NUTRITION AND HEALTH

Dietary Components and Immune Function

Edited by

Ronald Ross Watson
Sherma Zibadi
Victor R. Preedy

Humana Press

Dietary Components and Immune Function

Nutrition ◊ and ◊ Health

Adrianne Bendich, PhD, FACN, Series Editor

For other titles published in this series, go to
www.springer.com/series/7659

Dietary Components and Immune Function

Edited by

Ronald R. Watson, PhD
Division of Health Promotion Science, Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ, USA

Sherma Zibadi, MD, PhD
Division of Health Promotion Science, Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ, USA

Victor R. Preedy, PhD
King's College London, Department of Nutrition and Dietetics, UK

Humana Press

Editors
Ronald R. Watson
Division of Health Promotion Sciences,
Mel and Enid Zuckerman
College of Public Health,
University of Arizona,
Tucson, AZ,
USA
rwatson@u.arizona.edu

Sherma Zibadi
Division of Health Promotion Sciences,
Mel and Enid Zuckerman
College of Public Health,
University of Arizona,
Tucson, AZ,
USA
shermazib@yahoo.com

Victor R. Preedy
King's College London
Department of Nutrition and Dietetics
UK
victor.preedy@kcl.ac.uk

Series Editor
Adrianne Bendich
GlaxoSmithKline Consumer Healthcare
Parsippany, NJ

ISBN 978-1-60761-060-1 e-ISBN 978-1-60761-061-8
DOI 10.1007/978-1-60761-061-8
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2010931836

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Acknowledgments

The work of editorial assistant, Bethany L. Stevens, in communicating with authors, working with the manuscripts and the publisher was critical to the successful completion of the book and is much appreciated. Her daily responses to queries and collection of manuscripts and documents were extremely helpful. Support for her work was graciously provided by Elwood Richard and the National Health Research Institute in support of its mission to communicate with scientists about bioactive foods and dietary supplements was vital (http://www.naturalhealthresearch.org). This was part of their efforts to educate scientists and the lay public on the health and economic benefits of nutrients in the diet as well as supplements. Finally, Nguyen T. Nga and Mari Stoddard of the Arizona Health Sciences library were instrumental in finding the authors and their addresses in the early stages of the book's preparation.

A special appreciation is given to Adrianne Bendich, PhD, the Series Editor for her time and thought which resulted in important modification in the order of the chapters in this book. Also to the staff of Humana Press a branch of Springer who have helped the editors over many years provide interesting scientific reference books to other researchers.

Series Editor Introduction

The Nutrition and Health series of books has, as an overriding mission, to provide health professionals with texts that are considered essential because each includes: (1) a synthesis of the state of the science, (2) timely, in-depth reviews by the leading researchers in their respective fields, (3) extensive, up-to-date fully annotated reference lists, (4) a detailed index, (5) relevant tables and figures, (6) identification of paradigm shifts and the consequences, (7) virtually no overlap of information between chapters, but targeted, interchapter referrals, (8) suggestions of areas for future research, and (9) balanced, data-driven answers to patients/health professionals and questions which are based upon the totality of evidence rather than the findings of any single study.

The series volumes are not the outcome of a symposium. Rather, each editor(s) has the potential to examine a chosen area with a broad perspective, both in subject matter as well as in the choice of chapters. The international perspective, especially with regard to public health initiatives, is emphasized where appropriate. The editors, whose trainings are both research and practice oriented, have the opportunity to develop a primary objective for their book; define the scope and focus, and then invite the leading authorities from around the world to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research, and relate the research findings to potential human health consequences. Because each book is developed de novo, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

"Dietary Components and Immune Function", edited by Ronald R. Watson, Ph.D., Sherma Zibadi, M.D., and Victor Preedy, Ph.D. exemplifies the goals of the Nutrition and Health Series. Unlike many other books in the area of nutritional immunology, this text provides a critical assessment of the field based upon recent in vitro, laboratory animal studies as well as epidemiological and clinical intervention studies. Each of the editors has extensive experience in clinical immunology and the combined experiences in academia, and clinical practice provides a broad perspective on the role of food and food components, diet and diet modifications, nutrients and the multitude of nonessential components of the diet on critical aspects of human immune responses.

The editors have chosen 85 internationally recognized experts who are active investigators of the impact of overall diet on the risks of infection, cancer, autoimmune disease and environmental stressors in different age groups, in different countries throughout the world, and in both sexes. This important text provides practical, data-driven resources, including over 4,000 up-to-date references and more than 40 well-organized tables and figures that assist the reader in evaluating the nutritive value of the immunomodulatory vitamins and minerals and other dietary constituents, such as probiotics, long chain fatty acids, conjugated linoleic acid, traditional Chinese medicines, plant polyphenols, tannins, and many other components of foods. Moreover, the critical

value of nutrition for at-risk populations, including those living with cancer, allergies and/or asthma, autoimmune diseases, the very young and the very old are extensively reviewed in several unique chapters.

Each chapter begins with comprehensive bulleted Key Points followed by the list of key words, and includes an overview and historic review, examination of the literature with critical focus on comparisons between studies, discussion of the chemical composition of actives, where appropriate and conclusions and perspectives on future research areas. The overarching goal of the editors is to provide fully referenced information to health professionals so they may have a balanced perspective on the value of many dietary components that are routinely consumed by patients and clients with the hope that immune responses will be enhanced. This important volume provides health professionals with balanced, data-driven answers to numerous questions about the validity of the science to date and also provides researchers with opportunities to clarify areas where many questions still exist about the effects of specific nutrients/dietary factors on human immune responses.

The editors have organized the volume into six sections that reflect the breadth and depth of current knowledge in the area of dietary factors that affect immune responses. In the first section entitled Development of Human Immune Responses, the editors have wisely included an introductory chapter that clearly outlines the embryonic development of the human immune system, and this chapter as well as subsequent chapters review in depth the value of breast milk for the development of neonatal as well as lifetime immunity. Unique areas of focus include an analysis of the effects of maternal undernutrition on immune responses in neonates, and of relevance is the chapter on the effects of undernutrition on parasitic invasion. The second section on Nutrients and Immunomodulation contains complementary chapters on the role of fatty acids, especially long chain fatty acids on the immune system in general, and these chapters are followed by reviews of the specific effects with regard to the brain, fat tissue, and obesity, and effects on cancer cells and immune responses to tumors. There is a separate chapter on vitamins and minerals and another on trace minerals and the third examines the effects of vitamin supplements in women with HIV infections. The third section provides in-depth, separate chapters devoted to the Role of the Immune System in Cancer Prevention and Treatment and the effects of some of the thousands of dietary bioactive compounds including, but not limited to the vitamins, carotenoids, and minerals. Certain immune cells can directly kill tumor cells, and this potential can be enhanced by certain components of the diet. Fruit and vegetable intake above the average has been associated with decreased risk of several cancers, and the components of fruits and vegetables are reviewed with regard to enhanced tumor cell killing by cytotoxic immune cells. Lactoferrin, one of the whey proteins in milk, also has anticancer and immunoenhancing effects in vitro and in animal models that are outlined in a separate chapter. Other unique chapters review in detail the plant-derived drugs and semisynthetic derivatives that are used to treat cancer and compare these with bioactive plant compounds used in Traditional Chinese Medicine (TCM). Some of these compounds are directly cytotoxic to cancer cells, whereas many stimulate immune cells to kill tumor cells. The differences between the emphasis of TCM on restoring balance and prolonging life compared to treating the tumor in Western medicine is clearly described in the two complementary chapters.

The fourth section on Dietary Components in Allergy and Asthma includes chapters that examine controversial areas such as genetic factors and interactions between fetal and early neonatal exposure to allergens from the maternal sources versus diet and the increased risk of asthma in childhood. The importance of the neonatal colonization by gut microflora and the development of immune function resulting in tolerance of environmental antigens are also reviewed. The value of breast feeding is emphasized as is the delay of complementary feeding until 4–6 months of age. Dietary factors considered to be associated with the reduction in the risk of allergy/asthma development include long chain omega-3 polyunsaturated fatty acids, antioxidants, and certain probiotics. Preliminary evidence of the potential for an extract from a specific mushroom to affect the development of IgE responses is also included in a separate chapter. Authors of these chapters have been particularly inclusive and objective; extensive references to the published literature are provided. The fifth section looks mainly at the preliminary data from in vitro and small animal studies on the effects of Botanical Extracts and Bioactive Foods and includes separate chapters on resveratrol and on other bioactive flavonoids; immunoactive components of cocoa; extracts used in TCM; microalgae; extracts from edible mushrooms, including their immunoenhancing polysaccharides; soy sauce and its bioactive polysaccharides, and anti-inflammatory actions of cinnamon extracts. These chapters contain extensive, detailed tables that bring the reader up-to-date on the state of the science linking the compounds to immune function, specific immune cell interaction effects on allergy, antiviral activity, and disease resistance. The important emphasis on ethnopharmacology and the potential role of plants and their constituents as direct antivirals, anticancer, and immunostimulatory agents is of great value, especially as the data presented are balanced and objectively tabulated for the reader. Bioactive foods can also be considered as those that contain factors that can be immunotoxic such as aflatoxin, alcohol, methyl mercury, nitrosamines, polychlorinated compounds, as examples. This section ends with a critically important and unique chapter on immunotoxicology of foods. The last section includes four chapters that review the data on pre- and probiotics. The chapters examine the important role of the gastric mucosal immune system and the significance of gut bacteria in health as well their potential beneficial effects for those suffering from pancreatitis or irritable bowel diseases in separate chapters.

Understanding the complexities of the human immune system and the effects of food/environment/age/sex/concomitant disease/drugs/stressors certainly is not simple and the interactions can often seem daunting. However, the editors and authors have focused on assisting those who are unfamiliar with this field in understanding the critical issues and important new research findings that can impact their fields of interest. Drs. Watson, Zibadi, and Preedy have carefully chosen the very best researchers from around the world who can communicate the relevance of dietary components in both the maintenance of a healthy immune system and the potential for bioactive food components to affect the course of infections and chronic diseases. The authors have worked hard to make their information accessible to health professionals interested in public health, those practicing in medical specialties from pediatrics to geriatrics, those in general medical practice, nursing, pharmacy, educators, students as well as nutrition-related allied health professionals. The editors have taken special care to use the same terms and abbreviations between chapters, and provide a clearly written glossary of terms as well as a list of abbreviations used throughout the volume.

In conclusion, “Dietary Components and Immune Function”, edited by Ronald Ross Watson, Sherma Zibadi, and Victor Preedy provides health professionals in many areas of research and practice with the most up-to-date, well-referenced volume on the importance of dietary factors for optimal immune function. This volume will serve the reader as the most authoritative resource in the field to date and is a very welcome addition to the Nutrition and Health Series.

Parsippany, NJ *Adrianne Bendich, PhD, FACN*

Preface

The Strategic Plan of the United States' National Institute of Health stresses the pursuit of mechanistic studies as an overarching priority. Among the basic mechanisms identified as needing exploration are immune-focused studies. Many dietary components are believed to exert their activities by modulating immune function. These are highly sought by patients in the absence of an effective standard pharmaceutical therapy. Numerous botanical extracts as well as high dose vitamin supplements are used by the public with the expectation that they will boost and/or modulate immune responses. As the number of seniors grows interest in their loss of immune defenses (immunosenescence) increases and is linked to longevity or lack thereof. The desire to maintain wellness by preventing and treating infectious diseases are among the key reasons for responses. Normal functioning of the immune system is critical to health. One new tumor cell appears about every day and is eliminated by the immune system. Aging, stress, diseases like AIDS, autoimmune reactions, chemical treatments to suppress immune responses in arthritis, and transplants can facilitate the survival of a cancer, leading to clinical disease. Can dietary modulation thereafter help treat cancer? Putative immune-modulating agents and practices are also being used with the belief that they will maintain wellness by reversing the immune decline-associated aging and the immunosuppression associated with cancer, its treatment, and with HIV/AIDS. Thus, an increased focus on understanding the efficacy of botanicals and other dietary supplements on immune function is warranted.

This book focuses on dietary modalities that modulate immune function. The first section discusses various nutrients that alter innate and/or adaptive immunity humoral and cellular responses affecting both immune mechanisms and disease endpoints. The second section investigates the role of nutriceuticals in immune-mediated cancer resistance. The third one investigates their role in asthma and allergy. The fourth segment reviews the role of botanical extracts and supplements in enhancing responses to pathogens, which should have significant public health value. Indeed, the usage of foods and their extracts as therapeutic tools appear in ancient and modern cultures. Thus, the first set of reviews investigates bioactive foods in immunodeficiency diseases. The next section looks at the role of nonpathogenic bacteria, prebiotics, and probiotics in immune modulation. Finally, the authors review dietary supplements in viral diseases. Historically, famine preceded disease and likely was treated by dietary therapy of the immunodeficiency due to dietary insufficiency.

Clearly, information is vital for the researcher, physician, and particularly the lay public as they are exposed to increased availability and media evidence that they may have efficacy. Importantly, in the USA the use of botanicals and their extracts are widely available, part of a 20 billion dollar business. The majority of seniors use dietary supplements and nutrients to promote health. How effective are these agents in doing so via

immune restoration or regulation? Therefore, information from scientific research is critical to help people make decisions on their benefits, risks, or value in the prevention and treatment of immune dysfunction with loss of resistance.

Tucson, AZ *Ronald R. Watson*
Tucson, AZ *Sherma Zibadi*
London, UK *Victor R. Preedy*

Contents

Contributors

Salomon Amar, DMD, PHD · *Professor, Associate Dean for Research School of Dental Medicine, Boston University, 650 Albany Street, X343D, Boston, MA 02118, USA*
samar@bu.edu

Cristina Andres-Lacueva · *Pharmacy Faculty, Nutrition and Food Science Department, XaRTA. INSA, University of Barcelona, Barcelona, 08028, Spain*
candres@ub.edu

Joanne E. Arsenault, PHD, RD · *Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston 02115, MA, USA*
joanne.arsenault09@gmail.com

Hitoshi Asakura · *The Koukann Clinics, Koukanndoori 1-2-3, Kawasakiku, Kawasaki 210-0852, Japan*
hitoshi-asakura@koukankai.or.jp

Spiridoula Athanasiadou, BVETMED PHD, MRCVS · *Disease Systems, Animal Health, Scottish Agricultural College, Bush Estate, Penicuik EH26 0PH, UK*
Spiridoula.Athanasiadou@sac.ac.uk

Günther Boehm · *Danone Research – Centre for Specialised Nutrition, Friedrichsdorf, GermanySophia Children's Hospital, Erasmus University Rotterdam, Rotterdam, The Netherlands*
Guenther.Boehm@Danone.com

Heping Cao · *US Department of Agriculture, Commodity Utilization Research Unit, Southern Regional Research Center, Agricultural Research Service, 1100 Robert E. Lee Blvd, New Orleans, LA 70124, USA*
Heping.Cao@ars.usda.gov; peacetd2003@yahoo.com

Margarida Castell · *Dept. Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, Barcelona, 08028, Spain*
margaridacastell@ub.edu

Cristina Castellote, PHD · *Professor, Faculty of Pharmacy Department of Physiology, University of Barcelona, Barcelona, Spain*
cristinacastellote@ub.edu

Susantha N. Chandersekera · *Department of Chemistry, University of Illinois at Chicago, IL 60616, USA*

Xin Chen, CMD, PHD · *Scientist II, SAIC-Frederick, Inc., Lab of Molecular Immunoregulation, Basic Research Program, Cancer and Inflammation Program, CCR, NCI-Frederick, P.O. Box B, Bldg 560, Rm 31-19, Fort Detrick, Frederick, MD 21702-1201, USA*
chenxin@mail.nih.gov

Antonio Cherubini · *Department of Clinical and Experimental Medicine, Institute of Gerontology and Geriatrics, University of Perugia, Perugia, Italy*
Jean-Pierre Chouraqui, md · *Gastro-entérologie, Hépatologie et Nutrition Pédiatriques, Centre Hospitalier Universitaire de Grenoble, Grenoble Cedev 09 217-38043, France*
jpchouraqui@chu-grenoble.fr
Manuel A. de Pablo Martínez · *Universidad de Jaén, Facultad de Ciencias Experimentales, Dpto. de Ciencias de la Salud, Área de Microbiología, 23071, JAEN, Spain*
mapablo@ujaen.es
Pedro Manoel Mendes Moraes-Vieira · *Associate research, Laboratory of Transplantation Immunobiology, Institute of Biomedical Science IV, Rua Prof. Lineu Prestes 1730, 05508-900, Cidade Universitaria, University of São Paulo, São Paulo, Brazil*
pedrommmv@gmail.com
Ye Deng · *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
Jean-Luc Desseyn, phd · *Clinique de Pédiatrie, CHRU de Lille, EA3925, IFR114, Faculté de Médecine, Université de Lille2, Lille, France*
Ligia Esperanza Díaz · *Department of Metabolism and Nutrition, Immunonutrition Research Group, Spanish National Research Council (CSIC), Institute Frio-ICTAN, Madrid, Spain*
Janice M. Dietert · *Performance Plus Consulting, Lansing, NY, 14882, USA*
Rodney R. Dietert · *Professor of Immunotoxicology, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, France*
rrd1@cornell.edu
O.N. Donkor · *Faculty of Health School of Biomedical and Health Sciences, Engineering and Science, Hopper Lane, Werribee, Victoria 3030, Australia*
A. Douglas Kinghorn, phd. d.sc · *Jack L Beal Professor and Chair College of Pharmacy Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue Columbus, OH 43210, USA*
kinghorn.4@osu.edu
Anais Duffaud, phd · *Psychoneuroimmunology, Nutrition and Genetic (PsyNuGen), INRA 1286, CNRS, 5226, Université Bordeaux 2, 146 Rue Leo Saignat, Bordeaux, Cedex 33076, France*
percival@ufl.edu
Nina D'Vaz · *School of Paediatrics and Child Health Research, University of Western Australia, Perth, Australia*
Zuleica B. Fortes, phd · *Department of Farmacology, Institute of Biomedical Sciences, Av Lineu Prestes, University of São Paulo, 1754, São Paulo, CEP 05508-000, Brazil*
zbfortes@icb.usp.br

Àngels Franch, phd · *Tenured Lecturer, Faculty of Pharmacy Department of Physiology, University of Barcelona, Barcelona, Spain*
angelsfranch@ub.edu
Johan Garssen, phd · *Professor, Immunopharmacology of Nutrition, Utrecht Institute for Pharmaceutical Sciences, Utrecht, The Netherlands*
Department of Pharmacology and Pathophysiology, Utrecht University, Wageningen, The Netherland
Director Platform Immunology, Danone Research Centre for Specialised Nutrition, Wageningen, The Netherlands
J.Garssen@UU.nl; Johan.Garssen@Donone.com
H. S. Gill · *Professor, Department of Primary Industries, 600 Sneydes Road, Werribee, VIC, 3000, Australia*
harsharn.gill@dpi.vic.gov.au
Sonia Gómez-Martínez S · *Department of Metabolism and Nutrition, Immunonutrition Research Group, Spanish National Research Council (CSIC), Institute Frio-ICTAN, Madrid, Spain*
Frédéric Gottrand, md, phd · *Clinique de Pédiatrie, CHRU de Lille, EA3925, IFR114, Faculté de Médecine, Université de Lille2, Lille, France*
frederic.gottrand@chru-lille.fr
Corinne Grangette · *Lactic Acid Bacteria and Mucosal Immunity, Pasteur Institute of Lille, Center for Infection and Immunity of Lille (CIIL) INSERM U1019 – CNRS UMR 8204 1, rue du Pr Calmette, 59019 Lille Cedex, France*
Corinne.grangette@ibl.fr
Ah-Reum Han · *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
Refaat A. Hegazi, md, phd · *Medical Director Adult Nutrition Research and Development, Abbott Nutrition, 3300 Stelzer Rd, Columbus, OH 43219, USA*
Refaat.Hegazi@abbott.com
Axel R. Heller, md, mba-hcm, deaa · *Senior Executive Physician Klinik und Poliklinik für Anaesthesiologie und Intensivtherapie, Universitätsklinikum Carl Gustav Carus, an der Technischen Universität Dresden, Anstalt des öffentlichen Rechts des Freistaates Sachsen, Fetscherstraße 74, Dresden 01307, Germany*
Axel.Heller@uniklinikum-dresden.de
Shanmugam Hemaiswarya · *Department of Biotechnology, Indian Institute of Technology-Madras, Chennai, Tamil Nadu 600 036, India*
iswarya_hema@yahoo.co.in
Jos G.M. Houdijk · *Disease Systems, Animal Health, Scottish Agricultural College, Bush Estate, Penicuik EH26 0PH, UK*
Shalini Jain · *Bio-organic Chemistry Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA*
Sarah Jennings · *School of Paediatrics and Child Health Research, University of Western Australia, Perth, Australia*
Henning Johannsen · *School of Paediatrics and Child Health Research, University of Western Australia, Perth, Australia*
Nasiruddin Khan · *Pharmacy Faculty Nutrition and Food Science Department, XaRTA. INSA, University of Barcelona, Barcelona 08028, Spain*

Makio Kobayashi · *Higashimaru Shoyu Co., Ltd., Research Laboratory, 100-3, Tominaga, Tatsuno, Hyogo, 679-4167, Japan*
mkobayashi@higashimaru.co.jp
Manoj Kumar · *Dairy Microbiology Division, National Dairy Research Institute, Karnal, Haryana, India*
Maristella A. Landgraf · *Department of Farmacology, Institute of Biomedical Sciences, University of São Paulo, Av Lineu Prestes, 1754, São Paulo CEP 05508-000, Brazil*
mvitta@usp.br
Richardt G. Landgraf, phd · *Senior associate research, Department of Immunology, Institute of Biomedical Sciences, Av Lineu Prestes, 1730, University of São Paulo, São Paulo, CEP 05508-000, Brazil*
Institute of Biomedical Science IV, Rua Prof. Lineu Prestes 1730, 05508-900 Cidade Universitaria, University of São Paulo, São Paulo, Brazil
richardt@usp.br
Sophie Layé, phd · *Psychoneuroimmunology, Nutrition and Genetic (PsyNuGen), INRA 1286, CNRS, 5226, Université Bordeaux 2, 146 Rue Leo Saignat, Bordeaux Cedex 33076, France*
sophie.laye@bordeaux.inra.fr
Jie Li, phd · *Guang An Men Hospital of Academy of Chinese Medical Science, No. 5 BeiXianGe St, XuanWu District, Beijing, China*
drjieli2007@gmail.com
Hongsheng Lin, md · *Vice President of Chinese Society of Oncology of Traditional Chinese Medicine, Vice Director of China Cancer Center of Traditional Chinese Medicine, Director of Principal Laboratory of China, China Academy of Chinese Medical Sciences, Dean of Oncology Department, Guang An Men Hospital of CATCM, China Academy of Chinese Medical Sciences, Beijing, China*
zylzy501@gmail.com
Rafael Llorach · *Pharmacy Faculty Nutrition and Food Science Department, XaRTA. INSA, University of Barcelona, Barcelona, 08028, Spain*
Silvia Maggini · *Senior Global Science Manager, Bayer Consumer Care AG Global Research & Development, Nutritionals SBU, Peter Merian Strasse 84, P.O. Box, 4002, Basel, Switzerland*
silvia.maggini.sm@bayer.ch
Ascensión Marcos · *Department of Metabolism and Nutrition, Immunonutrition Research Group, Spanish National Research Council (CSIC), Institute Frio-ICTAN, Madrid, Spain*
Javier Romeo Marin, phd · *Immunonutrition Research Group, Department of Metabolism and Nutrition, Institute of Food Science, Institute Frío-ICTAN, C/José Antonio Novais, Madrid10 28040, Spain*
jromeo@if.csic.es
Fracesco Marotta · *NEmergency Unit, S. Giuseppe Hosital, Milano, Italy*
David Martino · *School Paediatrics and Child Health Research, University of Western Australia, Perth, Australia*

James P. McClung, phd · *Military Nutrition Division, United States Army Research Institute of Environmental Medicine, Kansas St., Building 42, Natick, MA 01760, USA*
james.mcclung@amedd.army.mil
Rajendra G. Mehta · *Drug Discovery Division, IIT Research Institute, Chicago, IL, USA*
Carcinogenesis Chemoprevention Division, IIT Research Institute, Chicago, IL, USA
Saji Menon · *Department of Biotechnology and Bioinformatics, Birla Institute of Scientific Research, Jaipur, India*
Ken-ichiro Minato, phd · *Department of Applied Biological Chemistry, Meijo University, Aichi Prefecture, Japan,*
minato@meijo-u.ac.jp
Masashi Mizuno · *Professor Laboratory of Glyco-chain Biochemistry, Graduate School of Agricultural Science, Kobe University, Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan*
mizuno@kobe-u.ac.jp
Dheeraj Mohania · *Animal Biochemistry Division, National Dairy Research Institute, Karnal, Haryana, India*
Robert M. Moriarty · *Drug Discovery Division, IIT Research Institute, Chicago, IL, USA*
Department of Chemistry, University of Illinois, Chicago, IL, USA
Carol A. Morris, phd · *Director Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, NSW 2480, Australia*
carol.morris@scu.edu.au
Ann M. Mulder, phd · *Student Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, NSW 2480, Australia*
ann.mulder@scu.edu.au
Ravinder Nagpal · *Department of Biotechnology, School of Technology and Science, Lovely Professional University, Phagwara, Punjab, India*
Rajesh Naithani, phd, mba · *Senior Scientist, Drug Discovery Division, IIT Resrach institute, Chicago, IL 60616, USA*
rnaithani@iitri.org
Laetitia Niers · *Department of Pediatrics, Wilhelmina Children's Hospital, University Medical Center, Utrecht, The Netherlands*
E. Nova · *Department of Metabolism and Nutrition, Immunonutrition Research Group, Spanish National Research Council (CSIC), Institute Frio-ICTAN, Madrid, Spain*
Niels Olsen Saraiva Camara · *Associate Professor of Immunology, Laboratory of Transplantation Immunobiology, Institute of Biomedical Science IV, Rua Prof. Lineu Prestes 1730, 05508-900, Cidade Universitaria, University of São Paulo, São Paulo, Brazil*
niels@icb.usp.br
Li Pan · *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University*, *Columbus, OH 43210*, USA
Susan S. Percival, phd · *Professor Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611, USA*

Francisco J. Pérez-Cano, phd · *Reader, Faculty of Pharmacy Department of Physiology, University of Barcelona, Barcelona, Spain*
franciscoperez@ub.edu
Daniel G. Peterson, phd · *Associate Professor Department of Animal Science, California Polytechnic State University, San Luis Obispo, CA 93407, USA*
dpeterso@calpoly.edu
Susan L. Prescott, bmedsci(hons), mbbs, fracp, phd · *Professor and Paediatric Allergist and Immunologist. Princess Margaret Hospital for Children, School of Paediatrics and Child Health, University of Western Australia, GPO Box D 184, Perth, WA 6840, Australia*
sprescott@meddent.uwa.edu.au
Elena Puertollano · *Faculty of Experimental Sciences, Unit of Microbiology, University of Jaen, Jaen23071, Spain*
mpvacas@ujaen.es
Maria A. Puertollano · *Faculty of Experimental Sciences Division of Microbiology, Department of Health Sciences, University of Jaén, E-23071, Jaén, Spain*
mangvacas@hotmail.com
Rathinam Raja · *Department of Biotechnology, Indian Institute of Technology – Madras, Chennai, Tamil Nadu 600 036, India*
rraja307@yahoo.co.in
Yulin Ren · *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, 43210, USA*
Ger T. Rijkers, phd · *grijkers@umcutrecht.nl Department of Medical Microbiology and Immunology, St. Antonius Hospital, PO Box 2500, Nieuwegein, EM 3430, The Netherlands*
g.rijkers@antoniusziekenhuis.nl
Frans M. Rombouts · *Laboratory of Food Microbiology, Wageningen University, Wageningen, The Netherlands*
Deepak Shukla · *Department of Microbiology and Immunology, University of Illinois, Chicago, IL, USA*
Department of Ophthalmology and Visual Sciences, University of Illinois, Chicago, IL, USA
Marianne Stasse-Wolthuis · *Stasse Consultancy, Udenhout, The Netherlands*
Kenji Suzuki, md · *Division of Gastroenterology and Hepatology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan*
Mireia Urpi-Sarda · *Pharmacy Faculty Nutrition and Food Science Department, XaRTA. INSA, University of Barcelona, Barcelona 08028, Spain*
Belinda van't Land, phd · *Utrecht Institute for Pharmaceutical Sciences (UIPS), University of Utrecht, The Netherlands*
Danone Research Centre for Specialisied Nutrition, PO Box 6700 PH, Wageningen, The Nertherlands
Belinda.vantland@danone.com, B.vantland@uu.nl
T. Vasiljevic · *Faculty of Health Engineering and Science, School of Biomedical and Health Sciences, Hopper Lane, Werribee, Victoria 3030, Australia*

A. Veses · *Department of Metabolism and Nutrition, Immunonutrition Research Group, Spanish National Research Council (CSIC), Institute Frio-ICTAN, Madrid, Spain*

Eduardo Villamor · *Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA*
evillamo@hsph.harvard.edu

Julia Wärnberg · *Department of Metabolism and Nutrition, Immunonutrition Research Group, Spanish National Research Council (CSIC), Institute Frio-ICTAN, Madrid, Spain*
Department of Preventive Medicine & Public Health, University of Navarra, Pamplona, Spain

Martina Wendel · *Prinzregent Luitpold Hospital, Scheidegg, Germany*
MartinaWendel@gmx.de

Dhananjay Yadav · *School of Studies in Biochemistry, Jiwaji University, Gwalior, Madhya Pradesh, India*

Hariom Yadav · *Diabetes Branch, Clinical Research Center, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA*
yadavh@mail.nih.gov

Mukesh Yadav · *Department of Biotechnology, College of Advanced Studies, Datia, Madhya Pradesh, India*

Ying Zhang, phd · *Guang An Men Hospital of Academy of Chinese Medical Science, No.5 BeiXianGe St, XuanWu District, Beijing, China*
zylzy501@gmail.com

Raul Zamora-Ros · *Pharmacy Faculty, Nutrition and Food Science Department, XaRTA. INSA, University of Barcelona, Barcelona, 08028, Spain*

Qingde Zhou · *School of Dental Medicine, Boston University, 650 Albany Street, X343D, Boston, MA 02118, USA*

Abbreviations

AA	Arachidonic acid
AGI	Astragulus injection
AKT	Protein kinase b
ALA	Alpha linolenic acid
AP-1	Activator protein-1
APC	Antigen-presenting cells
ARE	Adenylate and uridylate-rich element
ASP	*Astragulus* polysaccharide
BMDCs	Bone marrow-derived DCs
BZYQT	Bu Zhong Yi Qi Tang
CD14	CD14 surface receptor
CE	Cinnamon extract
CLA	Conjugated linoleic acid
Con A	Concanavalin A
COX	Cyclooxygenase
CP	Cinnamon polyphenol
CRP	C-reactive protein
CTL	Cytotoxic T lymphocyte
DCs	Dendritic cells
DGLA	Dihomo-g-linoleic acid
DHA	Docosahexaenoic acid
EGF	Epidermal growth factor
EPA	Eicosapentaenoic acid
ErbB2	Leukemia viral oncogene homolog 2 ErbB2
ERK	Extracellular signal regulated protein-kinase
ES-PL	Extract of the seeds of *Plantago asiatica* L.
FA	Fatty acids
FAO	Food and Agricultural Organization
FGF	Fibroblast growth factor
GALT	Gut-associated lymphoid tissue
GL	*Ganoderma lucidum*
GLA	Gamma linolenic acid
GL-M	GL mycelium extract
GL-P	GL mycelium polysaccharides
GL-S	GL spore extracts
GLUT	Glucose transporter
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPCR	G protein-coupled receptor

HETE	Hydroxyeicosatetraenoic acid
HPEP	Hydroperoxyeicosapentaenoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
HSV-1	Herpes simplex virus type-1
i.p.	Intraperitoneal
i.r.	Intrarectal
i.v.	Intravenous
IBD	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule
IFN-g	Interferon-g
IGF	Insulin growth factor
IKK	IkB-kinase
IL	Interleukin
IL-2R	IL-2 receptor
iNOS	Inductible nitric oxide synthase
IkB	Inhibitory subunit of nuclear factor kB
JNK	c-jun N-amino terminal kinase
LA	Linoleic acid
LBP	*L. barbarum* polysaccharide–protein complex
LBP3p	The third fraction of LBP
LC	Liquid chromatography
LCPUFA	Long chain polyunsaturated fatty acids
LDH	Lactate dehydrogenase
LNs	Lymph nodes
5-LOX	Lipooxygenase
LPS	Lipopolysaccharide
LT	Leukotriene
LTB_4	Leukotriene B_4
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinases
MAPKK1	MAPK-kinase-1
MCP	Monocyte chemoattractant protein
MEK	Mitogen activated kinase-kinase
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory proteins
MLN	Mesenteric lymph nodes
MLR	Mixed leukocyte reaction
MMP	Matrix metalloproteinases
MoDCs	Monocyte-derived DCs
mRNA	Messenger RNA
MS	Mass spectrometry
NF-kB	Nuclear factor-kB
NK cells	Natural killer cells
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOD	Nucleotide-binding oligomerization domain receptors

NOD2	Nucleotide-binding oligomerization domain 2
OVA	Ovalbumin
p.o.	Per os (oral route)
PAMP	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cell
PDGF	Platelet-derived growth factor
PG	Prostaglandin
PGE_2	Prostaglandin E_2
PGN	Peptidoglycan
PHA	Phytohemagglutinin
PMA	Phorbol 12-myristate 13-acetate
PP	Peyer's patches
PPAR	Peroxisome proliferator-activated receptor
PRR	Pattern recognition receptors
PS-G	Polysaccharide from GL
PUFA	Polyunsaturated fatty acids
RAR	Retinoic acid receptors
RIG-like helicases	Retinoic acid-inducible gene-like helicases
ROS	Reactive oxygen species
RT-PCR	Real-time polymerase chain reaction
RXR	Retinoid X receptors
SOD	Superoxide dismutase
STAT	Activator of transcription
TCM	Traditional Chinese medicines
TCR	T cell receptor
TF	Transcription factor
TGF	Transforming growth factor
Th	T helper
Th1/Th2	T lymphocytes helper 1/2
TKR	Tyrosine kinase receptors
TLRs	Toll-like receptors
TNBS	Trinitrobenzene sulfonate
TNF	Tumor necrosis factor
TTP	Tristetraprolin
TX	Thromboxane
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
ZFP36	Zinc finger protein 36

Section A
Development of Human Immune Responses

1 Nutrition, The Infant and the Immune System

Ger T. Rijkers, Laetitia Niers, Marianne Stasse-Wolthuis, and Frans M. Rombouts

Key Points

- The human newborn possesses a functional but immature immune system in order to provide defense against a world teeming with microorganisms.
- Breast milk contains a number of biological active compounds which support the infant's immune system.
- These include secretory IgAs, which confer specific protection against enteric pathogens, as well as many other immunological active ingredients.
- A number of these ingredients can and are being used as supplements for infant nutrition formulas.
- The strength of the evidence for immunostimulating effects of selected minerals, vitamins, fatty acids, pre- and probiotics and nucleotides is reviewed.

Key Words: Infant immune system, cordblood, breast milk, pre- and probiotics, nutrition.

1.1 THE INFANT IMMUNE SYSTEM

1.1.1 Development of the Immune System

The development of the immune system starts during embryogenesis when the first hematopoietic cells develop outside the embryo, in the yolk sac. Then, in the 6th week of gestation, the first committed hematopoietic stem cells can be detected in the mesoderm of the fetus, the so-called aorta-gonad-mesonephros (1). Next, these hematopoietic stem cells migrate to the fetal liver and there they initiate erythropoiesis (2). During the 7th week of gestation, progenitor cells seed the developing thymus. Seeding into the bone marrow occurs much later (by week 20) (3, 4). The T lymphocytes develop in the thymus,

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_1

a process which involves rearrangement of the T cell receptors, followed by selection for functionality and negative selection against self-antigens. The development of natural killer (NK) cells as well as various dendritic cell (DC) populations also takes place in the thymus. The B lymphocytes, granulocytes, monocytes, and DC develop in the bone marrow. The development of lymphoid cells and organs is a complex process that requires timely expression of growth factors (cytokines, chemokines), receptors as well as adhesion molecules. Apart from maternal-fetal transfer, the development of the immune system is independent of antigenic stimulation (either bacterial, viral, or allergenic).

Birth marks a fundamental change in the demand put upon the immune system. When a baby is born, it emerges from the relatively sterile environment of the uterus into a world teeming with bacteria. Within the first few days of life, mucosal surfaces of the gastrointestinal as well as respiratory tract become colonized with bacterial communities (5).

As stated above, at birth, the immune system, although being developed, is not yet fully matured. All T and B lymphocytes are in the so-called naïve state, that is, they have not yet encountered antigen, and memory lymphocytes therefore are not yet present. Some studies suggest that especially for allergens, transplacental priming (of mother to child) of fetal T lymphocytes may occur, but this is still controversial (6). Activation of the neonatal T lymphocytes results in a response that is dominated by TH2 cytokine production (IL-4 and IL-5) with relatively little production of the TH1 cytokine g-interferon (7). The reasons for this dysbalance are unclear; it may be that the default differentiation pathway in the absence of antigenic stimuli is biased to TH2. Alternatively, the immature phenotypes of antigen-presenting cells, or differential expression of cell-signaling molecules or transcription factors may also contribute to this bias (8).

Like T lymphocytes, B lymphocytes also are naive and immature at birth, and memory B lymphocytes have not developed yet (9). Still, the neonate is able to mount an antibody response upon primary infection or upon primary vaccination with protein-based vaccines. Neonates, however, are unable to respond to polysaccharide antigens, making them extra vulnerable to infections with polysaccharide encapsulated bacteria such as group B Streptococci and pneumococci. The polysaccharide-specific B lymphocytes are already present, but they fail to express a co-receptor (CD21) which is necessary for the response to these antigens (10, 11).

Postnatally, the gastrointestinal tract as well as the respiratory tract becomes rapidly colonized with microorganisms (5). The spectrum of commensal and pathogenic microorganisms (and the corresponding pathogen-associated molecular patterns, PAMPs) to which the immune system is exposed is immense. The broad repertoire of the adaptive immune system allows a specific response to virtually every triggering antigen. It is now realized, however, that the immune system does not respond to every stimulus, but rather responds to "danger signals" from the environment (12). The emerging mechanism is that PAMPs are recognized by a polymorphic repertoire of receptors of the innate and adaptive immune system (13), and this will shape the direction of the development of the immune system of childhood and adulthood.

1.1.2 Infections Early in Life

During pregnancy, the immune system of the fetus operates in coexistence with the mother's immune system. After birth, the immune system of the newborn must switch to

protection against invading pathogens and develop tolerance to harmless nonself antigens such as food antigens. The competence of the newborn's immune system develops progressively during the first few months of life. Specific features of the newborn's immune system and its development clarifies the susceptibility to different types of infectious diseases. First, T cell mediated immunity in newborns is predominantly naive. Memory T lymphocytes develop gradually in healthy infants during the first years of life. The response of T lymphocytes at birth or in cordblood to specific antigens is largely absent unless intrauterine exposure occurred. This indicates that after birth every encounter with a pathogen can result in a primary infection. The neonatal immune system will be capable to clear pathogens, but neonates and young infants are prone to experience more morbidity from viral infections due to a lower responsiveness of T cell mediated immunity compared to older children. Respiratory syncytial virus, enteroviruses, influenza virus are only a few examples of viruses which may cause severe illness. Immunological memory will be present during the second encounter, and the ensuing improved clearance will result in less morbidity.

A second immunological factor which contributes to the increased susceptibility for infections is the immature antibody production in newborns and young infants. As a result newborns and young infants are more vulnerable to serious bacterial infections. Pathogens acquired from the maternal genital tract such as group B streptococcus and enteric organisms like *Escherichia coli* are major causative agents for bacterial infections in neonates. In young infants, the production of antibodies to polysaccharide antigens especially is underdeveloped. Polysaccharide-encapsulated bacteria such as *Streptococcus pneumoniae* are therefore major causative agents of lower and upper respiratory tract infections (pneumonia and otitis media, respectively) in young infants. Routine immunization with conjugate vaccines for encapsulated bacteria such as *Haemophilus influenzae* type b and the pneumococcal conjugate vaccine (PCV7) have resulted in a decrease of invasive disease in young infants (14). In general, infants are at risk for serious infections due to their developing immune system, and this warrants for vaccination against pathogens which would otherwise cause a high morbidity and mortality.

1.1.3 Allergy

The immune system is tightly controlled by its own regulatory network to prevent inappropriate immune reactions which would result in pathologic conditions. Failure or breakdown of regulatory networks is believed to result in allergic and/or autoimmune diseases. Genetic as well as environmental factors contribute to susceptibility to autoimmune and allergic disease. Autoimmune diseases are relatively rare in children but allergic disease adds considerably to childhood morbidity. The cumulative prevalence during childhood is estimated to be 20–30%. The pathogenesis of allergic diseases is multifactorial. One of the factors is a positive family history, whose contributions have been associated with allergic disease. Furthermore, among other environmental factors, feeding and nutritional composition play an important role in gastrointestinal function, host defense and exposure to food allergens (15). Breastfeeding may protect against the development of allergic disease, although this is still subject a of discussion. Breastfeeding appears to be protective against the development of food allergy. Sensitization to allergens only develops upon exposure. The cow's milk protein b-lactoglobulin, which is a major allergen in cow's milk protein allergy can be detected in breast milk, but in much lower

amounts compared to cow's milk and infant formulas. Besides less exposure to cow's milk protein with breastfeeding, oligosaccharides present in breast milk affect the intestinal bacterial flora by promoting the presence of bifidobacteria and lactobacilli. The reduced presence of bifidobacteria in infants' stool early in life is associated with the development of allergic disease later in life. When breast milk is insufficient or lacking, infant formula is needed. Feeding with hydrolyzed formulas, especially extensively hydrolyzed formulas, significantly reduces cow's milk protein allergy when compared to feeding with conventional cow's milk formulas (16).

1.2 CONTRIBUTION OF BREAST MILK TO HOST DEFENSE OF THE BABY

The immature immune system of the baby is supported by passive acquired immunity transferred from mother to child. Passive acquired immunity is provided by maternal immunoglobulins and by breast milk.

During the last semester of pregnancy there is active transport of maternal IgG across the placenta so that after a full-term pregnancy, the IgG levels in the neonate equal that of the mother (Fig. 1.1). IgM and IgA antibodies are not able to be transported transplacentally and therefore the newborn lacks these immunoglobulin isotypes. The IgG from the mother disappears with a half-life of 21 days so that most of maternal IgG has gone by the age of 3 months. Prolonged support of passive immunity can be provided by breastfeeding (18). On a world-wide scale, it has been estimated that optimal breastfeeding behavior (defined as exclusive breastfeeding for at least 6 months and continuation for the first year) could prevent the death of 1.3 million children annually (19). Breast milk contains 0.4–1.0 g/l secretory IgA. The antibody specificities of this IgA are directed against enteric and respiratory pathogens from the environment of the mother

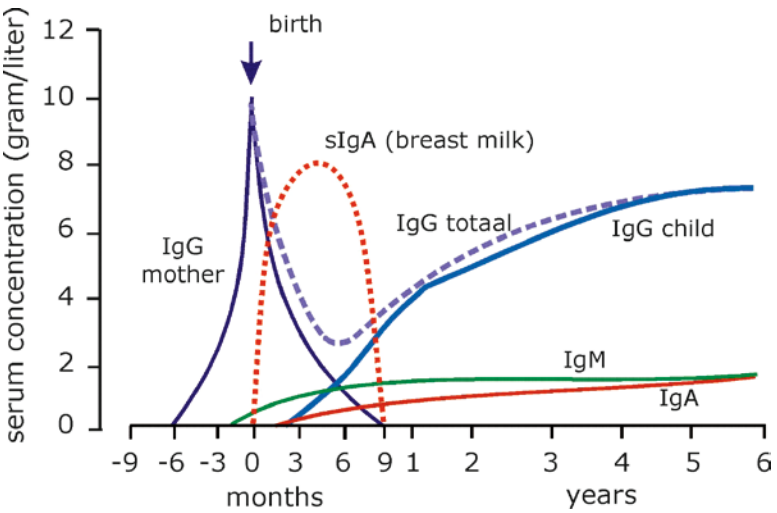


Fig. 1.1. Development of serum immunoglobulins in early life. Note that secretory IgA delivered by breastfeeding remains confined to the intestine and does not contribute to the serum IgA levels. Partly based on (17).

and child. Apart from IgA, breast milk contains many other components which directly or indirectly support the baby's ability to resist infections (Table 1.1). Lactoferrin and lysozyme are proteins with antibacterial activity. Lactoferrin is a ferric iron-binding glycoprotein which inhibits the growth of pathogens by competing with bacteria for

Table 1.1
Immunological active ingredients of human milk (per 100 kcal)

Class	*Compound*	*Concentration (19)*	*Recommended (22) (min–max)*
Immunoglobulins	sIgA	69–153 mg	
Antimicrobial proteins	Lactoferrin	139–264 mg	
	Lysozyme	260–347 mg	
Leukocytes		0.5 × 10^6/ml	
Cytokines, chemokines	IL-1, IL-6, TNF-a g-IFN, IL-12, IL-10 IL-8, CCL5		
Hormones	GM-CSF, EPO Cortisol		
Fatty acids	DHA	9.8–12.5 mg	Optional
	AA	16.7–23.6 mg	Optional
Oligosaccharides	FOS	1.53–1.67 g	Optional
	GOS	0.28–0.42 g	Optional
Minerals	Zn	360–460 mg	500–1,500
	Se	1.1–2.6 mg	1–9
	Fe	86–130 mg	30(60)–130(170)
	Cu	51–60 mg	35–80
	Mn	2–3.5 mg	1–50
	Ca	44–50 mg	50–140
Vitamins	Vitamin A	56–106 mg	66–198
	Vitamin E	0.39–0.54 IU	0.75–7.5
	Vitamin D3	<0.01 mg	1–2.5 (3)
	Vitamin C	4.3–6.3 mg	10–30
	Vitamin B12	0.01 mg	0.10–0.50
Nucleotides	Total	7.38–8.06 mg	–5 (optional)
	AMP	0.42–0.48 mg	–1.5
	CMP	2.19–2.37 mg	–2.5
	GMP	1.35–1.55 mg	–0.5
	IMP	1.23–1.29 mg	–1.0
	UMP	2.19–2.37 mg	–1.75
Various	Sialic acid	41.7–208 mg	
	Gangliosides	1.1–1.4 mg	
	L-carnitine	0.94–1.31 mg	1.2 -

Figures between brackets: follow-on formulas
Addition of probiotics is optional for follow-on formulas (10^6–10^8 CFU/g)

ferric iron (20). The amino-terminal peptide of lactoferrin, lactoferricin, also exerts chelation-independent bactericidal activity. Because lactoferrin is resistant to digestion by proteolytic enzymes, most of the ingested lactoferrin survives throughout the gastrointestinal tract of infants. Lysozyme, an antimicrobial peptide which acts by cleaving peptidoglycans in the cell walls of bacteria, is present in a 300-fold higher concentration in breast milk as in cow's milk.

Breast milk contains at least 80 different oligosaccharides. Many of these oligosaccharides function as receptor analogs that inhibit the binding of bacterial or viral pathogens, or toxins, to gut epithelial cells. The structure of the oligosaccharide determines the specificity of binding to adherence receptors of bacteria or bacterial toxins. GM1 gangliosides are receptor analogs for toxins produced by *Vibrio cholerae* and *E. coli*, whereas lacto-*N*-fucopentaose II (the oligosaccharide which forms the Lewis X blood-group antigen) prevents HIV1 transfer. Furthermore, certain glycosylated proteins, such as the mucin MUC1, interfere with bacterial or viral adherence. Lactadhedrin, a component of milk-fat globules, protects against rotavirus infections (21). Free fatty acids and monoglycerides, generated by enzymatic digestion of triglycerides, can disrupt enveloped viruses (21).

An important characteristic of breast milk oligosaccharides is that they promote the outgrowth of commensal *Bifidobacterium sp.* and lactobacilli in the intestinal tract. These bacteria, which are termed probiotic bacteria, are generally believed to have a health-promoting effect probably because, among other things, they produce organic acids that retard the growth of enteric pathogens.

Human milk contains leukocytes, including neutrophils (40–65% of total leukocytes), monocytes/macrophages (35–55%) and, mainly activated, $CD8^+$ T lymphocytes (5–10%). It is unknown whether any of these cells can transfer functional cellular immunity from mother to child during breastfeeding.

Human milk also contains a number of cytokines and chemokines. These include the pro-inflammatory cytokines interleukin-1 (IL-1), IL-6, and tumor-necrosis factor-a (TNF-a), the TH1 cytokines g-interferon and IL-12, the TH2 cytokines IL-4, IL-5 and IL-13, the regulatory cytokines IL-10 and transforming growth factor-b (TGF-b) and the chemokines IL-8 and CCL5. Granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin (EPO) and cortisol are also detectable in breast milk.

Apart from the components listed above with a direct effect on the immune system, breast milk contains a number of other ingredients which indirectly support the infant's immune system, including vitamins, minerals, and nucleotides (Table 1.1).

Breast milk has been termed an irreplaceable immunological resource because it supports both passive and active immunity during the vulnerable first months and years of life (21). A number of components of breast milk indeed are irreplaceable, notably IgA (23, 24). These antibodies are formed by the mother in response to environmental exposure to a range of potential pathogens. Because the mother and child share the same environment, they are exposed to the same range of micro-organisms. The spectrum of IgA antibody specificities in the breast milk of the mother thus matches the requirements of the baby. While IgA therefore is irreplaceable, other components can be used as additive in infant nutrition formulas, as will be detailed below.

1.3 NUTRITION AND DEVELOPMENT OF THE IMMUNE SYSTEM

Because the immune system is immature at birth, malnutrition during childhood might have long-term effects on health status. Lack of adequate macronutrients or undernutrition impairs the development and differentiation of a normal immune system. The combination of chronic undernutrition and infection further weakens the immune response. Micronutrient deficiencies will affect the adaptive antibody and cellular immune response, as well as the innate immune response (25). Concurrent deficiency of micronutrients may attenuate or aggravate effects on the different components of the immune system. Therefore, when infants are deficient for one or more micronutrients, it is likely that their immune function is also impaired.

Unfortunately there is no single test or biomarker which can adequately define the overall immune status of a given individual (26). However, the measurement of several parameters of the separate components of the immune system in combination can be used to assess functional capacity: (a) measuring specific cell functions *ex vivo*, (b) measuring *in vivo* responses to challenge, for example change in the antibody levels in the peripheral blood or response to antigens, and (c) determining the incidence and severity of infection in target populations during naturally occurring episodes or in response to attenuated pathogens (26, 27).

The focus of this chapter is on replaceable ingredients of human milk and their (possible) relationships with immune function.

1.4 STIMULATION OF THE IMMUNE SYSTEM BY NUTRITIONAL INGREDIENTS

Table 1.1 summarizes data from studies that have been performed on immune stimulation through selected ingredients. It must be realized that published RCTs are usually short-termed and not many longer term RCTs are available. When data from RCTs in babies and infants are lacking, data from human adult trials and *in vitro* and animal studies have been evaluated because they can give some clues about the activity of different ingredients.

The role of individual nutritional factors on function and development of the immune system is difficult to quantify because: (a) different ingredients may affect different components of the immune system, (b) numerous interactions between separate nutrition factors do exist, (c) dose–effect relationships are difficult to establish in young children because of methodological aspects (significant non-nutritional confounding variables) and also because of ethical issues in designing intervention trials in infants, and (d) the relevance of subclinical deficiency of a given nutrient is often difficult to judge (subclinical inflammation or disease may result in lower plasma micronutrient concentration that may be misinterpreted as deficiency) (28). Low concentrations of other nutrients such as ascorbate and iron may not necessarily impair immune function. The same holds true for the relevance of the effects of short-term supplementation with supraoptimal doses as compared to the amounts usually contained within infant formulas. For many micronutrients, excessive intake is associated with impaired immune function (29).

1.4.1 Minerals

A number of minerals, especially zinc, and also selenium, copper, and iron are essential for normal immune function. It must be noted that some minerals can compete for absorption in the body and an abundance of a given micronutrient might lead to a deficiency in another. Therefore, these potential interactions between minerals should be taken into account. While suppletion with minerals may have a positive effect on the development and function of the immune system it should be realized that many microorganisms also require iron and other trace elements for their survival and replication in the host.

Zn deficiency clearly is associated with a state of immunodeficiency: lymphopenia, thymic atrophy, and altered T-lymphocyte subsets and cytokine response profiles (25, 30–32). The clinical symptoms of Zn deficiency include an increased susceptibility to infections, skin lesions, and diarrhea (25, 30). Zn is an essential cofactor for many enzymes, including thymic hormone, and Zn depletion decreases the functional capacity of a variety of cells of the immune system. Zn supplementation reversed the impaired immune functions, including cytokine production and reduced the incidence of diarrhea and pneumonia, both in adults and children (33, 34). Zn should not be overdosed, because this can have negative effects on the immune status (32). Zn deficiency often is a component of protein calorie malnutrition (PCM) and under those circumstances the suppletion of Zn alone is insufficient to restore the immune status.

Se deficiency, which like Zn can be associated with PCM and also with vitamin E deficiency, impairs antioxidant defense systems and leads to rapid progression of viral and other infections as well as to cardiomyopathy (25, 35). Model studies show that Se influences many components of the immune system (35), because of its critical role for the function of selenoproteins. It has been demonstrated that the fortification of infant formulas with Se improves the Se status of infants (36). Attention must be paid to the risk of subclinical Se deficiency especially in premature infants. Furthermore, the supplementation of Se should be carefully monitored because of the small range between inadequate and excessive Se intake (37).

Iron has diverse and partly opposing roles in host defense against infectious diseases. Within the gut lumen, bacterial growth depends on the bioavailability of Fe, and lactoferrin has potent antibacterial effects because of its Fe-sequestering properties. On the other hand, Fe deficiency causes T lymphocyte dysfunction, manifested in impaired delayed type hypersensitivity reactions as well as a number of other immune defects (decreased IgG levels and phagocytic activity) (25, 38, 39). Different components of the immune system are affected in different ways depending on the degree of Fe deficiency and concomitant infections. Clinically, Fe-deficiency anemia is frequently associated with infections. Oral Fe supplementation can have different effects in malaria regions as compared with nonmalaria regions; morbidity/mortality may be worsened by Fe supplementation during infection with Fe-dependent organisms. Excess Fe intake should be avoided because it can induce free-radical–mediated damage and may have negative effects on Cu and Zn status.

Cu (and Zn) deficiency are fairly common in children with hypoproteinemia and anemia. Cu deficiency may cause lymphopenia and a decreased IL-2 response. the clinical signs of Cu deficiency are anemia, neutropenia, depressed growth, and abnormal bone development (25, 40). Cu is an essential cofactor of a number of antioxidant enzymes. Cu intake should be carefully controlled because excess Cu can

induce free-radical-mediated damage. Supraoptimal Zn and Fe intake, however, can cause a lower Cu status (40, 41).

Mn is needed for normal immune function because mitochondria and a number of cellular enzymes are Mn dependent (42). The direct clinical consequence of Mn deficiency, however, is not well known, and no recent RCTs have been published. It should be noted that excessive Mn may induce neurotoxicity in neonates receiving parenteral nutrition.

Ca, while being an obvious essential nutrient, probably does not have a specific role in the function of the immune system. In adults, Ca supplementation reduced the severity of enterotoxigenic *E. coli* -induced diarrhea (43). It has been speculated that low Ca intake may impair host resistance to food-borne intestinal infections. RCTs in infants have not been published.

1.4.2 Vitamins

Largely supportive evidence exists for an important role for a number of vitamins in the development and function of the immune system. Lack of adequate intake of antioxidant vitamins can lead to clinically significant immune deficiency and infections in children (25). It thus has been demonstrated that vitamin A deficiency leads to the impairment of the activity of TH2 lymphocytes, phagocytes, and NK cells. Clinical signs of vitamin A deficiency include night blindness, mucosal damage, and dry skin (latter two conditions contributing to the loss of barrier function for entry of pathogens) and hyperkeratosis (22, 25). Vitamin A supplementation has been shown to reduce the risk of mortality and morbidity from some forms of diarrhea, measles, malaria, and HIV (44, 45). In an RCT in infants 5–15 months old, it was demonstrated that the regulation of the mucosal immune response depends on the type of enteric pathogen. Furthermore, the effect of vitamin supplementation in infected children was significantly different from uninfected children (46). This may explain the variable and inconsistent effects of vitamin supplementation on the incidence of diarrhoeal disease. Overdosing of vitamin A can, among other things, lead to loss of appetite, dermal dryness, and loss of hair (22).

In experimental animals, vitamin E deficiency is associated with specific defects in the immune function and increased susceptibility to infections. In humans too, severe vitamin E deficiency is associated with impaired T lymphocyte function (47). The correction of the deficient state may reverse these abnormalities (47). Clinical signs of milder vitamin E deficiency in man include atopic diseases as well as neurological symptoms (25).

Increase of intake of vitamin E can have an immune-stimulating effect (48–50).

A poor vitamin D status has been reported to be associated with chronic mycobacterial disease, and vitamin D may augment the function of regulatory T lymphocytes (51).

Vitamin D appears to be a selective regulator of the immune system and the outcome of vitamin D treatment or deficiency of vitamin D (receptor) depends on the nature of the immune response (e.g., infectious disease, asthma, or autoimmune disease) (52, 53). An additional factor that determines the effect of vitamin D status on immune function is dietary calcium.

Vitamin C is a good example of a nutrient wherein experimental animal deficiencies have shown a consistently increased susceptibility to infection, yet the evidence in human studies of disadvantage from deficiency or benefit from supplementation is at best contradictory.

Overdosing of vitamins appears to have no negative effects on the function of the immune system, except for vitamin E, which at high doses may depress phagocytosis and intracellular killing of bacteria (49). The reports on the relation between excess vitamin D supplementation and the increased risk of food allergy and asthma in later life have methodological flaws: confounding factors, relative high doses used, and an extreme long period between supplementation and outcome assessment (54, 55).

1.4.3 Long-Chain Polyunsaturated Fatty Acids

Long-chain poly-unsaturated fatty acids (LC-PUFA) improve growth, visual acuity, and the neurodevelopmental performance of infants. Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are the main LC-PUFA in breast milk. LC-PUFA of the n-3 and n-6 series are metabolic competitors with differential effects, for example, on eicosanoid metabolism, membrane physiology, as well as on immune function. Eicosapentaenoic acid (EPA, 20:5n-3) is found in only minor concentrations in breast milk and infant tissues and is a direct metabolic competitor of AA.

It has been demonstrated in *in vitro* studies and in experimental animals that DHA and AA can alter immune function in several ways (56). Thus, fatty acids can affect T lymphocyte functions by increasing or decreasing the production of eicosanoids from AA. n-3 LC-PUFA decrease the production of these mediators (57). Furthermore, EPA is a substrate for cyclooxygenase and lipoxygenase enzymes leading to eicosanoids of altered structure and reduced biological potency. A novel family of EPA derived eicosanoid-like mediators, termed E- and D- resolvins, have been shown in cell culture and animal models to be anti-inflammatory and inflammation resolving, respectively (58). Fatty acids can also alter gene expression profiles through the modification of transcription factor activity as well as by incorporation of n-3LC-PUFA into membrane phospholipids and the subsequent modulation of membrane structure and function. N-3 LC-PUFAs are potentially potent anti-inflammatory agents and they may be of therapeutic use in acute and chronic inflammatory diseases (57). From a different perspective, it has been hypothesized that increased n-6 PUFA and decreased n-3 PUFA dietary intakes have contributed to the recent increases in asthma and other allergic diseases (59). The associations with n-6 and n-3 PUFA, however, appear to be very complex and might differ between asthma and atopic dermatitis. In atopic dermatitis, a mild enzyme deficiency has been proposed, resulting in altered PUFA metabolism that compromises epithelial structure and function (60).

There are only few data on the effects of PUFA supplementation in infants. In preterm babies, the addition of LC-PUFA to infant formula resulted in lymphocyte populations, phospholipid composition, and cytokine production that are more consistent with that in breast milk–fed infants (61, 62).

Although large amounts of LC-PUFA may increase lipid peroxidation and oxidative stress, there is no evidence that concentrations within the range found in human milk are harmful. For instance, a recent RCT in preterm infants demonstrated that plasma LC-PUFA levels similar to those of breast-fed babies can be achieved with LC-PUFA supplemented formula without evidence of adverse effects (63).

In 1998, the Life Science Research Office (LSRO) expert committee did not set minimum and maximum values for the addition of LC-PUFA to infant formulas, but later on

European expert reports supported the optional addition of DHA and AA (22). At this moment, there is no sufficient documentation of the benefits and safety of the addition of DHA to infant formula at levels above 0.5% of total fat content, or of DHA without concomitant addition of AA. If LC-PUFA is added, proper balance between n-6/n-3 should be taken care of. Note that the requirements established by the Scientific Committee on Food are slightly different from the European Society of Pediatric Gastroenterology, Hepatology and Nutrition, ESPGHAN (22, 64).

Clearly, there is a need for larger, properly designed, and controlled studies with longer follow-up with respect to functional outcomes in relation to intake levels of LC-PUFA in healthy term infants (64). Also more studies are needed for the potential prophylactic effect of n-3 fatty acid supplementation on the development of asthma (60, 65).

1.4.4 Prebiotics

As already indicated above, the intestinal microbiota plays an important role in postnatal development of the immune system. The lower incidence of (gastrointestinal) infections found in breast-fed infants may be related in part to the early pattern of microbial colonization. The colonizing bifidobacteria and lactobacilli may inhibit the growth of pathogenic microorganisms through the production of lactic, acetic, and other organic acids, with a consequent decrease of the intraluminal pH that inhibits the growth of some bacterial pathogens. In contrast, formula feeding tends to favor microbiota associated with a near neutral pH of the feces. Moreover, bifidobacteria and lactobacilli compete with potentially pathogenic bacteria for nutrients and epithelial adhesion sites. Accumulating evidence also indicates that the gut microbiota modulates mucosal physiology, barrier function, and systemic immunologic and inflammatory responses (66).

Two different approaches can be taken to modify the development and balance of intestinal microbiota: the first one is the addition of live lactic acid bacteria and bifidobacteria (probiotics; see below) and the second one is the addition of oligosaccharides that survive passage through the small intestine and are used by colonic bacteria (prebiotics).

Breast milk contains many different oligosaccharides that may have prebiotic activity and thus have an effect on the composition of the intestinal microbiota. Because of the variety, variability, complexity, and polymorphism of the structure of breast milk oligosccharides, it is currently not feasible to add a similar oligosaccharide composition as contained in human milk to infant formulas. Alternatively, the addition of a more simple mixture of galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) to infant formulas and to follow-on formulas has been proposed (67, 68).

Accumulating evidence indicates that the addition of oligosaccharides to infant formula may induce a composition and metabolic activity of the intestinal microbiota which closely resembles that of breast-fed infants (69). Most studies thus far have been performed with a combination of 90% short chain GOS and 10% long chain FOS. While the bifidogenic effect of prebiotics has convincingly been demonstrated, limited data exist on the direct effects on the infants immune system (70–72). There is evidence that prebiotics may improve the antibody response to vaccination and may reduce the incidence of atopic dermatitis (70, 73, 74). Expert committee reports conclude that at present there is insufficient conclusive evidence that a bifidobacteria-dominated microbiota is related to health and well-being and offers protection against enteric infections

(64, 66). On the other hand, the Scientific Committee on Food had no objections against the addition of a combination of 90/10% GOS/FOS. An upper level of supplementation has been proposed (Table 1.1) based on indications from animal studies that above a certain threshold concentration, oligosaccharides may have a negative effect on water balance (64). Clearly, additional research is needed on optimal composition and the dosage of (combinations of) different oligosaccharides.

1.4.5 Probiotics

Probiotics are live, microbial food ingredients that, when administered in sufficient amounts, are beneficial to health. The bacterial strains with these properties most frequently belong to the genera *Bifidobacterium* and *Lactobacillus*. Probiotics can have a health-promoting effect by interaction with gut microbiota, fortification of gut barrier function, and modulation of the immune system. The net outcome of these combined activities is an increase of host defense against infections. In this respect, several studies have shown that the addition of probiotics to infant formulas has beneficial effects, especially in reducing the severity of acute diarrhea (75). Probiotics also have been demonstrated to be effective in the prevention of antibiotic-associated diarrhea, to reduce the incidence and severity of necrotizing enterocolitis and to reduce recurrent *Clostridium difficile* infections (76–78). The interaction of probiotics with cells of the immune system, in particular, dendritic cells results in improved function of regulatory T cells (Fig. 1.2) (79, 80). Probiotics therefore may be effective in the prevention of atopic dermatitis, asthma and other allergic and immune-mediated diseases. Clinical studies suggest a beneficial effect of selected probiotics in the primary prevention of atopic diseases (81, 82). So far, no effects have been shown in the management or prevention of IgE-mediated allergic disease (83). If the efficacy of probiotics in the treatment of allergic symptoms can be confirmed in subsequent studies, it is reasonable to expect that this may be more obvious in or even limited to infancy and early childhood, that is, before the immune responses to allergens and immune regulatory

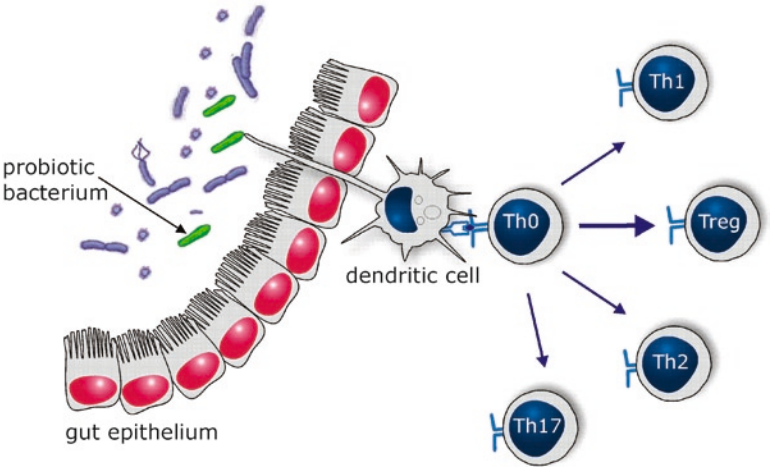


Fig. 1.2. Interaction between probiotics and the mucosal immune system in the gut.

networks have been fully developed and at a time when the complex ecosystem of gut microbiota is not yet established (window of opportunity) (84).

It should be noted that in most studies different (combinations of) strains and species of probiotic bacteria have been used. "Not all probiotics are created equal" and therefore different strains may have different effects and more research is needed into mechanisms of action of individual probiotic strains (84). The administration of probiotics to (even preterm) infants appears to be safe and no serious adverse effects, including no cases of pathogenic infections caused by a probiotic organism, have been reported (85–89). Yet, probiotics are not indicated for immunocompromised neonates (malnourished, ill), and their long-term effects on the development and function of the immune system have not been studied in detail (78).

1.4.6 Nucleotides

In breast milk, nucleotides are present as nucleic acids, nucleosides, nucleotides, and related metabolic products. An exogenous supply of nucleotides may be important during infancy, when nucleotides requirements are increased to provide for nucleic acid synthesis. This may be especially important for infants born prematurely, since preterm birth is associated with the limitations of many metabolic functions and breastfeeding is not always possible. In addition to serving as nucleic acid precursors, nucleotides play roles as intercellular and intracellular biological mediators. Infant formulas supplemented with nucleotides are currently being marketed. Animals fed nucleotides-supplemented versus non-supplemented diets show enhanced indices of humoral and cellular immunity, as well as enhanced survival rates following infection with pathogens (90).

A number of randomized studies in pre-term as well as healthy full-term babies show an improved antibody response after vaccination and enhanced lymphocyte maturation by the addition of free nucleotides to infant formulas (91–93). However, the results of recent large RCTs are not always consistent. For instance, in a large RCT supplementation of cow's milk-based formula at a lower dosage level of nucleotides resulted in only a modest improvement in certain antibody responses in healthy-term infants, with no effect on other markers of immune status and growth (94). The authors speculate that the most of the benefits of nucleotides may be obtained by high-risk infants such as those born prematurely and those from socially disadvantaged backgrounds (94). Some studies in infants indicate that the addition of nucleotides may protect against diarrhoeal disease (90, 94, 95). However, there are few data from well-controlled studies to demonstrate that nucleotides may reduce the risk of infections.

There are no studies available that evaluate a dose–response relationship between the concentrations of nucleotides in infant formula and relevant outcomes in infants. In different regions of the world, the recommended maximum doses of nucleotides vary (64, 65, 96). A recent RCT suggested a negative effect of nucleotides supplementation on the incidence of upper respiratory tract infections throughout the 1st year of life (95).

Whether the observed immunomodulating effects may be translated into clinical benefits in well-nourished infants requires further study. Also, more research is needed into the relative contribution of individual nucleotides and into optimum dosage levels and working mechanisms.

1.4.7 Miscellaneous Ingredients

Apart from the nutrition factors discussed above, there is circumstantial evidence for the immunostimulating effects of several other food ingredients, such as vitamin B12 (49).

Furthermore, breast milk contains a number of proteins with antibacterial activity, which potentially could be added to infant formula. It is technically feasible to add bovine lactoferrin or transgenic human transferrin, but bovine lactoferrin does not bind consistently to human lactoferrin receptors and does not increase Fe absorption. Bovine lactoferrin has, however, been shown to reduce late-onset sepsis in very low-birth-weight infants (97). Efficacy and safety studies of human lactoferrin are currently ongoing.

Gangliosides are glycosphingolipids that contain sialic acid (N-acetylneuraminic acid) as part of their carbohydrate moiety. GM, a ganglioside present in human milk, binds to *E. coli* and *Vibrio cholerae* toxins and thus may contribute to infant protection against infection by those enteropathogens (98, 99).

Breast milk is a rich source of sialic acid-containing oligosaccharides. Animal studies documented favorable effects of supplemental sialic acid on learning, even in well-nourished animals. In infants who died of sudden infant death syndrome, higher brain ganglioside and glycoprotein sialic acid concentrations were found in infants fed breast milk, suggesting increased synaptogenesis and differences in neurodevelopment. (100). The same research group reported that the saliva of preterm breast-fed infants contains twice the level of sialic acid as that in formula-fed infants. The higher sialic acid level may suggest greater viscosity and enhanced protection of the mucosal surfaces in breast-fed infants (100, 101). In the absence of sufficient data, no recommendations can be made on the addition of sialic acid, which, unless supplemented, is lower in infant formula than in human milk (64).

L-carnitine is considered an indispensable nutrient for newborn infants because of a temporarily compromised synthesizing capacity. Its function is the transport across membranes of carboxylic acids that have been activated to the co-enzyme A level, thereby delivering substrates for oxidation and removing toxic compounds. Infants receiving unsupplemented soy showed lower serum levels of carnitine, higher levels of free fatty acids, and an increased excretion of medium-chain dicarboxylic acids. The minimal dietary carnitine requirement of a newborn infant has been estimated to be 1.7 mg/kg/day due to the almost absent endogenous synthesis. Because cow's milk is rich in carnitine compared to human milk, carnitine addition to cow's milk-based formula is not necessary. Supply from appropriate complementary food and from endogeneous synthesis should be sufficient in older infants (64).

ESPGHAN experts set a minimum L-carnitine content of 1.2 mg/100 kcal. In the absence of indications of any untoward effects of higher L-carnitine intakes in infants, no maximum level is needed to be set (63).

1.5 CONTRIBUTION OF INFANT FORMULA TO THE DEVELOPMENT AND FUNCTION OF THE IMMUNE SYSTEM

1.5.1 Compositional Requirements of Infant Formulas

Different scientific and regulatory bodies have established compositional requirements of infant formulas (63, 64, 96). The first guiding principle is that human breast milk is the gold standard. However, the levels of the various (immunomodulatory)

components in human milk are not easily to be translated into composition guidelines for infant formula, because of possible differences in bioavailability and the fact that substances other than components found in human milk may need to be used to achieve the desired effects in infants. The second principle is that all infant formulas must be safe and nutritionally adequate, meeting the normal nutritional requirements of babies. The establishment of minimum and maximum values also must take into account the differences in bioavailability and losses during processing and shelf-life. Thirdly, maximum nutrient values are based on available scientific data on infants' requirements and the absence of adverse effects. The immature organ system should not be charged with ingredients without reasonable evidence of efficacy. Infant formulas should therefore contain components only in such amounts that serve a nutritional purpose, provide another benefit, or are necessary for technological reasons.

1.5.2 Assessment Composition of Infant Formulas

In order to evaluate how the accumulating knowledge on immunomodulation by nutrition ingredients has been translated into the incorporation of these ingredients in infant formula currently available, we have assessed the composition of five infant formula products available on the Asian market. This specific geographic area and market was chosen because children born in this region experience a high infection pressure due to climatic and social–economic circumstances. In Asia, there is a high awareness among parents for protecting their children against infectious diseases. Because of the awareness and concern of parents for the health status of their baby, a broad spectrum of infant formulas supplemented with ingredients which may support the infant's immune system is available on the market.

The compositional data were evaluated, taking into consideration the estimated strength of evidence for immunostimulating effects of separate nutrition factors, and in comparison with the requirements for infant formulas (Fig. 1.3). Note that ingredients which from a nutritional viewpoint may be important for growth and development, but have no impact on the immune system, are not listed in the figure.

We used nutrient requirement levels established by the most recent expert consultation by ESPGHAN (63) except for specific values for separate nucleotides and oligosaccharides that were taken from the earlier report of the Scientific Committee on Food (64); see Fig. 1.3. Specific nutritional requirements for infant formula for 1–3 years old toddlers have not been established. We therefore extrapolated figures for nutrient requirement of formula products for this age group from the Scientific Committee on Food data on follow-on formulas, albeit follow-on formulas actually have been defined for 6–12 months old babies (64), and realizing that the daily doses of formula products consumed by this age group – next to increasing amounts of complementary feeding – are highly variable. This approach allowed to at least obtain a gross indication of the contribution of these formula products to the immune system in toddlers.

The assessment shows (Fig. 1.3) that all leading brands have added fatty acids, vitamins, and minerals and four out of five also nucleotides. Prebiotics and probiotics are not yet widely used and in most cases these ingredients are not added to 0–6 months formula products.

