents following hyposensitization). Münch Med Wochenschr 1996; 138(8):45–48.
19. Stewart GE, Lockey RF. Systemic reactions from allergen immunotherapy. J Allergy Clin Immunology 1992; 90:567–578.
20. Finkelman FD. Anaphylaxis: lessons from mouse models. J Allergy Clin Immunol 2007; 120:506–515.
21. Goetzl EJ, Wasserman SI, Austin KF. Eosinophil polymorphonuclear leukocyte function in immediate hypersensitivity. Arch Pathol 1975; 99:1–4.
22. Lieberman P. Anaphylaxis and anaphylactoid reactions. In: Adkinson NF Jr., Yunginger JW, Busse WW, et al., eds. Middleton's Allergy: Principles and Practice. 6th ed. St. Louis: Mosby-Year Book, 2003:1497–1522.
23. Erjefält JS, Korsgren M, Malm-Erjefält M, et al. Acute allergic responses induce a prompt luminal entry of airway tissue eosinophils. Am J Respir Cell Mol Biol 2003; 29:439–448.
24. Kaliner M, Sigler R, Summers R, et al. Effects of infused histamine: analysis of the effects of H-1 and H-2 receptor antagonists on cardiovascular and pulmonary responses. J Allergy Clin Immunol 1981; 68:365–371.
25. Chrusch C, Sharma S, Unruh H, et al. Histamine H3 receptor blockade improves cardiac function in canine anaphylaxis. Am J Respir Crit Care Med 1999; 160:142–149.
26. Schwartz LB. Effector cells of anaphylaxis: mast cells and basophils. Novartis Found Symp 2004; 257:65–74.
27. Yunginger JW, Nelson DR, Squillace DL, et al. Laboratory investigations of death due to anaphylaxis. J Forensic Sci 1991; 36:857–865.
28. Mertes PM, Laxenaire MC, Alla F. Anaphylactic and anaphylactoid reactions occurring during anesthesia in France in 1999–2000. Anesthesiology 2003; 99:536–545.
29. Lin RY, Schwartz LB, Curry A, et al. Histamine and tryptase levels in patients with acute allergic reactions: an emergency department-based study. J Allergy Clin Immunol 2000; 106:65–71.
30. Smith PL, Kagey-Sobotka A, Bleecker ER, et al. Physiologic manifestations of human anaphylaxis. J Clin Invest 1980; 66:1072–1080.
31. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium derived relaxing factor. Nature 1987; 327:524–526.

32. Coleman JW. Nitric oxide: a regulator of mast cell activation and mast cell-mediated inflammation. Clin Exp Immunol 2002; 129:4–10.
33. Mitsuhata H, Shimizu R, Yokoyama MM. Role of nitric oxide in anaphylactic shock. J Clin Immunol 1995; 15:277–283.
34. DeSousa RL, Short T, Warman GR, et al. Anaphylaxis with associated fibrinolysis reversed with tranexamic acid and demonstrated by thromboelastography. Anaesth Intensive Care 2004; 32:580–587.
35. Marone G, Bova M, Detoraki A, et al. The human heart as a shock organ in anaphylaxis. Novartis Found Symp 2004; 257:133–149.
36. Raper RF, Fisher MM. Profound reversible myocardial depression after anaphylaxis. Lancet 1988; 1:386–388.
37. Wittstein IS, Thiemann DR, Lima JAC, et al. Neurohumoral features of myocardial stunning due to sudden emotional stress. N Engl J Med 2005; 352:539–548.
38. Bristow MR, Ginsburg R, Harrison DC. Histamine and the human heart: the other receptor system. Am J Cardiol 1982; 49:249–251.
39. Fisher MM. Clinical observations on the pathophysiology and treatment of anaphylactic cardiovascular collapse. Anaesth Intensive Care 1986; 14:17–21.
40. Pumphrey RS. Fatal posture in anaphylactic shock. J Allergy Clin Immunol 2003; 112:451–452.
41. Kovanen PT, Kaartinen M, Paavonen T. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. Circulation 1995; 92:1084–1088.
42. Kounis NG. Kounis syndrome (allergic angina and allergic myocardial infarction): a natural paradigm? Int J Cardiol 2006; 110:7–14.
43. Abela GS, Picon PD, Friedl SE, et al. Triggering of plaque disruption and arterial thrombosis in an atherosclerotic rabbit model. Circulation 1995; 91:776–784.
44. Steffel J, Akhmedov A, Greutert H, et al. Histamine induces tissue factor expression: implications for acute coronary syndromes. Circulation 2005; 112:341–349.
45. Rubin LE, Levi R. Protective role of bradykinin in cardiac anaphylaxis: coronary-vasodilating and antiarrhythmic activities mediated by autocrine/paracrine mechanisms. Circ Res 1995; 76:434–440.
46. Schuligoi R, Amann R, Donnerer J, et al. Release of calcitonin gene-related peptide in cardiac anaphylaxis. Naunyn Schmiedeberg Arch Pharmacol 1997; 355:224–229.
47. Rang WQ, Du YH, Hu CP, et al. Protective effects of calcitonin gene-related peptide-mediated evodiamine on guinea-pig cardiac anaphylaxis. Naunyn Schmiedeberg Arch Pharmacol 2003; 367:306–311.
48. van der Linden P-WG, Struyvenberg A, Kraaijenhagen RJ, et al. Anaphylactic shock after insect-sting challenge in 138 persons with a previous insect-sting reaction. Ann Intern Med 1993; 118:161–168.
49. Brown SGA, Blackman KE, Stenlake V, et al. Insect sting anaphylaxis: prospective evaluation of treatment with intravenous adrenaline and volume resuscitation. Emerg Med J 2004; 21:149–154.
50. Schadt JC, Ludbrook J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. Am J Physiol 1991; 260:H305–H318.
51. Demetriades D, Chan LS, Bhasin P, et al. Relative bradycardia in patients with traumatic hypotension. J Trauma 1998; 45:534–539.
52. Soreide E, Harboe S. Severe anaphylactic reactions outside hospital: etiology, symptoms and treatment. Acta Anaesthesiol Scand 1988; 32:339–342.
53. Viner NA, Rhamy RK. Anaphylaxis manifested by hypotension alone. J Urol 1975; 113:108–110.
54. Brown AFT, McKinnon D, Chu K. Emergency department anaphylaxis: a review of 142 patients in a single year. J Allergy Clin Immunol 2001; 108:861–866.
55. Webb LM, Lieberman P. Anaphylaxis: a review of 601 cases. Ann Allergy Asthma Immunol 2006; 97:39–43.
56. Vander Leek TK, Liu AH, Stefanski K, et al. The natural history of peanut allergy in young children and its association with serum peanut-specific IgE. J Pediatr 2000; 137:749–755.
57. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001; 107:891–896.
58. Horan RF, DuBuske LM, Sheffer AL. Exercise-induced anaphylaxis. Immunol Allergy Clin North Am 2001; 21:769–782.
59. Ditto AM, Harris KE, Krasnick J, et al. Idiopathic anaphylaxis: a series of 335 cases. Ann Allergy Asthma Immunol 1996; 77:285–291.
60. James LP Jr., Austen KF. Fatal and systemic anaphylaxis in man. N Engl J Med 1964; 270:597–603.
61. Abraham D, Grammer L. Idiopathic anaphylaxis. Immunol Allergy Clin North Am 2001; 21:783–794.
62. Shadick NA, Liang MH, Partridge AJ, et al. The natural history of exercise-induced anaphylaxis: survey results from a ten year follow-up study. J Allergy Clin Immunol 1999; 104:123–127.
63. Bacale E, Patterson R, Zeiss CR. Evaluation of severe (anaphylactic) reactions. Clin Allergy 1978; 8:295–304.
64. Stricker WE, Anorve-Lopez E, Reed CE. Food skin testing in patients with idiopathic anaphylaxis. J Allergy Clin Immunol 1986; 77:516–519.

65. Slater JE, Raphael G, Cutler GB, et al. Recurrent anaphylaxis in menstruating women: treatment with a luteinizing hormone-releasing hormone agonist—a preliminary report. Obstet Gynecol 1987; 70:542–546.
66. Meggs WJ, Pesconitz OH, Metcalfe D, et al. Progesterone sensitivity as a cause of recurrent anaphylaxis. N Engl J Med 1984; 311:1236–1238.
67. Kemp SF. The post-anaphylaxis dilemma: how long is long enough to observe a patient after resolution of symptoms? Current Allergy Asthma Rep 2008; 8:45–48.
68. Lieberman P. Biphasic anaphylactic reactions. Ann Allergy Asthma Immunol 2005; 95:217–26.
69. Stark BJ, Sullivan TJ. Biphasic and protracted anaphylaxis. J Allergy Clin Immunol 1986; 78:76–83.
70. Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med 1992; 327:380–384.
71. Lieberman P, Kemp SF, Oppenheimer J, et al. Joint Task Force on Practice Parameters. The diagnosis and management of anaphylaxis: an updated practice parameter. J Allergy Clin Immunol 2005; 115: S483–S523.
72. American Heart Association in collaboration with International Liaison Committee on Resuscitation. 2005 American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care. Anaphylaxis. Circulation 2005; 112(suppl 4):IV143–IV145.
73. Soar J, Deakin CD, Nolan JP, et al. European Resuscitation Council guidelines for resuscitation 2005. Section 7. Cardiac arrest in special circumstances. Resuscitation 2005; 67(suppl 1):S135–S170.
74. Gompels LL, Bethune C, Johnston SL, et al. Proposed use of adrenaline (epinephrine) in anaphylaxis and related conditions: a study of senior house officers starting accident and emergency posts. Postgrad Med J 2002; 78:416–418.
75. Clark S, Bock SA, Gaeta TH, et al. Multicenter study of emergency department visits for food allergies. J Allergy Clin Immunol 2004; 113:347–352.
76. Haymore BR, Carr WW, Frank WT. Anaphylaxis and epinephrine prescribing patterns in a military hospital: underutilization of the intramuscular route. Allergy Asthma Proc 2005; 26:361–365.
77. WHO Position Paper. Allergen Immunotherapy. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998; 53:S1–S42.
78. Cox L, Li JT, Nelson H, et al. Joint Task Force on Practice Parameters. Allergen immunotherapy: a practice parameter second update. J Allergy Clin Immunol 2007; 120:S25–S85.
79. Caroline NL. Emergency Care in the Streets. 2nd ed. Boston: Little, Brown, 1983:57–98.
80. Sicherer SH, Simons FER. Section on Allergy and Immunology, American Academy of Pediatrics. Self-injectable epinephrine for first-aid management of anaphylaxis. Pediatrics 2007; 119:638–646.
81. Lieberman P. Use of epinephrine in the treatment of anaphylaxis. Curr Opin Allergy Clin Immunol 2003; 3:313–318.
82. Korenblatt P, Lundie MJ, Dankner RE, et al. A retrospective study of epinephrine administration for anaphylaxis: how many doses are needed? Allergy Asthma Proc 1999; 20:383–386.
83. Uguz A, Lack G, Pumphrey R, et al. Allergic reactions in the community: a questionnaire survey of members of the anaphylaxis campaign. Clin Exp Allergy 2005; 35:746–750.
84. Kelso JM. A second dose of epinephrine for anaphylaxis: how often needed and how to carry. J Allergy Clin Immunol 2006; 117:464–465.
85. Yunginger JW, Sweeney KG, Sturner WQ, et al. Fatal food-induced anaphylaxis. JAMA 1988; 260:1450–1452.
86. Pumphrey RSH, Gowland MH. Further fatal allergic reactions to food in the United Kingdom, 1999–2006. J Allergy Clin Immunol 2007; 119:1018–1019.
87. Bock SA, Muñoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. J Allergy Clin Immunol 2007; 119:1016–1018.
88. Simons FER, Roberts JR, Gu X, et al. Epinephrine absorption in children with a history of anaphylaxis. J Allergy Clin Immunol 1998; 101:33–37.
89. Simons FER, Gu X, Simons KJ. Epinephrine absorption in adults: intramuscular versus subcutaneous injection. J Allergy Clin Immunol 2001; 108:871–873.
90. Song TT, Nelson MR, Chang JH, et al. Adequacy of the epinephrine autoinjector needle length in delivering epinephrine to the intramuscular tissues. Ann Allergy Asthma Immunol 2005; 94:539–542.
91. Westfall TC, Westfall DP. Adrenergic agonists and antagonists. In: Brunton LL, ed. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill, 2006: 215–268.
92. Barach EM, Nowak RM, Lee TG, et al. Epinephrine for treatment of anaphylactic shock. JAMA 1984; 251:2118–2122.
93. Austen KF. Systemic anaphylaxis in the human being. N Engl J Med 1974; 291:661–664.
94. Lin RY, Curry A, Pitsios VI, et al. Cardiovascular responses in patients with acute allergic reactions treated with parenteral epinephrine. Am J Emerg Med 2005; 23:266–272.
95. Horak A, Raine R, Opie LH, et al. Severe myocardial ischaemia induced by intravenous adrenaline. BMJ 1983; 286:519.

96. Sullivan TJ. Cardiac disorders in penicillin-induced anaphylaxis. Association with intravenous epinephrine therapy. JAMA 1982; 248:2161–2162.
97. Butte MJ, Nguyen BX, Hutchison TJ, et al. Pediatric myocardial infarction after racemic epinephrine administration. Pediatrics 1999; 104:9–11.
98. Saff R, Nahhas A, Fink JN. Myocardial infarction induced by coronary vasospasm after self-administration of epinephrine. Ann Allergy 1993; 70:396–398.
99. Davis CO, Wax PM. Prehospital epinephrine overdose in a child resulting in ventricular dysrhythmias and myocardial ischemia. Pediatr Emerg Care 1999; 15:116–118.
100. Goldhaver-Fiebert S, Grecu L. Postoperative ST-segment elevation: was vasospasm caused by anaphylaxis or by its treatment with epinephrine? Ann Allergy Asthma Immunol 2006; 97:449–453.
101. Ersoz N, Firestone SC. Adrenaline-induced pulmonary edema and its treatment: a report of two cases. Br J Anesth 1971; 43:709–712.
102. Freedman BJ. Accidental adrenaline overdosage and its treatment with piperoxan. Lancet 1955; 2:575–578.
103. Ackerman MJ, Khositseth A, Tester DJ, et al. Epinephrine induced QT interval prolongation: a gene specific paradoxical response in congenital long QT syndrome. Mayo Clin Proc 2002; 77:413–421.
104. Horowitz BZ, Jadallah S, Derlet RW. Fatal intracranial bleeding associated with prehospital use of epinephrine. Ann Emerg Med 1996; 28:725–727.
105. Lewis MA. Treatment and survival after massive overdose of adrenaline. BMJ 1967; 4:38.
106. Novey HS, Meleyco LN. Alarming reaction after intravenous administration of 30 ml of epinephrine. JAMA 1969; 207:2435–2436.
107. American Heart Association in collaboration with International Liaison Committee on Resuscitation. 2005 American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care. Management of cardiac arrest. Circulation 2005; 112(suppl 4):IV58–IV66.
108. American Heart Association. Advanced Cardiovascular Life Support Provider Manual. Dallas: American Heart Association, 2006:48.
109. Simons FE. Advances in H1-antihistamines. N Engl J Med 2004; 351:2203–2217.
110. Thomas M, Crawford I. Best evidence topic report. Glucagon infusion in refractory anaphylactic shock in patients on beta-blockers. Emerg Med J 2005; 22:272–273.
111. American Heart Association. Advanced Cardiovascular Life Support Provider Manual Supplementary Material. Dallas: American Heart Association, 2006:1–86.
112. Schatz M, Hoffman CP, Zeiger RS, et al. Asthma and allergic diseases during pregnancy. In: Adkinson NF Jr., Yunginger JW, Busse WW, et al., eds. Middleton's Allergy: Principles and Practice. 6th ed. St. Louis: Mosby-Year Book, 2003:1303–1322.
113. Pumphrey RSH. Lessons for management of anaphylaxis from a study of fatal reactions. Clin Exp Allergy 2000; 30:1144–1150.
114. Grouhi M, Alshehri M, Hummel D, et al. Anaphylaxis and epinephrine auto-injector training: who will teach the teachers? J Allergy Clin Immunol 1999; 104:190–193.
115. Sicherer SH, Forman JA, Noone SA. Use assessment of self-administered epinephrine among food-allergic children and pediatricians. Pediatrics 2000; 105:359–362.
116. Kemp SF, Lockey RF, Wolf BL, et al. Anaphylaxis: a review of 266 cases. Arch Intern Med 1995; 155:1749–1754.
117. Rosen JP. Empowering patients with a history of anaphylaxis to use an epinephrine autoinjector without fear. Ann Allergy Asthma Immunol 2006; 97:418.
118. Sampson HA. Food allergy. Part 2: Diagnosis and management J Allergy Clin Immunol 1999; 103:981–989.
119. deShazo RD, Kemp SF. Allergic reactions to drugs and biologic agents. JAMA 1997; 278:1895–1906.
120. Kemp SF. Anaphylaxis: current concepts in pathophysiology, diagnosis, and management. Immunol Allergy Clin North Am 2001; 21:611–634.