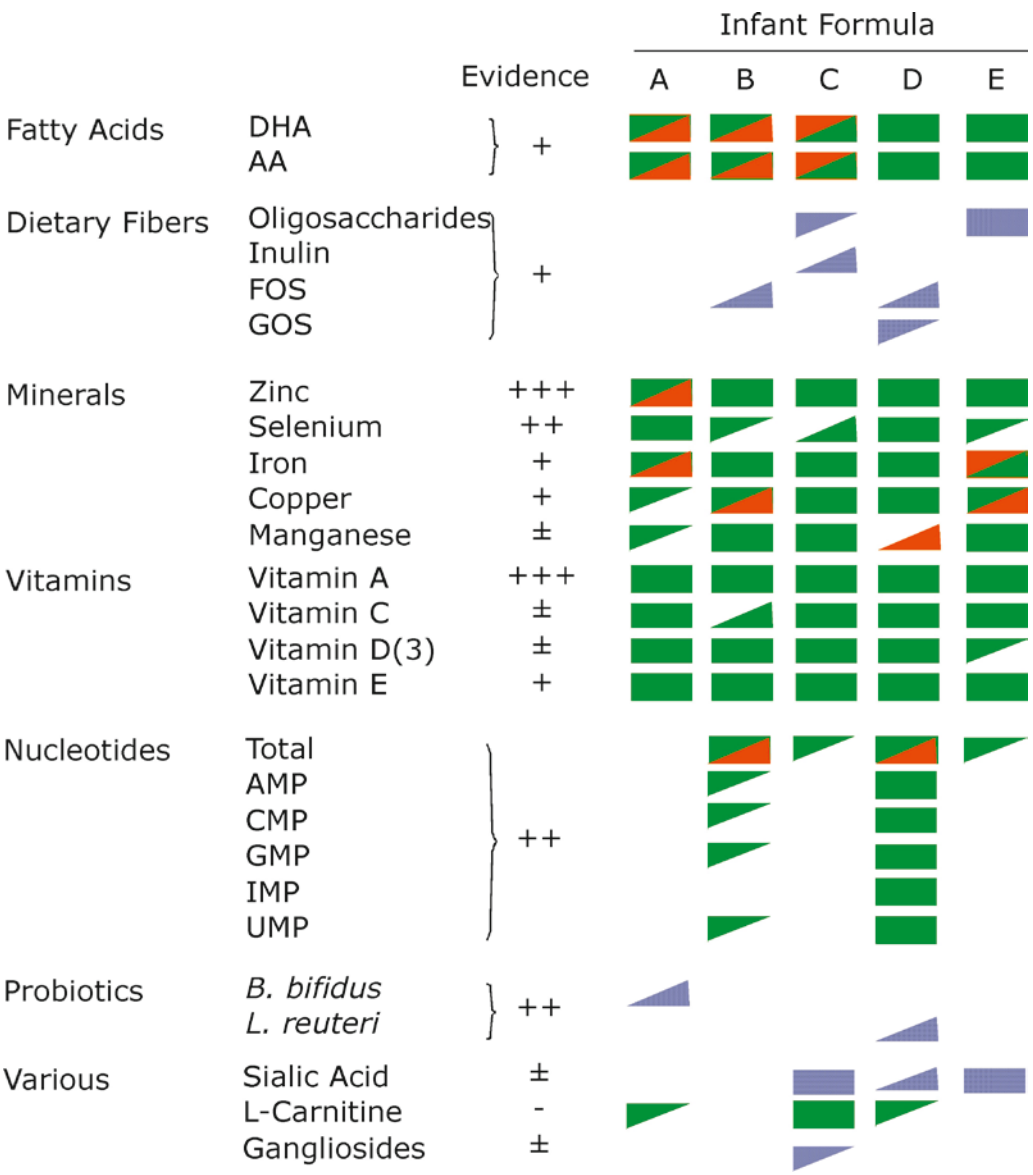


Fig. 1.3. Comparison of immunomodulating ingredients in infant formula. Composition in comparison with requirements, taking into account the strength of evidence for immune stimulating effects. *Green rectangles* () indicate that the compound is present within the recommended range (see Table 1.1); *Orange* (): concentration above maximal recommended level or ratio DHA/AA outside the range. *Blue symbols* () indicate the presence of compounds for which no recommendations exist. Open spaces indicate absence of the particular compound (i.e., not listed on the label) or a concentration below the minimal recommended concentration. *Upper half rectangles* (): 0–6 months formula, *lower half rectangles* (): 1–3 years formula. Columns A–E represent infant formulas from different brands. Strength of evidence graded as ±: Circumstantial evidence: data from *in vitro*/animal studies only or data from a single study; +: Some supportive evidence, including data from a small number of human trials; ++: Fairly supportive evidence: data from different type of studies, for example, in adult subjects or pre-term infants; +++: Largely supportive evidence: consistent results from different type of studies, including RCTs in (malnourished) infants.

1.6 CONCLUSIONS AND PERSPECTIVES

During the first 6 months of life, the infant's immune system develops gradually. Every primary infection the baby experiences, induces a response which leads to the elimination of the invading pathogen and to the generation of specific memory T and B lymphocytes which protect against the recurrence of infection with the same pathogen. As a general rule, the number of infectious episodes decreases with age. Especially during the first 6 months, but also thereafter, it remains important to sustain the infant's immune system in order to protect against infections. In order to be able to grow up and develop in a world teeming with micro-organisms, the infant depends on an adequate function of the immune system. Breastfeeding and infant formula supplemented with specific ingredients as indicated in this chapter, support the infant's immune system.

Acknowledgements Parts of this chapter have been published in another form (102).

REFERENCES

1. Le Douarin NM (1988) The claude bernard lecture, 1987. Embryonic chimeras: a tool for studying the development of the nervous and immune systems. Proc R Soc Lond B Biol Sci 235:1–17
2. Marshall CJ, Thrasher AJ (2001) The embryonic origins of human haematopoiesis. Br J Haematol 112:838–850
3. Bodey B, Bodey B Jr, Siegel SE, Kaiser HE (1998) Intrathymic non-lymphatic hematopoiesis during mammalian ontogenesis. In Vivo 12:599–618
4. Plum J, De Smedt M, Verhasselt B, Kerre T, Vanhecke D, Vandekerckhove B, Leclercq G (2000) Human T lymphopoiesis. In vitro and in vivo study models. Ann N Y Acad Sci 917:724–731
5. Favier CF, Vaughan EE, de Vos WM, Akkermans ADL (2002) Molecular monitoring of succession of bacterial communities in human neonates. Appl Environ Microbiol 68:219–216
6. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, Holt PG (1998) Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol 160:4730–4737
7. Liew FY (2002) T(H)1 and T(H)2 cells: a historical perspective. Nat Rev Immunol 2002(2):55–60
8. Adkins B (2005) Neonatal T cell function. J Pediatr Gastroenterol Nutr 40(Suppl 1):S5–S7
9. Agematsu K, Nagumo H, Yang FC, Nakazawa T, Fukushima K, Ito S, Sugita K, Mori T, Kobata T, Morimoto C, Komiyama A (1997) B cell subpopulations separated by CD27 and crucial collaboration of CD27+ B cells and helper T cells in immunoglobulin production. Eur J Immunol 27:2073–2079
10. Rijkers GT, Dollekamp I, Zegers BJ (1988) 8-Mercaptoguanosine overcomes unresponsiveness of human neonatal B cells to polysaccharide antigens. J Immunol 141:2313–2316
11. Peset LM, Harms G, Hardonk MJ, Timens W (1996) Human immune response to pneumococcal polysaccharides, complement- mediated localization preferentially on CD21-positive splenic marginal zone B cells and follicular dendritic cells. J Allergy Clin Immunol 97:1015–1024
12. Matzinger P (2002) The danger model: a renewed sense of self. Science 296:301–305
13. Medzhitov R (2001) Toll-like receptors and innate immunity. Nat Rev Immunol 1:135–145
14. Straetemans M, Sanders EA, Veenhoven RH, Schilder AG, Damoiseaux RA, Zielhuis GA (2004) Pneumococcal vaccines for preventing otitis media. Cochrane Database Syst Rev 1:CD001480
15. Upham JW, Holt PG (2005) Environment and development of atopy. Curr Opin Allergy Clin Immunol 5:167–172
16. Friedman NJ, Zeiger RS (2005) The role of breast-feeding in the development of allergies and asthma. J Allergy Clin Immunol 115:1238–1248
17. Zinkernagel RM (2001) Maternal antibodies, childhood infections, and autoimmune diseases. N Engl J Med 345:1331–1335

18. Chirico G, Marzollo R, Cortinovis S, Fonte C, Gasparoni A (2008) Anti-infective properties of human milk. J Nutr 138(9):1801S–1806S
19. Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS (2003) Bellagio child survival study group. How many child deaths can we prevent this year? Lancet 362:65–71
20. Jenssen H, Hancock RE (2009) Antimicrobial properties of lactoferrin. Biochimie 91:19–29
21. Labbok MH, Clark D, Goldman AS (2004) Breastfeeding: maintaining an irreplaceable immunological resource. Nat Rev Immunol 4:565–572
22. Koletzko B, Baker S, Cleghorn G et al (2005) Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. J Pediatr Gastroenterol Nutr 41:584–599
23. Michaelsen KF (2008) Breastfeeding and the infant's immune system. Gesundheitswesen 70(Suppl 1):S20–S21
24. Hosea Blewett HJ, Cicalo MC, Holland CD, Field CJ (2008) The immunological components of human milk. Adv Food Nutr Res 54:45–80
25. Cunningham-Rundles S, McNeeley DF, Moon A (2005) Mechanisms of nutrient modulation of the immune response. J Allergy Clin Immunol 115:1119–1128
26. Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartin S, Sanderson IR, Van Loo J, Vas Dias FW, Watzl B (2005) Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr 94:452–481
27. Cummings JH, Antoine JM, Azpiroz F (2004) PASSCLAIM–gut health and immunity. Eur J Nutr 43(Suppl 2):II118–II173
28. Klein CJ, Heird WC (2005). Summary and comparison of recommendations for nutrient contents of low-birth-weight infant formulas. Bethesda, Maryland: Life Sciences Research Office, 2005. Available at: www.lsro.org/articles/lowbirthweight_rpt.pdf
29. Thurnham DI (1997) Micronutrients and immune function: some recent developments. J Clin Pathol 50:887–891
30. Fischer Walker C, Black RE (2004) Zinc and the risk for infectious disease. Annu Rev Nutr 24:255–275
31. Shankar AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68(2 Suppl):447S–463S
32. Ibs KH, Rink L, Zinc-altered immune function (2003) J Nutr 133(5 Suppl 1):1452S–1456S
33. Wieringa FT, Dijkhuizen MA, West CE, van der Ven-Jongekrijg J, van der Meer JW, Muhilal (2004). Reduced production of immunoregulatory cytokines in vitamin A- and zinc-deficient Indonesian infants. Eur J Clin Nutr 58:1498–1504
34. Rahman MJ, Sarker P, Roy SK, Ahmad SM, Chisti J, Azim T, Mathan M, Sack D, Andersson J, Raqib R (2005) Effects of zinc supplementation as adjunct therapy on the systemic immune responses in shigellosis. Am J Clin Nutr 81:495–502
35. Arthur JR, McKenzie RC, Beckett GJ (2003) Selenium in the immune system. J Nutr 133(5 Suppl 1):1457S–1459S
36. Van Dael P, Davidsson L, Ziegler EE, Fay LB, Barclay D (2002) Comparison of selenite and selenate apparent absorption and retention in infants using stable isotope methodology. Pediatr Res 51:71–75
37. Navarro-Blasco I, Alvarez-Galindo JI (2004) Selenium content of Spanish infant formulae and human milk: influence of protein matrix, interactions with other trace elements and estimation of dietary intake by infants. J Trace Elem Med Biol 17:277–289
38. Brock JH, Mulero V (2000) Cellular and molecular aspects of iron and immune function. Proc Nutr Soc 59:537–540
39. Oppenheimer SJ (2001) Iron and its relation to immunity and infectious disease. J Nutr 131(2S-2):616S–633S
40. Failla ML (2003) Trace elements and host defense: recent advances and continuing challenges. J Nutr 133(5 Suppl 1):1443S–1447S
41. Bonham M, O'Connor JM, Hannigan BM, Strain JJ (2002) The immune system as a physiological indicator of marginal copper status? Br J Nutr 87:393–403
42. Aschner JL, Aschner M (2005) Nutritional aspects of manganese homeostasis. Mol Aspects Med 26:353–362

43. Bovee-Oudenhoven IM, Lettink-Wissink ML, Van Doesburg W, Witteman BJ, van der Meer R (2003) Diarrhea caused by enterotoxigenic Escherichia coli infection of humans is inhibited by dietary calcium. Gastroenterology 125:469–476
44. Huiming Y, Chaomin W, Meng M (2005) Vitamin A for treating measles in children. Cochrane Database Syst Rev 4:CD001479
45. Villamor E, Fawzi WW (2005) Effects of vitamin a supplementation on immune responses and correlation with clinical outcomes. Clin Microbiol Rev 18:446–464
46. Long KZ, Estrada-Garcia T, Rosado JL, Ignacio Santos J, Haas M, Firestone M, Bhagwat J, Young C, DuPont HL, Hertzmark E, Nanthakumar NN (2006) The effect of vitamin A supplementation on the intestinal immune response in Mexican children is modified by pathogen infections and diarrhea. J Nutr 136:1365–1370
47. Kowdley KV, Mason JB, Meydani SN, Cornwall S, Grand RJ (1992) Vitamin E deficiency and impaired cellular immunity related to intestinal fat malabsorption. Gastroenterology 102:2139–2142
48. Meydani SN, Beharka AA (1998) Recent developments in vitamin E and immune response. Nutr Rev 56:S49–S58
49. Calder PC, Kew S (2002) The immune system: a target for functional foods? Br J Nutr 88(Suppl 2):S165–S177
50. Mahalanabis D, Basak M, Paul D, Gupta S, Shaikh S, Wahed MA, Khaled MA (2006) Antioxidant vitamins E and C as adjunct therapy of severe acute lower-respiratory infection in infants and young children, a randomized controlled trial. Eur J Clin Nutr 60:673–680
51. Hayes CE, Nashold FE, Spach KM, Pedersen LB (2003) The immunological functions of the vitamin D endocrine system. Cell Mol Biol 49:277–300
52. Cantorna MT, Zhu Y, Froicu M, Wittke A (2004) Vitamin D status, 1, 25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 80:1717S–1720S
53. James WP (2008) 22 Marabou Symposium: the changing faces of vitamin D. Nutr Rev 66(Suppl 2):S213–S217
54. Milner JD, Stein DM, McCarter R, Moon RY (2004) Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma. Pediatrics 114:27–32
55. Hypponen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, Jarvelinb MR (2004) Infant vitamin D supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Ann N Y Acad Sci 1037:84–95
56. Willemsen LE, Koetsier MA, Balvers M, Beermann C, Stahl B, van Tol EA (2008) Polyunsaturated fatty acids support epithelial barrier integrity and reduce IL-4 mediated permeability in vitro. Eur J Nutr 47:183–191
57. Calder PC (2006) N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83:1505S–1519S
58. Serhan CN (2005) Novel eicosanoid and docosanoid mediators: resolvins, docosatrienes, and neuroprotrectins. Curr Opin Clin Nutr Metab Care 8:115–121
59. Devereux G (2006) The increase in the prevalence of asthma and allergy: food for thought. Nat Rev Immunol 6:869–874
60. Devereux G, Seaton A (2005) Diet as a risk factor for atopy and asthma. J Allergy Clin Immunol 115:1109–1117
61. Field CJ, Thomson CA, Van Aerde JE, Parrott A, Euler A, Lien E, Clandinin MT (2003) Lower proportion of CD45R0+ cells and deficient interleukin-10 production by formula-fed infants, compared with human-fed, is corrected with supplementation of long-chain polyunsaturated fatty acids. J Pediatr Gastroenterol Nutr 31:291–299
62. Field CJ, Van Aerde JE, Robinson LE, Clandinin MT (2008) Effect of providing a formula supplemented with long-chain polyunsaturated fatty acids on immunity in full-term neonates. Br J Nutr 99:91–99
63. Koletzko B, Sauerwald U, Keicher U et al (2003) Fatty acid profiles, antioxidant status, and growth of preterm infants fed diets without or with long-chain polyunsaturated fatty acids. A randomized clinical trial. Eur J Nutr 42:243–253
64. Scientific Committee on Food (2003). Report of the Scientific Committee on Food on the revision of essential requirements of infant formulae and follow-on formulae. Brussels: European Commission,

2003. (SCF/CS/NUT/IF/65 Final. 2003). Available at: http://europa.eu.int/comm/food/fs/sc/scf/out199_en.pdf
65. Schachter HM, Reisman J, Tran K, Dales B, Kourad K, Barnes D, Sampson M, Morrison A, Gaboury I, Blackman J (2004) Health effects of omega-3 fatty acids on asthma. Evid Rep Technol Assess Summ 91:1–7
66. Agostoni C, Axelsson I, Goulet O et al (2004) ESPGHAN committee on nutrition. Prebiotic oligosaccharides in dietetic products for infants: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 39:465–473
67. Boehm G, Jelinek J, Stahl B, van Laere K, Knol J, Fanaro S, Moro G, Vigi V (2004) Prebiotics in infant formulas. J Clin Gastroenterol 38:S76–S79
68. Kunz C, Rudloff S, Baier W, Klein N, Strobel S (2000) Oligosaccharides in human milk: structural, functional, and metabolic aspects. Annu Rev Nutr 20:699–722
69. Knol J, Scholtens P, Kafka C, Steenbakkers J, Gro S, Helm K, Klarczyk M, Schopfer H, Bockler HM, Wells J (2005) Colon microflora in infants fed formula with galacto- and fructo-oligosaccharides: more like breast-fed infants. J Pediatr Gastroenterol Nutr 40:36–42
70. van Hoffen E, Ruiter B, Faber J, M'Rabet L, Knol EF, Stahl B, Arslanoglu S, Moro G, Boehm G, Garssen J (2009) A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in infants at high risk for allergy. Allergy 64:484–487
71. Shadid R, Haarman M, Knol J, Theis W, Beermann C, Rjosk-Dendorfer D, Schendel DJ, Koletzko BV, Krauss-Etschmann S (2007) Effects of galactooligosaccharide and long-chain fructooligosaccharide supplementation during pregnancy on maternal and neonatal microbiota and immunity – a randomized, double-blind, placebo-controlled study. Am J Clin Nutr 86:1426–1437
72. Lomax AR, Calder PC (2009) Prebiotics, immune function, infection and inflammation: a review of the evidence. Br J Nutr 101:633–658
73. Fanaro S, Boehm G, Garssen J, Knol J, Mosca F, Stahl B, Vigi V (2005) Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. Acta Paediatr 94:22–26
74. Rao S, Srinivasjois R, Patole S (2009) Prebiotic supplementation in full-term neonates: a systematic review of randomized controlled trials. Arch Pediatr Adolesc Med 163:755–764
75. Thibault H, Aubert-Jacquin C, Goulet O (2004) Effects of long-term consumption of a fermented infant formula (with Bifidobacterium breve c50 and Streptococcus thermophilus 065) on acute diarrhea in healthy infants. J Pediatr Gastroenterol Nutr 39:147–152
76. Alfaleh K, Anabrees J, Bassler D (2009) Probiotics reduce the risk of necrotizing enterocolitis in preterm infants, a meta-analysis. Neonatology 97:93–99
77. NASPGHAN Nutrition Report Committee, Michail S, Sylvester F, Fuchs G, Issenman R (2006) Clinical efficacy of probiotics: review of the evidence with focus on children. J Pediatr Gastroenterol Nutr 43:550–557
78. Parkes GC, Sanderson JD, Whelan K (2009) The mechanisms and efficacy of probiotics in the prevention of Clostridium difficile-associated diarrhoea. Lancet Infect Dis 9:237–244
79. Kapsenberg ML (2003) Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol 3:984–993
80. van Baarlen P, Troost FJ, van Hemert S, van der Meer C, de Vos WM, de Groot PJ, Hooiveld GJ, Brummer RJ, Kleerebezem M (2009) Differential NF-kappaB pathways induction by Lactobacillus plantarum in the duodenum of healthy humans correlating with immune tolerance. Proc Natl Acad Sci U S A 106:2371–2376
81. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E (2001) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 357:1076–1079
82. Kopp MV, Salfeld P (2009) Probiotics and prevention of allergic disease. Curr Opin Clin Nutr Metab Care 12:298–303
83. Kalliomäki M, Antoine JM, Herz U, Rijkers GT, Wells JM, Mercenier A (2010) Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of allergic diseases by probiotics. J Nutr 140:713S–721S
84. Rijkers GT, Bengmark S, Enck P, Haller D, Herz U, Kalliomaki M, Kudo S, Lenoir-Wijnkoop I, Mercenier A, Myllyluoma E, Rabot S, Rafter J, Szajewska H, Watzl B, Wells J, Wolvers D, Antoine

JM (2010) Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. J Nutr 140:671S–676S
85. Saavedra JM, Abi-Hanna A, Moore N, Yolken RH (2004) Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. Am J Clin Nutr 79:261–267
86. Boyle RJ, Robins-Browne RM, Tang ML (2006) Probiotic use in clinical practice: what are the risks? Am J Clin Nutr 83:1256–1264
87. Dugoua JJ, Machado M, Zhu X, Chen X, Koren G, Einarson TR (2009) Probiotic safety in pregnancy: a systematic review and meta-analysis of randomized controlled trials of Lactobacillus, Bifidobacterium, and Saccharomyces spp. J Obstet Gynaecol Can 31:542–552
88. Vlieger AM, Robroch A, van Buuren S, Kiers J, Rijkers G, Benninga MA, te Biesebeke R (2009) Tolerance and safety of Lactobacillus paracasei ssp. paracasei in combination with Bifidobacterium animalis ssp. lactis in a prebiotic-containing infant formula: a randomised controlled trial. Br J Nutr 102:869–875
89. Snydman DR (2008) The safety of probiotics. Clin Infect Dis 46(Suppl 2):S104–S111
90. Carver JD (1999) Dietary nucleotides: effects on the immune and gastrointestinal systems. Acta Paediatr Suppl 88:83–88
91. Maldonado J, Navarro J, Narbona E, Gil A (2001) The influence of dietary nucleotides on humoral and cell immunity in the neonate and lactating infant. Early Hum Dev 65:S69–S74
92. Buck RH, Thomas DL, Winship TR, Cordle CT, Kuchan MJ, Baggs GE, Schaller JP, Wheeler JG (2004) Effect of dietary ribonucleotides on infant immune status. Part 2: immune cell development. Pediatr Res 56:891–900
93. Schaller JP, Kuchan MJ, Thomas DL, Cordle CT, Winship TR, Buck RH, Baggs GE, Wheeler JG (2004) Effect of dietary ribonucleotides on infant immune status. Part 1: humoral responses. Pediatr Res 56:883–890
94. Hawkes JS, Gibson RA, Roberton D, Makrides M (2006) Effect of dietary nucleotide supplementation on growth and immune function in term infants: a randomized controlled trial. Eur J Clin Nutr 60:254–264
95. Yau KI, Huang CB, Chen W, Chen SJ, Chou YH, Huang FY, Kua KE, Chen N, McCue M, Alarcon PA, Tressler RL, Comer GM, Baggs G, Merritt RJ, Masor ML (2003) Effect of nucleotides on diarrhea and immune responses in healthy term infants in Taiwan. J Pediatr Gastroenterol Nutr 36:37–43
96. Raiten DJ, Talbot JM, Waters JH (eds) (1998). Life Sciences Research Office Report. Assessment of nutrient requirements for infant formulas. J Nutr 128:2059S–2293S
97. Manzoni P, Rinaldi M, Cattani S, Pugni L, Romeo MG, Messner H, Stolfi I, Decembrino L, Laforgia N, Vagnarelli F, Memo L, Bordignon L, Saia OS, Maule M, Gallo E, Mostert M, Magnani C, Quercia M, Bollani L, Pedicino R, Renzullo L, Betta P, Mosca F, Ferrari F, Magaldi R, Stronati M, Farina D, Italian Task Force for the Study and Prevention of Neonatal Fungal Infections, Italian Society of Neonatology (2009) Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA 302:1421–1428
98. Koletzko B, Aggett PJ, Bindels JG, Bung P, Ferre P, Gil A, Lentze MJ, Roberfroid M, Strobel S (1998) Growth, development and differentiation: a functional food science approach. Br J Nutr 80(Suppl 1):S5–S45
99. Rueda R (2007) The role of dietary gangliosides on immunity and the prevention of infection. Br J Nutr 98(Suppl 1):S68–S73
100. Wang B, McVeagh P, Petocz P, Brand-Miller J (2003) Brain ganglioside and glycoprotein sialic acid in breastfed compared with formula-fed infants. Am J Clin Nutr 78:1024–1029
101. Wang B, Miller JB, Sun Y, Ahmad Z, McVeagh P, Petocz P (2001) A longitudinal study of salivary sialic acid in preterm infants: Comparison of human milk-fed versus formula-fed infants. J Pediatr 138:914–916
102. Niers L, Stasse-Wolthuis M, Rombouts FM, Rijkers GT (2007) Nutritional support for the infant's immune system. Nutr Rev 65:347–360

2 Breast Milk: Components with Immune Modulating Potential and Their Possible Role in Immune Mediated Disease Resistance

Belinda van't Land, Günther Boehm, and Johan Garssen

Key Points

- Breast milk contains several interesting immune modulating components with specific modulating potentials, which are known to have a clear role in immune mediated disease resistance later in life.
- The development and deterioration of our immune defenses show differences as well as similarities in immunological challenges throughout life.
- Each phase in life puts specific requirements on nutrition, although no clear statement can be made based on literature as to what the exact dietary requirements are in order to fully support the immune system during life.

Key Words: Immune-modulation, protection, breast milk components, infant, adult, disease.

2.1 INTRODUCTION

The ontogeny of the immune system starts early in gestation but is not completed at birth. The highly protective germ-free environment and the need to avoid immunological interactions of the infant against the mother seems to be the main reason for this "physiological" immaturity of the immune system in newborn infants. The immaturity is characterized by deficiencies of the innate and the adaptive immune responses. The insufficient innate immune response is evident by an improper chemical barrier (1), a weak mucosal barrier integrity (2), reduced NK cell responsiveness, defective APC

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_2

function (3), and reduced gene expression in DCs (i.e., IL-12) (4), which is ccompanied by DCs biased against Th1 response induction (5). In addition, the pathogen recognition apparatus, although present does not respond properly in human neonates, resulting in, for example, impaired TLR responsiveness and pathogen recognition (1, 6). Furthermore, it is increasingly recognized that regulatory T cells, which inhibit excessive immune responses thereby maintaining peripheral T-cell tolerance, are particularly abundant and potent at birth (7). The adaptive immune system still needs to mature completely and get in contact with pathogens in order to build upon a proper memory. T-cell generation (8) and function (9) in neonates is not fully developed. In addition, neonatal B cells are capable of switching to IgG1 and IgG3 during the first 2 years of life, but the switch to IgG2 and IgG3 is inadequate during this period. To compensate the lack of protection in the fetus and newborn, microbe-specific maternal IgG antibodies move across the placental barrier to provide some vital protection. After birth, breastfeeding maintains the maternal–fetal immunological link by transferring immune competence of the mother to her infant. Anti-infective compounds provide directly defense potential and immune modulating compounds stimulate the postnatal development of all levels of the immune system. Both groups of factors contribute to the protection during lactation but there is broad consensus that the immune modulation of the infant during lactation has also consequences for the immune response after lactation. The present review describes the protective and immune modulatory factors in breast milk which are considered important for the neonate defense system during the very vulnerable period immediately after birth and also for the programming of the immune system for later life.

2.2 NATURAL DEFENSES THROUGHOUT LIFE

In principle, the human body is protected by various nonspecific defense mechanisms. Pathogens that break through the mucosal surface barrier encounter two additional levels of defense, the innate and acquired immune responses. The efficient cross-talk between innate and acquired immunity enables the powerful host defense protecting us from immune-related disorders and pathogenic invaders. The innate immune response is immediately activated after infection, while the acquired immune response takes at least several days for full development. Increased susceptibility to infections and decreased immune responsiveness are indeed present during the first years of life and are in part related to the incapacity of the infant's immune system to respond properly. It is important to maintain proper levels of immune defenses throughout life as they are challenged daily. During the different phases of life, several factors influence our immune system and immune responsiveness, such as nutrition, hormonal changes during adolescence and so on. A vast amount of literature is available on the role that nutrients, that is, as present in breast milk (as reviewed in this article), have on the development of the immune system; a lot less is known about the exact requirements in the phases thereafter, that is, in toddlers, during adolescence, and in the later stages of life. Although it is clear that each phase in life puts specific requirements on nutrition, no clear statement can be made based on literature as to what the exact dietary requirements are in order to fully support the immune system during these stages in life.

The aging processes induce multiple changes in metabolism, the hormones network, the immune system, which can modulate the efficiency and effectiveness of the immune system, determining a response to stressors. For example, normal aging is associated with deregulated immune and inflammatory responses, which results in increased susceptibility toward infections in the elderly. Host resistance in general undergoes changes in both a qualitative and quantitative manner with aging as a declined T cell function is the best-characterized feature of immunosenescence. The development and deterioration of our immune defenses is schematically represented in Fig. 2.1, illustrating differences and also similarities in immune challenges throughout life.

2.3 ROLE OF BREASTFEEDING

The human immune system can be easily modified during the first years of life, which is necessary to complete protection against infections and sufficiently tolerate non-harmful environmental agents. Breastfeeding is adapted to the infant's requirements and may compensate for the relative inefficiency of host defense by providing considerable amounts of both nonspecific as well as pathogen-specific secretory IgA (sIgA). These antibodies, which are formed as a consequence of the previous exposure to infectious agents by the mother, can bind and inactivate potential harmful pathogens. In addition to the antibodies, breast milk contains several other nonspecific factors that have antimicrobial effects, or provide protection to the infant through alternative routes. These factors with immunological, hormonal, enzymatic, trophic, and/or bioactivity present in breast milk may offer passive protection (10). Other factors like macrophages and leukocytes, largely present at the beginning of lactation, may exert a more modulatory effect on the neonatal immune system and provide additional protection (11). Breast milk contains several immune-modulatory compounds, including the antibodies, IgGs, IgMs, isoforms of immunoglobulins (sIgA), nucleotides, specific amino acids (taurine, polyamines), PUFA's (eicosapentaenoic acid, docosahexaenoic acid), monoglycerides, lauric acid, linoleic acid, cytokines and chemokines, soluble receptors (CD14, sTLR2), antibacterial proteins/peptides (lactoferrin, lysosyme, β-lactoglobulin, casein), prebiotics, oligosaccharides, and intact immune cells as well reviewed by M'Rabet et al. (12). Several of these components have been extensively studied on their specific immune modulating potencies in general as reviewed by Calder et al. (13).

2.4 IMMUNE MODULATION PROPERTIES OF BREAST MILK

Several reports confirm that the immunological components of human milk can influence the infant's immune response. The influence of breastfeeding compared to formula feeding on immune modulation has been studied in infants by measuring, for instance, vaccination responses, but also incidences of infections, allergies, etc. As recommended by the WHO and the International Life Science Institute, the immune response after vaccination can be taken as an objective measurement or model to evaluate the immune response (14). A vaccination response can be measured by vaccine-specific *ex vivo* cell proliferation and cytokine production and by the level of neutralizing antibodies (9) cov-

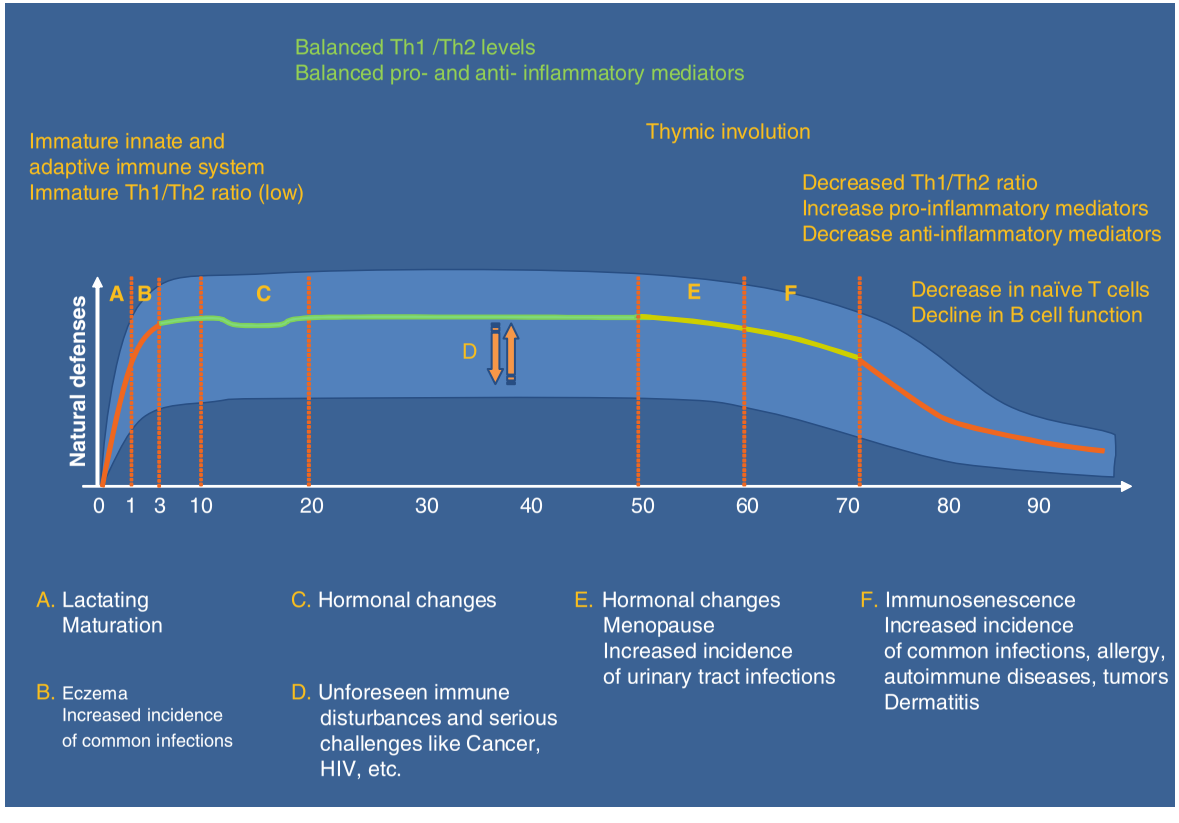


Fig. 2.1. Immunity throughout life. This figure schematically represents the level of human natural immune defenses throughout life, from the developmental stages during infancy until immune-senescence at the latest stages in life. Several stages can be identified, with unique immunological features and influences of nutrition as discussed within this review.

ering both the efficiency and magnitude of antigen-specific T and B cell responses. For example in 12-month-old children receiving breast milk, an increased production of IFN-gamma and increased percentage of CD56+CD8+ cells (activated cytotoxic T cells) after measles–mumps–rubella vaccination were seen, as compared with formula-fed infants. In addition, cytokine responses to measles, mumps, and rubella vaccination differed between the two types of nutrition (15). These results are suggestive of a more Th1 type of responsiveness in breastfed infants as compared to formula-fed infants.

2.5 ANTI-INFECTIVE PROPERTIES OF HUMAN MILK

Several studies have shown the protective capacity of breast milk. It is known that breastfeeding reduces the incidence of gastrointestinal and nonenteric infections in infants because of its antimicrobial activity against several viruses, bacteria, and protozoa as reviewed by Chirico et al. (16). It was shown in a recent meta-analysis that infants who were breastfed for more than 4 months showed a significant reduced incidence of respiratory tract infection requiring hospitalization, as compared to infants who were not breastfed (17). In addition, the risk for infectious diarrhea is higher in nonbreastfed infants, than for infants receiving human milk. Other studies showed that breastfeeding provides protection against urinary tract infections and otitis media (18, 19). It can reduce infant mortality, and protect, for instance, against neonatal meningitis and septicemia. In addition, protection is also clearly demonstrated against respiratory infections. These studies show the clear protective nature of human milk against all sorts of invasive pathogens. In addition to that, human milk has been shown to reduce the development of inflammatory conditions such as allergy (20), Crohn's disease, ulcerative colitis, and protect possibly against certain other immunological diseases such as insulin dependent diabetes and tumors in infancy (21, 22). This, moreover, emphasizes the diversity of activity and active components present in human breast milk.

2.6 ANTI-INFLAMMATORY PROPERTIES OF BREAST MILK

During the bacterial colonization of the newborn's mucosal surfaces, including the skin and gut, a huge amount of microbial components are brought in acute contact with the sterile neonate. The coordination of the inflammatory response developed after this first contact is of vital importance. The epithelial layer, together with the intra-epithelial and lamina propria immune competent cells, is the most important player in regulating the recognition of microorganisms and maintenance of gut homeostasis. Spontaneous integrin expression on several lymphocytes at 6 months of age was reported significantly lower in breastfed than formula-fed infants, which is indicative for the anti-inflammatory potency of human breast milk.

2.7 THE ONSET OF ALLERGIES VS. BREAST MILK

According to the hygiene hypothesis, for instance, the exposure of microbial components including TLR agonists early in life serves to polarize the immune response away from Th2 responses toward a more Th1 type of response, thereby reducing the

onset of allergy and/or atopy. Consistent with this hypothesis is the inverse epidemiological relationship between the decreasing rate of common infections (in industrialized countries) and the parallely increasing rate of allergy and autoimmune diseases. However, the improving vaccination strategies occurring simultaneously may hamper this view. It is clear, however, that the onset of allergy is influenced by breast milk as well as atopy-related disorders (23–25) although some controversy exists regarding the beneficial length of breastfeeding (26–28). In a multidisciplinary review of the literature (1966–2001) van Odijk et al. (23) showed that exclusive breastfeeding reduces the risk of asthma, particularly, strong effects in infants with atopic heredity. These protective effects increase with the duration of breastfeeding (up to at least 4 months). These protective effects of human breast milk seem to persist at least during the first decade of life.

2.8 DURATION OF BREASTFEEDING

While discussing maternal factors and their influence on the immune system, it is important to recognize that human milk composition differs in time elapsed postpartum, in density, and composition. The favorable duration of breastfeeding differs between country and continent, due to the environmental challenges and cultural opinions. Several studies have been performed to determine the optimal duration of breastfeeding. In general, it can be stated that breastfeeding for less than 2 months may be deleterious because of lack of exposure to the protective factors in breast milk. In addition, there are some indications that breastfeeding for longer than 8 months is associated with increased BMI and percentage of body fat in later life (29). Currently, it is recommended to breastfeed exclusively during the first 6 months of infant life. Although breastfeeding can prevent 13–15% of child deaths in low-income countries, in some circumstances, breastfeeding can present a terrible dilemma, for instance, in the risk for transition of pathogens like HIV. Through breastfeeding, over 300,000 children are infected with HIV every year, as estimated by UNAIDS. However, a significant increase in early mortality was identified in studies in Kenya (11% vs. 9%) and Botswana (9.3% vs 4.9%; $P = .003$) in formula-fed versus breastfed infants (30). When HIV-positive mothers breastfeed exclusively, infection with HIV of their babies is relatively low (4%). This risk is lower than that in babies who receive other food or liquids in addition to breast milk before 6 months of age. Exclusive breastfeeding protects the integrity of the intestinal mucosa of the infant, which may thereby be a more effective barrier to HIV infection. Therefore, it has been advised that in developing countries, early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival (31). However, in better resourced areas, these differences have not been reported. This reinforces the UNAIDS guidelines on breastfeeding for HIV-infected mothers – namely, "where replacement feeding is acceptable, feasible, affordable, sustainable, and safe, avoidance of all breastfeeding is recommended, otherwise exclusive breastfeeding is recommended for the first few months of life." The WHO established a global determined goal, which states that in 2010 within the healthy population at least an initiation rate of 75% breastfeeding should be possible to reach. In addition, a breastfeeding rate of 50% at 6 months and 25% at 1 year is favorable. Although initiation rates approach 70%, rates at 6 months (33%) and 1 year (18%) currently remain low; in 2009, these goals have not been reached yet.

2.9 IMMUNE MODULATION CAPACITY OF SPECIFIC COMPONENTS AND EFFECT LATER IN LIFE

Researchers are actively investigating how dietary modifications that influence the immune system can be used to reduce the risk of various diseases or to improve their management. Most host defense mechanisms are impaired in malnutrition, even if the nutritional deficiency is only moderate in severity. Protein-energy malnutrition is often accompanied by deficiencies of micronutrients such as vitamin A, vitamin E, vitamin B6, vitamin C, folate, zinc, iron, copper, and selenium. For example, the rapid proliferating T cells responding to pathogens are especially affected by the lack of essential nutrients, resulting in a decrease in their numbers. Severe and chronic malnutrition may even lead to atrophy of the thymus and other lymphoid organs affecting the basis of our immune apparatus. The possibility that supplementation with certain nutrients, like vitamin C, D, or E, at levels above the Recommended Dietary Allowances (RDA), and food constituents such as probiotics and prebiotics may improve immune function in vulnerable individuals, like the elderly or immune compromised, but also in the general population is subject to increasing research. Compounds with specific immune

Table 2.1
Immune modulating breast milk components[a]

Components	*Activity*	*References (infant)*	*References (adult)*
Carbohydrates			
Oligosaccharides, glycoconjugates	Modulation of microbiota and immune function, antiadhesive function	(66–68)	(69, 82)
Antioxidants			
Vitamin A, C, E, catalase, glutathione peroxidase, lutein, etc.	Radical scavenging, anti-inflammatory activity	(63, 70)	(62)
Lipids/PUFAs			
Free fatty acids, monoglycerides	Antimicrobial and antiviral effects	(10)	(73)
Arachidonic acid, docosahexaenoic acids, etc.	Modulation of prostaglandin production Immune modulation	(71, 72)	
Carrier proteins			
Lactoferrin, transferrin, vitamin B-12 binding protein, steroid binding protein, α-lactalbumin, κ-casein	Antimicrobial activity, immune modulation, microbicidal effect and iron binding capacity	(74–76)	(35, 36, 77).
Bacteria			
Bifidobacteria, Lactobacillus	Modulation of microbiota and immune function	(78–81)	(55, 59)

[a]The list is a small selection of immune modulating compounds present in breast milk, with the focus on immune-modulating ingredients which are also used in daily diet/supplements to improve immune functions

modulation capacity present in breast milk and used to supplement daily diet are depicted in Table 2.1 and some of which are discussed below in more detail.

2.9.1 Proteins

Major nutrient groups with several important bioactive factors are the proteins, including immune-globulins, lactoferrin, lysozyme, α-lactalbumin and casein. Lactoferrin is a proteolysis-resistant glycoprotein and one of the most abundant proteins in human milk. An impressive range of effects have contributed to lactoferrin which include direct antimicrobial activities against a large panel of microorganisms, including bacteria, viruses, fungi, and parasites, and anti-inflammatory effects in addition to anticancer activities. Lactoferrin limits bacterial and fungal growth by competing for essential iron and may act as an alarmin to promote the recruitment and activation of APCs and antigen-specific immune responses (32–34). In addition, epithelial growth-promoting activities have been associated with lactoferrin. Ingesting lactoferrin relieves some symptoms of H. pylori gastric infection and increases the eradication of H. pylori in the stomach (35). In addition, benefits have been shown on rotaviral gastroenteritis and the possible inhibition of colorectal adenoma development. This indicates that protective immune modulatory proteins are present in human milk and may be of use in disease-specific conditions. The exact mechanism of action as well as the benefit for healthy adults still needs to be addressed.

Besides lactoferrin other interesting proteins are present in human breast milk including the enzyme lysozyme which inhibits the growth of many bacterial species by disrupting the bacterial cell wall, more specifically, the proteoglycan layer. In addition, casein may inhibit the adhesion of various bacteria at different epithelial sites. Lactalbumin is part of an enzyme complex that synthesizes lactose, and upon modification seems to contribute to the apoptosis of malignant cells. The anti-secretory factor is a protein present in most tissues in the body, and the plasma levels of anti-secretory factor can be increased by exposure to bacterial enterotoxins and to specially processed cereals. The anti-secretory effect has been shown in patients with secretory diarrhea, and the additional anti-inflammatory effect of anti-secretory factor has been demonstrated in ulcerative colitis and Crohn's disease (36).

2.9.2 Lipids

Another major nutrient and energy source in breast milk are the lipids, including long chain polyunsaturated fatty acids (PUFA), triglycerides, glycosphingolipids and free fatty acids (FFA). Monoglycerides, digestive products of triglycerides and some FFAs may have some lytic effect on viruses. Glycosphingolipids have been hypothesized to be one of the non-immunoglobulin compounds in human milk that can contribute to the protection against pathogens. Preterm infants given an adapted milk formula with gangliosides had fewer *E. coli* in feces and higher bifido-bacterial count than infants fed normal control formula (37). PUFAs such as arachidonic acid (AA) of the omega-6 (n-6) family and eicosapentaenoic acid (EPA), gamma-linoleic acid (GLA), and docosahexaenoic acid (DHA) of the omega-3 (n-3) family can potently alter the functioning of immune cells. In general, diets rich in n-3 PUFA tend to inhibit excessive immune responses which are associated with chronic inflammatory diseases such as asthma and

rheumatoid arthritis among others, as nicely reviewed by Riediger et al. (38). Whereas diets rich in n-6 PUFA tend to promote immune responses, which can lead to inflammation affecting chronic inflammatory diseases. The ratio of n-6 to n-3 PUFA may therefore be more important than the absolute amount of each of these classes of fatty acids in the diet. The basis for the anti-inflammatory properties of dietary fatty acids like EPA and GLA is their ability to replace AA ultimately as substrate for the synthesis of eicosanoids.

n-3 fatty acids are required for normal conception, growth, and development of an embryo. During the third trimester, approximately 50–60 mg/day of maternal DHA stores are transferred to a fetus via the placenta. Fish oil supplementation modulates immune function in healthy infants (39). Human milk contains DHA, and several organizations recommend supplementing infant formulas with DHA for infants and premature infants. DHA is particularly highly concentrated in the brain and retinal membranes, especially in photoreceptors, and is therefore assumed to play a critical role in both vision and cognitive function. However, several studies show either no improvement or slight improvement in visual acuity of infants receiving DHA supplemented to the infant formula compared to controls (40, 41).

Inappropriate immunologic activity, including inflammation, is a characteristic of many common human disorders. The oral supplementation of n-3 PUFAs has been evaluated in various clinical studies for their immunomodulatory capacity. Despite some inconsistencies in literature, it is clear that the addition of n-3 PUFAs like EPA and GLA to the diet leads to marked decreases in AA-derived eicosanoids and pro-inflammatory cytokines. Moreover, oral EPA and GLA have shown to be beneficial in patients with chronic inflammatory bowel disease not only in extending episodes of relapses but also reduced mucosal inflammation and production of local inflammatory mediators. In addition, it has been suggested that n-3 LCPUFA may inhibit atherosclerosis development by blocking the production of cytokines which promote inflammation. For example, the supplementation of 1.5 g/day AA for 7 weeks increased PGE2 production by leukocytes. In another study, 18 g/day of fish oil (equivalent to 5 g of n-3 LCPUFAs) suppressed several indices of non-specific and specific immune responses in healthy men. The dietary intake of fish, containing high levels of n-3 LCPUFAs, may as well be protective against asthma. It is believed that the production of mediators involved in allergic responses is affected by the balance between the two types of PUFA. However, evidence from clinical trials with n-3 LCPUFA supplementation is not conclusive herein. In conclusion, EPA- and GLA-rich supplements have been used to attenuate inflammatory processes in various chronic and autoimmune diseases, for example, rheumatoid arthritis (RA), inflammatory bowel disease (IBD), asthma, psoriasis, and multiple sclerosis. In each of these situations, the benefits of decreased production of PGE2 and proinflammatory cytokines are evident. In addition, EPA and DHA give rise to resolvins, which are anti-inflammatory and inflammation resolving. Human immune cells are typically rich in AA, but AA, EPA, and DHA contents can be altered through the oral administration of EPA and DHA already within few days (42). This results in a changed fatty acid composition of the immune cells which also affects phagocytosis, T cell signaling, and antigen-presentation capability. The immune cell-membrane is important in lipid raft structure and function, and membrane trafficking, suggesting an important role for fatty acids affecting the immune cell function through a variety of complex mechanisms.

2.9.3 Carbohydrates

Carbohydrates in breast milk mainly function as important nutrients for energy production and consist of lactose, oligosaccharides, and glycoconjugates. Prebiotics are nondigestible food ingredients, generally oligosaccharides, that modify intestinal microbiota balance by stimulating the growth of beneficial bacteria, such as bifidobacteria and lactobacilli (43). A clear bifidogenic effect has been ascribed to the nondigestible oligosaccharides present in human breast milk on the gut microflora, and a positive effect on the incidence of infections at short term and possibly also long term. In addition, however, the direct effects of human oligosaccharides on immune cells cannot be excluded. Human milk derived nondigestible sialylated and fucosylated oligosaccharides, may directly inhibit the adhesion of pathogens to the mucosal surfaces (44) and protect the infant against, for instance, infectious diarrhea (45). They may as well indirectly produce a protective and immune modulatory result through a prebiotic effect on the infant intestinal microflora (46). These responses/mechanisms whereby carbohydrates and prebiotics can modulate the immune response were reviewed in depth by Vos et al., and are therefore not discussed in detail herein (47).

The complex prebiotic carbohydrate are found only in trace amounts in cow's milk but are a substantial portion of human milk sugars. The addition of carbohydrates as prebiotics to infant formula is a subject of increasing investigation, since these oligosaccharides may create a bifidogenic effect on the flora of the gut, in an attempt to reduce the number of latent invasive bacteria. In addition, several studies indicate that a mixture of oligosaccharides (fructo-oligosaccharides and galacto-oligosaccharides) was shown to reduce the incidence of infections and atopic dermatitis during the first 6 months of life (48). This protective effect of oligosaccharide supplementation early in life was still present beyond the intervention period up to 2 years of life (49, 50).

These data clearly illustrate the protective and immune modulatory factors in breast milk as important to the neonate defense system during the vulnerable period immediately after birth but as well as for the programming of the immune system for later life.

Impairment in microbiota composition can be addressed by using prebiotics. It is indicated in some studies that the oligofructose consumed by toddlers increases fecal Bifidobacteria counts and decreases fecal Clostridia counts during consumption. Besides the microflora changes, some but limited evidence is available on the immunological effect of given prebiotics in adults (83); few studies are even available in adolescents. Other benefits relate to improving bone health, reducing the risk of colorectal cancer, boosting immunity, and enhancing satiety and aiding weight management. In terms of bone health, studies in humans (51, 52) have shown that inulin/oligofructose supplementation to a diet results in more absorption of calcium, accumulation of bone mineral and improved trabecular network structure. In addition to reported immune modulation induced through the ingestion of prebiotics, more health benefits are reported through modulation of the gut microbiota via prebiotic ingestion which may improve or prevent the disruption of intestinal permeability in humans (53, 54).

The glycoconjugates although not that abundantly present in breast milk may be essential in inactivation and binding to specific bacterial (V. cholera) and viral ligands (rotavirus).

2.9.4 Bacteria

At the time the fetus leaves the protective germ-free environment, it lacks antigenic experience and stable flora on all mucosal sites, including the skin and the gastrointestinal tract. The intestinal microbiota is important in several aspects related to the digestion of food and establishment, and the maintenance of gastrointestinal immune defensive barrier. A stable microbiota composition will improve the resistance to colonization by pathogens; it controls proliferation and differentiation of epithelial cells. By definition, a probiotic is a life microorganism which has a health benefit on the host. Specific strains of probiotic bacteria exert different effects on the immune system. Therefore, a generalization of the category would be inaccurate and misleading. The effects of probiotic strains on gut health in adults is well established, as well indicated by a meta-analysis in The Lancet (55). Feeding with breast milk may add to the development of a healthy intestinal microflora. At the age of 1 year, differences still exist in microbiota composition of formula and breastfed infants (56). The intestinal microbiota composition of breastfed infants is less diverse, more bifidogenic than bottle-fed infants. Several components of breast milk can contribute to this observation, including the presence of some bacteria or bacterial fragments (57) as well as prebiotics like nondigestible oligosaccharides or certain peptides. Positive effects have been described as to the development of the gut toward a more bifidogenic environment (58).

One of the most common gastrointestinal complications in premature babies is necrotizing entrocolitis. A meta-analysis of different organisms used as probiotics in this situation has shown that results are generally positive [Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotizing enterocolitis in preterm neonates with very low birthweight: a systematic review of randomized controlled trials. Lancet 2007; 369:1614–20]. Again, caution is needed in that it may not be applicable for all probiotics. As the inexperienced newborn is stabilizing its microbiota in conjunction with immune responsiveness during the first months of life, a disturbance of this delicate balance may involve serious risks; additionally, the effects on immune responsiveness later in life are not defined.

The immune-enhancing potential of probiotics has been reported frequently; the mechanism by how these effects may be occurring, however, has not been elucidated. Recently, some evidence of how probiotics may influence the immune system in humans in the short term, that is, directly after intake, has been published by the group of Kleerebezem (59). The study identified changes in mucosal gene expression patterns and cellular pathways, through the ingestion of life or death *bacteria*, in healthy adults. Although the differences indicated are minimal, the difference between the form of which these probiotics are supplemented (i.e., life vs. death) is remarkable, although not surprising since the immune system in the gastrointestinal tract is highly trained to identify /respond to possible life intruders, and neglect non-harmful agents present in our daily diet. Unfortunately, no differences between different probiotic strains have been studied yet, and the effect on longer term remains to be elucidated.

The consumption of a combination of probiotics and prebiotics also called synbiotics may approach the composition of breast milk even more, and may add individual value to the development of improved gut health and immune health (60). One recent study reports in elderly at least an additive effect on the use of a probiotic strain in addition

to a prebiotic disaccharide, increasing the levels of bifidobacteria and improving mucosal functions like bowel movement (61). However, clinical studies are clearly required to identify the individual effects of the probiotics, probiotic strains, and prebiotics in order to establish the existence of real synbiotic benefits.

2.9.5 Antioxidants

Human milk has tremendously important anti-inflammatory effects, which are in part mediated through oxygen radical scavenging. Factors contributing to the antioxidant capacity of breast milk are α-tocopherol, β-carotene, ascorbic acid, and l-histidine. Vitamins including vitamin A, C, and E have anti-inflammatory effects due to oxygen radical scavenging, and may be immunomodulatory. These antioxidants act both at the mucosal level and after absorption systemically (α-tocopherol, β-carotene). Glutathione peroxidase can decrease inflammation by preventing lipid peroxidation. The effects of vitamin E supplementation have been shown to be variable and dependent on several confounding factors like the severity of vitamin E deficiency, dosage used, and age of individuals and other factors. In the elderly, an enhanced cell-mediated immune response and decreased prostaglandin E2 production were seen when they were given high concentrations of vitamin E. A mechanistic explanation for the enhanced immune function with vitamin E supplementation in the elderly could be besides the prevention of oxidative damage in immune cell membranes, the fact that high concentrations of prostaglandin E2 may inhibit T cell function and proliferation.

Like vitamin E, vitamin C is an antioxidant and present in several vegetables and fruits. A high concentration of Vitamin C is found in immune cells and is used rapidly during infection. The mechanisms whereby vitamin C affects the immune system are hardly understood. Vitamin C may modulate the functions of phagocytes, production of cytokines, proliferation of T lymphocytes, and gene expression of monocyte adhesion molecules. Numerous controlled trials in human volunteers have been conducted to evaluate the effect of vitamin C on the incidence and severity of common cold. People who regularly take high dosages of vitamin C seem to have a slightly shorter duration of common colds (about 10%) than those who do not. Although, no effect of vitamin C supplementation were seen on the incidence of common colds in the normal population, a clear reduction (up to 50%) was seen among people that are regularly stressed by, for instance, physical activity (like marathon runners) (62).

Like vitamins C and E, carotenoids (including the precursor of vitamin A, β-carotene) are antioxidants. In contrast to vitamin C and E, Vitamin A is an example of a dietary component that enhances the immune system and has become a part of standard medical practice. Vitamin A is found to enhance the regeneration of damaged mucosal epithelium and improves phagocytic activity of neutrophils and macrophages. In vitamin A deficiency, the ability of gut immune cells to produce antibodies (IgA and IgG) against bacterial toxins is compromised. Supplementation with vitamin A restores this defect, and is known to reduce severe illness induced by measles and shorten the duration of the infection. However, the benefit of vitamin A supplementation seems not to be limited to deprived populations, but is rather effective during vitamin A-depriving infections like measles. Moreover, carotenoids including β-carotene, which is widely distributed in plants, and lycopene, a carotenoid found in tomatoes, are found to be beneficial for individuals with a compromised immune system. Epidemiological studies

suggest that the risk of respiratory infections is reduced when diets are rich in carotenoids. In particular, in the elderly, a recovery of declined NK cell activity to normal levels was observed following β-carotene supplementation. In contrast, in healthy adults with adequate carotenoid intake and normal immune responses supplementation with carotenoids did not further improve immune responses.

One of the vitamins important for infant's development, but not supplied in high amounts through breast milk, is vitamin D (63). Exclusively breastfed infants are indeed at higher risk of vitamin D deficiency than formula-fed infants. Therefore, some guidelines subscribe the supplementation of Vitamin D drops containing 200 IU to be given to all breastfed infants starting in the first 2 months of life. Vitamin D-deficient or insufficient neonates are at an increased risk of being affected by hypocalcemia and rickets. Serum calcium concentrations and bone metabolism in adults are related to Vitamin D levels. Although vitamin D deficiency increases neonatal hypocalcemia risk, it is unclear whether vitamin D insufficiency causes hypocalcemia. Increasing evidence from observational studies in infants at older ages, indicate that vitamin D insufficiency and deficiency might increase the risk of chronic diseases such as type 1 diabetes and multiple sclerosis. However, clear randomized trials on this association need to be conducted before clinicians can recommend vitamin D supplementation to reduce the incidence of type 1 diabetes. In the last decades, observations accumulated that vitamin D deficiency leads to more often and more serious respiratory infections than in individuals with sufficient vitamin D plasma levels. It has even been speculated that the increasing incidence of respiratory infections in the winter season could originate from a latent vitamin D deficiency, since solar radiation beyond 45° latitude is considerably lower in winter than in summer. Specific immune cells, that is, the macrophages, contain enzymatic capacity to make biologically active forms of vitamin D in addition to the liver and kidney. Interestingly, toll-like receptor stimulation in macrophages enhances the conversion of vitamin D precursor into active vitamin D as well as the expression of the vitamin D receptor. Vitamin D in macrophages regulates the production of an endogenous antibiotic called cathelicidin and modulates the pattern of cytokine secretion. Both cathelicidin and the cytokines enhance the defense against pathogens. Obviously, vitamin D is a key link between toll-like receptor activation and antibacterial responses in innate immunity.

2.9.6 Nucleotides

Nucleotides, nucleosides, nucleic acids, and related products in human milk are important in a number of cellular functions and have been shown to enhance immune function in infants. A variety of different roles have been assigned to ingested nucleotides, including supporting energy metabolism (ATP), nucleic acid production and their messengers (RNA,DNA and cAMP, cGMP, ADP respectively), coenzymes in metabolic processes (NAD, CoA), signal transduction molecules (cAMP), and carrier molecules in synthetic reactions (UDP, GDP, CMP). Although nucleotides are not essential nutrients, they are important in situations of increased demand and metabolic activity such as infection, or rapid growth (64). Mean values of the total potentially available nucleosides as measured in breast milk in different populations are used to guide the addition of nucleotides to infant formula. In some clinical studies, small benefits have been attributed to the addition of nucleotides to infant formulas, including

fewer episodes of diarrhea and higher plasma levels of IgA and IgM. The proposed mechanisms of action contributed to nucleotide-induced effects include increased iron absorption, increased growth of Bifidobacterium, improved development, and repair of the gastrointestinal mucosa, in addition to improved systemic immune responses including increased NK cell activity and IL-2 production.

2.10 CONCLUSIONS AND PERSPECTIVES

The list of bioactive factors present in human breast milk is still incomplete as investigators are continuously identifying new components like cathelicidin antimicrobial peptides (65). In addition the specific action and contribution toward the protective and immune modulatory capacity of breast milk from the individual components with their potential as ingredients supplemented to daily diet is still to be determined. The current knowledge of immune modulating components present in breast milk and their immune modulating potential including their possible role in immune mediated disease resistance later in life remains an increasingly important subject for research.

REFERENCES

1. Levy O (2007) Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol 7(5):379–390
2. Blumer N, Pfefferle PI, Renz H (2007) Development of mucosal immune function in the intrauterine and early postnatal environment. Curr Opin Gastroenterol 23(6):655–660
3. Velilla PA, Rugeles MT, Chougnet CA (2006) Defective antigen-presenting cell function in human neonates. Clin Immunol 121(3):251–259
4. Goriely S, Vincart B, Stordeur P et al (2001) Deficient IL-12(p35) gene expression by dendritic cells derived from neonatal monocytes. J Immunol 166(3):2141–2146
5. Langrish CL, Buddle JC, Thrasher AJ, Goldblatt D (2002) Neonatal dendritic cells are intrinsically biased against Th-1 immune responses. Clin Exp Immunol 128(1):118–123
6. De Wit D, Olislagers V, Goriely S et al (2004) Blood plasmacytoid dendritic cell responses to CpG oligodeoxynucleotides are impaired in human newborns. Blood 103(3):1030–1032
7. Godfrey WR, Spoden DJ, Ge YG et al (2005) Cord blood CD4(+)CD25(+)-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. Blood 105(2):750–758
8. Schonland SO, Zimmer JK, Lopez-Benitez CM et al (2003) Homeostatic control of T-cell generation in neonates. Blood 102(4):1428–1434
9. Marchant A, Goldman M (2005) T cell-mediated immune responses in human newborns: ready to learn? Clin Exp Immunol 141(1):10–18
10. Hamosh M (2001) Bioactive factors in human milk. Pediatr Clin North Am 48(1):69–86
11. Xanthou M (1997) Human milk cells. Acta Paediatr 86(12):1288–1290
12. M'Rabet L, Vos AP, Boehm G, Garssen J (2008) Breast-feeding and its role in early development of the immune system in infants: consequences for health later in life. J Nutr 138(9):1782S–1790S
13. Calder PC, Krauss-Etschmann S, de Jong EC et al (2006) Early nutrition and immunity – progress and perspectives. Br J Nutr 96(4):774–790
14. Albers R, Antoine JM, Bourdet-Sicard R et al (2005) Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr 94(3):452–481
15. Pabst HF, Spady DW, Pilarski LM, Carson MM, Beeler JA, Krezolek MP (1997) Differential modulation of the immune response by breast- or formula-feeding of infants. Acta Paediatr 86(12):1291–1297
16. Chirico G, Marzollo R, Cortinovis S, Fonte C, Gasparoni A (2008) Antiinfective properties of human milk. J Nutr 138(9):1801S–1806S

17. Bachrach VR, Schwarz E, Bachrach LR (2003) Breastfeeding and the risk of hospitalization for respiratory disease in infancy: a meta-analysis. Arch Pediatr Adolesc Med 157(3):237–243
18. Lawrence RM, Pane CA (2007) Human breast milk: current concepts of immunology and infectious diseases. Curr Probl Pediatr Adolesc Health Care 37(1):7–36
19. Hanson LA (2007) Session 1: feeding and infant development breast-feeding and immune function. Proc Nutr Soc 66(3):384–396
20. Fiocchi A, Martelli A, De Chiara A, Moro G, Warm A, Terracciano L (2003) Primary dietary prevention of food allergy. Ann Allergy Asthma Immunol 91(1):3–12, quiz 5, 91
21. Martin RM, Gunnell D, Owen CG, Smith GD (2005) Breast-feeding and childhood cancer: a systematic review with metaanalysis. Int J Cancer 117(6):1020–1031
22. Martin RM, Ben-Shlomo Y, Gunnell D, Elwood P, Yarnell JW (2005) Davey Smith G. Breast feeding and cardiovascular disease risk factors, incidence, and mortality: the Caerphilly study. J Epidemiol Community Health 59(2):121–129
23. van Odijk J, Kull I, Borres MP et al (2003) Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. Allergy 58(9):833–843
24. Gdalevich M, Mimouni D, David M, Mimouni M (2001) Breast-feeding and the onset of atopic dermatitis in childhood: a systematic review and meta-analysis of prospective studies. J Am Acad Dermatol 45(4):520–527
25. Gdalevich M, Mimouni D, Mimouni M (2001) Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. J Pediatr 139(2):261–266
26. Sears MR, Greene JM, Willan AR et al (2002) Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. Lancet 360(9337):901–907
27. Bergmann RL, Diepgen TL, Kuss O et al (2002) Breastfeeding duration is a risk factor for atopic eczema. Clin Exp Allergy 32(2):205–209
28. Oberle D, Von Kries R, Von Mutius E (2001) Asthma and breast feeding. Thorax 56(11):896
29. O'Tierney PF, Barker DJ, Osmond C, Kajantie E, Eriksson JG (2009) Duration of breast-feeding and adiposity in adult life. J Nutr 139(2):422S–425S
30. Thior I, Lockman S, Smeaton LM et al (2006) Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. JAMA 296(7):794–805
31. Iliff PJ, Piwoz EG, Tavengwa NV et al (2005) Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. AIDS 19(7):699–708
32. de la Rosa G, Yang D, Tewary P, Varadhachary A, Oppenheim JJ (2008) Lactoferrin acts as an alarmin to promote the recruitment and activation of APCs and antigen-specific immune responses. J Immunol 180(10):6868–6876
33. Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, Mazurier J (2008) Lactoferrin structure and functions. Adv Exp Med Biol 606:163–194
34. Lopez Alvarez MJ (2007) Proteins in human milk. Breastfeed Rev 15(1):5–16
35. Okuda M, Nakazawa T, Yamauchi K et al (2005) Bovine lactoferrin is effective to suppress Helicobacter pylori colonization in the human stomach: a randomized, double-blind, placebo-controlled study. J Infect Chemother 11(6):265–269
36. Eriksson A, Shafazand M, Jennische E, Lonnroth I, Lange S (2003) Antisecretory factor-induced regression of Crohn's disease in a weak responder to conventional pharmacological treatment. Inflamm Bowel Dis 9(6):398–400
37. Rueda R, Maldonado J, Narbona E, Gil A (1998) Neonatal dietary gangliosides. Early Hum Dev 53(Suppl):S135–S147
38. Riediger ND, Othman RA, Suh M, Moghadasian MH (2009) A systemic review of the roles of n-3 fatty acids in health and disease. J Am Diet Assoc 109(4):668–679
39. Damsgaard CT, Lauritzen L, Kjaer TM et al (2007) Fish oil supplementation modulates immune function in healthy infants. J Nutr 137(4):1031–1036
40. Hoffman DR, Birch EE, Castaneda YS et al (2003) Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. J Pediatr 142(6):669–677

41. Dunstan JA, Simmer K, Dixon G, Prescott SL (2008) Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 93(1):F45–F50
42. Faber J, Berkhout M, Vos AP, Calder PC, Garssen J, van Helvoort A (2010) Supplementation of healthy elderly with a fish-oil enriched sip feed leads to fast incorporation of EPA into white and red blood cells and results in improved immune responses within one week. Clinical Nutrition Supplements 5(1):1–16
43. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125(6):1401–1412
44. Coppa GV, Zampini L, Galeazzi T, Gabrielli O (2006) Prebiotics in human milk: a review. Dig Liver Dis 38(Suppl 2):S291–S294
45. Morrow AL, Rangel JM (2004) Human milk protection against infectious diarrhea: implications for prevention and clinical care. Semin Pediatr Infect Dis 15(4):221–228
46. Boehm G, Jelinek J, Knol J et al (2004) Prebiotics and immune responses. J Pediatr Gastroenterol Nutr 39(Suppl 3):S772–S773
47. Vos A, M'Rabet L, Stahl B, Boehm G, Garssen J (2007) Immune-modulatory effects and potential working mechanisms of orally applied nondigestible carbohydrates. Crit Rev Immunol 27(2):97–140
48. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G (2006) A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Arch Dis Child 91(10):814–819
49. van Hoffen E, Ruiter B, Faber J et al (2009) A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in infants at high risk for allergy. Allergy 64(3):484–487
50. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G (2008) Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. J Nutr 138(6):1091–1095
51. Weaver CM (2005) Inulin, oligofructose and bone health: experimental approaches and mechanisms. Br J Nutr 93(Suppl 1):S99–S103
52. Watzl B, Girrbach S, Roller M (2005) Inulin, oligofructose and immunomodulation. Br J Nutr 93(Suppl 1):S49–S55
53. Spindler-Vesel A, Bengmark S, Vovk I, Cerovic O, Kompan L (2007) Synbiotics, prebiotics, glutamine, or peptide in early enteral nutrition: a randomized study in trauma patients. JPEN J Parenter Enteral Nutr 31(2):119–126
54. Commane DM, Shortt CT, Silvi S, Cresci A, Hughes RM, Rowland IR (2005) Effects of fermentation products of pro- and prebiotics on trans-epithelial electrical resistance in an in vitro model of the colon. Nutr Cancer 51(1):102–109
55. Caffarelli C, Bernasconi S (2007) Preventing necrotising enterocolitis with probiotics. Lancet 369(9573):1578–1580
56. Harmsen D, Rothganger J, Singer C, Albert J, Frosch M (1999) Intuitive hypertext-based molecular identification of micro-organisms. Lancet 353(9149):291
57. Perez PF, Dore J, Leclerc M et al (2007) Bacterial imprinting of the neonatal immune system: lessons from maternal cells? Pediatrics 119(3):e724–e732
58. Neu J, Caicedo R (2005) Probiotics: protecting the intestinal ecosystem? J Pediatr 147(2):143–146
59. van Baarlen P, Troost FJ, van Hemert S et al (2009) Differential NF-{kappa}B pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. Proc Natl Acad Sci USA 106(7):2371–2376
60. Schouten B, van Esch BC, Hofman GA et al (2009) Cow milk allergy symptoms are reduced in mice fed dietary synbiotics during oral sensitization with whey. J Nutr 139(7):1398–1403
61. Pitkala KH, Strandberg TE, Finne Soveri UH, Ouwehand AC, Poussa T, Salminen S (2007) Fermented cereal with specific bifidobacteria normalizes bowel movements in elderly nursing home residents. A randomized, controlled trial. J Nutr Health Aging 11(4):305–311
62. Douglas RM, Hemila H, Chalker E, Treacy B (2007) Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev (3):CD000980

63. Kovacs CS (2008) Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr 88(2):520S–528S
64. Carver JD (1999) Dietary nucleotides: effects on the immune and gastrointestinal systems. Acta Paediatr Suppl 88(430):83–88
65. Murakami M, Dorschner RA, Stern LJ, Lin KH, Gallo RL (2005) Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. Pediatr Res 57(1):10–15
66. Peterson JA, Hamosh M, Scallan CD et al (1998) Milk fat globule glycoproteins in human milk and in gastric aspirates of mother's milk-fed preterm infants. Pediatr Res 44(4):499–506
67. Newburg DS, Ruiz-Palacios GM, Morrow AL (2005) Human milk glycans protect infants against enteric pathogens. Annu Rev Nutr 25:37–58
68. Eiwegger T, Stahl B, Schmitt J et al (2004) Human milk-derived oligosaccharides and plant-derived oligosaccharides stimulate cytokine production of cord blood T-cells in vitro. Pediatr Res 56(4):536–540
69. Bruzzese E, Volpicelli M, Squaglia M, Tartaglione A, Guarino A (2006) Impact of prebiotics on human health. Dig Liver Dis 38(Suppl 2):S283–S287
70. Garofalo RP, Goldman AS (1999) Expression of functional immunomodulatory and anti-inflammatory factors in human milk. Clin Perinatol 26(2):361–377
71. Duchen K (2001) Are human milk polyunsaturated fatty acids (PUFA) related to atopy in the mother and her child? Allergy 56(7):587–592
72. Gottrand F (2008) Long-chain polyunsaturated fatty acids influence the immune system of infants. J Nutr 138(9):1807S–1812S
73. Sijben JW, Calder PC (2007) Differential immunomodulation with long-chain n-3 PUFA in health and chronic disease. Proc Nutr Soc 66(2):237–259
74. Chipman DM, Sharon N (1969) Mechanism of lysozyme action. Science 165(892):454–465
75. Ellison RT 3rd, Giehl TJ (1991) Killing of gram-negative bacteria by lactoferrin and lysozyme. J Clin Invest 88(4):1080–1091
76. Sanchez L, Calvo M, Brock JH (1992) Biological role of lactoferrin. Arch Dis Child 67(5):657–661
77. Tanaka K, Ikeda M, Nozaki A et al (1999) Lactoferrin inhibits hepatitis C virus viremia in patients with chronic hepatitis C: a pilot study. Jpn J Cancer Res 90(4):367–371
78. Lara-Villoslada F, Olivares M, Sierra S, Rodriguez JM, Boza J, Xaus J (2007) Beneficial effects of probiotic bacteria isolated from breast milk. Br J Nutr 98(Suppl 1):S96–S100
79. Martin R, Heilig GH, Zoetendal EG, Smidt H, Rodriguez JM (2007) Diversity of the Lactobacillus group in breast milk and vagina of healthy women and potential role in the colonization of the infant gut. J Appl Microbiol 103(6):2638–2644
80. Diaz-Ropero MP, Martin R, Sierra S et al (2007) Two Lactobacillus strains, isolated from breast milk, differently modulate the immune response. J Appl Microbiol 102(2):337–343
81. Gueimonde M, Laitinen K, Salminen S, Isolauri E (2007) Breast milk: a source of bifidobacteria for infant gut development and maturation? Neonatology 92(1):64–66
82. van't Land B, Benlhassan-Chahour K, Rizzardini G, Vriesema A, Garssen J, Trabattoni D, Bray D, Gori A, Clerici M (CROI 2008) A Specific Mixture of Prebiotic Oligosaccharides Reduces Hyper-Immune Activation and Improves NK Cell Cytolytic Activity in HAART-naive HIV Positive Adults, Poster # 723
83. Gori A, Rizzardine G, Van't Land B, Ben Amor K, Van Schaik J, Moroni M, Torti C, Quirino T, Trabattoni D, Tincati C, Bandera A, Benlhassan-Chabour K, Bray D, Knol J, Vriesema A, Welling G, Garssen J, Clerici M (submitted 2010). Specific prebiotics positively modify gut microbiota and immune activation in HAART-naïve HIV infected adults

3 Role of Maternal and Infant Malnutrition on the Development of the Inflammatory Response

Maristella A. Landgraf, Richardt G. Landgraf, and Zuleica B. Fortes

Key Points

- The thrifty phenotype hypothesis holds that intrauterine malnutrition leads to an adaptive response that alters the fetal metabolic and hormonal milieu designed for intrauterine survival. This fetal programming predisposes individuals to several diseases in adulthood.
- Fetal nutrition is an important key regulator of fetal growth and thus an obvious candidate as a possible programming influence.
- Fetal overexposure to maternal glucocorticoids triggers programming events in utero; these effects appear to be relevant to changes in utero because there are strong correlations between birth weight, plasma cortisol concentrations, and the development of hypertension and type 2 diabetes.
- Malnutrition, an important cause of immunosuppression and undernutrition during critical periods of gestation, neonatal maturation, and weaning, can lead to clinically significant immune deficiency and infections in children.
- Micronutrients have a relationship to antibody formation and the development of the immune system, and micronutrient deficiencies are related to poor growth, impaired intellect, and increased mortality, and susceptibility to infection.