32 Instructions and Consent Forms for Subcutaneous Allergen Immunotherapy

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INTRODUCTION

The primary objective of allergy skin test and immunotherapy forms is to provide sufficient information about the procedures to allow accurate interpretation by physicians and other health care professionals, even when they are in an office other than that of the prescribing physician. The immunotherapy and allergy skin test forms also should be sufficiently detailed to enable all physicians to make treatment decisions.

The recommended information to be included on the forms has been outlined in the *Guidelines for Reporting Immediate Allergy Skin Test Results* by the American Academy of Allergy, Asthma, and Immunology's Immunotherapy Committee (AAAAI's ICOM) (1) and in the Joint Task Force on Practice Parameters' *Allergen Immunotherapy: A Practice Parameter Second Update (JAIPP)*(2). The purpose of these guidelines is to optimize the practice of clinical allergy through objective, scientific, and reproducible documentation and standardization of skin testing and immunotherapy procedures. The intended outcome is enhanced safety, accuracy, and efficacy of allergy diagnostic testing and allergen immunotherapy (AIT).

This chapter reviews these guidelines and recommendations and includes examples of the standardized immunotherapy and allergy skin test forms developed by the AAAAI's ICOM and the Joint Task Force on Practice Parameters. The immunotherapy forms are also included in the *Allergen Immunotherapy: A Practice Parameter Second Update* and are provided as a courtesy of the Joint Task Force on Practice Parameters (2). The forms integrate the guidelines into editable documents. Also included are examples of consent and instruction forms. Readers can obtain copies of the Allergy Skin Test form, Immunotherapy Prescription and Administration forms, Consent and Instruction forms on the AAAAI website (www.aaaai.org/members).

Physicians may use these forms as a template and customize them for their practice, while maintaining the basic information recommended in the published guidelines. Utilization of these forms will potentially lead to uniformity of allergy skin testing and immunotherapy procedures and improve the safety and accuracy of allergy care, particularly if there is a transfer or change in a patient's allergy care (e.g., new location, new nursing staff).

ALLERGY SKIN TEST FORMS

The use of immediate hypersensitivity (allergy) skin testing as a diagnostic tool in clinical allergy dates to the late 1800s with the work of Charles Blakely (1820–1900), an English physician who proved, through experiments on himself, that hay fever was a result of pollen. During these experiments, he discovered that he could elicit a response if he rubbed the pollen into his scratched skin (3). Since its inception more than 100 years ago, allergy skin testing has continued to be the primary diagnostic tool for patients with allergic diseases and for clinical trials of AIT. Until the latter part of the 20th century, there was little effort to ensure that the clinical practice of allergy skin testing is uniform and consistent. As a result, there is considerable variability in how allergy skin test results are performed and recorded. This variability can adversely impact patient care, particularly individuals who may require

a transfer of their allergy care. Physicians may have difficulty interpreting allergy skin test results performed in another facility and recommend the transferring patient undergo a repeat allergy evaluation. The AAAAI's ICOM developed guidelines to provide parameters for allergy skin reporting to help the allergy community move toward a more uniform practice. The goal of these guidelines is to improve the quality of allergy skin testing and reduce undesirable variation by documenting the results in an objective, scientific, and reproducible manner.

The completed allergy skin test form should provide enough information to allow other physicians to understand the type of test and how it was performed. Many variables can potentially affect allergy skin test results and are included in the skin test form developed by the AAAAI's Immunotherapy Committee. These variables include location of the testing site (4), skin test device (5–7), testing technician, patient age (8), sun damage of the skin (8), and medications. These variables can influence the interpretation of the allergy skin test results. Therefore, it is important to include details about these variables on the allergy skin test form.

The key purpose of the allergy skin test report is to convey information about the test results. The skin test results should be recorded in a manner permitting other physicians to readily interpret the patient's positive and negative allergy skin test profile. The two most commonly used methods of reporting allergy skin test results are

- *Quantitative results*: These are reported as measurement in millimeters of the longest diameter of wheal and diameter of erythema/flare or the sum of the longest diameter plus the widest perpendicular diameter (orthogonal).
- *Semiquantitative scoring*: This is reported on a scale of 0 to 4+. The key to scoring must be included and must be based on measurement of wheal and flare.

One of the limitations of the semiquantitative scoring method is that it fails to provide specific information about the degree of skin test reactivity (i.e., a 4+ could represent a wide range of wheal sizes in many of the currently used scoring systems). Reporting the skin test response in millimeters of the longest diameters of the wheal and erythema/flare may provide more specific information. The longest diameter of the skin test response correlates well with the actual area in a study that used a graphics tablet interfaced to a microcomputer to compare computer-determined areas of 50 randomly selected skin test responses with measured skin test responses (9). In this study, the areas measured by the computer were highly correlated with the actual areas ($r = 0.999$). The sums and products of orthogonal diameters were also highly correlated with the actual areas ($r = 0.953$ and $r = 0.988$, respectively) and added little to the correlation of the maximum (longest) diameter ($r = 0.923$). This study also compared area and maximum diameter measurements of prick skin test responses applied by different persons and found significant differences in the area measurements between testers, which were not found when maximum diameter measurements were compared.

There are some clinical implications associated with the degree of skin test reactivity that are pertinent to patients on AIT.

- High degree of skin test reactivity may be associated with greater risk during immunotherapy (10).
- Starting dose of immunotherapy may be based on skin test reactivity (10).
- Change in skin test reactivity with immunotherapy may predict who is less likely to experience clinical relapse after discontinuing immunotherapy, although this is controversial (11).

Recording the allergy skin test results as measurements of the wheal and erythema in millimeters will provide any physician with precise, reproducible information about the patient's degree of allergen sensitivity.

An objective test protocol for quality assurance should be used to assess the allergy skin test technician's testing proficiency. One suggested protocol for quality assurance and proficiency testing is to have the skin testing technician test 20 alternating positive and negative controls on the back (Fig. 1A) (12). The longest diameters of the wheal and the longest diameters of the flare should be measured and recorded in millimeters. The mean wheal

Suggested Proficiency Testing/Quality Assurance Technique for Skin Prick Testing

- Using desired skin test device, perform skin testing with positive and negative controls in an alternate pattern on a subject's back (histamine 1-10 [10 mg/mL] and saline 1-10)

- Record histamine results at 8 minutes by outlining wheals with a felt-tip pen and transferring results with transparent tape to a blank sheet of paper

- Record saline results at 15 minutes by outlining wheal and flares with a felt-tip pen and transferring results with transparent tape to a blank sheet of paper

- Calculate the mean diameter X = (D+d)/2; D=largest diameter and *d=perpendicular diameter at midpoint of D*

- **Histamine**

Calculate the mean and standard deviations of each mean wheal diameter
Determine coefficient of variation = standard deviation/mean
Quality standard should be less than 30%

- **Saline**

(A) All negative controls should be <3-mm wheals and <10-mm flares

(B)

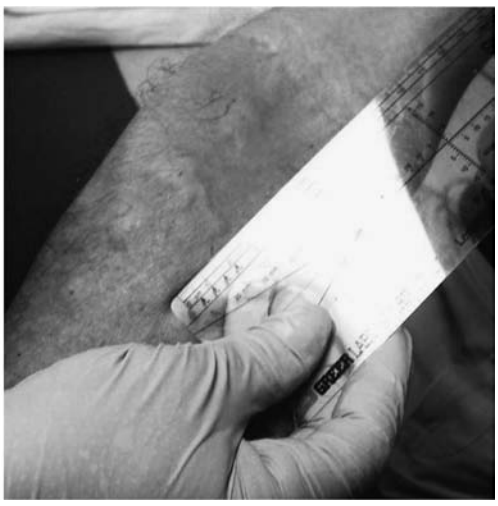


Figure 1 (**A**) Skin test proficiency test. (**B**) (*See color insert.*) Reporting allergy skin test results as a measurement in millimeters of longest diameter of the wheal and flare/erythema. *Source*: From Ref. 12.

diameter with the following formula can be used to calculate the coefficient of variation, which is a measure of skin test reproducibility: standard deviation of wheal divided by mean wheal diameter. Ideally, the coefficient of variation should be less than 30%.

The skin testing form should contain the following information:

A. Patient and prescribing physician information
   1. Patient name, date of birth, and identifying number (if applicable)
   2. Prescribing physician name, address, and telephone number
   3. Testing date
   4. Last time of administration of medications, which can interfere with interpretation or increase the risk of skin testing (e.g., antihistamine or β-blocker)

B. Allergy skin test methods
   1. Skin test technician (the clinic should have some documentation of the quality assurance evaluation of skin test technician's skin test technique)
   2. Location of test (e.g., back or arm)
   3. Type of test (e.g., percutaneous and/or intradermal)
   4. Instrument used (e.g., testing device, needle size and commercial kit)
   5. Elapsed time between application of tests and reading of tests
   6. Amount injected with intradermal technique

C. Testing materials
   1. Positive and negative controls
   2. Manufacturing company or source of reagents
   3. Common name (scientific name optional) of allergens
   4. Concentration used in testing
   5. Dilution and diluent where applicable
   6. Contents, concentrations, and diluents of any mixtures

D. Recording of results
   1. Quantitative: The method recommended by the Joint Task Force's *Allergy Diagnostic Testing: An Updated Practice Parameter* (13) and the AAAAI Immunotherapy and Allergy Diagnostic Committee's skin test reporting guidelines is recording results as the longest diameters of wheal and erythema/flare in millimeters *or* recording both the longest diameters of wheal and erythema/flare and the widest perpendicular diameter (orthogonal) (Fig. 1B).
   2. Semi-quantitative method: Results are recorded by a numerical scale from 0 through 4+. This method is *not recommended* because it is more variable and lacks precision. If this method is used, each score must include a measure of wheal and flare/erythema in millimeters.

E. Actual forms

   The following forms were developed by the AAAAI's ICOM and include the information recommended by its earlier published guidelines. Figure 2 is an example of an allergy skin test form and Figure 3 represents an example of a completed allergy skin test form.

AIT PRESCRIPTION FORMS

The immunotherapy prescription form should provide specific information about the contents of the AIT vaccine. Precise details are necessary for any other physician or health care professional to replicate the prescription without significant variation from the previous vaccine, aside from known differences of lots and manufacturers. AIT vaccines differ when there are changes in the constituents of the mixture, including the diluent, manufacturer, and extract type (aqueous vs. glycerinated) (2). The AIT vaccine label is important and should contain sufficient detail to allow physicians, other health care professionals and the patient to recognize for whom the AIT vaccine is indicated as well as pertinent information about its content (Figs. 4 and 5). The JAIPP has proposed a nomenclature system for allergen vaccine dilutions (Table 1, Figs. 4 and 5). Uniform adoption of this system should reduce errors in administration of AIT, particularly when administered outside of the prescribing physician's office.

Practice name Ordering physician:
Street address **City** **State** **Zip**
Telephone **Fax**

Patient name: ____________________________ **Date of birth**: __/__/__ **Patient number**:___________________________
Testing Technician: ________________________
Last use of antihistamine (or other med affecting response to histamine): ___ days
Testing Date (s) and Time: Percutaneous __/__/____________**AM PM Intradermal** __/__/____________**AM PM**

1) General information about skin test protocol

1. **Percutaneous** reported as: **Allergen: Testing concentration: Extract company** (*see below)
   Location: back___arm___ **Device**: ____
2. **Intradermal**: 0.__ml injected, **Location**: arm **Testing concentration**: 1:_____w/v or BAU or AU/ml, PNU
3. **Results** Longest diameter **or** longest diameter and orthogonal diameter (perpendicular diameters) of wheal (W) and erythema (flare) (F) measured in millimeters at 15 minutes

Blank in results column indicates test was not performed, 0=negative

* Extract manufacturer abbreviations: G=Greer, AL=Allergy Labs, Ohio, LO Allergy Labs, Oklahoma, AK=ALK, HS=Hollister–Stier, , NE=Nelco, AM=Allermed, AT=Antigen Labs

Allergen: Concentration: Extract Manufacturer. *	**Percutaneous W (mm) F**		**Intradermal W (mm) F**		**Allergen: Concentration: Extract Manufacturer. ***	**Percutaneous W (mm) F**		**Intradermal W (mm) F**	
					Controls				
					Percutaneous				
					Negative:				
					Positive:				
					Intradermal				
					Negative				
					Positive:				

Comments:

Figure 2 Allergy skin test form. *Source*: From AAAAI website.

Immunotherapy prescription forms should contain the following information:

A. Patient information
   1. Patient name, patient number (if applicable), birth date, telephone number, and picture (optional but helpful)
B. Preparation information
   1. Name and signature of person preparing the vaccine
   2. Date of preparation
   3. Vial name

      For example, a name could be "Trees and Grasses." If abbreviations are used, a legend should be included to describe the meaning of abbreviations.
C. AIT extract content information (The following information for each allergen should be included on the form in a separate column.)
   1. Content of the allergen extract including common name or genus and species of individual allergens and detail of all mixes

Dr. Ah Choo, M.D.
Address: 665 Rosebud Lane
Hollywood, Fl. 33424
Telephone: 645-123-4444 Fax: 645-123-4567

Patient name: **Jerry Cleanex** **Date of birth: 05/05/90** **Patient number**: **23456**
Testing Technician: Mary Lancet
Last use of antihistamine (or other med affecting response to histamine): **10** days ago medication _____
Testing Date (s) and Time: **Percutaneous 5/30/02 10:30 AM** **Intradermal** 6/2/02 11:15 **AM**

General information about skin test protocol

1. **Percutaneous** reported as: **Allergen: Testing concentration: Extract compan**y (*see below)
   Location: back_X__arm_____ **Device**: HS Quintip_____
2. **Intradermal**: 0.02ml injected, **Location**: arm **Testing concentration**: 1:500 w/v, 100 BAU or AU/ml, 400 PNU
3. **Results** Longest diameter *(Left in this example)* **or** longest diameter and orthogonal diameter (*Right in this example*) of wheal (W) and erythema (flare) (F) measured in millimeters at 15 minutes

Blank in results column indicates test was not performed, O=negative

* Extract manufacturer abbreviations: G=Greer, AL= Allergy Labs (Oklahoma), AK=ALK Abello, AD=ALK (Denmark), H=Hollister–Stier, AG=Antigen, N=Nelco, AM=Allermed,, AT=Antigen

Allergen: Concentration: Extract Manufacturer. *	**Percutaneous W (mm) F**		**Intradermal W (mm) F**		**Allergen: Concentration: Extract Manufacturer. ***	**Percutaneous W (mm) F**		**Intradermal W (mm) F**	
Trees					**Weeds**				
Ulmaceae					***Composite family***				
1. American Elm 1:20 G	**0**	**0**			21. Mugwort 10,000 PNU AD	**4/6**	**18/15**		
Cupressaceae					22. Short Ragweed 1:10 H	**10/6**	**20/20**		
2. Mountain Cedar 1:10 AL	**0**	**0**			***Chenopod***				
Betulaceae					23. Russian Thistle 1:20 AG	**3/7**	**10/15**		
3. Paper Birch 1:20 AK	**3**	**15**			24. Burning Bush 20,000 PNU N	**4/6**	**15/20**		
4. Red Alder 1:20 AD	**3**	**10**			25. Lamb's Quarter 1:40 AM	**6/10**	**15/20**		
Fagaceae					***Amaranth***				
5. White Oak 1:10 H	**0**	**0**	**10**	**20**	26. Red Root Pigweed G	**8/10**	**20/30**		
6. Red Oak 1:10 AG	**5**	**15**			***Plantaginaceae***				
Aceraceae					27. English Plantain AK	**10/9**	**20/18**		
7. Box Elder 1:20 N	**0**	**0**			**Molds/Fungi**				
Oleaceae					28. Alternaria alternata AD	**10/9**	**20/18**		
8. White Ash 1:20 AM	**0**	**0**			29. Cladosporium herbarum H	**0**	**0**	**15/18**	**25/20**
9. Olive 1:20 G	**5**	**20**			30. Cladosporium cladosporioides AG	**0**	**0**	**18/22**	**30/35**
Salicaciae					31. Penicillium chrysogenum N	**4/5**	**15/10**		
10. Cottonwood Eastern 1:40 AL	**6**	**25**			32. Aspergillus fumigatus AM	**5/7**	**20/16**		
Moraceae					33. Epicoccum nigrum G	**0**	**0**		
11. Mulberry 1:20 AK	**7**	**30**			34. Helminthosporium solani AL	**0**	**0**		
Juglandaceae									
12. Pecan 1:20 AD	**0**	**0**			**Animals/Mites /Cockroach/Others**				
13. Black Walnut 1:20 H	**0**	**0**			35. D. Pteronyssinus AK	**20/30**	**40/30**		
Plantaceae					36. D. Farinae AD	**15/9**	**32/40**		
14. Sycamore 1:40 AG	**0**	**0**			37. American Cockroach H	**5/6**	**12/10**		
					38. German Cockroach AG	**7**	**18**		
Grasses					39. Cat Epithelium N	**15**	**30**		
15. Bahia 1:20 N	**20**	**40**			40. Dog Epithelium 1:20 AM	**0**	**0**	**15**	**25**
16. Bermuda 10,000 BAU/ml AM	**15**	**35**			**Controls**				
17. Sweet Vernal 1:20 G	**25**	**40**			**Percutaneous**				
18. Timothy 100,000BAU/ml AL	**30**	**45**			**Negative:** 50% glycerine-saline G	**0**	**0**		
19. Johnson 1:10 AK	**15**	**30**			**Positive:** Histamine 1mg/ml AL	**5/7**	**20/15**		
Weeds					**Intradermal**				
Polygonaceae					**Negative**: 0.05 % glycerine-saline AK			**0**	**7/8**
20. Sheep sorrel H	**4/9**	**15/12**			**Positive:** Histamine 1mg/ml AD			**15/20**	**25/15**

Interpretation:

Figure 3 Example of a completed allergy skin test form. *Source*: From AAAAI website.

2. Concentration of manufacturer's extract
3. Volume of manufacturer's extract to add to achieve a selected volume of the projected effective concentration

This can be calculated by dividing the projected effective concentration by the concentration of available manufacturer's extract and multiplying by the selected volume. For example, the recommended maintenance dose for cat is 1000 to 4000 BAU. To deliver 2000 BAU in a 0.5 mL maintenance injection, the final concentration would be 4000 BAU/mL (projected effective concentration). To calculate how much cat extract should be added to a mixture with a final volume of 5 mL the following formula can be used

$$4000\ (\text{projected effective concentration}) \div 10{,}000\ \text{BAU/mL}\ (\text{available manufacturer's extract concentration}) \times 5\ \text{mL}\ (\text{final volume}) =$$

Figure 4 (*See color insert.*) Picture of color-coded labeled allergen immunotherapy extracts with dilutions from maintenance concentrate.

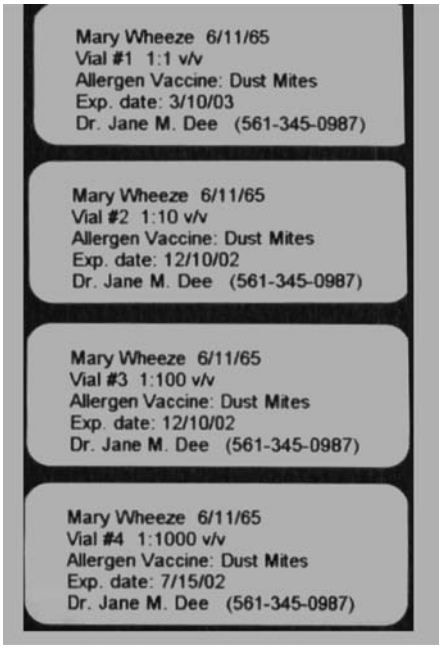


Figure 5 Example of a completed allergen extract label.

Table 1 Suggested nomenclature for labeling dilutions from the maintenance concentrate

Dilution from maintenance concentrate	[a]Vol/vol label	[b]Number	Color
Maintenance concentrate	1:1	1	Red
10-fold	1:10	2	Yellow
100-fold	1:100	3	Blue
1000-fold	1:1000	4	Green
10,000-fold	1:10,000	5	Silver

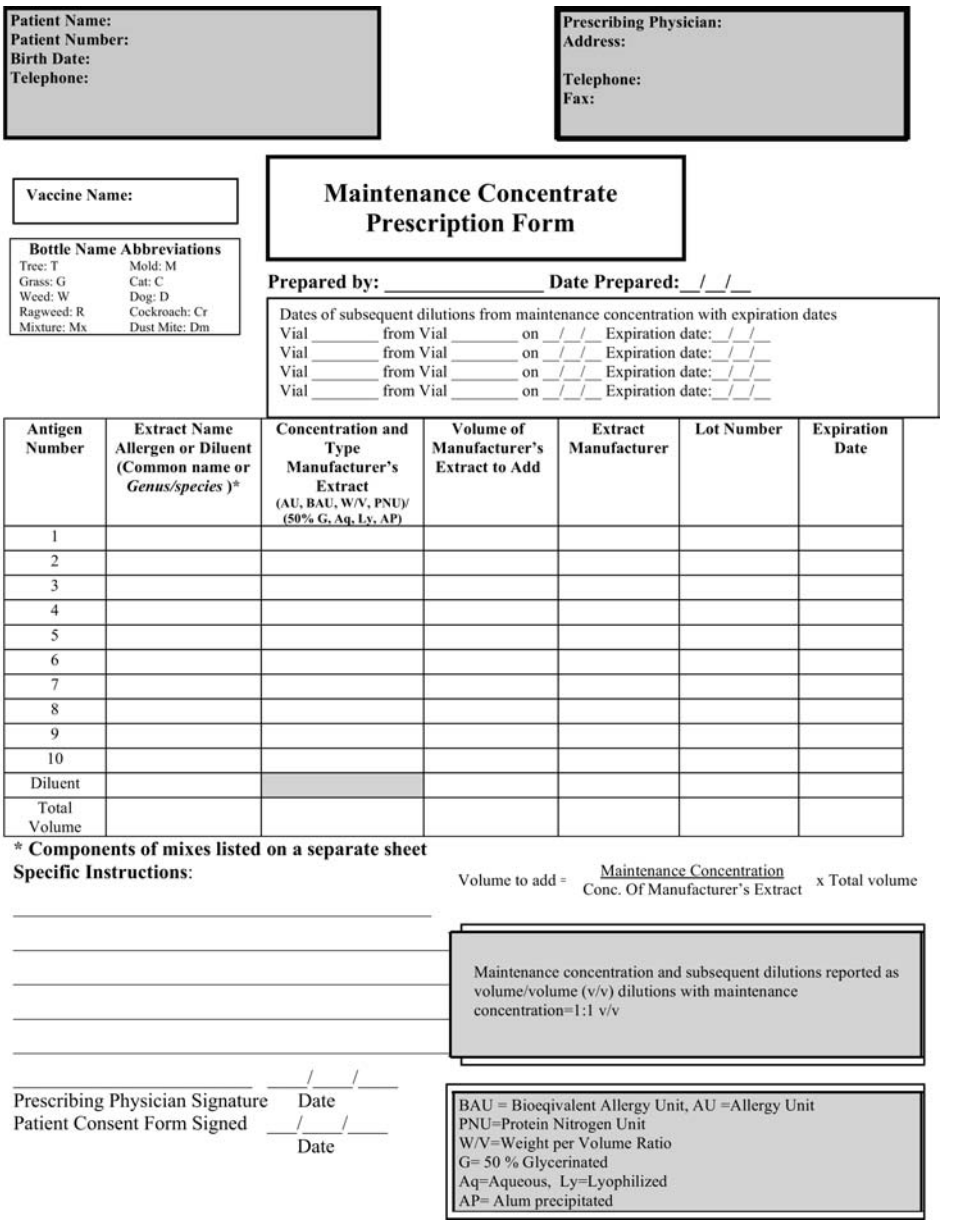
[a]Vol/vol refers to volume per volume dilution with 1:1 being the maintenance concentrate and subsequent dilutions based on the maintenance concentrate.
[b]It is recommended that the numbering system begin with the highest concentration, the maintenance concentrate. This will provide consistency in labeling in the event a greater number of dilutions are needed.
Source: From JAIPP.

2.00 mL (amount of cat extract to be added to the diluent or other allergen extract to make a final volume of 5 mL).

4. The type of diluent (if used)
5. Extract/vaccine manufacturer
6. Lot number
7. Expiration date (This date should not be later than the expiration date of any of the individual components.)

D. Actual forms

The AAAAI's ICOM developed standardized forms for immunotherapy prescription writing. These forms are included in the JAIPP *Allergen Immunotherapy: A Practice Parameter* (14). One form (Fig. 6) is primarily used for the build-up phase of immunotherapy, because it includes a section to document subsequent dilutions



Patient Name:
Patient Number:
Birth Date:
Telephone:

Prescribing Physician:
Address:

Telephone:
Fax:

Vaccine Name:

Maintenance Concentrate Prescription Form

Bottle Name Abbreviations

Tree: T	Mold: M
Grass: G	Cat: C
Weed: W	Dog: D
Ragweed: R	Cockroach: Cr
Mixture: Mx	Dust Mite: Dm

Prepared by: _____ **Date Prepared:**__/__/__

Dates of subsequent dilutions from maintenance concentration with expiration dates
Vial _____ from Vial _____ on __/__/__ Expiration date:__/__/__
Vial _____ from Vial _____ on __/__/__ Expiration date:__/__/__
Vial _____ from Vial _____ on __/__/__ Expiration date:__/__/__
Vial _____ from Vial _____ on __/__/__ Expiration date:__/__/__

Antigen Number	Extract Name Allergen or Diluent (Common name or *Genus/species*)*	Concentration and Type Manufacturer's Extract (AU, BAU, W/V, PNU)/ (50% G, Aq, Ly, AP)	Volume of Manufacturer's Extract to Add	Extract Manufacturer	Lot Number	Expiration Date
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
Diluent						
Total Volume						

*** Components of mixes listed on a separate sheet**

Specific Instructions:

Volume to add = $\frac{\text{Maintenance Concentration}}{\text{Conc. Of Manufacturer's Extract}}$ x Total volume

Maintenance concentration and subsequent dilutions reported as volume/volume (v/v) dilutions with maintenance concentration=1:1 v/v

_____ ___/___/____
Prescribing Physician Signature Date
Patient Consent Form Signed ___/___/____
Date

BAU = Bioeqivalent Allergy Unit, AU =Allergy Unit
PNU=Protein Nitrogen Unit
W/V=Weight per Volume Ratio
G= 50 % Glycerinated
Aq=Aqueous, Ly=Lyophilized
AP= Alum precipitated

Figure 6 Immunotherapy prescription form for build-up phase. *Source*: From AAAAI website.

Patient Name:
Patient Number:
Birth Date:
Telephone:

Prescribing Physician:
Address:

Telephone:
Fax:

Vaccine Name:

Maintenance Concentrate Prescription Form

Bottle Name Abbreviations

Tree: T	Mold: M
Grass: G	Cat: C
Weed: W	Dog: D
Ragweed: R	Cockroach: Cr
Mixture: Mx	Dust Mite: Dm

Prepared by: _____ **Date Prepared:**__/__/__

Antigen Number	Extract Name Allergen or Diluent (*Genus, species* or Common name)*	Concentration and Type Manufacturer's Extract (AU, BAU, W/V, PNU)/ (50% G, Aq, Ly, AP)	Volume of Manufacturer's Extract to Add	Extract Manufacturer	Lot Number	Expiration Date
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
Diluent						
Total Volume						

*** Components of mixes listed on a separate sheet**

Specific Instructions:

Volume to add = $\frac{\text{Maintenance Concentration}}{\text{Conc. Of Manufacturer's Extract}}$ x Total volume

Maintenance concentration and subsequent dilutions reported as volume/volume (v/v) dilutions with maintenance concentration=1:1 v/v

BAU = Bioeqivalent Allergy Unit, AU =Allergy Unit
PNU=Protein Nitrogen Unit
W/V=Weight per Volume Ratio
50%G= 50 % Glycerinated
Aq=Aqueous, Ly=Lyophilized
AP= Alum precipitated

Figure 7 Maintenance Immunotherapy prescription form. *Source*: From AAAAI website.

from the maintenance concentration. The second form (Fig. 7) does not include the build-up section and is intended for the maintenance phase of immunotherapy treatment. The third form (Fig. 8) is used to document the components of any mixes used in the immunotherapy vaccine and would accompany the primary immunotherapy prescription. Figure 9 is an example of a completed immunotherapy prescription form.