Key Words: Programming, thrifty genotype, inflammation, intrauterine malnutrition, glucocorticoids, insulin, predictive adaptive response, immune system.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_3

3.1 PROGRAMMING

3.1.1 The Early Origins of Adult Disease: Programming Hypothesis

The hypothesis that adult diseases are related to fetal origins was originally put forward by David Barker and colleagues in Southampton in the United Kingdom. The hypothesis stated that environmental factors, particularly nutrition, act in early life to program the risks for the early onset of cardiovascular and metabolic disease in adult life and premature death (1). However, before Barker, other authors related early life events to diseases developed in adult life (2, 3). In 1914, it had been suggested by the chief medical officer to the Board of Education in Britain that, "recent progress has shown that the health of the adult is dependent upon the health of the child and that the health of the child is dependent upon the health of the infant and its mother" (4). Anders Forsdahl attempted to explain the association between poverty during adolescence and diseases in adult life (5, 6). In Norway in the 1970s, Forsdahl, in an epidemiological study, found a significant positive correlation between the county age-adjusted mortality from arteriosclerotic heart disease in people aged between 40 and 69 years and county infant mortality relating to the early years in the same cohorts. He concluded that great poverty in childhood and adolescence followed by prosperity was a risk factor for arteriosclerotic heart disease (3). In 1986, Barker and Osmond (7) observed that there were different rates of mortality from stroke and cardiovascular diseases in geographical regions of England and Wales. They noted that the geographical distribution of mortality rates from stroke and cardiovascular diseases in 1968–1978 was closely related to neonatal mortality in 1921–1925, and they concluded that poor nutrition in early life increased susceptibility and was an important determinant of the risk of stroke in their offspring. Barker and colleagues presented several studies that detailed the relationship between low birth weight and subsequent adult cardiovascular disease. In these studies, it was hypothesized that adverse environmental factors in early life cause disruption of normal growth and development, leading to an adult phenotype that was more susceptible to cardiovascular diseases (8–11). Experimental studies in animals have documented many examples of fetal programming (12–14), which increased the credibility of this hypothesis.

Programming, or imprinting, reflects the action of a factor during a sensitive developmental period or "window" that affects the development and organization of specific tissues that are concurrently vulnerable, producing effects that persist throughout life. Of course, different cells and tissues are sensitive at different times, so the effects of environmental challenges will have distinct effects depending not only on the challenge involved, but also upon its timing (15).

This adaptive response includes changes in hemodynamics, metabolism, hormone production, and tissue sensitivity and may affect the development of various organs, thereby predisposing individuals to cardiovascular, metabolic, and endocrine diseases in adult life (11). In industrialized countries, an adverse fetal environment may be caused by maternal life style habits, including suboptimal dietary intake, smoking, and alcohol consumption (16), but nutrition is a major determinant of body size in utero and later life (17). The association between low birth weight and later disease risk should be interpreted as reflecting the long-term consequences of fetal adaptive responses. In this model, low birth weight itself is not the causal factor per se but merely a marker of fetal adaptive responses to suboptimal exposures. These adaptive responses are not necessarily evident at birth but may result in diseases in later life (16).

Poor people in developing countries can be viewed as having been exposed to four phases during which nutritional deprivation may have imprinted itself on their metabolic make-up: an evolutionary phase with frequent famines selecting what Neel (18) termed a *thrifty genotype*; an intergenerational phase in which the failure of women to grow to their full genetic potential imposes uterine restraint on the developing fetuses; a fetal phase; and a postnatal phase. J.V. Neel had proposed that "thrifty" genes were selected during evolution at a time when food resources were scarce and that they resulted in a "fast insulin trigger" and thus an enhanced capacity to store fat, which placed the individual at risk for insulin resistance and type 2 diabetes (18, 19).

An intergenerational phase in which the failure of women to grow to their full genetic potential imposes uterine restraint on the developing fetuses, a fetal phase and a postnatal phase might create what Hales and Barker (20) have termed a "*thrifty phenotype*." This hypothesis suggested that adult insulin resistance and type 2 diabetes could result from the persistence of a fetal glucose-conserving adaptation in response to intrauterine hypoglycemia. During periods of maternal undernutrition, the fetus reduces insulin secretion and increases peripheral insulin resistance, thus directing more glucose to the brain and the heart and less to insulin-dependent tissues, such as skeletal muscle (21, 22). When nutrient availability is abundant in postnatal life, this pancreatic β-cell defect and peripheral insulin resistance could then cause glucose intolerance and eventually diabetes. This would explain why it is mainly thin babies who then become overweight during childhood who are prone to developing type 2 diabetes later in life (23).

All the adaptations observed in the offspring due to the intrauterine and infancy environments are manifestations of the general phenomenon of developmental plasticity. Like other living creatures, human beings are "plastic" and able to adapt to their environment. The development of the sweat glands provides a simple example of this. All humans have similar numbers of sweat glands at birth, but none of them function. In the first 3 years after birth, a proportion of the glands became functional, depending on the temperature to which the child is exposed. The hotter the conditions, the greater the number of sweat glands that are programmed to function. After 3 years, the process is complete, and the number of sweat glands is fixed. Thereafter, the child who has experienced hot conditions will be better equipped to adapt to similar conditions in later life because people with more functioning sweat glands cool down faster (24).

This brief description encapsulates the essence of developmental plasticity: a critical period when a system is plastic and sensitive to the environment, followed by the loss of plasticity and a fixed functional capacity. For most organs and systems, the critical period occurs in utero (24). The formal definition of developmental plasticity is the ability of a single genotype to produce more than one alternative structural form, physiological state or behavior in response to environmental conditions (25).

3.1.2 The Predictive Adaptive Response

Gluckman and Hanson (26) revised and extended the thrifty phenotype hypothesis. They proposed that when there is a change in the intrauterine environment, for example, nutrient restriction or high glucocorticoid levels, the fetus will adapt to improve its immediate chances of survival. These adaptations are often reversible. However, if the environmental changes persist, the fetus is forced to make irreversible adaptations that may not be immediately beneficial, but that will manifest themselves later in life.

In this way, the fetus is preparing itself for life in an extrauterine environment with, for example, low food availability or high levels of stress. Gluckman and Hanson (27) coined the term "predictive adaptive response" for this phenomenon. When this response is appropriate, the phenotype is normal; however, where mismatch occurs between the predicted and actual environment, diseases manifest. There are examples of predictive adaptive responses from the animal world. For example, the meadow vole pup is born with a thicker coat in the autumn than in the spring. In this case, the dams exerted strong control over the development of photosensitive traits in offspring because changes in day length, signaled to the pup in utero by maternal melatonin levels, result in adaptive changes in coat thickness in anticipation of the extra-uterine environment being cold or warm (28).

Unlike previous programming hypotheses, contends that in response to a given in utero or early postnatal nutritional plane (either high or low), cellular processes are tuned to cope with the predicted environment and that these adaptations are not necessarily advantageous in utero. Thus, it is proposed that disease only manifests when the actual adult diet diverges from this plane which the fetus has predicted (29).

Such decreases in structure, and hence the lifelong functional capacity of an organ system, may be an inadvertent consequence of a decrease in energy supply across the placenta or a selective trade-off to maintain the development of more important tissues, such as the brain. At this stage, it is not clear that such responses are either adaptive or predictive, although it is clear that they will result in the programming of a reduced functional capacity for life (30). So, in this chapter we will use programming as a term that allows the inclusion of such developmental deficits and their consequences.

3.1.3 Possible Mechanisms Involved in Developmental Origin Hypothesis

The most widely accepted phenomenon proposed to underlie the developmental origin hypothesis is that of programming. This is the process whereby a stimulus or insult during a sensitive or critical period has irreversible long-term effects on development. Well-recognized mechanisms include altered fetal nutrition and increased glucocorticoid exposure. However, there may also be genetic and epigenetic links in addition to other hormonal factors, such as insulin (22).

3.1.3.1 Fetal Malnutrition

Fetal nutrition is a key regulator of fetal growth and thus an obvious candidate as a possible programming influence (31). Studies investigating the historical cohort of the Dutch winter famine (32, 33), which was a 5-month period of malnutrition experienced by pregnant women during the winter of 1944–1945 in Amsterdam, the Netherlands, found that individuals who were exposed to the famine as an embryo or fetus during early gestation had an increased prevalence of coronary heart disease, increased body mass index, and glucose intolerance due to a deficit in insulin secretion.

Offspring of dams exposed to caloric restriction during pregnancy are generally born with low birth weight (34, 35), increased blood pressure (35), and impaired glucose tolerance (36). Variations in dietary patterns and micronutrient intake also seem to be relevant. Studies showed that low folate acid levels and high homocysteine levels are associated with both low birth weight and increased blood pressure in children (37, 38).

Folate levels also seem to be associated with endothelial dysfunction in children, which is an early risk factor for the development of atherosclerosis in adulthood (39). There are many possible mechanisms by which altered fetal nutrition might lead to the increased risk of disease in the offspring (40), but it is useful to think of altered fetal nutrition as leading, directly or indirectly, to altered growth and maturation of various fetal organ systems. Permanent changes in the homeostatic regulation of these systems could then lead to an increased risk of subsequent disease, especially when placed under increased stress after birth by additional risk factors, such as aging and obesity (22).

3.1.3.2 Glucocorticoids

Fetal overexposure to maternal glucocorticoids in both human and animal models triggers programming events in utero (13) that establish an increase in glucocorticoid action throughout life (41). These effects of the glucocorticoids appear to be relevant to changes in utero because there are strong correlations between birth weight, plasma cortisol concentrations, and the development of hypertension and type 2 diabetes (42). This may occur if exogenous synthetic glucocorticoids are administered or if the placental barrier that protects the fetus from high levels of maternal glucocorticoids is impaired. In rats, the offspring of dams given dexamethasone during pregnancy have reduced birth weight, increased blood pressure (43), and glucose intolerance in adulthood (44). Repeated doses of betamethasone given to pregnant sheep have similar effects (45).

Fetal glucocorticoid levels are lower than maternal levels, and the fetus is normally protected, to some degree, from maternal glucocorticoids by the presence of the barrier enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2). However, this enzyme is downregulated by maternal undernutrition, thus potentially exposing the fetus to increased glucocorticoids (46) and causing retarded growth and programming responses that lead to later adult disease. In support of this proposal, deleterious mutations of the 11β-HSD-2 gene in man are associated with reduced birth weight (47) Fig. 3.1.

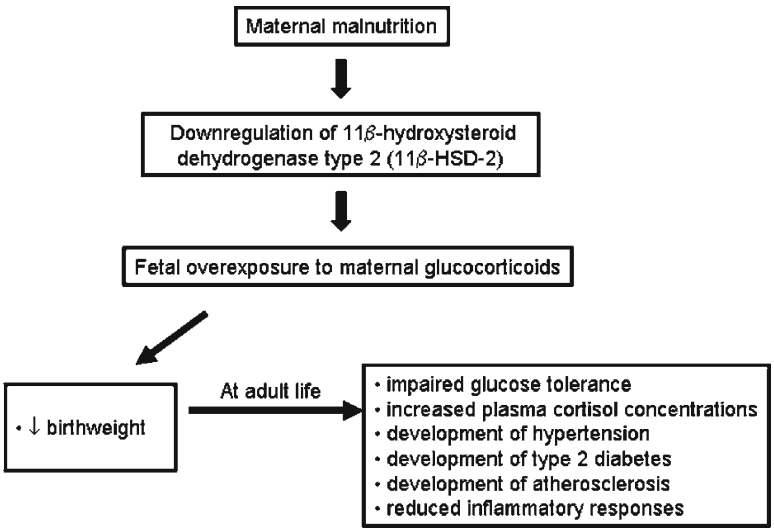


Fig. 3.1. Intrauterine programming by maternal malnutrition and its consequences at adult life.

3.1.3.3 Fetal Insulin Hypothesis

Lower birth weight is associated with an increased risk of adult coronary heart disease, insulin resistance, type 2 diabetes, and metabolic syndrome (48). Another hypothesis for this situation is that genes that either increase insulin resistance or reduce insulin secretion result in both impaired insulin-mediated fetal growth and later coronary heart disease and diabetes (the "fetal insulin hypothesis") (49). Since insulin is an important regulator of fetal growth, affected individuals with impaired insulin secretion would have impaired growth before birth and would also go on to have impaired glucose tolerance in adulthood. However, these relatively rare changes seem unlikely to explain the very widespread relationship between birth size and later glucose tolerance described in many different populations and across the range of normal birth weights (22). Currently, there is little direct evidence for this hypothesis, although two studies have shown an inverse association between a father's risk of diabetes and the birth weight of his offspring (50, 51). Findings related to paternal diabetes are of particular interest because they cannot directly reflect an influence of the intrauterine environment. Conversely, impaired glucose tolerance in pregnant women is associated with greater offspring birth weight, and, as women with impaired glucose tolerance during pregnancy are at an increased risk of developing diabetes, the expectation is of a positive association between offspring birth weight and maternal diabetes risk (22). This evidence suggests a link between birth weight and diabetes that is not dependent on the intrauterine environment.

3.1.3.4 Genetic and Epigenetic Links

Fetal nutrition exposure may lead to fetal adaptive responses by epigenetic modifications. Epigenetic refers to covalent modifications of DNA and core histones that regulate gene activity without altering the nucleotide sequence of DNA (52). During early embryogenesis, DNA undergoes demethylation and remethylation, a process that involves "labeling" of some genes as being of maternal or paternal origin, and marks these genes for subsequent inactivation. This epigenetic process of imprinting is thought to particularly affect many of the genes regulating fetal and placental growth (53). Study in sheep showed that lower levels of maternal folate and vitamin B12 supplementation are associated with alterations in the methylation status of CpG islands (a short stretch of DNA in which the frequency of the CG sequence is higher than in other regions; the "p" in CpG island simply indicates that the "C"-cytosine and "G"-guanine are connected by a phosphodiester bond) in the offspring, which leads to widespread epigenetic modifications to the genome associated with increased adiposity, insulin resistance, altered immune function, and high blood pressure in adult offspring (54). Mice treated with an inhibitor of DNA methylation (5-azacytidine) in early postnatal life have altered allelic expression of insulin-like growth factor II (IGF-2) (55). More recently, persistent impaired methylation of the IGF-2 gene was reported in adults who were exposed to the Dutch Famine. These results suggest that epigenetic modifications induced by adverse nutritional exposures in the periconceptional period may persist throughout postnatal life and could affect the development of risk factors for cardiovascular disease and type 2 diabetes. This is because IGF-2 has a role in pancreatic β-cell development, and the altered expression of this gene could affect insulin secretion (56). Thus, changes in the intrauterine environment may ultimately lead to altered gene expression via alterations

in DNA methylation and other epigenetic mechanisms, resulting in increased susceptibility to chronic disease in adulthood (52).

3.1.3.5 Early Post Natal Malnutrition

The early postnatal period is also a period of physiological plasticity, although the timing of this "window of opportunity" may differ depending on the outcome of interest and may be species- and gender-specific (57). The composition of milk understandably has a major impact on the developing neonate. As would be expected, the maternal diet is a primary determinant of milk composition. Feeding dams a "cafeteria diet" that is composed of highly palatable junk foods increases the long-chain and decreases the medium-chain fatty acid content of their milk. Intake of such a diet has an additive effect to the presence of maternal obesity in lowering the protein content and raising the long-chain fatty acid content of the milk (58). Feeding dams a high-fat diet also accelerates the onset of independent feeding in neonates by 1–2 days (59) in association with increased weight gain (60) and the development of hypertension and abnormal glucose homeostasis as adults (61). Rats exposed to a high-fat diet in utero and during the suckling period are somewhat protected from developing endothelial dysfunction if they are maintained on the same high-fat diet throughout life (62). We conclude that manipulations of the early postnatal environment can have profound effects on the development of offspring. The mechanisms underlying these effects are currently unknown. Changes in the composition and amount of maternal milk may be among the most important of these, although maternal-pup behavioral interactions are also likely to affect the outcome (63).

3.1.3.6 Intergenerational Effects

It is now becoming clear that adverse events during pregnancy can affect not only the offspring of that pregnancy, but also the next generation (22). Feeding rats a protein-deficient diet over 12 generations resulted in progressively greater fetal growth retardations over the subsequent generations. When the rats were refed with a normal diet, it then took three generations to normalize their growth and development (64). A similar effect was seen during the Dutch hunger winter of 1944–1945, a 5-month period of severe famine at the end of the Second World War. Women who were severely undernourished during the first trimester of pregnancy gave birth to babies who were, on average, of normal birth weight; however, those babies themselves then went on to give birth to smaller babies in the next generation (65). In the rat where the F1 generation is exposed in utero to a low-protein diet, the F2 generation demonstrates insulin resistance, especially if the F1 generation was fed a high-fat diet (66). There are several possible mechanisms for these intergenerational effects. First, growth-retarded fetuses have smaller uteri as adults, constraining the growth of their fetuses further (67). Second, any epigenetic changes to the genome may be passed on to the second generation (68).

3.2 IMMUNE SYSTEM AND NUTRITIONAL DEFICIENCY

It has long been accepted that immunity depends to some extent on nutrition. In fact, nutritional deficiency is commonly associated with an impaired immune response (69). Primary malnutrition caused by an inadequate supply of either macronutrients or selected micronutrients can lead to clinically significant immune deficiency and infections in

children. An inadequate dietary intake leads to weight loss, lowered immunity, mucosal damage, invasion by pathogens, and impaired growth and development in children (70).

The immune system protects the host against pathogenic organisms, and highly complex pathways of recognition, response, elimination, and memory have evolved in order to fulfill this role. A breakdown in these pathways or their inefficient operation can lead to increased susceptibility to, and increased morbidity and mortality as a result of, infectious disease (71).

The immune system consists of organs and several cell types that recognize foreign antigens. The primary immunological organs consist of the bone marrow and the thymus, and the secondary organs include the spleen, mesenteric lymph nodes and Payer's patches. The immune cells can be grouped into two categories: lymphocytes and phagocytes. The latter group includes monocytes, macrophages, and neutrophils (72). In an immune response, the antigen is processed and presented to lymphocytes; lymphocytes not only need to recognize the antigen with their receptor, but also need to engage a costimulation molecule. This is followed by the activation of signaling molecules, which leads to the engagement of nuclear factor-κB, gene activation, and mRNA transcription, followed by the synthesis and secretion of various cytokines. The secreted cytokines bind their appropriate receptors, leading to the clinical manifestations of various diseases (73).

Various alterations, such as impaired immune responses, particularly cell-mediated immunity, phagocyte function, cytokine production, the complement system, secretory antibody responses and antibody affinity can occur in nutritional deficiency (74–76).

Severe protein malnutrition in newborns and small children causes atrophy of the thymus with reduced cell numbers and subsequently ill-developed peripheral lymphoid organs, i.e. the lymph nodes and spleen. This phenomenon is largely, but not exclusively, due to changes in the lymphoid compartment. In fact, thymocyte depletion appears as an outcome of both acute and chronic experimental protein malnutrition. The overall malnutrition-related thymocyte depletion seems to result from enhanced thymocyte death plus decreased thymocyte proliferation (77). This causal chain leads to long-lasting immune defects characterized by leucopenia, a decreased CD4 to CD8 ratio, and increased numbers of CD4/CD8 double-negative T cells and, therefore, the appearance of immature T cells in the periphery (78). However, previous reports have indicated that the impaired immune response observed in severely malnourished and infected children is mainly related to altered lymphocyte function rather than decreased numbers of T cell subsets (79, 80). It has also been suggested that T lymphocytes are unable to secrete normal quantities of cytokines that mediate and regulate the activation, differentiation, and proliferation of lymphocytes required to achieve an adequate immunological response (81).

Although T and B lymphocytes represent effector cells of the immune system, the functional capacities of lymphocytes, especially the induction and function of antigen-specific lymphocytes, are regulated by antigen-presenting dendritic cells (82). Niiya et al. (83) demonstrated that in protein calorie malnourished mice, the number of spleen dendritic cells, the T lymphocyte-stimulatory capacities of dendritic cells, and their production of IL-12p70 and IFN-γ were reduced. The authors suggested that chronic undernutrition disrupts antigen-specific immune responses and that this disruption can be attributed, at least in part, to reduced frequencies and impaired functions of dendritic cells.

Macrophages, cells that occupy a vital role in regulating both the innate and adaptive immune systems, are dysfunctional under conditions of protein calorie malnutrition. This dysfunction includes significantly diminished proinflammatory cytokine production,

decreases in respiratory burst activity, impaired phagocyte activity, and significant decreases in the cell yield of peritoneal macrophages (84, 85). Anstead et al. (86) demonstrated that macrophages from a murine model of multinutrient undernutrition that were stimulated with IFN-γ/LPS showed increased IL-6 production (a potentially immunosuppressive mediator) and decreased IL-10 and TNF-α production. Neutralization of TNF-α in macrophage cultures from the control mice mimicked the effect of malnutrition on NO and IL-10 production (where these productions are reduced), whereas supplemental TNF-α added to cultures of macrophages from malnourished mice increased NO secretion. Redmond et al. (85) also demonstrated that superoxide anion production in resident and activated (LPS, IFN-γ and bacille Calmette-Guérin infection) peritoneal macrophages were significantly reduced in malnourished mice. Candida phagocytosis and killing were also both depressed.

Complement is part of the innate immune system and underlies one of the main effector mechanisms of antibody-mediated immunity. It has three overarching physiologic activities: defending against pyogenic bacterial infection, bridging innate and adaptive immunity, and disposing of immune complexes and the products of inflammatory injury (87). In protein malnutrition, the concentrations and activity of most complement components are decreased. The best documented of these is a reduction in C3, C5, factor B and total hemolytic activity. There was a slight reduction in the opsonin activity of plasma. Furthermore, metabolic activation and intracellular destruction of bacteria were also reduced (88)**.**

IgG from the mother acquired through placental transfer is the principal immunoglobulin in cord blood. All four subclasses of IgG are detected in fetal sera as early as 16 weeks of gestation, the bulk consisting of IgG1. In small for gestation infants, the cord blood levels of IgG1 are reduced much more than in those of the other subclasses; the number of immunoglobulin-producing cells and the amount of immunoglobulin secreted is decreased in small for gestation infants who are symptomatic, that is, those who have recurrent infections (89).

3.2.1 Micronutrients and Immunity

Worldwide, almost two billion people are affected by micronutrient deficiencies, including vitamins A, C, and E and the minerals zinc, iron, and iodine. The effects are poor growth, impaired intellect, increased mortality, and susceptibility to infection. Micronutrients have a relationship to antibody formation and the development of the immune system (90).

Vitamin A deficiency impairs innate immunity by impeding normal regeneration of the mucosal barriers damaged by infection and by diminishing the function of neutrophils, macrophages, and natural killer cells. Vitamin A is also required for adaptive immunity and plays a role in the development of both helper T (Th) cells and B cells. In particular, vitamin A deficiency diminishes antibody-mediated responses directed by Th2 cells, although some aspects of Th1-mediated immunity are also diminished (91).

Vitamin E is a strong antioxidant that can support monocyte/macrophage-mediated response, and this deficiency causes increases in the susceptibility of animals to infectious pathogens due to decreases in spleen lymphocyte proliferation, natural killer cell activity, specific antibody production following vaccination, and phagocytosis by neutrophils (92).

Vitamin C can affect phagocyte function, T cell proliferation, and the production of inflammatory cytokines. Li et al. (93) demonstrated that vitamin C-deficient mice had greater lung pathology late after infection. This could be due to reduced immune responses: vitamin C-deficient male mice had less *RANTES and MCP-1* mRNA production in the lungs and decreased immune cell infiltration. Less production of chemokines and cytokines at an early stage of the infection and changes in the migration ability of inflammatory cells caused by vitamin C-deficiency might be involved.

Low serum vitamin D concentrations have also been associated with complications, such as tuberculosis, cancer (prostate, breast, and colon), multiple sclerosis, and diabetes, indicating that vitamin D possesses important pleiotropic actions besides calcium homeostasis and bone metabolism. A recent epidemiological study clearly demonstrates the link between vitamin D deficiency and the increased incidence of respiratory infections (94). Besides, vitamin D deficiency has been linked to several different diseases, including the immune system-mediated diseases ulcerative colitis and Crohn's disease (95).

Zinc is crucial for the normal development and function of cells mediating the innate immunity, including neutrophils and NK cells. Macrophages, phagocytosis, and intracellular killing are affected by zinc deficiency. Additionally, Th1 cytokines and thymic hormone activity are reduced. Zinc deficiency adversely affects the bone marrow environment, reducing the number of nucleated cells and the number of and proportion of cells which are lymphoid precursors (96). Low plasma zinc levels predicted the subsequent development of lower respiratory tract infections and diarrhea among Indian infants (97). Zinc administration to preterm low-birth-weight infants increased the number of circulating T lymphocytes and lymphocyte proliferation. However, excessive zinc intake causes copper depletion and impairs the immune response, decreasing lymphocytes and phagocyte function (98).

Copper has an important role in immune system function, and copper deficiency causes reduced T cell proliferation and neutropenia and decreases the ability of neutrophils to generate superoxide anion and kill ingested microorganisms (99). Both copper deficiency and a high intake over longer periods can modulate several aspects of the immune response (100).

It is well established that iron is essential to several immune system functions, and a deficiency results in impaired cell-mediated immunity. NK activity and neutrophil functions, such as myeloperoxidase activity, which is involved in the killing process of bacteria, are impaired in iron deficiency, along with the reduced T-cell response and IL-2 production. On the other hand, pathogens such as infectious microorganisms and viruses require iron and other micronutrients for replication and survival, and *in vitro* studies have shown that the provision of iron to rodents increases the pathogenicity of a number of bacteria (101–103). Whereas, it seems essential to restrict the access of the infecting microorganism to iron, it is important to maintain a suitable concentration of iron that allows the host to mount an optimum immune response. Avoiding excess amounts of iron is also important since it may induce free radical-mediated damage (102).

Selenium is essential for optimum immune responses and influences the innate and acquired immune systems. In both animal and human studies, selenium supplementation has been shown to increase T-cell proliferation and natural killer cell cytotoxicity (103, 104). On the other hand, selenium deficiency has been demonstrated to result in more severe viral infections, including HIV (105, 106).

3.3 DEVELOPMENT OF THE INFLAMMATORY RESPONSE AND LUNG ALLERGIC INFLAMMATION IN INTRAUTERINE MALNUTRITION

The activation and migration of leukocytes is an essential step in the inflammatory response, and polymorphonuclear neutrophils represent the first-line in host defense, acting to eliminate invading bacteria at the site of infections (107, 108). Leukocyte extravasation is orchestrated by the combined action of cellular adhesion receptors and chemotactic factors and involves radical morphological changes in both leukocytes and endothelial cells. Selectins, integrins, and the immunoglobulin gene superfamily of adhesion receptors mediate different steps of leukocyte migration from the bloodstream toward the inflammatory foci (109) Fig. 3.2.

A reduction in the inflammatory response has been observed in children with nutritional deficiencies and, in particular, malnutrition. Studies have shown that nutritional deficiencies and other influences that reduce growth during critical periods of life can permanently affect the structure and physiology of a variety of organs and tissues.

Several infectious diseases are more severe in nutritionally deprived children. Children with kwashiorkor[1] presented with defective leukocyte mobilization, manifest as an early delayed macrophage migration into dermal abrasions, in addition to severe changes in liver metabolism, a reduction of fractions of the complement system, reduced phagocytic activity, and a reduction of circulating T3 and T4 hormones (110, 111). In fact, most children with severe protein-energy malnutrition have asymptomatic infections because their immune system fails to respond with chemotaxis, opsonization, and phagocytosis of bacteria, viruses, or fungi (112). Protein calorie malnutrition contributes to increased morbidity and mortality through the impairment of host defense mechanisms and reduced macrophage function. Peritoneal macrophages from mice with protein calorie malnutrition produced significantly less TNF-alpha and IL-6 and had significantly less cell-associated IL-6 when compared to macrophages from control mice (113). In laboratory animals subjected to protein deficiency, protozoan and helminth infections are typically facilitated (114).

Malnourished people, small for gestational age neonates, and low birth weight babies often have higher rates of infection by enteropathogens, such as *Shigella* spp. The incidence and severity of pneumonia and diarrhea are higher with high mortality rates (115). Most of the data suggests increased susceptibility to infectious diseases in individuals with protein energy malnutrition, increases that may be related to the reduction of activation and leukocyte migration (116).

In experimental studies of mice and guinea pigs, it was demonstrated that protein energy malnutrition reduced the granuloma formation and macrophage activation induced by the administration of bacillus Calmette-Guerin vaccine and impaired effector mechanisms, including intracellular pathogen rejection (117, 118). In rats, such malnutrition was found to decrease monocyte migration and to limit the inflammatory response induced by chemical irritants (119, 120).

[1]Kwashiorkor is a type of severe malnutrition that usually manifests with edema, changes to hair and skin color, anemia, hepatomegaly, lethargy, severe immune deficiency, and early death (136).

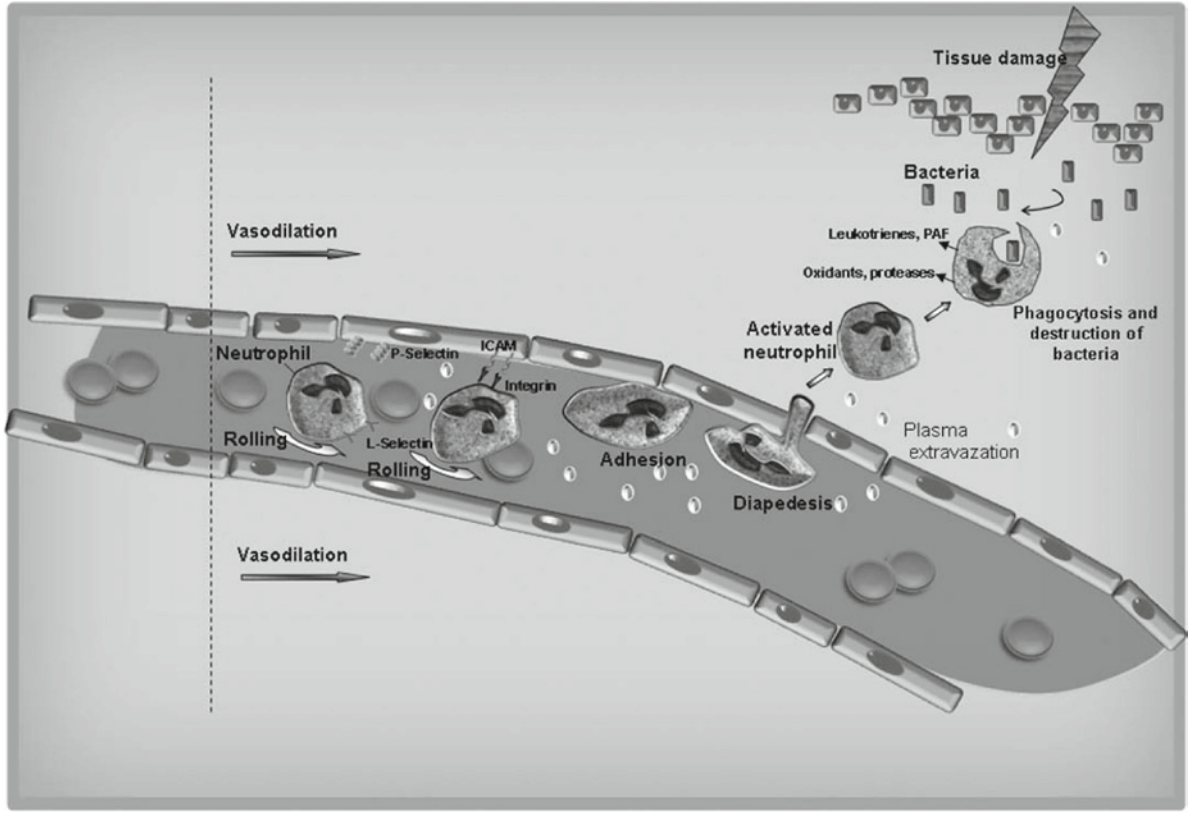


Fig. 3.2. Inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. The response includes the attraction of phagocytes in a chemotactic gradient of microbial products, movement of the phagocyte to the inflammatory site and contact with organism and phagocytosis of the organism.

Malnutrition, an important cause of immunosuppression (121) and undernutrition in critical periods of gestation and neonatal maturation and weaning, impairs the development and differentiation of a normal immune system. Intrauterine and early postnatal environments have long-term consequences for the risk of infectious diseases and other diseases with an immunologic component, particularly in developing countries (122).

Postnatal malnutrition in rats can reduce macrophage spreading and phagocytosis of opsonized yeast (123) as well as impairing the inflammatory response (124).

Recently, interest in prenatal conditions, and the effect they may have on health status in later life, has increased. Failure of the materno-placental supply line to satisfy fetal nutrient requirements results in a range of fetal adaptations and developmental changes and may lead to permanent alterations in the body's structure and metabolism (125). Although these adaptations may be beneficial for short-term survival, they can lead to permanent alterations in body structure and metabolism, and thereby to cardiovascular and metabolic disease in adult life (126).

Intrauterine undernourished rats have hypocellularity in the bone marrow and peripheral blood. This factor, associated with the downregulated expression of adhesion molecules, such as L-selectin, P-selectin, and intercellular adhesion molecule-1 (ICAM-1), alters the composition of the basal membrane with a decreased type IV collagen concentration and a reduction of leukotriene B4 (LTB4) release in response to inflammatory stimulus and caused reduced leukocyte migration in these animals. This altered inflammatory response may be involved in the increased predisposition to infections in undernourished subjects (127, 128).

Epidemiological studies have indicated that in humans the incidence of alterations in lung functions can be associated with birth weight and specifically with maternal malnutrition (129, 130). However, with respect to lung allergic inflammation, the data are controversial.

Villamor et al. (131) examined the association between birth weight and asthma during childhood and adult life in Swedish twins and concluded that there is a negative association between birth weight and asthma. Hagstrom et al. (132) analyzed characteristics, such as birth weight, birth height, head circumference, placental weight, and gestational age and concluded that they were not associated with a "programming" factor for asthma. In contrast, other environmental factors in childhood seemed more important than fetal malnutrition for the development of asthma in adult life.

On the other hand, some authors have demonstrated that asthma symptoms are inversely associated with birth weight. Svanes et al. (133) observed that asthma symptoms in a random sample of young Norwegian adults were strongly and inversely associated with birth weight. This was consistent when adjusting for gestational age, other birth characteristics, and adult factors, and the association was similar in smokers and nonsmokers as well as in people with and without hay fever. The association between birth weight and asthma symptoms remained when births complicated by prematurity, low birth weight, or asphyxia were excluded; the association was driven by the full-term births within the normal birth weight range. Kitchen et al. (134) concluded that increased bronchial responsiveness is common in school children who were born substantially preterm.

In a study that investigated the development of asthma in intrauterine undernourished rats, Landgraf et al. (135) observed that rats challenged with ovalbumin at 9 weeks of age had significant decreases in the allergic inflammatory response compared with rats

challenged with ovalbumin at 5 weeks of age. These data indicate that in intrauterine undernourishment models the intensity of the allergic inflammatory response depended on the age at which the organism was challenged.

3.4 CONCLUSIONS AND PERSPECTIVES

Programming is the process whereby a stimulus or insult that occurs during a sensitive or critical period has irreversible long-term effects on development. Well-recognized mechanisms include fetal nutrition and increased corticosteroid exposure. However, there may also be genetic and epigenetic links in addition to other hormonal factors, such as insulin.

Immunity depends to some extent on nutrition. Nutritional deficiency is commonly associated with depression of immune responses in relation to cell-mediated immunity, phagocyte function, cytokine production, the complement system, the secretory antibody response, and antibody affinity. In humans, the incidence of alterations in lung functions can be associated with birth weight and specifically with maternal malnutrition. Intrauterine nutrition is fundamental for the development and functioning of organs and tissues, and intrauterine undernourishment reduced allergic lung inflammation in the offspring. In an intrauterine undernourishment model, the intensity of the allergic inflammatory response depends on the age at which the organism was challenged. It is likely that leptin affects the immune system (i.e. the decrease in allergic inflammatory responses) both via a direct effect and via interference with glucocorticoid levels.

Acknowledgments The authors are grateful to Rafael Larocca for expert assistance with the illustration. The authors acknowledge Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisa e Tecnologia/Programa de apoio a Núcleos de Excelência (CNPq/PRONEX) for the financial support.

REFERENCES

1. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME (1989) Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 298:564–567
2. Kermack WO, McKendrick AG, McKinlay PL (1934) Death-rates in Great Britain and Sweden. Some general regularities and their significance. Lancet I:698–703
3. Forsdahl A (1977) Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? Br J Prev Soc Med 31:91–95
4. Anon (1914) Annual report for 1913 of Chief Medical Officer of the Board of Education, Cd 7330. HMSO, London
5. Forsdahl A (1973) Points which enlighten the high mortality rate in the county of Finnmark. Can the high mortality rate today be a consequence of bad conditions of life in childhood and adolescence? Tidsskr Nor Lwgeforen 93:661–667
6. Forsdahl A, Salmi H, Forsdahl F (1974) Finnish descendants in the municipality of Sor-Varanger-II. An investigation of blood pressure, height, weight, cholesterol, triglycerides and lipoproteins among Finnish descendant men – and the effect of a diet change. Tidsskr Nor Lwgeforen 94:1565–1572
7. Barker DJ, Osmond C (1986) Infant mortality, childhood nutrition, and ischemia heart disease in England and Wales. Lancet 1:1077–1081
8. Barker DJ, Osmond C, Law CM (1989) The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. J Epidemiol Community Health 43:237–240

9. Barker DJ, Bull AR, Osmond C (1990) Fetal and placental size and risk of hypertension in adult life. BMJ 301:259–262
10. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS (1993) Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938–941
11. Barker DJ (1995) Fetal origins of coronary heart disease. BMJ 311:171–174
12. Langley SC, Jackson AA (1994) Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin Sci 86:217–222
13. Langley-Evans SC, Phillips GJ, Benediktsson R, Gardner DS, Edwards CR, Jackson AA, Seckl JR (1996) Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. Placenta 17:169–172
14. Langley-Evans SC, Sherman RC, Welham SJ, Nwagwu MO, Gardner DS, Jackson AA (1999) Intrauterine programming of hypertension: the role of the renin-angiotensin system. Biochem Soc Trans 27:88–93
15. Seckl JR (2004) Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 151:U49–U62
16. Jaddoe VW (2008) Fetal nutritional origins of adult diseases: challenges for epidemiological research. Eur J Epidemiol 23:767–771
17. Yajnik CS, Deshmukh US (2008) Maternal nutrition, intrauterine programming and consequential risks in the offspring. Rev Endocr Metab Disord 9:203–211
18. Neel JV (1962) Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet 14:353–362
19. Adair LS, Prentice AM (2004) A critical evaluation of the fetal origins hypothesis and its implications for developing countries. J Nutr 134:191–193
20. Hales CN, Barker DJ (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35:595–601
21. Phillips DI (1996) Insulin resistance as a programmed response to fetal undernutrition. Diabetologia 39:1119–1122
22. de Boo HA, Harding JE (2006) The developmental origins of adult disease (Barker) hypothesis. Aust N Z J Obstet Gynaecol 46:4–14
23. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ (2003) Early adiposity rebound in childhood and risk of type 2 diabetes in adult life. Diabetologia 46:190–194
24. Barker DJP (2007) The origins of the developmental origins theory. J Inter Med 261:412–417
25. Barker DJP (2004) Developmental origins of adult health and disease. J Epidemiol Community Health 58:114–115
26. Gluckman PD, Hanson MA (2004) The developmental origins of the metabolic syndrome. Trends Endocrinol Metab 15:183–187
27. Gluckman PD, Hanson MA (2004) Developmental origins of disease paradigm: a mechanism and evolutionary perspective. Pediatr Res 56:311–317
28. Lee TM, Spears N, Tuthill CR, Zucker I (1989) Maternal melatonin treatment influences rates of neonatal development of meadow vole pups. Biol Reprod 40:495–502
29. Armitage JA, Taylor PD, Poston L (2005) Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. J Physiol 565:3–8
30. McMillen IC, Robinson JS (2005) Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 85:571–633
31. Harding JE (2001) The nutritional basis of the fetal origins of adult disease. Int J Epidemiol 30:15–23
32. de Rooij SR, Painter RC, Roseboom TJ, Phillips DI, Osmond C, Barker DJ, Tanck MW, Michels RP, Bossuyt PM, Bleker OP (2006) Glucose tolerance at age 58 and decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine. Diabetologia 49:637–643
33. Painter RC, de Rooij SR, Bossuyt PM, Simmers TA, Osmond C, Barker DJ, Bleker OP, Roseboom TJ (2006) Early onset of coronary artery disease after prenatal exposure to Dutch famine. Am J Clin Nutr 84:322–327

34. Woodall SM, Breier BH, Johnston BM, Gluckman PD (1996) A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. J Endocrinol 150:231–242
35. Franco MDC, Arruda RM, Fortes ZB, de Oliveira SF, Carvalho MH, Tostes RC, Nigro D (2002) Severe nutritional restriction in pregnant rats aggravates hypertension, altered vascular reactivity, and renal development in spontaneously hypertensive rats offspring. J Cardiovasc Pharmacol 39:369–377
36. Ozanne SE, Hales CN (1999) The long-term consequences of intra-uterine protein malnutrition for glucose metabolism. Proc Nutr Soc 58:615–619
37. Takimoto H, Mito N, Umegaki K, Ishiwaki A, Kusama K, Abe S, Yamawaki M, Fukuoka H, Ohta C, Yoshiike N (2007) Relationship between dietary folate intakes, maternal plasma total homocysteine and B-vitamins during pregnancy and fetal growth in Japan. Eur J Nutr 46:300–306
38. Huemer M, Vonblon K, Födinger M, Krumpholz R, Hubmann M, Ulmer H, Simma B (2006) Total homocysteine, folate, and cobalamin, and their relation to genetic polymorphisms, lifestyle and body mass index in healthy children and adolescence. Pediatr Res 60:764–769
39. Martin H, Lindblad B, Norman M (2007) Endothelial function in newborn infants is related to folate levels and birth weight. Pediatrics 119:1152–1158
40. Buckley AJ, Jaquiery AL, Harding JE (2005) Nutritional programming of adult disease. Cell Tissue Res 322:73–79
41. Lesage J, Blondeau B, Grino M, Breant B, Dupouy JP (2001) Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. Endocrinology 142:1692–1702
42. Phillips DI, Walker BR, Reynolds RM, Flanagan DE, Wood PJ, Osmond C, Barker DJ, Whorwood CB (2000) Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. Hypertension 35:1301–1306
43. Levitt NS, Lindsay RS, Holmes MC, Seckl JR (1996) Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevated blood pressure in the adult offspring in the rat. Neuroendocrinology 64:412–418
44. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR (1998) Glçucocorticoid exposure in the late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J Clin Invest 101:2174–2181
45. Moss TJ, Sloboda DM, Gurrin LC, Harding R, Challis JR, Newnham JP (2001) Programming effects in sheep prenatal growth restriction and glucocorticoid exposure. Am J Physiol 281:R960–R970
46. Seckl JR (1997) Glucocorticoids, feto-placental 11-beta-hydroxysteroid dehydrogenase type 2, and the early life origins of adult disease. Steroids 62:89–94
47. Dave-Sharma S, Wilson RC, Harbison MD, Newfield R, Azar MR, Krozowski ZS, Funder JW, Shackleton CH, Bradlow HL, Wei JQ, Hertecant J, Moran A, Neiberger RE, Balfe JW, Fattah A, Daneman D, Akkurt HI, De Santis C, New MI (1998) Examination of genotype and phenotype relationship in 14 patients with apparent mineralocorticoid excess. J Clin Endocrinol Metab 83:2244–2254
48. Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS (1993) Type II (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to fetal growth. Diabetologia 36:62–67
49. Hattersley AT, Tooke JE (1999) The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789–1792
50. Linsday RS, Dabelea D, Roumain J, Hanson RL, Bennett PH, Knowler WC (2000) Type 2 diabetes and low birth weight: the role of paternal inheritance in the association of low birth weight and diabetes. Diabetes 49:445–449
51. Hypponen E, Smith GD, Power C (2003) Parenteral diabetes and birth weight of offspring: intergenerational cohort study. Br Med J 326:19–20
52. Waterland RA, Michels KB (2007) Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr 27:363–388
53. Reik W, Dean W, Walter J (2001) Epigenetic programming in mammalian development. Science 293:1089–1093

54. Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA, Lea RG, Craigon J, McEvoy TG, Young LE (2007) DNA methylation, insuline resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. Proc Natl Acad Sci USA 104:19351–19356
55. Hu JF, Nguyen PH, Pham NV, Vu TH, Hoffman AR (1997) Modulation of IGF-2 genomic imprinting in mice induced by 5-azacytidene, an inhibitor of DNA methylation. Mol Endocrinol 11:1891–1898
56. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey PH (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci USA 105:17046–17049
57. Buckley AJ, Jaquiery AL, Harding JE (2005) Nutritional programming of adult disease. Cell Tissue Res 322:73–79
58. Rolls BA, Gurr MI, van Duijvenvoorde PM, Rolls BJ, Rowe EA (1986) Lactation in lean and obese rats: effect of cafeteria feeding and of dietary obesity on milk composition. Physio Behav 38:185–190
59. Doerflinger A, Swithers SE (2004) Effects of diet and handling on initiation of independent ingestion in rats. Dev Psychobiol 45:72–82
60. Swithers SE, Melendez RI, Watkins BA, Davis J (2001) Metabolic and behavioral responses in preweanling rats following alteration of maternal diet. Physiol Behav 72:147–157
61. Khan IY, Dekou V, Douglas G, Jensen R, Hanson MA, Poston L, Taylor PD (2005) A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. Am J Physiol Regul Integr Comp Physiol 288:R127–R133
62. Khan IY, Dekou V, Hanson MA, Poston L, Taylor PD (2004) Predictive adaptive responses to maternal high-fat diet prevent endothelial dysfunction but not hypertension in adult rats offspring. Hypertension 110:1097–1102
63. Levin BE (2006) Metabolic imprinting: critical impact of the perinatal environment on the regulation of energy homeostasis. Philos Trans R Soc Lond B Biol Sci 361:1107–1121
64. Stewart RJC, Sheppard HG, Preece RF, Waterlow JC (1980) The effect of rehabilitation at different stages of development of rats marginally malnourished for ten to twelve generations. Br J Nutr 43:403–411
65. Stein AD, Lumey LH (2000) The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: the Dutch Famine Birth Cohort Study. Hum Biol 72:641–654
66. Martin JF, Johnston CS, Han CT, Benyshek DC (2000) Nutritional origins of insulin resistance: a rat model for diabetes-prone human populations. J Nutr 130:741–744
67. Ibanez L, Potau N, Enriquez G, de Zegher F (2000) Reduced uterine and ovarian size in adolescent girls born small for gestational age. Pediatr Res 47:575–577
68. Reik W, Santos F, Dean W (2003) Mammalian epigenomics: reprogramming the genome for development and therapy. Theriogenology 59:21–32
69. Chandra RK, Newberne PM (1977) Nutrition, immunity and infection mechanisms of interactions. Plenum, New York
70. Scrimshaw NS (2003) Historical concepts of interactions, synergism and antagonism between nutrition and infection. J Nutr 133:316S–322S
71. Calder PC, Krauss-Etschmann S, de Jong EC, Dupont C, Frick JS, Frokiaer H, Heinrich J, Garn H, Koletzko S, Lack G, Mattelio G, Renz H, Sangild PT, Schrezenmeir J, Stulnig TM, Thymann T, Wold AE, Koletzko B (2006) Early nutrition and immunity – progress and perspectives. Brit J Nutr 96:774–790
72. Kelly D, Coutts GP (2000) Early nutrition and the development of immune function in the neonate. Proc Nutr Soc 59:177–185
73. Ballas ZK (2008) Immunomodulators: a brave new world. J Allergy Clin Immunol 121:331–333
74. Gershwin ME, Beach RS, Hurley LS (1984) Nutrition and immunity. Academic, New York
75. Bendich A, Chandra RK (1990) Micronutrients and immune functions. New York Academy of Science, New York
76. Chandra RK (1992) Nutrition and immunology. St John's, ARTS Biomedical, Canada
77. Savino W (2002) The thymus gland is a target in malnutrition. Eur J Clin Nutr 56:S46–S49
78. Schaible VE, Kaufmann SH (2007) Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 5:e115

79. Nájera O, Gonzalez C, Cortes E, Toledo G, Ortiz R (2007) Effector T lymphocytes in well nourished and malnourished infected children. Clin Exp Immunol 148:501–506
80. Najera O, Gonzalez C, Toledo G, Lopez L, Ortiz R (2004) Flow cytometry study of lymphocyte subsets in malnourished and well-nourished children with bacterial infections. Clin Diagn Lab Immunol 11:577–580
81. Rodriguez L, Gonzalez C, Flores L, Jimenez-Zamudio L, Graniel J, Ortiz R (2005) Assessment by flow cytometry of cytokine production in malnourished children. Clin Diagn Lab Immunol 12:502–507
82. McMurray DN, Bartow RA (1992) Immunosuppression and alteration of resistance to pulmonary tuberculosis in guinea pigs by protein undernutrition. J Nutr 122:738–743
83. Niiya T, Akbar F, Yoshida O, Miyake T, Matsuura B, Murakami H, Abe M, Hiasa Y, Onji M (2007) Impaired dendritic cell function resulting from chronic undernutrition disrupts the antigen-specific immune response in mice. J Nutr 137:671–675
84. McCarter MD, Naama HA, Shou J, Kwi LX, Evoy DA, Calvano CE, Daly JM (1998) Altered macrophage intracellular signaling induced by protein calorie malnutrition. Cell Immunol 183:131–136
85. Redmond HP, Shou J, Kelly CJ, Schreiber S, Miller E, Leon P, Daly JM (1991) Immunosuppressive mechanisms in protein-calorie malnutrition. Surgery 110:311–317
86. Anstead GM, Chandrasekar B, Zhang Q, Melby PC (2003) Multinutrient malnutrition dysregulates the resident macrophage proinflammatory cytokine network, nuclear factor-κB activation, and nitric oxide production. J Leuk Biol 74:982–991
87. Walport MJ (2001) Complement. First of two parts. N Engl J Med 344:1058–1066
88. Chandra RK, Newberne PM (1977) In nutrition, immunity and infection. Mechanisms of interactions. Plenum, New York
89. Chandra RK (2002) Nutrition and the immune system from birth to old age. Eur J Clin Nutr 56:S73–S76
90. Katona P, Katona-Apte J (2008) The interaction between nutrition and infection. Clin Infec Dis 46:1582–1588
91. Stephensen CB (2001) Vitamin A, infection, and immune function. Annu Rev Nutr 21:167–192
92. Erickson KL, Medina EA, Hubbard NE (2000) Micronutrients and innate immunity. J Inf Disease 182:S5–S10
93. Li W, Maeda N, Beck MA (2006) Vitamin C deficiency increases the lung pathology of influenza virus-infected Gulo$^{-/-}$ mice. J Nutr 136:2611–2616
94. Walker VP, Modlin RL (2009) The vitamin D connection to pediatric infections and immune function. Pediatr Res 65(5 Pt 2):106R–113R
95. Cantorna MT, Zhu Y, Froicu M, Wittke A (2004) Vitamin D status, 1, 25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 80:1717S–1720S
96. Shankar AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68:447S–463S
97. Calder PC, Kew S (2002) The immune system: a target for functional foods? Br J Nutr 88:S165–S176
98. Chandra RK (1991) 1990 McCollum Award lecture. Nutrition and immunity: lessons from the past and new insights into the future. Am J Clin Nutr 53:1087–1101
99. Percival SS (1998) Copper and immunity. Am J Clin Nutr 67:1064S–1068S
100. Bonham M, O'Connor JM, Hannigan BM, Strain JJ (2002) The immune system as a physiological indicator of marginal copper status? Br J Nutr 87:393–403
101. Sussman M (1974) Iron and infection. In: Jacobs A, Worwood AM (eds) Iron in biochemistry and medicine. Academic Press, New York, pp 649–679
102. Oppenheimer SJ (2001) Iron and its relation to immunity and infectious disease. J Nutr 131:616S–635S
103. Maggini S, Wintergerst ES, Beveridge S, Hornig DH (2007) Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Br J Nutr 98:S29–S35
104. Kiremidjian-Schumacher L, Roy M, Wishe HI, Cohen MW, Stotzky G (1994) Supplementation with selenium and human immune cell functions. II. Effect on cytotoxic lymphocytes and natural killer cells. Biol Trace Elem Res 41:115–127

105. Gladyshev VN, Stadtman TC, Hatfield DL, Jeang KT (1999) Levels of major selenoproteins in T cells decrease during HIV infection and low molecular mass selenium compounds increase. Proc Natl Acad Sci USA 96:835–839
106. Baum MK, Miguez-Burbano MJ, Campa A, Shor-Posner G (2000) Selenium and interleukins in persons infected with human immunodeficiency virus type 1. J Infect Dis 182:S69–S73
107. Moreland JG, Bailey G (2006) Neutrophil transendothelial migration in vitro to Streptococcus pneumoniae is pneumolysis dependent. Am J Physiol Lung Cell Moll Physiol 290:L833–L840
108. Patarroyo M (1991) Leukocyte adhesion in host defense and tissue injury. Clin Immunol Immunopathol 60:333–348
109. Golias C, Tsoutsi E, Matziridis A, Makridis P, Batistatou A, Charalabopoulos K (2007) Leukocyte and endothelial cell adhesion molecules in inflammation focusing on inflammatory heart disease. In vivo 21:757–769
110. Kulapongs P, Edelman R, Suskind R, Olson R (1977) Defective local leukocyte mobilization in children with kwashiorkor. Am J Clin Nutr 30:367–370
111. Schopfer K, Douglas SD (1975) Neutrophil functions in children with kwashiorkor. J Lab Clin Med 88:450–461
112. Bhan MK, Bhandari N, Bahl R (2003) Management of the severely malnourished child: perspective from developing countries. BMJ 326:146–151
113. McCarter MD, Naama HA, Shou J, Kwi LX, Evoy DA, Calvano SE, Daly JM (1998) Altered macrophage intracellular signaling induced by protein-calorie malnutrition. Cell Immunol 183:131–136
114. Ing R, Su Z, Scott ME, Koski KG (2000) Suppressed T helper 2 immunity and prolonged survival of a nematode parasite in protein-malnourished mice. Proc Natl Acad Sci USA 97:7078–7083
115. Reinert P (1993) Infections in malnourished infants and children. Dev Sante 103:4–6
116. Chandra RK (1980) Fetal nutrition. In: Immunology of nutritional disorders. Year Book Medical Publishers, Chicago, pp 60–68
117. Bhuyan UN, Ramalingaswami V (1973) Immune response of the protein-deficient guinea pig to BCG vaccination. Am J Pathol 72:489–502
118. Reynold JV, Redmond HP, Ueno N, Steigman C, Ziegler MM, Daly JM, Johnston RB Jr (1992) Impairment of macrophage activation and granuloma formation by protein deprivation in mice. Cell Immunol 139:493–504
119. Leme-Brasil MR, Collares EF, Veríssimo de Mello SB, Garcia-Leme J (1980) Acute inflammatory responses in rat with protein-calorie malnutrition. Agents Actions 10:445–450
120. Tchervenkov JI, Latter DA (1988) The influence of long-term protein deprivation on in vitro phagocytic cell delivery to inflammatory lesions. Surgery 103:463–469
121. Peters-Golden M, Canetti C, Mancuso P, Coffrey MJ (2004) Leukotrienes: underappreciated mediators of innate immune responses. J Immunol 173:589–594
122. McDade TW, Beck MA, Kuzawa C, Adair LS (2001) Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence. Am J Clin Nutr 74:543–548
123. Prestes-Carneiro LE, Laraya RD, Silva PR, Moliterno RA, Felipe I, Mathias PC (2006) Long-term effect of early protein malnutrition on growth curve, hematological parameters and macrophage function of rats. J Nutr Sci Vitaminol 52:414–420
124. Barja-Fidalgo C, Souza EP, Silva SV, Rodrigues L, Anjos-Valotta EA, Sannomyia P, DeFreitas MS, Moura AS (2003) Impairment of inflammatory response in adult rats submitted to maternal undernutrition during early lactation: role of insulin and glucocorticoid. Inflamm Res 52:470–476
125. Zadik Z (2003) Maternal nutrition, fetal weight, body composition and disease in later life. J Endocrinol Invest 26:941–955
126. Godfrey KM, Barker DJ (2000) Fetal nutrition and adult disease. Am J Clin Nutr 71:1344S–1352S
127. Landgraf MA, Martinez LL, Rastelli VMF, Franco MCP, Soto-Suazo M, Tostes RCA, Carvalho MHC, Nigro D, Fortes ZB (2005) Intrauterine undernutrition in rats interferes with leukocyte migration, decreasing adhesion molecule expression in leukocytes and endothelial cells. J Nutr 135:1480–1485
128. Landgraf MA, Tostes RCA, Borelli P, Zorn TMT, Nigro D, Carvalho MHC, Fortes ZB (2007) Mechanisms involved in the reduced leukocyte migration in intrauterine undernourishment. Nutrition 23:145–156

129. Langley-Evans SC, Phillips GJ, Jackson AA (1997) Fetal exposure to low protein maternal diet alters the susceptibility of young adult rats to sulfur dioxide-induced lung injury. J Nutr 27:202–209
130. Edwards CA, Osman LM, Godden DJ, Campbell DM, Douglas JG (2003) Relationship between birth weight and adult lung function: controlling for factors. Thorax 58:1061–1065
131. Villamor E, Iliadou A, Cnattingius S (2009) Is the association between low birth weight and asthma independent of genetic and shared environmental factors? Am J Epidemiol 169(11):1337–1343. doi:DOI 10.1093/aje/kwp054
132. Hangstron B, Nyberg P, Nilsson PM (1998) Asthma in adult life – is there an association with birth weight? Scan J Prim Health Care 16:117–120
133. Svanes C, Omenaas E, Heuch JM, Irgens LM, Gulsvik A (1998) Birth characteristics and asthma symptoms in young adults: results from a population-based cohort study in Norway. Eur Respir J 12:1366–1370
134. Kitchen WH, Olinsky A, Doyle LW, Ford GW, Murton LJ, Slonim L, Callanan C (1992) Respiratory health and lung function in 8-yearold children of very low birth weight: a cohort study. Pediatrics 89:1151–1158
135. Landgraf MA, Landgraf RG, Jancar S, Fortes ZB (2007) Influence of age on the development of immunological lung response in intrauterine undernourishment. Nutrition 24:262–269
136. Muller O, Krawinkel M (2005) Malnutrition and health in developing countries. CMAJ 173:279–286

4 Nutrition and Immunity in Animal Disease: Lessons from Parasitic Gastroenteritis

Spiridoula Athanasiadou and Jos G. M. Houdijk

Key Points

- Macro- and micro-nutrients, such as dietary protein and zinc, and plant secondary compounds, such as condensed tannins, can affect the manifestations of immunity to parasites; the relationship between nutrition and immunity to parasites may be quantitative and/or qualitative.
- The former is often demonstrated under nutrient scarcity, where immunity may be penalized and the penalties in immune response can be rectified through nutritional supplementation.
- The latter is frequently related to specific nutrients and/or food compounds acting as triggers for gene expression responsible for immunity to nematodes, even under conditions of nutrient adequacy.
- Implementation of novel methodologies in the investigation of nutrition and immunity to parasites can help towards exploiting nutrition alone, or in combination with other treatments to improve protection towards parasitism, in animals and man.

Key Words: Bioactive forages, dietary protein, gastrointestinal parasites, immunity, immunomodulation, macro-nutrients, micro-nutrients, nematodes, nutrition, plant metabolites, condensed tannins.

4.1 INTRODUCTION

As well as offering micro- and macro-nutrients for maintenance, growth and reproduction, diet can also influence health of all living organisms. Intake of dietary components can contribute to regulating host micro- and macro-parasite populations in

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_4

a variety of ways. *Firstly*, nutrition may directly affect the pathogen fitness, through the ingestion of compounds that are toxic for the pathogen. *Secondly*, the consumption of certain nutrients, or their lack from host's diet, may alter the conditions in the micro-environment from beneficial to detrimental and even toxic for the survival of the pathogen. *Finally*, dietary nutrients can affect host resistance, i.e. the ability of the host to regulate establishment, development, reproduction and survival of the pathogen. Host resistance is regulated and mediated through immunity and the consequences of nutrition on immunity to pathogens are the subject of this paper. While host nutrition can also ameliorate the detrimental effects of infection on host productivity, by improving its resilience to infection, this may not necessarily have any direct consequences on host health, and will not be further considered in this chapter.

The immunomodulatory effects of nutrition, i.e. effects of dietary components on the immune responses to pathogens, are not yet fully understood. A variety of approaches have been used to investigate them. In medical research, for example, most of the evidence is derived from studies on innate immune responses, often in the absence of specific pathogens or from *in vitro* studies. In animal science, many examples arise from studies where hosts have been infected with specific pathogens, and the relevant responses are investigated. The dietary components that have been shown to affect immunity to pathogens include vitamins and minerals, fatty acids, protein and individual amino acids, and they may be effective in a variety of ways. For example, recent studies have suggested a potential role for dietary amino acids by regulating lymphocyte and macrophage activation, lymphocyte proliferation, antibody production, and also hormone excretion (1). Other studies have monitored the consequences of nutrient supplementation on antibody titres and cytokine profiles. For example, dietary supplementation with branched chain amino acids, such as leucine and valine, have resulted in changes on the blood cytokine profile in healthy subjects, such as an increase in Interferon-γ production and a decrease in interleukin-4 release (2), leading towards an improved Th1 immune response.

One of the areas that has received a lot of attention over the last 20 years in veterinary research is the consequences of nutrition towards gastrointestinal nematodes. Gastrointestinal parasitism has been classified as a major health and welfare problem for grazing herbivores; nematodiasis in particular, impairs health by causing inappetence, diarrhea, poor growth, anemia and in severe cases even death (3). In the current review, we draw our examples from interactions between nutrition and immunity to gastrointestinal parasitism, but the same principals may be relevant for bacterial and viral pathogens. Examples of nutrients that regulate immunity to bacterial and viral disease have also been reported, such as the consequences of vitamin D (4) and non-digestible carbohydrates on bacterial infection (5) and other nutrients in smaller extend (for additional reviews see 6, 7). Often in parasitological papers, immunogenic consequences of nutrition are indirectly demonstrated through reduction in parasite establishment, fecundity and survival. However, the lack of direct measurements of immune responses makes difficult the interpretation of the results. It is then unclear whether, for example, observed effects are attributed to immunogenic effects of nutrition or to other type of effects (8, 9). Thus, in the present review, we present examples of studies where direct measurements on immune responses (i.e. effector molecules, involving cells or immunoregulating compounds) have been performed. It is noted that these are, almost by definition, correlated responses and thus they may not necessarily be protective

against the specific pathogen, or they may be partially protective (10). We consider a variety of nutritional compounds, macro- and micro-nutrients and secondary plant metabolites. In the first part of our paper, we concentrate on the effects of macro-nutrients, and in particular protein, and micro-nutrients, such as zinc and molybdenum on immune responses to gastrointestinal nematodes. In the second part, we examine the available evidence on the effects of plant secondary metabolites (PSM) and in particular condensed tannins on immunoregulation and discuss the implications of these interactions. In the final part, we look into possible mechanisms of action of nutrients on immune functions and propose directions on how we can further advance our knowledge on the interactions between nutrition and immunity.

4.2 EFFECTS OF MACRO- AND MICRO-NUTRIENTS ON IMMUNITY TO PARASITES

A considerable amount of research on effects of host nutrition on immunity to pathogens has focused on the relationship between host protein nutrition and gastrointestinal nematode parasitism. Such a focus may arise from the long standing knowledge that nutrition and gastrointestinal parasitism are intimately linked through, on the one hand parasites affecting hosts via affecting feed intake, while on the other hand, host diet affecting parasites through direct or indirect antiparasitic action (11). In particular, a focus on protein nutrition seems sensible, as the immune system can be expected to draw heavily on protein resources due to the highly proteinaceous nature of its effector molecules (12–14), which would support the view that protein supply can be limiting for immune functions. However, like any bodily function, the immune system would also have requirements for energy and micro-nutrients. In theory, an increased availability of each of these resources can be expected to increase immune responses to gastrointestinal nematodes provided that they were scarce in the absence of supplementation. For example, improved immune responses, including circulating and intestinal antibodies, circulating eosinophils, intestinal granulocyte numbers, *in vitro* proliferation of lymphocytes, cytokine levels and neutrophil and lymphocyte functions, have been observed for an increased intake of energy, zinc, iron, selenium, vitamin E, vitamin A and molybdenum (e.g. 15–20).

A large body of evidence shows that protein supplementation reduces gastrointestinal parasitism in growing hosts, especially during later stages of infection, as well as in periparturient hosts, as was speculated through a nutrient partitioning framework (21). This evidence has recently been extensively reviewed (11, 22). While such reductions in parasitism indirectly suggest that an increased protein supply improved expression of immunity to parasites, a range of studies that directly assessed immune effector responses are in support of this view (Table 4.1). In general, protein supplementation to growing hosts results in an increased concentration of circulating and local inflammatory cells, mast cell proteases and circulating antibodies. Apparent inconsistencies may be related to the timing of sampling in relation to the development of the infection and the type of immune response assessed. For example, effects of protein nutrition on immune responses are often more pronounced during the phase of expression of immunity (e.g. 23). This would be related to the fact that immune responses will differ in relation to the development of the infection as e.g. eosinophilia occurs at later stages in the

Table 4.1
Effects of protein supplementation on immune responses in parasitised growing hosts

	Response	*Effect*[a]	*References*
Sheep			
Oesophagostomum columbianum	Goblet cells	↑	(65)
	Mast cells	↑	
	Eosinophils	↑	
	Globule leukocytes	↑	
Trichostrongylus colubriformis	Circulating eosinophils	↑, =	(23, 66)
	Plasma antibodies	=	(67)
	Lymphocyte stimulation	↑	
	Circulating T cells	↑	
	Sheep mast cell proteases	↑	
Nematodirus battus	Mucosal mast cells	=	(68)
	Sheep mast cell proteases	=	
	Globule leukocytes	↑	
	Mucosal eosinophils	↑	
	Circulating eosinophils	=	
	Plasma antibodies	↑	
Teladorsagia circumcincta	Mucosal mast cells	↑	(14, 69)
	Globule leukocytes	↑	
	Sheep mast cell proteases	↑	
Haemonchus contortus	Plasma antibodies	↑	(31)
Mice			
Heligmosomoides bakeri	Cytokines	↑, =, ↓	(17, 58, 70, 71)
	Circulating antibodies	↑, =, ↓	(24, 71)
	Serum mast cell proteases	↑	
	Pro-inflammatory cytokines	↓	
	Intestinal mast cells	↑	
	Circulating eosinophils	↑	
	Intestinal eosinophils	↑	
Trichuris muris	Circulating antibodies	↓	(72, 73)
Pigs			
Trichuris suis + Ascaris sum	Circulating eosinophils	↑	(74)

[a]Relative to the unsupplemented group, ↓: decrease, ↑: increase, =: no change

infection. Protein supplementation also affects different types of effector arms differently. Detailed studies in mice, infected with *Heligmosomoides bakeri* are showing that protein supplementation reduces worm survival by increasing gut-associated Th2 responses, while reducing Th1 responses (24).

In addition to the growing animals, there is an increasing body of evidence in support of the view that protein supplementation affects effector responses in periparturient hosts (Table 4.2). Reproducing animals are crucial for the epidemiology of the parasitic infection; they experience a breakdown in their immunity to parasite around parturition

Table 4.2
Effects of protein supplementation on immune responses in parasitised periparturient hosts

	Response	*Effect*[a]	*References*
Ewes			
Teladorsagia circumcincta	Mucosal mast cells	=	(26, 55, 75–77)
	Globule leukocytes	↑	(78)
	Circulating eosinophils	=	
	Mucosal eosinophils	=	
	Plasma antibodies	↑, =, ↓	
Trichostrongylus colubriformis	Mucosal mast cells	=	(57)
	Globule leukocytes	↑	
T. circumcincta + *T. colubriformis*	Mucosal mast cells	↑	(79)
	Mucosal eosinophils	=	
	Globule leukocytes	↑, =	
	Serum interleukin-5	↓	
	Plasma antibodies	=, ↓	
Rats			
Nippostrongylus brasiliensis	Mucosal mast cells	↑	(29)
	Goblet cells	↑	
	Eosinophils	=	
	Mucosal antibodies	↑	

[a]Relative to the unsupplemented group, ↓: decrease, ↑: increase, =: no change

and are the main source of infection for the young animals. Although, to date, such responses have been rather variable for some effector responses, e.g. circulating antibodies, the presence of an effect of protein supplementation on globule leukocytes appears consistent. The latter may reflect the long known importance of this type of inflammatory cell in expression of immunity to gastrointestinal nematodes (25). Effects of protein nutrition on plasma antibodies may be more variable, since plasma antibody concentration depends on the balance between antibody production, utilization and excretion in milk. However, as milk volume is also sensitive to protein supply, the outcome of effects of protein nutrition on circulating antibodies may not always follow an expected dose-response relationship (26).

The sensitivity of immunity to parasites to host protein nutrition has been demonstrated through the use of a wide range of protein sources, including soybean meal (e.g. 26), fish meal (e.g. 27), cottonseed meal (e.g. 28), casein (e.g. 29), ovalbumin (e.g. 17), sunflower meal (e.g. 30) and urea (31). This would suggest that immune responses may not necessarily be sensitive to variation in the quality of the supplemented protein (i.e. amino acid composition). However, in order to demonstrate an effect on immune responses, the level of protein supplementation used in most studies above was considerably higher than expected host protein requirement. Therefore, we argue that if levels of protein supplementation are reduced, then impact of protein quality becomes more important. This hypothesis still needs to be tested, but it is supported with an increasing body of evidence on immunomodulating effects of specific amino acids (32), and would suggest that protein nutrition could potentially be tailored to elicit specific immune responses in parasitised hosts.

4.3 CONSEQUENCES OF PLANT SECONDARY COMPOUNDS ON IMMUNE REGULATION

Plant secondary metabolites are organic compounds that do not appear to have a function in the primary plant metabolism, i.e. in growth, reproduction or development. More than 80,000 different PSM compounds have been described, whereas more than 100,000 compounds are believed to exist in plants resulting in a great compound diversity (33). Although PSM are present in all plants in small and moderate amounts, only a small proportion of plant species contain PSM in high amounts and those are referred to as PSM-rich plants (34). Saponins, alkaloids, lactones, glycosides and polyphenols are all PSM and some immunomodulatory effects have been reported, although not always in the presence of a pathogen. For example, caffeine is a well-known alkaloid, with reported negative immunomodulatory effects, such as suppression of neutrophil and monocyte chemotaxis and suppressed the production of pro-inflamatory cytokine TNF-a factor (35). Similarly digoxin, which is a cardiac glycoside, also suppresses the production of TNF-α, and plays a role in regulating the NF-κβ pathway, which is a central regulator of inflammation (36). Saponins, on the other hand, are known for their immunogenic abilities, such as Quil A, which has been used as adjuvant for a variety of veterinary vaccines for a number of years (37).

Although many PSMs are known for their activity against a variety of micro- and macro-organisms, for the majority of them it is still not known whether such effects are mediated through immunity or through direct toxic effects on the pathogen or its environment. For example, a particular class of PSM are the polyphenols, which are the most abundant antioxidants in human diets and have long been known for their immunomodulatory activity (38). As immune cells are particularly susceptible to oxidative stress, it is believed that these effects may be exerted, at least partly, through the antioxidant activity (39). A specific class of polyphenols are the condensed tannins (CT), which are polymers of flavonoid units with reported antimicrobial and antiparasitic activity. The consumption of CT has resulted in a reduction of the level of gastrointestinal nematode parasitism in sheep and goats (40–45). The antiparasitic activity of CT is attributed, at least partly, to direct toxicity against various stages of nematodes, which has been confirmed with a number of *in vitro* and *in vivo* studies (for reviews see 46, 47). In addition, CTs appear to exert immunomodulatory effects and enhance an effective immune response against parasites. Although the immunomodulatory effects of CT have not (yet) been investigated in a systematic way, a limited number of studies have looked at the effects of CT on effector molecules that have been associated with immunity to parasites. For example, the administration of a CT extract, Quebracho extract, in goats parasitised with the small-intestinal nematode *Trichostrongylus colubriformis* resulted in an increase in mucosal mast cell numbers (48). Proliferation of mucosal mast cells and globule leukocytes has been associated with the expulsion of nematodes from the gastrointestinal tract (49, 50). It appears, however, that the effects on mucosal mast cells may not be consistent throughout the gastrointestinal tract; the same CT extract did not affect the number of mast cells in the abomasum (true stomach) of sheep infected with the abomasal parasite *Haemonchus contortus* (43). This inconsistency is also apparent in the observed anthelmintic activity of CT; the administration of CT resulted in a reduction in the intestinal parasite burden in sheep, but no effect was observed in

an abomasal parasite population (41). This lack of an immunogenic and an anthelmintic effect in the abomasum may be related to the reduced bioavailability of CT in the abomasum. It has been previously suggested that as CT are in complexes with dietary protein or other macro-molecules in the aboamsaum (51), they may be unavailable to exert any type of activity. Appropriate pH and other conditions in the small intestine may enable the disassociation of CT from complexes and likely allow them exert their activity, both immunogenetic and anthelmintic.