Suggested nomenclature for labeling allergen immunotherapy extract dilutions is based upon the system proposed by the JAIPP in *Allergen Immunotherapy: A Practice Parameter* (Figs. 4 and 5) (14)

Physician/Practice name
Address:

Telephone Number **Fax Number**

Allergen Immunotherapy Mixture Components

Patient Name:_____

Name of Mix:

Extract Name Allergen or Diluent (Common name or *Genus/species*)*	Concentration and Type Manufacturer's Extract (AU, BAU, W/V, PNU)/ (50% G, Aq, Ly, AP)	Volume of Manufacturer's Extract Added	Extract Manufacturer	Lot Number	Expiration Date
		Total:			

Date Mix Prepared (if mix prepared in the office): __/__/__
Prepared by: _____

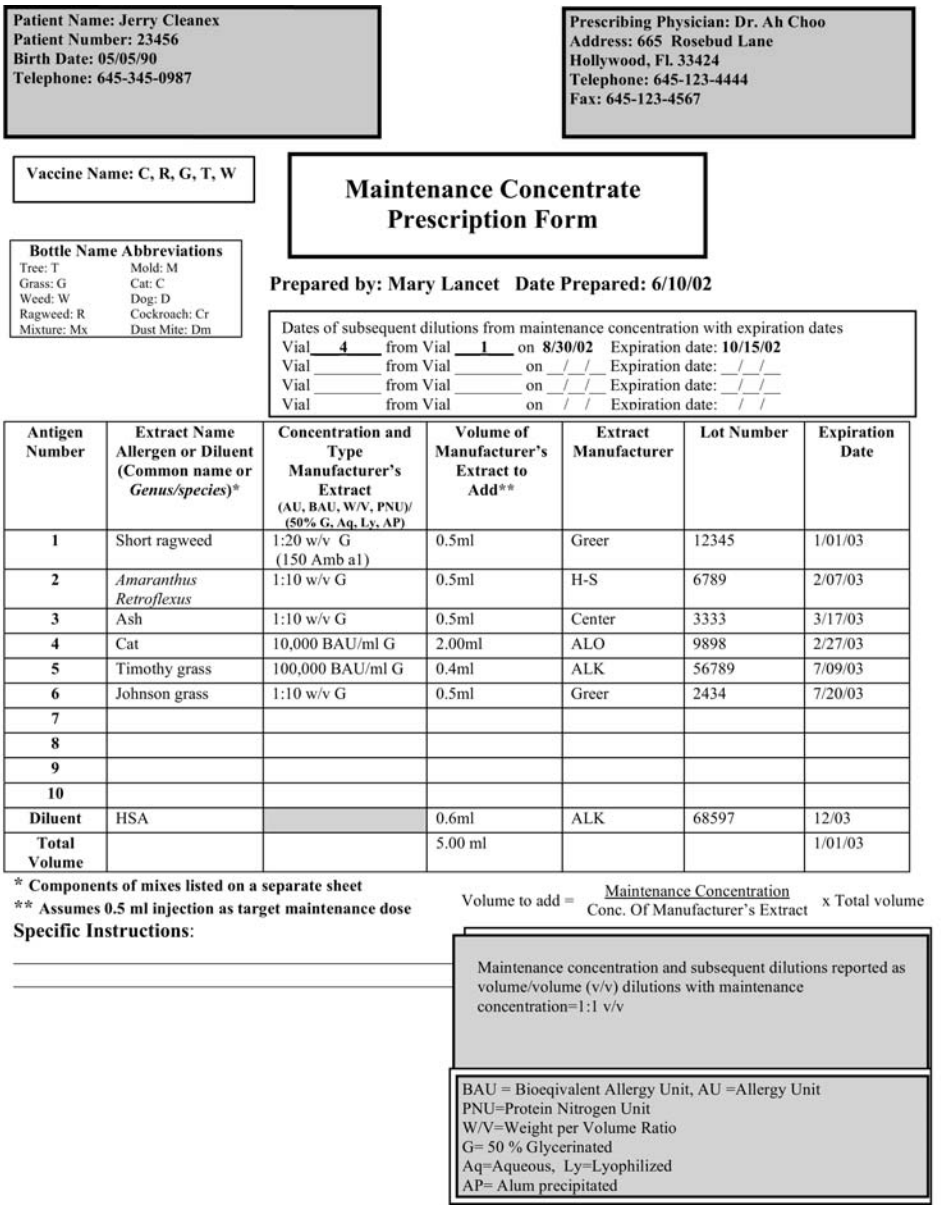
BAU = Bioeqivalent Allergy Unit, AU =Allergy Unit
PNU=Protein Nitrogen Unit
W/V=Weight per Volume Ratio
50%G= 50 % Glycerinated
Aq=Aqueous, Ly=Lyophilized
AP= Alum precipitated

Figure 8 Immunotherapy prescription mix-components form. *Source*: From AAAAI website.

IMMUNOTHERAPY ADMINISTRATION FORMS

The fundamental purpose of the immunotherapy administration form is to provide enough information to enable physicians and other health care personal to know precisely before dosing and to furnish a detailed record of the patient's immunotherapy history to include

- a record of any systemic reaction and treatment administered; and
- other adverse reactions encountered during immunotherapy, such as large local reactions, bruises, or sore arm.



Patient Name: Jerry Cleanex
Patient Number: 23456
Birth Date: 05/05/90
Telephone: 645-345-0987

Prescribing Physician: Dr. Ah Choo
Address: 665 Rosebud Lane
Hollywood, Fl. 33424
Telephone: 645-123-4444
Fax: 645-123-4567

Vaccine Name: C, R, G, T, W

Maintenance Concentrate Prescription Form

Bottle Name Abbreviations

Tree: T	Mold: M
Grass: G	Cat: C
Weed: W	Dog: D
Ragweed: R	Cockroach: Cr
Mixture: Mx	Dust Mite: Dm

Prepared by: Mary Lancet Date Prepared: 6/10/02

Dates of subsequent dilutions from maintenance concentration with expiration dates
Vial ___4___ from Vial ___1___ on **8/30/02** Expiration date: **10/15/02**
Vial _____ from Vial _____ on __/__/__ Expiration date: __/__/__
Vial _____ from Vial _____ on __/__/__ Expiration date: __/__/__
Vial _____ from Vial _____ on / / Expiration date: / /

Antigen Number	Extract Name Allergen or Diluent (Common name or *Genus/species*)*	Concentration and Type Manufacturer's Extract (AU, BAU, W/V, PNU)/ (50% G, Aq, Ly, AP)	Volume of Manufacturer's Extract to Add**	Extract Manufacturer	Lot Number	Expiration Date
1	Short ragweed	1:20 w/v G (150 Amb a1)	0.5ml	Greer	12345	1/01/03
2	*Amaranthus Retroflexus*	1:10 w/v G	0.5ml	H-S	6789	2/07/03
3	Ash	1:10 w/v G	0.5ml	Center	3333	3/17/03
4	Cat	10,000 BAU/ml G	2.00ml	ALO	9898	2/27/03
5	Timothy grass	100,000 BAU/ml G	0.4ml	ALK	56789	7/09/03
6	Johnson grass	1:10 w/v G	0.5ml	Greer	2434	7/20/03
7						
8						
9						
10						
Diluent	HSA		0.6ml	ALK	68597	12/03
Total Volume			5.00 ml			1/01/03

* Components of mixes listed on a separate sheet
** Assumes 0.5 ml injection as target maintenance dose

Volume to add = $\frac{\text{Maintenance Concentration}}{\text{Conc. Of Manufacturer's Extract}}$ x Total volume

Specific Instructions:

Maintenance concentration and subsequent dilutions reported as volume/volume (v/v) dilutions with maintenance concentration=1:1 v/v

BAU = Bioeqivalent Allergy Unit, AU =Allergy Unit
PNU=Protein Nitrogen Unit
W/V=Weight per Volume Ratio
G= 50 % Glycerinated
Aq=Aqueous, Ly=Lyophilized
AP= Alum precipitated

Figure 9 Example of a completed immunotherapy prescription. *Source*: From AAAAI website.

Several risk factors for immunotherapy have been identified and include symptomatic asthma (15), high degree of hypersensitivity (10,16), use of β-blockers, dosing errors, and injections giving during periods of symptom exacerbation (16,17). With the exception of dosing errors and high degree of hypersensitivity, these risk factors can be minimized with a preinjection health screen before the administration of the allergy vaccine. This preinjection evaluation may include a peak flow measurement for asthmatic patients and a health inquiry administered verbally or as a written questionnaire. The health inquiry is to determine if there were any recent health changes that may require modifying or withholding that patient's

immunotherapy treatment (e.g., the addition of a β-blocker medication to treat hypertension). The immunotherapy administration form is used to document an evaluation of the patient's health status before administering the allergy vaccine. The form was created by the AAAAI's ICOM and is based on the recommendations of the JAIPP. The information recommended on an immunotherapy administration form is summarized below.

Summary Points of AIT Administration Forms

A. Patient information
   1. Patient name, date of birth, telephone number, patient photograph (optional but helpful)

B. AIT vaccine information
   1. AIT vaccine name and dilution from maintenance concentrate in vol/vol (Table 1), vial letter (e.g., A, B), color or number if used.
   2. Expiration date of all dilutions

C. Administration information in separate columns
   1. Date of injection
   2. Patient's health before injection

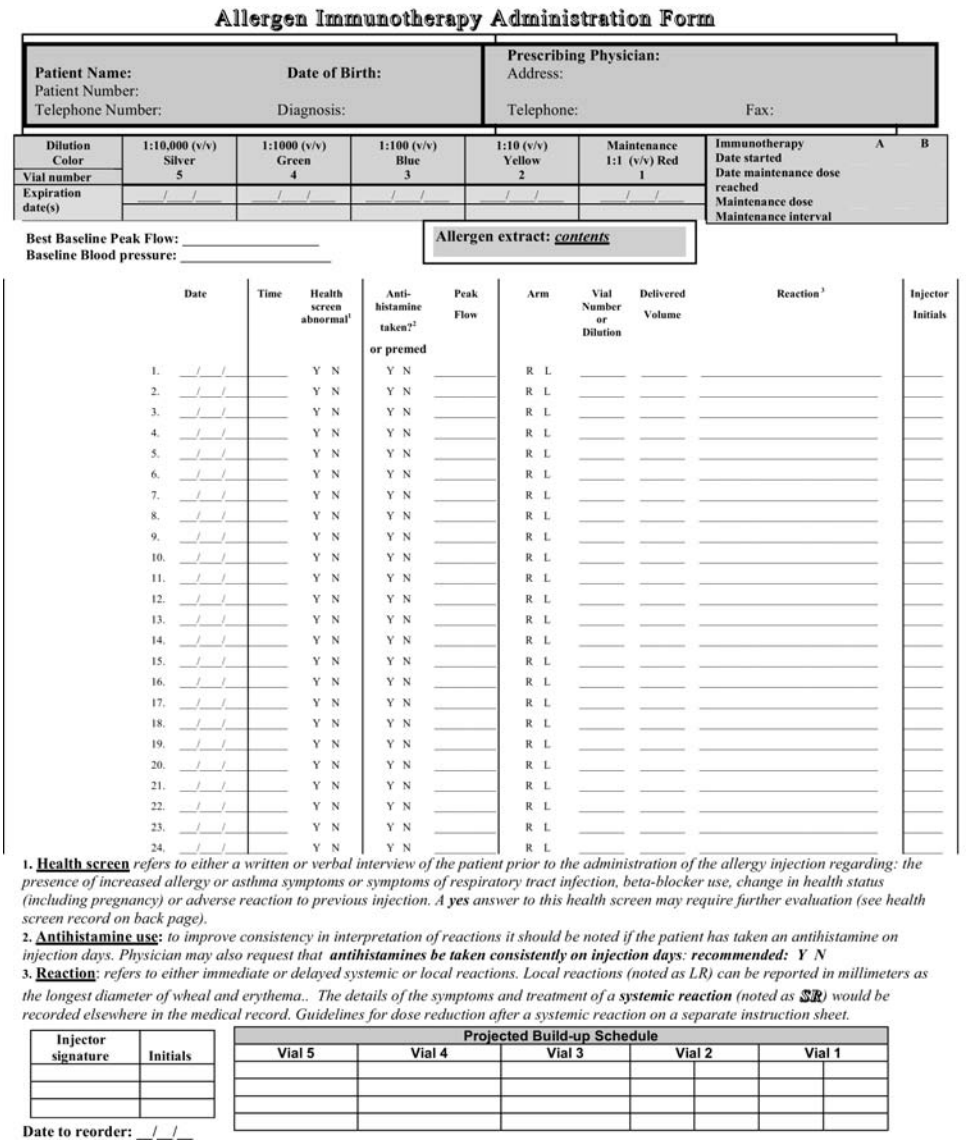
      This is obtained via a verbal or written interview of the patient before administering the immunotherapy injection. Among other issues, the patient is questioned about increased asthma or allergy symptoms, β-blocker use, change in health status (including pregnancy), or an adverse reaction to previous injection (including delayed large local reactions). Patients with a significant systemic illness, such as influenza, usually should not receive an allergy injection.
   3. Antihistamine use

      There have been very few studies that have investigated the effect of premedication on conventional immunotherapy build-up schedules. One concern is that antihistamines taken before each injection during a conventional immunotherapy buildup might mask a minor reaction that would otherwise alert a physician to an impending systemic reaction. However, one randomized-controlled study demonstrated that premedication with fexofenadine reduced the frequency of severe systemic reactions during a conventional immunotherapy buildup as well as increasing the proportion of patients who achieved the target maintenance dose (18). The JAIPP suggests noting if the patient is taking an antihistamine in order to consistently interpret reactions. It may also be desirable for a patient to be consistent and either take or not take an antihistamine on the day they receive their injection. The immunotherapy administration form is a means by which antihistamine use is documented and reflects specific instructions from the treating physician about an antihistamine on injections days.
   4. Peak flow reading

      Symptomatic asthma is a risk factor for immunotherapy (10,15). Obtaining a peak flow measurement prior to the immunotherapy injection may help screen patients with active asthma who should not receive their immunotherapy injection on that day. The form should provide the patient's best peak flow baseline as a reference and the health care professional giving the injection should be provided with specific guidelines about the degree of diminished peak flow for which an injection should be withheld.
   5. Baseline blood pressure

      It is desirable to record the patient's baseline blood pressure for future reference.
   6. Arm administered

      Noting into which arm each vaccine is injected facilitates identification of the cause of a large local reaction and, therefore, which vaccine may be modified.
   7. Projected build-up schedule
   8. Delivered volume reported in milliliters
   9. Injection reaction



Allergen Immunotherapy Administration Form

Patient Name: **Date of Birth:**
Patient Number:
Telephone Number: Diagnosis:

Prescribing Physician:
Address:
Telephone: Fax:

Dilution Color	1:10,000 (v/v) Silver	1:1000 (v/v) Green	1:100 (v/v) Blue	1:10 (v/v) Yellow	Maintenance 1:1 (v/v) Red
Vial number	5	4	3	2	1
Expiration date(s)	___/___/___	___/___/___	___/___/___	___/___/___	___/___/___

Immunotherapy A B
Date started
Date maintenance dose reached
Maintenance dose
Maintenance interval

Best Baseline Peak Flow: _____
Baseline Blood pressure: _____

Allergen extract: *contents*

	Date	Time	Health screen abnormal[1]	Anti-histamine taken?[2] or premed	Peak Flow	Arm	Vial Number or Dilution	Delivered Volume	Reaction[3]	Injector Initials
1.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
2.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
3.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
4.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
5.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
6.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
7.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
8.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
9.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
10.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
11.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
12.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
13.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
14.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
15.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
16.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
17.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
18.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
19.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
20.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
21.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
22.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
23.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____
24.	___/___/___	_____	Y N	Y N	_____	R L	_____	_____	_____	_____

1. **Health screen** *refers to either a written or verbal interview of the patient prior to the administration of the allergy injection regarding: the presence of increased allergy or asthma symptoms or symptoms of respiratory tract infection, beta-blocker use, change in health status (including pregnancy) or adverse reaction to previous injection. A* ***yes*** *answer to this health screen may require further evaluation (see health screen record on back page).*
2. **Antihistamine use:** *to improve consistency in interpretation of reactions it should be noted if the patient has taken an antihistamine on injection days. Physician may also request that* ***antihistamines be taken consistently on injection days: recommended: Y N***
3. **Reaction**: *refers to either immediate or delayed systemic or local reactions. Local reactions (noted as LR) can be reported in millimeters as the longest diameter of wheal and erythema.. The details of the symptoms and treatment of a* ***systemic reaction*** *(noted as* ***SR****) would be recorded elsewhere in the medical record. Guidelines for dose reduction after a systemic reaction on a separate instruction sheet.*

Injector signature	Initials

Date to reorder: __/__/__

Projected Build-up Schedule						
Vial 5	Vial 4	Vial 3	Vial 2		Vial 1	

Figure 10 Allergen immunotherapy administration form. *Source*: From AAAAI website.

The details of any treatment given in response to either a systemic or large reaction should be documented on the health screen (second page of the administration form) or elsewhere in medical record and referenced on the administration form.

The initials of the individual who gives the injection should be included. The immunotherapy administration forms were developed as a two-part form (Figs. 10 and 11) and are included in the JAIPP. Figure 10 is used to document the preinjection screening and allergy immunotherapy injection administration for up to two vaccines. A modification of this form with columns for three vaccines is available but not included in this book. The second page is used to note the results of the pre-injection screen including any delayed immunotherapy reactions or immediate systemic reactions. Figures 12 and 13 are the pre-immunotherapy injection

Patient name:_____ **Date of birth:**_____ **Patient number**_____

Health Screen Record

1. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

2. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

3. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

4. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

5. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

6. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

7.Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

8.Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

9.Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any) : _____

10. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

11. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

12. Date of immunotherapy injection visit: __/__/___
Patient's response to pre-injection screening questions: _____
Staff action taken (if any): _____

Figure 11 Health screen record form for immunotherapy administration page 2. *Source*: From AAAAI website.

questionnaire and the systemic reaction reporting forms (developed by the AAAAI's Immunotherapy and Anaphylaxis Committee).

IMMUNOTHERAPY INSTRUCTION AND CONSENT FORMS

Instruction Forms

There are two types of instruction forms pertinent to immunotherapy treatment. One form is designed to instruct physicians and other health care professionals from offices outside the prescribing allergist's office if the patient transfers their immunotherapy treatment. The other is directed at the patient or patient's guardian. If a patient's immunotherapy treatment is transferred from one physician to another, there is an added risk for a systemic reaction

Immunotherapy Pre-Injection Questionnaire

Patient Name:_____ **Date:**_____

This questionnaire is designed to optimize safety precautions already in place for your allergen immunotherapy injection (s) (allergy shot). Please review and answer the following questions. The nursing staff will review your responses and notify your physician if they have any questions or concerns about whether you should receive your injection(s) today. **If you are pregnant or have been diagnosed with a new medical condition, please notify the staff.** (Please circle the appropriate answer.)

1. Have you had increased asthma symptoms (chest tightness, increased cough, wheezing, or shortness of breath) in the past week? **Yes No**

2. Have you had increased allergy symptoms (itching eyes or nose, sneezing, runny nose, post-nasal drip, or throat-clearing) in the past week? **Yes No**

3. Have you had a cold, respiratory tract infection, or flu-like symptoms in the past two weeks? **Yes No**

4. Did you have any problems such as increased allergy or asthma symptoms, hives, or generalized itching within 12 hours of receiving your last injection or swelling that persisted into the next day? **Yes No**

5. Are you on any new medications? Any new eyedrops? Please specify._____

Staff intervention/office visit:

_____

_____

_____

Staff Signature:_____

Figure 12 Preimmunotherapy health screen form. *Source*: From AAAAI website.

because of the multiple variables that may change with the transfer of care. Changes in the allergen extract components, such as the extract manufacturer, may be one of the reasons for the added risk following transfer of immunotherapy treatment. Additional risk may come from staff unfamiliar with the prescribing allergist's immunotherapy schedule, allergy immunotherapy vial color-coding and nomenclature system. Therefore, it is important that immunotherapy transfer forms provide clear, specific instructions, and information. When such documentation is provided and there is no change in the allergy vaccine components or immunotherapy schedule, the risk of a systemic reaction from transferring care is minimized.

It is important to provide patients with information about immunotherapy prior to starting treatment. Compliance with immunotherapy treatment is historically poor (19), which should improve by enhancing a patient's understanding of the immunotherapy process. A study of patients receiving AIT ($N = 134$ patients: mean age 30 ± 13 with male to female ratio of 1:2 and mean duration of immunotherapy 30 ± 60 months) demonstrates that a substantial number of patients have poor knowledge, many misconceptions and unfounded expectations

Allergen Immunotherapy Systemic Reaction/Anaphylaxis Treatment Record

Name:_____ **Date:**_____

Date of Birth:_____ **Prescribing Physician:**_____

Allergens: Tree-Grass-Weed-Mites-Cockroach-Animal Dander-Mold-Hymenoptera

Prior systemic rxn:_____ **Hx of asthma?**_____

Date/time of injection:_____ **Date/time of rxn:**_____

Dilution (Vial #): _____ **New? Yes No**

History of the systemic reaction (SR):

Immediate measures:
__Assess airway, breathing, circulation, and orientation
__Epinephrine IM into thigh
__Activate EMS (call 911 or local rescue squad) Y/N Time called:_____AM/PM
__Management algorithm reviewed (as needed)

Signs and Symptoms

Respiratory:	**Skin:**	**Eye/Nasal:**	**Vascular:**	**Other:**
Shortness of Breath	Hives	Runny Nose	Hypotension	Difficulty Swallowing
Wheezing	Angioedema	Red Eyes	Chest Discomfort	Abdominal pain, nausea, diarrhea
Cough	Generalized Itch	Congestion	Dizziness	Diaphoresis
Stridor	Flushing	Sneezing	Headache	

Time	Resp. rate/ PEFR	Pulse/ O2 Saturation	BP	Intervention, Medications, Exam Comments

Time of discharge from the office:_____ **Condition upon release:** _____

_____

Patient instructions:

_____

Follow-up call to patient:
Time_____
Comments:

_____

_____

_____

_____

Clinical impression: True SR **Questionable SR** **No SR**

Systemic reaction severity classification: EAACI_____ **Portnoy** _____

Dosage adjustment? _____

Signatures_____ **RN** _____**MD/DO**

Figure 13 Systemic reaction reporting sheet. *Source*: From AAAAI website.

of various important aspects of immunotherapy (20). Immunotherapy patients should be familiar with the potential risks involved and the time commitment necessary to receive such therapy. They should also have an understanding of the time necessary before they will begin to improve. Consent and Instructions Forms for Allergy Skin testing and Immunotherapy (Figs. 14–20).

Immunotherapy Instruction Forms for Physicians Supervising Immunotherapy Prescribed by Another Physician

Instruction forms (Figs. 14, 16, and 20) for patients transferring from one physician to another should provide the following:

1. Detailed documentation of the patient's previous immunotherapy treatment, which should include specific information about the components of the AIT

<Insert Date>

<NAME AND ADDRESS OF SUPERVISING PHYSICIAN>

Re: Patient Allergen Immunotherapy

Dear Supervising Physician:

We understand that (Patient) will receive his allergen immunotherapy injections in your office/clinic. Allergen Immunotherapy has demonstrated in controlled studies to be effective in the treatment of allergic rhinitis, asthma and hymenopteria hypersensitivity. But the treatment is not without risk. We have explained the risk and benefits of immunotherapy to (Patient) prior to obtaining (his/her) consent to begin this treatment. (Patient) has requested that immunotherapy treatment be administered in your office because of (reason e. g. convenience of location). Serious adverse reactions to immunotherapy are rare but do occur. There are some safety measures you should implement to ensure this treatment is administered under the safest circumstances. The following are some of the identified risk factors for adverse reactions to allergen immunotherapy.

Risk Factors for immunotherapy[1]

Some of the primary common risk factors in administering immunotherapy treatments are as follows:

1. Error in dosage
2. Presence of symptomatic asthma
3. High degree of hypersensitivity
4. Use of beta-blockers
5. Injections from new vials
6. Injections made during periods of exacerbation of symptoms

Minimizing Risk with Immunotherapy[2]

The following is an excerpt of a Position Statement on the administration of immunotherapy outside of the prescribing allergist's office. A copy of the full Position Statement is enclosed herewith for your information. Please review this and the accompanying documentation before administering immunotherapy to (Patient):

"It has been recommended that allergen immunotherapy should be given in settings where emergency resuscitative equipment and trained personnel are immediately available to treat systemic reactions under the supervision of a physician or licensed physician extender. The trained personnel should be familiar with the following procedures:

- Adjustment of dose of allergen immunotherapy extract to minimize reactions.
- Recognition and treatment of local and systemic reactions to immunotherapy injections.
- Basic cardiopulmonary resuscitation.
- Ongoing patient education in recognition and treatment of local and systemic reactions that occur outside the physician's office."

Recommended emergency resuscitative equipment

- Stethoscope and sphygmomanometer.
- Tourniquet, syringes, hypodermic needles (14-gauge) and large bore needles.
- Aqueous epinephrine HCL 1:1000.
- Equipment to administer oxygen by mask.
- Intravenous fluid set-up.
- Antihistamine.
- Corticosteroids for intravenous injection.
- Vasopressor
- Oral airway.
- Equipment to maintain an airway appropriate for the supervising physician expertise and skill.

"and trained personnel are immediately available to trea t systemic reactions under the supervision of a physician or licensed physician extender. The trained personnel should be familiar with the following procedures: "

Figure 14 A letter to physician who will be supervising immunotherapy outside of the prescribing allergist's office. *Source*: From AAAAI and modified with permission by Richard Lockey, MD.

Please execute and date a duplicate copy of this letter and return it to our office to confirm that your office will be administering (Patient's) immunotherapy under your supervision.

We appreciate your assistance and cooperation in this matter and look forward to receiving the request confirmation from you in the near future.

Sincerely,

[PRESCRIBING PHYSICIAN SIGNATURE]

I have reviewed the allergen immunotherapy information and nstructions set forth herein and enclosed herewith and I hereby give permission for (Patient) to receive (his/her) allergy immunotherapy injections in my office under my supervision. I further agree to administer same in accordance with the information and instructions set forth herein or enclosed herewith.

Name and Address of physician:

_____

_____

_____

Physician's signature: _____ Date: _____

Patient's signature: _____ Date: _____

1. Bousquet J., Lockey R., Malling H.J. WHO Position Paper Allergen immunotherapy: therapeutic vaccines for allergic diseases Allergy Eur. J of Allergy Clin Immunol 1998; Number 44 Volume 53

2. Position Statement on: Administration of immunotherapy outside of the prescribing allergist facility *Drug and Anaphylaxis Committee of ACAAI. Ann Allergy Asthma and Immunol 1998;81: 101-102.*

The readers can obtain copies of this position statement through the American College of Allergy, Asthma and Immunology website www. ACAAI.org

Modified with permission of Dr. Richard Lockey, M.D.

Figure 14 (*Continued*)

extract, details of any adverse reaction to immunotherapy, schedule, and allergy skin test results
2. Specific instructions for administering immunotherapy and treatment of immunotherapy large local and systemic reactions
3. Guidelines for dosage adjustments for unexpected interruptions in immunotherapy injections and systemic reactions (see Figure 20 for an example of a dose reduction schedule for interruptions in treatment).

Patient Skin Test and Immunotherapy Instruction and Consent Forms (Figs. 15,17–19)

Instructions for patients beginning immunotherapy should provide the following information:

1. Description of what immunotherapy treatment involves and what alternative treatments are available
2. Potential benefits to be expected from the treatment and the expected timing of these benefits
3. Potential risk of immunotherapy, including the remote possibility of death
4. Costs associated with immunotherapy and who pays these costs
5. The anticipated duration of treatment

<Insert date>

Re: Receiving allergy injections outside prescribing allergist's office

Dear: <patient name>

We understand that you will receive your allergy injections at another health care facility outside of this office. For your continued safety and well-being, we want to make sure that you are fully aware of several important issues about allergy shots. To do so, I have enclosed a copy of the American College of Allergy Asthma and Immunology 's position statement on this issue for your information.

Allergy injections, when appropriately administered, effectively alleviate symptoms caused by allergic diseases, such as hay fever, eye symptoms, allergic asthma, and insect allergy. However, as is true with any form of treatment, there are potential side effects. By injecting allergic patients with the very things to which they are allergic, it is possible to cause an allergic reaction. Some of the symptoms of such a reaction can include shortness of breath, hives, drop in blood pressure, and even loss of consciousness. Very rarely, these reactions can be life-threatening and result in death.

If you experience any increased allergy symptoms, difficulty breathing, light-headedness or any unusual symptoms after receiving your allergy injection please report them to the physician or staff immediately.

It is for these reasons that patients are not permitted to receive their allergy injections at home. Also; the physician supervising your allergy injections should be prepared to treat an adverse reaction and have available various medications and equipment. (please see the enclosed American College of Allergy, Asthma and Immunology's Position Statement).

It is also important that you remain in the office where you receive your injection for 30 minutes following your injection and that a physician be present during that time adjustments in your dosage sometimes are necessary when you have worsening of your nasal symptoms or asthma from a cold or allergen exposure. Similarly, dosage adjustments are sometimes necessary if you have large bumps at your injection site or have hay fever symptoms, asthma, or other symptoms following your injection. The nurse or physician monitoring your injections will ask questions regarding your health status prior to administering your injection. Please inform him/her if you are having increased allergy or asthma symptoms, new medications or other change in your health status or problems with your previous injection. The instructions accompanying your allergen immunotherapy vials contain information about adjusting your dose if there has been a gap in your treatment or if you encounter difficulties with large local reactions.

Together, we can control your allergic problems. Help us help you by receiving your shots on a regular basis. Please feel free to call on us if you have any questions about your injections or injection schedule. We look forward to seeing you at your next office visit.

Sincerely,

<Prescribing allergist>

Figure 15 A letter to a patient who will receive immunotherapy in an outside office. *Source*: From AAAAI website and modified with permission by Richard Lockey, MD.

6. Any specific office policies regarding immunotherapy, such as deferment of immunotherapy injections with acute illness

Examples of consent and instruction forms, allergy skin test forms, AIT, and administration forms can be downloaded from www.aaaai.org Members Only section.

ALLERGY INJECTIONS ADMINISTERED AT AN OUTSIDE MEDICAL FACILITY

Please complete the following if the allergen vaccine will be administered at an outside medical facility.

I have read (if new patient) or re-read (if established patient) all the information about allergy injections, and I agree that I will not attempt to administer my vaccines to myself nor will I permit anyone who is not a licensed physician or under the supervision of a licensed physician to administer these vaccines.

Patient (or parent/guardian if minor) _____ Date:_____

Witness_____

FACILITY WHERE IMMUNOTHERAPY INJECTIONS WILL BE ADMINISTERED:

_____

_____

_____

Figure 16 Consent form for patient to sign if receiving immunotherapy outside of the prescribing allergist's office. *Source*: From AAAAI website and modified with permission by Richard Lockey, MD.

SALIENT POINTS FOR INSTRUCTIONS AND CONSENT FORMS FOR ALLERGEN IMMUNOTHERAPY (AIT)

- AIT administration forms should include patient information, allergen vaccine information, and administration information.
- Patient information on AIT forms should include patient name and sufficient of the following data to allow identification of patient. These data may include date of birth, telephone number, record number, or patient photograph.
- Allergen vaccine information on AIT form should convey the contents of the maintenance vaccine with sources of components, the expiration date, and the dilution from maintenance in volume per volume of each vial. The dilutions may be designated with numbers (e.g., 1:1 v/v, 1:10 v/v), letters (e.g., A, B) or color (e.g., red, yellow). A combination of two is desirable.
- Administration information on AIT form should include date, dose, arm in which dose administered, side effects, current health status, and initials of the professional administering the dose. Additional information desirable in select cases includes blood pressure, peak flow and premedication. Subsequent doses and dosing interval should also be clearly displayed.
- Forms for administration of AIT by another, nonprescribing physician should include vaccine contents, schedule and dose of AIT, history of significant prior reactions, and guidelines for change following local or systemic reactions or following interruptions in treatment schedule. Contact information for the prescribing physician should also be clearly displayed. Recommendations for required observation time after treatment and equipment and medication recommended on site should be considered.
- Patient instruction forms for AIT should include a simple description of treatment, treatment options other than AIT, potential benefits and risks, required waiting time after injections, estimated cost, estimated duration of treatment and specific office/clinic policies related to deferment of treatment for illness.