In some studies, the effects of condensed tannins have been studied under grazing conditions, where parasitised animals are given access to either CT-rich or conventional forages. Sheep infected with an abomasal nematode, *Teladorsagia circumcincta*, and grazing on sulla (*Hedysarum coronarium*), a legume rich in CT, had increased number of mucosal mast cells and globule leukocytes in their abomasum, when compared with sheep grazing on conventional pastures (52). In another study, humoral responses were measured in sheep grazing on sulla and conventional legumes (*Medicago sativa*); antibody titres against specific parasite antigens were higher in sheep grazing on sulla compared to the conventional legumes (45). Furthermore, the consumption of sainfoin (*Onobrychis viciifolia*), another CT-rich legume appeared to enhance immune cell development in the small intestine of sheep, such as mucosal eosinophils, mast cells and Paneth cells, when compared to those grazing on conventional pastures (53). Despite the clarity of the above results, it is not always easy to distinguish whether the immunomodulatory effects observed are attributed to the activity of CT or to the nutritional superiority of the CT-rich grazing, often reported. CTs are macro-molecules with great affinity to dietary protein; they form stable compounds with the protein, protect it from rumen degradation, and then released in the small intestine for absorption (54). Thus, CT consumption has the ability to increase host protein availability, and it may very well be that the immunogenic effects observed arise from an improved level of protein nutrition, as discussed above, rather than the CT per se. However, it should be noted that in most of the cases mentioned above, control grazing was of similar nutritional quality as CT-rich grazing and that animal productivity did not differ between grazing treatments. Although this supports the view that immunogenic effects observed directly from CT, it cannot be excluded that increased immune responses are the consequences of the increased protein supply. Parasitized animals have higher protein requirement than their non-parasitized counterparts (55); in addition, it has been suggested that allocation of scarce protein to growth and reproductive functions may be prioritized over expression of immunity to parasites (21). Thus, even at time of apparent protein abundance, an increased protein supply could affect selected immune responses, as observed in parasitized sheep (26, 56, 57) as well as mice (58).

4.4 CONCLUSIONS AND PERSPECTIVES

We have showed that the nutritional environment of the host can affect the manifestations of immunity to parasites, although in many cases, it is not yet clear how this is achieved. Most of the evidence available to date is supportive of a quantitative relationship between nutrition and immunity to parasites. As mentioned earlier, host's nutrition is responsible to cover the requirements for effective immune response to parasites, in addition to maintenance and growth/reproduction. As a consequence, under nutrient

scarcity, immunity may be penalised and the penalties in immune response can be rectified through nutritional supplementation (21). In addition to the quantitative relationship, it seems that a qualitative relationship between certain nutrients and effector arms of immunity may also be also possible, i.e. specific nutrients may affect the expression of genes that are responsible for immunity to nematodes. For example, although such a relationship has not yet been demonstrated in livestock, there is evidence from rodent models that certain nutrients, such as branched chained amino acids may be acting as regulators of gene expression via modulation of the initiation phase of mRNA translation (59). Other nutrients have been shown to regulate the expression of specific proteins that have been associated with the regulation of disease occurrence; for example, zinc has been shown to regulate uroguanylin, a peptide hormone, which increases the occurrence of diarrhoea (60). Thus, it is possible that even under conditions of nutrient adequacy, certain nutrients may signal and trigger the synthesis of components of the immune response to parasites, which may consequently result in an enhanced immune response. Whether such relationships are responsible for the aforementioned observed immunogenic effects of CT-rich forages in the absence of effect on host performance (i.e. under apparent nutrient adequacy), remains to be investigated. In the absence of any pathogens, gene expression analysis revealed that protein supplementation resulted in several hundreds of genes being differentially expressed in specific tissue of rats compared to unsupplemented ones (61). In the presence of intestinal nematode parasites, increased dietary protein supply has increased protein synthesis in the small intestine of hosts, in rodent and ruminant models (62, 63). If the cells that benefit from nutritionally enhanced gene expression are those actively synthesizing molecules for the immune response, this would be expected to enhance host immunity, and would give the prospect of modulating immunity by manipulating the diet of parasitized hosts. This last hypothesis still remains to be tested.

There is already evidence on the effects of nutrition on gene expression and such investigations have been greatly advanced by the exploitation of high throughput technologies. Gene expression profiling and biomics have revealed genes related to aging, cancer, metabolic disease and diabetes that can be affected by host nutrition. Similar advancements have been reported in parasite immunology following the implementation of high throughput technologies. We believe that the latest advancements in genomic and proteomic developments will enable the investigation of nutritional regulation of expression of genes responsible for the immune responses that control parasitism in mammals. In the longer term, the investigation of the effects of nutrients on gene expression could allow effective dietary interventions to prevent diet-related disease. Exploitation of biomic technologies in rodent models has greatly advanced our knowledge in mechanisms, effector molecules, functional pathways and their importance in expression of immunity. For example, recent gene profiling in murine model systems has identified a number of molecules that are up-regulated during a Th2 response, which is responsible for the expulsion of gastrointestinal nematodes (64). By bringing together basic immunology and nutrition and by exploring the novel technologies that can assure a global unbiased approach, we can further advance our understanding on the immunomodulatory effects of nutrition. To this effect, ongoing gene expression studies on interactive effects between host protein nutrition and parasitism are starting to show nutritional enhancement of expression of selected genes related to immunity to parasites

(Athanasiadou, unpublished). By identifying such pathways and associated molecules that are sensitive to host nutrition, we can make significant contributions in the formation of recommendations for the use of nutrition alone, or in combination with other treatments to improve protection towards parasitism, in animals and man. In man, in particular, this route will lead to mapping individual's health which can lead to personalized nutrition for health and immunity.

REFERENCES

1. Stephen AI, Avenell A (2006) A systematic review of multivitamin and multimineral supplementation for infection. J Hum Nutr Diet 19:179–190
2. Bassit RA, Sawada LA, Bacurau RF, Navarro F, Martins E Jr, Santos RV, Caperuto EC, Rogeri P, Costa Rosa LF (2002) Branched-chain amino acid supplementation and the immune response of long-distance athletes. Nutr 18:376–379
3. Athanasiadou S, Kyriazakis I (2004) Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. Proc Nutr Soc 63:631–639
4. Bikle DD (2008) Vitamin D and the immune system: role in protection against bacterial infection. Curr Opin Nephrol Hypertens 17:348–352
5. Watzl B, Girrbach S, Girrbach S, Roller M (2005) Inulin, oligofructose and immunomodulation. Br J Nutr 93:S49–S55
6. Spears JW, Weiss WP (2008) Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet J 176:70–76
7. Volman JJ, Ramakers JD, Plat J (2008) Dietary modulation of immune function by beta-glucans. Dietary modulation of immune function by beta-glucans. Physiol Behav 94:276–284
8. Petkevicius S, Murrell KD, Bach Knudsen KE, Jorgensen H, Roepstorff A, Laue A, Wachmann H (2004) Effects of short chain fatty acids and lactic acids on survival of *Oesophagostomum dentatum* in pigs. Vet Parasitol 122:293–301
9. Petkevicius S, Thomsen LE, Bach Knudsen KE, Murrell KD, Roepstorff A (2007) The effect of inulin on new and pattent infections of *Trichuris suis* in growing pigs. Parasitology 134:121–127
10. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE (2004) Helminth parasites-masters of regulation. Immunol Rev 201:89–116
11. Houdijk JGM, Athanasiadou S (2003) Direct and indirect effects of host nutrition on ruminant gastrointestinal nematodes. In: 't Mannetje L, Ramírez-Avilés L, Sandoval-Castro CA, Ku-Vera JC (eds) 6th international symposium on the nutrition of herbivores. Universidad Autónoma de Yucatán, Mérida, pp 213–236
12. Lewis RA, Austen KF (1981) Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. Nature 293:103–108
13. MacRae JC (1993) Metabolic consequences of intestinal parasitism. Proc Nutr Soc 52:121–130
14. Coop RL, Holmes PH (1996) Nutrition and parasite interaction. Int J Parasitol 26:951–962
15. McClure SJ, McClure TJ, Emery DL (1999) Effects of molybdenum intake on primary infection and subsequent challenge by the nematode parasite *Trichostrongylus colubriformis* in weaned Merino lambs. Res Vet Sci 67:17–22
16. Finch JM, Turner RJ (1996) Effects of selenium and vitamin E on the immune responses of domestic animals. Res Vet Sci 60:97–106
17. Boulay M, Scott ME, Conly SL, Stevenson MM, Koski KG (1998) Dietary protein and zinc restrictions independently modify a *Heligmosomoides polygyrus* (Nematoda) infection in mice. Parasitology 116:449–462
18. Koski KG, Scott ME (2001) Gastroinetstinal nematodes, nutrition and immunity: breaking the negative spiral. Annu Rev Nutr 21:297–321
19. Calder PC, Field CJ, Gill HS (2002) Nutrition and immune function. CABI Publishing, CAB International, Wallingford, UK
20. Koski KG, Su Z, Scott ME (1999) Energy deficits suppress both systemic and gut immunity during infection. Biochem Biophys Res Commun 264:796–801

21. Coop RL, Kyriazakis I (1999) Nutrition-parasite interaction. Vet Parasitol 84:187–204
22. Sykes AR, Kyriazakis I (2008) Opportunities to control herbivore nematodes through manipulation of the grazing environment. In: Meng QX, Ren LP, Cao ZJ (eds) Proceedings of the 7th international symposium on the nutrition of herbivores: herbivore nutrition for the development of efficient, safe and sustainable livestock production. China Agricultural University Press, Beijing, pp 329–353
23. van Houtert MFJ, Barger IA, Steel JW, Windon RG, Emery DL (1995) Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. Vet Parasitol 56:163–180
24. Ing R, Su Z, Scott ME, Koski KG (2000) Suppressed T helper 2 immunity and prolonged survival of a nematode parasite in protein malnourished mice. Proc Natl Acad Sci 97:7078–7083
25. O'Sullivan BM, Donald AD (1973) Responses to infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in ewes of different reproductive status. Int J Parasitol 3:521–530
26. Houdijk JGM, Kyriazakis I, Jackson F, Huntley JF, Coop RL (2003) Is the allocation of metabolisable protein prioritised to milk production rather than to immune functions in *Teladorsagia circumcincta* infected lactating ewes? Int J Parasitol 33:329–338
27. Donaldson J, van Houtert MFJ, Sykes AR (1998) The effect of nutrition on the periparturient parasite status of mature ewes. Anim Sci 67:523–533
28. Shaw KL, Nolan JV, Lyneh JJ, Coverdole OR, Gill HS (1995) Effect of weaning supplementation and gender on acquired immunity to *Haemonchus contortus* in lambs. Int J Parasitol 25:381–387
29. Jones LA, Houdijk JGM, Knox DP, Kyriazakis I (2009) Immunomodulatory effects of dietary protein during *Nippostrongylus brasiliensis* infection in lactating rats. Parasite Immunol 31:412–421
30. van Houtert MFJ, Barger IA, Steel JW (1995) Dietary protein for grazing sheep: interaction with gastrointestinal parasites. Vet Parasitol 60:283–295
31. Strain SAJ, Stear MJ (2001) The influence of protein supplementation on the immune response to *Haemonchus contortus*. Parasite Immunol 23:527–531
32. Li P, Yin YL, Li D, Kim SW, Wu G (2007) Amino acids and immune function. Br J Nutr 98:237–252
33. Acamovic T, Brooker JD (2005) Biochemistry of plant secondary metabolites and their effects in animals. Proc Nutr Soc 64:403–412
34. Taiz L, Zeiger E (2002) Secondary metabolites and plant defences. In: Taiz L, Zaiger E (eds) Plant physiology. Sinauder, Sunderland
35. Horrigan LA, Kelly JP, Connor TJ (2006) Immunomodulatory effects of caffeine: friend or foe? Pharmacol Ther 111:877–892
36. Yang Q, Huang W, Jozwik C, Lin Y, Glasman M, Caohuy H, Srivastava M, Esposito D, Gillette W, Hartley J, Pollard HB (2005) Cardiac glycosides inhibit TNF-alpha/NF-kappaB signaling by blocking recruitment of TNF receptor-associated death domain to the TNF receptor. Proc Natl Acad Sci 102:9631–9636
37. Potter A, Volker G, van Drunen S, van den Hurk L (2008) Veterinary vaccines: alternatives to antibiotics? Anim Health Res Rev 9:187–199
38. Spears JW, Weiss WP (2008) Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet J 176:70–76
39. Neyestani TR (2008) Polyphenols and immunity. In: de Meester F, Watson RR (eds) Wild-type food in health promotion and disease prevention. Humana, Totowa, NJ
40. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL (2000) Effects of short-term exposure to condensed tannins on adults *Trichostrongylus colubriformis*. Vet Rec 146:728–732
41. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL (2001) Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. Vet Parasitol 99:205–219
42. Paolini V, De La Farge F, Prevot F, Dorchies P, Hoste H (2005) Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. Vet Parasitol 127:277–283
43. Paolini V, Bergeaud JP, Grisez C, Prevot F, Dorchies P, Hoste H (2003) Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. Vet Parasitol 113:253–261
44. Marley CL, Cook R, Barrett J, Keatinge R, Lampkin NH (2006) The effects of birdsfoot trefoil (*Lotus corniculatus*) and chicory (*Cichorium intybus*) when compared with perennial ryegrass (*Lolium perenne*) on ovine gastrointestinal parasite development, survival and migration. Vet Parasitol 138:280–290

45. Niezen JH, Charleston WAG, Robertson HA, Shelton D, Waghorn GC, Green R (2002) The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Vet Parasitol 105:229–245
46. Athanasiadou S, Githiori J, Kyriazakis I (2007) Medicinal plants for helminth parasite control: facts and fiction. Animal 1:1392–1400
47. Hoste H, Jackson F, Athanasiadou S, Thamsborg SM, Hoskin SO (2006) The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol 22:253–261
48. Paolini V, Frayssines A, De La Farge F, Dorchies P, Hoste H (2003) Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. Vet Res 34:331–339
49. Huntley JF, Newlands GF, Jackson F, Miller HR (1992) The influence of challenge dose, duration of immunity, or steroid treatment on mucosal mast cells and on the distribution of sheep mast cell proteinase in *Haemonchus*-infected sheep. Parasite Immunol 14:429–440
50. Harrison GB, Pulford HD, Gatehouse TK, Shaw RJ, Pfeffer A, Shoemaker CB (1999) Studies on the role of mucus and mucosal hypersensitivity reactions during rejection of *Trichostrongylus colubriformis* from the intestine of immune sheep using an experimental challenge model. Int J Parasitol 29:459–468
51. Mueller-Harvey I (2006) Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agric 86:201–2037
52. Tzamaloukas O, Athanasiadou S, Kyriazakis I, Huntley JF, Jackson F (2006) The effect of chicory (*Cichorium intybus*) and sulla (*Hedysarum coronarium*) on larval development and mucosal cell responses of growing lambs challenged with *Teladorsagia circumcincta*. Parasitology 132:419–426
53. Ríos-de Alvarez L, Greer AW, Jackson F, Athanasiadou S, Kyriazakis I, Huntley JF (2008) The effect of dietary sainfoin (*Onobrychis viciifolia*) on local cellular responses to *Trichostrongylus colubriformis* in sheep. Parasitology 135:1117–1124
54. Min BR, Hart SP (2003) Tannins for suppression of internal parasites. J Anim Sci 81:E102–E109
55. Houdijk JGM, Kyriazakis I, Jackson F, Coop RL (2001) The expression of immunity to *Teladorsagia circumcincta* and its relationship to protein nutrition depend on body protein reserves. Parasitology 122:661–672
56. Bricarello PA, Rocha RA, Silva MB, Houdijk JGM, Almeida FA, Cardia DFF, Amarante AFT (2009) Effects of protein supply on immunological responses to *Haemonchus contortus* in Santa Ines and Ile de France ewes. Proceedings of the 22nd international conference of the world association for the advancement of veterinary parasitology, p 167
57. Houdijk JGM, Jackson F, Kyriazakis I (2009) Nutritional sensitivity of resistance to *Trichostrongylus colubriformis* in lactating ewes. Vet Parasitol 160:258–266
58. Coltherd JC, Babayan S, Bünger L, Kyriazakis I, Allen JE, Houdijk JGM (2009) Effect of genetic growth potential and nutrition on immune response to *Heligmosomoides bakeri* infection. Abstract book British Society of Parasitology Spring and Maleria meeting 2009, p 116
59. Kimball SR, Jefferson LS (2006) New functions for amino acids: effects on gene transcription and translation. Am J Clin Nutr 83:500S–507S
60. Blanchard RK, Cousins RJ (2000) Regulation of intestinal gene expression by dietary zinc: induction of uroguanylin mRNA by zinc deficiency. J Nutr 130:1393S–1398S
61. Kato H, Kimura T (2003) Evaluation of the effects of the dietary intake of proteins and amino acids by DNA microarray technology. J Nutr 133:2073S–2077S
62. Symons LE, Jones WO (1981) Distribution of 14C-L-leucine on organs and tissues of guinea pigs infected with *Trichostrongylus colubriformis*. Int J Parasitol 11:229–233
63. Yu F, Bruce LA, Calder AG, Milne E, Coop RL, Jackson F, Horgan GW, MacRae AC (2000) Subclinical infection with the nematode *Trichostrongylus colubriformis* increases gastrointestinal tract leucine metabolism and reduces availability of leucine for other tissues. J Anim Sci 78:380–390
64. Artis D (2006) New weapons in the war on worms: identification of putative mechanisms of immune-mediated expulsion of gastrointestinal nematodes. Int J Parasitol 36:723–733
65. Dobson C, Bawden RJ (1974) Studies on the immunity of sheep to *Oesophagostomum columbianum*: effects of low-protein diet on resistance to infection and cellular reactions in the gut. Parasitology 69:239–255

66. Kambara T, McFarlane RG (1996) Changes in T cell subpopulations of sheep due to age and dietary protein intake: association with protective immunity to *Trichostrongylus colubriformis*. Vet Immunol Immunopathol 51:127–135
67. Kahn LP, Kyriazakis I, Jackson F, Coop RL (2000) Temporal effects of protein nutrition on the growth and immunity of lambs infected with *Trichostrongylus colubriformis*. Int J Parasitol 30:193–205
68. Israf DA, Coop RL, Stevenson LM, Jones DG, Jackson F, Jackson E, MacKellar A, Huntley JF (1996) Dietary protein influences upon immunity to *Nematodirus battus* infection in lambs. Vet Parasitol 61:273–286
69. Coop RL, Huntley JF, Smith WD (1995) Effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. Res Vet Sci 59:24–29
70. Tu T, Koski KG, Scott ME (2007) Mechanisms underlying reduced expulsion of a murine nematode infection during protein deficiency. Parasitology 135:81–93
71. Tu T, Koski KG, Wykes LJ, Scott ME (2007) Re-feeding rapidly restores protection against *Heligmosomoides bakeri* (Nematoda) in protein-deficient mice. Parasitology 134:899–909
72. Michael E, Bundy DAP (1992) Protein content of CBA/Ca mouse diet: relationship with host antibody responses and the population dynamics of *Trichuris muris* (Nematoda) in repeated infection. Parasitology 105:139–150
73. Michael E, Bundy DAP (1992) Nutrition, immunity and helminth infection: effect of dietary protein on the dynamics of the primary antibody response to *Trichuris muris* (Nematoda) in CBA/Ca mice. Parasite Immunol 14:169–183
74. Pedersen S, Saeed I, Michaelsen KF, Friis H, Murrell KD (2002) Impact of protein energy malnutrition on *Trichuris suis* infection in pigs concomitantly infected with *Ascaris suum*. Parasitology 124:561–568
75. Houdijk JGM, Kyriazakis I, Jackson F, Huntley JF, Coop RL (2000) Can an increased metabolizable protein intake affect the periparturient relaxation in immunity against *Teladorsagia circumcincta* in sheep? Vet Parasitol 91:43–62
76. Houdijk JGM, Kyriazakis I, Jackson F, Huntley JF, Coop RL (2005) Effects of protein supply and reproductive status on local and systemic immune responses to *Teladorsagia circumcincta* in sheep. Vet Parasitol 129:105–117
77. Houdijk JGM, Jackson F, Kyriazakis I (2006) Rapid improvement of immunity to *Teladorsagia circumcincta* is achieved through a reduction in the demand for protein in lactating ewes. Int J Parasitol 36:219–227
78. Huntley JF, Jackson F, Coop RL, Macaldowie C, Houdijk JGM, Familton AS, Xieh HL, Stankiewicz M, Sykes AR (2004) The sequential analysis of local inflammatory cells during abomasal nematode infection in periparturient sheep. Vet Immunol Immunopathol 97:163–176
79. Sykes AR, Xie HL, Stankiewicz M, Huntley JF, MacKellar A, Sedcole JR, McAnulty RW, Green R (2007) The effect of vaccinating infection during pregnancy and dietary protein supply on the periparturient immune response of sheep to infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* larvae. Animal 1:249–260

5 Neuroimmunomodulation, Stress–Nutrition Interactions and Diet

Javier Romeo Marin, Julia Wärnberg, E. Nova, Sonia Gómez-Martínez S, Ligia Esperanza Díaz, A. Veses, and Ascansión Marcos

Key Points

- The immune system requires a constant supply of nutrients for its optimal function and performance.
- Under stress conditions, the immune response may be suppressed and is modulated by the central nervous system through a complex network of signals.
- Communication between the neuroendocrine and immune systems has been well established, and there is ample evidence to indicate that stress-associated immune dysregulation is sufficiently intense for repercussions on health.
- Psychological stress contributes to many disease states with the clear involvement of immune mechanisms that can be modulated or conditioned by food components and nutrients.
- Understanding interactions among diet, the central nervous system and the immune system provides insight on health of situations of psychological stress.

Key Words: Nutrition, stress, immune system, neuroendocrine system.

5.1 INTRODUCTION

Stress is a term that means different things to different people, but in general has long been suspected to play a role in the etiology of many diseases. Stress has been defined as a constellation of events that starts with a stimulus (stressor), which triggers a reaction

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_5

in the brain (stress perception) that in turn activates physiological systems in the body (stress response) (1). Stress is triggered by a variety of unexpected environmental stimuli, such as aggressive behavior, fear, forced physical activity, sudden environmental changes, social isolation or illness (2). In general terms, it can be described as the consequence of the body's reaction to abnormal external conditions that requires some sort of physiological regulation often associated with an immune response.

The immune system protects the body from bacteria, viruses, fungi, and other harmful organisms and to correctly carry out its functions requires a constant supply of nutrients. While under normal circumstances, the immune system is highly efficient with its multiple defense systems (nonspecific and specific immune responses) against the onslaught of outside invaders, under stress conditions the immune response may be suppressed (3). The influence of stress on immune responses has long been addressed in animals and humans and results have revealed that stress affects thymus-derived lymphocytes (T-cells) and plays a role in cell–cell interactions and the release of mediators from activated lymphocytes (4). Results have also shown that stress-induced immune dysregulation can have significant impacts on health, including an impaired immune response to antigens such as bacteria and viruses.

5.2 STRESS AND THE IMMUNE SYSTEM

Communication between the neuroendocrine and the immune systems has been well established, and there is sufficient evidence to indicate that stress-associated immune dysregulation may have implications for the health of an individual. As part of the adaptive response to stress, body systems such as the autonomic, cardiovascular, gastrointestinal and immune systems may be affected (3). From a review of the literature, it becomes clear that psychological stress may suppress or enhance immune functions, depending on the nature of the stressor (acute or chronic). Thus, it is important to distinguish between the different features of stress such as its duration and intensity. Acute stress has been defined as stress that lasts for a period of minutes to hours (e.g., that triggered by a speech task, taking school exams) and chronic stress as stress that persists for days to months (e.g. when facing bereavement, unemployment, divorce or caring for patients with Alzheimer's disease, etc.) (5).

5.2.1 Acute Stress

The first stage of the acute stress response is characterized by changes in the activity and levels of cytokines and immune cells such as B-lymphocytes and natural killer (NK) cells (1, 6). Acute psychological stress is generally associated with immune stimulation, enhancing the delayed type hypersensitivity (DTH) response, NK cell activity, immune cell numbers (7) such as CD8+ lymphocytes, B-cells (CD19+), NK cells, and tumor necrosis factor-alpha (TNF-α) levels, interleukine (IL)-6, IL-4, IL-10, IL-13 production and salivary secretion of immunoglobulin A (IgA) (8, 9). Acute psychological stress experienced by medical students during exams resulted in a decrease in the synthesis of interferon-gamma (IFN-γ) accompanied by increased IL-10 production by isolated peripheral blood lymphocytes (PBL_s) (10). Collectively, these findings suggest that acute stress has an activating effect on immune factors (11, 12).

5.2.2 Chronic Stress

Contrary to acute stress, chronic stress can lead to decreases in B-cells, lymphocyte levels, proliferative responses of lymphocytes to several mitogens, as well as NK activity (13, 14). Chronic stress has also been associated with a reduction in the DTH response, CD4+ and CD8+ lymphocytes, and IL-2 and IFN-γ cytokine production, leading to an increased risk of upper respiratory tract infections (8, 15).

In response to chronic stress, the findings of several studies indicate inhibition of T-cell responses to mitogens such as concavaline A (Con A), phytohemagglutinin (PHA) and a monoclonal antibody used to stimulate the T-cell receptor, a poorer proliferative (memory) response to herpes simplex virus-1 (HSV-1), and inhibition of the ability of NK cells to respond to recombinant IL-2 and IFN-γ (16–20).

De Gucht et al. (21) examined the effects of chronic work-related stress (nurses working at a university hospital) on immunity in two groups of subjects classified as high stress/low psychopathology and high stress/high psychopathology according to the scores obtained in tests. These authors observed activation of CD4+ cells and expansion of the CD3+CD16CD56+ compartment in the group with a low psychopathology score, and a reduced suppressor CD8+CD11b+subset in the other group. Serum IL-2 levels were higher in the high stress/low psychopathology score group.

Other studies have also addressed the relationship between job-related stress and the immune system. Thus, caregivers of dementia patients have been shown to display changes in several immune parameters, including mitogen-induced proliferation (22), NK cell activity (23) and antibody responses following influenza vaccination relative to age-matched noncaregivers (24, 25). A drop in lymphocyte and cytotoxicity markers (CD56, CD8) has also been observed in elderly caregivers (23). In summary, chronic stress is associated with serious impairment of immune functions, with possible consequences on health (8).

5.3 NEUROIMMUNOMODULATION AND STRESS

In stress conditions, the immune system is modulated by the central nervous system (CNS) through a complex network of signals. In effect, stress has been linked to diminished resistance to infection.

The neuroendocrine and immune systems communicate bidirectionally. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal medullary (SAM) axis are pathways via which psychological stress can modulate the immune system. Arginine vasopressin (AVP) plays an important role in stress-induced activation of the HPA axis.

Researchers have shown that stress-induced activation of the HPA axis by the release of neuroendocrine hormones from the pituitary gland, results in dysregulation of the immune system (26). The SAM axis and the endogenous opioid system have also been incriminated in the immune dysfunction that occurs in response to stress.

Crosstalk between the neuroendocrine and the immune systems is now well established (27). Neurotransmitters and hormones can modify the actions of immune cells and conversely, cytokines secreted by immune cells can alter CNS homeostasis (28). The link with stress is manifested by experimental studies showing a relationship between stress and resistance to infection. For example, chronic psychological stress induces activation of the HPA axis and sympathetic nervous system (SNS) causing the release of cortisol

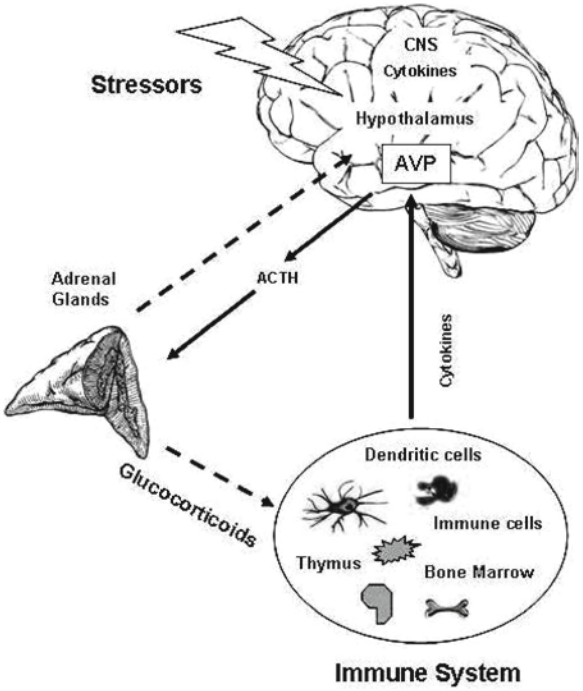


Fig. 5.1. Diagram showing communication pathways between the brain and immune system and cytokine feedback.

and hormones such as corticotrophin-releasing hormone (CRH) (29). These in turn may dysregulate the immune response and probably lead to impaired immune function (30) (Fig. 5.1). What has been clearly established is that the neuroendrocrine system plays an essential role in the pathophysiology of stress-associated immune disorders (31).

In summary, stress affects the HPA axis, brain neurotransmitter systems and SNS, all of which may affect the neural regulation of immune function (2). Indeed, neuroimmunomodulation seems to be the main mediator of the immune alterations associated with conditions of stress. It is important to remember that the bidirectional interactions between the endocrine, CNS, and immune systems constitute a finely tuned regulatory system or homeostasis required for health maintenance (30, 32).

5.4 STRESS–NUTRITION INTERACTIONS

The available evidence indicates that exposure to stressful situations induces a highly complex set of neuroendocrine changes that depends on individual characteristics and physiological factors such as nutritional status (33). Thus, the extent of stress-associated immune dysregulation produced in an individual is sufficient to have serious effects on health such that it is essential to consider nutritional aspects.

Energy intake imbalance is considered a physiological stressor involving potential biological mechanisms (e.g. chronic inflammation and oxidative stress) (34). Several studies have shown that calorie restriction and obesity are physiological stress models (35–38).

The nutritional stress of a 36-h fast has been shown to increase the number of neutrophils in the peripheral blood of elderly and young adult subjects (35). Fasting seems

also to affect immunity (decreasing CD4 cell subsets and increasing NK cell activity), at least to some extent, through changes in adrenal gland-related hormones (39).

This immunomodulatory effect observed after stress due to calorie restriction seems to be related to elevated plasma cortisol levels causing lymphocytes to be drawn into regional lymph nodes (6). The lymphopenia caused by stress due to calorie restriction is reversible within minutes of alleviating the stress (40).

On the other hand, several studies have reported that psychological stress is associated with a greater consumption of high fat foods, leading to overweight and obesity (41). There is also mounting evidence that stress-related chronic stimulation of the HPA axis and the consequent excess exposure to glucocorticoids could play a role in the development of visceral obesity (42–44). Some authors have hypothesized a role for cortisol in promoting calorie-dense food intake and identified the potential contribution of neuroendocrine/peptide mediators, such as leptin, insulin, and neuropeptide Y, to interact between stress and eating behavior (45).

The bidirectional relationship between stress and energy intake imbalance, leading to reduced calorie intake or obesity is well documented. Thus, stress may lead to neurobiological adaptations that promote the compulsive nature of overeating (45), finally leading to obesity. In addition, obesity is considered a physiological stress state related to inflammatory and oxidative stress status of the individual (38, 46, 47). Stress may also have the opposite effect on food behavior, leading to reduced calorie intake. Moreover, repeated dietary patterns often induce addictive behavior, which could lead to eating disorders such as obesity. In effect, addictive behavior patterns have been correlated to stressful life events (48).

5.5 DIET EFFECTS ON STRESS-RELATED NEUROIMMUNOMODULATION

Interactions among diet, CNS, and the immune system may help our understanding of their roles in physiological stress situations. Several articles have addressed the relationship among diet, stress, and immunity.

Many disease situations involve psychological stress, with the clear participation of immune mechanisms that can be modulated or conditioned by food components and nutrients (49). Thus, vitamins and amino acids (AA) have been described to play a possible role in antiphysiological stress in animal and human models developed to examine HPA regulation and immunomodulatory effects (49–53). In the following sections, we briefly describe nutrition effects on stress situations.

5.5.1 Can Dietary Fatty Acid Supplementation Help Control Stress?

Fatty acids are directly involved in tolerance to stress reactions. These acids form part of the brain's structure and account for 20% of the membranes of nerve cells.There is evidence that a high intake of eicosapentaenoic acid (EPA) (1 g/day) increases the chances of recovery from depression caused by stress (54). Some researchers have also observed that EPA induces neutrophilia and increases the CD4/CD8 ratio (55).

Supplementation with EPA (1 g/day) in combination with other compounds such as dexamethasone (0.001 g/day) has also been suggested to help to control stressful situations (55, 56).

In a double blind intervention study, the authors reported reduced stress-related violence after 9 months of supplementing the diet with vitamins, minerals, and essential fatty acids (EPA, docosahexaenoic acid (DHA), and linoleic acid) (57). A drop in aggressiveness was also observed in alcoholics with significant levels of anxiety after 3 weeks of DHA and EPA fatty acid supplements (2 g/day) (58). There is thus enough evidence to support the benefits of omega-3 fatty acid supplementation in some stress situations.

5.5.2 Micronutrients and Stress

Vitamin supplementation has been linked to beneficial effects on immune function (59, 60). Beta-carotene (doses of 15, 30, 45, or 60 mg of β-carotene per day for 2 months) raises the number of NK cells and the percentage of cells expressing the IL-2 receptor with increasing doses (59).

Changes in environmental factors such as heat, cold, high-altitude, etc., cause stress and metabolic adaptations might be, in some instances, also accompanied by changes in nutrient requirements and energy expenditure. Thus, vitamin B-cofactor requirements increase proportionally to energy expenditure. Additional doses of vitamins with antioxidant properties may be beneficial for reducing oxidative stress associated with exposure to heat, cold, or high-altitude outdoor environments (61).

Vitamins are also considered to play an important role in physiological stress situations. The effects of micronutrient supplementation on the neuroimmunoendocrine system have been explored in several physiological stress situations (e.g., post exercise or job associated stress). Davison et al. (62) examined the effects of daily vitamin C (L-ascorbic acid, 1,000 mg/day) and vitamin E (RRR-alpha-tocopherol, 400 IU/day) supplements on immunoendocrine responses over a 4-week period in healthy adults undergoing prolonged exercise. Their results suggest that four weeks of vitamin supplementation may blunt the cortisol response independently of changes in oxidative stress or plasma IL-6 concentrations.

One of the first studies performed in the 1990s in experimental animals, addressed the effect of vitamin E and selenium (Se) supplements for a period of 12 weeks. Supplementation reduced the oxidative reactions and the ensuing lipid peroxidation in lung tissue when the animals were subjected to strenuous physical exercise (63). Similar beneficial effects on oxidant compound levels under acute exercise with antioxidant vitamin and mineral supplements have been documented in several human studies (64–67).

Oxidative stress has also been incriminated in the pathogenesis of a number of diseases such as aging, diabetes, neurodegenerative disorders and chronic diseases. In a study of the effects of 2 months of therapy based on a combination of vitamins (vitamin E, 800 IU; vitamin C, 250 mg; vitamin B6, 100 mg; vitamin B12, 250 mg; and folic acid, 10 mg) conducted in end-stage renal disease (ESRD) patients, the authors concluded that adding a potent antioxidant cocktail to conventional vitamin supplements had no effect on the severity of ESRD-induced oxidative stress, and also unaffected reactive protein C and IL-6 levels in these patients (68).

Vitamin E (200 mg/day) has been reported to reduce oxidative stress in type 2 diabetes (69). A recent study has shown that supplementation with vitamin E and selenium (140 mg alpha-tocopherol and 200 μg selenium) reduces oxidative stress and improves the

overall antioxidant status of patients with pulmonary tuberculosis treated with standard chemotherapy (70). Vitamin E (400 IU) and selenium (600 μg) co-supplementation also seems to attenuate oxidative stress in hemodialysis patients undergoing intradialysis iron infusion (71).

5.5.3 Amino Acids

There is mounting evidence to suggest that, besides their role as building blocks of proteins and polypeptides, some AA are important regulators of key metabolic pathways that are necessary for growth, reproduction, and immunity in humans (72). Indeed, protein deficiency has long been known to impair immune function and increase the susceptibility of animals to disease (73).

While examining the relationship between dietary AA intake and stress, Sung and Chang (74) observed a negative relationship between taurine intake and the scores obtained in a self-administered life stress questionnaire. Dietary tyrosine supplementation is thought to reduce some of the adverse effects of stress situations (cold conditions) through CNS activation (75). Glutamine also seems to play an important role during metabolic stress (e.g. surgery) (76). Dietary supplementation with glutamine was found to reduce the production of glucocorticoids in weanling pigs (77).

Glutamine and arginine are both used as nutritional supplements in critically ill patients. Both AA affect similar organ systems although they differ in their targets. For example, glutamine serves as fuel for immune cells, increases human leukocyte antigen-DR expression on monocytes, enhances neutrophil phagocytosis, and increases heat shock protein expression (78). Arginine affects the immune system by directly or indirectly stimulating the proliferation of immune cells. The indirect effect is possibly mediated by nitric oxide, which also enhances macrophage cytotoxicity. Further, glutamine serves as a precursor for the de novo production of arginine through the citrulline-arginine pathway. Glutamine has been shown to be beneficial in the surgical and critically ill patient, while the benefits of arginine supplementation are still under debate (78).

In summary, several studies have indicated the utility of AA supplementation in stress situations such as those suffered by postsurgery or critically ill patients. The possible benefits of AA supplements in healthy subjects subjected to stress conditions have yet to be explored.

5.5.4 Nucleotides

Many foods are dietary sources of nucleotides, e.g. lamb, liver, mushrooms (but not other vegetables or fruit). In stress situations, dietary nucleotides have been described as "semi-essential," optimizing the functions of the gastrointestinal and immune systems (79). The benefits of nucleotide supplementation have been mainly studied in exercise-related stress situations.

Mc Naughton et al. (80) determined the effect of a nucleotide supplement on salivary immunoglobulin A and cortisol responses after prolonged endurance cycling exercise (90-min cycle ergometer trials; 60% VO_2max) and proposed that this supplementation could offset the cortisol response associated with acute exercise. The same authors also reported that chronic intake of a nucleotide supplement blunts the response of

the hormones associated with physiological stress produced after short-duration high-intensity exercise (2 min) in trained male subjects (81).

Blouet et al. (82) investigated the effects of dietary cysteine supplementation (5.8 or 20 g *N*-acetylcysteine per kilogram of food) on oxidative stress and glucose homeostasis in rats fed a high-sucrose diet. Their results indicated improved impairment of glucose homeostasis mediated by a reduction in oxidative stress.

5.5.5 Probiotics

To understand how the CNS connects psychological and physical manifestations in the form of diseases, the case of inflammation related to gastrointestinal alterations resulting from stress may be used as an example.

Diop et al. (83) noted that the consumption of probiotics [*Lactobacillus acidophilus* Rosell-52 and *Bifidobacterium longum* Rosell-175; 3×10^9 colony-forming units (cfu)] over 3 weeks could have a beneficial effect on the gastrointestinal symptoms experienced by individuals with chronic stress (abdominal pain, nausea).

Gastrointestinal diseases may provoke mood changes. The clearest example is that of irritable bowel syndrome (IBS), which is considered both a gastrointestinal and a psychological disturbance (84) and is frequently accompanied by depression symptoms (85). Studies performed in gnotobiotic animal models have shown that intestinal health is a prerequisite for the normal function of both the immune system and CNS. Microorganisms are responsive to the host's neuroendocrine environment and conversely, bacteria can influence the neuroendocrine environment by producing neurochemicals such as gamma amino butyric acid (GABA), serotonin, and several biologically active peptides (86). Germ-free animals show several signs of an underdeveloped immune system and also exhibit hyperactivity of the HPA axis and reduced monoaminergic activity (87). In this context, probiotics might be regarded as a beneficial dietary strategy for two reasons. First, as modulators of the immune response at the level of the intestinal mucosa, they maintain the balance between proinflammatory and anti-inflammatory cytokines; and secondly, probiotics have been shown to modulate impaired afferent neuronal activity, as in the case of the visceral hyperalgesia typical of IBS (88–90). Hence, certain metabolites or other chemical compounds generated by probiotic organisms could directly affect neuronal function. Effectively, certain microorganism species have been shown to elaborate neurotransmitter peptides and nitric oxide (91).

In double-blind, placebo-controlled studies, *Lactobacillus plantarum* 299v (92, 93) was reported to relieve some IBS symptoms, while *Lactobacillus rhamnosus* GG and *Lactobacillus reuteri* were unable to modify or improve IBS symptoms (94, 95). Moreover, Verdu et al. (89) showed that *Lactobacillus paracasei* may prevent visceral pain, stress, and antibiotic-induced visceral hypersensitivity in rats. Other authors have reported a beneficial effect on abdominal pain/discomfort in patients with IBS of probiotic supplementation (*Lactobacillus salivarius* UCC4331 or *Bifidobacterium infantis* 35624, each as a dose of 1×10^{10}) (96) through normalization of the ratio of anti- to proinflammatory cytokines. As already mentioned, since stress induces several disorders, including gastrointestinal symptoms, probiotics may be useful to help regulate or modulate gastrointestinal functions (83).

In other stress situations such as psychological stress in university students undergoing exams, probiotics such as *Lactobacillus casei DN-114001* may modulate the altered

immune response caused by stress (97). In this latter study, supplementation with probiotics prevented the decrease in NK cells observed in the placebo group during the examination period.

5.6 CONCLUSIONS AND PERSPECTIVES

While acute and chronic stress induces physiological alteration of the neuroendocrine and immune systems, there is increasing evidence that nutrition may serve as a useful tool against stress.

Existing data suggest that dietary intake may play an important role in the general relationship between psychological stress and immune function (98). However, although there is evidence to suggest that nutrition interventions are promising approaches to modulating the immune alterations induced by both acute and chronic stress, this issue requires further investigation and the use of nutritional therapy to restore the neuroendocrine and immune alterations induced by specific stress conditions is still a distant goal.

Based on the evidence we have so far, eating a well-balanced, moderate diet and avoiding rapid weight loss, weight fluctuations, or inadequate weight increases, may be a promising strategy to regulate stress-induced immune–endocrine interactions and optimize or maintain health.

REFERENCES

1. Dhabhar FS, McEwen BS (1997) Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav Immun 11:286–306
2. Bellinger DL, Lubahn C, Lorton D (2008) Maternal and early life stress effects on immune function: relevance to immunotoxicology. J Immunotoxicol 5:419–444
3. McEwen BS, Stellar E (1993) Stress and the individual: Mechanisms leading to disease. Arch Intern Med 153:2093–2101
4. Arora S, Bhattacharjee J (2008) Modulation of immune responses in stress by Yoga. Int J Yoga 1:45–55
5. Dhabhar FS (2000) Acute stress enhances while chronic stress suppresses skin immunity. The role of stress hormones and leukocyte trafficking. Ann NY Acad Sci 917:876–893
6. Dhabhar FS, McEwen BS (1999) Enhancing versus suppressive effects of stress hormones on skin immune function. Proc Natl Acad Sci 96:1059–1064
7. Landmann RM, Muller FB, Perini C, Wesp M, Erne P, Buhler FR (1984) Changes of immunoregulatory cells induced by psychological and physical stress: relationship to plasma catecholamines. Clin Exp Immunol 58:127–135
8. Irwin M, Patterson T, Smith TL, Caldwell C, Brown SA, Gillin JC, Grant I (1990) Reduction of immune function in life stress and depression. Biol Psychiatry 27:22–30
9. Shephard RJ (2002) Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. Crit Rev Immunol 22:165–182
10. Marshall GD Jr, Agarwal SK, Lloyd C, Cohen L, Henninger EM, Morris GJ (1998) Cytokine dysregulation associated with exam stress in healthy medical students. Brain Behav Immun 12:297–307
11. Brosschot JF, Benschop RJ, Godaert GL, Olff M, De Smet M, Heijnen CJ, Ballieux RE (1994) Influence of life stress on immunological reactivity to mild psychological stress. Psychosom Med 56:216–224
12. Benschop RJ, Godaert GL, Geenen R, Brosschot JF, De Smet MB, Olff M, Heijnen CJ, Ballieux RE (1995) Relationships between cardiovascular and immunological changes in an experimental stress model. Psychol Med 25:323–327
13. Olff M (1999) Stress, depression and immunity: the role of defence and coping styles. Psychiatry Res 85:7–15

14. Dhabhar FS (2000) Acute stress enhances while chronic stress suppresses skin immunity. The role of stress hormones and leukocyte trafficking. Ann NY Acad Sci 917:876–893
15. Besedovsky H, Del Ray A (1991) Physiological implication of the immune-neuro-endocrine network. In: Ader R, Felte DL, Cohe N (eds) Psychoneuroimmunology, 2nd edn. Academic, San Diego, pp 589–608
16. Kiecolt-Glaser JK, Dura JR, Speicher CE, Trask OJ, Glaser R (1991) Spousal caregivers of dementia victims: longitudinal changes in immunity and health. Psychosom Med 53:345–362
17. Esterling BA, Kiecolt-Glaser JK, Bodnar JC, Glaser R (1994) Chronic stress, social support, and persistent alterations in the natural killer cell response to cytokines in older adults. Health Psychol 13:291–298
18. Esterling BA, Kiecolt-Glaser JK, Glaser R (1996) Psychosocial modulation of cytokine-induced natural killer cell activity in older adults. Psychosom Med 58:264–272
19. Malarkey WB, Pearl DK, Demers LM, Kiecolt-Glaser JK, Glaser R (1995) Influence of academic stress and season on 24-hour mean concentrations of ACTH, cortisol, and beta-endorphin. Psychoneuroendocrinology 20:499–508
20. Glaser R, Kiecolt-Glaser JK (1997) Chronic stress modulates the virus-specific immune response to latent herpes simplex virus type 1. Ann Behav Med 19:78–82
21. De Gucht V, Fischler B, Demanet C (1999) Immune dysfunction associated with chronic professional stress in nurses. Psychiatry Res 85:105–111
22. Kiecolt-Glaser JK, Dura JR, Speicher CE, Trask OJ, Glaser R (1991) Spousal caregivers of dementia victims: longitudinal changes in immunity and health. Psychosom Med 53:345–362
23. Castle S, Wilkins S, Heck E, Tanzy K, Fahey J (1995) Depression in caregivers of demented patients is associated with altered immunity: impaired proliferative capacity, increased CD8+, and a decline in lymphocytes with surface signal transduction molecules (CD38+) and a cytotoxicity marker (CD56+ CD8+). Clin Exp Immunol 101:487–493
24. Kiecolt-Glaser JK, Glaser R, Gravenstein S, Malarkey WB, Sheridan J (1996) Chronic stress alters the immune response to influenza virus vaccine in older adults. Proc Natl Acad Sci 93:3043–3047
25. Vedhara K, Cox NK, Wilcock GK, Perks P, Hunt M, Anderson S, Lightman SL, Shanks NM (1999) Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. Lancet 353:627–631
26. Yang EV, Glaser R (2002) Stress induced immunomodulation and the implications for health. Int Immunopharmacol 2:315–324
27. Butts CL, Sternberg EM (2008) Neuroendocrine factors alter host defense by modulating immune function. Cell Immunol 252:7–15
28. Klein TW (1993) Stress and infections. J Fla Med Assoc 80:409–411
29. Hamer M, Wolvers D, Albers R (2004) Using stress models to evaluate immuno-modulating effects of nutritional intervention in healthy individuals. J Am Coll Nutr 23:637–646
30. Yang EV, Glaser R (2002) Stress induced immunomodulation and the implications for health. Int Immunopharmacol 2:315–324
31. Makino S, Hashimoto K, Gold PW (2002) Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress. Pharmacol Biochem Behav 73:147–158
32. Eskandari F, Webster JI, Sternberg EM (2003) Neural immune pathways and their connection to inflammatory diseases. Arthritis Res Ther 5:251–265
33. Mormède P, Lemaire V, Castanon N, Dulluc J, Laval M, Le Moal M (1990) Multiple neuroendocrine responses to chronic social stress: interaction between individual characteristics and situational factors. Physiol Behav 47:1099–1105
34. McTiernan A (2000) Associations between energy balance and body mass index and risk of breast carcinoma in women from diverse racial and ethnic backgrounds in the U.S. Cancer 88:1248–1255
35. Walrand S, Moreau K, Caldefie F, Tridon A, Chassagne J, Portefaix G, Cynober L, Beaufrere B, Vasson MP, Boirie Y (2001) Specific and nonspecific immune responses to fasting and refeeding differ in healthy young adult and elderly persons. Am J Clin Nutr 74:670–678
36. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P (2003) Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 52: 2882–2887

37. Ferroni P, Basili S, Falco A, Dari G (2004) Inflammation, insulin resistance and obesity. Curr Atheroscler Rep 6:424–431
38. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R (2005) Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 111:1448–1454
39. Komaki G, Kanazawa F, Sogawa H, Mine K, Tamai H, Okamura S, Kubo C (1997) Alterations in lymphocyte subsets and pituitary-adrenal gland-related hormones during fasting. Am J Clin Nutr 66:147–152
40. Dhabhar FS, Miller AH, McEwen BS, Spencer RL (1995) Effects of stress on immune cell distribution: dynamics and hormonal mechanisms. J Immunol 154:5511–5527
41. Sims R, Gordon S, Garcia W, Clark E, Monye D, Callender C, Campbell A (2008) Perceived stress and eating behaviors in a community-based sample of African Americans. Eat Behav 9:137–142
42. Pasquali R, Anconetani B, Chattat R, Biscotti M, Spinucci G, Casimirri F (1996) Hypothalamic-pituitary-adrenal axis activity and its relationship to the autonomic nervous system in women with visceral and subcutaneous obesity: effects of the corticotropin-releasing factor/arginine-vasopressin test and of stress. Metabolism 45:351–356
43. Rosmond R, Dallman MF, Bjorntorp P (1998) Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab 83:1853–1859
44. Nieuwenhuizen AG, Rutters F (2007) The hypothalamic-pituitary-adrenal-axis in the regulation of energy balance. Physiol Behav 94:169–177
45. Adam TC, Epel ES (2007) Stress, eating and the reward system. Physiol Behav 91:449–458
46. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P (2003) Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 52:2882–2887
47. Ferroni P, Basili S, Falco A, Dari G (2004) Inflammation, insulin resistance, and obesity. Curr Atheroscler Rep 6:424–431
48. Rojo L, Conesa L, Bermudez O, Livianos L (2006) Influence of stress in the onset of eating disorder: data from a two stage epidemiologic controlled study. Psychosom Med 68:628–635
49. Marcos A, Nova E, Montero A (2003) Changes in the immune system are conditioned by nutrition. Eur J Clin Nutr 57:66–69
50. Bassit RA, Sawada LA, Bacurau RF, Navarro F, Costa Rosa LF (2000) The effect of BCAA supplementation upon the immune response of triathletes. Med Sci Sports Exerc 32:1214–1219
51. Calder PC, Kew S (2002) The immune system: a target for functional foods? Br J Nutr 88:165–176
52. Gruenwald J, Graubaum HJ, Harde A (2002) Effect of a probiotic multivitamin compound on stress and exhaustion. Adv Ther 19:141–150
53. Lippolis JD (2008) Immunological signalling networks: Integrating the body's immune response. J Anim Sci 86:53–63
54. Casper CR (2004) Nutrients, neurodevelopment and mood. Curr Psychiatry Rep 6:425–429
55. Murck H, Song C, Horrobin DF, Uhr M (2004) Ethyl-eicosapentaenoate and dexamethasone resistance in therapy-refractory depression. Int J Neuropsychopharmacol 7:341–349
56. Hibbeln JR, Bissette G, Umhau JC, George DT (2004) Omega-3 status and cerebrospinal fluid corticotrophin releasing hormone in perpetrators of domestic violence. Biol Psychiatry 56:895–897
57. Gesch CB, Hammond SM, Hampson SE, Eves A, Crowder MJ (2002) Influence of supplementary vitamins, minerals and essential fatty acids on the antisocial behaviour of young adult prisoners. Br J Psychiatry 181:22–28
58. Budens-Branchey L, Branchey M, Hibbeln JR (2008) Associations between increases in plasma n-3 polyunsaturated fatty acids following supplementation and decreases in anger and anxiety in substance abusers. Prog Neuropsychopharmacol Biol Psychiat 32:568–575
59. Watson RR (1991) Beta-carotene's effects on serum lipoproteins and immunologic indices in humans. Am J Clin Nutr 54:609–660
60. Santos MS, Meydani SN, Leka L, Wu D, Fotouhi N, Meydani M, Hennekens CH, Gaziano JM (1996) Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. Am J Clin Nutr 64:772–777

61. Askew EW (1995) Environmental and physical stress and nutrient requirements. Am J Clin Nutr 61:631S–637S
62. Davison G, Gleeson M, Phillips S (2007) Antioxidant supplementation and immunoendocrine responses to prolonged exercise. Med Sci Sports Exerc 39:645–652
63. Veera Reddy K, Charles Kumar T, Prasad M, Reddanna P (1992) Exercise-induced oxidant stress in the lung tissue: role of dietary supplementation of vitamin E and selenium. Biochem Int 26:863–871
64. Kanter MM (1994) Free radical, exercise and antioxidant supplementation. Int J Sports Nutr 4:205
65. Machlin LJ, Bendich A (1987) Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1:441
66. Keith RE (1997) Ascorbic acid. In: Wolinsky I, Driskell JA (eds) Sports nutrition: vitamins and trace elements. CRC Press, Boca Raton, FL, p 29
67. Machefer G, Groussard C, Vincent S, Zouhal H, Faure H, Cillard J, Radák Z, Gratas-Delamarche A (2007) Multivitamin-mineral supplementation prevents lipid peroxidation during "the Marathon des Sables". J Am Coll Nutr 26:111–120
68. Kamgar M, Zaldivar F, Vaziri ND, Pahl MV (2009) Antioxidant therapy does not ameliorate oxidative stress and inflammation in patients with end-stage renal disease. J Natl Med Assoc 101:336–344
69. Bartlett HE, Eperjesi F (2008) Nutritional supplementation for type 2 diabetes: a systematic review. Ophthalmic Physiol Opt 28:503–523
70. Seyedrezazadeh E, Ostadrahimi A, Mahboob S, Assadi Y, Ghaemmagami J, Pourmogaddam M (2008) Effect of vitamin E and selenium supplementation on oxidative stress status in pulmonary tuberculosis patients. Respirology 13:294–298
71. Ardalan MR, Tubbs RS, Shoja MM (2007) Vitamin E and selenium co-supplementation attenuates oxidative stress in haemodialysis patients receiving intra-dialysis iron infusion. Nephrol Dial Transplant 22:973–975
72. Suenaga R, Tomonaga S, Yamane H (2008) Intracerebroventricular injection of L-arginine induces sedative and hypnotic effects under an acute stress in neonatal chicks. Amino Acids 35:139–146
73. Wu G (2009) Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1–17
74. Sung MJ, Chang KJ (2009) Correlations between dietary taurine intake and life stress in Korean college students. Adv Exp Med Biol 643:423–428
75. Mahoney CR, Castellani J, Kramer FM, Young A, Lieberman HR (2007) Tyrosine supplementation mitigates working memory decrements during cold exposure. Physiol Behav 92:575–582
76. Boelens PG, Nijveldt RJ, Houdijk AP, Meijer S, van Leeuwen PA (2001) Glutamine alimentation in catabolic state. J Nutr 131:2569–2577
77. Li P, Yin YL, Li DF, Kim SW, Wu G (2007) Amino acids and immune function. Br J Nutr 98:237–252
78. Vermeulen MA, van de Poll MC, Ligthart-Melis GC, Dejong CH, van den Tol MP, Boelens PG, van Leeuwen PA (2007) Specific amino acids in the critically ill patient-exogenous glutamine/arginine: a common denominator? Crit Care Med 35:568–576
79. Maldonado J, Navarro J, Narbona E, Gil A (2001) The influence of dietary nucleotides on humoral and cell immunity in the neonate and lactating infant. Early Hum Dev 65:69–74
80. Mc Naughton L, Bentley DJ, Koeppel P (2006) The effects of a nucleotide supplement on salivary IgA and cortisol after moderate endurance exercise. J Sports Med Phys Fitness 46:84–89
81. Mc Naughton L, Bentley D, Koeppel P (2007) The effects of a nucleotide supplement on the immune and metabolic response to short term, high intensity exercise performance in trained male subjects. J Sports Med Phys Fitness 47:112–118
82. Blouet C, Mariotti F, Azzout-Marniche D, Mathé V, Mikogami T, Tomé D, Huneau JF (2007) Dietary cysteine alleviates sucrose-induced oxidative stress and insulin resistance. Free Radic Biol Med 42:1089–1097
83. Diop L, Guillou S, Durand H (2008) Probiotic food supplement reduces stress-induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial. Nutr Res 28:1–5
84. Levy RL, Olden KW, Naliboff BD, Bradley LA, Francisconi C, Drossman DA, Creed F (2006) Psychosocial aspects of the functional gastrointestinal disorders. Gastroenterology 130:1447–1458
85. Whitehead WE (2007) Twin studies used to prove that the comorbidity of major depressive disorder with IBS is not influenced by heredity. Am J Gastro 102:2230–2231

86. Logan AC, Katzman M (2005) Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 64:533–538
87. Sudo N (2006) Stress and gut microbiota: does postnatal microbial colonization programs the hypothalamic-pituitary–adrenal system for stress response? Int Cong Ser 1287:350–354
88. Eutamene H, Bueno L (2007) Role of probiotics in correcting abnormalities of colonic flora induced by stress. Gut 56:1495–1497
89. Verdú EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, Mao Y, Wang L, Rochat F, Collins SM (2006) Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut 55:182–190
90. Kamiya LW, Forsythe P, Goettsche G, Mao Y, Wang Y et al (2006) Inhibitory effects of *Lactobacillus reuteri* on visceral pain induced by colorectal distension in Sprague-Dawley rats. Gut 55:191–196
91. Ait-Belgnaoui A, Han W, Lamine F, Eutamene H, Fioramonti J, Bueno L, Theodorou V (2006) Lactobacillus farciminis treatment suppresses stress-induced visceral hypersensitivity: a possible action through interaction with epithelial cells cytoskeleton contraction. Gut 55:1090–1094
92. Niedzielin K, Kordecki H, Birkenfeld B (2001) A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol 13:1143–1147
93. Sen S, Mullan MM, Parker TJ, Woolner JT, Tarry SA, Hunter JO (2002) Effect of Lactobacillus plantarum 299v on colonic fermentation and symptoms of irritable bowel syndrome. Dig Dis Sci 47:2615–2620
94. O'Sullivan MA, O'Morain CA (2000) Bacterial supplementation in the irritable bowel syndrome. A randomised double-blind placebo-controlled crossover study. Dig Liver Dis 32:294–301
95. Niv E, Naftali T, Hallak R, Vaisman N (2005) The efficacy of Lactobacillus reuteri ATCC 55730 in the treatment of patients with irritable bowel syndrome – a double blind, placebo-controlled, randomized study. Clin Nutr 24:925–931
96. O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K (2005) Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 128:541–551
97. Marcos A, Wärnberg J, Nova E, Gómez S, Alvarez A, Alvarez R, Mateos JA, Cobo JM (2004) The effect of milk fermented by yoghurt cultures plus Lactobacillus casei DN-114001 on the immune response of subjects under academic examination stress. Eur J Nutr 43:381–389
98. Hamer M, Wolvers D, Albers R (2004) Using stress models to evaluate immuno-modulating effects of nutritional intervention in healthy individuals. J Am Coll Nutr 23:637–646

6 The Intricate Role of Adipokines in Immune-Mediated Diseases

Pedro Manoel Mendes Moraes-Vieira, Richardt Gama Landgraf, and Niels Olsen Saraiva Câmara

Key Points

- Food intake and nutritional status modify the physiological response of the immune system to illness and influence the development of chronic inflammatory processes.
- Adipose tissue secretes immune-related proteins called adipokines that are pleiotropic factors acting in the immune system and in the neuroendocrine system, linking metabolism and immune physiology.
- Obesity and adipokines display a variety of immune and physiologic functions, participating in several immune responses, such as autoimmunity, antitumor responses, transplanted organ rejection, infectious diseases, and many others.
- The understanding and, more important, the ability to modulate these adipose tissue-derived hormones may have a great impact on the treatment of several immune- and metabolic-related diseases.

Key Words: Adipokines, obesity, hormones, immune diseases, metabolic disease.

6.1 INTRODUCTION

Obesity, a pathological condition accompanied by excessive fat deposits, is currently a major public health issue and an important risk factor contributing to the development of different types of diseases worldwide. Obesity is correlated with low-grade inflammation of white adipose tissue accompanied by chronic activation of the innate immune system, insulin resistance, impaired glucose tolerance, and diabetes (1). In addition, obesity has been associated with hypertension, hypercholesterolemia, several types of

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_6

tumors, cardiovascular diseases, and immune dysfunctions accompanied by higher infection rates and delayed wound healing. The alteration of energy homeostasis during obesity has been attributed both to physiological imbalances, which can occur from a number of causes, such as neuroendocrine factors, metabolic disturbances, and genetic traits, as well as nonphysiological influences such as changes in lifestyle, excessive energy intake, and/or reduced physical activity (2).

6.2 ENERGY BALANCE, IMMUNITY AND DISEASE

It is well known that body compositions depend on the balance between food intake and energy expenditure, and they are apparently under the control of three components: (1) food intake, (2) energy utilization and thermogenesis, and (3) adipocyte metabolism.

A multifactorial and mutual interaction exists among nutrition, immune function, and pathological disorders. Food intake and nutritional status may influence the physiological response of the immune system to illness and infection, resulting in the rapid clearance of a pathogen or even in the development of a chronic inflammatory process. On the other hand, a disruption in immune function may result in detrimental effects on nutrient utilization, which can lead to malnutrition and immunodeficiency (3). This close relationship between the immune system and nutrition indicates that nutritional monitoring during illness is an important factor in helping to clear diseases in a timely manner.

Clinical and epidemiological data indicate that immunocompetence depends on nutritional status because impaired immune responses are, in some cases, connected to malnutrition, thus contributing to the increased prevalence of infectious disorders (4). It has been observed that the rapid turnover in lymphoid tissues appears to be highly affected by nutrient imbalances, thus linking metabolic pathways and the immune system. Moreover, infection is associated with decreased food intake, intestinal catabolic stress and increased nutrient loss through urine and sweat, which work together with the impaired synthesis of proteins such as immunoglobulins and impaired cell proliferation (5). This impaired protein synthesis and the decrease in cell proliferation may lead to some degree of immunodeficiency, resulting in illness and chronic inflammatory responses.

A number of immunological measures can be used to assess nutritional status, such as leukocytes counts, proliferation assays induced by several antigens, leukocyte migration, and phagocytosis, delayed-type hypersensitivity reactions (DTH), plasma concentrations of different immunoglobulins, the production or activity of complement compounds, cytokines, etc. Although all of these assays can be used clinically, lymphocyte counts are one of the most commonly applied tests in nutritional evaluation (6). It is important to keep in mind, once one of the immune measures is investigated, that a determined immune function that is altered in one individual may be normal in another, meaning that, depending on the case, more than one immune parameter may be necessary.

6.3 ADIPOKINES: MEDIATORS LINKING IMMUNITY AND NEUROENDOCRINOLOGY

Adipose tissue is not only used to store energy. Recently, it has become clear that this tissue is involved in a number of functions as an endocrine organ. The immune-related proteins fashioned by adipocyte tissue include leptin, adiponectin, resistin, visfatin, TNF, adipsin, IL-6, and others. Moreover, leukocytes have been suggested to contribute to adipose tissue metabolism because of their ability to influence fat deposits. Furthermore, preadipocytes might function as macrophage-like cells, raising the possibility that adipose tissue might participate in the inflammatory process (7). In addition, the protein macrophage migration inhibitory factor (MIF), an inflammatory mediator involved in macrophage migration, is expressed in adipocytes, which indicates that it is involved in several biological events, such as wound healing, atopic dermatitis, and possibly in diabetes and obesity (8, 9). Therefore, a close relationship exists between adipocyte physiology and immune function, and, as we will discuss further, several adipokines participate in a variety of immune responses in both the innate and adaptive arms of the immune system (Fig. 6.1).

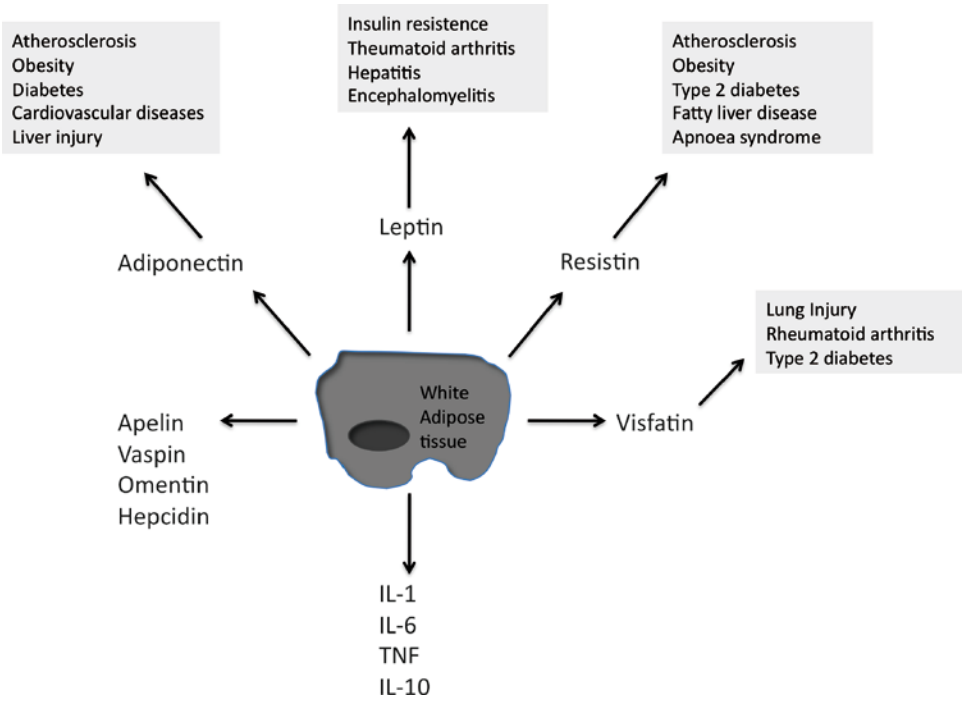


Fig. 6.1. The multiple functions of white adipose tissue. *Functions*: synthesis and secretion of adipokines, and the uptake, storage, and synthesis of lipids. White adipose tissue is also a source of pro-inflammatory factor that modulates the inflammatory response and promotes several diseases.

Evidence for a link between fat metabolism and immune system interactions comes from the fact that the fatty acid composition of phospholipids from splenocyte membranes are affected by dietary lipid manipulation, thereby modifying lymphocyte functions (10, 11). For example, higher levels of linoleic acids in spleen lymphocytes negatively correlate with CD25 (IL-2 α chain receptor) expression and proliferation rates. Thus, immunosuppressive effects induced by polyunsaturated fatty acids may be due to an increase in linoleic acid or a decrease in oleic acid-modifying components that are found during plasma membrane-associated events involved in lymphocyte activation (10).

6.3.1 Leptin

Several adipocyte-secreted products have been identified and some of these products are known as adipokines. This term is used to describe a cytokyne produced mainly by adipocytes (12). One of the adipokines that has gained enthusiastic attention from the scientific community is called leptin.

Leptin was first described in 1994 (13). Indeed, the study of leptin biology took place with the description of two mouse models, one obese (*ob*) and the other diabetic (*db*) (14, 15). In homozygotes (*ob/ob* and *db/db*), mutations in these loci led to hyperphagia and lower metabolic rates. Although genetic studies mapped these genes to different loci, their correlation became clear through parabiosis studies. The term parabiosis refers to the natural or surgical union of anatomical parts of two organisms, usually involving the exchange of blood, as in the development of Siamese twins or in certain transplant operations. In these studies, the parabiosis of *ob/ob* animals with both control littermates and *db/db* mice abrogated the phenotype observed in *ob/ob* animals, suggesting that the product of the ob gene was a circulating factor found in wild type and *db/db* mice, termed *db* (16, 17). In contrast with the results observed in *ob/ob* animals, the phenotype of the *db/db* mice was not modified by parabiosis with either wild type or *ob/ob* littermates, suggesting that the *db/db* animals, although possessing the circulating factor, were insensitive to it. These classical experiments lead to the assumption that the ob receptor gene was not functional in the *db/db* mice.

The hypothesis brought out by these classical experiments was confirmed by cloning the ob gene and identifying its product as leptin (14, 15). Shortly after cloning and identifying the ob gene, its receptor was also cloned (18). Once the leptin receptor gene was cloned, it was possible to observe that the gene responsible for leptin receptor transcription was mutated in the *db/db* mice (19).

There are several forms of the leptin receptor, all of which are a product of a single gene, *lepr*, and result from alternative splicing and photolytic cleavage (20). The leptin receptor can be divided into three categories: secreted form, short form, and long form (20, 21). Although the roles of the short forms of the leptin receptor are not well know, the long form, *OBRb*, is of greater importance in leptin function. *OBRb* is a receptor that activates Jak kinases, thus inducing STAT3 phosphorylation and activation (22).

After these initial studies, it became well known that leptin, the mouse ob gene product, is an unglycosylated peptide hormone belonging to the cytokine class I superfamily, is primarily synthesized by adipocytes, and acts in the hypothalamus to regulate energy expenditure and food intake (23). Blood levels of leptin are associated directly with the quantity of adipose tissue. The action of leptin in the central nervous system leads to a

decrease in food intake and an increase in energy expenditure. Moreover, leptin levels are gender dependent, being higher in females. In summary, leptin is a cytokine with hormonal characteristics and pleiotropic functions (24).

Because of leptin's role in the regulation of metabolism, it is a mediator of both the neuroendocrine system and immune responses (25). In the immune system, the lack of leptin or the inability to synthesize it promotes a variety of alterations. The *db/db* and *ob/ob* animals showed thymic atrophy and a tendency to become immunodeficient (26). Some of the actions of leptin in the immune system include the modulation of monocytes/macrophages, neutrophis, eosinophis, basophis, natural killer cells, dendritic cells, and lymphocytes (27–31). Leptin may induce the activation of T cells and modify the balance between Th1 and Th2 cells, favoring the production of Th1-pattern cytokines, thus indicating its pro-inflammatory role (28). Leptin might be produced by inflammatory cells and its gene transcription and transduction may be increased by LPS, IL-1, and IL-6 stimulation (32, 33).

In the innate immune system, leptin apparently participates in monocyte and macrophage activation, favoring phagocytosis and the production of B4 leukotriene, cyclooxygenase, nitric oxide, and the proinflammatory cytokines TNF and IL-1 (34, 35). In neutrophils, leptin increases chemotaxis and the release of oxygen radicals, and in humans, these effects are apparently indirect and promoted by the production of TNF by macrophages (36–38). Leptin affects the activation and development of NK cells, both *in vivo* and *in vitro* (39, 40). Due to the fact that the NK cells express OBRb and that *db/db* mice display an NK cell deficit, it is possible that leptin acts on both the development and maintenance of these cells (39–41).

Regarding adaptive immunity, leptin has been principally investigated in $CD4^+$ cells. Leptin increases the activation of $CD4^+$ T cells and their migration to inflammatory sites, possibly through an increase in the expression of adhesion molecules such as ICAM (*intercellular adhesion molecules*) and VLA2 (*very late antigen 2*) in response to IFN-γ (42).

Another role for leptin was observed in *ob/ob* mice. The lack of leptin in these animals is associated with thymic atrophy and immunosuppression (26, 42). Also, a leptin deficiency correlates with decreased hypersensitivity reactions. These alterations can be reversed by leptin administration (26, 43).

The *ob/ob* animals show only minor secretion levels of IL-2, IFN-γ, TNF and IL-18 and an increase in Th2 cytokines, such as IL-4 and IL-10, after mitogenic stimulation, which can cause these animals to be protected from induced autoimmune diseases (28, 44–46). Because leptin favors the Th1 profile and these cells have been associated with autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), it is possible that leptin neutralization at least partially protects the development of EAE and other autoimmune diseases, such as type 1 diabetes and antigen induced arthritis (47). The participation of leptin and other adipokines in autoimmune diseases will be further discussed (Fig. 6.2).

6.3.2 Adiponectin

Adiponectin is an adipose tissue-derived cytokine presenting homology to collagens VIII and X and to complement factor C1q. This adipokine is found in the peripheral blood in vast quantities and is found as several molecular isoforms (48). It acts through two

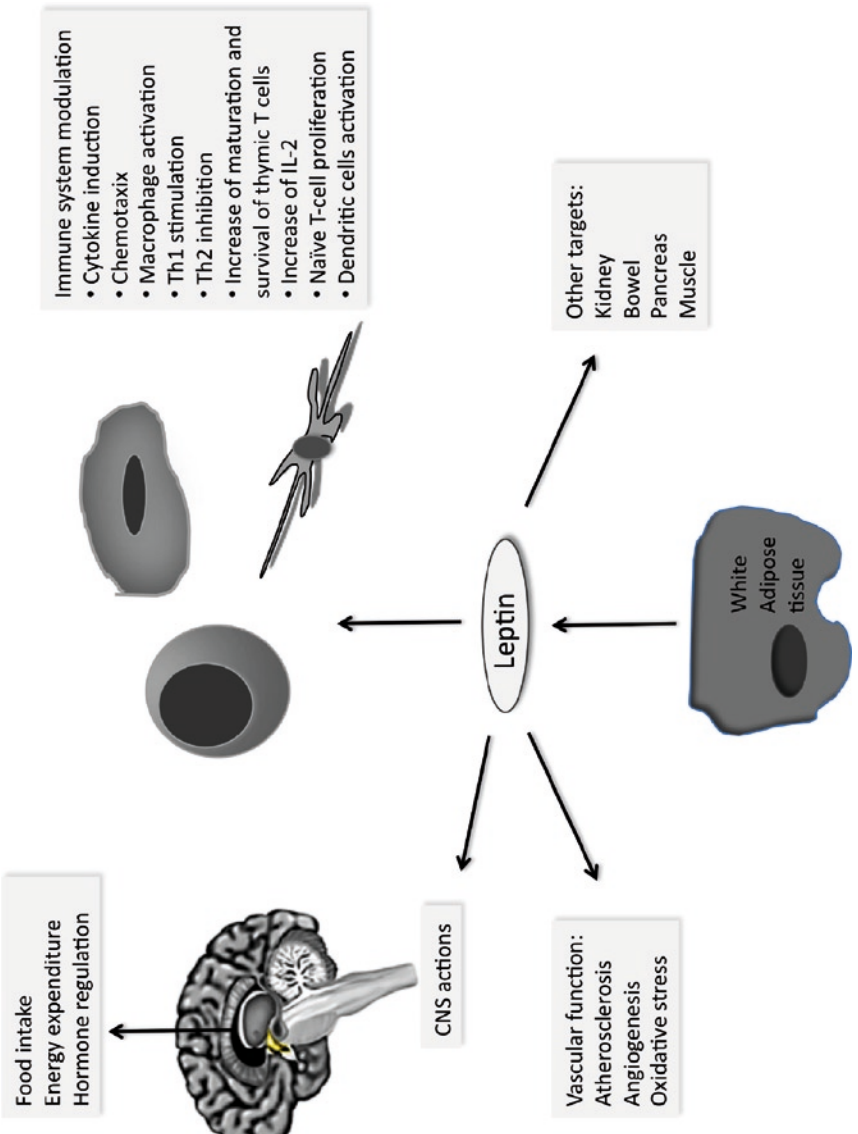


Fig. 6.2. A schematic representation of the pleiotropic function of leptin.

receptors, AdipoR1 (presented preferentially in skeletal muscle) and AdipoR2 (presented in the liver). The binding of this adiponectin to AdipoR1 and R2 leads to the activation of the kinase proteins AMPK, PPARα and PPARγ, as well as other molecules (49).