<Practice letterhead>

Patient Instruction/Consent Sheet for Allergy Skin Testing

Skin Test: Skin tests are methods of testing for allergic antibodies. A test consists of introducing small amounts of the suspected substance, or allergen, into the skin and noting the development of a positive reaction (which consists of a wheal, swelling, or flare in the surrounding area of redness). The results are read at 15 to 20 minutes after the application of the allergen. The skin test methods are:

Prick Method: The skin is pricked with a needle where a drop of allergen has already been placed.

Intradermal Method: This method consists of injecting small amounts of an allergen into the superficial layers of the skin.

Interpreting the clinical significance of skin tests requires skillful correlation of the test results with the patient's clinical history. Positive tests indicate the presence of allergic antibodies and are not necessarily correlated with clinical symptoms.

You will be tested to important (location) airborne allergens and possibly some foods. These include, trees, grasses, weeds, molds, dust mites, and animal danders and, possibly some foods. The skin testing generally takes 45 minutes. Prick (also known as percutaneous) tests are usually performed on your back but may also be performed on your arms. Intradermal skin tests may be performed if the prick skin tests are negative and are performed on your arms. If you have a specific allergic sensitivity to one of the allergens, a red, raised, itchy hive (caused by histamine release into the skin) will appear on your skin within 15 to 20 minutes. These positive reactions will gradually disappear over a period of 30 to 60 minutes, and, typically, no treatment is necessary for this itchiness. Occasionally local swelling at a test site will begin 4 to 8 hours after the skin tests are applied, particularly at sites of intradermal testing. These reactions are not serious and will disappear over the next week or so. They should be measured and reported to your physician at your next visit. You may be scheduled for skin testing to antibiotics, caines, venoms, or other biological agents. The same guidelines apply.

DO NOT

1. No prescription or over the counter antihistamines should be used 4 to 5 days prior to scheduled skin testing. These include cold tablets, sinus tablets, hay fever medications, or oral treatments for itchy skin. Over the counter allergy medications, such as Actifed, Dimetapp, Benedryl, and many others. If you have any questions whether or not you are using an antihistamine, lease please asks the nurse or the doctor. Some allergy eye medications have antihistaminic activity and will need to be discontinued prior to testing. Patients on new prescription antihistamines such as Claritin, Clarinex, Alavert, Allegra, and Zyrtec should be off these medications for at least 5 days prior to testing. In some instances a longer period of time off these medications may be necessary.

2. Medications such as over the counter sleeping medications (e.g. Tylenol PM) and other prescribed drugs, such as amytriptyline hydrochloride (Elavil), hydroxyzine (Atarax), doxepin (Sinequan), and imipramine (Tofranil) have antihistaminic activity and should be discontinued at least 2 weeks prior to receiving skin test after consultation with your physician. Please make the doctor or nurse aware of the fact that you are taking these medications so that you may be advised as to how long prior to testing you should stop taking them.

Figure 17 Allergy skin test consent form. *Source*: From AAAAI website and modified with permission by Richard Lockey, MD. (*continued on next page.)*

YOU MAY

1. You may continue to use your intranasal allergy sprays such as Flonase Rhinocort, Nasonex, Nasacort and Nasarel. Afrin and Sudafed may be used temporarily but not the day of testing.

2. Asthma inhalers (inhaled steroids and bronchodilators), leukotriene antagonist s (e.g. Singulair, Accolate) and oral theophylline (Theo-Dur,T-Phyl, Uniphyl, Theo-24, etc.) do not interfere with skin testing and should be used as prescribed.

3. Most drugs do not interfere with skin testing but make certain that your physician and nurse know about every drug you are taking (bring a list if necessary)..

Skin testing will be administered at this medical facility with a medical physician or other health care professional present since occasional reactions may require immediate therapy. These reactions may consist of any or all of the following symptoms: itchy eyes, nose, or throat; nasal congestion; runny nose; tightness in the throat or chest; increased wheezing; lightheadedness; faintness; nausea and vomiting; hives; generalized itching; and shock, the latter under extreme circumstances. Please let the physician and nurse know if you are pregnant or taking beta-blockers. Allergy skin testing may be postponed until after the pregnancy in the unlikely event of a reactions to the allergy testing and beta-blockers are medications they may make the treatment of a reaction to skin testing more difficult.

Please note that these reactions rarely occur but in the event a reaction would occur, the staff is fully trained and emergency equipment is available.

After skin testing, you will consult with your physician or other health care professional who will make further recommendations regarding your treatment

We request that you do not bring small children with you when you are scheduled for skin testing unless they are accompanied by another adult who can sit with them in the reception room.

Please do not cancel your appointment since the time set aside for your skin test is exclusively yours for which special allergens are prepared. If for any reason you need to change your skin test appointment, please give us at least 48 hours notice, due to the length of time scheduled for skin testing, a last minute change results in a loss of valuable time that another patient might have utilized.

I have read the patient information sheet on allergy skin testing and understand it. The opportunity has been provided for me to ask questions regarding the potential side effects of allergy skin testing and these questions have been answered to my satisfaction. I understand that every precaution consistent with the best medical practice will be carried out to protect me against such reactions.

Patient_____ Date signed_____

Parent or legal guardian*_____ Date signed_____
*as parent or legal guardian, I understand that I must accompany my child throughout the entire procedure and visit.

Witness _____ Date signed_____

Figure 17 (*Continued*)

BACKGROUND INFORMATION ABOUT IMMUNOTHERAPY

(ALLERGY SHOTS) FOR PATIENTS

Allergy shots

Allergen immunotherapy injections or "allergy shots" are prescribed for patients with allergic rhinitis (hay fever), allergic asthma or life threatening reactions to insect stings. Immunotherapy is the only medical treatment that could potentially modify allergic disease. Some studies have shown that it may have a preventive role in allergic children, possibly preventing asthma from developing in some patients with allergic rhinitis. Immunotherapy would be considered for individuals, who have moderate or severe symptoms not adequately controlled by environmental control measures and/or medications.

Effectiveness

Allergen immunotherapy (allergy shots) may "turn down" allergic reactions to common allergens including pollens, molds, animal dander and dust mites . In most cases, the initial 6 to 12 month course of allergy shots is likely to gradually decrease sensitivity to airborne allergens and continuation of injections leads to further improvement. The injections do not cure patients but diminish sensitivities, resulting in fewer symptoms and use of fewer medications. It is important to maintain shots at the proper time interval; missing your shots for a short time may be acceptable but an appropriate adjustment in the dose of vaccine may be necessary for long lapses in injections. Please see us if you miss receiving your injections for longer than what is recommended for your current vial.

How long are shots given?

There are generally two phases to immunotherapy: a build-up phase and a maintenance phase

- **Build-up phase**: involves receiving injections with increasing amounts of the allergens. The frequency of injections during this phase generally ranges from 1 to 2 times a week, though more rapid build-up schedules are sometimes used. The duration of this phase depends on the frequency of the injections but generally ranges from 3 to 6 months (at a frequency of 2 times and 1 time a week, respectively).
- **Maintenance phase:** This phase begins when the effective therapeutic dose is reached. The effective therapeutic dose is based on recommendations from a national collaborative committee called the **Joint Task Force for Practice Parameters: Allergen Immunotherapy: A Practice Parameter** and was determined after review of a number of published studies on immunotherapy. The effective maintenance dose may be individualized for a particular person based on their degree of sensitivity (how 'allergic they are' to the allergens in their vaccine) and their response to the immunotherapy build-up phase. Once the target maintenance dose is reached, the intervals between the allergy injections can be increased. The intervals between maintenance immunotherapy injections generally ranges from every 2 to every 4 weeks but should be individualized to provide the best combination of effectiveness and safety for each person. Shorter intervals between allergy injections may lead to fewer reactions and greater benefit in some people and some individuals may tolerate intervals longer than four weeks between injections.

Figure 18 Patient immunotherapy information sheet. *Source*: From AAAAI and modified with permission by Richard Lockey, MD. (*continued on next page.)*

Reactions to allergy infections

It is possible to have an allergic reaction to the allergy injection itself. Reactions can be local (swelling at the injection site) or systemic (affecting the rest of the body). Systemic reactions include hay fever type symptoms, hives, flushing, lightheadedness, and/or asthma, and rarely, life threatening reactions. Some conditions can make allergic reactions to the injections more likely: heavy natural exposure to pollen during a pollen season and exercise after an injection. Serious systemic reactions can occur in patients with asthma that has worsened and is not well controlled on recommended medications. Therefore, if you have noted worsening of your asthma symptoms, notify your nurse or physician before receiving your scheduled injections! Reactions to injections can occur, however, even in the absence of these conditions.

Please inform the nursing staff if you have been diagnosed with a new medical condition or prescribed any new medications since yourlast visit. If any symptoms occur immediately or within hours of your injection, please inform the nurse before you receive your next injection.

Figure 18 (*Continued*)

ALLERGEN IMMUNOTHERAPY PATIENT CONSENT FORM

Immunotherapy, hyposensitization, or allergy injections should be administered at a medical facility with a medical physician present since occasional reactions may require immediate therapy. These reactions may consist of any or all the following symptoms: itchy eyes, nose, or throat; nasal congestion; runny nose; tightness in the throat or chest; coughing; increased wheezing; lightheadedness; faintness; nausea and vomiting; hives; generalized itching; and shock, the last under extreme conditions. Reactions, even though unusual, can be serious and rarely, fatal. You are required to wait in the medical facility in which you receive the injections for 30 minutes after each injection. If the patient is 17 years of age or younger, a parent or legal guardian must be present during the waiting period. I verify that I (or patient) am not taking beta blocker medications or that if I am, I have discussed the risks/benefits of doing so with my physician (see information sheet).

I have read (if new patient) or re-read (if established patient) the patient information sheet on immunotherapy and understand it. The opportunity has been provided for me to ask questions regarding the potential side effects of immunotherapy and these questions have been answered to my satisfaction. I understand that every precaution consistent with the best medical practice will be carried out to protect me against such reactions. I also agree that if I have an allergic reaction to the injections that the physician-in-charge has permission to treat said reaction.

I acknowledge the fact with my signature that I am authorizing the office to bill for allergen vaccines, even if, for any reason, I decide not to initiate the allergen immunotherapy program after the vaccine has been made. Vaccines may be prepared up to 1½ weeks prior to my appointment. I agree to obtain prior authorization, if needed, from my insurance plan.

PATIENT_____ **DATE** _____

PARENT or LEGAL GUARDIAN_____ **DATE** _____

As parent or legal guardian, I understand that I must accompany my child throughout the entire 30-minute wait.

WITNESS_____ **DATE**_____

Figure 19 Patient Immunotherapy consent form. *Source*: From AAAAI and modified with permission by Richard Lockey, MD.

Immunotherapy Administration instructions

NAME _____ DATE _____

1) Use a 1 ml disposable syringe, graduated to 0.01 cc and a 26 to 27 gauge, ½-inch needle.
2) Carefully withdraw the proper amount from the appropriate vial.
3) Check to make sure that it is the correct patient, dilution dose and that the patient did not have any problems with the previous injection.
4) Allergy vaccines should be refrigerated. Avoid vaccine exposure to sunlight, extreme heat or freezing. Do not administer expired vaccines
5) Cleanse the skin area with an alcohol swab before injecting.
6) Give the injection **subcutaneously** in the posterior aspect of the middle third of the arm.
    a. Gently draw back the plunger before injecting and if blood appears, withdraw the needle and select a new site.
    b. Slowly inject the vaccine, withdraw the needle, and apply pressure over the injection site to prevent bleeding.
    c. Do not massage the area.
    d. Either arm may be used or the arms may be alternated.
7) **30 minute wait**: Each patient is required to wait 30 minutes in a medical facility after receiving allergy infection treatment so that he or she can be checked for local and systemic reactions.

Note: Do not give allergy injection unless a appropriate medical supervision is present during the 30 minute waiting period.

Management of local reactions: recent literature suggests that individual local reactions do not predict systemic reaction.[1, 2] However, one study found that the rate of large local reactions, defined as ≥ 25 mm was almost 4 times higher (35.2% vs 8.9% of all visits and 19.5% vs 5.3% of all injections; P <.001 for each) among patients who subsequently experienced a systemic reaction compared with those who had never experienced a systemic reaction.[3] For patient comfort dosage adjustments should be considered if large local reactions develop.

Guidelines for administration of allergy injection based on reaction size.

- Negative: Swelling (as in a welt NOT the redness) <15 mm (dime size) - progress according to schedule.
- Swelling 15-20 mm (dime to nickel size) - repeat same dosage.
- Swelling 20-25 mm (quarter size) - return to the last dosage, which caused no reaction.
- Swelling persisting more than 12 hours or over 25 mm (quarter size or larger) - decrease dosage by 50%*

*If reduced dose is tolerated, increase dose by 0.05 to 0.1 cc weekly and resume schedule. If local reaction occurs again, patient should be seen in our office with dosage sheet.

Example of Immunotherapy Dose Adjustments for Unscheduled Gaps in Allergen Immunotherapy Injection Intervals

There have been no studies that have investigated the effect of dosage modification for gaps in immunotherapy injection intervals. Below is a suggested approach to modification of doses of allergen immunotherapy because of gaps between treatment during the build-up and maintenance phase

Build-up phase for weekly or biweekly injections (time intervals from missed injection)

- Up to 7 days, continue as scheduled (i.e. if on weekly build-up then it would be up to 14 days after administered injection or 7 days after the missed scheduled injection):
- 8 to 13 days after missed scheduled injection; repeat previous dose.
- 14 to 21 days after missed scheduled injection; reduce dose 25%
- 21 to 28 days after missed scheduled injection; reduce previous dose 50%

Then increase dose each injection visit as directed on the immunotherapy schedule until therapeutic maintenance dose is reached.

Figure 20 Guidelines for administration of immunotherapy. *Source*: From AAAAI and modified with permission by Richard Lockey, MD. (*continued on next page.)*

Maintenance phase (time intervals from missed injection)

- Up to 10 days, repeat last dose.
- 11 to 20 days, reduce dose by 25%. *
- 21 to 28 days, reduce dose by 50%. *
- Over 28 days, contact physician for orders.

Increase dose by 0.05 cc one injection per week (each vial, if more than one vial) until maintenance is reached, and then resume maintenance schedule.

Systemic reactions: Systemic reactions resulting from injections can occur in the course of treating allergic patients. Most reactions occur within 30 minutes after an injection. Symptoms may include itching of the palms of the hands or other parts of the body, sneezing, coughing, hives (welts), swelling of the lips or other areas, and shortness of breath. At the first sign of any systemic reaction **epinephrine 1: 1.000 w/v** should be administered intramuscularly (about 0.3 cc in adults and 0.15 ml in a child over 30 kg and 12 years and 0.01 mg/kg in children under 30 kg). With severe reactions, acute asthma or a drop in blood pressure (anaphylaxis) may occur. Fatalities can occur with allergy injections. Epinephrine should be repeated if improvement does not occur within minutes, almost immediately. A venous tourniquet applied above the injection site may decrease absorption of the allergen. Any hypotension or loss of consciousness should be treated first with epinephrine, followed by rapid intravenous infusion of normal saline solution. Epinephrine, 1:10,000 w/v, can be given, as needed, intravenously, with severe anaphylaxis. Oxygen by mask or cannula should be administered if respiratory or circulatory compromise occurs. Antihistamines. glucocorticosteroids, vasopressors, and other medications may be necessary for a severe reaction after treatment with epinephrine. After a systemic reaction additional allergy injections should not be given. The patient must return to our office with all records for re-evaluation before injections are resumed.

First sign of systemic reaction: Epinephrine 1:1000 w/v (1 mg/ml): intramuscularly
Adults: 0.3 ml epinephrine 1:1000 w/v IM
Children: < 30 kg give: 0.01 mg/kg 1:1000 w/v IM
: >30 kg and 12 yrs give: 0.15 ml 1:1000 w/v IM

Note: If patient has a history of previous systemic reactions or severe asthma from injection therapy, discuss with the attending physician if any additional reduction is necessary.

If you have any questions, please call our office at: prescribing allergists/immunologist's office number_____

Additional considerations:

- **Refrigeration:** If vaccine is exposed to extreme heat or cold or if serum becomes cloudy, do not administer and notify the office.
- **Expiration date**: Allergen vaccines have an expiration date and should be replaced after this date.
- **Beta-blockers:** Oral and eye drop beta-blockers used concomitantly with allergen immunotherapy are a potential problem because the medications can worsen anaphylaxis by limiting the effectiveness of epinephrine.
    - **Advise the physician if patient is taking any of these or other beta blockers**:
        - Blocadren, Brevibloc, Corgard, Inderal, Inderal-LA, Lopressor, Normozide, Sectral Tenoretoz, Tenormin Visken , Normodyne, Tenoretic, Ziac, Corzide, Timolide, Sectral Tenormin Visken, and Inderide.
- **Pregnant**: If the patient becomes pregnant, do not administer any further injections. Have her schedule an appointment with our office and bring vials and all dosage sheets for this visit.
- **Wheezing**: do not give allergy shots if the patient is having asthma symptoms.
- **Exercise**: no exercise for at least 2 hours after receiving injection.

Figure 20 (*Continued*)

- **Wheezing:**: **Do not give allergy shots** if patient is having *any asthma symptoms*.
- **Asthmatic patients:** Peak flow measurements or another form of breathing test should ideally be done prior to all injections. If the peak flow measurement is **less than 70%** of the patient's baseline, the allergy injection should **not be given until the patient is further evaluated by physician**..
- **Exercise**: **No exercise** for at least **2 hours** after receiving injection.

Always send the dosage sheet(s) and remaining vials with the patient when he or she is returning to the office for new vials and/or dosage adjustments.

1. Tankersley MS, Butler KK, Butler WK, Goetz DW. Local reactions during allergen immunotherapy do not require dose adjustment. J Allergy Clin Immunol 2000;106:840-3.
2. Kelso JM. The rate of systemic reactions to immunotherapy injections is the same whether or not the dose is reduced after a local reaction. Ann Allergy Asthma Immunol 2004;92:225-7.
3. Roy SR, Sigmon JR, Olivier J, Moffitt JE, Brown DA, Marshall GD. Increased frequency of large local reactions among systemic reactors during subcutaneous allergen immunotherapy. Ann Allergy Asthma Immunol 2007;99:82-6.

Figure 20 (*Continued*)

REFERENCES

1. American Academy of Allergy Asthma. American Academy of Allergy, Asthma and Immunology's Immunotherapy Committee's Guidelines for Reporting Immediate Allergy Skin Test Results. Academy News 1999.
2. Cox L, Li J, Nelson H, Lockey R. Allergen immunotherapy: a practice parameter second update. J Allergy Clin Immunol 2007; 116:120:525–585.
3. Simons EF. Ancestors of Allergy. New York: New York Global Medical Communications Ltd, 1994.
4. Carr WW, Martin B, Howard RS, et al. Comparison of test devices for skin prick testing. J Allergy Clin Immunol 2005; 116:341–346.
5. Nelson HS, Lahr J, Buchmeier A, McCormick D. Evaluation of devices for skin prick testing. J Allergy Clin Immunol 1998; 101:153–156.
6. Nelson HS, Oppenheimer J, Buchmeier A, et al. An assessment of the role of intradermal skin testing in the diagnosis of clinically relevant allergy to timothy grass. J Allergy Clin Immunol 1996; 97:1193–1201.
7. Nelson HS, Rosloniec DM, McCall LI, et al. Comparative performance of five commercial prick skin test devices. J Allergy Clin Immunol 1993; 92:750–756.
8. King MJ, Lockey RF. Allergen prick-puncture skin testing in the elderly. Drugs Aging 2003; 20:1011–1017.
9. Ownby DR. Computerized measurement of allergen-induced skin reactions. J Allergy Clin Immunol 1982; 69:536–538.
10. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol 1998; 102:558–562.
11. Des Roches A, Paradis L, Knani J, et al. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. V. Duration of the efficacy of immunotherapy after its cessation. Allergy 1996; 51:430–433.
12. Oppenheimer J, Nelson HS. Skin testing: a survey of allergists. Ann Allergy Asthma Immunol 2006; 96:19–23.
13. Bernstein IL, Li JT, Bernstein DI, et al. Allergy diagnostic testing: an updated practice parameter. Ann Allergy Asthma Immunol 2008; 100:S1–148.
14. American Academy of Allergy, Asthma and Immunology. American College of Allergy, Asthma and Immunology. Allergen immunotherapy: a practice parameter. Ann Allergy Asthma Immunol 2003; 90:1–40.
15. Bernstein DI, Wanner M, Borish L, et al. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990–2001. J Allergy Clin Immunol 2004; 113:1129–1136.
16. Lockey RF, Benedict LM, Turkeltaub PC, et al. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol, 1987:660–677.
17. Amin HS, Liss GM, Bernstein DI. Evaluation of near-fatal reactions to allergen immunotherapy injections. J Allergy Clin Immunol 2006; 117:169–175.
18. Ohasi Y, Nakai Y, Murata K. Effect of pretreatment with fexofenadine on the safety of immunotherapy in patients with allergic rhinitis. Ann Allergy Asthma Immunol 2006; 96:600–605.
19. Cohn JR, Pizzi A. Determinants of patient compliance with allergen immunotherapy. J Allergy Clin Immunol 1993; 91:734–737.
20. Sade K, Berkun Y, Dolev Z, et al. Knowledge and expectations of patients receiving aeroallergen immunotherapy. Ann Allergy Asthma Immunol 2003; 91:444–448.

33 Information and Consent Forms for Sublingual Immunotherapy

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INTRODUCTION

The intrusion of the legal system into the relationship between doctors and patients is a phenomenon that started in the United States, but now is growing in Europe. Improved patient information and communication help to avoid legal actions and is desirable for optimal care.

In the field of clinical trials, the criteria for information for study subjects are considerably more explicit than in the past. The perceived importance of proper informed consent has resulted in legislation in many countries. The subject's consent to participate in a clinical trial must be accompanied by straightforward and comprehensive information about the study objective, the expected results, and the possible risks involved. The style and public expectations of everyday medical practice have also evolved. Previously, doctors sometimes acted with paternalism and benevolence, deciding what was best for their patients. Many patients now seek active involvement in diagnostic and treatment decisions.

With respect to allergen immunotherapy, if sublingual immunotherapy (SLIT) is approved, patient information should be communicated in simple terms, should avoid medical jargon, and should include the following elements:

- The diagnosis of allergic disease and a brief description of its cause and the known mechanisms;
- Symptomatic treatment options and the inherent risks and disadvantages of each;
- Etiological treatment choices, i.e., allergen avoidance and immunotherapy;
- Routes of administration of specific immunotherapy and advantages and disadvantages of each;
- Comparison of the efficacy and safety of subcutaneous immunotherapy (SCIT) and SLIT;
- Recommended available allergens for SCIT and SLIT;
- Expected consequences of immunotherapy on the disease and its course;
- Comparative costs of treatment, including SCIT and SLIT; and
- Documentation that information has been shared with the patient.

INFORMATION ABOUT THE DISEASE

Compliance of patients with their treatment is much better when they have a good understanding of their disease. Patients with allergy should be informed that their symptoms are caused by genetic immune dysregulation, which is responsible for an increase in a type of antibodies produced in response to various common foreign molecules called allergens. Contact between these allergens and immunoglobulin E (IgE) antibodies in mucous membranes induces the release of mediators and triggers allergic inflammation.

In allergic rhinitis, for example, interaction of the allergen with specific IgE induces the release of mediators, including histamine, which stimulates nerves, causing sneezing and itching, increases secretions, resulting in rhinorrhea, and vasodilates, causing congestion. The result is a blocked nose caused by tissue inflammation and dilatation of blood vessels. The

itching, tearing, and redness of allergic conjunctivitis and the inflammation and bronchoconstriction of allergic asthma can be explained by similar mechanisms.

TREATMENT OPTIONS AVAILABLE TO LIMIT SYMPTOMS AND THEIR INHERENT RISKS AND DISADVANTAGES

The symptoms of respiratory allergy, including bronchial, nasal and ocular symptoms, can be rapidly, but not always completely, relieved by using drugs acting on the pathogenic mechanisms of the disease. For example, H1 antihistamines used systemically (oral) and/or topically (nasal and conjunctival) limit the effect of histamine. Antihistamines are minimally effective in asthma, and their positive therapeutic effects disappear rather quickly after stopping treatment if the allergen is still present. Regular use of these drugs is therefore essential and must be continued year after year in the cases of persistent or recurrent allergies. First generation antihistamines can cause adverse effects in some patients, including drowsiness, weight gain, reduced seizure threshold, and extremely rare cardiac conduction abnormalities, particularly with first generation antihistamines. Most of the newer, second generation H1 antihistamines have minimal or no significant side effects. Topical corticosteroids (eye, nose, bronchi) effectively control allergic inflammation, but their effect is limited, they have to be repeatedly administered, and they do not treat the cause but rather the consequences of the disease. Adverse effects, although minimal for most topical dosage forms, are a persisting, mostly groundless, concern for some patients or the parents of pediatric patients.

Another treatment option is cromones. These agents are less commonly used in asthma, but are occasionally used in rhinitis and conjunctivitis. They act by limiting the release of mediators. Cromones are almost devoid of adverse effects, but their main disadvantage is the need for multiple daily doses to achieve efficacy.

Bronchodilators are essential to reverse or prevent bronchospasm in asthma. These agents have a potential to cause tachycardia, a decrease in serum potassium, and an increase in blood glucose. Temporary action necessitates repeated use. Regular use of inhaled bronchodilators, particularly without inhaled corticosteroids, may result in an increased susceptibility to severe asthma.

AVAILABLE ETIOLOGIC TREATMENTS

Only three etiological treatments are currently available for the treatment of allergic diseases: allergen avoidance, administration of monoclonal antihuman IgE, and specific immunotherapy. Allergen avoidance is obviously the first measure to be taken. Patients who are allergic to cats should avoid having one in the house. However, this can cause psychological or emotional difficulties, not only in children but also in adults. Even when personal allergen avoidance can be achieved, the patient may encounter animal allergens outside the home, for example, in cinemas, department stores, airplanes, or schools. Mold allergy may benefit from reduction of excess water or humidity inside homes or buildings, but mold exposure is usually greatest outside. Pollen allergy may require avoidance of outdoor activities during the pollen season, the use of pollen filters in cars, or personal inhalation filters. The efficacy of house-dust mite avoidance is controversial. The use of mattress covers combined with washing the bedding in 130°F (54.4°C) have a certain degree of efficacy on reduction of allergen exposure. Sensitization to dust mites occurs in atopic subjects when the Der p1 major allergen content is greater than 2 μg/g of house dust, but respiratory symptoms secondary to this sensitization occur at concentrations higher than 10 μg/g. Thus, persistent dust-mite reduction efforts may result in reduced symptoms.

The second etiological treatment is administration of humanized, monoclonal anti-IgE (omalizumab). Studies show a reduction of exacerbations of asthma and a reduced need for medications, particularly inhaled or oral corticosteroid therapy. The impact of omalizumab on reduction of oral corticosteroid therapy is somewhat controversial. There is no evidence of a persisting benefit after omalizumab is discontinued, necessitating the long-term, indefinite use of this expensive, parenteral therapy. Currently, omalizumab is approved only for asthma.

The third etiological treatment of allergy is specific immunotherapy. This technique, invented a century ago, was initially intended for use as an anti-infective vaccination (1). It has demonstrated efficacy, although the mechanisms of action remain incompletely understood.

ROUTES OF ADMINISTRATION OF SPECIFIC IMMUNOTHERAPY

For the last century (1), immunotherapy has been administered primarily by subcutaneous injection (SCIT). Doses are gradually increased during the initial phase of treatment before starting maintenance treatment that ideally should be continued for three to five years. Although there are few studies demonstrating the dosage of this treatment, efficacy is known to be dose related (2), with an upper limit associated with adverse reactions that can sometimes be very severe and even cause death. Anaphylaxis, the most severe adverse effect, was the main reason leading to the search for other alternative routes of administration.

As the simplest route of drug administration is the oral route, SLIT has been used for decades, usually with subtherapeutic doses. Initial clinical trials, sometimes reporting positive results (3), were performed during the 1980s. These studies were first initiated because of the possibility of inducing oral tolerance with simplified administration. Limited evidence of immune modulation partially supports this mechanism of action with oral immunotherapy (4).

Another topical form of immunotherapy, nasal immunotherapy, was performed almost exclusively in Italy. Nasal immunotherapy has now been almost completely abandoned because of the risk of accidental, simultaneous bronchial administration and the possibility of aggravating rhinitis.

Sublingual antigen administration, followed either by expectoration or swallowing, is also an old technique purported to be useful for diagnosis and treatment. This method as previously practiced was challenged by the American Academy of Allergy, Asthma, and Immunology in 1981 and documented to be ineffective (5). Using low dose of antigens, a small number of clinical studies using sublingual-spit immunotherapy failed to validate efficacy.

In some European countries, such as Spain, France, and Italy, the most frequent form of specific immunotherapy is now based on high-dose SLIT. Like SCIT, a dose effect is also observed for SLIT (6–8). High-dose SLIT is efficacious, but low dose is not. SLIT usually includes a dose-titration phase followed by maintenance treatment, but attempts have been made to simplify the process by eliminating the dose-titration phase (9). Treatment can be administered perennially, but pre- and coseasonal administration can also be used for the treatment of pollen allergies.

COMPARISON OF THE EFFICACY AND SAFETY OF SCIT AND SLIT

In countries in which health authorities have approved both SCIT and SLIT, ethical and legal requirements imply that the patient must be informed about the expected efficacy of treatment, as well as the possible risks involved.

Other chapters of this book deal with the efficacy and safety of these two treatment modalities, but comparative patient information on the two techniques must summarize this information. A meta-analysis, according to the Cochrane method, demonstrates the efficacy of SCIT in terms of reduction of symptoms compared with placebo (10). The difference expressed as the standardized mean difference (SMD) is -0.73 lower than placebo. In a similar comparison, performed according to the same method but comparing placebo and SLIT (11), the SMD is also statistically significant, but with a value of -0.42 lower than placebo. The efficacy of the SLIT therefore is less than that of SCIT, but head to head trials are necessary to confirm and quantify the magnitude of this difference. There is only one small study, using a double-dummy methodology, comparing the two routes of administration (12). In this study of birch pollen-allergic patients, the efficacy of both forms of immunotherapy was statistically superior to that of placebo, with no significant difference between the two routes of administration. However, this study lacks power because of the limited number of patients. In the comparison with placebo, the area under the curve of symptoms was reduced by 45% for the subcutaneous route versus a 27% reduction for the sublingual route. This difference is not statistical but suggests that SCIT is slightly more effective than the SLIT.

However, the choice of immunotherapy treatment is not based solely on efficacy but must also consider adverse effects. In the only well-designed comparative study available (12), a case of anaphylactic shock was observed in the SCIT study group, while no serious adverse effects were observed in the SLIT group. SCIT may cause serious adverse effects, even in the secured environments of clinical trials. Estimates are one death every 2 to 2.5 million injections in clinical practice (13,14). Anaphylaxis has been anecdotally observed with SLIT using nonstandardized allergen extracts, mixtures of allergen extracts, or latex (15–17).