In the liver, adiponectin acts by increasing fatty acid oxidation and reducing liver glucose synthesis. Knock-out mice lacking adiponectin did not show any severe affects under regular conditions, but, under a high fat diet, the mice displayed severe insulin resistance and muscle lipid accumulation. Circulating concentrations of adiponectin have a tendency to be lower in morbidly obese individuals and increase with weight loss and the use of thiazolidinediones, which enhance sensitivity to insulin (48, 50).

Although adiponectin and leptin were identified around the same time, compared to leptin, less data exists about adiponectin in obesity and obesity related disorders. Adiponectin's participation in some obesity related pathologies was only recently recognized. Some examples of adiponectin participation include cardiovascular diseases, type 2 diabetes, and metabolic syndrome. Adiponectin displays relevant actions on both the innate and adaptive immune responses. It inhibits the phagocytic activity and IL-6 and TNF production of macrophages. Moreover, it may act to diminish T cell responses and B cell lymphopoiesis. Furthermore, adiponectin can induce the production of important anti-inflammatory mediators such as IL-10 and IL-1RA by human monocytes, dendritic cells and macrophages (51). Thus, adiponectin may have an important role in protecting against obesity and vascular diseases and, as it inhibits leptin-induced cytokines such as TNF and IL-6, adiponectin may be a negative regulator of leptin, although further evidence for this hypothesis needs to be discovered.

6.3.3 Resistin

Resistin has been attributed to mediating insulin resistance, but it appears that this role is limited to animal models, specifically rodents. Resistin received its name because of its ostensible induction of insulin resistance. It is a dimeric protein belonging to the FIZZ (found in inflammatory zones) family. Resistin, also recognized as FIZZ3, is chiefly found in macrophages and adipocytes (52).

As resistin is secreted in considerable quantities by mononuclear cells, it was suggested to be involved in inflammatory conditions. Resistin levels correlate with the levels of cell adhesion molecules, such as ICAM1, in individuals with sleep apnea. Moreover, in individuals with atherosclerosis, resistin correlates with other inflammation-related molecules, such as TNF-RII and a phospholipase A2-associated lipoprotein (53, 54). In addition, LPS was shown to induce resistin transcription in murine macrophages by a mechanism involving the production of several proinflammatory cytokines. In human PBMCs, resistin seems to induce and be induced by TNF and IL-6 by a mechanism that depends on the activation of the NFκB pathway (55). Therefore, even though limited data is available, resistin appears to be induced under inflammatory conditions.

6.3.4 Visfatin

Visfatin was identified in the liver, skeletal muscle and bone marrow as a growth factor for B cells precursors and has also been recognized as a pre-B-colony enhancing factor (PBEF). Visfatin circulating levels are closely related to white adipose tissue

deposits and increases with the differentiation of adipocytes. Vifastin transcription is regulated by TNF, IL-6 and glucocorticoids. This adipokine accumulates during lung injury and sepsis and is not exclusively produced by adipose tissue (56). Neutrophils can produce visfatin after being stimulated with endotoxin, leading to the caspase 3 and 8 mechanisms of preventing apoptosis (56). Individuals suffering from inflammatory bowel diseases display elevated levels of circulating visfatin. In monocytes, visfatin acts to stimulate the production of TNF, IL-6, IL-1β and costimulatory molecules and to promote chemotaxis. Also, it may act to augment the monocyte-mediated alloresponses in lymphocytes (57). Therefore, visfatin, another adipokine, is considered to be a pro-inflammatory mediator and might participate in a variety of inflammatory conditions.

6.3.5 Other Adipokines

Several other adipokines have been identified and their roles in immune-mediated diseases are currently being explored. Some of these adipokines include apelin, vaspin, hepcidin, and omentin.

Apelin was identified as a peptide and an endogenous binder of the orphan G-protein-coupled receptor APJ. Apelin secretion in adipose tissue is increased by TNF. In mice with diet-induced obesity, the levels of proinflammatory factors and the macrophage count rise progressively in adipose tissue and it is possible that apelin plays a role in promoting this condition. There is a lack of information about the participation of apelin in immune responses, although some data supports its participation in tumor neovascularization, as it increases the proliferation of endothelial cells (58).

Vaspin (visceral adipose tissue derived serine protease inhibitor) was first described in 2005 as a serine protease inhibitor produced by visceral adipose tissue (59). The administration of vaspin to obese mice improved glucose tolerance and insulin sensitivity (59). The induction of vaspin by adipose tissue may constitute a compensatory mechanism in response to obesity. Moreover, vaspin is modulated by the energy status of the placenta, indicating that this protein may be involved in the regulation of placental metabolic functions (60).

Hepcidin, described in 2001 as an antimicrobial urinary peptide that is produced in the liver, was later characterized as an adipokine (61). It appears to be an important regulator of iron homeostasis. The production of hepcidin does not exclusively depend on iron metabolism, but can also be stimulated by hypoxia and inflammatory stimuli (62). The levels of this adipokine are higher in disorders involving generalized inflammation that results in hypoferremia, which is caused by decreased iron absorption and increased sequestration of iron by macrophages. In mice, the stimulus to produce hepcidin in an acute inflammation model was described as depending on the IL-6 and the STAT3 pathway (63). Also, hepcidin may act against invading microorganisms as it decreases the extracellular levels of iron, thus limiting the amount of iron available to the microorganisms. Due to the characteristic of being induced by IL-6 and STAT3, hepcidin may be induced by leptin and, if so, a higher body mass index and obesity could lead to the production of hepcidin.

The 40 kDa protein omentin, produced by omental adipose tissue, was previously identified as intelectin, a $Ca2^{+}$-dependent lectin with high affinity for galactofuranosyl residues, which are found in pathogens (64). One possible physiological function for

omentin is the specific recognition of pathogens and bacterial components, presenting a role for omentin in the innate immune response (65). Furthermore, omentin levels are modified by inflammation and obesity. In patients with asthma, the levels of omentin are increased, although the mechanism leading to this is still unknown (66). The levels of omentin are also increased in type I diabetes mellitus (67). Another aspect of omentin is that its mRNA levels are differentially expressed in the omental adipose tissue of patients suffering from Crohn's disease, indicating that omentin may be a new factor involved in chronic inflammatory diseases and autoimmunity in humans (64).

6.4 IMMUNE FUNCTION IN OBESE MODELS

In genetic animal models, there is data showing that obesity and being overweight are associated with alterations in immunocompetence (68), and studies conducted in animal models showed that obesity caused impairments in the immune response (68, 69). In agreement with this data, obese leptin-deficient *ob/ob* mice show a decrease in body temperature, hyperphagia, infertility, and evidence of immune defects such as lymphoid organ atrophy, which mainly affects thymic size and cellularity (70). Therefore, *ob/ob* animals present immune disturbances characterized by reduced thymus proportions, lower numbers of lymphocytes and natural killer (NK) cells and lower cytotoxic activity (69). Regarding the thymus, although the size is reduced, the percentage of double positive and single positive cells is maintained at levels that are comparable to the wild type littermates (71). Hyperlipidemia and hyperglycemia are commonly observed and cortisol and ACTH (adrenocorticotropic hormone) may provide clues about some of the abnormalities observed in the immune response of *ob/ob* mice (72). Higher systemic levels of cortisol may lead to systemic immunosuppression. This immunosuppression may also be a result of the thymus atrophy because, although the percentages of the cells are similar, the atrophy results in a lower number of cells that differentiate and, as a consequence, the turnover of T lymphocytes may be affected.

Obese strains produce macrophages with lower phagocytic activity and also have lower expression levels of proinflammatory-related cytokines (73). Macrophages obtained from either *ob/ob* or *db/db* mice have shown some difficulties in destroying intracellular parasites, such as *Candida albicans*, as compared to macrophages isolated from normal animals, indicating that the adipokine leptin participates in the phagocytic process and influences immunocompetence (74). On the other hand, the intraperitoneal administration of recombinant leptin into *ob/ob* animals appears to contribute to the improvement of some immune functions (74). Thus, because of the importance of macrophages in the general aspects of inflammation and immunity, alterations in their functions may contribute to obesity-related pathophysiology. Rat models of obesity, which lack the leptin receptor, display lymphopenia, with low levels of CD4 and CD8 positive T cells in the thymus, spleen, and peripheral blood (68). These animals also showed reduced macrophage function. Moreover, the spleen proliferative response to mitogen is decreased and is apparently associated with lower glucose uptake mediated by the GLUT-1 transporter (75). These observations, from different animal models, indicate that these alterations are evolutionarily maintained and the study of such models may contribute to understanding the physiology of being overweight and related disorders.

6.5 IMMUNE FUNCTION IN OBESE INDIVIDUALS

There are few studies analyzing and comparing the immune responses of obese individuals, and almost all clinical trials analyze a limited number of subjects and immune parameters.

About 38% of obese children have an impairment in cell-mediated immune responses, exemplified by delayed cutaneous hypersensitivity, abnormal lymphoproliferative responses to mitogens, and a reduction in the capacity of polymorphonuclear leukocytes to clear intracellular bacterial infections (76). These data may be a result of altered macrophage functions, as previously mentioned in animal models, such as *ob/ob* mice. These same findings were also reported in adults suffering from morbid obesity (77). In addition, obesity apparently reduces lymphocyte immune function and NK cell activity in the elderly. This data shows that aging is an additional risk factor for obese humans, in respect to immune functions (78).

Regarding obesity in adult populations, it is observed that being overweight is associated with elevated lymphocyte and leukocyte subset counts, excluding NK, cytotoxic and suppressor T cells (presently called regulatory T cells). Being overweight is also associated with lower mitogen proliferation-induced of T and B cell lymphocytes, which is accompanied by higher monocyte and granulocyte phagocytosis, as well as being associated with oxidative burst activity and normal NK cell functions (79). Also, in adults, serum cholesterol, triglycerides, and glucose levels may be associated with several aspects of immunity and a positive correlation between body mass index (BMI) and total leukocytes has been observed.

Moderate immune stimulation in obese humans might be implicated in the development of insulin resistance with a possible role of TNF and melanocortin peptides (80). In this regard, type 2 diabetes mellitus is associated with an increase in the main cytokine mediator of the acute-phase response, IL-6, which could be a major contributor to the biochemical and clinical features of the metabolic syndrome and central obesity (81).

The moderate energy restriction in obese females affects a variety of parameters in both the innate and adaptive immune systems. However, weight loss, even though it was relatively moderate, was associated with significant decreases in several measurements of the functions of T cells, B cells, monocytes, and granulocytes relative to nonobese individuals, indicating that mild-to-moderate obesity is associated with alterations in immune function. Therefore, weight loss, even at a moderate rate, is related to a decrease in certain aspects of the function of the immune system (82). Lower numbers of NK cells and immunoglobulins were observed in overweight women following a restricted diet, and women who were on a restricted diet combined with a supervised program of aerobic activity were able to alleviate the apparent decrease in NK cell cytotoxicity as well as other distortions associated with weight loss, for example, the reduction of CD2 positive expressing IL-2Rα cells (83). Moreover, surgically-induced weight reduction leads to a decrease in IL-13, a Th2 cytokine and TNF, which could be one of the factors leading to altered immune function (84). Thus, the nutritional treatment of obesity by calorie restriction, surgery and physical activities may affect some of the altered immune functions observed in obese individuals, thereby improving the immune system and its capacity to control immune-related diseases such as diabetes, autoimmune diseases, and infections.

Weight reduction, as mentioned, may restore some of the physiological functions of the immune system. The safety of acute weight loss on immune functions, for example, was investigated by a supervised program involving 7–24 weeks with a very low calorie diet, during which the numbers of peripheral leukocytes did not change significantly, although a decrease in IgM serum levels during the program was observed (85). Therefore, diet programs, even with very low calorie levels, appear to be suitable for preoperative weight reduction in morbid obesity without seeming to alter the immune functions during this period.

Remarkably, acute nutritional deprivation frequently occurs in individuals suffering from obesity, thus affecting their immunocompetence (86). The bactericidal activity of blood monocytes and the cytolytic activity of NK cells were enhanced by fasting. Starvation also enhanced humoral immunity, as observed by an elevation in the serum concentrations of IgG, IgA, and IgM. The lymphocyte mitogen-induced proliferative responses modestly decreased, while the blood leukocytes did not appear to be significantly affected. Thus, fasting displays a variety of influences on immune functions rather than a patterned deleterious effect. These alterations in nutritional status appear to be potentially important, as they can actually enhance the functions of certain effectors of the host defense system in obese individuals, thereby improving the immune response.

Some studies have observed a lower production of antibodies after hepatitis B vaccination in obese patients (87). Moreover, the incidence and severity of infectious diseases was higher in obese patients than in lean individuals. Therefore, bacteremia and clinical sepsis occurred more often with obese individuals than with nonobese individuals, and antibiotic therapy was required for twice as many days for obese individuals (88). These are examples of some of the immune alterations observed in obese individuals.

6.6 ROLE OF ADIPOKINES IN CANCER

Recent international cancer prevention guidelines recommend weight loss for the purpose of reducing the risk of cancer. However, limited research is available relating weight loss to subsequent cancer incidence, mostly due to the difficulty of achieving long-term weight loss maintenance among large groups of patients (89). There are several reports linking adipokines, obesity and cancer occurrence. As there are multiple types of tumors, we chose to use breast cancer as an example of how these factors may be related.

Among several cancers, obesity is a strong risk factor for breast cancer in postmenopausal women and an adverse prognostic indicator regardless of menopausal status. Preexisting obesity and postoperative weight gain are associated with a poor prognosis in breast cancer, regardless of menopausal status. The relationship between adiposity and estrogen receptor-negative tumors that display a propensity for distant metastasis is of biological significance, especially in younger women. Insulin and some adipokines also stimulate breast cancer growth and metastasis, directly and possibly indirectly by augmenting angiogenesis. Thus, clinical methods for weight control are extremely important, not only for breast cancer occurrence and progression, but also for decreasing the nonbreast cancer mortality risk associated with excess adiposity (90). Also, in a

population-based study consisting mostly of European-American women, leptin polymorphisms were characterized as a risk factor for the development of breast cancer, with the associations being stronger in obese postmenopausal women (91). Therefore, a common variant of the leptin gene may be associated with the risk of developing breast cancer and may be involved in breast carcinogenesis. As mentioned, obesity is a risk factor for breast cancer development. A recent hypothesis suggests that the adipokines adiponectin and leptin may be involved in this process. Regarding this hypothesis, an interaction exists between leptin and adiponectin signaling pathways in cancer cells in which proliferation is stimulated by leptin and suppressed by adiponectin (92). This shows that on the one hand, leptin stimulates tumorigenesis and angiogenesis, while adiponectin might act as a negative regulator of this process.

6.7 ADIPOKINES AND IMMUNE-MEDIATED DISEASES

Over the past several years, a number of molecules known to play a role in metabolism have also been shown to have an important role in immune response regulation. Regarding this, the adipocyte hormone leptin regulates the immune response in normal and pathological conditions. More specifically, conditions of reduced leptin production, such as a genetic leptin deficiency, nervous anorexia, and malnutrition, are associated with increased susceptibility to infections. On the other hand, immune-mediated disorders such as autoimmune diseases are associated with increased leptin levels and the production of proinflammatory cytokines. Therefore, it could be argued that leptin represents the "missing link" between the immune responses, metabolic function, and nutritional status. In agreement with this idea, *ob/ob* mice, which do not produce leptin, were recently shown to be resistant to a series of experimentally induced autoimmune disorders, including EAE, an animal model of multiple sclerosis (93). Wild-type mice have increased secretion of leptin in serum during EAE induction and their brain inflammatory infiltrates stained positive for leptin. Lastly, leptin neutralization improves EAE, altering the intracellular signaling of auto-reactive T cells and increasing the number of Foxp3 positive T cells, thus leading to the idea that leptin could be considered a link between immune tolerance, metabolic state, and autoimmunity (94). The influence of leptin on autoimmunity and EAE susceptibility, which is induced by immunization with a myelin-derived peptide, demonstrates that a switch from a Th2 to a Th1 pattern of cytokine release could be deleterious and may increase the development of EAE. Thus, leptin is most likely required for the induction and maintenance of an effective pro-inflammatory immune response in the CNS (93). Moreover, as leptin promotes a switch toward a Th1 phenotype, its absence, as observed in *ob/ob* animals, could reverse this polarization and support an increase in Th2 immune responses, a mechanism that could aid in making these animals resistant to the induction of EAE.

Type 1 diabetes, which usually develops as an autoimmune disease, is increasing in incidence worldwide. The adipocyte hormone leptin may be involved in this process by promoting the polarization of a Th1 immune response in individuals at risk for the development of diabetes. Although leptin has been studied in animal models of autoimmune diseases, in humans, the data is not as clear. For example, elevated leptin levels do not appear to be a major determinant of whether an individual develops autoimmunity. If a high body mass index and elevated leptin levels are risk factors for the initiation

and/or progression of autoimmunity, they may act more as moderate factors in this process, probably acting to a certain degree in individuals that possess some kind of genetic predisposition (95).

As regards the studies of spontaneous autoimmunity, a role for leptin in nonobese diabetic (NOD) mice, an animal model for the study of human insulin-dependent diabetes mellitus (type 1 diabetes), demonstrates that the expression of serum leptin increased soon before the onset of hyperglycemia and diabetes in susceptible females. The intraperitoneal administration of leptin accelerates autoimmunity and the destruction of beta cells, promoting an increase in the IFN-γ production of peripheral T-cells. Thus, leptin can favor proinflammatory cellular responses and directly influence the development of autoimmune diseases that are mediated by Th1 responses (96). Unlike what is observed in humans, leptin appears to have an important role in development in animal models. As mentioned, due to the differences in HLA antigens and the distinct immune experiences of humans during their lives, it is most likely that in humans, as is observed in animal models, leptin functions during the course of autoimmune diseases, at least in susceptible individuals.

6.8 OBESITY, ADIPOKINES, AND KIDNEY DISEASES

Most forms of glomerulonephritis are immune-mediated. Accelerated nephrotoxic nephritis is a model of immune complex glomerulonephritis, in which an immune response is raised against foreign antiglomerular basement membrane antibodies deposited in the glomerulus. This leads to proliferative glomerulnephritis, characterized by albuminuria, leukocyte infiltration, glomerular capillary thrombosis, glomerular crescent formation, and renal impairment. $CD4^+$ T cell responses, especially Th1 responses, are important in mediating diseases in these situations (97, 98). Leukocytes, particularly macrophages, are involved in the effector phase of nephrotoxic nephritis (99, 100). Thus, as mentioned before, due to the action of leptin on leukocytes and macrophages, this adipokine appears to participate in the pathophysiology of several glomerulonephritis diseases.

Leptin is required for the induction and maintenance of immune-mediated glomerulonephritis, and blocking leptin appears to provide an attractive therapeutic possibility. Leptin-deficient mice are strongly protected from histological renal injury and albuminuria during accelerated nephrotoxic nephritis. Leptin-deficient mice present defects in humoral immunity and probably have additional defects in the innate immune response (101).

Focal and segmental glomerulosclerosis that leads to nephritic syndrome may be found in patients who are morbidly obese. Whether chronic stimulation of the glomerular cells by leptin contributes to glomerular remodeling and what mechanism could participate in this process are important questions. It appears that glomerular endothelial cells express Ob-R. The treatment of endothelial cells with leptin induces TGF-β synthesis. Although TGF-β is considered to be a suppressive immune growth factor, it also participates in tissue remodeling and fibrosis, which can lead to a loss of kidney function, a factor that may be found in chronic kidney diseases such as glomerulonephritis. Leptin stimulates glomerular endothelial cell proliferation *in vitro* and *in vivo* and leptin administration in rats causes proteinuria and glomerula mesangial matrix

expansion. A link between the hormonal system controlling body fat stores and glomerular size and matrix content appears to exist and implicates leptin as a causal factor for segmental glomerulosclerosis (102).

Obesity is an important cause of renal dysfunction. One of the clinical features of obese individuals, without systemic disorders but with apparent renal disorders, called obesity-related glomerulopathy, is slow progressive proteinuria. Several structural changes, such as glomerulomegaly, focal segmental glomerulosclerosis and damage to the glomerular basement membrane can be seen in biopsy sections of the kidney (103–105).

Obesity may increase kidney dysfunction in patients with glomerulonephritis by IgA-mediated nephropathy and, in fact, individuals suffering from IgA nephropathy have more severe forms of the disease and proteinuria (106). In addition to the information mentioned above about TGF-β, this growth factor may also promote a switch toward the secretion of IgA by B cells, a process that is likely to occur in the presence of high levels of leptin, such as in obesity, linking these three factors. Obesity worsens urine protein levels and glomerular damage in individuals suffering from several nephropathies, such as those mediated by IgA, basement membrane disease, and benign nephrosclerosis. Additionally, hypertension, a common feature of obesity-related alterations, is an important factor that precipitates kidney diseases in obesity-related nephropathies (107). Thus, obesity worsens chronic kidney diseases, leading to proteinuria and renal structural changes, and it promotes the progression of chronic renal diseases.

End-stage renal disease is a well-known trigger of cardiovascular complications because endothelial dysfunction and atherosclerosis are, for unknown reasons, common in this condition. Adiponectin and leptin are inversely related to the glomerular filtration rate (108, 109). Furthermore, it has also been shown that adiponectin and leptin are markedly increased in patients with end-stage renal disease (109). Nephrotic syndrome is a high-risk situation because, in spite of renal function, abundant urinary protein loss triggers hypoalbuminemia, hyperfibrinogenemia and hypercholesterolemia. Plasma adiponectin is apparently much higher in patients with nephrotic syndrome than in patients with mild or moderate proteinuria. Increased levels of adiponectin during heavy proteinuria is a counter-regulatory response aimed at attenuating the atherosclerotic effect of the biochemical alterations that are associated with this condition (110).

All of these kidney diseases have one thing in common: upon the loss of kidney function, the treatment of choice is hemodialysis and/or transplantation. In respect to kidney and other organ transplants, obesity and adipokines may interfere with both the graft and the patient's survival, linking metabolic regulation and the immune responses.

6.9 THE ROLE OF ADIPOKINES IN TRANSPLANT TOLERANCE AND REJECTION

In the context of organ transplants, there is limited information about the influence of adipose tissue-derived products on graft evolution. In transplants, the balance between Th1 and Th2 cells has an important role in the graft outcome, for both tolerance and rejection. The Th2 cells are described as having a protecting effect in relation to graft rejection responses, participating in the process of graft homeostasis (111–113). The Th1 cells, on the other hand, have a deleterious effect on graft survival, regulating

the inflammatory response to the graft, leading to the occurrence of both acute and chronic rejection. The physiological role of leptin and the observation that a lack of leptin favors the development of Th2 responses indicates that this adipokine could display an important role in organ transplants. Thus, obese individuals who possess elevated levels of serum leptin may be more susceptible to the occurrence of rejection as compared to normal individuals, as leptin favors the polarization toward Th1 responses. However, although this hypothesis can be supported, there is limited data describing whether the levels of leptin in the serum are correlated with a higher incidence of rejection.

Apparently, obesity itself is not associated with worse kidney transplant outcomes, although it appears to be associated with factors that lead to worse graft outcomes and patient survival. Being underweight is associated with late graft failure, mainly due to chronic allograft nephropathy (114). Moreover, fat mass correlated positively with C-reactive protein, suggesting that obesity may increase the risk of cardiovascular disease and chronic allograft rejection in kidney-transplant patients (115).

The graft function in normal-weight recipients is apparently better than that of obese individuals, as normal grafted individuals live longer than obese transplanted individuals. Graft survival is significantly inferior among obese and extremely obese patients, and average graft survival is higher in recipients with a normal body mass index than for overweight, obese, and extremely obese individuals. Thus, an increased body mass index is associated with inferior patient and graft survival (116). By analyzing graft and patient survival rates, the body mass index may be considered a risk factor and the treatment of obesity is of great importance in minimizing the side effects of organ transplants.

Additionally, endothelial dysfunction is strongly linked to cardiovascular disease and the outcome of patients with chronic kidney disease. It appears that increased inflammatory responses and increased adiponectin levels following transplants could be a mechanism for better endothelial health, as previously mentioned. However, concerning this aspect, endothelial function improved during the first month after the transplant, and the degree of improvement is associated with reductions in circulating levels of visfatin and adiponectin, two recently described adipokines (117). Thus, the levels of circulating adipokines, such as visfatin, adiponectin and leptin, among others, may influence a series of immune responses and disorders and may be of considerable importance in the response to grafted organs. Moreover, at least in relation to graft outcome, adiponectin appears to have a deleterious effect, although it improves endothelial function.

Another type of transplant, stem cell transplant, was also revealed to be modulated by adipokines, albeit to a lesser degree. Enhanced endothelial cell loss and diminished functions of endothelial stem cell progenitors in diabetic subjects are well-known phenomena. Bone marrow stem cell transplants that include mesenchymal cells may restore insulin sensitivity and glucose tolerance. In view of this, combined with the induction of HO-1 in the recipient, stem cell transplants appear to improve bone marrow function. HO-1 stimulation enhanced the ability of bone marrow stem cells to prevent diabetes. Thus, transplanting bone marrow stem cells and mesenchymal stem cells together with the induction of HO-1 can eradicate type 2 diabetes, and the beneficial effect of HO-1 leads to the hypothesis that the abnormality in endothelial progenitor cells is due to a

mesenchymal stem cell-stromal cell disorder, which is exacerbated by oxidative stress and decreased by adiponectin (118).

In the area of heart transplants, adiponectin appears to participate in impairing metabolic syndrome because renal failure is frequent among these patients. In contrast with the high frequency of metabolic syndrome in heart transplant patients, adiponectin levels were usually in the normal or high range, probably as a consequence of renal failure. Therefore, adiponectin is not a major determinant of insulin resistance among these patients (119). Among patients without chronic kidney disease, resistin, an adipokine, has been related to inflammatory markers, coronary artery disease and cardiovascular disease in metabolic syndrome. Moreover, resistin upregulates adhesion molecules. Because inflammation and endothelial cell damage or injury are invariably associated with thrombosis, atherosclerosis, and their major clinical consequences, resistin may play a role in linking inflammation and chronic disease. Moreover, triglycerides, creatinine, IL-6, TNF, vWF, prothrombin fragments 1 + 2, and resistin are elevated among kidney transplant recipients. Kidney allograft recipients with coronary artery disease displayed resistin levels that were elevated as compared to individuals without this complication. The resistin levels in kidney allograft recipients appear to be related to IL-6, thrombomodulin, red blood cell counts, white blood cell counts, platelet counts, creatinine, urea, and VCAM. Moreover, resistin is only independently related to creatinine and white blood cell counts in kidney allograft recipients. Therefore, the relation between elevated resistin levels to markers of inflammation may represent a link between these conditions and adipokines and, importantly, renal function was a major determinant of elevated resistin levels in kidney allograft recipients (120).

Leptin, which is primarily produced by adipocytes, has its receptors expressed in a variety of tissues, including the heart. Higher plasma leptin levels foretell acute myocardial infarction and it has been shown to be an acute phase reactant and a risk factor for coronary heart disease. An important question is whether or not a relationship exists between serum leptin levels and the grade of acute cellular rejection. In this regard, there is a positive correlation between serum leptin concentrations and body mass index, diastolic blood pressure, total cholesterol, and low-density lipoprotein levels. Therefore, elevated leptin levels in heart transplant patients may be related to the results of steroid therapy (121).

These transplant studies have a major implication. As leptin and other adipokines have an important metabolic function, any alteration in these adipokines might result in systemic pathophysiological modifications. Thus, it is difficult to conclude whether the effect of an adipokine is secondary to the metabolic alteration presented or primary, acting directly to modulate the immune system or the graft function. Thus, it is feasible to understand why almost all of the studies on transplant patients also include some alteration of metabolic aspects, such as metabolic syndrome or insulin resistance.

Insulin resistance and anthropometrical parameters of serum leptin levels after renal transplant demonstrated that circulating leptin levels dramatically decreased immediately after the transplant was performed and significantly correlated with serum insulin levels. An increase in serum leptin levels 6 months after the transplant is probably due to an increase in fat mass, insulin resistance, and steroid use in the renal transplant recipients (122). This reinforces what was mentioned before, that the effect of leptin may be secondary to the metabolic changes in the individual studied.

As mentioned earlier, weight reduction appears to have a beneficial effect on graft and recipient survival. In addition, in order to prevent graft loss and rejection, a large variety of immunosuppression agents are used by the clinics. One of these agents is rapamycin, an immunosuppressive drug used extensively to prevent graft rejection in transplant patients and to inhibit adipogenesis *in vitro*. This drug appears to display important antiobesity effects in mice on a high-fat diet. Rapamycin-treated animals display reduced body weight and epididymal fat pads/body weight, reduced daily food efficiency and lower levels of leptin and insulin in the serum. However, rapamycin-treated mice are hyperphagic, demonstrating an increase in food intake, and the dissection of rapamycin-treated mice revealed a marked reduction in fatty liver scores, average fat cell size and percentage of large retroperitoneal adipocytes and epididymal white adipose tissue (123). Therefore, rapamycin may prevent the effects of a high-fat diet on the rate of increase in body weight via reducing lipid accumulation, despite greater food intake, and makes it likely that rapamycin might serve as a potential therapy for body weight control and/or antiobesity therapy in transplant patients.

6.10 CONCLUSIONS AND PERSPECTIVES

Obesity and adipokines display a variety of immune and physiologic functions. They link the immune system to the neuroendocrine system and, as a consequence, the cytokines produced mainly by adipose tissue participate in several immune responses, such as autoimmunity, antitumor responses, transplants, infectious diseases, and many others. By doing so, understanding and modulating these adipose hormones may have a great impact on the treatment of several immune- and metabolic-related diseases.

REFERENCES

1. Bastard JP, Maachi M, Lagathu C et al (2006) Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 17:4–12
2. Martinez JA (2000) Obesity in young Europeans: genetic and environmental influences. Eur J Clin Nutr 54(Suppl 1):S56–S60
3. Chandra RK (1991) McCollum Award lecture. Nutrition and immunity: lessons from the past and new insights into the future. Am J Clin Nutr 53:1087–1101
4. Chandra RK (1997) Nutrition and the immune system: an introduction. Am J Clin Nutr 66:460S–463S
5. Chandra RK (1997) Graying of the immune system. Can nutrient supplements improve immunity in the elderly? JAMA 277:1398–1399
6. Keith ME, Jeejeebhoy KN (1997) Immunonutrition. Baillières Clin Endocrinol Metab 11:709–738
7. Cousin B, Munoz O, Andre M et al (1999) A role for preadipocytes as macrophage-like cells. FASEB J 13:305–312
8. Nishihira J (1998) Novel pathophysiological aspects of macrophage migration inhibitory factor (review). Int J Mol Med 2:17–28
9. Nishihira J (1997) The novel pathophysiological function of macrophage migration inhibitory factor. Seikagaku 69:1026–1029
10. Moussa M, Le Boucher J, Garcia J et al (2000) In vivo effects of olive oil-based lipid emulsion on lymphocyte activation in rats. Clin Nutr 19:49–54
11. Moussa M, Tkaczuk J, Ragab J et al (2000) Relationship between the fatty acid composition of rat lymphocytes and immune functions. Br J Nutr 83:327–333
12. MacLaren R, Cui W, Cianflone K (2008) Adipokines and the immune system: an adipocentric view. Adv Exp Med Biol 632:1–21

13. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432
14. Flier JS (1995) The adipocyte: storage depot or node on the energy information superhighway? Cell 80:15–18
15. Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. Nature 395:763–770
16. Coleman DL (1973) Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9:294–298
17. Coleman DL, Hummel KP (1969) Effects of parabiosis of normal with genetically diabetic mice. Am J Physiol 217:1298–1304
18. Tartaglia LA, Dembski M, Weng X et al (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263–1271
19. Chua SC Jr, White DW, Wu-Peng XS et al (1996) Phenotype of fatty due to Gln269Pro mutation in the leptin receptor (Lepr). Diabetes 45:1141–1143
20. Chua SC Jr, Koutras IK, Han L et al (1997) Fine structure of the murine leptin receptor gene: splice site suppression is required to form two alternatively spliced transcripts. Genomics 45:264–270
21. Tartaglia LA (1997) The leptin receptor. J Biol Chem 272:6093–6096
22. Baumann H, Morella KK, White DW et al (1996) The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proc Natl Acad Sci USA 93:8374–8378
23. Halaas JL, Gajiwala KS, Maffei M et al (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546
24. Lago F, Dieguez C, Gomez-Reino J, Gualillo O (2007) Adipokines as emerging mediators of immune response and inflammation. Nat Clin Pract Rheumatol 3:716–724
25. Guzik TJ, Marvar PJ, Czesnikiewicz-Guzik M, Korbut R (2007) Perivascular adipose tissue as a messenger of the brain-vessel axis: role in vascular inflammation and dysfunction. J Physiol Pharmacol 58:591–610
26. Howard JK, Lord GM, Matarese G et al (1999) Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. J Clin Invest 104:1051–1059
27. Dovio A, Caramello V, Masera RG et al (2004) Natural killer cell activity and sensitivity to positive and negative modulation in uncomplicated obese subjects: relationships to leptin and diet composition. Int J Obes Relat Metab Disord 28:894–901
28. Busso N, So A, Chobaz-Peclat V et al (2002) Leptin signaling deficiency impairs humoral and cellular immune responses and attenuates experimental arthritis. J Immunol 168:875–882
29. Fujita Y, Murakami M, Ogawa Y et al (2002) Leptin inhibits stress-induced apoptosis of T lymphocytes. Clin Exp Immunol 128:21–26
30. Li L, Renier G (2007) Adipocyte-derived lipoprotein lipase induces macrophage activation and monocyte adhesion: role of fatty acids. Obesity (Silver Spring) 15:2595–2604
31. Macia L, Delacre M, Abboud G et al (2006) Impairment of dendritic cell functionality and steady-state number in obese mice. J Immunol 177:5997–6006
32. Landman RE, Puder JJ, Xiao E, Freda PU, Ferin M, Wardlaw SL (2003) Endotoxin stimulates leptin in the human and nonhuman primate. J Clin Endocrinol Metab 88:1285–1291
33. Sarraf P, Frederich RC, Turner EM et al (1997) Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med 185:171–175
34. Mancuso P, Gottschalk A, Phare SM, Peters-Golden M, Lukacs NW, Huffnagle GB (2002) Leptin-deficient mice exhibit impaired host defense in Gram-negative pneumonia. J Immunol 168:4018–4024
35. Zarkesh-Esfahani H, Pockley G, Metcalfe RA et al (2001) High-dose leptin activates human leukocytes via receptor expression on monocytes. J Immunol 167:4593–4599
36. Caldefie-Chezet F, Poulin A, Tridon A, Sion B, Vasson MP (2001) Leptin: a potential regulator of polymorphonuclear neutrophil bactericidal action? J Leukoc Biol 69:414–418
37. Caldefie-Chezet F, Poulin A, Vasson MP (2003) Leptin regulates functional capacities of polymorphonuclear neutrophils. Free Radic Res 37:809–814
38. Zarkesh-Esfahani H, Pockley AG, Wu Z, Hellewell PG, Weetman AP, Ross RJ (2004) Leptin indirectly activates human neutrophils via induction of TNF-alpha. J Immunol 172:1809–1814

39. Zhao Y, Sun R, You L, Gao C, Tian Z (2003) Expression of leptin receptors and response to leptin stimulation of human natural killer cell lines. Biochem Biophys Res Commun 300:247–252
40. Tian Z, Sun R, Wei H, Gao B (2002) Impaired natural killer (NK) cell activity in leptin receptor deficient mice: leptin as a critical regulator in NK cell development and activation. Biochem Biophys Res Commun 298:297–302
41. Siegmund B, Lear-Kaul KC, Faggioni R, Fantuzzi G (2002) Leptin deficiency, not obesity, protects mice from Con A-induced hepatitis. Eur J Immunol 32:552–560
42. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394:897–901
43. Lord GM, Matarese G, Howard JK, Lechler RI (2001) The bioenergetics of the immune system. Science 292:855–856
44. Frisullo G, Mirabella M, Angelucci F et al (2007) The effect of disease activity on leptin, leptin receptor and suppressor of cytokine signalling-3 expression in relapsing-remitting multiple sclerosis. J Neuroimmunol 192:174–183
45. Faggioni R, Jones-Carson J, Reed DA et al (2000) Leptin-deficient (ob/ob) mice are protected from T cell-mediated hepatotoxicity: role of tumor necrosis factor alpha and IL-18. Proc Natl Acad Sci USA 97:2367–2372
46. Matarese G, Procaccini C, De Rosa V (2008) The intricate interface between immune and metabolic regulation: a role for leptin in the pathogenesis of multiple sclerosis? J Leukoc Biol 84:893–899
47. Ozata M, Ozdemir IC, Licinio J (1999) Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J Clin Endocrinol Metab 84:3686–3695
48. Oh DK, Ciaraldi T, Henry RR (2007) Adiponectin in health and disease. Diabetes Obes Metab 9:282–289
49. Kadowaki T, Yamauchi T, Kubota N (2008) The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett 582:74–80
50. Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. Endocr Rev 26:439–451
51. Tilg H, Moschen AR (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6:772–783
52. Steppan CM, Bailey ST, Bhat S et al (2001) The hormone resistin links obesity to diabetes. Nature 409:307–312
53. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ (2005) Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 111:932–939
54. Harsch IA, Koebnick C, Wallaschofski H et al (2004) Resistin levels in patients with obstructive sleep apnoea syndrome – the link to subclinical inflammation? Med Sci Monit 10:CR510–CR515
55. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A (2005) Resistin, an adipokine with potent proinflammatory properties. J Immunol 174:5789–5795
56. Jia SH, Li Y, Parodo J et al (2004) Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest 113:1318–1327
57. Moschen AR, Kaser A, Enrich B et al (2007) Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol 178:1748–1758
58. Masri B, van den Berghe L, Sorli C, Knibiehler B, Audigier Y (2009) Apelin signalisation and vascular physiopathology. J Soc Biol 203:171–179
59. Hida K, Wada J, Eguchi J et al (2005) Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci USA 102:10610–10615
60. Caminos JE, Bravo SB, Garces MF et al (2009) Vaspin and amylin are expressed in human and rat placenta and regulated by nutritional status. Histol Histopathol 24:979–990
61. Park CH, Valore EV, Waring AJ, Ganz T (2001) Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 276:7806–7810
62. Fleming RE (2007) Hepcidin activation during inflammation: make it STAT. Gastroenterology 132:447–449
63. Pietrangelo A, Dierssen U, Valli L et al (2007) STAT3 is required for IL-6-gp130-dependent activation of hepcidin in vivo. Gastroenterology 132:294–300

64. Schaffler A, Neumeier M, Herfarth H, Furst A, Scholmerich J, Buchler C (2005) Genomic structure of human omentin, a new adipocytokine expressed in omental adipose tissue. Biochim Biophys Acta 1732:96–102
65. Gerwick L, Corley-Smith G, Bayne CJ (2007) Gene transcript changes in individual rainbow trout livers following an inflammatory stimulus. Fish Shellfish Immunol 22:157–171
66. Kuperman DA, Lewis CC, Woodruff PG et al (2005) Dissecting asthma using focused transgenic modeling and functional genomics. J Allergy Clin Immunol 116:305–311
67. Tan BK, Pua S, Syed F, Lewandowski KC, O'Hare JP, Randeva HS (2008) Decreased plasma omentin-1 levels in Type 1 diabetes mellitus. Diabet Med 25:1254–1255
68. Tanaka S, Isoda F, Yamakawa T, Ishihara M, Sekihara H (1998) T lymphopenia in genetically obese rats. Clin Immunol Immunopathol 86:219–225
69. Tanaka S, Isoda F, Kiuchi Y, Ikeda H, Mobbs CV, Yamakawa T (2000) T lymphopenia in genetically obese-diabetic Wistar fatty rats: effects of body weight reduction on T cells. Metabolism 49:1261–1266
70. Mandel MA, Mahmoud AA (1978) Impairment of cell-mediated immunity in mutation diabetic mice (db/db). J Immunol 120:1375–1377
71. Palmer G, Aurrand-Lions M, Contassot E et al (2006) Indirect effects of leptin receptor deficiency on lymphocyte populations and immune response in db/db mice. J Immunol 177:2899–2907
72. Ahima RS, Prabakaran D, Mantzoros C et al (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252
73. Matarese G (2000) Leptin and the immune system: how nutritional status influences the immune response. Eur Cytokine Netw 11:7–14
74. Loffreda S, Yang SQ, Lin HZ et al (1998) Leptin regulates proinflammatory immune responses. FASEB J 12:57–65
75. Moriguchi S, Kato M, Sakai K, Yamamoto S, Shimizu E (1998) Decreased mitogen response of splenic lymphocytes in obese Zucker rats is associated with the decreased expression of glucose transporter 1 (GLUT-1). Am J Clin Nutr 67:1124–1129
76. Chandra RK, Kutty KM (1980) Immunocompetence in obesity. Acta Paediatr Scand 69:25–30
77. Palmblad J, Hallberg D, Engstedt L (1980) Polymorphonuclear (PMN) function after small intestinal shunt operation for morbid obesity. Br J Haematol 44:101–108
78. Fernandes G (2008) Progress in nutritional immunology. Immunol Res 40:244–261
79. Nieman DC, Henson DA, Nehlsen-Cannarella SL et al (1999) Influence of obesity on immune function. J Am Diet Assoc 99:294–299
80. Mountjoy KG, Wong J (1997) Obesity, diabetes and functions for proopiomelanocortin-derived peptides. Mol Cell Endocrinol 128:171–177
81. Pickup JC, Crook MA (1998) Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 41:1241–1248
82. Nieman DC, Nehlsen-Cannarella SI, Henson DA et al (1996) Immune response to obesity and moderate weight loss. Int J Obes Relat Metab Disord 20:353–360
83. Scanga CB, Verde TJ, Paolone AM, Andersen RE, Wadden TA (1998) Effects of weight loss and exercise training on natural killer cell activity in obese women. Med Sci Sports Exerc 30:1666–1671
84. Kyzer S, Binyamini J, Chaimoff C, Fishman P (1999) The effect of surgically induced weight reduction on the serum levels of the cytokines: interleukin-3 and tumor necrosis factor. Obes Surg 9:229–234
85. Pekkarinen T, Mustajoki P (1997) Use of very low-calorie diet in preoperative weight loss: efficacy and safety. Obes Res 5:595–602
86. Wing EJ, Stanko RT, Winkelstein A, Adibi SA (1983) Fasting-enhanced immune effector mechanisms in obese subjects. Am J Med 75:91–96
87. Weber DJ, Rutala WA, Samsa GP, Bradshaw SE, Lemon SM (1986) Impaired immunogenicity of hepatitis B vaccine in obese persons. N Engl J Med 314:1393
88. Gottschlich MM, Mayes T, Khoury JC, Warden GD (1993) Significance of obesity on nutritional, immunologic, hormonal, and clinical outcome parameters in burns. J Am Diet Assoc 93:1261–1268
89. Adams TD, Hunt SC (2009) Cancer and obesity: effect of bariatric surgery. World J Surg 33:2028–2033
90. Rose DP, Vona-Davis L (2009) Influence of obesity on breast cancer receptor status and prognosis. Expert Rev Anticancer Ther 9:1091–1101

91. Cleveland RJ, Gammon MD, Long CM et al (2010) Common genetic variations in the LEP and LEPR genes, obesity and breast cancer incidence and survival. Breast Cancer Res Treat 120:745–752
92. Jarde T, Caldefie-Chezet F, Goncalves-Mendes N et al (2009) Involvement of adiponectin and leptin in breast cancer: clinical and in vitro studies. Endocr Relat Cancer 16:1197–1210
93. Matarese G, Di Giacomo A, Sanna V et al (2001) Requirement for leptin in the induction and progression of autoimmune encephalomyelitis. J Immunol 166:5909–5916
94. De Rosa V, Procaccini C, La Cava A et al (2006) Leptin neutralization interferes with pathogenic T cell autoreactivity in autoimmune encephalomyelitis. J Clin Invest 116:447–455
95. Gilliam LK, Jensen RA, Yang P, Weigle DS, Greenbaum CJ, Pihoker C (2006) Evaluation of leptin levels in subjects at risk for type 1 diabetes. J Autoimmun 26:133–137
96. Matarese G, Sanna V, Lechler RI et al (2002) Leptin accelerates autoimmune diabetes in female NOD mice. Diabetes 51:1356–1361
97. Huang XR, Tipping PG, Shuo L, Holdsworth SR (1997) Th1 responsiveness to nephritogenic antigens determines susceptibility to crescentic glomerulonephritis in mice. Kidney Int 51:94–103
98. Kitching AR, Holdsworth SR, Tipping PG (1999) IFN-gamma mediates crescent formation and cell-mediated immune injury in murine glomerulonephritis. J Am Soc Nephrol 10:752–759
99. Nikolic-Paterson DJ (2003) A role for macrophages in mediating tubular cell apoptosis? Kidney Int 63:1582–1583
100. Nikolic-Paterson DJ, Atkins RC (2001) The role of macrophages in glomerulonephritis. Nephrol Dial Transplant 16(suppl 5):3–7
101. Tarzi RM, Cook HT, Jackson I, Pusey CD, Lord GM (2004) Leptin-deficient mice are protected from accelerated nephrotoxic nephritis. Am J Pathol 164:385–390
102. Wolf G, Hamann A, Han DC et al (1999) Leptin stimulates proliferation and TGF-beta expression in renal glomerular endothelial cells: potential role in glomerulosclerosis [see comments]. Kidney Int 56:860–872
103. Kambham N, Markowitz GS, Valeri AM, Lin J, D'Agati VD (2001) Obesity-related glomerulopathy: an emerging epidemic. Kidney Int 59:1498–1509
104. Praga M, Morales E (2006) Obesity, proteinuria and progression of renal failure. Curr Opin Nephrol Hypertens 15:481–486
105. Henegar JR, Bigler SA, Henegar LK, Tyagi SC, Hall JE (2001) Functional and structural changes in the kidney in the early stages of obesity. J Am Soc Nephrol 12:1211–1217
106. Ross WR, McGill JB (2006) Epidemiology of obesity and chronic kidney disease. Adv Chronic Kidney Dis 13:325–335
107. Kato S, Nazneen A, Nakashima Y et al (2009) Pathological influence of obesity on renal structural changes in chronic kidney disease. Clin Exp Nephrol 13:332–340
108. Wallace AM, McMahon AD, Packard CJ et al (2001) Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 104:3052–3056
109. Zoccali C, Mallamaci F, Tripepi G et al (2002) Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J Am Soc Nephrol 13:134–141
110. Hayakawa K, Ohashi H, Yokoyama H, Yoshida G, Okada M, Minatoguchi S (2009) Adiponectin is increased and correlated with the degree of proteinuria, but plasma leptin is not changed in patients with chronic glomerulonephritis. Nephrology (Carlton) 14:327–331
111. Lee WC, Jeng LB, Chiang YJ, Wang HC, Huang CC (2000) Dendritic cell progenitors prolong allograft survival through T-helper 2 deviation of the Th1/Th2 paradigm. Transplant Proc 32:2076–2077
112. Davidson C, Verma ND, Robinson CM et al (2007) IL-13 prolongs allograft survival: association with inhibition of macrophage cytokine activation. Transpl Immunol 17:178–186
113. Waaga AM, Gasser M, Kist-van Holthe JE et al (2001) Regulatory functions of self-restricted MHC class II allopeptide-specific Th2 clones in vivo. J Clin Invest 107:909–916
114. Chang SH, Coates PT, McDonald SP (2007) Effects of body mass index at transplant on outcomes of kidney transplantation. Transplantation 84:981–987
115. Ewers B, Gasbjerg A, Zerahn B, Marckmann P (2008) Impact of vitamin D status and obesity on C-reactive protein in kidney-transplant patients. J Ren Nutr 18:294–300
116. Ghahramani N, Reeves WB, Hollenbeak C (2008) Association between increased body mass index, calcineurin inhibitor use, and renal graft survival. Exp Clin Transplant 6:199–202

117. Yilmaz MI, Saglam M, Carrero JJ et al (2009) Normalization of endothelial dysfunction following renal transplantation is accompanied by a reduction of circulating visfatin/NAMPT. A novel marker of endothelial damage? Clin Transplant 23:241–248
118. Abraham NG, Li M, Vanella L, Peterson SJ, Ikehara S, Asprinio D (2008) Bone marrow stem cell transplant into intra-bone cavity prevents type 2 diabetes: role of heme oxygenase-adiponectin. J Autoimmun 30:128–135
119. Ambrosi P, Oddoze C, Nicolay A et al (2009) Plasma adiponectin in heart transplant recipients. Clin Transplant 23:83–88
120. Malyszko J, Malyszko JS, Pawlak K, Mysliwiec M (2006) Resistin, a new adipokine, is related to inflammation and renal function in kidney allograft recipients. Transplant Proc 38:3434–3436
121. Zwirska-Korczala K, Zakliczynski M, Berdowska A, Zembala M, Kukla M (2006) Serum leptin concentration in patients after heart transplantation. Clin Transplant 20:102–105
122. Kayacan SM, Yildiz A, Kazancioglu R, Sahin S, Sever MS, Ark E (2003) The changes in serum leptin, body fat mass and insulin resistance after renal transplantation. Clin Transplant 17:63–68
123. Chang GR, Chiu YS, Wu YY et al (2009) Rapamycin protects against high fat diet-induced obesity in C57BL/6J mice. J Pharmacol Sci 109:496–503

7 Obesity and Immune Functions

Qingde Zhou and Salomon Amar

Key Points

- The chapter reviews the literature pertaining to the role of obesity in modulating immune function.
- Obesity is found in both genetic and diet-induced obese animal models as well as in obese humans to impair immune response; both innate and acquired immune functions are affected.
- The immune dysfunction during obesity increases host susceptibility to infections and to chronic inflammatory diseases.

Key Words: Adipokines, adipose tissue, immune-related diseases, immunity.

7.1 INTRODUCTION

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse affect on health. The US National Institutes of Health and the WHO use body mass index (BMI), which compares weight and height, to define a person as overweight when his or her BMI is between 25 and 30 kg/m^2, and as obese when it is greater than 30 kg/m^2 (1). Obesity has become a global problem affecting over one billion adults and 17.6 million children under 5 years of age (2, 3). Since 1980, a threefold increase in obesity has been reported in much of the world, and more than 65% of the US population is categorized as either overweight or obese (3). Given the increasing trends of obesity in younger ages, a large group of the individuals will carry and express obesity-related health risks for a longer period of their lifespan. How this growing number of the adult obese population will age may have significant health and social implications.

Obesity represents a pathological state of energy imbalance accompanied by excessive fat deposition. Energy balance depends upon energy intake and expenditure. Three components controlling energy balance are (1) food intake, (2) fuel utilization and thermogenesis, and (3) adipocyte metabolism. Factors influencing these components include neuroendocrine factors, metabolic disturbances, genetic traits, and psychological

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_7

influences, as well as lifestyle habits such as excessive calorie intake or reduced physical activity. Among these, excessive calorie intake and reduced physical activity leading to diet induced obesity (DIO) are predominant and preventable and thus attract significant attention from the public.

Obesity has been linked with a wide variety of health problems, including hypertension, dyslipidemia, atherosclerosis, diabetes, nonalcoholic fatty liver disease, periodontal disease, certain cancers, and asthma (4–7). The extent to which obesity affects immune health is a topic of increasing interest to researchers. For example, clinical and epidemiological evidence indicates that the incidence and severity of specific types of infectious illness are higher in obese people compared with lean people: obesity has been identified as the risk factor for infection and poor wound healing after surgical procedures (8, 9), and overweight patients with burns have been shown to be at greater risk for infection and bacteremia than their nonobese counterparts (10). Obesity has also been associated with a poor antibody response to vaccine (11) and with an impaired innate immune response to infections (12–14). In addition to this indirect evidence of reduced immunocompetence among obese individuals, recent research has established the molecular basis for the impaired immune functioning in obese animals. Obesity is now seen as resulting in a dysregulation of the immune response to various infections, leading to attenuated host anti-infection capability (12, 14).

7.2 ADIPOSE TISSUE AND IMMUNE CELL INTERACTIONS

White adipose tissue (WAT) functions not only as an energy store, but also as an important endocrine organ capable of secreting a number of adipokines, including leptin, adiponectin, visfatin, apelin, vaspin, omentin, resistin, and hepcidin, and is involved in the regulation of many pathological processes. Many of the receptors for these adipokines are expressed on immune cells (15–18), providing a route of direct impact on immune cell function from the expansion of adipose tissue and adipose-derived products. The interactions between adipose tissue and the immune system can occur by two different mechanisms: (1) direct effects of adipokines and cytokines in circulation via specific receptor binding on adipocytes and leukocytes and (2) cell–cell interactions within adipose and lymphoid tissue microenvironments of bone marrow, thymus, and spleen.

7.2.1 Adipokine-Mediated Interactions

The role of adipose tissue in immunity has been elucidated by a number of cytokine-like hormones produced by adipocytes – called adipokines. Currently, the most relevant adipokines are thought to be leptin, adiponectin, resistin, and visfatin, all of which have marked effects on metabolic and immune function.

Leptin is a 16 kDa nonglycosylated peptide hormone encoded by the gene *LEP* (19); the murine homolog is called obese (*ob*). Leptin is mainly secreted by adipocytes, and circulating leptin levels are directly correlated with WAT mass. Leptin decreases food intake and increases energy consumption by inducing anorexigenic factors. Leptin sustains Th1 immunity by promoting effector T cell proliferation and by constraining regulatory T cell expansion. Leptin has different effects on proliferation and cytokine production by human naive (CD45RA+) and memory (CD45RO+) CD4+ T cells. While

promoting proliferation and IL-2 secretion by naive T cells, leptin minimally affects the proliferation of memory cells. Leptin deficiency in *ob/ob* mice or during acute starvation causes increased apoptosis of CD4+CD8+ T cells in the cortex of the thymus, leading to reduced delayed-type hypersensitivity (DTH) responses and thymic atrophy (20). In innate immunity, leptin promotes activation of and phagocytosis by monocytes/macrophages and their secretion of leukotriene B4 (LTB4), cyclooxygenase 2 (COX2) metabolites, NO, and proinflammatory cytokines (21). The products of the inducible form of COX2 and NO are involved in the regulation of inflammation, chemotaxis, and cytokine production, and therefore markedly impact the inflammatory immune response, which may provide a common pathogenetic mechanism that contributes to several of the major complications of obesity (22). Moreover, leptin can induce chemotaxis of neutrophils and the release of oxygen radicals (22). These mediators can be particularly harmful to cells, as they can denature proteins and damage membrane lipids, carbohydrates, and nucleic acids. NK cell development and activation are also affected in leptin receptor deficient (*db/db*) mice (23). Moreover, lymphocytes express receptor for growth hormone (GH) and also produce GH, which is apparently similar to its pituitary counterpart (24–26). GH increases the migration of fresh and activated lymphocytes and augments T cell adhesion via β1 and β2 integrins (27). Leptin has been reported to stimulate the production of GH in German Landrace gilts by peripheral blood mononuclear cells through protein kinase C- and NO-dependent pathways (28). This effect of leptin on GH production might be important in immune homeostasis.

Adiponectin is a 244-residue protein that is produced largely by WAT. It has structural homology with collagens VIII and X and complement factor C1q, and circulates in the blood in relatively large amounts in different molecular forms (29). It increases fatty acid oxidation and reduces the synthesis of glucose in the liver. Adiponectin has a wide range of effects in pathologies with immune and inflammatory components. It interferes with macrophage function by inhibiting phagocytic activity and IL-6 and TNF production. This anti-inflammatory action of adiponectin is exerted on monocytes/macrophages by inducing IRAK-M expression via adiponectin receptors 1 and 2 (30). In addition, it reduces B-cell lymphopoiesis, decreases effector T-cell response, and induces the production of important anti-inflammatory factors, such as IL-10 and IL-1RA, by human monocytes, macrophages, and dendritic cells (DC) (31).

Resistin is a dimeric protein that received its name from its apparent induction of insulin resistance in mice. It belongs to a gene family FIZZ (Found in inflammatory zone) and has been found in adipocytes, macrophages, and other cell types (32). Resistin is engaged in inflammatory conditions in humans due to its secretion in substantial quantities by mononuclear cells. Also, resistin levels are mutually correlated with those of cell adhesion molecules such as ICAM1 in patients with obstructive sleep apnea, and in atherosclerotic patients resistin levels are positively associated with other markers of inflammation, such as soluble TNFR type II and lipoprotein-associated phospholipase A2 (33). In addition, LPS has been reported to induce resistin gene expression in primary human and murine macrophages via a cascade involving the secretion of proinflammatory cytokines, and in human peripheral blood mononuclear cells resistin seems both to induce IL-6 and TNF via the NFκB pathway, and be induced by IL-6 and TNF (34).

Visfatin secreted by visceral fat is an insulin-mimetic adipokine (35). Circulating visfatin levels are closely correlated with WAT accumulation, visfatin mRNA levels

increase in the course of adipocyte differentiation, and visfatin synthesis is regulated by several factors, including glucocorticoids, TNF, IL-6, and growth hormone. However, visfatin was originally discovered in liver, skeletal muscle and bone marrow as a growth factor for B lymphocyte precursors. Visfatin is not only produced by WAT, but also by endotoxin-challenged neutrophils, in which it prevents apoptosis through a mechanism mediated by caspases 3 and 8 (36). Also, circulating visfatin levels are elevated in patients with inflammatory bowel diseases and visfatin mRNA expression levels are increased in their intestinal epithelium. Visfatin has been shown to induce chemotaxis and the production of IL-1β, TNF, IL-6, and costimulatory molecules by $CD14^+$ monocytes, and to increase their ability to induce alloproliferative responses in lymphocytes, effects which are mediated intracellularly by p38 and MEK1 (37).

7.2.2 Cell–Cell Interactions

The interaction between adipose tissue and the immune system is not only mediated by adipokines and cytokines via specific receptor binding on adipocytes and leukocytes, but also occurs through direct cell–cell contact within the specific tissue microenvironments of bone marrow, thymus, lymph nodes, and adipose tissue. Dense aggregations of lymphoid tissue, including lymph nodes, omentum, thymus and bone marrow, are anatomically associated with adipose tissue. The majority of smaller adipose depots enclose one or more lymph nodes, or less structured lymphoid tissues, including omental "milky spots." The close anatomic relationship between adipose tissue and lymph tissue provides an apparent venue for direct or paracrine interactions. Adipocytes, which both produce molecules containing various fatty acids derived from lipolysis and also release lipids directly, can fuel the adjacent lymphoid cells in response to local immune stimuli (38). The communication between adipocytes and lymphoid cells is bidirectional: lymph tissue-derived DC can suppress lipolysis in perinodal adipocytes, but those that permeate adipose tissue stimulate lipolysis, especially after minor, local immune stimulation (38).

7.2.2.1 Adipose Resident Immune Cells

Immune cells found in adipose tissues include macrophages, CD4 and CD8 T cells and multipotent mesenchymal stem cells. Among these, macrophages are the immune cells best studied in the adipose tissue microenvironment. Adipose tissue macrophages exist in a lipid-rich environment and fatty acids can broadly activate the macrophage inflammatory program through the pattern recognition receptors TLR2 and TLR4 (13, 39, 40). Adipose tissue macrophages were found to be responsible for the majority of TNF expression in adipose tissue and to contribute significantly to the inflammatory state of obese individuals. However, the high level of fatty acids in obesity can suppress bone marrow macrophage immune response when obese individuals are infected with bacteria or viruses (12, 14). The mechanism by which fatty acids inhibit macrophage immune responses involves induction by fatty acids of carboxy-terminal modulator protein (CTMP), which suppresses Akt phosphorylation and subsequently leads to the inhibition of NFκB activation and finally results in attenuated NO production and cytokine secretion (13).

Adipose tissue macrophages display heterogeneous inflammatory behavior, such that discrete cellular populations can be identified. Macrophages recruited to adipose tissue

during the induction of obesity with a high fat diet (HFD) are proinflammatory, as indicated by increased expression of cytokines (e.g., TNF) and inflammatory pathway genes, and are classified as M1 macrophages. In contrast, the macrophages resident in adipose tissue before HFD feeding are anti-inflammatory in nature (41) and are classified as M2 macrophages. M1 macrophages are induced by proinflammatory factors and display increased inflammatory gene expression, with enhanced reactivity to fatty acids and LPS (42). In obesity, the major component of the increased adipose tissue macrophage content is composed of proinflammatory macrophages that are triple positive for the surface markers F4/80, CD11b, and CD11c (39). Accordingly, because of their proinflammatory properties, these macrophages are implicated in the pathogenesis of insulin resistance. M2 macrophages display low levels of inflammatory gene expression, secrete high levels of anti-inflammatory factors, such as IL-10 and IL-4, and are poorly activated by fatty acids and LPS (42). Similar to M1 macrophages, they are positive for F4/80 and CD11b, but they are negative for CD11c (39). Importantly, the PPAR-γ nuclear receptor appears to be required for the maturation of M2 macrophages: treatment with PPARγ ligands (e.g., thiazolidinediones) polarizes macrophages toward the M2 state, thus providing a potential mechanism by which activation of macrophage PPARγ reduces inflammation and enhances insulin sensitivity (43, 44).

Recent studies also suggest that obesity leads to increased T cell infiltration in adipose tissue as CD4 and CD8 cells are increased in adipose tissue from obese mice (45). Since activation of T cells in the absence of costimulatory signals leads to generation of immune suppressive CD4+CD25+ T regulatory (Treg) cells (46), the local activation of immunity in adipose tissue would theoretically be associated with reduced costimulatory molecule expression by the M2 macrophages, which may predispose to Treg generation. Conversely, it is known that Tregs are involved in maintaining macrophages in the M2 phenotype (47). Supporting the possibility of Treg in adipose tissue also comes from the high concentration of local mesenchymal stem cells (MSC) which are known to secrete TGF-beta and IL-10, both involved in Treg generation (48). Indeed, studies have demonstrated the ability of MSC to induce Treg cells; a threefold increase in the CD4+CD25+ Treg cells were found in adipose tissue as compared to lymph node and spleen tissues (49). Functionally, Treg cells are capable of suppressing immune responses from other immune cells.

The mononuclear fraction of adipose tissue, referred to as the stromal vascular fraction (SVF), was originally described as a mitotically active source of adipocyte precursors (50). The SVF contains large numbers of MSC-like cells that could be induced to differentiate into adipogenic, chondrogenic, myogenic, and osteogenic lineages (51) and the notion of "adipose-derived stem cells" was then widely recognized. The SVF derived cells have surface marker expression similar to bone marrow derived MSC, comprising cells positive for CD29, CD44, CD71, CD90, CD105/SH2, and SH3 and lacking CD31, CD34, and CD45 expression (52). The CD31 negative cells exhibit mesenchymal properties and can be expanded *in vitro*, whereas the CD31 positive cells possess endothelial-like properties with poor *in vitro* expansion capacity (53).

7.2.2.2 Bone Marrow Adipocytes

Adipocytes are constituents of the bone marrow stromal cell microenvironment, but their exact origin and functional relevance remain unknown. Adipocytes in the bone marrow play four potential roles: (1) Adipocytes act as "filler" cells serving to occupy excess

space in marrow cavity; (2) Adipocytes play a role in regulating systemic lipid metabolism and energy storage; (3) Adipocytes are a reservoir and a local source of the energy required for hematopoiesis; and (4) Adipocytes may be required as "support" cells required for maturation of hematopoietic lineages and regulation of osteogenesis (54).

With increasing age, the bone marrow is progressively replaced with adipocytes with a concomitant reduction in osteoblasts and increased osteoporosis. Increased numbers of bone marrow adipocytes with aging are correlated with a reduction in pre-B-cell number, decreased B-cell generation, and immunoglobin diversity (45). Reciprocal bone marrow chimera experiments revealed that the production rates of pre-B cells are controlled primarily by bone marrow microenvironmental factors, rather than intrinsic events (45). The bone marrow adipocytes may arise from preferential skewing of multipotent marrow mesenchymal stem cells toward the generation of adipocytes rather than osteoblasts or stromal cells. Bone marrow adipogenesis may impair erythropoiesis, resulting in certain forms of anemia. Considering that adipocytes are the predominant cell type in an aging bone marrow microenvironment, the cellular interactions critical for the development of hematopoietic cells may be compromised with aging.

Non-aged adult bone marrow also contains adipocytes, the number of which correlates inversely with the hematopoietic activity of the marrow. Fatty infiltration of hematopoietic red marrow follows irradiation or chemotherapy and is a diagnostic feature in biopsies from patients with marrow aplasia. A recent study shows that hematopoietic stem cells and short-term progenitors are reduced in frequency in the adipocyte-rich vertebrae of the mouse tail relative to the adipocyte-free vertebrae of the thorax. In lipoatrophic A-ZIP/F1 "fatless" mice, which are genetically incapable of forming adipocytes, and in mice treated with the PPARγ inhibitor bisphenol A diglycidyl ether, which inhibits adipogenesis, marrow engraftment after irradiation is accelerated relative to wild type or untreated mice. These data implicate adipocytes as predominantly negative regulators in the bone-marrow microenvironment, and indicate that antagonizing marrow adipogenesis may enhance hematopoietic recovery in clinical bone-marrow transplantation (55).

7.2.2.3 Thymic Adipocytes

One of the most dramatic immune changes with aging is found in the thymic environment, which undergoes a progressive increase in adipocytes in the thymic parenchyma, septa, cortex, and medulla with the loss of epithelial and T lymphopoietic thymic zones into adipose tissue (56). The thymus is the only organ whose loss of function with increasing age is related to almost complete replacement of its microenvironment with adipose tissue. In contrast to a young thymus, where a large number of thymocytes are the major contributors to the thymic environment, the situation is reversed in aging, where the adipocytes constitute the bulk of the thymic space, thus altering the thymic milieu.

In obese animals, the thymi present a considerably different distribution of thymocyte subpopulations compared to lean mice. The absolute number of thymocytes are reduced by about tenfold, which is accompanied by a relative increase in $CD4^{-}CD8^{-}$ and $CD4^{+}CD8^{-}$ cells and by a relative decrease in $CD4^{+}CD8^{+}$, resulting in a decreased ratio of $CD4^{+}CD8^{+}/CD4^{-}CD8^{-}$ cells (57). Moreover, when exposed to a nonspecific stimulus, thymocytes from obese mice proliferate to a significantly lesser extent than do cells from lean mice counterparts (58).

It is conceivable that the presence of a large number of adipocytes in the thymus would significantly alter and influence the intrathymic milieu required to support the development of lymphoid progenitors into competent, mature lymphocytes. Interestingly, despite dramatic changes in thymic architecture with age and the large number of adipocytes, the aging human thymus retains thymopoietic potential, albeit to a limited extent. This suggests that interactions of adipocytes with lymphoid progenitors, developing T cells, and other stromal cells may have important consequences for thymic biology. Forestalling adipocyte development in the thymus or bone marrow could thus be an important strategy to preserve or prevent the decline in immune function with age.

7.2.2.4 Lymph Node Adipocytes

Adipose and lymphoid tissues develop in parallel in fetal and neonatal mammals. Adipocytes anatomically associated with lymph nodes and omental milky spots have many special properties, including fatty acid composition and the control of lipolysis, that equip them to interact locally with lymphoid cells. Lymph node lymphocytes and tissue DC acquire their fatty acids from the contiguous adipocytes. Lymph node-derived DC suppresses lipolysis in perinodal adipocytes but those that permeate the adipose tissue stimulate lipolysis, especially after minor, local immune stimulation. Inflammation changes the composition of fatty acids incorporated into DC, and the adipocytes of node-containing adipose tissue, counteracting the effects of dietary lipids. Thus, these specialized adipocytes partially emancipate the immune system from fluctuations in the abundance and composition of dietary lipids (38). Paracrine interactions between adipose and lymphoid tissues are enhanced by diets rich in n-6 fatty acids and attenuated by fish oils. The latter improve immune function and body conformation in humans and other animals (59).

7.3 IMMUNE FUNCTION IN OBESE ANIMAL MODELS

Different animal models are used to analyze how obesity influences immune status: genetically obese rodents characterized by mutations in the leptin gene (*ob/ob* mice) or leptin receptor gene (*db/db* mice and *fa/fa* rats) and DIO rodents are the most heavily employed models.

7.3.1 Genetically Obese Animal Models

Obese leptin-deficient *ob/ob* mice possess a spontaneous mutation of the leptin gene that inhibits the secretion of mature leptin. They display hyperphagia, hypothermia, hypercorticosteronemia, hyperglycemia, hypothyroidism, growth hormone deficiency, hyperinsulinemia, reduced energy expenditure, decreased linear growth, infertility, and early-onset morbid obesity. The *db/db* mice have a similar phenotype to *ob/ob* mice, but they are resistant to leptin because of a mutated leptin receptor. These animals display immune disturbances such as reduced thymus proportion, lower lymphocyte and NK cell numbers as well as decreased cytotoxic activity. Lymphocyte responsiveness to different mitogens is lower in these animals compared with wild type counterparts. Macrophages obtained either from *ob/ob* mice or from *db/db* mice have less phagocyte activity and a lower expression of proinflammatory-related cytokines and thus are less

active in destroying *Candida* than those isolated from control wild type animals (22). The impairment in the phagocytic activity of macrophages from genetically obese animals may also be associated with high levels of TNF, which are known to alter iNOS and cytokine production (13, 60). These genetically obese animals also produce less IL-2 than lean animals, which could partly explain the lower capacity of T cells to proliferate in obese animals (61). Elevated serum free fatty acid concentrations may inhibit T-lymphocyte signaling, while decreased lymphocyte proliferation may be due to the impairment of glucose uptake by lymphocytes (62).

In obese Zucker rats, lymphopenia is found in the thymus and the spleen, as well as in the peripheral blood (61). The proliferation response of spleen cells to mitogen is decreased, which has been associated with a lower glucose uptake mediated by the glucose transporter (GLUT)-1 transporter (62). NK cell activity has been reported to be suppressed, but this effect was found to be reversible through exercise training, which correlated with improved lymphocyte glucose uptake and enhanced GLUT-1 expression. On the other hand, phagocytosis was not affected by obesity, but obese Zucker rats had diminished ability to kill phagocytosed bacteria compared to control rats, due to lower oxidative burst activity (63).

7.3.2 Diet-Induced Obese Animal Models

In DIO animals, similar results concerning the impairment of immune function have been found, although the effects are less pronounced than in genetically obese animals. DIO mice display aberrant innate immune responses to both bacterial and viral infections.

DIO mice are more susceptible than lean mice to morbidity and mortality during influenza infection. Following infection with influenza, DIO mice display elevated lung pathology and aberrant innate immune responses, characterized by minimal induction of the Type I interferons (IFN) IFN-α and IFN-β, delayed expression of proinflammatory cytokines and chemokines, and impaired NK cell cytotoxicity (14). In addition, DIO mice have a delayed mononuclear cell entry at the site of infection, with a marked decrease in DC throughout the infection. Although obesity does not interfere with DC migration or antigen uptake, it does impair DC antigen presentation, and thereby alters antigen-specific CD8+ T-cell responses (64).

Obesity is also associated with an increased susceptibility to bacterial infection, such as that associated with periodontal disease. Following oral infection with *Porphyromonas gingivalis*, DIO mice exhibit a significantly higher level of alveolar bone loss than lean controls do. Oral microbial sampling discloses higher levels of *P. gingivalis* in mice with DIO vs. lean mice during and after infection. When exposed to systemic inoculation of *P. gingivalis*, DIO mice develop a blunted inflammatory response with reduced expression of TNF, IL-6, and SAA at all time points compared to lean mice (12). In addition, the induction of inducible NO synthase (iNOS) is also suppressed systemically in mice with DIO, and in bone marrow macrophages from mice with DIO exposed to *P. gingivalis*. Bone marrow macrophages from lean mice pretreated with free fatty acids (FFAs) and exposed to *P. gingivalis* exhibit a diminished induction of iNOS and cytokines. Bone marrow macrophages from lean and DIO mice exposed to *P. gingivalis* and analyzed by a phosphorylation protein array showed a reduction of Akt in bone

marrow macrophages from mice with DIO, but not from lean mice. This reduction was found to be responsible for diminished NF-κB activation and diminished induction of iNOS and cytokines. Moreover, TLR2 responses are suppressed in bone marrow macrophages from DIO mice, whereas the level of CTMP, a known suppressor of Akt phosphorylation, is elevated. This elevation stems from defective TLR2 signaling. In bone marrow macrophages from lean mice, both FFAs and TNF, via separate pathways, induce an increase in CMTP. However, in bone marrow macrophages from DIO mice, TLR2 can no longer inhibit the TNF-induced increase in CTMP caused by *P. gingivalis* challenge. This defect can then be restored by transfecting exogenous TLR2 into bone marrow macrophages from DIO mice. Thus, feeding mice a HFD over time elevates the CTMP intracellular pool, initially via the action of FFAs to activate TLR2, and later when the defective TLR2 is unable to inhibit TNF-induced CTMP. These findings describe a link between obesity and impaired innate immunity (13).

Poor oxidative burst activity has also been found in DIO animals, related to the increased uncoupling protein-2 (UCP2) mRNA levels in spleens of obese rats. A mild uncoupling of respiration by UCP2 may regulate ROS production by modulating proton leakage through the inner mitochondrial membrane, suggesting a greater capacity of macrophages to generate ROS in the absence of UCP2 in the mitochondria (65). Other genes implicated in obesity, such as PPARγ, which is highly expressed in adipose tissue, may be key modulators of adipogenesis, and also involved in macrophage and T-helper functions.