In summary, we conclude that because of its added safety and with a current follow-up of 20 years, the benefit/risk balance possibly favors SLIT, at least if single allergen extracts are used. Long-term benefits from SLIT are not as well established as from SCIT. The patient should be informed that SLIT is likely less effective but safer than SCIT. Oral or local side effects are relatively common with SLIT, but are generally of limited severity. However, systemic adverse reactions, including anaphylaxis, may occur with SLIT. This is a concern, despite infrequent occurrence, as the patient usually self-administers SLIT at home and would be denied immediate, professional treatment.

RECOMMENDED AVAILABLE ALLERGENS

Immunotherapy consists of the administration of vaccines made of natural extracts. These extracts usually contain a complex mixture of different molecules that are responsible for sensitization and symptoms. Not all patients are sensitized to all of the allergens in the extract. Thus, some components of a vaccine are useful and some are not.

The appropriately treated patient is exposed to two main risks with allergen immunotherapy. The first risk is lack of efficacy due to treatment with an insufficient quantity, or even none, of the individually relevant, allergenic molecules. The second risk is adverse effects, most notably anaphylaxis. This risk is inherent to an allergic individual receiving complex allergen mixtures. Natural products are subject to variability of the constituent molecules. Consequently, when initiating treatment and when changing vials during maintenance immunotherapy, the patient may be exposed to unexpected amounts of an allergen leading to reactions, including systemic reactions. Standardization of allergens is designed to limit this risk. Depending on the country, monitoring of production standards for allergen extracts is performed either by the manufacturer's in-house control laboratory or by health authorities like the Food and Drug Administration (FDA) in the United States. Biological activity is generally measured by an in vivo method such as skin test titration. This method is limited by the number and variability of the allergic subjects involved in the titration. Antigen content can be measured by a total allergen assessment using enzyme-linked immunosorbent assay (ELISA) or radioallergosorbent test (RAST) inhibition. More precise control tests include content determination of one major allergen, which may not predict the concentration variation of other major or minor allergens. These alternative allergens may be important sensitizers for an individual patient. Despite these limitations, immunotherapy ideally should be performed with standardized allergen extracts. Unfortunately, all extracts are not available in standardized formulations, and some patients are reactive to non-standardized allergens, allergens that are not standardized because of the relatively low frequency of their clinical utility. The information intended for patients must communicate these limitations.

EXPECTED CONSEQUENCES OF ALLERGEN IMMUNOTHERAPY ON ALLERGIC DISEASE

The overall efficacy of SCIT in asthma and rhinitis in allergic adults has been demonstrated with the highest level of proof (A), on the basis of meta-analyses that have been regularly updated since 1995 (10,18–21). SCIT has a significant effect (improvement of specific bronchial hyperresponsiveness, reduced need for medications, improved symptoms) on dust mite and pollen allergies (birch, grasses, cypress, olive tree, ragweed, *Parietaria*), but less effect on mammalian allergens. Some data also indicate that specific immunotherapy can modify the natural history of allergy (level of proof = B) (22–26). Some of these studies report persistence

of efficacy on asthma and rhinitis symptoms three years after stopping treatment, while others suggest prevention of the development of new sensitizations to respiratory allergens and a significant reduction of the incidence of asthma in children with rhinitis. There are criticisms of the methodologies used in some of these studies, recognizing that long-term investigations are challenging. However, SCIT is not commonly used before the age of five years because of the trauma induced by injection therapy and the risk of systemic reactions that may be more difficult to detect and manage at this age.

A meta-analysis of SLIT has been conducted in asthma and rhinitis (11) in adults and children. These studies showed (level of proof = A) a significant effect on asthma and rhinitis symptoms and a reduction of medication intake for dust mite and pollen (birch, grasses, cypress, olive tree, *Parietaria*). Studies investigating the effect of SLIT on the course of allergic disease are ongoing (27,28).

The objectives of SLIT must be explained in simple terms: reduction of clinical scores (symptoms, medications, functional scores), ease of use, improvement of the allergic patient's quality of life, and less convincing evidence of disease modification.

COMPARATIVE COST OF TREATMENT WITH SCIT OR SLIT

The cost of immunotherapy for the community or for the individual is difficult to compare because of the very marked differences between health care systems throughout the world. Even within the same country, Italy, for example, patients may be reimbursed for immunotherapy in one region and not in another region. SLIT has a reduced professional cost because of the opportunity for the patient to self-administer the treatment. However, the costs of the allergen vaccine for SLIT are sufficiently increased to minimize cost differences between SCIT and SLIT.

A patient should be informed about the cost of treatment. For example, in France, the retail price of two 5 mL bottles of maintenance treatment, necessary for one year of treatment, with a mixture of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* is about US $80; but in Germany and Scandinavian countries, the daily cost of SLIT is about US $5. If we assume that the cost of residual symptomatic treatments and the costs related to diagnosis leading to the decision to prescribe SLIT remain the same, SLIT should allow for a considerable reduction of the costs of medical visits and the number of days off school or work for each injection, although it does not eliminate the need for regular medical follow-up. Cost-analyses will depend upon the health care system and the perspective of societal cost, borne by the health plan, versus individual cost, borne by the patient. Thus, each physician will need to assess these issues for each patient.

PROOF THAT INFORMATION HAS BEEN GIVEN TO THE PATIENT

Regardless of differences in international legal requirements, it is recommended that written proof be obtained that information about any form of immunotherapy has been given to the patient. This is particularly true since the addition of SLIT, in some countries, has added options for clinical practice.

The importance of patient information is provided by the history of French legal practice. Previously, in a case of a serious adverse event related to treatment or diagnosis, proof was required that the patient had received no information prior to the therapy. This situation is now reversed, and, according to French law, the physician must provide proof that accurate and complete information is provided to the patient prior to the event. The written text should be signed by the patient or the patient's legal representative and by the physician. In other countries, the patient's signature may be replaced by that of an observer or witness. This requirement does not eliminate the need for the physician and other health care professionals to educate the patient about the treatment in question. Information obtained in the context of clinical trials in emergencies, such as myocardial infarction, although a very different situation, offers additional insight about this problem. In this emergent setting, 19% of the participants in this type of study report having read the information and consent forms, while 81% report they understood the information given orally by the investigator (29). Thus, both written and oral exchange of information is desirable, but documentation realistically requires written confirmation.

SPECIFIC SUBLINGUAL IMMUNOTHERAPY

PATIENT INFORMATION SHEET

Your doctor has proposed to treat you with specific immunotherapy to decrease your symptoms related to allergy: allergic nasal and/or eye symptoms and allergic asthma, especially when pulmonary functions are minimally decreased.

OBJECTIVE OF TREATMENT

Specific immunotherapy decreases and can even eliminate allergic reactions triggered by contact with the allergen(s) to which you are sensitized. These include dust mites, pollens, cat, as well as molds and others. Such treatment is long-term and addresses the cause. This treatment must be combined, at least at the beginning, with other medications used to relieve allergic diseases.

DESCRIPTION

Specific immunotherapy is administered in two ways. One option is injecting an allergen mixture into your arm, initially once or twice weekly, and then gradually at longer intervals until a maintenance dose is given every month. A 30-minute wait with a physician present is required after each injection to monitor and ensure you do not experience a severe allergic reaction (called anaphylaxis). If allergic reactions occur, they are merely treated. Severe allergic reactions and even death have been associated with injection therapy.

The second option of administration consists of placing the allergens under the tongue, holding them in your mouth for two minutes, and then swallowing them. This is a form of treatment being proposed to you or your family member. The dose administered under the tongue and then swallowed are increased over a short period of time, and when maintenance is reached, given on a regular basis, while fasting in the morning. All therapy is administered at home. When this form of therapy is initiated using high dose of allergen, it should be done so in the presence of a doctor. Thereafter, such therapy can be administered at home.

Specific immunotherapy should be administered for at least 3, and ideally for 5 years, to ensure lasting benefits. Unlike injection immunotherapy, which is usually administered continuously throughout the year, sublingual immunotherapy can be limited to pre- and co-seasonal periods.

Few studies have compared the therapeutic efficacy of the injection form of therapy to the sublingual administration, but both methods are effective when optimal doses are used for treatment. In any event, the indication for immunotherapy must be reviewed when treatment is not effective after one year for perennial allergens and after two seasons for pollens. Whatever the form of immunotherapy, patients should be monitored while they continue such therapy.

POSSIBLE RISKS

Immunotherapy is associated with risks of allergic reactions. Reactions are primarily local, causing swelling, redness, and itching of the mouth and tongue. More serious reactions can involve the gastrointestinal tract (swelling of the tongue and lips, itchiness of the mouth, diarrhoea, cramps, and abdominal pain) after oral administration. Much less frequently, oral immunotherapy can cause deterioration of allergic symptoms or can induce hives or swelling of the skin.

To date, serious but exceptional adverse reactions such as anaphylactic shock or death have been observed with the injection route (death has been observed once every 2 to 2.5 million injections). This type of reaction is usually characterized by generalized itching, flushing, shortness of breath, dizziness, or severe weakness. This is potentially life threatening, and you should administer the epinephrine injector in this event and you should call 911 or notify emergency services. Anaphylaxis occurs but is much less common with sublingual immunotherapy.

INFORMED CONSENT FORM FOR PATIENTS OR LEGAL GUARDIANS

PATIENT'S LAST NAME and FIRST NAME:_____

My doctor, Doctor_____**, ha s provided me with detailed information about the treatment of allergy by specific sublingual immunotherapy.**

He has explained to me the objectives, modalities and risks of this treatment as well as the other available treatment options, and I have understood this information.

I hereby give my consent to receive this treatment.

PLACE:_____**,** **DATE:**_____

SIGNATURE:_____

Document to be kept in the patient's medical records.

SALIENT POINTS

The essentials of the information outlined in this chapter should be given to the patient orally and presented in abridged form in an information sheet. This information sheet and especially the informed consent form should include the following points:

- Immunotherapy treats the cause of respiratory allergy and asthma;
- Severe asthma is a relative contraindication to immunotherapy, at least via the subcutaneous route;
- Historically, the presence of an autoimmune disease also constitutes a relative contraindication;
- The use of β-blocker therapy may decrease the efficacy of treatment in case of anaphylaxis caused by immunotherapy, increasing the risk to the patient;
- The duration of treatment and the possible disadvantages, benefits, and risks must be clearly specified;
- Both SCIT and SLIT are effective when optimal doses are used for treatment;
- SCIT modifies the course of allergic disease, data are pending with respect to SLIT; and
- Anaphylaxis occurs more frequently with SCIT than with SLIT.

The information sheet must be written in terms that are easily understood. Sentences should be short, concise, and avoid negative expressions, in particular, double negatives. Technical terms must be explained in layman's terms (avoid excessively complicated terms). Information should be formulated directly for the patient, avoiding the use of third person. Medical jargon and the use of abbreviations are discouraged. When abbreviations are used, they should be explained in full when used for the first time. Choose a font that is easy to read and sufficiently large (e.g., Times 12 or even 14). Break the text into paragraphs or use bulleted points. If several pages are necessary, number the pages (page/number of pages). Finally, for minors, information must also be given to the parents or legal guardians.

REFERENCES

1. Noon L, Cantab BO. Prophylactic inoculation against hay fever. Lancet 1911; 1: 1572–1573.
2. Haugaard L, Dahl R, Jacobsen L. A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: Clinical efficacy and side effects. J Allergy Clin Immunol 1993; 91:709–722.

3. Taudorf E, Laursen LC, Lanner A, et al. Oral immunotherapy in birch pollen hay fever. J Allergy Clin Immunol 1987; 80:153–161.
4. Roth-Walter F, Jensen-Jarolim E. Oral immunotherapy against type 1 allergy. Allergy Clin Immunol Int 2007; 19:21–26.
5. American Academy of Allergy. Position statements. Controversial techniques: provocation testing (sublingual). J Allergy Clin Immunol 1981; 67:337–338.
6. Andre C, Perrin-Fayolle M, Grosclaude M, et al. A double-blind placebo-controlled evaluation of sublingual immunotherapy with a standardized ragweed extract in patients with seasonal rhinitis. Evidence for a dose-response relationship. Int Arch Allergy Immunol 2003; 131:111–118.
7. Didier A, Melac M, Combebias A, et al. Efficacy and safety of sublingual immunotherapy (SLIT) tablets in grass pollen rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117:721.
8. Durham SR, Yang WH, Pedersen MR, et al. Sublingual immunotherapy with once-daily grass allergen tablets: A randomised controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117:802–809.
9. Dahl R, Kapp A, Colombo G, et al. Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhiniconjunctivitis. J Allergy Clin Immunol 2006; 118:434–440.
10. Calderon MA, Alves B, Jacobson M, et al. Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev 2007; 1:CD001936.
11. Wilson DR, Torres Lima M., Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005; 60:4–12.
12. Khinchi MS, Poulsen LK, Carat F, et al. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. Allergy 2004; 59:45–53.
13. Bernstein DI, Wanner M, Borish L, et al. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990 to 2001. J Allergy Clin Immunol 2004; 113:1129–1136.
14. Reid MJ, Lockey RF, Turkeltaub PC, et al. Survey of fatalities from skin testing and immunotherapy 1985 to 1989. J Allergy Clin Immunol 1993; 92:6–15.
15. Cox LS, Linnemann DL, Nolte H, et al. Sublingual immunotherapy. A comprehensive review. J Allergy Clin Immunol 2006; 117:1021–1035.
16. Marogna M, Spadolini I, Massolo A, et al. Effects of sublingual immunotherapy for multiple or single allergens in polysensitized patients. Ann Allergy Asthma Immunol 2007; 98:274–280.
17. Dunsky E, Goldstein MF, Dvorin MJ, et al. Anaphylaxis due to sublingual immunotherapy. Allergy 2006; 61:1235.
18. Eifan AO, Keles S, Bahceciler NN, et al. Anaphylaxis to multiple pollen allergen sublingual immunotherapy. Allergy 2007; 62:567–568.
19. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. Am J Respir Crit Care Med 1995; 151:969–974.
20. Abramson MJ, Puy RM, Weiner JM. Immunotherapy in asthma: an updated systematic review. Allergy 1999; 54:1002–1041.
21. Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. Cochrane Database Syst Rev 2003; 4:CD001186.
22. Des Roches A, Paradis L, Menardo JL, et al. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. J Allergy Clin Immunol 1997; 99:450–453.
23. Durham SR, Walker SM, Varga EM, et al. Long term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999; 341:468–475.
24. Eng PA, Reinhold M, Gnehm HPE. Long term efficacy of preseasonal grass pollen immunotherapy in children. Allergy 2002; 57:306–312.
25. Eng PA, Borer-Reinhold M, Heijnen IA, et al. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. Allergy 2006; 61:198–201.
26. Moller C, Dreborg S, Ferdousi Ha, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis. J Allergy Clin Immunol 2002; 109:251–256.
27. Novembre E, Galli E, Landi F, et al. Coseasonal sublingual immunotherapy reduces the development of asthma in children with allergic rhinoconjunctivitis. J Allergy Clin Immunol 2004; 114:851–857.
28. Marogna M, Spadolini I, Massolo A, et al. Randomized controlled open study of sublingual immunotherapy for respiratory allergy in real-life: clinical efficacy and more. Allergy 2004; 59: 1205–1210.
29. Williams BF, French JK, White HD, et al. Informed consent during the clinical emergency of acute myocardial infarction (HERO-2 consent substudy): a prospective observational study. Lancet 2003; 361:918–922.

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