7.4 IMMUNE FUNCTION IN OBESE HUMANS

Epidemiological data support the idea that obesity is associated with alterations in immune function, but data are controversial for some parameters. One of the problems in analyzing immune function in obese individuals is that the effects of obesity itself on the immune system can be hidden by the coexistence of hyperglycemia and dyslipidemia. Studies in which obese individuals with diabetes, insulin resistance or hyperlipidemia are excluded may eliminate these confounding factors. As occurs in animal models, most investigations confirm a lower capacity of lymphocytes to proliferate in response to mitogen activation. Insulin receptor synthesis by T-lymphocytes after *in vitro* stimulation is reduced in obese subjects when compared to nonobese individuals (66), and it is possible that this lower expression may play a role in the impairment of T-lymphocyte functions. Although neutrophil, monocyte, T lymphocyte, and B lympnocyte were reported elevated in obese individuals (66), other investigation has reported a T-lymphopenia in obese patients (67). This lymphopenia is apparently related to higher body mass index and TNF production. It appears that in elderly men and women, obesity is also related to a reduced activity of NK cells. A negative correlation between body fat and NK cell activity in elderly women and adult men has been established (66). In infants, a positive relationship between body weight and infection of the lower respiratory tract has been observed. Possible reasons for the higher incidence would include mechanical factors that affect pulmonary function and impaired immune status concerning cell-mediated immunity and phagocyte function. Monocyte and granulocyte phagocytosis were not found to be influenced by obesity, while basal and activated monocyte and basal granulocyte oxidative bursts were higher in obese subjects (66). Moreover, in

several studies that have assessed the immune response in obese patients after weight loss or nutritional deprivation, results suggest that immune impairments can be corrected with adequate weight control (67–69). Obese patients after weight reduction have increased T-cell response and higher proliferation response to mitogen stimulation, but have decreased monocyte oxidative burst as well as NK cell counts, but not T- and B-cell counts at baseline level. While total lymphocyte numbers did not change after a weight reduction program, the response of T-lymphocytes to different mitogens was increased as was B-lymphocyte blastogenesis at the end of the dietary restriction period. During the slimming period, fasting blood glucose and serum triglyceride concentrations were slightly reduced. Therefore, these results suggest that an improvement in the physiological milieu may contribute to an improvement in immune function during weight loss. A positive effect on immune response might be observed over the long-term period after subjects have achieved and maintained normal weight. Further research is warranted in this area before meaningful conclusions can be drawn, however, because most of the studies have analyzed the effects of short-term weight loss on immunity in obese subjects. Furthermore, the interactive effect of changes in psychological stress with weight loss on immune function needs to be addressed. These results indicate that nutritional restriction appears to enhance certain effector functions of the host defense system in the obese patient.

7.5 OBESITY AND IMMUNE RELATED DISEASES (FIG. 7.1)

7.5.1 Obesity and Infections

Obesity increases morbidity and mortality through its multiple effects on nearly every human system. Several epidemiological studies of obese individuals found evidence of increased susceptibility to infections, including post-operative infectious complications (70), and a positive correlation between BMI and the incidence of nosocomial infections such as pneumonia, wound infection, bacteremia, and *Clostridium difficile* colitis (71, 72). The incidence of community-acquired respiratory tract infections has been found higher in obese patients. A large population study has demonstrated that the BMI is directly associated with an increased risk of community-acquired pneumonia among women (73), and women who gained weight (18 kg or more) during follow-up observation had a twofold increase in the risk for community-acquired pneumonia than those who maintained their weight (74). Overweight children also have twice the risk of acute respiratory infections than children with a normal BMI (75). In patients with chronic hepatitis C infection, markers of obesity such as the BMI and waist-to-hip ratio correlate with the extent of steatosis in this population independently of the presence of diabetes (76). Since steatosis affects the natural course of hepatitis C infection, it is not surprising that hepatitis C infection progresses more rapidly in obese compared with nonobese patients (77). Moreover, obese people are more prone to cutaneous infections and display reduced wound-healing capabilities (78, 79). Intertigo, candidiasis, furunculosis, erythrasma, tinea cruris, and folliculitis are skin infections frequently encountered among obese patients. Fungal foot infections, such as tinea pedis and toenail onychomycosis, are more common in obese than nonobese patients, and in the long run may predispose the affected patients to acute bacterial cellulitis of the lower extremities (80, 81). Obesity-linked impairment of immunity has also been noted in various animal

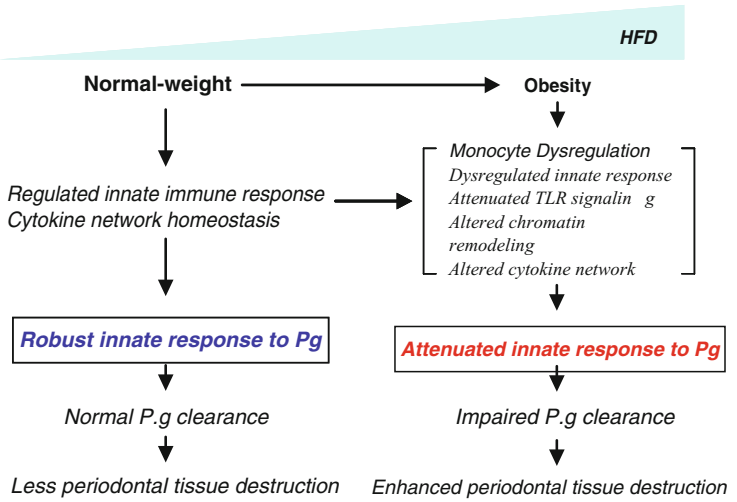


Fig. 7.1. *Model for the proposed effect of obesity on innate response to P.g:* This illustrates how we interpret the effect of obesity on peripheral innate immune response to *P.g* infection. We propose that in normal mice, a homeostatic cytokine network maintains a regulated response to bacterial challenge through a cycle of transient inflammation, followed by downmodulation with anti-inflammatory cytokines. As obesity develops, monocyte dysregulation develops. Together, these perturbations alert the homeostatic network that normally counters inflammation associated with infections. We postulate that obesity becomes associated with an altered pro- and anti-inflammatory network, an altered gene expression profile in monocyte/macrophage, an altered capacity for signaling through TLRs and other microbially induced pathways, and an altered chromatin status definable at specific cytokine loci. A state of attenuated innate response to *P.g*. with an impaired *P.g* clearance is established leading to an enhanced tissue damage.

models, such as increased susceptibility to *Candida albicans* infections (63) and impaired response to *Listeria monocytogenes* in obese leptin-deficient *ob/ob* and leptin-resistant *db/db* mice (82).

7.5.2 Obesity and Periodontal Disease

Since 1998, several epidemiological studies have found an association between obesity and an increased incidence of periodontal disease. After adjusting for potentially confounding factors such as age, gender, oral hygiene status, and smoking, the relative risk of periodontitis was 3.4 in persons with BMI of 25–29.9 kg/m^2, and 8.6 in those with BMI above 30 kg/m^2 (83). Another study not only corroborated this association, but also found that older men who had sustained a large (>30%) increase in weight during adulthood had an even higher risk of poor periodontal condition (7). A third study in a younger population also linked overall and abdominal obesity with an increased prevalence of periodontal disease, noting that underweight (BMI < 18.5) was associated with a decreased prevalence (84). As in metabolic syndrome X, the pattern of fat distribution plays a crucial role in the association with periodontitis, with both total body fat elevation and upper body obesity correlated with a higher risk of periodontal disease (84, 85). Among people with periodontal disease, obesity is also associated with deep periodontal pockets, and BMI is positively correlated with the severity of periodontal attachment

loss. Individuals who maintained a normal weight, pursued regular exercise, and consumed a diet in conformity with the Dietary Guidelines for Americans and the Food Guide Pyramid recommendations were 40% less likely to have periodontitis (86–88). Moreover, obesity significantly contributed to the severity of periodontal disease in an animal model. In a ligature-induced periodontitis rodent model, alveolar bone resorption was greater in obese rats compared with nonobese rats (89). Proinflammatory cytokines may be part of the link between periodontitis and obesity, as high TNF in the gingival crevice fluid closely correlates to high body mass index (90).

7.5.3 Obesity in Diabetes and Cardiovascular Disease

The growing prevalence of type 2 diabetes and cardiovascular disease is tied to overweight or obesity (91). About 90% of type 2 diabetes is attributable to excess weight. Furthermore, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome. This number is expected to increase to 420 million by 2025. Population-based surveys of 75 communities in 32 countries show that diabetes is rare in communities in developing countries where a traditional lifestyle has been preserved. By contrast, some Arab, migrant Asian Indian, Chinese, and the U.S. Hispanic communities that have undergone westernization and urbanization are at higher risk; in these populations, the prevalence of diabetes ranges from 14 to 20%. In addition, most of the population growth in the developing world is taking place in urban areas.

Obesity is a critical risk factor in the development of insulin resistance, which is characterized by an inability of insulin to inhibit glucose output from the liver and to increase glucose uptake into skeletal muscle (92, 93). Recent research unveils several mechanisms, whereby obesity causes diabetes: (1) Obesity increases hepatic endoplasmic reticulum (ER) stress, leading to an exhaustion of activating transcription factor 6 alpha (ATF6α). Since ATF6α reduces hepatic glucose output by inhibiting CREB regulated transcription coactivator 2 (CRTC2) occupancy over the promoters of gluconeogenic genes via disrupting the interaction between CREB and CRTC2, gluconeogenic genes are upregulated when hepatic ATF6α protein amounts are reduced, therefore leading to the increase of hepatic glucose output and insulin resistance (94, 95); (2) Adipocytes release a protein called pigment epithelium-derived factor (PEDF), which causes the muscle and liver to become desensitized to insulin, leading to the development of Type 2 diabetes (96, 97). (3) Elevated inflammatory factors (such as TNF-alpha, IL-1, IL-6, leptin, and resistin) in obesity inhibit insulin signaling in hepatocytes by activating suppressor of cytokine signaling (SOCS) proteins, several kinases (such as JNK, IKKβ, and PKC) and protein tyrosine phosphatases (such as PTP1B and PTEN), that in turn impair insulin signaling at both the insulin receptor and insulin receptor substrate (IRS) levels (98).

The risk of cardiovascular disease is considerably greater among obese people, and this group has an incidence of hypertension that is five times the incidence among people of normal weight. Hence, overweight and obesity are contributing to a global increase in hypertension: one billion people had hypertension in 2000, and 1.56 billion people are expected to have this condition by 2025 (99). The effect of obesity and diabetes on complications of cardiovascular disease is also more severe among members of most ethnic

minority groups in Western countries as well as among the populations of developing countries, where an increased waist-to-hip ratio is a strong predictor of ischemic heart disease and stroke. The estimated risk of cardiovascular disease is higher among South Asians than among white Westerners or persons of African origin; this difference is attributable to earlier onset of obesity and diabetes and to higher blood pressure (91).

7.6 CONCLUSIONS AND PERSPECTIVES

Although obesity is a hyperinflammation state characterized with expanded macrophages, leukocytes, and lymphocytes infiltration into adipose tissue and with activated cytokine network, the immune system seems paralyzed in response to various infections. This immune paralysis finds its basis in epidemiological studies of obese individuals which found the evidence of increased susceptibility to infections, including post-operative infectious complications, and a positive correlation between body weight index and the incidence of both community and nosocomial infections. Recent evidence points to the high fat diets which interfere with the ability of the immune system to appropriately respond to *P. gingivalis* infection and causes a higher mortality rate in mice following infection with influenza virus.

Several lines of evidence have supported a link between adipose tissue and immunocompetent cells. The activated interaction between adipocytes and immune cells is mediated by adipokines or by direct cell–cell contact. Immune cell subsets expand within adipose tissue during obesity causing chronic inflammation and insulin resistance, and an increase in the number of adipocytes in the thymus, bone marrow, and lymph nodes is associated with reduced immunity. In obesity, excess adiposity and impaired immune function have also been described in both human and diet-induced or genetically obese rodents. Thus, the direct interactions between immune cells and adipocytes by cell–cell contact and via their secreted products could potentially be the significant pathological processes for obese-related immune diseases, such as infections, periodontitis, diabetes, and cardiovascular diseases.

Acknowledgments This work was supported by a grant from the National Institute of Dental and Craniofacial Research DE15989 (SA).

REFERENCES

1. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr (1999) Body-mass index and mortality in a prospective cohort of U.S. adults. N Engl J Med 341:1097–1105
2. WHO (2004) Global strategy on diet, physical activity and health. Obesity and overweight. http://www.who.int/dietphysicalactivity/media/en/gsfsobesity.pdf
3. National Health Center for Statistics (2003–2004) Prevalence of overweight and obesity among adults. www.CDC.gov/nchs http://www.CDC.gov/nchs
4. Dalla Vecchia CF, Susin C, Rosing CK, Oppermann RV, Albandar JM (2005) Overweight and obesity as risk indicators for periodontitis in adults. J Periodontol 76:1721–1728
5. Miranda PJ, DeFronzo RA, Califf RM, Guyton JR (2005) Metabolic syndrome: definition, pathophysiology, and mechanisms. Am Heart J 149:33–45
6. Miranda PJ, DeFronzo RA, Califf RM, Guyton JR (2005) Metabolic syndrome: evaluation of pathological and therapeutic outcomes. Am Heart J 149:20–32

7. Linden G, Patterson C, Evans A, Kee F (2007) Obesity and periodontitis in 60–70-year-old men. J Clin Periodontol 34:461–466
8. Stallone DD (1994) The influence of obesity and its treatment on the immune system. Nutr Rev 52:37–50
9. Fasol R, Schindler M, Schumacher B, Schlaudraff K, Hannes W, Seitelberger R, Schlosser V (1992) The influence of obesity on perioperative morbidity: retrospective study of 502 aortocoronary bypass operations. Thorac Cardiovasc Surg 40:126–129
10. Gottschlich MM, Mayes T, Khoury JC, Warden GD (1993) Significance of obesity on nutritional, immunologic, hormonal, and clinical outcome parameters in burns. J Am Diet Assoc 93:1261–1268
11. Weber DJ, Rutala WA, Samsa GP, Santimaw JE, Lemon SM (1985) Obesity as a predictor of poor antibody response to hepatitis B plasma vaccine. JAMA 254:3187–3189
12. Amar S, Zhou Q, Shaik-Dasthagirisaheb Y, Leeman S (2007) Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge. Proc Natl Acad Sci USA 104:20466–20471
13. Zhou Q, Leeman SE, Amar S (2009) Signaling mechanisms involved in altered function of macrophages from diet-induced obese mice affect immune responses. Proc Natl Acad Sci USA 106:10740–10745
14. Smith AG, Sheridan PA, Harp JB, Beck MA (2007) Diet-induced obese mice have increased mortality and altered immune responses when infected with influenza virus. J Nutr 137:1236–1243
15. Dunn SL, Bjornholm M, Bates SH, Chen Z, Seifert M, Myers MG Jr (2005) Feedback inhibition of leptin receptor/Jak2 signaling via Tyr1138 of the leptin receptor and suppressor of cytokine signaling 3. Mol Endocrinol 19:925–938
16. Taildeman J, Perez-Novo CA, Rottiers I, Ferdinande L, Waeytens A, De Colvenaer V, Bachert C, Demetter P, Waelput W, Braet K, Cuvelier CA (2009) Human mast cells express leptin and leptin receptors. Histochem Cell Biol 131:703–711
17. Vernooy JH, Drummen NE, van Suylen RJ, Cloots RH, Moller GM, Bracke KR, Zuyderduyn S, Dentener MA, Brusselle GG, Hiemstra PS, Wouters EF (2009) Enhanced pulmonary leptin expression in patients with severe COPD and asymptomatic smokers. Thorax 64:26–32
18. Zarkesh-Esfahani H, Pockley G, Metcalfe RA, Bidlingmaier M, Wu Z, Ajami A, Weetman AP, Strasburger CJ, Ross RJ (2001) High-dose leptin activates human leukocytes via receptor expression on monocytes. J Immunol 167:4593–4599
19. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432
20. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394:897–901
21. La Cava A, Matarese G (2004) The weight of leptin in immunity. Nat Rev Immunol 4:371–379
22. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM (1998) Leptin regulates proinflammatory immune responses. FASEB J 12:57–65
23. Tian Z, Sun R, Wei H, Gao B (2002) Impaired natural killer (NK) cell activity in leptin receptor deficient mice: leptin as a critical regulator in NK cell development and activation. Biochem Biophys Res Commun 298:297–302
24. Bresson JL, Jeay S, Gagnerault MC, Kayser C, Beressi N, Wu Z, Kinet S, Dardenne M, Postel-Vinay MC (1999) Growth hormone (GH) and prolactin receptors in human peripheral blood mononuclear cells: relation with age and GH-binding protein. Endocrinology 140:3203–3209
25. Poppi L, Dixit VD, Baratta M, Giustina A, Tamanini C, Parvizi N (2002) Growth hormone secretagogue (GHS) analogue, hexarelin stimulates GH from peripheral lymphocytes. Exp Clin Endocrinol Diabetes 110:343–347
26. Hattori N, Shimatsu A, Sugita M, Kumagai S, Imura H (1990) Immunoreactive growth hormone (GH) secretion by human lymphocytes: augmented release by exogenous GH. Biochem Biophys Res Commun 168:396–401
27. Taub DD, Tsarfaty G, Lloyd AR, Durum SK, Longo DL, Murphy WJ (1994) Growth hormone promotes human T cell adhesion and migration to both human and murine matrix proteins in vitro and directly promotes xenogeneic engraftment. J Clin Invest 94:293–300

28. Dixit VD, Mielenz M, Taub DD, Parvizi N (2003) Leptin induces growth hormone secretion from peripheral blood mononuclear cells via a protein kinase C- and nitric oxide-dependent mechanism. Endocrinology 144:5595–5603
29. Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. Endocr Rev 26:439–451
30. Zacharioudaki V, Androulidaki A, Arranz A, Vrentzos G, Margioris AN, Tsatsanis C (2009) Adiponectin promotes endotoxin tolerance in macrophages by inducing IRAK-M expression. J Immunol 182:6444–6451
31. Tilg H, Moschen AR (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6:772–783
32. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA (2001) The hormone resistin links obesity to diabetes. Nature 409:307–312
33. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ (2005) Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 111:932–939
34. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A (2005) Resistin, an adipokine with potent proinflammatory properties. J Immunol 174:5789–5795
35. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I (2005) Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 307:426–430
36. Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD, Marshall JC (2004) Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest 113:1318–1327
37. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, Tilg H (2007) Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol 178:1748–1758
38. Pond CM (2005) Adipose tissue and the immune system. Prostaglandins Leukot Essent Fatty Acids 73:17–30
39. Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, Liu-Bryan R, Glass CK, Neels JG, Olefsky JM (2007) A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem 282:35279–35292
40. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116:3015–3025
41. Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR (2007) Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes 56:16–23
42. Martinez FO, Sironi M, Vecchi A, Colotta F, Mantovani A, Locati M (2004) IL-8 induces a specific transcriptional profile in human neutrophils: synergism with LPS for IL-1 production. Eur J Immunol 34:2286–2292
43. Hevener AL, Olefsky JM, Reichart D, Nguyen MT, Bandyopadyhay G, Leung HY, Watt MJ, Benner C, Febbraio MA, Nguyen AK, Folian B, Subramaniam S, Gonzalez FJ, Glass CK, Ricote M (2007) Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. J Clin Invest 117:1658–1669
44. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vats D, Brombacher F, Ferrante AW, Chawla A (2007) Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 447:1116–1120
45. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV (2008) Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes (Lond) 32:451–463
46. Zhang X, Li M, Lian D, Zheng X, Zhang ZX, Ichim TE, Xia X, Huang X, Vladau C, Suzuki M, Garcia B, Jevnikar AM, Min WP (2008) Generation of therapeutic dendritic cells and regulatory T cells for preventing allogeneic cardiac graft rejection. Clin Immunol 127:313–321
47. Tiemessen MM, Jagger AL, Evans HG, van Herwijnen MJ, John S, Taams LS (2007) CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. Proc Natl Acad Sci USA 104:19446–19451
48. Askenasy N, Kaminitz A, Yarkoni S (2008) Mechanisms of T regulatory cell function. Autoimmun Rev 7:370–375

49. Gonzalez-Rey E, Gonzalez MA, Varela N, O'Valle F, Hernandez-Cortes P, Rico L, Buscher D, Delgado M (2009) Human adipose-derived mesenchymal stem cells reduce inflammatory and T-cell responses and induce regulatory T cells in vitro in rheumatoid arthritis. Ann Rheum Dis 69(1):241–248
50. Hollenberg CH, Vost A (1969) Regulation of DNA synthesis in fat cells and stromal elements from rat adipose tissue. J Clin Invest 47:2485–2498
51. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7:211–228
52. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279–4295
53. Boquest AC, Shahdadfar A, Fronsdal K, Sigurjonsson O, Tunheim SH, Collas P, Brinchmann JE (2005) Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after in vitro cell culture. Mol Biol Cell 16:1131–1141
54. Gimble JM, Robinson CE, Wu X, Kelly KA (1996) The function of adipocytes in the bone marrow stroma: an update. Bone 19:421–428
55. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ (2009) Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature 460:259–263
56. Taub DD, Longo DL (2005) Insights into thymic aging and regeneration. Immunol Rev 205:72–93
57. Howard JK, Lord GM, Matarese G, Vendetti S, Ghatei MA, Ritter MA, Lechler RI, Bloom SR (1999) Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. J Clin Invest 104:1051–1059
58. Palmer G, Aurrand-Lions M, Contassot E, Talabot-Ayer D, Ducrest-Gay D, Vesin C, Chobaz-Peclat V, Busso N, Gabay C (2006) Indirect effects of leptin receptor deficiency on lymphocyte populations and immune response in db/db mice. J Immunol 177:2899–2907
59. MacQueen HA, Sadler D, Mattacks C (2000) Dietary fatty acids influence the appearance of tumour necrosis factor-alpha receptors on adipocytes following an immune challenge. Br J Nutr 84:387–392
60. Moshyedi AK, Josephs MD, Abdalla EK, Mackay SL, Edwards CK 3rd, Copeland EM 3rd, Moldawer LL (1998) Increased leptin expression in mice with bacterial peritonitis is partially regulated by tumor necrosis factor alpha. Infect Immun 66:1800–1802
61. Tanaka S, Isoda F, Yamakawa T, Ishihara M, Sekihara H (1998) T lymphopenia in genetically obese rats. Clin Immunol Immunopathol 86:219–225
62. Moriguchi S, Kato M, Sakai K, Yamamoto S, Shimizu E (1998) Decreased mitogen response of splenic lymphocytes in obese Zucker rats is associated with the decreased expression of glucose transporter 1 (GLUT-1). Am J Clin Nutr 67:1124–1129
63. Plotkin BJ, Paulson D (1996) Zucker rat (fa/fa), a model for the study of immune function in type-II diabetes mellitus: effect of exercise and caloric restriction on the phagocytic activity of macrophages. Lab Anim Sci 46:682–684
64. Smith AG, Sheridan PA, Tseng RJ, Sheridan JF, Beck MA (2009) Selective impairment in dendritic cell function and altered antigen-specific CD8+ T-cell responses in diet-induced obese mice infected with influenza virus. Immunology 126:268–279
65. Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, Goubern M, Surwit R, Bouillaud F, Richard D, Collins S, Ricquier D (2000) Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat Genet 26:435–439
66. Nieman DC, Henson DA, Nehlsen-Cannarella SL, Ekkens M, Utter AC, Butterworth DE, Fagoaga OR (1999) Influence of obesity on immune function. J Am Diet Assoc 99:294–299
67. Tanaka R, Yoshikawa N, Kitano Y, Ito H, Nakamura H (1993) Long-term ciclosporin treatment in children with steroid-dependent nephrotic syndrome. Pediatr Nephrol 7:249–252
68. Nieman DC, Nehlsen-Cannarella SI, Henson DA, Butterworth DE, Fagoaga OR, Warren BJ, Rainwater MK (1996) Immune response to obesity and moderate weight loss. Int J Obes Relat Metab Disord 20:353–360
69. Tanaka S, Isoda F, Ishihara Y, Kimura M, Yamakawa T (2001) T lymphopaenia in relation to body mass index and TNF-alpha in human obesity: adequate weight reduction can be corrective. Clin Endocrinol (Oxf) 54:347–354

70. Espejo B, Torres A, Valentin M, Bueno B, Andres A, Praga M, Morales JM (2003) Obesity favors surgical and infectious complications after renal transplantation. Transplant Proc 35:1762–1763
71. Choban PS, Heckler R, Burge JC, Flancbaum L (1995) Increased incidence of nosocomial infections in obese surgical patients. Am Surg 61:1001–1005
72. Canturk Z, Canturk NZ, Cetinarslan B, Utkan NZ, Tarkun I (2003) Nosocomial infections and obesity in surgical patients. Obes Res 11:769–775
73. Curhan GC, Chertow GM, Willett WC, Spiegelman D, Colditz GA, Manson JE, Speizer FE, Stampfer MJ (1996) Birth weight and adult hypertension and obesity in women. Circulation 94:1310–1315
74. Baik I, Curhan GC, Rimm EB, Bendich A, Willett WC, Fawzi WW (2000) A prospective study of age and lifestyle factors in relation to community-acquired pneumonia in US men and women. Arch Intern Med 160:3082–3088
75. Jedrychowski W, Maugeri U, Flak E, Mroz E, Bianchi I (1998) Predisposition to acute respiratory infections among overweight preadolescent children: an epidemiologic study in Poland. Public Health 112:189–195
76. Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL (2002) Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. Hepatology 36:729–736
77. Ortiz V, Berenguer M, Rayon JM, Carrasco D, Berenguer J (2002) Contribution of obesity to hepatitis C-related fibrosis progression. Am J Gastroenterol 97:2408–2414
78. Janniger CK, Schwartz RA, Szepietowski JC, Reich A (2005) Intertrigo and common secondary skin infections. Am Fam Physician 72:833–838
79. Scheinfeld NS (2004) Obesity and dermatology. Clin Dermatol 22:303–309
80. Chan MK, Chong LY (2002) A prospective epidemiologic survey on the prevalence of foot disease in Hong Kong. J Am Podiatr Med Assoc 92:450–456
81. Roujeau JC, Sigurgeirsson B, Korting HC, Kerl H, Paul C (2004) Chronic dermatomycoses of the foot as risk factors for acute bacterial cellulitis of the leg: a case-control study. Dermatology 209:301–307
82. Ikejima S, Sasaki S, Sashinami H, Mori F, Ogawa Y, Nakamura T, Abe Y, Wakabayashi K, Suda T, Nakane A (2005) Impairment of host resistance to Listeria monocytogenes infection in liver of db/db and ob/ob mice. Diabetes 54:182–189
83. Saito T, Shimazaki Y, Sakamoto M (1998) Obesity and periodontitis. N Engl J Med 339:482–483
84. Al-Zahrani MS, Bissada NF, Borawskit EA (2003) Obesity and periodontal disease in young, middle-aged, and older adults. J Periodontol 74:610–615
85. Saito T, Shimazaki Y, Koga T, Tsuzuki M, Ohshima A (2001) Relationship between upper body obesity and periodontitis. J Dent Res 80:1631–1636
86. Al-Zahrani MS, Bissada NF, Borawski EA (2005) Diet and periodontitis. J Int Acad Periodontol 7:21–26
87. Al-Zahrani MS, Borawski EA, Bissada NF (2005) Increased physical activity reduces prevalence of periodontitis. J Dent 33:703–710
88. Al-Zahrani MS, Borawski EA, Bissada NF (2005) Periodontitis and three health-enhancing behaviors: maintaining normal weight, engaging in recommended level of exercise, and consuming a high-quality diet. J Periodontol 76:1362–1366
89. Perlstein MI, Bissada NF (1977) Influence of obesity and hypertension on the severity of periodontitis in rats. Oral Surg Oral Med Oral Pathol 43:707–719
90. Lundin M, Yucel-Lindberg T, Dahllof G, Marcus C, Modeer T (2004) Correlation between TNFalpha in gingival crevicular fluid and body mass index in obese subjects. Acta Odontol Scand 62:273–277
91. Hossain P, Kawar B, El Nahas M (2007) Obesity and diabetes in the developing world – a growing challenge. N Engl J Med 356:213–215
92. Kahn BB, Flier JS (2000) Obesity and insulin resistance. J Clin Invest 106:473–481
93. Saltiel AR (2001) New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell 104:517–529
94. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi N, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306:457–461
95. Wang Y, Vera L, Fischer WH, Montminy M (2009) The CREB coactivation CRTC2 links hepatic ER stress and fasting gluconeogenesis. Nature 460:534–537

96. Crowe S, Wu LE, Economou C, Turpin SM, Matzaris M, Hoehn KL, Hevener AL, James DE, Duh EJ, Watt MJ (2009) Pigment epithelium-derived factor contributes to insulin resistance in obesity. Cell Metab 10:40–47
97. Nakamura K, Yamagishi S, Adachi H, Kurita-Nakamura Y, Matsui T, Inoue H (2009) Serum levels of pigment epithelium-derived factor (PEDF) are positively associated with visceral adiposity in Japanese patients with type 2 diabetes. Diabetes Metab Res Rev 25:52–56
98. Ruan H, Pownall HJ (2009) The adipocyte IKK/NFkappaB pathway: a therapeutic target for insulin resistance. Curr Opin Investig Drugs 10:346–352
99. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J (2005) Global burden of hypertension: analysis of worldwide data. Lancet 365:217–223

Section B
Nutrients and Immunomodulation

8 Host Immune Resistance and Dietary Lipids

Manuel A. de Pablo Martínez, Maria A. Puertollano, and Elena Puertollano

Key Points

- Both *n*-3 polyunsaturated fatty acids and *n*-9 monounsaturated fatty acids contribute to the suppression of several immune system functions, exerting important anti-inflammatory properties.
- *n*-3 polyunsaturated acids are the most immunosuppressive fatty acids, and they have been applied in the resolution of diseases characterized by an overactivation of immune response.
- Different factors and mechanisms are involved in the execution of these properties.
- The administration of high amounts of *n*-3 polyunsaturated fatty acids reduces host defense to bacteria, viruses, parasites, or fungi.
- Inappropriate administration of *n*-3 polyunsaturated fatty acids in patients at risk of sepsis may cause adverse effects due to an increase in the susceptibility to infection.

Key Words: Olive oil, immune system, lymphocytes, cytokines, autoimmune diseases, infection, immune resistance.

8.1 INTRODUCTION

Mammals and other vertebrates have developed, during evolution, the immune system, a specialized, organized, and sophisticated mechanism of defense to combat and eliminate harmful microorganisms. This important protective system may be defined as a highly effective and complex network of cells and factors, perfectly orchestrated and coordinated, responsible for the host defense from infectious and pathogenic agents. Immune system is divided into innate and acquired immunity. The innate arm of immunity prevents the entry of foreign microorganisms into the body, playing a crucial role in the early control of infectious agents, as well as in the initiation and subsequent course

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_8

of the acquired immunity. Therefore, innate response constitutes the first line (early phase) of defense against pathogens. It is obvious that innate immunity represents an important mechanism, which rapidly destroys and eliminates microorganisms through different procedures such as phagocytosis or engulfment of undesirable agents, direct destruction by the production of complement or toxic substances released from phagocytic cells or natural killer (NK) cells. In fact, innate immunity is the most efficient mechanism in order to eliminate intracellular growth microorganisms. Innate resistance does not distinguish among microorganisms and does not change in intensity upon reexposure. On the other hand, acquired immunity requires the identification of molecules from an invading agent. The recognition of antigens is carried out by B lymphocytes and T lymphocytes, which constitute the main arm of acquired immunity and produce antibodies (B cells) or recognize the antigens on the surface of cells (B or T cells). Adaptive immunity leads to the generation of immunological memory, which is characterized for the capacity to respond better and quicker upon reexposure to the same antigen.

The innate immune system is capable of recognizing a limited group of conserved components of bacteria, parasites, fungi, or viruses, known as pathogen-associated molecular patterns (PAMP), which have therefore been called pattern recognition receptors (PRR). Host cells express various PRR that detect diverse pathogen-associated molecular patterns, varying from lipids, lipopolysaccharides (LPS), lipoproteins, proteins, and nucleic acids. As a consequence, identification of these PAMP by PRR promotes the activation of intracellular signaling pathways that conclude in the release of proinflammatory cytokines, chemokines, or interferons; thus, preparing the organism to the presence of infection (1). PRR comprise the members of the Toll-like receptors (TLRs) family, the nucleotide-binding oligomerization domain receptors (NOD-like receptors, NLRs) and the retinoic acid-inducible gene-like helicases (RIG-like helicases, RLHs) (2). Both TLRs and NLRs have shown a critical role in host protection against microbial infections and in homeostasis of commensal microbiota (3). It has been demonstrated that saturated fatty acids activate TLRs, whereas *n*-3 polyunsaturated fatty acids inhibit agonist-induced TLR activation. Therefore, it is important to remark that the induction of Toll-mediated immune responses can also be affected by the balance between saturated and unsaturated fatty acids (4).

Numerous experimental studies have determined that certain dietary lipids are involved in the modulation of several immune functions. These alterations include the reduction of lymphocyte proliferation, which is modified by polyunsaturated fatty acids (mainly *n*-3 polyunsaturated fatty acids) or monounsaturated fatty acids (*n*-9 monounsaturated fatty acids). Studies carried out in both humans and animals have revealed that the administration of high amounts of *n*-3 polyunsaturated fatty acids or *n*-9 monounsaturated fatty acids in the diet, or the inclusion in parenteral regimens of lipid emulsions rich in *n*-3 or *n*-9 fatty acids are related to the reduction of lymphocyte proliferation during the supplementation. Cytokine production is reduced by the action of *n*-3 polyunsaturated fatty acids or *n*-9 monounsaturated fatty acids, natural killer (NK) activity is significantly suppressed, phagocytic activity of macrophages is modified, surface molecule expression is altered, and the antigen-presenting function of human monocytes is inhibited (5, 6). Based on these experimental observations, we can affirm that *n*-3 polyunsaturated fatty acids rather than *n*-6 polyunsaturated or *n*-9 monounsaturated

fatty acids are directly associated with the suppression of immune and inflammatory response. These effects, due mainly to *n*-3 polyunsaturated fatty acids, may be beneficial for a variety of inflammatory disorders, including rheumatoid arthritis, Crohn´s disease, atherosclerosis, psoriasis, systemic lupus erythematosus, multiple sclerosis, and asthma (7, 8), which are characterized by an overactivation of immune response. It is important to note that the immunomodulation attributed to fatty acids depends on different factors, such as the nature of fatty acids added to diets, the concentration of fatty acids, the duration of supplementation with dietary lipids, or differences among animal species fed dietary lipids (9).

It is clearly established that resistance to infection is strongly influenced by the effectiveness of the immune system in protecting the host against pathogenic microorganisms. The immune system of both humans and animals may be influenced by several essential nutrients, which appear to play a crucial role in preserving an optimal immune response. Indeed, deficient and excessive intakes of nutrients can have adverse effects on immune functions, and consequently may impair host defense to a broad variety of infectious microorganisms. The first confirmation that several dietary lipids might have health benefits came from epidemiological studies, which revealed that populations that consumed large amounts of fish as part of their traditional lifestyle, such as Greenland Eskimos, showed low incidence of myocardial infarction, and autoimmune disorders (10). Therefore, it was clear that the administration of this diet was directly related to a substantial reduction of inflammatory processes and autoimmune disorders. Taking into account that polyunsaturated fatty acids, particularly the *n*-3 class, exert immunosuppressive effects, it is reasonable to expect that dietary intake of these fatty acids would compromise host response to infectious microorganisms.

8.2 TYPES OF FATTY ACIDS AND LIPID MEDIATORS

Fatty acids are hydrocarbon chains with a terminal carboxyl group and a methyl group at the other end. They are characterized by the number of C atoms, the number of double bonds and the position of the first double bond. Therefore, fatty acids constitute a class of fat or lipid, whereas the concept of lipid may be defined as a variety of compounds that share similar properties of hydrophobicity. Thus, dietary lipids may include mono-, di-, and triglycerides, sphingolipids, free fatty acids (saturated, mono- or polyunsaturated), cholesterol, plant sterols, various pigments, and fat-soluble vitamins. Three major groups of dietary fatty acids are oleic, linoleic, and linolenic acid. They are not interconvertible and serve as precursors for the biosynthesis of polyunsaturated fatty acids through a series of desaturation and chain elongation steps.

Plants possess enzyme Δ9-desaturase which enables to insert double bonds into oleic acid to produce linoleic acid, from which *n*-6 fatty acids are derived, and α-linolenic acid from which *n*-3 fatty acids are derived. By contrast, animals lack the enzyme Δ9-desaturase and are therefore unable to synthesize linoleic acid and α-linolenic acid, which should be incorporated in the animal diet. For this reason, these last fatty acids are called essential fatty acids. In addition, the administration of dietary *n*-3 fatty acids causes a reduction of arachidonic acid (AA) levels in plasma membrane of tissues. This fact is produced by competence with AA to serve as a substrate for both cyclooxygenase

(COX) and lipooxygenase (LOX) activities. Thus, the modification of dietary fatty acids can lead to alteration of phospholipid profiles in plasma membranes and, in turn, changes in cellular responses.

Polyunsaturated fatty acids with 20 C (AA, eicosapentaenoic acid [EPA], and dihomo-γ-linoleic acid [DGLA]) can be metabolized to a variety of eicosanoids or biological mediators, which play important roles in the regulation of immune and inflammatory responses. Two potent inflammatory eicosanoids are prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), which are produced from AA by the enzymes COX and 5-lipoxygenase (5-LOX), respectively. Other 20-C fatty acids, EPA, and DGLA compete with AA for these enzymes, and thus, decrease the production of PGE_2 and LTB_4. The eicosanoids produced from EPA and DGLA have only weak effects on the immune cells. Docohexaenoic acid (DHA) is not a substrate for cyclooxygenase and lipoxygenase, but does suppress the synthesis of the *n*-6 eicosanoids by inhibiting the release of membrane AA. It can also be retroconverted to EPA. The reduction in the production of inflammatory eicosanoids by DHA, EPA, and DGLA forms the basis for their use in the management of inflammatory diseases. Hence, their modes of action are similar to those of many nonsteroidal anti-inflammatory drugs.

8.3 FATTY ACIDS AND IMMUNE SYSTEM FUNCTIONS: PROPOSED MECHANISMS OF IMMUNOMODULATION

Several potential mechanisms have been proposed to explain the immunomodulatory effects of dietary lipids on immune functions by direct and indirect effects on the cells of the immune system, which are not mutually exclusive. The biological consequences derived from these changes are not totally elucidated, but it is probable that the alterations observed by lymphocyte population after dietary lipid administration enhance the host susceptibility against microorganisms. Figure 8.1 illustrates the different mechanisms capable of modulating immune system functions.

8.3.1 Alteration of Membrane Fluidity and Lipid Rafts

Fatty acids constitute important structural components of the plasma membrane. Therefore, the administration of a specific type of dietary fat implies pivotal changes in the fatty acid composition leading to an alteration of cell membrane fluidity (11). As a result, diets containing unsaturated fatty acid increase the fluidity of the plasma membrane, whereas diets containing saturated fatty acid reduce the fluidity of the cell membrane. For this reason, numerous cellular functions may be affected, such as intercellular interaction, nutrient transport, receptor expression, or signal transduction. It is likely that such changes will influence lipid–protein interactions and membrane lateral organization (12). Thus, the binding of numerous cytokines to their respective receptors placed in the membrane surface may depend on fatty acid structure (13). Similarly, the expression of major histocompatibility complex (MHC) class II molecules and intercellular adhesion molecule-1 (ICAM-1) are altered after dietary lipid administration, which leads to a significant inhibition of antigen-presenting function (14).

Initiation and transmission of the signaling events taking place in immune cells occur in specialized membrane regions called lipid rafts. Currently, the study of membrane

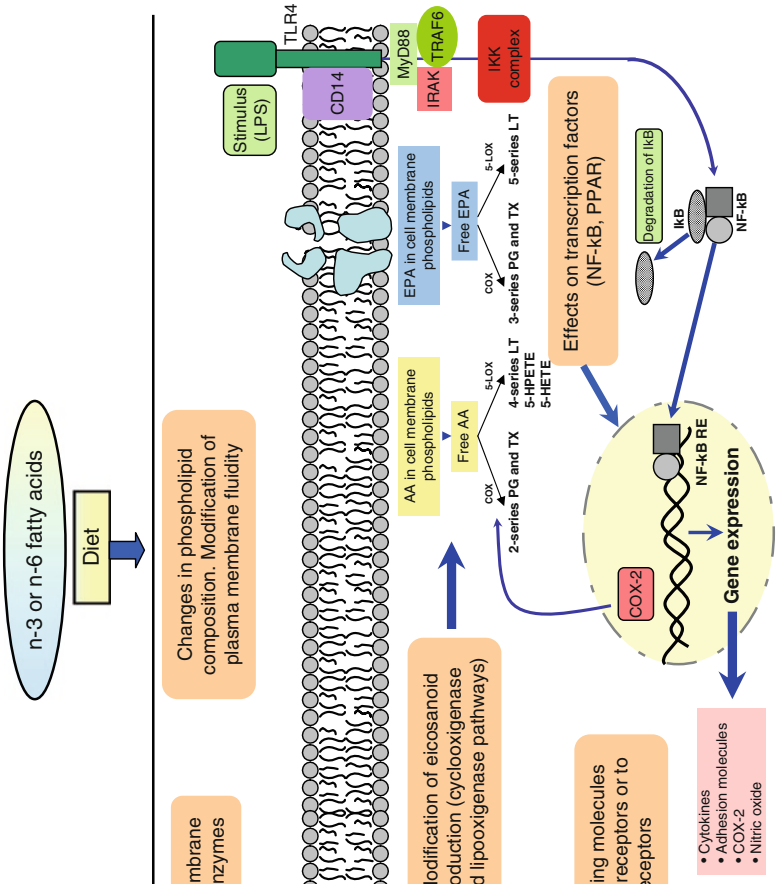


Fig. 8.1. Schematic diagram of proposed mechanisms of action of *n*-3 or *n*-6 polyunsaturated fatty acids, whereby these fatty acids are involved in the modulation of immune system functions. Outline of the LPS-induced signaling pathway of inflammatory gene expression via nuclear factor-κB that might be influenced by polyunsaturated fatty acids. LPS initially binds to CD14 and TLR4, and it activates a number of intracellular signaling pathways, including the IκB kinase (IKK)–nuclear factor κB (NF-κB) complex. These pathways phosphorylate and activate various transcription factors, such as NF-κB/Rel proteins, allowing the expression of inflammatory mediators. Abbreviations: *5-LOX* 5-lipooxygenase, *AA* arachidonic acid, *CD14* CD14 surface receptor, *COX* cyclooxygenase, *EPA* eicosapentaenoic acid, *HETE* hydroxyeicosatetraenoic acid, *HPETE* hydroperoxyeicosatetraenoic acid, *IκB* NF-κB inhibitory protein, *LPS* lipopolysaccharide, *LT* leukotriene, *NF-κB* nuclear factor-κB, *PG* prostaglandin, *PPAR* peroxisome proliferator-activated receptor, *TLR* Toll-like receptors, *Tx* thromboxane.

lipid microdomains, so-called rafts, is acquiring considerable attention, to explain the modulatory mechanisms of certain fatty acids on T cell signal transduction (15, 16). Membrane lipid rafts may be defined as specialized regions within plasma membrane composed of high concentrations of cholesterol and sphingolipids (sphingomyelin and glycolipids) and a polar region that contains saturated fatty acid residues. Different proteins involved in T cell signaling are concentrated in rafts, and alterations of raft lipid fatty acyl composition is a crucial event that has a relevant role in the inhibitory effects of polyunsaturated fatty acids on T cell activation and in the modulation of immune system functions by certain dietary lipids (17). Overall, several fatty acids incorporated in human diets can modify membrane organization, and thereby influence a variety of cellular events, including the expression of surface proteins.

8.3.2 Eicosanoid Synthesis

Eicosanoids are 20 carbon bioactive lipid mediators of inflammation, and they comprise an important family of oxygenated derivatives of DGLA, AA (the major eicosanoid precursor), or EPA that act as a second group of chemical messengers within the immune system. The main eicosanoids are prostaglandins (PG), thromboxanes (Tx), leukotrienes (LT), hydroperoxyeicosatetraenoic acids (HPETEs), hydroxyeicosatetraenoic acids (HETEs), and lipoxins, as well as newly identified families of lipid mediators generated from *n*-3 polyunsaturated fatty acids, called resolvins. Two enzymes acquire a great importance in the biosynthesis of eicosanoids: (1) cyclooxygenase, which produces the PG and Tx, and (2) lipoxygenase, which produces the HPETEs, HETEs, lipoxins, and resolvins. In general, eicosanoids participate in critical physiological functions (induction of fever, increase of vascular permeability, vasodilatation, regulation of smooth muscle tone, neuronal function, platelet aggregation, and chemotaxis) and, in particular, the most significant actions of eicosanoids are focused on their participation as inflammatory regulators and on the modulation of immune system (8). The administration of diets containing fish oil (which is mainly composed of EPA or DHA) to both humans and animals produces a significant reduction in the amount of AA in the phospholipid membrane of cells that participate in the immune functions. This event modifies the production of different eicosanoid types, and therefore, immune system functions are also altered. In general, eicosanoids derived from *n*-3 polyunsaturated fatty acids are less reactive than those derived from *n*-6 polyunsaturated fatty acids. Thus, 3-series PG (PGE_3) and 5-series LT (LTB_5) (both derived from EPA) are more potent than eicosanoids derived from AA (2-series PG [PGE_2] and 4-series LT [LTB_4]) in inhibiting lymphocyte proliferation (18). In addition, prostaglandins inhibit production of interleukin-1 (IL-1) and tumor necrosis factor (TNF), whereas leukotrienes increase NK cell activity, IL-1 production by macrophages, and interferon-γ (IFN-γ) synthesis by lymphocytes (19). Therefore, fatty acids derived from fish oil may inhibit the metabolism of AA by two pathways: (a) reduction of substrate availability because the administration of *n*-3 polyunsaturated fatty acids results in a significant decrease in the amount of AA in the plasma membrane of immune system cells, and (b) competition with AA to serve as a substrate for both cyclooxygenase and lipoxygenase activities.

8.3.3 Oxidative Stress

The antioxidant nutrient status of the subjects is of crucial relevance in the determination of the effects of polyunsaturated fatty acids on immune system functions because the incorporation of polyunsaturated fatty acids into plasma membrane increases lipid peroxidation, as well as the requirements for antioxidant substances that protect plasma membrane from lipid peroxidation. It is clearly established that lipid peroxidation modifies the expression of surface molecules (11), suppressing human leukocyte antigen (HLA-DR) expression due to free radical production (20). In fact, the immunosuppression attributed to certain dietary lipids might be in part associated with an increase of lipid peroxidation, and consequently a substantial reduction of antioxidants (particularly vitamin E). Antioxidants from extra virgin olive oil have received particular attention for their protective effects against the damage from biological oxidants. Among the antioxidant agents that constitute olive oil are hydroxytyrosol, oleuropein, and caffeic acid. These natural compounds are capable of scavenging free radicals and of breaking peroxidative chain reactions (21).

8.3.4 Regulation of Gene Expression

Recent studies have suggested that the modulatory properties of *n*-3 polyunsaturated fatty acids are attributed in part to a reduction in the activity of nuclear factor- κB (NF-κB), which is an important transcription factor involved in the promotion of several important proteins (22). The association of stimulus – CD14 receptor- MD2 adaptor protein – TLR4 induces dimerization of TLR4 and initiates signaling cascade, which results in activation of phosphorylation kinases of NF-κB transcription factor inhibitor (IκB). This process induces a transcription of genes whose products take part in inflammatory response (e.g. proinflammatory cytokines TNF-α, IL-1, IL-6, IL-12, COX, and adhesion molecules) (23). Other transcription factors, including peroxisome proliferator-activated receptors (PPARs), a group of key nuclear receptors involved in the regulation of lipid homeostasis, can bind to DNA sequence elements and are involved in the regulation of inflammatory processes by modulating the expression of target genes (24, 25).

8.3.5 Apoptosis

Apoptosis has been defined as a genetic program crucial for the development and homeostasis of the immune system, in which the activation of catabolic processes and enzymes occurs before cytolysis, thereby facilitating the recognition, uptake, and digestion of the apoptotic cell by phagocytes. Different studies performed by *in vitro* assays or by administration of dietary lipids in both animals and humans have described an important role for several fatty acids in the induction or inhibition of apoptosis. Thus, polyunsaturated fatty acids such as EPA, DHA, saturated fatty acids such as palmitic acid, or fats such as fish oil administered in the diet have been defined as substances capable of inducing cell death via a mitochondrial process, or by downregulation of Bcl-2 (an antiapoptotic protooncogene product) (26–28). In addition, another possibility that explains in part the induction of apoptosis by long-chain *n*-3 polyunsaturated fatty acids is the direct action of these substances on the cells and by activation of the caspase

cascade through cytochrome *c* release coupled with a modulation of mitochondrial membrane depolarization (29). Recent studies have also determined the crucial importance of dietary fatty acids in the reduction of Bcl-2 expression as well as an increase of Fas ligand (Fas-L) expression. Therefore, when the concentration of polyunsaturated fatty acids augments, Bcl-2 expression is reduced, and cell death occurs (28). More recently, a novel mechanism has been proposed to establish a relationship between fatty acids and apoptosis. This mechanism suggests that *n*-3 fatty acids modulate T cell-mediated immunity by selective deletion of Th1-like cells, while maintaining or increasing the Th2-mediated humoral immune response (17). While *n*-6 polyunsaturated fatty acids are involved in apoptosis induction, acting on mitochondrial depolarization and oxygen radical production, oleic acid is less toxic and is involved in caspase-3 activation (30).

8.3.6 Antigen Presentation

Diets containing fish oil are capable of diminishing the expression of MHC class II antigen-presenting molecules on peripheral blood monocytes or dendritic cells (14, 31, 32). Similarly, the expression of MHC class I antigen-presenting molecules was decreased with a treatment *in vitro* of B lymphocytes in the presence of AA or DHA. Recently, it has been demonstrated that the treatment of human B lymphoblasts with polyunsaturated fatty acids involves a modification of antigen presentation (33). Thus, the culture of B lymphoblasts with either AA or DHA as free fatty acids reduced the lysis of antigen-presenting cells. This effect may be attributed to a small reduction in MHC class I surface expression and a significant decrease in the rate of antigen-presenting cells-T cell association after incorporation of polyunsaturated fatty acids (33). These findings suggest that polyunsaturated fatty acids are modifying the adhesion properties of antigen-presenting cells (APC)-T cell conjugates. However, MHC class I expression was only restricted to *in vitro* studies. Therefore, it would be necessary to examine these effects with regard to *in vivo* models.

8.3.7 Modulation of Gastrointestinal Microbiota

Resident intestinal microbiota plays a pivotal role in the prevention of infections caused by potential pathogens. Several studies have determined that high concentrations of polyunsaturated fatty acids exert an important effect on gastrointestinal microbiota, showing that they inhibit the growth and mucus adhesion of lactobacilli, whereas low concentrations of γ-linolenic acid and AA promoted the growth and mucus adhesion of *Lactobacillus casei* (34). In addition, the administration of probiotics supplemented infant formula results in altered plasma lipid polyunsaturated fatty acid composition, modifying the absorption and utilization of dietary polyunsaturated fatty acids (35). Polyunsaturated fatty acids can alter intestinal microbiota increasing the number of probiotic microorganisms. As a direct consequence, polyunsaturated fatty acids might be recommended in addition to probiotics for the prevention and/or maintenance treatment of colitis (36). It has been demonstrated that long-chain polyunsaturated fatty acids, by enhancing the adhesion of probiotic organisms to the gut mucosal cells, may increase the development of gut-associated lymphoid tissue by direct interaction

between the probiotics and the lymphoid tissue and by the ability of long-chain polyunsaturated fatty acids and probiotics to augment certain growth factors such as transforming growth factor-β (TGF-β) and various cytokines (37).

8.4 DIETARY LIPIDS AND HOST SUSCEPTIBILITY TO INFECTIOUS MICROORGANISMS

After explaining how dietary lipids affect immune functions, we examine the action of dietary lipids on host susceptibility to infection. It is clearly recognized that nutrient intake may commonly be considered as a critical determinant of immunocompetence because of the impact of certain micronutrients and macronutrients on immune system functions. Accordingly, nutrients play a critical role in the appropriate development and maintenance of optimal health in both animals and humans (38). Many investigations have reported the modulatory role that certain fatty acids play on the immune system and the clinical benefits exerted by dietary lipid supplementation with fish oil, olive oil, or other fats in both humans and animals (6, 39). As a result, diets containing fish oil or olive oil have traditionally been applied in the resolution, or at least in the attenuation of incidence of diseases characterized by an overactivation of immune system (40, 41) because unsaturated fatty acids (mainly *n*-3 or *n*-9 fatty acids) are able to reduce the levels of many biological mediators associated with the promotion of the inflammatory mechanisms that participate in an inappropriate immune response.

For obvious reasons, the altered resistance to infectious microorganisms has been analyzed in animal models in which the administration of diets containing fish oil generally reduces the clearance of bacteria from either liver or spleen and significantly diminishes survival during the course of an experimental infection with different types of pathogens. Consequently, the elimination of microbial agents (bacteria, fungi, viruses, or parasites) is more difficult (reviewed in 42–44). Different reports have described in the last years the clinical consequences derived from dietary supplementation with *n*-3 polyunsaturated fatty acids, which are characterized by suppressing immune system functions. Nevertheless, the studies focused on the action promoted by fatty acids on immune system functions, and the modulation of host resistance to infectious microorganisms has generated many discrepancies that may be directly attributed to multiple factors, such as type and amount of diet consumed, time of feeding before the challenge with the microorganism, and the type, dose, and route of infection (9). The main studies investigating the impact of dietary lipids (particularly *n*-3 polyunsaturated fatty acids) on the host immune resistance of different animal models are summarized in Table 8.1.

8.4.1 Bacteria

Listeria monocytogenes, is a gram-positive food-borne pathogen that invades through the intestinal epithelium, causing the potentially fatal disease, listeriosis. This microorganism serves as an important model for understanding host immune resistance against intracellular bacteria, and it has been extensively used in numerous investigations associated with dietary lipids and infection. Initially, it is important to emphasize that an early study showed an inhibition in host resistance as measured by susceptibility to

Table 8.1
Studies showing the effects of dietary lipid administration on immune resistance to microorganism infection or other treatments

Pathogens	*Treatments*	*Species*	*Time (week)*	*Main results*	*References*
Bacteria					
Listeria monocytogenes	Diet high in lard, cholesterol and sucrose	C57BL/6 mice	26	Impairment of specific immunity to *L. monocytogenes* infection. Persistence of *L. monocytogenes* in livers	(46)
	High-fat diet	ddN mice	1–2	Reduction to *L. monocytogenes* resistance. Suppression of macrophage functions	(48)
	Diets containing lard (20%), soybean oil (20%) or menhaden fish oil (17% + 3% corn oil)	C3H/Hen mice	4	Reduction of survival in the groups fed soybean and fish oil diets. Reduction of bacteria counts from spleen of mice fed fish oil, but no differences in bacteria counts from liver	(52)
	Diets containing lard (20%), soybean (20%) or fish oil (20%)	C3H/ HeNMice	4	Reduction of both IL-12 and IFN-γ production during the early phase of a *Listeria* infection	(55)
	Diets containing olive oil (20%), fish oil (20%) or hydrogenated coconut oil (20%)	Balb/c mice	4	Reduction of survival in mice fed a fish oil diet. Increase of bacteria counts from spleen	(51)
	Diets containing 5% conjugated linoleic acid or 5% corn oil	CD1 mice	2 or 4	No alteration of cellular immunity to infection	(53)
	Diets containing 200 g/kg olive oil, fish oil or hydrogenated coconut oil	Mouse	4	Invasion and adherence of *L. monocytogenes* to splenic cells was increased after infection	(50)
	Diets containing 200 g/kg olive oil, fish oil or hydrogenated coconut oil and treated with NAC	Balb/c mice	4	Administration of NAC (antioxidant function) exerted a moderate detrimental effect after challenge with *L. monocytogenes*	(58)
	Diets containing 200 g/kg olive oil, fish oil or hydrogenated coconut oil	Balb/c mice	4	Concentration of IL-12 from serum was reduced, but IL-4 production was increased after *L. monocytogenes* infection	(57)
	Diet containing olive oil, fish oil or hydrogenated coconut oil and treated with cyclophosphamide	Balb/c mice	4	Susceptibility to infection was increased in immunosuppressed animals, particularly fed a fish oil diet	(60)

Mycobacterium tuberculosis	Diets containing different concentrations of *n*-3 and *n*-6 polyunsaturated fatty acids	Guinea pigs	13	Increase in the number of bacteria from spleen in animals fed *n*-3 polyunsaturated rich diets	(66, 67)
	Diets containing corn oil or fish oil (4% by weight)	Guinea pigs	3 or 6	Augment in the number of bacteria from lungs in (*n*-3) fatty acid-fed animals compared with the number of bacteria from lungs in (*n*-6) fatty acid-fed animals	(68)
	Soy oil (3.5%), sunflower oil (7.41%) and menhaden oil (10%)	Balb/c mice	5	*n*-6 fatty acids increased the survival of bacteria in mice, while *n*-3-fatty acids increased pathogen killing	(69)
Pseudomonas aeruginosa, Listeria monocytogenes, Candida albicans, murine cytomegalovirus	Diet containing melted beef tallow (46%) or fish oil	(NZBxNZW) F1 mice	4	No differences in the susceptibility of animals after experimental infection. No association with an increased risk of infection	(47)
Pseudomonas aeruginosa, Salmonella typhimurium	Diets containing either coconut oil, olive oil, safflower oil or fish oil	CF1 mice	3	No changes were observed on the survival among different sources of fat. Manipulation of dietary fat did not affect outcome from infection	(71)
Pseudomonas aeruginosa	Diets containing 10 and 40% safflower oil or MaxEPA	Balb/c mice	2–3	The group fed fish oil diet had significantly higher mortality than those fed safflower oil. Fish oil diets reduced resistance to infection	(72)
	Diets containing n-3 polyunsaturated fatty acids (36.5%) or n-6 polyunsaturated fatty acids (32.2%)	C57BL/6 mice	5	Reduction of mortality in *n*-3 group after infection. Increase of distal alveolar fluid clearance and improvement of the inflammatory response	(70)
	Diets containing EPA (11.4%) + DHA (4.7%)	C57BL/6 mice		Reduction of pulmonary bacterial load and the decrease of mortality percentage. Modulation of pro- and anti-inflammatory cytokine balance	(73)

(continued)

Table 8.1
(continued)

Pathogens	*Treatments*	*Species*	*Time (week)*	*Main results*	*References*
Salmonella typhimurium	Diets containing corn oil (20%), hydrogenated coconut oil (20%) or fish oil (20%)	Swiss Webster mice	4	Reduction of survival in mice fed fish oil. A diet rich in fish oil decreased host resistance to infection	(74)
	Diets containing corn oil (20%), menhaden fish oil (17% + 3% corn oil)	Mouse	1	Reduction of Kupffer cell phagocytosis by fish oil. Diminution of CD18 expression in splenocytes	(75)
Klebsiella pneumoniae, Plasmodium berghei	Diets containing fish oil (14%), corn oil (15%) or palm oil (15%)	Swiss mice	6	Increase of the survival after challenge and resistance to infection. Indomethacin treatment did not alter the outcome in the two infectious processes	(62)
Klebsiella pneumoniae, Streptococcus pneumoniae	Diets containing fish oil (10%) or corn oil (10%)	Mice	6	Beneficial effects of fish oil on survival after infection with *K. pneumoniae*, but detrimental effects on survival were observed after *S. pneumoniae* infection	(65)
Klebsiella pneumoniae	Diets containing fish oil or olive oil	NMRI mice	6	Increase of the survival in the group fed a fish oil diet after challenge	(64)
	Diets containing fish oil or corn oil and treated with 5-lipooxygenase (5-LO) inhibitor	Mice	6	Survival of mice was increased after fish oil administration, but the neutralization of leukotrienes blocked the observed beneficial effects	(63)
Viruses					
Human immunodeficiency virus	Bars containing fish oil or safflower oil	Human	6	Reduction of CD4 cell counts after treatment with fish oil	(82)
	Diets containing corn oil (20%) or fish oil (17% + 3% corn oil)	Mice	10	Reduction of proinflammatory cytokine production and suppression of NF-kB activation	(81)

Influenza virus	Diets containing fish oil (17% + 3% sunflower oil) or beef tallow (17% + 3% sunflower oil)	Balb/c mice	2	Delay in viral clearance and reduction of IFN-γ production, IgG and IgA synthesis	(76)
Human rhinovirus	2 g/day conjugated linoleic acid	Human	4	No effects on the virological and clinical course of infection	(78)
Herpes simplex virus type I (HSV-1)	Diets containing corn oil (5.7% + 14.3% hydrogenated vegetable oil) or fish oil (20%)	Balb/c mice	2	Increase of herpes stromal keratitis progression	(79)
Reovirus	Diets containing 10 g/kg corn oil + 60 g/kg high oleic acid safflower oil or 10 g/kg corn oil + 60 g/kg DHA-enriched fish oil	Mice	4	Reduction of virus clearance from the intestinal tract, whereas DHA did not affect immunoglobulin production	(80)
Hepatitis C	Capsules. 1,800 mg/day of EPA	Human	48	Prevention of a decrease in the lymphocyte counts	(83)
Parasites					
Eimeria tenella, Eimeria maxima	Diets containing menhaden oil (5%), medium chain triglyceride oil (5%) or flaxseed oil (15%)	Chickens	3	Suppression of *E. tenella* development, whereas fish oil diet administration was not efficient in the reduction of *E. maxima* infection. This fat may exacerbate lesions at high parasite doses	(84)
Plasmodium berghei	Diets containing fish oil (14% FO and 1% corn oil), 15% corn oil, or 15% palm oil	C57/Bl/6J mice	6	Increase of resistance to infection in fish oil-fed group associated with an increase of *ex vivo* production of IL-1 and TNF-α by peritoneal cells	(62)
Plasmodium yoelii	Diets containing fish oil and 100 mg/kg vitamin E	Balb/c mice	4–6	Reduction of survival in mice fed fish oil and vitamin E. The oxidative process was crucial in parasite elimination	(85)
Trichinella spiralis	Diets containing fish oil (19%)	Rats	9	Reduction in the number of adult worms and larvae. Increase of both IFN-γ and IL-4 production	(86)

(continued)

Table 8.1
(continued)

Pathogens	*Treatments*	*Species*	*Time (week)*	*Main results*	*References*
Fungi					
Paracoccidioides brasiliensis	Different diets containing polyunsaturated (15%) and saturated fatty acids (15%)	Balb/c mice	4	Reduction of the host resistance to dietary supplementation with DHA, but not with EPA	(87)
Others					
Induction of polymicrobial sepsis by caecal ligation and puncture	Diets containing fish oil (7%) + soybean oil (3%)	ICR mice	3	Increase of the inflammatory reaction and neutrophil infiltration into tissues. Fish oil diet augmented Th2-type response	(90)
Escherichia coli LPS	Safflower oil (10%) or fish oil (10%) emulsions	Guinea pigs	24, 48 h	Increase of the survival to endotoxin in animals treated with parenteral fish oil	(89)
PrP^{Sc} formation	EPA (1 μM) and DHA (1 μM)	Three neuronal cultures	1	Increase of prion formation after fatty acid treatment *in vitro*	(91)

DHA docosaheaenoic acid, *EPA* eicosapentaenoic acid, *IFN*-γ interferon-γ, *IL* interleukin, *Ig* immunoglobulin, *LPS* lipopolysaccharide, *NAC* N-acetyl-l-cysteine, *NF*-κ*B* nuclear factor-κB, *TNF*-α tumor necrosis factor-α

L. monocytogenes infection in mice fed a hypercholesteremic diet (45), indicating an alteration of host response. However, the first report in the literature regarding *L. monocytogenes* resistance and dietary *n*-3 polyunsaturated fatty acids was the study by Kos et al. in which demonstrated an impairment of specific immunity to infection and persistence of this microorganism in the liver (46). Subsequently, another early study reported no differences in the susceptibility to this pathogen of animals fed fish oil diets (47), whereas other investigations found a reduction of host resistance to *L. monocytogenes* infection due mainly to a suppression of macrophage functions (48). Other more recent investigations have demonstrated a significant reduction of survival rates in hosts to *L. monocytogenes* infection after feeding experimental mice with a diet exclusively constituted by high concentrations of fish oil. Animals were inoculated with a lethal dose of a virulent *L. monocytogenes* strain and bacterial clearance from liver or spleen was increased in these models. Bactericidal activity of peritoneal cells was significantly reduced (49), and cytotoxic effects due to bacterial infection were aggravated (50), whereas the susceptibility of cells to adhesion or invasion by *L. monocytogenes* infection was substantially modified (50). Overall, these observations indicate that diets containing high amounts of fish oil promote an immunosuppressive state unable to protect the host from an infectious microorganism, or in other words, an ineffective capacity of the immune system to destroy and eliminate the infectious agents (50–52). Instead, Turnock et al. reported that conjugated linoleic acid (CLA), a potent immunomodulator that is used as a dietary supplement, did not alter antibacterial resistance to *L. monocytogenes* infection (53).

Fritsche and coworkers have attempted to explain in part the reasons for which *n*-3 polyunsaturated fatty acids reduce host defense against *L. monocytogenes* infection. Thus, consumption of EPA or DHA (both contained in fish oil) impairs the production of IL-12 and IFN-γ, cytokines that play an essential role in the innate and adaptive responses of host immune system (54). For this reason, the reduction of IL-12 levels may clarify the impaired bacterial clearance from spleen or livers, as well as the reduction of survival to *L. monocytogenes* infection in a murine model (55). Another possible explanation for the reduction of host resistance is based on the inhibition of MHC class II expression (called Ia in mice) that is reduced in mice fed a fish oil diet and infected with *L. monocytogenes* (56). Similarly, our research group and others have described an increase of mortality in the animals fed a fish oil diet as well as a diminution of bacteria counts from spleen after challenge with *L. monocytogenes* (51, 52). As mentioned above, this outcome may be promoted not only by a reduction of proinflammatory cytokine production, such as IL-12 and IFN-γ (55), but also by an increase in the synthesis of an anti-inflammatory cytokine as IL-4 during the early phase of *L. monocytogenes* infection (57). However, it is important to underline that the mortality of animals was not increased in mice fed an olive oil diet after the exposure to this bacterium (51, 58).

The combination of these dietary lipids with an antioxidant agent, such as N-acetyl-l-cysteine (NAC), was responsible for an adverse effect, leading to a reduction of mice survival and to an increase of viable bacteria from the spleen (58). Nevertheless, the administration of diets containing fish oil in immunosuppressed models treated with a neutrophil-depleting antibody (RB6-8C5) has demonstrated that *n*-3 polyunsaturated fatty acid-mediated reduction of host resistance to *L. monocytogenes* is independent of

neutrophil activity (59). In addition, *n*-3 polyunsaturated fatty acids contribute to exacerbating the susceptibility against *L. monocytogenes* infection in immunosuppressed animals, which had previously been treated with cyclophosphamide (a neutropenic agent) (60). Therefore, the results obtained in animal models clearly indicate that the administration of diets containing fish oil may exhibit an important immunosuppressive effect that may acquire a great relevance in clinical nutrition, when lipid emulsions constituted by polyunsaturated fatty acids are supplied to patients at risk of sepsis (59, 60).

Similarly, an early study reported that dietary supplementation with either *n*-3 or *n*-6 polyunsaturated fatty acids can have significant adverse effects in the elimination of *Staphylococcus aureus* from lungs of newborn rabbits (61). Nevertheless, these authors suggested that lipid administration did not alter lung neutrophil chemotaxis or alveolar macrophage phagocytic activity. Therefore, the interpretation of these results is highly difficult.

Several research groups have previously shown in mice fed with fish oil, an increased survival, compared to mice fed with corn oil, after infection with the Gram-negative bacteria *Klebsiella pneumoniae* (62). The explanation for this difference in survival is not absolutely known, but it may be attributed to an altered balance between various cytokines and chemical mediators. Therefore, it has been suggested that the consumption of fish oil suppresses the abundant and sometimes harmful immunological response to an overwhelming infection. Thus, several investigations have concluded that the effects of diets containing fish oil are mediated through altered production of leukotrienes, when mice were infected with a gram-negative bacterium such as *K. pneumoniae* (63), in fact, different studies have reported that the administration of fish oil diets exerts beneficial effects on survival of mice after experimental infection with *K. pneumoniae* (62–65). By a strict contrast, one of these studies has not observed any effect after infection with a gram-positive microorganism as *Streptococcus pneumoniae*, despite a beneficial effect on survival after experimental pneumoniae when mice were infected with *K. pneumoniae* (65).

The exposure of guinea pigs fed a diet containing fish oil to *Mycobacterium tuberculosis* produced an increase of host susceptibility to this pathogen. Therefore, an increase in the number of bacteria from spleen and lung was described, when animals were fed a diet containing fish oil (66–69). A possible explanation for these observed effects may be found in the reduction of TNF-α production by *M. tuberculosis*-infected macrophages (69).

The action of *Pseudomonas aeruginosa*, a gram-negative pathogen involved in a large number of nosocomial and opportunistic infections, has also been evaluated in different studies. Evidence from several findings have indicated that diets containing fish oil reduce mortality rates of hosts after the exposure to this pathogen (70), whereas other investigations have reported no differences in the susceptibility of animals to this microorganism (47, 71) or a significant reduction of mouse survival when were fed a fish oil diet after the challenge (72). Finally, a recent investigation has reported that the administration of a diet constituted by EPA along with DHA may be used as a preventive treatment against initial infection with *P. aeruginosa*, increasing host resistance after exposure to this pathogen. The combined administration of both EPA + DHA decreases pulmonary bacterial load and reduces mortality (73). In addition, these

authors have suggested that both EPA + DHA may be applied as a preventive agent against an initial colonization of *P. aeruginosa*, acting synergistically with antibiotics and reducing morbidity to this pathogen microorganism (73).

In spite of the fact that *Salmonella enterica* serovar Typhimurium has not been related to changes in survival after the administration of *n*-3 polyunsaturated fatty acids (71), other authors have found substantial differences in the survival of animals fed a diet containing fish oil and infected with this bacterium. Thus, mice fed *n*-3 polyunsaturated fatty acids showed an increase in mortality and diminished bacterial clearance, when this pathogen was administered by an oral route (74). In addition, the functionality of Kupffer cells and splenocytes were affected after challenge with this gram-negative microorganism (75).

8.4.2 Viruses

Viruses constitute intracellular obligate parasites that need the cellular machinery for their replication. Therefore, it is obvious that viruses will be eliminated through a cell-mediated immune response. Nevertheless, some viruses are able to evade this immune response because they use one or more mechanisms capable of disrupting the host's immune response. Experimental observations with viruses have also demonstrated that influenza virus infection in animals fed fish oil diet delayed the clearance, due to an impairment of primary virus-specific T cell cytotoxicity, but had no effect on NK cytotoxicity (76, 77). Recently, it has been reported that CLA dietary supplementation had no consistent effects on the virological or clinical course of experimental human rhinovirus infection (78). On the other hand, the infection in an animal model with ocular herpes simplex virus type 1 (HSV-1) has promoted the development of more severe lesions in mice fed a fish oil diet. Taking into account that the susceptibility of certain strains of mice to HSV-1 stromal keratitis is related to hyperresponsiveness of T lymphocyte to HSV-1 antigens, it is probable that the activation of T lymphocytes observed in the fish oil-fed group could be responsible for the reported exacerbation of this disease (79). However, other early studies have reported that the administration of *n*-3 polyunsaturated fatty acids did not affect survival after a lethal infection with murine cytomegalovirus (47). The infection of mice fed a diet containing DHA with an enteric reovirus produced a reduction of clearance of the virus from the intestinal tract, although the level of immunoglobulin A at 6 or 8 h of infection was not modified (80). On the other hand, the administration of diets containing fish oil in a murine model of acquired immunodeficiency syndrome (AIDS) produced a significant reduction in human immunodeficiency virus progression due mainly to a reduction of proinflammatory cytokines, and a diminution of NF-kB expression (81). Nevertheless, a previous study demonstrated that administration of a diet containing fish oil to patients suffering from AIDS is well tolerated, but decreased CD4 counts. Therefore, these authors suggest that clinical use of this diet is inappropriate in these patients (82). Finally, a recent investigation has reported that EPA supplementation does not reduce lymphocyte counts in patients suffering from hepatitis C receiving a combinatory therapy of pegylated interferon (PEG-IFN) and ribavirin (83).

8.4.3 Parasites

A study established that a fish oil diet is efficient in the elimination of *Eimeria tenella*, whereas this fat is not beneficial in the reduction of *Eimeria maxima* infection. In fact, fish oil may exert adverse effects because it exacerbates lesions at high parasite doses (84). The effect of dietary lipids on host resistance to infection has also been explored in models infected with *Plasmodium berghei*. The administration of a diet containing fish oil did not lead to decreased resistance to infection. This event was associated with an enhanced *ex vivo* production of proinflammatory cytokines, IL-1 and TNF, by peritoneal cells, whereas the reduction of prostaglandin synthesis did not appear to play an important role during the course of *P. berghei* infection (62). It has been determined that mice fed vitamin E-deficient diets containing *n*-3 polyunsaturated fatty acids survived to infection with lethal *Plasmodium yoelii*. Indeed, these results underline the importance of cellular oxidative processes in parasite elimination (85). Finally, another study reported a reduction in the number of both adult worms and larvae from *Trichinella spiralis* in rats fed a diet supplemented with fish oil, although a loss of weight was detected after infection in animals fed a fish oil-enriched diet (86).

8.4.4 Fungi

Little information about the impact of dietary lipids in infections promoted by fungi has been published. However, Oarada et al. investigated the action of either EPA or DHA on host resistance to *Paracoccidioides brasiliensis* infection. Thus, this investigation demonstrated that mice fed palm oil supplemented with DHA showed reduced antifungal activity in both spleen and liver, as compared with mice fed palm oil or soybean oil without supplementation with DHA. In addition, mice fed DHA-supplemented soybean oil also showed reduced antifungal activity in the liver, but the extent of reduction was less severe. Nevertheless, this reduction in antifungal activity was not observed with EPA-supplemented palm oil or EPA-supplemented soybean oil. Therefore, these authors conclude that DHA, but not EPA, reduces host resistance against this pathogenic fungus (87). On the other hand, intravenous administration to volunteers of an emulsion of medium-chain lipids, or an emulsion of pure long-chain lipids produced different responses to *Candida albicans* infection. These authors conclude that medium chain lipids, but not long-chain lipids increase susceptibility to *C. albicans*, and as a result they augment the risk for infections by this microorganism (88).

8.4.5 Others

Administration of *n*-3 polyunsaturated fatty acid is related to the reduction of the proinflammatory biological mediator induced by *Escherichia coli* LPS. These alterations may exert beneficial effects because *n*-3 polyunsaturated fatty acid intake, either via parenteral emulsion or dietary administration, increased the survival of guinea pigs after LPS injection, exerting a protective effect (89). The treatment with fish oil diet-induced leucocyte integrin expression, increased plasma ICAM-1 levels, and enhanced IL-6 production, as well as, mieloperoxidase activity in various organs from a murine model in which polymicrobial sepsis was induced (90). A recent study has determined that the treatment of different neuronal cells with EPA and DHA *in vitro* leads to an increase of prion formation, in spite of the fact that these polyunsaturated fatty acids are

related to a diminution of cholesterol content. There is not a convincing reason to explain these effects, but altered cell signaling activity within prion-infected cells occurs after long-chain *n*-3 polyunsaturated fatty acid administration. In addition, EPA and DHA are capable of increasing the activation of PLA_2 that is required for PrP^{Sc} formation (91).

8.5 CONCLUSIONS AND PERSPECTIVES

It should be obvious that any factors that increase the risk of infection are of clinical and public health importance. Consequently, it is also evident that *n*-3 polyunsaturated fatty acids are responsible for an important impact on immune system functions, although they represent two faces of the same coin because this potential action may cause either beneficial or adverse effects. Thus, polyunsaturated fatty acids have been applied in the resolution of diseases characterized by an overactivation of immune system due mainly to their anti-inflammatory properties. By a strict contrast, high amounts of polyunsaturated fatty acids could be responsible for an immunosuppressive state and for an increase in the susceptibility to infectious microorganisms. Whereas increasing the intake of polyunsaturated fatty acids to certain levels may not result in beneficial effects in healthy individuals, it may be effective in preventing and treating certain inflammatory disorders. In short, by modulating fatty acids in a regulated fashion, the therapeutic implications will be enormous. However, caution should be recommended because an excessive amount may impair host resistance to infection. This is particularly true for infants and the elderly, the major targets for *n*-3 polyunsaturated fatty acids supplementation, who constitute the main groups at risk of sepsis. Similarly, in critically ill patients, administration of *n*-3 polyunsaturated fatty acids is not associated with any apparent clinical benefits, and it may produce adverse effects in some subgroups of patients. Therefore, other alternatives to classical lipid emulsions are being developed for parenteral application in the clinical practice. The verification of main mechanisms by which certain dietary lipids may modulate immune functions, and the identification of products and optimal dosages applied in critically ill patients is crucial for clinical nutrition. On the other hand, the threshold dose of *n*-3 polyunsaturated fatty acids for causing impaired resistance in animals is not clearly established, and whether this result can be extrapolated to humans has yet to be verified. Therefore, it is necessary to determine the mechanisms of the effects of certain fatty acids contained in the diet on increased susceptibility to infection and when and how they can be reversed. In addition, *in vitro* studies should define the action of fatty acids on different types of cells, as well as precise the more appropriate dosage to be administered. Finally, it is important to know how *n*-3 polyunsaturated fatty acids affect host immune response to a broader diversity of human infectious pathogens.

REFERENCES

1. Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, Grangette C (2008) Feeding our immune system: impact on metabolism. Clin Dev Immunol 2008:639803
2. Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, Foy E, Loo YM, Gale M Jr, Akira S, Yonehara S, Kato A, Fujita T (2005) Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J Immunol 175:2851–2858
3. Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Annu Rev Immunol 21:335–376

4. Weatherill AR, Lee JY, Zhao L, Lemay DG, Youn HS, Hwang DH (2005) Saturated and polyunsaturated fatty acids reciprocally modulate dendritic cell functions mediated through TLR4. J Immunol 174:5390–5397
5. de Pablo MA, Alvarez de Cienfuegos G (2000) Modulatory effects of dietary lipids on immune system functions. Immunol Cell Biol 78:31–39
6. Fritsche K (2006) Fatty acids as modulators of the immune response. Annu Rev Nutr 26:45–73
7. Mills SC, Windsor AC, Knight SC (2005) The potential interactions between polyunsaturated fatty acids and colonic inflammatory processes. Clin Exp Immunol 142:216–228
8. Calder PC (2001) Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36:1007–1024
9. Fritsche K (2007) Important differences exist in the dose-response relationship between diet and immune cell fatty acids in humans and rodents. Lipids 42:961–979
10. Kromann N, Green A (1980) Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950–74. Acta Med 208:401–406
11. Stubbs CD, Smith AD (1984) The modification of mammalian polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim Biophys Acta 779:89–137
12. Stillwell W, Wassall SR (2003) Docosahexaenoic acid: membrane properties of a unique fatty acid. Chem Phys Lipids 126:1–27
13. Grimble RF, Tappia PS (1995) The modulatory influence of unsaturated fatty acid on the biology of tumor necrosis factor. Biochem Soc Trans 287:282–286
14. Hughes DA, Pinder AC, Piper Z, Johnson IT, Lund EK (1996) Fish oil supplementation inhibits the expression of major histocompatibility complex class II molecules and adhesion molecules on human monocytes. Am J Clin Nutr 63:267–272
15. Stulnig TM (2003) Immunomodulation by polyunsaturated fatty acids: mechanisms and effects. Int Arch Allergy Immunol 132:310–321
16. Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A (1999) T lymphocyte costimulation mediated by reorganization of membrane microdomains. Science 283:680–682
17. Switzer KC, McMurray DN, Morris JS, Chapkin RS (2003) (n-3) polyunsaturated fatty acids promote activation-induced cell death in murine T lymphocytes. J Nutr 133:496–503
18. Shapiro AC, Wu D, Meydani SN (1993) Eicosanoids derived from arachidonic and eicosapentaenoic acids inhibit T cell proliferative response. Prostaglandins 45:229–240
19. Marcinkiewicz J (1991) In vitro cytokine release by activated murine peritoneal macrophages: role of prostaglandins in the differential regulation of tumor necrosis factor alpha, interleukin 1, and interleukin-6. Cytokine 3:327–332
20. Gruner S, Volk HD, Falck P, Baehr RV (1986) The influence of phagocytic stimuli on the expression of HLA-DR antigens: role of reactive oxygen intermediates. Eur J Immunol 16:212–215
21. Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 247:60–64
22. Yaqoob P (2003) Fatty acids as gatekeepers of immune cell regulation. Trends Immunol 24:639–645
23. Kawai T, Akira S (2006) TLR signaling. Cell Death Differ 13:816–825
24. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W (1996) The PPARalpha-leukotriene B4 pathway to inflammation control. Nature 384:39–43
25. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci USA 94:4318–4323
26. Chiu LC, Wan JM (1999) Induction of apoptosis in HL-60 cells by eicosapentaenoic acid (EPA) is associated with downregulation of bcl-2 expression. Cancer Lett 145:17–27
27. de Pablo MA, Susin SA, Jacotot E, Larochette N, Costantini P, Ravagnan L, Zamzami N, Kroemer G (1999) Palmitate induces apoptosis via a direct effect on mitochondria. Apoptosis 4:81–87
28. Reddy Avula CP, Zaman AK, Lawrence R, Fernandes G (1999) Induction of apoptotic mediators in Balb/c splenic lymphocytes by dietary *n*-3 and *n*-6 fatty acids. Lipids 34:921–927
29. Arita K, Kobuchic H, Utsumia T, Takehara Y, Akiyama J, Horton AA, Utsumi K (2001) Mechanism of apoptosis in HL-60 cells induced by n-3 and n-6 polyunsaturated fatty acids. Biochem Pharmacol 62:821–828

30. Cury-Boaventura MF, Gorjão R, de Lima TM, Newsholme P, Curi R (2006) Comparative toxicity of oleic and linoleic acid on human lymphocytes. Life Sci 78:1448–1456
31. Sanderson P, MacPerson GG, Jenkins CH, Calder PC (1997) Dietary fish oil diminishes the antigen presentation activity of rat dendritic cells. J Leuk Biol 62:771–777
32. Shaikh SR, Edidin M (2006) Polyunsaturated fatty acids, membrane organization. T cells, and antigen presentation. Am J Clin Nutr 84:1277–1289
33. Shaikh SR, Edidin M (2007) Immunosuppressive effects of polyunsaturated fatty acids on antigen presentation by human leukocyte antigen class I molecules. J Lipid Res 48:127–138
34. Kankaanpää PE, Salminen SJ, Isolauri E, Lee YK (2001) The influence of polyunsaturated fatty acids on probiotic growth and adhesion. FEMS Microbiol Lett 194:149–153
35. Kankaanpää PE, Yang B, Kallio HP, Isolauri E, Salminen SJ (2002) Influence of probiotic supplemented infant formula on composition of plasma lipids in atopic infants. J Nutr Biochem 13:364–369
36. Hekmatdoost A, Feizabadi MM, Djazayery A, Mirshafiey A, Eshraghian MR, Yeganeh SM, Sedaghat R, Jacobson K (2008) The effect of dietary oils on cecal microflora in experimental colitis in mice. Indian J Gastroenterol 27:186–189
37. Das UN (2002) Essential fatty acids as possible enhancers of the beneficial actions of probiotics. Nutrition 18:786–789
38. Field CJ, Johnson IR, Schley PD (2002) Nutrients and their role in host resistance to infection. J Leukoc Biol 71:16–32
39. Puertollano MA, Puertollano E, Alvarez de Cienfuegos G, de Pablo MA (2007) Significance of olive oil in the host immune resistance to infection. Brit J Nutr 98:S54–S58
40. Kremer J, Lawrence DL, Jubiz W, DiGiacomo R, Rynes R, Bartholomew LE, Sherman M (1990) Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis: clinical and immunologic effects. Arthritis Rheum 33:810–820
41. Kelley DS (2001) Modulation of human immune and inflammatory responses by dietary fatty acids. Nutrition 17:669–673
42. Anderson M, Fritsche KL (2002) (n-3) Fatty acids and infectious disease resistance. J Nutr 132:3566–3576
43. de Pablo MA, Puertollano MA, Alvarez de Cienfuegos G (2002) Biological and clinical significance of lipids as modulators of immune system functions. Clin Diagn Lab Immunol 9:945–950
44. de Pablo MA, Puertollano MA, Alvarez de Cienfuegos G (2000) Immune cell functions, lipids and host natural resistance. FEMS Immunol Med Microbiol 29:323–328
45. Kos WL, Loria RM, Snodgrass MJ, Cohen D, Thorpe TG, Kaplan AM (1979) Inhibition of host resistance by nutritional hypercholesteremia. Infect Immun 26:658–667
46. Kos WL, Kos KA, Kaplan AM (1984) Impaired function of immune reactivity to *Listeria monocytogenes* in diet-fed mice. Infect Immun 43:1094–1096
47. Rubin RH, Wilkinson RA, Xu L, Robinson DR (1989) Dietary marine lipid does not alter susceptibility of (NZBxNZW)F1 mice to pathogenic microorganisms. Prostaglandins 38:251–262
48. Shinomiya N, Tsuru S, Fujisawa H, Taniguchi M, Zinnaka Y, Nomoto K (1988) Effect of a high-fat diet on resistance to *Listeria monocytogenes*. J Clin Lab Immunol 25:97–100
49. Puertollano MA, de Pablo MA, Alvarez de Cienfuegos G (2001) Immunomodulatory effects of dietary lipids alter host natural resistance of mice to *Listeria monocytogenes* infection. FEMS Immunol Med Microbiol 32:47–52
50. Puertollano MA, de Pablo MA, Alvarez de Cienfuegos G (2002) Relevance of dietary lipids as modulators of immune functions in cells infected with *Listeria monocytogenes*. Clin Diagn Lab Immunol 9:352–357
51. de Pablo MA, Puertollano MA, Gálvez A, Ortega E, Gaforio JJ, Alvarez de Cienfuegos G (2000) Determination of natural resistance of mice fed dietary lipids to experimental infection induced by *Listeria monocytogenes*. FEMS Immunol Med Microbiol 27:127–133
52. Fritsche KL, Shahbazian LM, Feng C, Berg JN (1997) Dietary fish oil reduces survival and impairs bacterial clearance in C3H/Hen mice challenged with *Listeria monocytogenes*. Clin Sci 92:95–101
53. Turnock L, Cook M, Steinberg H, Czuprynski C (2001) Dietary supplementation with conjugated linoleic acid does not alter the resistance of mice to *Listeria monocytogenes* infection. Lipids 36:135–138
54. Fritsche KL, Anderson M, Feng C (2000) Consumption of eicosapentaenoic acid and docosahexaenoic acid impair murine interleukin-12 and interferon-gamma production in vivo. J Infect Dis 182:S54–S61

55. Fritsche KL, Byrge M, Feng C (1999) Dietary omega-3 polyunsaturated fatty acids from fish oil reduce interleukin-12 and interferon-gamma production in mice. Immunol Lett 65:167–173
56. Huang SC, Misfeldt ML, Fritsche KL (1992) Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. J Nutr 122:1219–1231
57. Puertollano MA, Puertollano E, Ruiz-Bravo A, Jimenez-Valera M, de Pablo MA, Alvarez de Cienfuegos G (2004) Changes in the immune functions and susceptibility to *Listeria monocytogenes* infection in mice fed dietary lipids. Immunol Cell Biol 82:370–376
58. Puertollano MA, de Pablo MA, Álvarez de Cienfuegos G (2003) Antioxidant properties of N-acetyl-l-cysteine do not improve the immune resistance of mice fed dietary lipids to *Listeria monocytogenes* infection. Clin Nutr 22:313–319
59. Fritsche K, Irons R, Pompos L, Janes J, Zheng Z, Brown C (2005) Omega-3 polyunsaturated fatty acid impairment of early host resistance against *Listeria monocytogenes* infection is independent of neutrophil infiltration and function. Cell Immunol 235:65–71
60. Cruz-Chamorro L, Puertollano MA, Puertollano E, Álvarez de Cienfuegos G, de Pablo MA (2007) Examination of host immune resistance against *Listeria monocytogenes* infection in cyclophosphamide-treated mice after dietary lipid administration. Clin Nutr 26:631–639
61. D'Ambola JB, Aeberhard EE, Trang N, Gaffar S, Barrett CT, Sherman MP (1991) Effect of dietary (n-3) and (n-6) fatty acids on in vivo pulmonary bacterial clearance by neonatal rabbits. J Nutr 121:1262–1269
62. Blok WL, Vogels MT, Curfs JH, Eling WM, Buurman WA, van der Meer JW (1992) Dietary fish-oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. J Infect Dis 165:898–903
63. Thors VS, Erlendsdóttir H, Olafsson O, Gunnarsson E, Haraldsson A (2004) The improved survival of experimental animals fed with fish oil is suppressed by a leukotriene inhibitor. Scand J Immunol 60:351–355
64. Björnsson S, Hardardóttir I, Gunnarsson E, Haraldsson A (1997) Dietary fish oil supplementation increases survival in mice following *Klebsiella pneumoniae* infection. Scand J Infect Dis 29:491–493
65. Thors VS, Thórisdóttir A, Erlendsdóttir H, Einarsson I, Gudmundsson S, Gunnarsson E, Haraldsson A (2004) The effect of dietary fish oil on survival after infection with *Klebsiella pneumoniae* or *Streptococcus pneumoniae*. Scand J Infect Dis 36:102–105
66. Paul KP, Leichsenring M, Pfisterer M, Mayatepek E, Wagner D, Domann M, Sonntag HG, Bremer HJ (1997) Influence of n-6 and n-3 polyunsaturated fatty acids on the resistance to experimental tuberculosis. Metabolism 46:619–624
67. Mayatepek E, Paul K, Leichsenring M, Pfisterer M, Wagner D, Domann M, Sonntag HG, Bremer HJ (1994) Influence of dietary (n-3)-polyunsaturated fatty acids on leukotriene B4 and prostaglandin E2 synthesis and course of experimental tuberculosis in guinea pigs. Infection 22:106–112
68. McFarland CT, Fan YY, Chapkin RS, Weeks BR, McMurray DN (2008) Dietary polyunsaturated fatty acids modulate resistance to *Mycobacterium tuberculosis* in guinea pigs. J Nutr 138:2123–2128
69. Jordao L, Lengeling A, Bordat Y, Boudou F, Gicquel B, Neyrolles O, Becker PD, Guzman CA, Griffiths G, Anes E (2008) Effects of omega-3 and -6 fatty acids on *Mycobacterium tuberculosis* in macrophages and in mice. Microbes Infect 10:1379–1386
70. Pierre M, Husson MO, Le Berre R, Desseyn JL, Galabert C, Béghin L, Beermann C, Dagenais A, Berthiaume Y, Cardinaud B, Barbry P, Gottrand F, Guery BP (2007) Omega-3 polyunsaturated fatty acids improve host response in chronic *Pseudomonas aeruginosa* lung infection in mice. Am J Physiol Lung Cell Mol Physiol 292:1422–1431
71. Clouva-Molyvdas P, Peck MD, Alexander JW (1992) Short-term dietary lipid manipulation does not affect survival in two models of murine sepsis. JPEN J Parenter Enteral Nutr 16:343–347
72. Peck MD, Alexander JW, Ogle CK, Babcock GF (1990) The effect of dietary fatty acids on response to *Pseudomonas* infection in burned mice. J Trauma 30:445–452
73. Tiesset H, Pierre M, Desseyn JL, Guéry B, Beermann C, Galabert C, Gottrand F, Husson MO (2009) Dietary (n-3) polyunsaturated fatty acids affect the kinetics of pro- and antiinflammatory responses in mice with *Pseudomonas aeruginosa* lung infection. J Nutr 139:82–89
74. Chang HR, Dulloo AG, Vladoianu IR, Piguet PF, Arsenijevic D, Girardier L, Pechère JC (1992) Fish oil decreases natural resistance of mice to infection with *Salmonella typhimurium*. Metabolism 41:1–2

75. Eicher SD, McVey DS (1995) Dietary modulation of Kupffer cell and splenocyte function during a *Salmonella typhimurium* challenge in mice. J Leukoc Biol 58:32–39
76. Byleveld PM, Pang GT, Clancy RL, Roberts DC (1999) Fish oil feeding delays influenza virus clearance and impairs production of interferon-gamma and virus-specific immunoglobulin A in the lungs of mice. J Nutr 129:328–335
77. Byleveld M, Pang GT, Clancy RL, Roberts DC (2000) Fish oil feeding enhances lymphocyte proliferation but impairs virus-specific T lymphocyte cytotoxicity in mice following challenge with influenza virus. Clin Exp Immunol 119:287–292
78. Peterson KM, O'Shea M, Stam W, Mohede IC, Patrie JT, Hayden FG (2009) Effects of dietary supplementation with conjugated linoleic acid on experimental human rhinovirus infection and illness. Antivir Ther 14:33–43
79. Courreges C, Benencia F, Monserrat AJ (2001) Effect of dietary fish oil on mouse ocular herpes simplex type I infection. Nutr Res 21:229–241
80. Beli E, Li M, Cuff C, Pestka JJ (2008) Docosahexaenoic acid enriched fish oil consumption modulates immunoglobulin responses to and clearance of enteric reovirus infection in mice. J Nutr 138:813–819
81. Xi S, Cohen D, Barvec S, Chen LH (2001) Fish oil suppressed cytokines and nuclear factor-kappaB induced by murine AIDS virus infection. Nutr Res 21:865–878
82. Bell SJ, Chavali S, Bistrian BR, Connolly CA, Utsunomiya T, Forse RA (1996) Dietary fish oil and cytokine and eicosanoid production during human immunodeficiency virus infection. JPEN J Parenter Enteral Nutr 20:43–49
83. Kawashima A, Tsukamoto I, Koyabu T, Murakami Y, Kawakami T, Kakibuchi N, Takaguchi K, Kita K, Okita M (2008) Eicosapentaenoic acid supplementation for chronic hepatitis C patients during combination therapy of pegylated interferon-2b and ribavirin. Lipids 43:325–333
84. Allen PC, Danforth H, Levander OA (1997) Interaction of dietary flaxseed with coccidia infections in chickens. Poult Sci 76:822–827
85. Taylor DW, Levander OA, Krishna VR, Evans CB, Morris VC, Barta JR (1997) Vitamin E-deficient diets enriched with fish oil suppress lethal *Plasmodium yoelii* infections in athymic and scid/bg mice. Infect Immun 65:197–202
86. Gómez García V, Sanz Sampelayo MR, Fernández Navarro JR, Carmona López FD, Gil Extremera F, Rodríguez Osorio M (2003) Polyunsaturated fatty acids and parasitism: effect of a diet supplemented with fish oil on the course of rat trichinellosis. Vet Parasitol 117:85–97
87. Oarada M, Tsuduki T, Suzuki T, Miyazawa T, Nikawa T, Hong-quan G, Kurita N (2003) Dietary supplementation with docosahexaenoic acid, but not with eicosapentaenoic acid, reduces host resistance to fungal infection in mice. Biochim Biophys Acta 1622:151–160
88. Wanten GJ, Netea MG, Naber TH, Curfs JH, Jacobs LE, Verver-Jansen TJ, Kullberg BJ (2002) Parenteral administration of medium- but not long-chain lipid emulsions may increase the risk for infections by *Candida albicans*. Infect Immun 70:6471–6474
89. Mascioli E, Leader L, Flores E, Trimbo S, Bistrian B, Blackburn G (1988) Enhanced survival to endotoxin in Guinea pigs fed IV fish oil emulsion. Lipids 23:623–625
90. Hsu CS, Chiu WC, Yeh CL, Hou YC, Chou SY, Yeh SL (2006) Dietary fish oil enhances adhesion molecule and interleukin-6 expression in mice with polymicrobial sepsis. Brit J Nutr 96:854–860
91. Bate C, Tayebi M, Diomede L, Salmona M, Williams A (2008) Docosahexaenoic and eicosapentaenoic acids increase prion formation in neuronal cells. BMC Biol 12:6–39

9 Long Chain Polyunsaturated Fatty Acids: Immunomodulators in Disease

Jean-Luc Desseyn and Frédéric Gottrand

Key Points

- (*n*-3) LCPUFA metabolites induce eicosanoid and docosanoid production, alter gene expression and modify lipid raft composition altering T-cell signaling all contribute to immunological functional changes.
- The interventional studies that have been performed for prevention of allergy, infectious and inflammatory diseases confirmed influence on T-cell function and cytokine profiles but clinical beneficial effects are more conflicting.

Key Words: Immunity, polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid, allergy, infectious disease.

9.1 INTRODUCTION

The immune system protects the host against pathogenic organisms and also ensures tolerance to "self", to food and other environmental components, and to commensal bacteria. It is now recognized that regulation of tolerance and active immune responses are critical to health, and failure to regulate these responses can lead to recurrent infections, inflammatory diseases, allergies, and cancer. Long chain polyunsaturated fatty acids (LCPUFA) have indeed been shown to influence immune system via different mechanisms, which are been recently better described and understood.

9.2 DIETARY POLYUNSATURATED FATTY ACIDS, NOMENCLATURE, BIOSYNTHESIS, AND SOURCE

Unsaturated fatty acids (FA) consist of monounsaturated FA (series *n*-9) and polyunsaturated FA (PUFA). PUFA are divided between the two classes *n*-3 and *n*-6. These two classes or two series of unsaturated FA have in common a final carbon–carbon

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_9

double bond in either the *n*-3 or the *n*-6 position; that is, the third and the sixth bond from the end of the FA, respectively. The long-chain PUFA (LCPUFA) contain 16 or more carbons. Most natural FA are in the *cis* configuration while FA usually resulting from human processing like hydrogenation or produced naturally through the rumination process are *trans* FA (1). The conformational freedom is more restricted for *cis* FA than for *trans* isoform and *cis* bonds limit the ability of FA to be closely packed, particularly when they are part of the phospholipid bilayer in cell membranes.

The majority of FA in mammal tissues are nonessential. They are both endogenously synthesized and dietarily supplied. LCPUFA are indispensable for life and are not synthesized de novo by mammals that lack the enzyme to introduce a double bond at the *n*-3 position or *n*-6 position. A schematic representation of the three main LCPUFA is given in Fig. 9.1. Furthermore, LCPUFA from the *n*-3 and *n*-6 series cannot be interconverted due to the lack of the enzyme. However, mammals are capable of synthesizing LCPUFA by a common series of elongases and desaturases from the two precursors (Fig. 9.2), which are linoleic acid (LA, 18:2, *n*-6) and alpha linolenic acid (ALA, 18:3 *n*-3). Thus, ALA and LA are dietary essential. LA is the precursor of the arachidonic

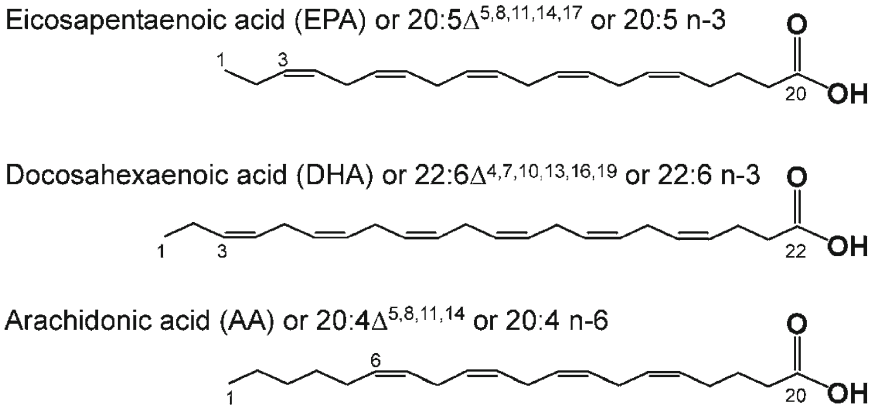


Fig. 9.1. Nomenclature schematic for the three main essential PUFA. First, last and carbon determining the serie (*n*-3 or *n*-6) are numbered. Only *trans* isomers are represented to gain space.

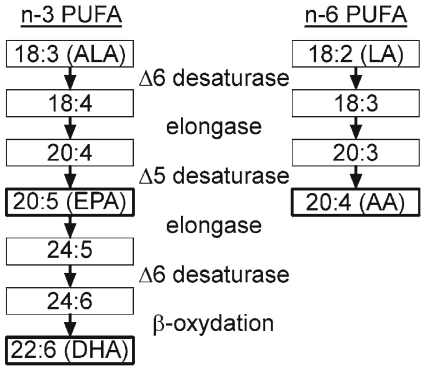


Fig. 9.2. Anabolic pathway of essential LCPUFA.

acid (AA, 20:4 *n*-6) while ALA is the precursor of the eicosapentaenoic acid (EPA, 20:5 *n*-3) and the docosahexaenoic acid (DHA, 22:6 *n*-3). The first step consists of the action of a Δ6 desaturase, which acts preferentially on *n*-3 PUFA over (*n*-6) PUFA. The two Δ desaturases are widely expressed in human tissues, with the highest expression in liver (2). Both Δ5 and Δ6 desaturases are suppressed by dietary PUFA and several works reported that these enzymes might be less active in elder people and in infant (3). Furthermore, it seems that whole body conversion of 18:3*n*-3 to 22:6*n*-3 is very low in humans (below 5%), and depends on the concentration of LCPUFA brought by the diet (4–6).

Tissue FA composition of cell membrane diet reflects FA composition of the diet (7–9). Furthermore, PUFA are metabolized to a number of competitive eicosanoids and docosanoids (see after), which are crucial in the regulation of the immune system. Therefore, the ratio between (*n*-6) and (*n*-3) PUFA in diet rather than their absolute amounts are highly important. Vegetable oils are the main sources of LA and the main sources of (*n*-3) PUFA for human consumption are vegetable oils and fish. LCPUFA in fish come mainly from marine microalgae. FA composition of fish is a reflection of the food available to the fish and the FA content is strongly influenced by the geographical area in which the animal lives and the season of the year (10). According to Larsson et al. (11) the total fat is very high for Pacific herring (18.5 g/100 g), Atlantic mackerel (16.0 g/100 g), Atlantic salmon (12.0 g/100 g), and sardines (14.8 g/100 g) with the highest ratios (*n*-3)/(*n*-6) FA for sardines (11.11), mackerel (7.14), and herring (5.88). The ratio is 3.85 for the Atlantic salmon while the ratio is 16.67 for the Pacific salmon, which contains less total fat (5.2 g/100 g). LA is found in the chloroplast of green leafy vegetables, with a high proportion comparing to the (*n*-3) LCPUFA in corn, sunflower, and safflower while flaxseed and canola are enriched in alpha-linolenic acid (18:3 *n*-3) (12).

As the concentrations of LCPUFA increase in organisms and as they move up the food chain, the ratio of (*n*-6)/(*n*-3) LCPUFA in human reflects the ratio absorbed in the diet. Since the dawn of civilization some 10,000 years ago, tribes of hunters and gatherers began to cultivateplant crops and eat domesticated animals. This leads to a major change of human food consumption with a change in the ratio (*n*-6)/(*n*-3) LC PUFA (13, 14). It is usually admitted that this ratio tends to be >15 while it used to be <5 or even equal to 1. The consumption of (*n*-6) LCPUFA increased remarkably during the last 100–150 years with the modern vegetable oil industry and new agriculture practices (3). This period of time was too short (<500 generations) for our genes to evolve and, consequently, we live in a nutritional environment that differs from that for which our genetic constitution was selected (14). Thus, a decrease in the amount of (*n*-3) LCPUFA and an increase of dietary (*n*-6) LCPUFA have led to an imbalance and an increase in the ratio of (*n*-6)/(*n*-3) LCPUFA.

9.3 MECHANISM OF ACTION AND BIOLOGICAL FUNCTIONS OF LCPUFA

Several molecular mechanisms whereby (*n*-3) LCPUFA act in the modulation of many biological functions have been demonstrated. These mechanisms include (1) alteration of cell membrane fluidity, (2) alteration of the raft lipid composition, (3) effects on eicosanoids, and (4) modification of transcription factor activity.

9.3.1 Cell Membrane Properties

The major LCPUFA in cell membranes is AA. LCPUFA play an important role in membrane structure and modification in the ratio (*n*-6)/(*n*-3) can thus affect membrane protein function. FA composition of membranes affects their fluidity (15). Furthermore, the FA environment likely affects the binding of many proteins to their receptor localized at the cell surface (3).

9.3.2 Raft Lipid Composition

Closely related to the cell membrane are microdomains called lipid rafts. These domains within the plasma membrane represent a platform that compartmentalizes and facilitates protein–protein interactions, and it has been suggested that LCPUFA composition of the lipid rafts may modulate these interaction and/or affect protein co- and posttranslational lipidation like *N*-myristoylation, which subsequently may modify the protein targeting to lipid rafts (16).

9.3.3 Effects on Eicosanoids and Docosanoids

The LCPUFA, particularly EPA (*n*-3) and AA (*n*-6), are converted into eicosanoids, which are lipids that modulate inflammatory and immune responses (Fig. 9.3). However, AA is the major precursor for eicosanoids because AA if found in higher levels in cell membranes compared to EPA. One particularity is that LCPUFA of the two series compete as substrates, after being released from membrane phospholipids

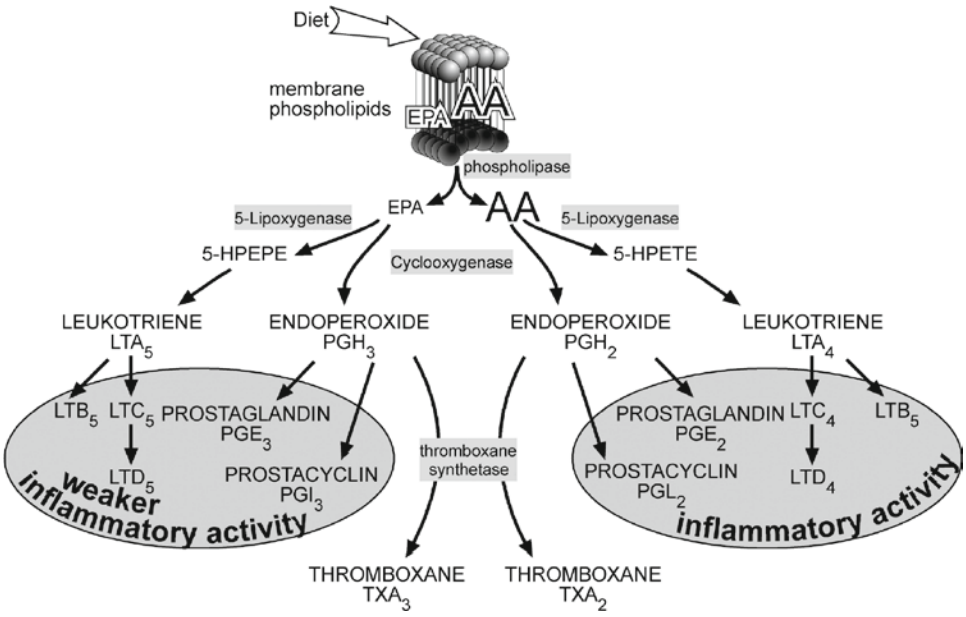


Fig. 9.3. Synthesis of eicosanoids with inflammatory activity. Membranes contain mainly AA compared to EPA. *HPEP* hydroperoxyeicosapentaenoic acid, *LT* leukotriene, *PG* prostaglandin, *HPETE* hydroperoxyeicosatetraenoic, *TX* thromboxane.

by various phospholipases, for cyclooxygenases to produce prostaglandins and thromboxanes, and lipoxygenases (high activity in neutrophils) to produce leukotrienes, hydroxy FA, and lipoxins (11). The simple picture is that EPA-derived eicosanoids (Fig. 9.3) are lipid mediators with anti-inflammatory effects while AA-derived eicosanoids have more potent biological functions with proinflammatory effects (17). However, some AA-derived eicosanoids like prostaglandin E2 (PGE2) may also show anti-inflammatory properties (18). The thromboxane synthetase synthesizes the thromboxane A2 (TXA2), which is derived from AA and TXA3, which is derived from EPA. TXA2 is a vasoconstrictor and a potent aggregator while TXA3 is biologically less active than TXA2 (19).

In addition to production of eicosanoids, EPA and DHA are precursors to potent bioactive mediators that possess both anti-inflammatory and protective properties. These mediators were coined resolvins, docosatrienes, and protectins as general classes, since each possesses unique chemical structures that are features of the new chemical classes and are biosynthesized by new pathways (20). Resolvins are specific lipid-derived mediators initiated by lipoxygenases that are involved in the resolution phase of acute inflammation (21). Docosatrienes contain conjugated triene structures generated from DHA as a defining feature. The protectins comprise docosatrienes and resolvins of the D series that have both a neuroprotective and an anti-inflammatory function (22).

9.3.4 Modification of Transcription Factor Activity

PUFA control gene transcription by two mechanisms (for review, see Schmitz et Ecker (23)). In the first one, LCPUFA and their metabolites interact with G protein-coupled receptor (GPCR). It has been reported that a deficient diet for (*n*-3) PUFA reduced the signaling efficiency in rat retinal rod outer segments (24). It has also been shown that PGE2 and leukotriene B4 (LTB4) interact with various GPCR implicated in physiological processes and inflammation in host defense (25, 26).

In the other mechanism, PUFA control gene transcription by direct interaction of PUFA with transcription factors. Several experimental data showed in cell culture that (*n*-3) LCPUFA inhibit nuclear factor kB (NFkB) directly though decreased degradation of IkB, the inhibitory subunit of NFkB (23, 27, 28). The team of JX Kang generated a transgenic mouse strain carrying the *fat-1* gene from *Clostridium elegans*. This gene encodes an enzyme that converts (*n*-6) LCPUFA to (*n*-3) LCPUFA (29). The authors have shown that the transgenic mice are protected from colitis through a decrease in NFkB activity (30). LCPUFA modulates also gene transcription activity via peroxysome proliferator activated receptors (PPAR). PPAR activation controls several genes implicated in lipid metabolism.

It has been reported that EPA and DHA are natural ligands of PPAR and that eicosanoids are much stronger activator of PPAR than LCPUFA (23). It has also been shown that DHA can bind retinoid X receptors (RXR) (31), which normally form homodimers or heterodimers with retinoic acid receptors (RAR). These receptors play multiple roles in the lipid metabolism and catabolism (31) and binding of FA stimulates an exchange of coactivators for corepressors on the chromatine-bound receptor (32).

9.4 BIOLOGICAL FUNCTIONS

9.4.1 Growth and Maturation of the Nervous System

During the pre and postnatal period (*n*-3) and (*n*-6) PUFA are required for normal growth and maturation of numerous organ systems, most importantly the brain and eye (33). As a major component of the cell membranes, DHA plays a critical role on the development of visual system and cognitive development (34, 35). Such properties have recently increased the interest of DHA in preventing cognitive decline in dementia such as Alzheimer disease (36) and various neurological diseases.

9.4.2 Anti-Inflammatory Properties

(*n*-3) LCPUFA have anti-inflammatory properties via various mechanisms. EPA and DHA, overall suppress eicosanoids associated with systemic inflammatory response syndrome and shift to the less biologically active 3-series prostaglandins and 5-series leukotrienes (37). In addition to (*n*-3) LCPUFA modulation of eicosanoids, a novel group of mediator termed E-series resolvins formed from EPA by cyclooxygenase (COX)-2 have been shown in cell culture and animal models to be anti-inflammatory (20, 38). Other mechanisms have been demonstrated, including surface receptor modulation, binding to transcription factors (e.g., NFκB), gene interactions and generation of growth factors (39).

9.4.3 Cancer

(*n*-3) LCPUFA consumption has long been associated with a lower incidence of colon, breast and prostate cancers in many human populations. Human trials have demonstrated that (*n*-3) LCPUFA have profound anti-inflammatory effects in those with cancer. Although results of interventional trials are conflicting, recent findings indicate that (*n*-3) LCPUFA act synergistically with chemotherapeutic agents and may also be used to enhance tumor radiosensitivity (40). *In vitro* and small animal studies have yielded a strong body of evidence establishing (*n*-3) FA as having anti-inflammatory, antiapoptotic, antiproliferative, and antiangiogenic effects (41).

9.4.4 Vascular Function

EPA and DHA can reduce blood pressure, improve arterial compliance in type 2 diabetics and dyslipidemics, and augment endothelium-dependent vasodilatation (42). Furthermore, EPA and DHA as ethyl esters inhibit platelet aggregability, and reduce serum triglycerides while leaving other serum lipids essentially unaltered. Proatherogenic cytokines are reduced, as are markers of endothelial activation. Endothelial function is improved; vascular occlusion is reduced; and the course of coronary atherosclerosis is mitigated. Heart rate is reduced, and heart rate variability is increased by EPA and DHA. An antiarrhythmic effect can be demonstrated on the supraventricular and the ventricular level. Several studies showed reductions in clinical endpoints such as sudden cardiac death or major adverse cardiac events (43).

9.4.5 Effect on Adipose Tissue

EPA and DHA have been shown to inhibit key enzymes responsible for lipid synthesis, such as FA synthase and stearoyl-CoA desaturase-1, enhance lipid oxidation and thermogenesis, and prevent free FA from entering adipocytes for lipogenesis. PUFA also exert suppressive effects on several key factors involved in adipocyte differentiation and fat storage (44).

(*n*-3) LCPUFA induce mitochondrial biogenesis and beta-oxidation in adipose tissue influencing systemic insulin sensitivity and adiposity. (*n*-3) LCPUFA ameliorate the low-grade inflammation of adipose tissue associated with obesity and induce changes in the pattern of secreted adipokines, resulting in improved systemic insulin sensitivity (45).

9.4.6 Effects of LCPUFA on the Immune System

Today, it is clear that LCPUFA play a key role in the modulation of the immune system in many diseases. In addition to proinflammatory effects, prostaglandin E_2 – produced from (*n*-6) PUFA – exerts effect on the Th1/Th2 balance. It decreases the production of the Th1-type cytokines interferon (IFNγ) and interleukin 2 (IL-2), enhances the production of Th2-type cytokines IL-4 and IL-5, and promotes IgE synthesis by B-cells (46, 47).

(*n*-6) PUFA are essential in relation to a thymus/thymocyte accretion of AA in early development, and the high requirement of lymphoid and other cells of the immune system for AA and LA for membrane phospholipids. Low (*n*-6) PUFA intakes enhance, whereas high intakes decrease certain immune functions. AA metabolites can limit or regulate cellular immune reactions and can induce deviation toward a Th2-like immune response (48). By competition with AA for several enzymatic pathways (i.e. prostaglandin production), (*n*-3) LCPUFA influence the Th1/Th2 balance.

As mentioned above, (*n*-3) LCPUFA induce also direct alteration of gene expression through modification of transcription factor activity such as nuclear factor kappa B (NFκB). NFκB plays a role in inducing a range of inflammatory genes, including cyclooxygenase (COX-2), intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, tumor necrozing factor (TNF-α), IL-1β, inductible nitric oxide synthase (iNOS), acute phase protein, in response to inflammatory stimuli. (*n*-3) LCPUFA decrease the activity of NFκB, via the inhibition of IκB, the inhibitory subunit of NFκB. (*n*-3) LCPUFA are natural ligands of nuclear receptors, such as PPAR α and γ PPAR, are ligand-activated transcription factors present in a variety of cell types, including inflammatory cells (49). (*n*-3) LCPUFA have also been shown to influence the expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin). Adhesion molecules direct the leucocyte-endothelium interactions, transendothelial migration of leucocytes and leucocyte trafficking in general (37).

9.4.7 (n-3) LCPUFA and T-cell Function

Several mechanisms by which (*n*-3) LCPUFA can affect T cells have been identified (Table 9.1) (46, 50–52). As mentioned above, (*n*-3) LCPUFA in membrane compete with AA as substrates for cyclooxygenase and lipoxygenase enzymes (Fig. 9.1). (*n*-3) LCPUFA decrease the production of AA-derived eicosanoids such as PGE2. PGE2 is

Table 9.1
Primary mechanisms by which (*n*-3) LCPUFA influence immunological responses

Biological properties of (n-3) LCPUFA	*Mediator*	*Mechanism*	*Immunological cells involved*	*Effect*
Membrane constituent	TCR clusters within lipid rafts on contact with an antigen-presenting cell	Modification of lipid rafts and caveolae structure	Th1 (Th2)	Inhibits T-cell response
Competition between (n-6) and (n-3) LCPUFA for the production of eicosanoids	Cyclooxygenase	Decreased PGE_2	Lymphocytes	Lymphocyte proliferation
	Lipoxygenase	Decreased LTB_4	Monocytes macrophages	NK cell activity
		Increased 3-series Prostaglandins	NK	Production of Th1 cytokines (IL-2, IFNγ)
		Increased 5-series leukotrienes	Leucocytes	MHC II expression and production of TNF-α, IL-1 and IL-6 by monocyte and macrophage
				IgE production
				Leucocyte activation, chemotaxis and degranulation
Direct action on gene expression	NF-κB (ligand of TLR4)	Inhibit expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin)	Endothelial cell	Reduced adherence to human blood monocytes
	PPAR γ (natural ligand)	Inhibit expression of inflammatory genes (COX-2, IL-1α, TNF-α, 5-LOX…)	T and B cells	Upregulate genes of fatty acid oxidation and thermogenesis
			Monocyte, macrophage	Inhibit proliferation of human T cells
				Decreased I-κB phosphorylation, Decreased endotoxin activation of NF-κb
				Reduce production of inflammatory eicosanoid by immune cells

TCR T-cell receptor, *LTB4* leukotriene, *MHC* major histocompatibility complex, *TLR4* Toll-like receptor, *COX-2* cyclooxygenase, *IL-1α, IL-2* interleukins, *I-κB* Inhibitor of NF-κB

immunosuppressive at high concentration, and low concentration is required for normal T-cell function. PGE-2 regulates cellular immune responses through distinct receptors on different immune cell populations: some receptors directly inhibit T-cell proliferation while others regulate antigen presenting cell functions (52).

Incorporation of (*n*-3) LCPUFA into membrane phospholipids modulates membrane structure and function. Indeed incorporation of EPA and DHA into lymphocyte membranes alters their fluidity and is associated with the effects of these FA on T-cell proliferation. Lipid rafts are crucial for T-cell activation besides fences and pickets and protein–protein interactions that take part in the formation of the immunological synapse as a highly organized structure at the T-cell contact site to the antigen-presenting cell (53). (*n*-3) LCPUFA treatment alters lipid rafts in altering the protein composition of the inner membrane lipid leaflet and inhibits T-cell responses (53, 54).

Both *in vitro* and animal feeding studies have reported that the (*n*-3) LCPUFA inhibit T-cell proliferation, production of IL-2 and IFNγ and surface expression of CD25 (17). Human studies provide more conflicting results suggesting the actions of (*n*-3) LCPUFA may differ according to the situation: age of the patients, Th1/Th2 balance, dose of FA, and experimental conditions (55).

9.5 LCPUFA AND IMMUNE FUNCTION IN EARLY LIFE

A few studies have suggested that early intervention with (*n*-3) LCPUFA may influence immune functioning and may affect the cytokine phenotype during development. Some of these studies demonstrated that early exposure to (*n*-3) LCPUFA during fetal and neonatal period has a prolonged impact on Th1/Th2 immune responses and T-cell cytokine profiles (56, 57). Maternal fish oil supplementation during the first 4 months of lactation resulted in an increased production of lipopolysaccharide-induced IFNγ upon stimulation in 2.5-year-old children, whereas IL-10 production was similar to the olive oil group. The IFN-γ/IL-10 ratio was twofold higher in the fish oil group and was positively correlated with EPA/DHA in erythrocytes at 4 months (56). Adding DHA + AA to a standard infant formula for healthy infants increased the proportion of antigen mature (CD45RO+) CD4+ cells, improved IL-10 production, and reduced IL-2 production to levels not different from those of human milk-fed infants (58). Healthy term infants receiving the same formula supplemented with ARA and DHA at age 2 weeks produced less TNF-α (unstimulated) and had a higher CD3+CD44+ cells before stimulation with phytohemagglutinin and higher CD11c+ cells poststimulation (59). Compared with formula-fed controls, the infants receiving LCPUFA had an immune cell distribution (higher percentage CD3+CD44+ and CD4+CD28+ cells) and cytokine profile (lower production of TNF-α poststimulation) that did not differ from breast-fed infants (59).

All these results demonstrate that early diet influences both the presence of specific cell types and function of infant blood immune cells.

9.6 LCPUFA: IMMUNOSUPPRESSIVE EFFECTS?

Since some classes of FA possess immunosuppressive properties, the issue of impairment of host resistance to infection and therefore undesirable effects of LCPUFA have been subject to controversy (50, 60). Studies conducted in animals, investigating the

influence of dietary FA on host survival and/or pathogen clearance in animals challenged with a live infectious agent are inconclusive, some showing that (*n*-3) LCPUFA improve host defense (61–63) and others showing impairment (64). However, most of them demonstrated that (*n*-3) PUFA induce modulation of the inflammatory response that can influence the response to a bacterial challenge (64), rather than producing a generalized immunosuppression (65).

9.7 LCPUFA AND ALLERGY

The increased prevalence of atopic disease observed in western countries during the last decades parallels with profound changes in the type of fat consumed. Intake of saturated FA decreased, while (*n*-6) PUFA, mainly LA increased. Several epidemiological studies support the hypothesis of the link between increased intake of LA and increased prevalence of allergic disease (66–69).

9.7.1 Observational Studies

Low levels of (*n*-3) LCPUFA in breast milk were associated with increased risk of infant atopy (70). Two-year children breast fed for the first 4 months of life by mothers with a high intake of (*n*-3) LCPUFA had a significant higher production of IFNγ upon stimulation of whole blood compared to a control group with low maternal (*n*-3) LCPUFA intake during lactation (56). In a case-control study, children born to mothers with a history of asthma, had an odd ratio of asthma of 0.20 (95% CI = 0.06–0.65) when mothers ate oily fish at least monthly during pregnancy compared with no consumption. Maternal oily fish consumption during pregnancy did not benefit children of nonasthmatic mothers in this study (71). Other studies did not show that fetal exposure to (*n*-6) and (*n*-3) FA was an important determinant of early childhood wheezing and atopic disease in the general population also suggesting that benefit should be limited to selected population of high risk of allergy (72). A study examined LCPUFA in serum cholesteryl esters in relation to asthma and lung function in children. Although there was a strong positive association between AA levels and current asthma and a negative association with forced expiratory volume, levels of EPA were not related to asthma and impaired lung function (72). In another study performed in 308 Korean children aged 4–6 years, it was found that red blood cell EPA+DHA were lower in children with atopy than controls while AA was greater (73). Several studies in adult population also demonstrated a negative association between allergic sensitization and fish and DHA consumption (74).

9.7.2 Intervention Trials

Treatment. A number of trials of dietary supplementation with (*n*-3) LCPUFA in patients with asthma have been performed. Their results were mainly disappointing showing no consistent effect on both clinical and functional respiratory parameters (75–78). The effects of dietary supplementation with fish oil for 10 months in children with bronchial asthma were investigated in a randomized controlled trial. Asthma symptom scores decreased and responsiveness to acetylcholine decreased in the fish oil group but not in

the control group plasma while EPA levels increased significantly only in the fish oil group (79). The Childhood Asthma Prevention Study included 6 month-old infants at risk of developing asthma to receive either EPA + DHA or placebo. Although no effects of fish oil was observed at 3 years of age on prevalence of asthma, wheeze, and atopic dermatitis, some beneficial effects on wheeze at 1.5 year of age (80) and cough at 3 years of age were observed (80, 81). Moreover, no effect of fish oil was observed on the prevalence of asthma, wheezing, eczema, or atopy at the age of 5 years (82). Data of intervention on atopic eczema in adult patients give also conflicting results (83).

Prevention. Since allergies appear to be determined early in life or even antenatally, (*n*-3) LCPUFA intervention should be more efficient early in life for preventing allergic disease rather than treating allergy already installed later in life (84). Several recent studies seem to support this hypothesis. Breast milk of atopic mothers supplemented during pregnancy with dietary fish oil contains higher levels of (*n*-3) LCPUFA and lower (*n*-6) LCPUFA than those of controls (85). In this study, the (*n*-3) PUFA concentration of breath milk on day 3 postpartum was positively associated with IgA, IL-10, and IL-6 and soluble CD14 levels (86). For these children, IL-13 (a predictor of allergic disease) was detected in 64% of cord plasma samples in the placebo group and 45% of samples in the fish oil group (87), and percentages of CD34+ cell numbers (that are hematopoietic progenitors altered in infants at risk of atopy) were higher after (*n*-3) LCPUFA treatment than placebo (88). In this study, although no difference was observed for food allergy, asthma, chronic cough, and angiodema, infants in the fish oil group were one-third as likely to have a positive skin prick test to egg at 1 year of age and less severe atopic dermatitis compared to the placebo group (89). A recent meta-analysis of the six randomized double bind studies assessing the efficacy of (*n*-3) and (*n*-6) i.e., gamma linolenic acid (GLA) PUFA concluded that supplementation with these nutrients is unlikely to be associated with a mark reduction in risk of developing allergic disease such as atopic dermatitis, asthma, allergic rhinitis, and food allergy (90). It is, however, to note that the number of well-conducted studies in this area is low, including heterogeneous groups of patients (lactating mothers, infants, high- and low-risk populations), and various combination and dose of PUFA (fish oil ± GLA). Moreover a recent study, not used in this meta-analysis, demonstrated that offspring of mother included in a randomized trial receiving fish oil capsule from 30 weeks of gestation to delivery had a reduced risk of asthma at the age of 16 years compared to the control group (91).

9.8 LCPUFA AND INFECTION IN HIGH-RISK PATIENTS

From experimental and adult studies, there is a strong rationale to study the effect of (*n*-3) LCPUFA in treatment and/or prevention of infections (i.e., intensive care unit, prematurity, cystic fibrosis) (60).

Excessive or inappropriate inflammation and immunosuppression are components of the response to surgery, trauma, injury, and infection in some individuals, and these can lead to sepsis and septic shock. Hyperinflammation is characterized by the production of inflammatory cytokines, AA-derived eicosanoids and other inflammatory mediators, while the immunosuppression is characterized by the impairment of antigen presentation

and of T-helper lymphocyte type-1 responses. (*n*-3) LCPUFA should indeed be helpful in such situations (92).

Several animal models support the rational of using (*n*-3) PUFA in the prevention of infection. We have shown that a 5-week diet enriched in (*n*-3) LCPUFA improved lung and host response to an experimental chronic infection with *Pseudomonas aeruginosa* with the improvement of the distal alveolar fluid clearance, the recruitment of the inflammatory cells, and the inflammatory response, and also to the increase of lean body mass (61). We also demonstrated that the enriched diet in DHA + EPA modulates, in our experimental model of lung infection, the balance between pro- and anti-inflammatory cytokines and alters the early response of the host to *P. aeruginosa* infection (93). Furthermore, lung infection with *P. aeruginosa* induced a disregulation of the large mucins *Muc5b* and *Muc4*, and we showed that if dietary LCPUFA did not influence mucin gene expression in lung of healthy mice after 5 weeks of diet, a (*n*-3) LCPUFA-enriched diet had a beneficial suppressive effect on mucin upregulation and (*n*-6) LCPUFA showed an opposite effect (enhanced expression of mucins) leading to mucus obstruction (63). Altogether, these experiments on mice exposed to high (*n*-3) or (*n*-6) LCPUFA intakes and infected with *P. aeruginosa* strongly supports that malnutrition can compromise pulmonary defenses against lung bacterial colonization and that a diet enriched in EPA and DHA may be used as a preventive treatment against the initial colonization of *P. aeruginosa*.

A large number of clinical trials have been performed in patients postsurgery and with severe sepsis (94). These studies using parenteral or enteral nutrition products showed that (*n*-3) LCPUFA may influence leukocyte function and plasma lipids in critical care patients, i.e., suppression of proinflammatory cytokines by mononuclear leukocytes (94). These studies report beneficial outcome, including decreased number and severity of infectious complications, decreased need for mechanical ventilation, decreased length in intensive care unit and/or total hospital stay (95), and even reduction of mortality (96, 97).

Today, there are very few studies assessing intervention with (*n*-3) LCPUFA in infection in the pediatric population (98–100). The administration of DHA during the acute phase of sepsis protected the nutritional status of neonates (99). Indeed, the DHA group presented increases in body mass and fat mass, whereas infants in the placebo group did not show an increase in any body composition components after 14 days of follow-up. In this study, no difference in outcome (mortality, mechanical ventilation) and/or severity of the sepsis (C-reactive protein, platelets) could be demonstrated (99). In a recent study, healthy Thai school children aged 9–12 years consumed milk containing placebo (soybean) oil ($n = 86$) or fish oil ($n = 94$) in 5 days per week for 6 months. The fish oil group showed fewer episodes and shorter duration of illness (mainly upper respiratory tract) than the placebo group (100).

9.9 LCPUFA AND IMMUNE DISEASE (IBD, RHEUMATOLOGIC, SKIN…)

In a review of randomized controlled trials from 1966 to 2004, it was shown that dietary supplementation with (*n*-3) LCPUFA provides modest symptomatic benefit in groups of patients with rheumatoid arthritis (101). In a meta-analysis of 17 randomized, controlled trials assessing the pain relieving effects of (*n*-3) PUFA in patients with

rheumatoid arthritis or joint pain secondary to inflammatory bowel disease and dysmenorrhea, it was shown that (*n*-3) PUFA reduce patient reported joint pain intensity, minutes of morning stiffness, number of painful and/or tender joints, and nonsteroid anti-inflammatory drug consumption (102).

Although epidemiological and experimental studies suggest that (*n*-3) LCPUFA supplementation may prevent or slow the progression of kidney disease, evidence from clinical trials is inconsistent. A recent meta-analysis of 17 trials (IgA nephropathy, diabetes, lupus nephritis) shows that (*n*-3) LCPUFA supplementation results in a significant reduction in urine protein excretion, a marker of kidney damage, and a trend (but no significant) slowing the decline of glomerular filtration rate, and a marker of kidney function (103).

In one previous double blind, placebo controlled study, 78 patients with Crohn's disease in remission received 1.8 g of EPA and 0.9 g of DHA per day for 1 year (104). A 33% absolute reduction in the 1-year risk of relapse was observed in the group of patients receiving (*n*-3) LCPUFA. Controversially, two independent trials performed with the same design in 374 and 379 patients, respectively, failed to demonstrate any benefit on the rate of relapse (105). A systematic review of six double-blind randomized controlled studies concluded that (*n*-3) LCPUFA are safe but probably ineffective for maintenance of remission in CD (106).

Fish oil has been shown to decrease colonic damage and inflammation, weight loss and mortality in animal models of colitis. Fish oil supplementation in patients with inflammatory bowel diseases results in (*n*-3) PUFA incorporation into gut mucosal tissue and modification of inflammatory mediator profiles (107). Clinical outcomes have been variably affected by fish oil, a recent Cochrane review of six randomized control studies in active ulcerative colitis patients suggesting some positive benefit on remission or secondary outcome. However, no definitive conclusion could be drawn due to small study size and poor study quality (108).

9.10 CONCLUSIONS AND PERSPECTIVES

There are strong data from experimental studies showing that (*n*-3) LCPUFA alter immune cells function and could influence immune system. (*n*-3) LCPUFA may influence the number and/or activity of certain subpopulations of cells, which could affect subsequent maturation and polarization of the immune system. Application during infancy should be the prevention of infection and allergy. However, mechanisms involved are complex since several modes of action have been described (reduction of synthesis of some type of eicosanoids, modification of gene expression, and modification of signaling process). Effects on immune system may also vary according to age and polarization Th1/Th2 immune status, dose of (*n*-3) LCPUFA, and type of T cells. Effects of (*n*-3) LCPUFA on naturally or adaptative Treg cells is a promising but largely unexplored area of research (109, 110). Supplementation of the maternal diet in pregnancy or early childhood with (*n*-3) PUFA may provide a noninvasive intervention with significant potential to prevent the development of allergic and possibly other immune-mediated diseases. Preventive effect deserves further studies in several inflammatory processes. One promising perspective could also be associated (*n*-3) PUFA with other pharmacological or nutritional approaches in preventing or limiting immune and inflammatory diseases.

REFERENCES

1. Arab L (2003) Biomarkers of fat and fatty acid intake. J Nutr 133(Suppl 3):925S–932S
2. Nakamura MT, Nara TY (2004) Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu Rev Nutr 24:345–376
3. Teitelbaum JE, Allan WW (2001) Review: the role of omega 3 fatty acids in intestinal inflammation. J Nutr Biochem 12(1):21–32
4. Vermunt SH, Mensink RP, Simonis MM, Hornstra G (2000) Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha-linolenic acid. Lipids 35(2):137–142
5. Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA (2003) Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. Br J Nutr 90(2):311–321
6. Emken EA, Adlof RO, Gulley RM (1994) Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1213(3):277–288
7. Garland M, Sacks FM, Colditz GA, Rimm EB, Sampson LA, Willett WC et al (1998) The relation between dietary intake and adipose tissue composition of selected fatty acids in US women. Am J Clin Nutr 67(1):25–30
8. Benatti P, Peluso G, Nicolai R, Calvani M (2004) Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties. J Am Coll Nutr 23(4):281–302
9. Mantzioris E, James MJ, Gibson RA, Cleland LG (1995) Differences exist in the relationships between dietary linoleic and alpha-linolenic acids and their respective long-chain metabolites. Am J Clin Nutr 61(2):320–324
10. Rose DP, Connolly JM (1999) Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther 83(3):217–244
11. Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A (2004) Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 79(6):935–945
12. James MJ, Gibson RA, Cleland LG (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 71(1 Suppl):343S–348S
13. Eaton SB, Konner M (1985) Paleolithic nutrition. A consideration of its nature and current implications. N Engl J Med 312(5):283–289
14. Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56(8):365–379
15. Spector AA, Yorek MA (1985) Membrane lipid composition and cellular function. J Lipid Res 26(9):1015–1035
16. Chapkin RS, Davidson LA, Ly L, Weeks BR, Lupton JR, McMurray DN (2007) Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. J Nutr 137(1 Suppl):200S–204S
17. Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA (2002) Fatty acids and lymphocyte functions. Br J Nutr 87(Suppl 1):S31–S48
18. Raud J, Dahlen SE, Sydbom A, Lindbom L, Hedqvist P (1988) Enhancement of acute allergic inflammation by indomethacin is reversed by prostaglandin E2: apparent correlation with in vivo modulation of mediator release. Proc Natl Acad Sci USA 85(7):2315–2319
19. Gil A (2002) Polyunsaturated fatty acids and inflammatory diseases. Biomed Pharmacother 56(8):388–396
20. Serhan CN (2005) Novel eicosanoid and docosanoid mediators: resolvins, docosatrienes, and neuroprotectins. Curr Opin Clin Nutr Metab Care 8(2):115–121
21. Merched AJ, Ko K, Gotlinger KH, Serhan CN, Chan L (2008) Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. FASEB J 22(10):3595–3606
22. Bazan NG (2007) Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Curr Opin Clin Nutr Metab Care 10(2):136–141
23. Schmitz G, Ecker J (2008) The opposing effects of n-3 and n-6 fatty acids. Prog Lipid Res 47(2):147–155

24. Niu SL, Mitchell DC, Lim SY, Wen ZM, Kim HY, Salem N Jr et al (2004) Reduced G protein-coupled signaling efficiency in retinal rod outer segments in response to n-3 fatty acid deficiency. J Biol Chem 279(30):31098–31104
25. Sugimoto Y, Narumiya S (2007) Prostaglandin E receptors. J Biol Chem 282(16):11613–11617
26. Lundeen KA, Sun B, Karlsson L, Fourie AM (2006) Leukotriene B4 receptors BLT1 and BLT2: expression and function in human and murine mast cells. J Immunol 177(5):3439–3447
27. Lo CJ, Chiu KC, Fu M, Lo R, Helton S (1999) Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NF kappa B activity. J Surg Res 82(2):216–221
28. Novak TE, Babcock TA, Jho DH, Helton WS, Espat NJ (2003) NF-kappa B inhibition by omega-3 fatty acids modulates LPS-stimulated macrophage TNF-alpha transcription. Am J Physiol Lung Cell Mol Physiol 284(1):L84–L89
29. Kang JX, Wang J, Wu L, Kang ZB (2004) Transgenic mice: fat-1 mice convert n-6 to n-3 fatty acids. Nature 427(6974):504
30. Hudert CA, Weylandt KH, Lu Y, Wang J, Hong S, Dignass A et al (2006) Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis. Proc Natl Acad Sci USA 103(30):11276–11281
31. de Urquiza AM, Liu S, Sjoberg M, Zetterstrom RH, Griffiths W, Sjovall J et al (2000) Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. Science 290(5499):2140–2144
32. Jump DB, Botolin D, Wang Y, Xu J, Demeure O, Christian B (2008) Docosahexaenoic acid (DHA) and hepatic gene transcription. Chem Phys Lipids 153(1):3–13
33. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I et al (2008) The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med 36(1):5–14
34. Carlson SE (2009) Early determinants of development: a lipid perspective. Am J Clin Nutr 89(5):1523S–1529S
35. Carlson SE (2009) Docosahexaenoic acid supplementation in pregnancy and lactation. Am J Clin Nutr 89(2):678S–684S
36. Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A et al (2006) Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. Arch Neurol 63(10):1402–1408
37. Grimm H, Mayer K, Mayser P, Eigenbrodt E (2002) Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. Br J Nutr 87(Suppl 1):S59–S67
38. Calder PC (2006) n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83(6 Suppl):1505S–1519S
39. Vedin I, Cederholm T, Freund LY, Basun H, Garlind A, Faxen IG et al (2008) Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. Am J Clin Nutr 87(6):1616–1622
40. Wendel M, Heller AR (2009) Anticancer actions of omega-3 fatty acids – current state and future perspectives. Anticancer Agents Med Chem 9(4):457–470
41. Spencer L, Mann C, Metcalfe M, Webb M, Pollard C, Spencer D et al (2009) The effect of omega-3 FAs on tumour angiogenesis and their therapeutic potential. Eur J Cancer 45(12):2077–2086
42. Hall WL (2009) Dietary saturated and unsaturated fats as determinants of blood pressure and vascular function. Nutr Res Rev 22(1):18–38
43. von Schacky C (2006) A review of omega-3 ethyl esters for cardiovascular prevention and treatment of increased blood triglyceride levels. Vasc Health Risk Manag 2(3):251–262
44. Li JJ, Huang CJ, Xie D (2008) Anti-obesity effects of conjugated linoleic acid, docosahexaenoic acid, and eicosapentaenoic acid. Mol Nutr Food Res 52(6):631–645
45. Flachs P, Rossmeisl M, Bryhn M, Kopecky J (2009) Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. Clin Sci (Lond) 116(1):1–16
46. Miles EA, Aston L, Calder PC (2003) In vitro effects of eicosanoids derived from different 20-carbon fatty acids on T helper type 1 and T helper type 2 cytokine production in human whole-blood cultures. Clin Exp Allergy 33(5):624–632
47. Prescott SL, Calder PC (2004) N-3 polyunsaturated fatty acids and allergic disease. Curr Opin Clin Nutr Metab Care 7(2):123–129

48. Harbige LS (2003) Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. Lipids 38(4):323–341
49. Sampath H, Ntambi JM (2005) Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu Rev Nutr 25:317–340
50. Yaqoob P (2003) Fatty acids as gatekeepers of immune cell regulation. Trends Immunol 24(12):639–645
51. Stulnig TM, Zeyda M (2004) Immunomodulation by polyunsaturated fatty acids: impact on T-cell signaling. Lipids 39(12):1171–1175
52. Nataraj C, Thomas DW, Tilley SL, Nguyen MT, Mannon R, Koller BH et al (2001) Receptors for prostaglandin E(2) that regulate cellular immune responses in the mouse. J Clin Invest 108(8):1229–1235
53. Zeyda M, Stulnig TM (2006) Lipid Rafts & Co.: an integrated model of membrane organization in T cell activation. Prog Lipid Res 45(3):187–202
54. Ma DW, Seo J, Switzer KC, Fan YY, McMurray DN, Lupton JR et al (2004) n-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. J Nutr Biochem 15(11):700–706
55. Calder PC, Krauss-Etschmann S, de Jong EC, Dupont C, Frick JS, Frokiaer H et al (2006) Early nutrition and immunity – progress and perspectives. Br J Nutr 96(4):774–790
56. Lauritzen L, Kjaer TM, Fruekilde MB, Michaelsen KF, Frokiaer H (2005) Fish oil supplementation of lactating mothers affects cytokine production in 2 1/2-year-old children. Lipids 40(7):669–676
57. Field CJ, Clandinin MT, Van Aerde JE (2001) Polyunsaturated fatty acids and T-cell function: implications for the neonate. Lipids 36(9):1025–1032
58. Field CJ, Thomson CA, Van Aerde JE, Parrott A, Euler A, Lien E et al (2000) Lower proportion of CD45R0+ cells and deficient interleukin-10 production by formula-fed infants, compared with human-fed, is corrected with supplementation of long-chain polyunsaturated fatty acids. J Pediatr Gastroenterol Nutr 31(3):291–299
59. Field CJ, Van Aerde JE, Robinson LE, Clandinin MT (2008) Effect of providing a formula supplemented with long-chain polyunsaturated fatty acids on immunity in full-term neonates. Br J Nutr 99(1):91–99
60. Sweeney B, Puri P, Reen DJ (2005) Modulation of immune cell function by polyunsaturated fatty acids. Pediatr Surg Int 21(5):335–340
61. Pierre M, Husson MO, Le Berre R, Desseyn JL, Galabert C, Beghin L et al (2007) Omega-3 polyunsaturated fatty acids improve host response in chronic *Pseudomonas aeruginosa* lung infection in mice. Am J Physiol Lung Cell Mol Physiol 292(6):L1422–L1431
62. Bjornsson S, Hardardottir I, Gunnarsson E, Haraldsson A (1997) Dietary fish oil supplementation increases survival in mice following *Klebsiella pneumoniae* infection. Scand J Infect Dis 29(5):491–493
63. Tetaert D, Pierre M, Demeyer D, Husson MO, Beghin L, Galabert C et al (2007) Dietary n-3 fatty acids have suppressive effects on mucin upregulation in mice infected with *Pseudomonas aeruginosa*. Respir Res 8:39
64. Anderson M, Fritsche KL (2002) (n-3) Fatty acids and infectious disease resistance. J Nutr 132(12):3566–3576
65. Barber MD, Fearon KC, Ross JA (2005) Eicosapentaenoic acid modulates the immune response but has no effect on a mimic of antigen-specific responses. Nutrition 21(5):588–593
66. Bolte G, Winkler G, Holscher B, Thefeld W, Weiland SK, Heinrich J (2005) Margarine consumption, asthma, and allergy in young adults: results of the German National Health Survey 1998. Ann Epidemiol 15(3):207–213
67. Trak-Fellermeier MA, Brasche S, Winkler G, Koletzko B, Heinrich J (2004) Food and fatty acid intake and atopic disease in adults. Eur Respir J 23(4):575–582
68. Kankaanpaa P, Sutas Y, Salminen S, Lichtenstein A, Isolauri E (1999) Dietary fatty acids and allergy. Ann Med 31(4):282–287
69. Sausenthaler S, Kompauer I, Borte M, Herbarth O, Schaaf B, Berg A et al (2006) Margarine and butter consumption, eczema and allergic sensitization in children. The LISA birth cohort study. Pediatr Allergy Immunol 17(2):85–93
70. Duchen K, Yu G, Bjorksten B (1998) Atopic sensitization during the first year of life in relation to long chain polyunsaturated fatty acid levels in human milk. Pediatr Res 44(4):478–484

71. Salam MT, Li YF, Langholz B, Gilliland FD (2005) Maternal fish consumption during pregnancy and risk of early childhood asthma. J Asthma 42(6):513–518
72. Newson RB, Shaheen SO, Henderson AJ, Emmett PM, Sherriff A, Calder PC (2004) Umbilical cord and maternal blood red cell fatty acids and early childhood wheezing and eczema. J Allergy Clin Immunol 114(3):531–537
73. Hwang I, Cha A, Lee H, Yoon H, Yoon T, Cho B et al (2007) N-3 polyunsaturated fatty acids and atopy in Korean preschoolers. Lipids 42(4):345–349
74. Schnappinger M, Sausenthaler S, Linseisen J, Hauner H, Heinrich J (2009) Fish consumption, allergic sensitisation and allergic diseases in adults. Ann Nutr Metab 54(1):67–74
75. Mickleborough TD, Rundell KW (2005) Dietary polyunsaturated fatty acids in asthma- and exercise-induced bronchoconstriction. Eur J Clin Nutr 59(12):1335–1346
76. Wong KW (2005) Clinical efficacy of n-3 fatty acid supplementation in patients with asthma. J Am Diet Assoc 105(1):98–105
77. Schwartz J (2000) Role of polyunsaturated fatty acids in lung disease. Am J Clin Nutr 71(1 Suppl):393S–396S
78. Devereux G (2007) Early life events in asthma – diet. Pediatr Pulmonol 42(8):663–673
79. Nagakura T, Matsuda S, Shichijyo K, Sugimoto H, Hata K (2000) Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. Eur Respir J 16(5):861–865
80. Mihrshahi S, Peat JK, Webb K, Oddy W, Marks GB, Mellis CM (2004) Effect of omega-3 fatty acid concentrations in plasma on symptoms of asthma at 18 months of age. Pediatr Allergy Immunol 15(6):517–522
81. Peat JK, Mihrshahi S, Kemp AS, Marks GB, Tovey ER, Webb K et al (2004) Three-year outcomes of dietary fatty acid modification and house dust mite reduction in the Childhood Asthma Prevention Study. J Allergy Clin Immunol 114(4):807–813
82. Marks GB, Mihrshahi S, Kemp AS, Tovey ER, Webb K, Almqvist C et al (2006) Prevention of asthma during the first 5 years of life: a randomized controlled trial. J Allergy Clin Immunol 118(1):53–61
83. Koch C, Dolle S, Metzger M, Rasche C, Jungclas H, Ruhl R et al (2008) Docosahexaenoic acid (DHA) supplementation in atopic eczema: a randomized, double-blind, controlled trial. Br J Dermatol 158(4):786–792
84. Dunstan JA, Prescott SL (2005) Does fish oil supplementation in pregnancy reduce the risk of allergic disease in infants? Curr Opin Allergy Clin Immunol 5(3):215–221
85. Dunstan JA, Mori TA, Barden A, Beilin LJ, Holt PG, Calder PC et al (2004) Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition. Eur J Clin Nutr 58(3):429–437
86. Dunstan JA, Roper J, Mitoulas L, Hartmann PE, Simmer K, Prescott SL (2004) The effect of supplementation with fish oil during pregnancy on breast milk immunoglobulin A, soluble CD14, cytokine levels and fatty acid composition. Clin Exp Allergy 34(8):1237–1242
87. Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG et al (2003) Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. Clin Exp Allergy 33(4):442–448
88. Denburg JA, Hatfield HM, Cyr MM, Hayes L, Holt PG, Sehmi R et al (2005) Fish oil supplementation in pregnancy modifies neonatal progenitors at birth in infants at risk of atopy. Pediatr Res 57(2):276–281
89. Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG et al (2003) Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. J Allergy Clin Immunol 112(6):1178–1184
90. Anandan C, Nurmatov U, Sheikh A (2009) Omega 3 and 6 oils for primary prevention of allergic disease: systematic review and meta-analysis. Allergy 64(6):840–848
91. Olsen SF, Osterdal ML, Salvig JD, Mortensen LM, Rytter D, Secher NJ et al (2008) Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. Am J Clin Nutr 88(1):167–175
92. Calder PC (2004) n-3 fatty acids, inflammation, and immunity – relevance to postsurgical and critically ill patients. Lipids 39(12):1147–1161

93. Tiesset H, Pierre M, Desseyn JL, Guery B, Beermann C, Galabert C et al (2009) Dietary (n-3) polyunsaturated fatty acids affect the kinetics of pro- and antiinflammatory responses in mice with *Pseudomonas aeruginosa* lung infection. J Nutr 139(1):82–89
94. Mayer K, Gokorsch S, Fegbeutel C, Hattar K, Rosseau S, Walmrath D et al (2003) Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. Am J Respir Crit Care Med 167(10):1321–1328
95. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H (2002) Immunomodulation by perioperative administration of n-3 fatty acids. Br J Nutr 87(Suppl 1):S89–S94
96. Heller AR, Rossler S, Litz RJ, Stehr SN, Heller SC, Koch R et al (2006) Omega-3 fatty acids improve the diagnosis-related clinical outcome. Crit Care Med 34(4):972–979
97. Pontes-Arruda A, Aragao AM, Albuquerque JD (2006) Effects of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in mechanically ventilated patients with severe sepsis and septic shock. Crit Care Med 34(9):2325–2333
98. Pastor N, Soler B, Mitmesser SH, Ferguson P, Lifschitz C (2006) Infants fed docosahexaenoic acid- and arachidonic acid-supplemented formula have decreased incidence of bronchiolitis/bronchitis the first year of life. Clin Pediatr (Phila) 45(9):850–855
99. Lopez-Alarcon M, Bernabe-Garcia M, Del Prado M, Rivera D, Ruiz G, Maldonado J et al (2006) Docosahexaenoic acid administered in the acute phase protects the nutritional status of septic neonates. Nutrition 22(7–8):731–737
100. Thienprasert A, Samuhaseneetoo S, Popplestone K, West AL, Miles EA, Calder PC (2009) Fish oil n-3 polyunsaturated fatty acids selectively affect plasma cytokines and decrease illness in Thai schoolchildren: a randomized, double-blind, placebo-controlled intervention trial. J Pediatr 154(3):391–395
101. Stamp LK, James MJ, Cleland LG (2005) Diet and rheumatoid arthritis: a review of the literature. Semin Arthritis Rheum 35(2):77–94
102. Goldberg RJ, Katz J (2007) A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. Pain 129(1–2):210–223
103. Miller ER III, Juraschek SP, Appel LJ, Madala M, Anderson CA, Bleys J et al (2009) The effect of n-3 long-chain polyunsaturated fatty acid supplementation on urine protein excretion and kidney function: meta-analysis of clinical trials. Am J Clin Nutr 89(6):1937–1945
104. Belluzzi A, Brignola C, Campieri M, Pera A, Boschi S, Miglioli M (1996) Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. N Engl J Med 334(24):1557–1560
105. Feagan BG, Sandborn WJ, Mittmann U, Bar-Meir S, D'Haens G, Bradette M et al (2008) Omega-3 free fatty acids for the maintenance of remission in Crohn disease: the EPIC Randomized Controlled Trials. JAMA 299(14):1690–1697
106. Turner D, Zlotkin SH, Shah PS, Griffiths AM (2009) Omega 3 fatty acids (fish oil) for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev (1):CD006320
107. Calder PC (2008) Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. Mol Nutr Food Res 52(8):885–897
108. De Ley M, de Vos R, Hommes DW, Stokkers P (2007) Fish oil for induction of remission in ulcerative colitis. Cochrane Database Syst Rev (4):CD005986
109. Prescott SL, Dunstan JA (2005) Immune dysregulation in allergic respiratory disease: the role of T regulatory cells. Pulm Pharmacol Ther 18(3):217–228
110. Yessoufou A, Ple A, Moutairou K, Hichami A, Khan NA (2009) DHA reduces suppressive and migratory functions of Treg cells. J Lipid Res 50(12):2377–2388

10 Immunomodulation by Fish Oil Derived Polyunsaturated Fatty Acids in Cancer

Axel R. Heller and Martina Wendel

Key Points

- Omega-3 fatty acids (ω-3 FA) have shown their efficacy in the treatment of chronic and acute diseases due to their pleiotropic effects on cell signaling pathways linked to inflammation, angiogenesis, and cell cycle progression.
- In a variety of human cancer cell lines derived from colonic, pancreatic, prostate, and breast cancer, omega-3 fatty acids attenuated growth and induced apoptosis. Recent findings likewise indicate that ω3-FA act synergistically with chemotherapeutic agents and may also be used to enhance tumor radiosensitivity.
- This chapter sheds light on all relevant known pathway systems today taking also in account recent epidemiologic studies on the nutritional role of ω-3 FA in the prevention of cancer development.

Key Words: Apoptosis, cancer, cyclooxygenase, docosahexaenoic acid, eicosanoids, eicosapentaenoic acid, lipid peroxidation, omega-3 fatty acids.

10.1 INTRODUCTION

Worldwide, cancer is newly diagnosed in about 11 million people per year and causes nearly nine million deaths (1). In developed countries, cancer contributes to one fourth of all deaths and is the second leading cause of mortality behind cardiovascular diseases.

In the last decades, the role of fatty acids in the context of cancer development and progression has thoroughly been investigated. It is clear from epidemiological studies that a high intake of saturated fat and/or animal fat increases the risk of colon and breast cancer (2, 3). On the other hand, certain polyunsaturated fatty acids, namely omega-3

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_10

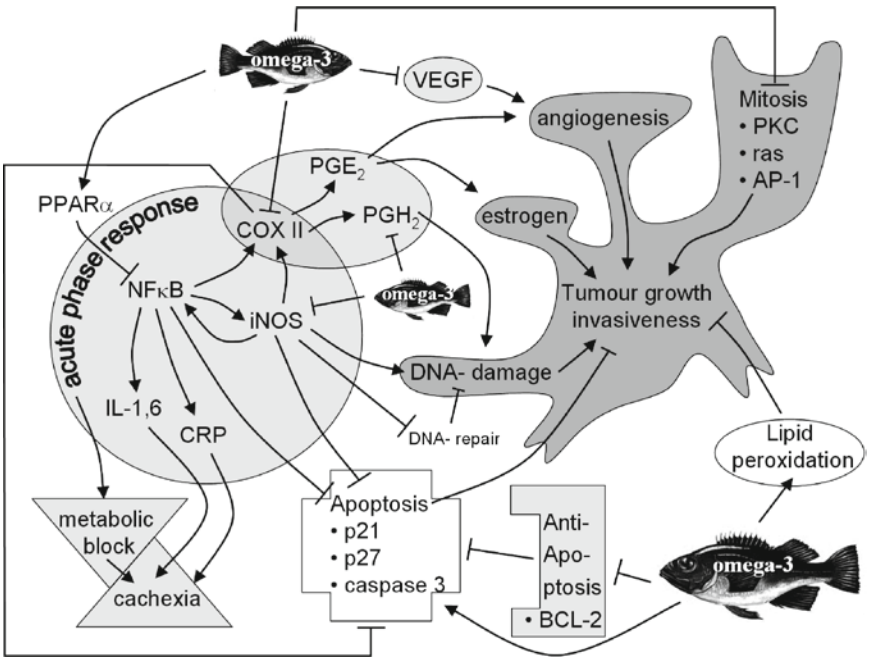


Fig. 10.1. Mechanisms of ω-3 FA action in tumor growth. Cyclooxygenase (COX) II and inducible NO synthase (iNOS) are key propagators of tumor development and inhibitors of tumoricidal activity. →favors activity; ⊥ blocks activity.

fatty acids (ω-3 FA), contained in fish oil were shown to inhibit the growth of a variety of human cancer cell lines *in vitro* (4). Diets rich in ω-3 FA were claimed to reduce the risk of several types of cancer, including prostate, colorectal, and breast cancer (5). A large number of *in vitro* experiments showing profound antitumor effects of ω-3 FA by suppressing neoplastic transformation, angiogenesis, and tumor cell growth (Fig. 10.1) supported this concept.

Early studies (6–8) demonstrated the potential to alter the fatty acid profile of tumor cells and to increase their content of ω-3 FA either by supplementation of growth medium or by feeding animals a diet enriched in ω-3 FA. This increase in ω-3 FA content also markedly affected tumor cell sensitivity toward cytotoxic drugs as is outlined below in more detail. Furthermore, the ω-3 FA eicosapentaenoic acid (EPA) decreased the uptake of saturated fatty acids, monounsaturated and ω-6 polyunsaturated fatty acids in MCF-7 breast cancer cells (9) and dietary ω-3 FA decreased the amount of linoleic acid uptake, the precursor of arachidonic acid, in hepatoma *in vivo* (10).

In contrast to ω-3 FA, the role of ω-6 FA seems to be double-edged as via different mechanisms they have the potential to both attenuate and promote tumor cell growth.

10.2 BIOLOGY OF UNSATURATED FATTY ACIDS: ω-3 AND ω-6 FA

Unsaturated fatty acids are divided into mono- and polyunsaturated fatty acids. From the first double bond of the polyunsaturated fatty acid (PUFA), regarded from the methyl end, the FA are further divided into ω-3, ω-6, and ω-9 FA. Oleic acid (C18:1) is

a monounsaturated FA, which can be synthesized by mammals, whereas ω-3 and ω-6 FA are essential for humans. From the ω-6 FA linoleic acid (C18:2n-6), arachidonic acid can be synthesized. In the case of ω-3 FA, the human organism is only capable of synthesizing small amounts of EPA from α-linoleic acid (C18:3n-3) that is present in vegetable oil, especially in linseed oil.

Omega-6 FA, such as linoleic acid (C18:2) and AA (C20:2) are found in plant oils and fatty tissues of mammals, which represent the major part of FA in the diet of industrialized societies. Long chain ω-3 FAs like EPA and docosahexaenoic acid (DHA) are produced by algae and plankton and are mainly found in maritime sources. EPA (C20:5) and DHA (C22:6) can be found in a concentration of 0.1–1.2% in deep-sea fish and therefore are the main ω-3 FA nutritional reservoir for humans. Diets enriched in ω-3 FA result in rapid incorporation of ω-3 FA into cell membranes (11), and thereby change the ratio of ω-3 FA to ω-6 FA.

Cellular lipid membranes represent a dynamic high turnover barrier system, which separate the intracellular from the extracellular space. They are about half lipids and half proteins by mass. In recent years, cellular lipid bilayers have been characterized to contain distinct lipid membrane microdomains, and consecutively, the so-called lipid-raft hypothesis has emerged. These lipid rafts were identified as detergent-resistant membrane (DRM) fractions isolated from cells and tissues and a large number of receptors and signaling proteins were found in association with this lipid fraction (12). These plasma membrane microdomains result from distinct biophysical properties of lipid bilayers depending on the incorporation of cholesterol and the kind of fatty acids present. This also affects the fluidity of the lipid bilayer, i.e. characterizes the degree of disorder and the rate of reorientation.

Studies in model membrane systems demonstrated that packing of cholesterol with saturated and monounsaturated phospholipids is entropically more favorable and leads to a tighter packing with reduced cross-sectional area per lipid compared to polyunsaturated phospholipids (13). Lipid rafts are characterized by a high concentration of cholesterol and sphingolipids, such as sphingomyelin and glycolipids, and their polar lipids, contain mainly saturated fatty acyl residues (14). Sphingolipids and glycosylphosphatidylinositol (GPI)-anchored proteins are attached exclusively to the outer leaflet of rafts (15), while transmembrane and intracellular proteins are targeted to membrane domains by acylation with fatty acyl moieties (16) like myristoylation and palmitoylation (17) whose high packing order facilitates their preferential interaction with membrane subdomains (18) and explains the enrichment of acylated proteins in lipid rafts under physiologic conditions.

Omega-3 FA incorporated into cell membranes alter the structural and functional state of lipid rafts due to their high degree of unsaturation. They are predominantly esterified into the sn-2 position of phosphatidylcholine and phosphatidyethanolamine phospholipids (19) and increase membrane fluidity (20) as the lipid order induced by the interaction of cholesterol with phosphatidylcholine and phosphatidyethanolamine is significantly reduced when these are esterified with polyunsaturated fatty acids in the sn-2 position compared to oleic acid (21). Incorporation of ω-3 FA into the cell membrane led to the displacement of cholesterol from the lipid raft fraction in endothelial cells (22). However, there may be cell-type specific differences, as cholesterol content of lipid rafts was unaffected by ω-3 FA in Jurkat T-cells (23).

Omega-3 FA also increase the transmembrane movement of lipids ("flip flop") with DHA, due to its high degree of polyunsaturation, being most effective (24). By these mechanisms, ω-3 FA affect the function and distribution of lipid raft-associated proteins both in the inner and outer leaflet of the cell membrane.

10.3 ω-3 AND ω-6 FA PUFAs ARE COMPETITIVE SOURCES FOR LIPID MEDIATOR GENERATION

10.3.1 Thromboxane, Prostaglandins, and Leukotrienes

Polyunsaturated fatty acids are incorporated into the lipid bilayer and serve as substrates for phospholipases, cyclooxygenases (COX), and lipoxygenases (LO) (Fig. 10.2). Upon dietary enrichment, the ω-3 FA EPA competes with ω-6 FA AA as a substrate for these enzymes, thereby affecting the profile of lipid mediators generated during inflammatory reactions (for review see (25)). The EPA-derived metabolites have lower biological activity compared to the analogous AA-derivatives. While AA is metabolized by COX to diene prostanoids (prostaglandins and thromboxane), by LOX to 4-series leukotrienes (tetraenoic leukotrienes) and hydroxyeicosatetraenoic acids (HETE), EPA is converted to triene-prostanoids by COX. Compared to the AA-derived TXA_2, the EPA-derived COX-product of the 3-series TXA_3 has considerably reduced pro-aggregatory and vasoconstrictive properties, while PGI_3 possesses similar anti-aggregatory and vasodilatative effects as PGI_2. Moreover, EPA is a preferred substrate

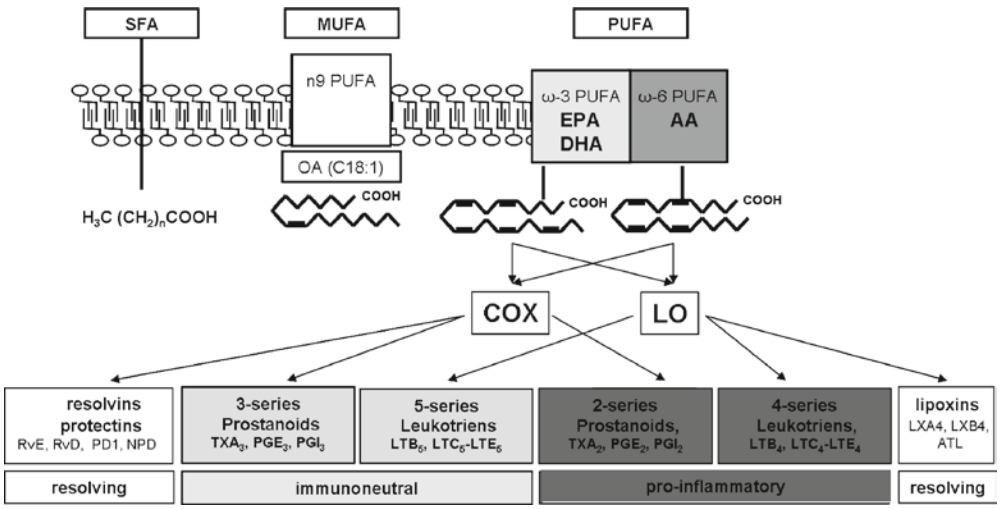


Fig. 10.2. Eicosanoids synthesis pathways. Depending on the fatty acid content of cellular membranes, lipid mediators with different pro-inflammatory potency or pro-resolving properties are generated from omega-3 or omega-6 polyunsaturated fatty acids via the cyclooxygenase or lipoxygenase pathway. Pro-inflammatory arachidonic acid (AA) -derived 5-series leukotrienes, 2-series prostanoids, and thromboxane A_2 and eicosapentaenoic acid derived 3-series prostanoids and 5-series leukotrienes with largely reduced inflammatory properties. Proresolving lipoxins are derived from AA while resolvins and protectins are generated from EPA.

of 5-LOX. EPA-derived 5-series leukotrienes have partially antagonistic biological effects compared to the respective AA-derivatives.

More recently, novel lipid mediators derived from polyunsaturated fatty acids with pro-resolving activities on inflammatory processes have been identified in exudates from resolving inflammation and comprise the lipoxins, resolvins, and protectins (for review see 26). Their synthesis is favored by the transcriptional upregulation of neutrophil 15-LO by PGE_2 andPGD_2, the so-called eicosanoid switch (27). They are highly stereospecific and act in the pico- to nanomolar range (28, 29). They affect PMN recruitment and trafficking, expression of pro-inflammatory genes, reduce leukocyte-mediated tissue injury, and take part in chemokine removal. Whether they also play a role in cancer development is currently unknown.

10.4 MODULATION OF THE INNATE IMMUNE RESPONSE BY ω-3 FATTY ACIDS

Recent work of Lee and coworkers demonstrated that the activation of general pro-inflammatory pathways, such as NF-κB and COX II expression, by saturated fatty acids and the inhibition of this induction by polyunsaturated fatty acids are mediated through a common signaling pathway derived from toll-like receptor (TLR)-4 (30). TLR-4 conveys signals as a part of innate immunity from the endotoxin receptor (CD14) on the surface of macrophages to the inner cell and may, likewise, be activated by saturated fatty acids. In recent years, a number of studies demonstrated attenuation of LPS-induced NF-κB activation and subsequent transcription of pro-inflammatory genes by ω-3 FA and several contributing mechanisms were identified.

First, ω-3 FA can interfere at the level of the initiation of the TLR-dependent signaling (Fig. 10.3). Lipid A, antigenic constituent of the walls of gram-negative bacterial species is the major determinant of LPS avidity to TLRs. Depending on the kind of fatty acid acylated to the disaccharide, this markedly affects the agonistic properties of Lipid A on TLR4. While Lipid A is maximally stimulatory when acylated with saturated fatty acids, it acts in fact as an antagonist at TLR4 when it is deacylated or acylated with unsaturated fatty acids (31). Further studies then identified free fatty acids as direct ligands for TLRs with saturated fatty acids acting as agonists. In contrast, unsaturated fatty acids did not induce NF-κB activation and DHA was the most potent inhibitor of LPS-induced NF-κB activation, (30). This inhibition was also demonstrated when a constitutively active TLR4 was expressed but not with constitutively active MyD88 or NF-κB inducing kinase (NIK) (30), suggesting that the inhibitory effect occurs upstream from MyD88. In addition, DHA blocked TLR4-induced PI3kinase/Akt activation that is also located upstream of MyD88 (32). All together, these findings imply that DHA acts at the level of TLR4 or associated signaling molecules like CD14 and MD2 that are recruited to the signaling complex upon LPS binding (33) but does not involve alteration of TLR homo- or heterodimerization (34). In this context, Chu and coworkers demonstrated reduced binding of fluorescently labeled LPS to THP-1 monocytes upon treatment with EPA and DHA that was accompanied by reduced expression of CD14 (35).

Chronic inflammation may be a risk factor for cancer development and the activation of NF-κB and upregulation of COX-2 could be contributing factors.

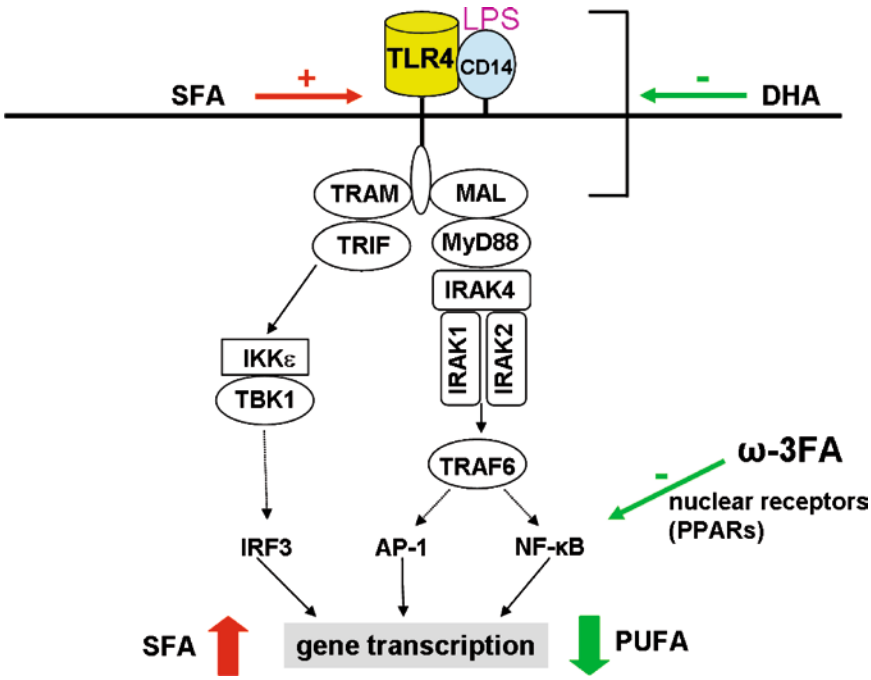


Fig. 10.3. Stimulatory effect of saturated fatty acids (SFA) and inhibitory effect of polyunsaturated fatty acids (PUFA) on TLR-4-dependent signal transduction and consecutive gene transcription. *DHA* docosahexaenoic acid, *TLR* toll-like receptor, *TIR* Toll/interleukin-1 receptor, *TRIF* TIR domain containing adaptor protein inducing IFN-β, *ISRE* interferon stimulatory response element, *TRAM* TRIF-related adapter molecule, *IRF* interferon regulatory factor, *IKK* IκB kinase, *TRAF* tumor necrosis factor receptor-associated factor, *TBK* TANK-binding kinase, *MAL* MyD88 adapter like, *IRAK* interleukin 1 receptor-associated kinase.

10.5 EFFECTS OF OMEGA-3 FATTY ACIDS ON IMMUNE CELL FUNCTIONS

A number of studies demonstrated immunosuppressive effects of ω-3 FA. This is associated with modulation of T-and B-cell activation and alteration of antigen presentation by MHC class I and II proteins that seem – at least in part – to be related to alterations of cell membrane composition and redistribution of signaling proteins. Key signaling proteins involved in T-cell activation such as the transmembrane linker for activation of T-cells (LAT) and src family protein-tyrosine kinases are highly concentrated in lipid rafts due to post-translational palmitoylation (36, 37). There is evidence from several studies that DHA directly modulates DRM that are considered representative for lipid rafts, and inhibits T-cell signaling. A recent *in vitro* study investigated the effects of DHA on IL-2 receptor-dependent signaling in Jurkat T-cells (38). Binding of IL-2 to the IL-2R leads to phosphorylation of Janus kinase that consecutively activates signal transducer and activators of transcription (STAT) proteins. DHA incorporation into Jurkat T-cells significantly altered the fatty acyl composition of DRM fractions that was accompanied by reduced cell surface expression of the IL-2R as well as displacement to detergent soluble membrane (DSM) fractions. Also, STAT5a and STAT5b proteins were redistributed from DRM to DSM fractions. Two studies in TCR-transgenic

mice investigated the effects of fish oil feeding on antigen-induced T-cell responses. Zhang and coworkers observed suppression of the proliferation of antigen-specific $CD4^+$ T-cells with a consecutive reduction of the total number of $CD4^+$ T-cells. However, a reduction in cell surface IL-2R was not observed (39). Similar results were obtained with splenocytes from TCR-transgenic mice fed a fish oil diet. Splenocytes isolated from these mice produced significantly less IL-2 upon antigen challenge compared with splenocytes from mice fed a control diet (40). A study by Anderson and Fritsche did not observe inhibition of antigen-induced $CD4^+$ T-cell expansion by ω-3 FA after adoptive transfer of TCR-transgenic T-cells but rather an increase in proliferation by omega-6 fatty acids (41). However, this study markedly differed in the mode and time scale of antigen-challenge and this might account for the differing findings.

Both, EPA and DHA seem capable of suppressing lymphocyte proliferation (42) and IL-2 production (43), thereby reducing clonal expansion of antigen-specific T-cells. But only EPA impaired natural killer (NK) cell activity (42). Results on the effects of ω-3 FA on T-cell apoptosis are controversial. Studies in naïve Th1 cells, murine $CD4^+$ T-cells and T-cell lines suggest that fish oil enhances activation-induced apoptosis (44). However, this was not observed in antigen-activated T-cells from TCR-transgenic mice (39). Several studies also demonstrated modulation of antigen-presenting cell (APC) functions by PUFAs. However, the underlying mechanisms are still incompletely understood. One hypothesis is that PUFAs alter the conformation of MHC class I, and thereby enhance antigen presentation. This view is supported by the observations that DHA incorporation into mouse EL4 lymphoma cells simultaneously altered the expression of two MHC I epitopes that might relate to a conformational change due to alterations in lipid bilayer organization (45).

In contrast to these limited data on MHC class I, there is clear evidence that PUFAs affect MHC class II expression. Most studies demonstrated decreased basal expression of MHC class II and inhibition of LPS-induced MHC class II upregulation by APC (46). In unstimulated monocytes, EPA and DHA exerted antagonistic regulatory effects as EPA inhibited and DHA increased HLA-DR expression. In IFN-γ-stimulated monocytes, however, both EPA and DHA alone or in combination decreased HLA-DR expression (46, 47). Few studies showed discrepant findings and either observed no effect of PUFAs on MHC class II expression (48) or an increase (49). This might relate to differences in cell types studied, oxidation status, and activation stimulus used.

Besides these mechanistic studies in animal models and cell cultures, there are a number of studies in humans that investigated the effects of dietary fish oil or purified DHA or EPA on immune cell functions. In healthy humans, Thies and coworkers observed decreased lymphocyte proliferation and NK cell activity by fish oil but not by DHA alone (50). Work by Kelley et al. using a higher dosage of DHA (6 g/d compared to 3.8 g/d) observed decreased NK activity and lower concentrations of TNF-α and IL-1β while lymphocyte proliferation, IL-2 production, antibody generation and delayed-type hypersensitivity were unaffected (51). After major abdominal surgery, omega-3 FA led to simultaneous increases in cytolytic T-lymphocytes, ($CD56^+3^+$), antigen presenting T-lymphocytes ($CD3^+HLADR^+$) and B-lymphocytes. Weiss and colleagues showed preservation of HLA-DR-positive cells (52) in postoperative patients receiving fish oil as opposed to soy bean oil while in the ω-6 FA group HLA-DR-positive cells significantly dropped.

10.6 EFFECTS OF OMEGA-3 FA ON THE COX-PATHWAY, TUMOR GROWTH, AND ANGIOGENESIS

COX-2 has been implicated to play an important role in tumorigenesis and tumor angiogenesis. Several studies showed enhanced COX-2 expression and PGE_2 production in gastric (53), colonic (54), lung (55), cervical (56), and pancreatic cancer (57), and in some studies high COX-2 expression was associated with poor prognosis (55, 56). While no consistent overexpression of COX-2 is seen in prostate cancer, Shappell and coworkers reported that enhanced immunostaining correlated with higher tumor grade (58). In addition, COX-2 was not only expressed in tumor cells, but also in tumor supplying vessels (59). Also, animal experimental studies suggest an important role for COX-2 in mediating tumor growth and angiogenesis (59). In this context, Liu and coworkers showed that overexpression of COX-2 alone led to mammary tumors in transgenic mice (60).

Extensive work studied the role of the COX/PGE_2 pathway in different cancer cell lines and identified putative mechanisms involved. Repasky and coworkers showed that Ras-mediated intestinal epithelial transformation required COX-2-induced PGE_2 signaling (61). In colon and prostate cancer cells, PGE_2 induced HIF-1α stabilization and nuclear translocation (62). Subsequently, upregulation of vascular endothelial growth factor (VEGF) expression was observed (62). In this context, the PGE_2-induced VEGF secretion in prostate cancer cells was shown to involve an EP2 receptor-mediated increase in cAMP (63). VEGF is supposed to be closely associated with neovascularization in developing tumors and increased levels of VEGF were observed both in preneoplastic lesions as well as in established colon cancer (64). Furthermore, PGE_2 affected survival and apoptosis pathways in cancer cells. It induced anti-apoptotic proteins Bcl-2 and Bcl-XL while levels of pro-apoptotic Bax were reduced (65), and promoted cell survival through the PI3kinase/Akt pathway (66). Also, synergism of PGE_2 with epidermal growth factor receptor-dependent signaling mediating growth and migration of colon tumor cells was reported (67).

10.7 MODULATION OF THE COX/PGE_2-PATHWAY BY POLYUNSATURATED FATTY ACIDS

Animal experimental studies demonstrated a strong inhibitory effect of diets containing ω-3 FA on tumor growth and angiogenesis of chemically induced tumors (68) as well as mammary and colonic cancer cells transplanted into mice (69, 70). Also, in mice transplanted with human prostate cancer cells, dietary increase in ω-3 FA impaired tumor cell proliferation, increased apoptosis, and reduced final tumor mass (71). These alterations were accompanied by decreased COX-2 protein and mRNA levels as well as reduced PGE_2 and VEGF levels. Both, EPA and DHA attenuated tumor cell growth and reduced the generation of PGE_2 and VEGF by cancer cells with EPA being more effective than DHA *in vitro* but equally effective *in vivo* (62). While a clear inhibiting effect of EPA on PGE_2 generation was observed by Dommels and coworkers in Caco-2 colonic cancer cells, they showed that administration of a stable PGE_2 analog did not reverse the EPA-induced growth inhibition (72). Furthermore, in their study also AA similar to EPA reduced tumor cell proliferation despite the competitive effect of EPA

on AA-induced PGE_2 generation. This is in line with the observations by Boudreau and coworkers who showed that ω-3 FA inhibited growth of the colon cancer cell line HCT-116 that does not express COX (73). Taken together, these observations strongly suggest that the growth inhibiting effect of ω-3 FA on individual cancer cells involves pathways independent from COX/PGE_2 while the inhibition of COX/PGE_2/VEGF pathway seems to be of major importance for the reduced formation of tumor microvessels and may also play a role in metastasis induction. In this context, Denkins and coworkers showed that AA increased PGE_2 production and invasiveness of melanoma cells while DHA and EPA had the opposite effects and also lowered expression of COX-2 (74). In line with that, targeting COX-2 is an emerging concept in anticancer therapy.

10.8 COX-INDEPENDENT PATHWAYS OF TUMOR GROWTH ATTENUATION BY ω-3 FA

10.8.1 Lipid Peroxidation

There is good evidence that enhanced lipid peroxidation may play an important role in tumor cell growth inhibition by polyunsaturated fatty acids. Both AA and EPA induced the formation of malondialdehyde that could be partially reversed by the COX-inhibitor indomethacin (72). In animal experimental studies, incorporation of ω-3 FA into the mitochondrial membrane coincided with the enhancement of colonocyte apoptosis (75, 76) and enhanced generation of reactive oxygen species was shown in response to feeding a fish oil diet and was accompanied by enhanced crypt cell apoptosis (77). The apoptosis-inducing potency of EPA and DHA can be further enhanced by conjugation of double bonds by alkaline treatment. Conjugated EPA and conjugated DHA induce strong lipid peroxidation and consecutive apoptosis as shown in DLD-1 colorectal adenocarcinoma cells either *in vitro* or after transplantation into nude mice (78).

In colon cancer cell lines, DHA incorporated into inner mitochondrial membrane phospholipids enhanced oxidative stress and induced pro-apoptotic signaling (75). Enhanced oxidative stress seems to be causally related to colonocyte apoptosis as the apoptosis-inducing action of DHA could partially be reverted by treatment with lipophilic antioxidants that target the inner mitochondrial membrane (76). Recently, Kolar and coworkers identified mitochondrial calcium accumulation to be involved in apoptosis induction of DHA-primed colonocytes exposed to butyrate (79).

10.8.2 p53 Activation

Apoptosis caused by lipid peroxidation has been related to the activity of p53 (80). In DLD-1 cells, conjugated EPA via increased lipid peroxidation activated mutant p53 and led to the induction of p53-dependent genes. This was followed by activation of both the mitochondrial as well as the receptor-mediated apoptosis inducing pathways (81). In prostate cancer cells that were treated with low dose DHA in combination with the COX-2 inhibitor celecoxib, Hsp70 and p53 activation were observed and accounted for enhanced tumor cell death (82). On the other hand, apoptosis induced by synergistic actions of DHA and butyrate through lipid peroxidation and mitochondrial calcium accumulation occurred independent from p53 (83).

10.8.3 DNA Polymerases and Topoisomerases

Very recently, ω-3 FA and their conjugated forms were shown to be strong inhibitors of DNA polymerases and topoisomerases that are critical to many cellular processes such as DNA replication, repair, and recombination (84). Among all, conjugated EPA had the strongest effect on the induction of apoptosis and inhibited both cell proliferation and cell cycle progression (84).

10.8.4 Impact of Pro-Survival Pathways Akt and NF-κB

The serine/threonine kinase Akt directly promotes cell survival and protects cells from apoptotic death by phosphorylating, and thereby inactivating components of the cell death machinery like caspase-9 and Bad. In addition, Akt by activating pro-survival transcription factors like NF-κB can also indirectly enhance cell survival as NF-κB is known to induce the transcription of pro-survival and anti-apoptotic genes (85) among them c-myc, cyclin-D1, VEGF, u-PA, and MMP-9 (86). In breast cancer cells, increased Akt activity has been reported to promote tamoxifen resistance (87). However, the treatment of Akt-transfected MCF-7 human mammary carcinoma cells with EPA resulted in decreased Akt-1 activity, whereas linoleic acid increased it (88). The EPA-induced decrease in Akt-1 activity coincided with decreased Akt-protein kinase activity and led to enhanced susceptibility toward tamoxifen. These observations are in line with previous reports of inhibition of protein kinase activity by ω-3 FA (89). Also, in the human breast cancer cell line MDA-MB-231, combined treatment with DHA and EPA resulted in the reduction of Akt-phosphorylation and nuclear NF-κB-DNA binding (90).

NF-κB has been found to be constitutively active (90) or to be induced by chemotherapy in different human tumor cell lines, and inhibition of NF-κB activation increased their radio- and chemosensitivity (91). It is now emerging that ω3-FA exert their inhibitory effects on inflammatory gene expression, at least in part, through direct actions on the intracellular signaling pathways which lead to activation of one or more transcription factors such as NF-κB. Several studies demonstrated that ω3-FA can down-regulate the activity of NF-κB. In cultured pancreatic cells, EPA prevented TNF-α-induced degradation of the inhibitory subunit of NF-κB (IκB) (92), and in human monocytes, EPA or fish oil decreased endotoxin-induced activation of NF-κB that was associated with decreased IκB phosphorylation (93). These observations suggest direct effects of long chain ω-3 FA on inflammatory gene expression via inhibition of NF-κB activation that may also contribute to their antitumor effects. Figure 10.4 summarizes the differential effects of ω-6 (AA) and ω-3 (EPA) fatty acids on these pathways and their effects on tumor cell survival, migration, and angiogenesis.

10.9 ROLE OF PPARs IN TUMORIGENESIS: IMPACT OF ω-3 FA

Polyunsaturated fatty acids are natural ligands of peroxisome proliferator activated receptors (PPARs) which seem to be involved in events associated with inflammation, tumor initiation and progression. However, the roles of the different PPAR subtypes – also depending on the model studied – seem to be ambiguous. PPARs belong to the family of nuclear hormone receptors (NR) that comprises up to now 42 known receptors. PPARs are expressed in a variety of tissues and different cell types. They heterodimerize with retinoid X receptors (RXRs), and then bind to PPAR-responsive

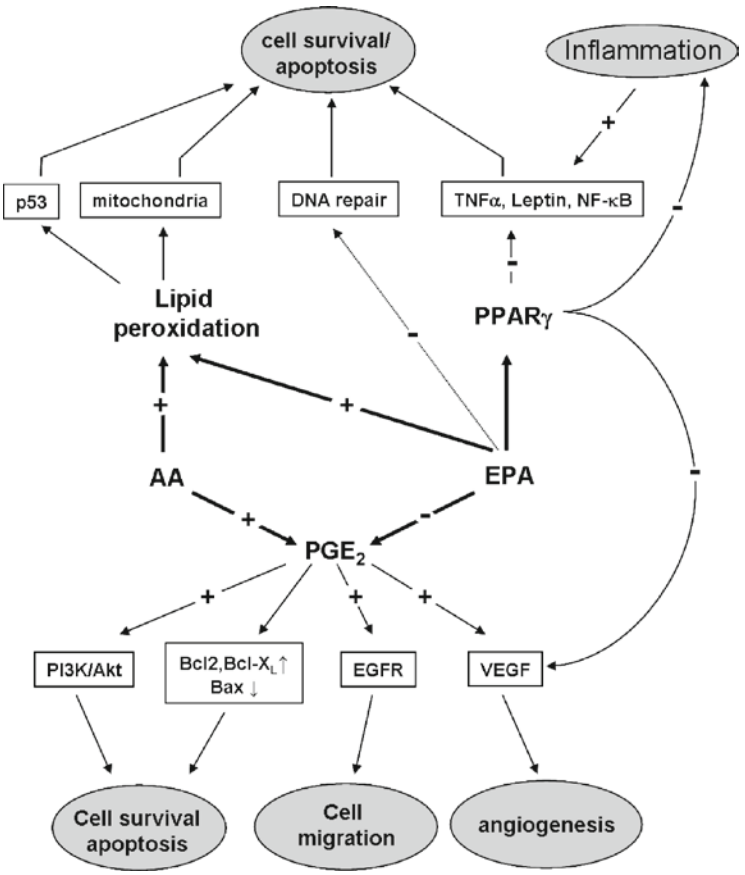


Fig. 10.4. Synergistic and antagonistic effects of omega-3 and omega-6 fatty acids on tumor cell survival, proliferation and tumor angiogenesis. Omega-3 fatty acids inhibit tumor cell growth via modulation of multiple signaling cascades that include reduced PGE_2 synthesis, impairment of DNA repair, and inhibition of inflammatory cascades. They act synergistically with omega-6 fatty acids by increasing lipid peroxidation that promotes tumor cell death but are antagonistic to them with respect to PGE_2-dependent tumor promoting signaling.

elements in target gene promoters. This usually leads to transcriptional activation. In the context of pro-inflammatory signaling, PPAR ligands were demonstrated to inhibit gene expression of pro-inflammatory mediators like tumor necrosis factor-α (TNFα), interleukin-1β, interleukin-6, inducible nitric oxide synthase (iNOS), COX-2, and matrix metalloproteinase-9 (MMP-9). This is accommodated by inhibiting various transcription factors like NF-κB by a process called "transrepression", and the pathways involved in this regulatory process are now emerging. In the case of LPS-induced activation of iNOS the PAPRγ ligand, rosiglitazone, recruited PPARγ to the NCoR-HDAC3-TBL1-TBLR1-repressor complex of the iNOS promoter and prevented its ubiquitination by SUMOylation (94), thereby keeping the iNOS gene in the repressed state.

In the context of cellular differentiation and proliferation, PPARδ and PPARγ were suggested to be regulated by the Wnt/β-catenin pathway that plays a central role in intestinal epithelial tissue renewal. However, concerning the roles of PPARs in the development and progression of intestinal cancer, available data are conflicting.

Wnt ligands act through a receptor complex consisting of a seven-transmembrane receptor, the Frizzled receptor and Lrp5/6, a member of the LDL receptor family. Wnt-initiated signaling targets the "destruction complex" consisting of two scaffolding proteins, tumor suppressor adenomatosis coli (APC) and axin that bind β-catenin and two kinases, CKI and GSK3β. In the absence of Wnt signaling, newly synthesized β-catenin is phosphorylated by the kinases and assigned to ubiquitination and proteasomal degradation. Upon Wnt activation, however, the kinase activity of the APC complex is inhibited and non-phosphorylated β-catenin accumulates and translocates into the nucleus, where it binds to lymphoid enhancer factor/T cell factor (LEF/TCF). While in the absence of Wnt signaling LEF/TCF acts as a transcriptional suppressor, binding of β-catenin converts it into an activator and the transcription of TCF target genes, among them c-myc, cyclin D1, PPARδ, and PPARγ, is induced.

Patients suffering from familial adenomatous polyposis were shown to carry one defective APC allele and the loss of APC frequently occurs in sporadic colorectal cancers leading to constitutive activation of the β-catenin/LEF/TCF pathway.

10.9.1 PPARδ

In line with these observations, PPARδ levels were higher in colorectal tumors than in normal mucosal tissue and were shown to increase in response to APC inactivation or azoxymethane treatment (95, 96). Furthermore, transplantation of PPARδ-null-HCT116 cells into nude mice resulted in the reduced tumor formation (97) and treating $Apc^{Min/+}$ mice with the specific PPARδ ligand GW501516 increased the number and size of intestinal adenomas (98). Similarly, Stephen and coworkers demonstrated that PPARδ activation stimulated proliferation of human breast and prostate cancer cell lines, but interestingly not colon cancer cells (99). These observations suggested a tumor-promoting effect of PPARδ activation. To further clarify the involvement of PPARδ on intestinal carcinogenesis, intercrossed mice were generated: $Apc^{Min/+}$ $PPARδ^{-/-}$ and $Mlh1^{-/-}PPARδ^{-/-}$ mice. $Mlh1^{-/-}$ mice carry a null mutation in the mismatch repair (MMR) gene Mlh1 (100) that leads to the development of multiple tumors in these mice (101). In humans, germline mutations of the Mlh1 gene are involved in nonpolyposis colorectal cancer (102). Interestingly, PPARδ deletion led to different effects depending on the genetic background: while the number of colon tumors increased in $Apc^{Min/+}$ $PPARδ^{-/-}$ (103), it was unaffected in $Mlh1^{-/-}PPARδ^{-/-}$ (100). Furthermore, PPARδ levels were lowered in $Apc^{Min/+}$ mice despite the increased levels of β-catenin. These observations suggest that PPARδ is not regulated by β-catenin (103) and that the involvement of PPARδ in tumorigenesis might be more complex as there seems to be a significant difference between lack of PPARδ activation and cellular PPARδ deficiency.

10.9.2 PPARγ

In normal colonic mucosa, PPARγ is differentially expressed along the crypt/villus axis with the highest levels of expression in postmitotic cells facing the intestinal lumen (104).

In cultured cancer cells, PPARγ has mainly been associated with the inhibition of tumor growth although there are also reports on tumor enhancement (105). PPARγ

ligands induced growth arrest and cellular differentiation of colon cancer cells (106). This growth inhibition is accomplished through reduced S-phase entry by the inhibition of E2F/DP DNA binding (107) and phosphorylation of the retinoblastoma gene (108). A further study demonstrated that PPARγ induced the cyclin-dependent kinase inhibitors p18 and p21, and decreased cyclin D1 expression (109). While these mechanisms were initially characterized in adipocyte differentiation, they were also observed in human lung carcinoma cell lines (110). Furthermore, PPARγ activation inhibited the proliferation of several other human cancer cell lines derived from liposarcoma, breast adenocarcinoma, prostate cancer, colorectal carcinoma, pancreatic carcinoma, bladder cancer, and gastric carcinoma (reviewed in (111)). While two studies in tissue samples from colorectal and thyroid carcinomas reported mutations of PPARγ that lead to impaired function (112), there is no evidence to support the hypothesis that carcinogenesis in general is due to impaired activity of PPARγ as a large study that analyzed clinical cancer samples originating from different tissues did not observe PPARγ gene mutations (113).

In addition to its modulating effect on cell cycle progression, PPARγ seems to attenuate tumor angiogenesis by both direct and indirect effects (for review see (114)). PPARγ activation reduces endothelial cell proliferation, differentiation, and migration. The endogenous PPARγ agonist 15d-PGJ2 reduced mRNA levels of VEGF receptors Flt1 and Flt 2. In addition to the modulation of VEGF receptor expression, several studies demonstrated that PPARγ activation also reduced the release of VEGF and basic fibroblast growth factor from tumor cells (115–117). In addition, impaired proteolysis due to reduced levels of urokinase plasminogen activator and increased plasminogen activator inhibitor type-1 (118) was observed. Also, the levels of leptin and TNFα, well known angiogenesis-inducing factors, were diminished (119, 120). Interestingly, the concentrations of thiazolidinediones usually reached under antidiabetic therapy (121) are sufficient to impair endothelial cell proliferation. In this context, the observation of a tendency toward lower colon cancer incidence in diabetic patients treated with thiazolidinediones deserves further attention.

Polyunsaturated fatty acids are natural ligands of PPARs as shown by the inhibition of vascular calcification by ω-3 FA via a PPARγ-p38 MAPK pathway (122). While native ω-3 FA are needed in relatively high doses to exert a biological effect and are not selective for PPAR subtypes, derivatives of fatty acids can also activate PPARs and convey PPAR-subtype specificity as shown in *in vitro* assays (123). In the context of inflammation, Sethi and colleagues showed that oxidized EPA and DHA were more potent than native fatty acids in reducing RNA levels of leukocyte adhesion receptors and adhesion of leukocytes to endothelial cells *in vitro* (124).

Taken together, a number of studies demonstrate antitumor effects of PPARγ activation at the level of cell cycle regulation and by the impairment of tumor angiogenesis, while studies aiming to define the role of PPARδ show conflicting results.

10.10 SYNERGISM OF ω-3 FA WITH ANTINEOPLASTIC RADIO- AND CHEMOTHERAPY

Omega-3 fatty acids were shown to enhance the antitumor effects of both radio- and chemotherapy (for review see (125)). The increased sensitivity of different tumor cell lines toward radiation therapy was mainly attributed to the increased lipid peroxidation

and was reversed by the addition of the antioxidant α-tocopherol. This was shown in rat astrocytoma cells (126), N-methylnitrosourea-induced mammary tumors (127) as well as in colon carcinoma cell lines (128). In contrast to these observations, feeding athymic mice transplanted with MD-MBA-231 mammary carcinoma cells, a diet supplemented with fish oil did not enhance the radiation response despite reductions in tumor growth rate, cell proliferation, and tumor blood vessel density (129).

Omega-3 FA enhance the sensitivity of tumor cells toward chemotherapeutic agents and can convey chemosensitivity to previously unresponsive tumor cells. The mechanisms by which ω-3 FA exert their sensitizing effects not only include enhanced lipid peroxidation and oxidative stress (130), but also involve alteration of transport proteins (131), inhibition of COX-2 (132), Akt (88), and protein kinase C (133). Furthermore, growth inhibition by cell cycle arrest was demonstrated in Caco-2 cells simultaneously treated with 5-fluorouracil and DHA (134) as well as in mammary carcinoma exposed to genistein and EPA (135). In the case of doxorubicin and vincristine, the synergistic effects of DHA with these chemotherapeutics were also due to the increased cellular drug uptake as shown in a variety of tumor cells like mouse leukemia (136), human lung carcinoma and glioblastoma (137), and human neuroblastoma (138). Mechanistically, one study showed that the increased uptake of doxorubicin by lymphoma cells was caused by altered membrane permeability (139).

10.11 EPIDEMIOLOGIC STUDIES ON CANCER PREVENTION BY NUTRITIONAL ω-3 FA

Despite the broad antitumor actions of ω-3 FA reported in *in vitro* experiments and animal studies, epidemiologic data are not as clear. A meta-analysis over 38 prospective cancer incidence studies observed only a trend toward the reduced cancer risk for colorectal cancer with increased intake of fish (5). Better evidence is provided by the European prospective investigation into cancer and nutrition study (140) which demonstrated a significant reduction of risk for colorectal cancer due to fish consumption. This study was also included in a recent meta-analysis (141) showing that each extra consumption of fish per week corresponded to a 4% lower risk for colorectal cancer, while the size of effect was clearly dependent on the contrast between the highest and lowest dose of fish consumption. So, the quantitative distribution of ω-3 FA intake in the study cohort seems to have an important influence on the ability to measure statistically significant effects. A similar effect was seen in the National Institutes of Health-AARP diet and health study cohort in which high intake of fat was associated with an increased risk of breast cancer in postmenopausal women (142).

The differential roles of ω-3 and ω-6 FA in breast and colonic carcinogenesis are of interest. Surely, there is need for more studies addressing this issue with adequate tools. However, one should keep in mind that both ω-3 and ω-6 FA confer cytotoxic effects on tumor cells by lipid peroxidation and oxidative stress. This may explain why intake of ω-6 FA compared to saturated fatty acids can reduce cancer risk. On the other hand, ω-3 and ω-6 FA markedly differ in their effects on cell proliferation and tumor vessel formation that are promoted by PGE2, and generation of PGE2 is dependent on COX-2 expression. So, in tumor cells lacking COX-2 expression or with low expression of the enzyme, ω-6 FA will rather be cytotoxic, and thereby prevent

cancer development, while in tumor cells expressing high levels of COX-2, ω-6 FA will enhance tumor cell proliferation and also tumor vessel formation. In the case of breast cancer, a clear role for COX-2 as an oncogene has been established (for review see (143)) and altering the ratio of ω-3 to ω-6 FA toward ω3-FA may be especially beneficial. Whether the differential expression of COX-2 in epithelial cells of breast cancer versus the stromal component of intestinal adenomas may also play a role has to be determined.

10.12 EFFECT OF NUTRITIONAL Ω-3 FA SUPPLEMENTATION ON CANCER CACHEXIA AND DISEASE PROGRESSION

Next to invasive or noninvasive tumor growth, weight loss and cancer cachexia are hallmarks of advanced cancer and major causes of morbidity and mortality. While it is possible to increase energy and protein intake via the enteral or parenteral route, this seems to have little impact on patients' progressive weight loss and life span prognosis (144). This has led to the suggestion of a partial metabolic block to the accretion of lean tissue in patients with cancer (145), which has been attributed to pro-inflammatory cytokines, alterations in the balance of neuroendocrine hormones, and specific tumor-derived proteolytic and lipid-mobilizing factors. In recent years, several clinical studies have provided evidence for beneficial effects of fish oil administration in cancer cachexia and during radio- and chemotherapy. It has been suggested that EPA is capable of downregulating the production and action of a number of mediators of cachexia, such as IL-1, IL-6, TNFα, and proteolysis-inducing factor (146, 147). On the other hand, soybean oil emulsions seem to impede tumoricidal activity as compared to EPA (148).

However, while more than 50 studies were published in this field, a recent Cochrane analysis (144) revealed only five studies that met the quality criteria to be included. Taken together, these studies did not demonstrate a beneficial effect of EPA on cancer cachexia, survival time, and quality of life. While the study by Gogos and coworkers reported improved survival of cancer patients who received EPA (149), this finding was limited by incomplete reporting and unconfirmed survival data. In a post-hoc analysis of their study on pancreatic cancer patients who received a protein supplement with or without EPA, Fearon and coworkers showed a dose-dependent effect on weight gain in patients who received the protein supplement with EPA that was not observed in patients receiving the protein supplement without EPA (150). Despite the limitations of a post-hoc analysis, this observation points to a possible beneficial effect of EPA that may be masked by insufficient intake of EPA.

10.13 CONCLUSIONS AND PERSPECTIVES

In the past decade, overlapping signaling pathways regulating both inflammation and cancer development via interconnected signal transduction pathways have been identified. Interestingly, the ω-3 FA EPA and DHA, well-known modulators of the immune response during inflammatory processes, are also potent inhibitors of tumor growth. By targeting different cell signaling pathways, ω-3 FAs can inhibit cell cycle progression of individual tumor cells and also attenuate the formation of tumor supplying vessels, thereby limiting the growth of established cancers.

However, the translation of the known antitumor effects of ω-3 FA into clinical protocols to prevent cancer development has to consider further cofactors as cancer development is a multifactorial process. In the case of colorectal cancer, intake of ω-3 FA should be combined with a diet rich in fibers and reduced intake of red and processed meat to maximize the preventive effects of nutritional means on cancer development.

For the future, well-designed epidemiologic studies on the cancer preventive effects of ω-3 FA are needed that should also include measurements of appropriate biomarkers to allow for sensitive determination and adjustment of individual ω-3 FA intake.

REFERENCES

1. Uniting to defeat cancer. International Union against cancer, Annual report 2007
2. Caygill CP, Charlett A, Hill MJ (1996) Fat, fish, fish oil and cancer. Br J Cancer 74(1):159–164
3. Hardman WE (2002) Omega-3 fatty acids to augment cancer therapy. J Nutr 132(11 Suppl): 3508S–3512S
4. Fearon KCH (2002) The anticancer and anticachectic effects of n-3 fatty acids. Clin Nutr 21(Suppl 2):69–73
5. MacLean CH, Newberry SJ, Mojica WA, Khanna P, Issa AM, Suttorp MJ, Lim Y-W, Traina SB, Hilton L, Garland R, Morton SC (2006) Effects of omega-3 fatty acids on cancer risk: a systematic review. JAMA 295(4):403–415
6. Hyman BT, Spector AA (1981) Accumulation of N-3 polyunsaturated fatty acids cultured human Y79 retinoblastoma cells. J Neurochem 37(1):60–69
7. Spector AA, Steinberg D (1967) Turnover and utilization of esterified fatty acids in Ehrlich ascites tumor cells. J Biol Chem 242(13):3057–3062
8. Burns CP, Spector AA (1987) Membrane fatty acid modification in tumor cells: a potential therapeutic adjunct. Lipids 22(3):178–184
9. Sauer LA, Dauchy RT, Blask DE, Krause JA, Davidson LK, Dauchy EM (2005) Eicosapentaenoic acid suppresses cell proliferation in MCF-7 human breast cancer xenografts in nude rats via a pertussis toxin-sensitive signal transduction pathway. J Nutr 135(9):2124–2129
10. Sauer LA, Dauchy RT, Blask DE (2001) Polyunsaturated fatty acids, melatonin, and cancer prevention. Biochem Pharmacol 61(12):1455–1462
11. Breil I, Koch T, Heller A, Schlotzer E, Grunert A, van Ackern K et al (1996) Alteration of n-3 fatty acid composition in lung tissue after short-term infusion of fish oil emulsion attenuates inflammatory vascular reaction. Crit Care Med 24(11):1893–1902
12. Wu C, Butz S, Ying Y, Anderson RGW (1997) Tyrosine kinase receptors concentrated in caveolae-like domains from neuronal plasma membrane. J Biol Chem 272:3554–3559
13. Sengupta P, Baird B, Holowka D. Lipid rafts, fluid/fluid phase separation, and their relevance to plasma membrane structure and function. Semin Cell Dev Biol 2007; doi:10.1016/semcdb2007.07.010
14. Hanada K, Nishijima M, Akamatsu Y, Pagano RE (1995) Both sphingolipids and cholesterol participate in the detergent insolubility of alkaline phosphatase, a glycosylphosphatidylinositol-anchored protein, in mammalian membranes. J Biol Chem 270:6254–6260
15. Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature 387:569–572
16. Melkonian KA, Ostermeyer AG, Chen JZ, Roth MG, Brown DA (1999) Role of lipid modifications in targeting proteins to detergent-resistant membrane rafts. J Biol Chem 274:3910–3917
17. Resh MD (1999) Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta 1451:1–16
18. Ahmed SN, Brown DA, London E (1997) On the origin of sphingolipid/cholesterol-rich detergent-insoluble cell membranes: physiological concentrations of cholesterol and sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes. Biochemistry 36:10944–10953
19. Robinson DR, Xu LL, Knoell CT, Tateno S, Olesiak W (1993) Modification of spleen phospholipids fatty acid composition by dietary fish oil and by n-3 fatty acid ethyl esters. J Lipid Res 34:1423–1434

20. Mitchell DC, Litman BJ (1998) Molecular order and dynamics in bilayers consisting of highly polyunsaturated phospholipids. Biophys J 74:879–891
21. Huster D, Arnold K, Gawrisch K (1998) Influence of docosahexaenoic acid and cholesterol on lateral lipid organization in phospholipids mixtures. Biochemistry 37:17299–17308
22. Hashimoto M, Hossain S, Tanabe Y, Shido O (2005) Effects of aging on the relation of adenyl purine release with plasma membrane fluidity of arterial endothelial cells. Prostaglandins Leukot Essent Fatty Acids 73:475–483
23. Stulnig TM, Huber J, Leitinger N, Imre EM, Angelisová P, Nowotny P, Waldhäusl W (2001) Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition. J Biol Chem 276:37335–37340
24. Armstrong VT, Brzustowicz MR, Wassall SR, Jenski LJ, Stillwell W (2003) Rapid flip-flop in polyunsaturated (docosahexaenoate) phospholipid membranes. Arch Biochem Biophys 414:74–82
25. Heller A, Koch T, Schmeck J (1998) van Ackern K:Lipid mediators in inflammatory disorders. Drugs 55(4):487–496
26. Serhan CN (2007) Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 25:101–137
27. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN (2001) Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol 2(7):612–619
28. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 192(8):1197–1204
29. Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA (2006) Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. J Immunol 176(3):1848–1859
30. Lee JY, Sohn KH, Rhee SH, Hwang D (2001) Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 276:16683–16689
31. Qureshi N, Takayama K, Kurtz R (1991) Diphosphoryl lipid A obtained from the nontoxic lipopolysaccharide of Rhodopseudomonas sphaeroides is an endotoxin antagonist in mice. Infect Immun 59:441–444
32. Lee JY, Ye J, Gao Z, Youn HS, Lee WH, WH ZL, Sizemore N, Hwang DH (2003) Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-Kinase/AKT by saturated and polyunsaturated fatty acids. J Biol Chem 278:37041–37051
33. Da Silva CJ, Soldau K, Christen U, Tobias PS, Ulevitch RJ (2001) Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. J Biol Chem 276:21129–21135
34. Lee JY, Zhao L, Youn HS, Weatherill AR, Tapping R, Feng L, Lee WH, Fitzgerald KA, Hwang DH (2004) Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. J Biol Chem 279:16971–16979
35. Chu AJ, Walton MA, Prasad JK, Seto A (1999) Blockade by polyunsaturated n-3 fatty acids of endotoxin-induced monocytic issue factor activation is mediated by the depressed receptor expression of THP-1 cells. Surg Res 87:217–224
36. Shenoy-Scaria AM, Dietzen DJ, Kwong J, Link DC, Lublin DM (1994) Cysteine3 of Src family protein tyrosine kinase determines palmitoylation and localization in caveolae. J Cell Biol 126:353–363
37. Zhang W, Trible RP, Samelson LE (1998) LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. Immunity 9:239–246
38. Li Q, Wang M, Tan L, Wang C, Ma J, Li N, Li Y, Xu G (2005) Li J (Docosahexaenic acid changes lipid composition and interleukin-2 receptor signaling in membrane rafts. J Lipid Res 46:1904–1913
39. Zhang J, Kim W, Zhou L, Wang N, Ly LH, McMurray DN, Chapkin RS (2006) Dietary fish oil inhibits antigen-specific muine Th1 cell development by suppression of clonal expansion. J Nutr 136:2391–2398
40. Pompos LJ, Fritsche KL (2002) Antigen-driven murine CD4+ T lymphocyte proliferation and interleukin-2 production are diminished by dietary (n) polyunsaturated fatty acids. J Nutr 132:3293–3300
41. Anderson MJ, Fritsche KL (2004) Dietary polyunsaturated fatty acids modulate in vivo, antigen-driven CD4+ T-cell proliferation in mice. J Nutr 134:1978–1983

42. Peterson LD, Jeffrey NM, Thies F, Sanderson P, Newsholme EA, Calder CA (1998) Eicosapentaenoic and docosahexaenoic acids alter rat spleen leukocyte fatty acid composition and prostaglandin E2 production but have different effects on lymphocyte functions and cell-mediated immunity. Lipids 33:171–180
43. Jolly CA, Jiang YH, Chapkin RS, McMurray DN (1997) Dietary (n-3) polyunsaturated fatty acids suppress murine lymphocyte proliferation, interleukin-2 secretion, and the formation of diacylglycerol and ceramide. J Nutr 127:37–43
44. Switzer KC, Fan YY, Wang N, McMurray DN, Chapkin RS (2004) Dietary n-3 polyunsaturated fatty acids promote activaito-induced cell death in Th1-polarized murine CD4+ T-cells. J Lipid Res 45:1482–1492
45. Pascale AW, Ehringer WD, Stillwell W, Sturdevant LK, Jenski LJ (1993) Omega-3 fatty acid modification of membrane structure and function. II. Alteration by docosahexaenoic acid of tumor cell sensitivity to immune cytolysis. Nutr Cancer 19:147–157
46. Hughes DA, Pinder AC (1997) N-3 Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes and inhibit antigen presentation in vitro. Clin Exp Immunol 110:516–523
47. Hughes DA, Pinder AC, Piper Z, Johnson IT, Lund EK (1996) Fish oil supplementation inhibits the expression of major histocompatibility complex class II molecules and adhesion molecules on human monocytes. Am J Clin Nutr 63:267–272
48. Zeyda M, Saemann MD, Stuhlmeier KM, Mascher DG, Nowotny PN, Zlabinger GJ, Waldhäusl W, Stulnig TM (2005) Polyunsaturated fatty acids block dendritic cell activation and function independently of NF-κB activation. J Biol Chem 280:14293–14301
49. Erickson BL, Howard AD, Chakrabarti R, Hubbard NE (1997) Alteration of platelet actvating factor-induced macrophage tumoricidal response, IA expression, and signal transduction by n-3 fatty acids. Adv Exp Med Biol 407:371–378
50. Thies F, Miles EA, Nebe-von-Caron G, Powell JR, Hurst TL, Newsholme EA, Calder PC (2001) Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. Lipids 36:1183–1193
51. Kelley DS, Taylor PC, Nelson GJ, Mackey BE (1998) Dietary docosahexaenoic acid and immnocompetence in young healthy men. Lipids 33:559–566
52. Weiss G, Meyer F, Matthies B, Pross M, Koenig W, Lippert H (2002) Immunomodulation by perioperative administration of n-3 fatty acids. Br J Nutr 87(Suppl 1):S89–S94
53. Lim HY, Joo HJ, Choi JH, Yi JW, Yang MS, Cho DY, Kim Nam DK, Lee KB, Him HC (2000) Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. Clin Cancer Res 6(2):519–525
54. Sheehan KM, Sheehan K, O'Donoghue DP, MacSweeeny F, Conroy RM, Fidzgerald DJ, Murray FE (1999) The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 282(13):1254–1257
55. Edelman MJ, Watson D, Wang X, Morrison C, Kratzke RA, Jewell S (2008) Hodgson, L, et al. Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy – Cancer and Leukemia Group B Trial 30203. J Clin Oncol 26(6):848–855
56. Ferrandina G, Lauriola L, Distefano MG, Zannoni GF, Gessi M, Legge F, Maggiano N, Mancuso S, Capelli A, Scambia G, Ranelletti FO (2002) Increased cyclooxygenase-2 expression is associated with chemotherapy resistance and poor survival in cervical cancer patients. J Clin Oncol 20(4):973–981
57. Kokawa A, Kondo H, Gotoda T, Ono H, Saito D, Nakadaira S, Kosuge T, Yoshida S (2001) Increased expression of cyclooxygenase-2 in human pancreatic neoplasms and potential for chemoprevention by cyclooxygenase inhibitors. Cancer (Phila) 91(2):333–338
58. Shappell SB, Manning S, Boeglin WE, Guan YF, Roberts RL, Davis L, Olson SJ, Jack GS, Coffey CS, Wheller TM (2001) Alterations in lipoxygenase and cyclooxygenase-2 catalytic activity and mRNA expression in prostate carcinoma. Neoplasia 3(4):287–303
59. Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ, Seibert K (2000) Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 60(5):1306–1311

60. Liu CH, Chang S-H, Narko K, Trifan OC, Wu M-T, Smith E, Haudenschild C, Lane TF, Hla T (2001) Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 276(21):18563–18569
61. Repasky GA, Zhou Y, Morita S, Der CJ (2007) Ras-mediated intestinal epithelial cell transformation requires cyclooxygenase-2-induced prostaglandin E2 signaling. Mol Carcinog 46(12):958–970
62. Calviello G, Di Nicuolo F, Gragnoli S, Piccioni E, Serini S, Maggiano N, Tringali G, Navarra P, Ranelletti FO, Palozza P (2004) n-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE2 induced ERK-1 and -2 and HIF-1 alpha induction pathway. Carcinogenesis 25(12):2303–2310
63. Wang X, Klein RD (2007) Prostaglandin E2 induces vascular endothelial growth factor secretion in prostate cancer cells through EP2 receptor-mediated cAMP pathway. Mol Carcinog 46(11):912–923
64. Takahashi Y, Kitaday Y, Bucana CD, Cleary KR, Ellis LM (1995) Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 55(18):3964–3968
65. Hong MY, Chapkin RS, Davidson LA, Turner ND, Morris JS, Carroll RJ, Lupton JR (2003) Fish oil enhances targeted apoptosis during colon tumor initiation in part by downregulating Bcl-2. Nutr Cancer 46(1):44–51
66. George RJ, Sturmoski MA, Anant S, Houchen CW (2007) EP4 mediates PGE2 dependent cell survival through the PI3 kinase/AKT pathway. Prostaglandins Other Lipid Mediat 83(1–2):112–120
67. Shao J, Evers BM, Sheng H (2004) Prostaglandin E2 synergistically enhances receptor tyrosine kinase-dependent signaling system in colon cancer cells. J Biol Chem 279(14):14287–14293
68. Reddy BS, Sugie S (1991) Effect of diets high in omega-3 and omega-6 fatty acids on initiation and postinitiation stages of colon carcinogenesis. Cancer Res 51(2):487–491
69. Mukutmoni-Norris M, Hubbard NE (2000) and Erickson, K.L. Modulation of murine mammary tumor vasculature by dietary n-3 fatty acids in fish oil. Cancer Lett 150(1):101–109
70. Anti M, Marra G, Armelao F, Bartoli GM, Ficarelli R, Percesepe A, De Vitis I, Maria G, Sofo L, Rapaccini GL et al (1992) Effect of omega-3 fatty acids on rectal mucosal cell proliferation in subjects at risk for colon cancer. Gastroenterology 103(3):883–891
71. Kobayashi N, Barnard RJ, Henning SM, Elashoff D, Reddy ST, Cohen P, Leung P et al (2006) Effect of altering dietary omega-6/omega-3 fatty acid ratios on prostate cancer membrane composition, cyclooxygenase-2, and prostaglandin E2. Clin Cancer Res 12(15):4662–4670
72. Dommels YE, Haring MMG, Keestra NGM, Alink GM, van Bladeren PJ, van Ommen B (2003) The role of cyclooxygenase in n-6 and n-3 polyunsaturated fatty acid mediated effects on cell proliferation, PGE(2) synthesis and cytotoxicity in human colorectal carcinoma cell lines. Carcinogenesis 24(3):385–392
73. Boudreau MD, Sohn KH, Rhee SH, Lee SW, Hunt JD, Hwang DH (2001) Suppression of tumor cell growth both in nude mice and in culture by n-3 polyunsaturated fatty acids: mediation through cyclooxygenase-independent pathways. Cancer Res 61(4):1386–1391
74. Denkins Y, Kempf D, Ferniz M, Nileshwar S, Marchetti D (2005) Role of omega-3 polyunsaturated fatty acids on cyclooxygenase-2 metabolism in brain-metastatic melanoma. J Lipid Res 46(6):1278–1284
75. Hong MY, Chapkin RS, Barhoumi R, Burghardt RC, Turner ND, Henderson CE, Sanders LM, Fan YY, Davidson LA, Murphy ME, Spinka CM, Carroll RJ, Lupton JR (2002) Fish oil increases mitochondrial phospholipid unsaturation, upregulating reactive oxygen species and apoptosis in rat colonocytes. Carcinogenesis 23(11):1919–1925
76. Malis CD, Weber PC, Leaf A, Bonventre JV (1990) Incorporation of marine lipids into mitochondrial membranes increases susceptibility to damage by calcium and reactive oxygen species: evidence for enhanced activation of phospholipase A2 in mitochondria enriched with n-3 fatty acids. Proc Natl Acad Sci USA 87(22):8845–8849
77. Sanders LM, Henderson CE, Hong MY, Barhoumi R, Burghardt RC, Wang N, Spinka CM, Carroll RJ, Turner ND, Chapkin RS, Lupton JR (2004) An increase in reactive oxygen species by dietary fish oil coupled with the attenuation of antioxidant defenses by dietary pectin enhances rat colonocyte apoptosis. J Nutr 134(12):3233–3238
78. Tsuzuki T, Igarashi M, Miyazawa T (2004) Conjugated eicosapentaenoic acid (EPA) inhibits transplanted tumor growth via membrane lipid peroxidation in nude mice. J Nutr 134(5):1162–1166

79. Kolar SSN, Barhoumi R, Lupton JR, Chapkin RS (2007) Docosahexaenoic acid and butyrate synergistically induce colonocyte apoptosis by enhancing mitochondrial Ca2+ accumulation. Cancer Res 67(11):5561–5568
80. Michalak E, Villunger A, Erlacher M, Strasser A (2005) Death squads enlisted by the tumour suppressor p53. Biochem Biophys Res Commun 331(3):786–798
81. Tsuzuki T, Kambe T, Shibata A, Kawakami Y, Nakagawa K, Miyazawa T (2007) Conjugated EPA activates mutant p53 via lipid peroxidation and induces p53-dependent apoptosis in DLD-1 colorectal adenocarcinoma human cells. Biochim Biophys Acta 1771(1):20–30
82. Narayanan NK, Narayanan BA, Bosland M, Condin M, Nargi D (2006) Docosahexaenoic acid in combination with celecoxib modulates HSP70 and p53 proteins in prostate cancer cells. Int J Cancer 119(7):1586–1598
83. Kolar S, Barhoumi R, Callaway ES, Fan YY, Wang N, Lupton JR, Chapkin RS (2007) Synergy between docosahexaenoic acid and butyrate elicits p53-independent apoptosis via mitochondrial Ca(2+) accumulation in colonocytes. Am J Physiol Gastrointest Liver Physiol 293(5):G935–G943
84. Yonezawa Y, Hada T, Uryu K, Tsuzuki T, Eitsuka T, Miyazawa T, Murakami-Nakai C, Yoshida H, Mizushina Y (2005) Inhibitory effect of conjugated eicosapentaenoic acid on mammalian DNA polymerase and topoisomerase activities and human cancer cell proliferation. Biochem Pharmacol 70(3):453–460
85. Nicholson KM, Anderson NG (2002) The protein kinase B/Akt signalling pathway in human malignancy. Cell Signal 14(5):381–395
86. Pahl HL (1999) Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18(49):6853–6866
87. Clark AS, West K, Streicher S, Denis PA (2002) Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. Mol Cancer Ther 1(9):707–717
88. DeGraffenried LA, Friedrichs WE, Fulcher L, Fernandez G, Silva JM, Peralba JM, Hidalgo M (2003) Eicosapentaenoic acid restores tamoxifen sensitivity in breast cancer cells with high Akt activity. Ann Oncol 14(7):1051–1056
89. Mirnikjoo B, Brown SE, Kim HF, Marangell LB, Sweatt JD, Weeber EJ (2001) Protein kinase inhibition by omega-3 fatty acids. J Biol Chem 276(14):10888–10896
90. Schley PD, Jijon HB, Robinson LE, Catherine J, Field CJ (2005) Mechanisms of omega-3 fatty acid-induced growth inhibition in MDA-MB-231 human breast cancer cells. Breast Cancer Res Treat 92(2):187–195
91. Cusack JC Jr, Liu R, Baldwin AS Jr (2000) Inducible chemoresistance to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothe cin (CPT-11) in colorectal cancer cells and a xenograft model is overcome by inhibition of nuclear factor-kappaB activation. Cancer Res 60(9):2323–2330
92. Ross JA, Maingay JP, Fearon JC, Sangster K, Powell JJ (2003) Eicosapentaenoic acid perturbs signalling via the NFkappaB transcriptional pathway in pancreatic tumour cells. Int J Oncol 23(6):1733–1738
93. Novak TE, Babcock TA, Jho DH, Helton WS, Espat NJ (2003) NF-kappa B inhibition by omega -3 fatty acids modulates LPS-stimulated macrophage TNF-alpha transcription. Am J Physiol Lung Cell Mol Physiol 284(1):L84–L89
94. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature 473(7059):759–763
95. He TC, Chan TA, Vogelstein B, Kinzler KW (1999) PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. Cell 99(3):335–345
96. Gupta RA, Tan J, Krause WF, Geraci MW, Wilson TM, Dey SK, DuBois RN (2000) Prostacyclin-mediated activation of peroxisome proliferator-activated receptor delta in colorectal cancer. Proc Natl Acad Sci 97(24):13275–13280
97. Park BH, Vogelstein B, Kinzler W (2001) Genetic disruption of PPARdelta decreases the tumorigenicity of human colon cancer cells. Proc Natl Acad Sci 98(5):2598–2603
98. Gupta RA, Wang D, Katkuri S, Wang H, Dey SK, DuBois RN (2004) Activation of nuclear hormone receptor peroxisome proliferator-activated receptor-delta accelerates intestinal adenoma growth. Nat Med 10(3):245–247

99. Stephen RL, Gustafsson MC, Jarvis M, Tatoud R, Marshall BR, Knight D, Ehrenborg E, Harris AL, Wolf CR, Palmer CN (2004) Activation of peroxisome proliferator-activated receptor delta stimulates the proliferation of human breast and prostate cancer cell lines. Cancer Res 64(9):3162–3170
100. Reed KR, Sansom OJ, Hayes AJ, Gescher AJ, Peters JM, Clarke AR (2006) PPARdelta status and mismatch repair mediated neoplasia in the mouse intestine. BMC Cancer 6:113
101. Prolla TA, Baker SM, Harris AC, Tsao LJ, Yao X, Brommer CE, Zheng B, Gordon M, Reneker J, Arnheim M, Shibata D, Bradley A, Liskay RM (1998) Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. Nat Genet 18(3):276–279
102. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368(6468):258–261
103. Reed KR, Sansom OJ, Hyes AJ, Gescher AJ, Wintin DJ, Peters JM, Clarke AR (2004) PPARdelta status and Apc-mediated tumourigenesis in the mouse intestine. Oncogene 23(55):8992–8996
104. Mansen A, Guardiola-Diaz H, Rafter J, Branting C, Gustafsson JA (1996) Expression of the peroxisome proliferator-activated receptor (PPAR) in the mouse colonic mucosa. Biochem Biophys Res Commun 222(3):844–851
105. Saez E, Tontonoz P, Nelson MC, Alvarez JG, Ming UT, Baird SM, Thomazy VA, Evans RM (1998) Activators of the nuclear receptor PPARgamma enhance colon polyp formation. Nat Med 4(9):1058–1061
106. Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB, Holden SA, Chen LB, Singer S, Fletcher C, Spiegelman BM (1998) Differentiation and reversal of malignant changes in colon cancer through PPARgamma. Nat Med 4(9):1046–1052
107. Altiok S, Xu M, Spiegelman BM (1997) PPARgamma induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. Genes Dev 11(15):1987–1998
108. Shao D, Lazar MA (1997) Peroxisome proliferator activated receptor gamma, CCAAT/enhancer-binding protein alpha, and cell cycle status regulate the commitment to adipocyte differentiation.J Biol Chem 272(34):21473–21478
109. Morrison RF, Farmer SR (1999) Role of PPARgamma in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18(INK4c) and p21(Waf1/Cip1), during adipogenesis. J Biol Chem 274(24):17088–17097
110. Han S, Sidell N, Fisher PB, Roman J (2004) Up-regulation of p21 gene expression by peroxisome proliferator-activated receptor gamma in human lung carcinoma cells. Clin Cancer Res 10(6):1911–1919
111. Debril MB, Renaud JP, Fajas L, Auwerx J (2001) The pleiotropic functions of peroxisome proliferator-activated receptor gamma. J Mol Med 79(1):30–47
112. Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA (2000) PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected]. Science 289(5483): 1357–1360
113. Ikezoe T, Miller CW, Kawano S, Heaney A, Williamson EA, Hisitake J, Green E, Hofmann W, Taguchi H, Koeffler HP (2001) Mutational analysis of the peroxisome proliferator-activated receptor gamma gene in human malignancies. Cancer Res 61(13):5307–5310
114. Margeli A, Kouraklis G, Theocharis S (2003) Peroxisome proliferator activated receptor-gamma (PPAR-gamma) ligands and angiogenesis. Angiogenesis 6(3):165–169
115. Panigrahy D, Singer S, Shen LQ, Butterfield CE, Chen EJ, Moses MA, Kilroy S, Duensing S, Fletcher C, Fletcher JA, Hlatky L, Hahnfeldt P, Folkman J, Kaipainen A (2002) PPARgamma ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis. J Clin Invest 110(7):923–932
116. Guan YF, Zhang YH, Breyer RM, Davis L, Breyer MD (1999) Expression of peroxisome proliferator-activated receptor gamma (PPARgamma) in human transitional bladder cancer and its role in inducing cell death. Neoplasia 1(4):330–339
117. Fauconnet S, Lascombe I, Chabannes E, Adessi GL, Desvergene B, Wahli W, Bittard H (2002) Differential regulation of vascular endothelial growth factor expression by peroxisome proliferator-activated receptors in bladder cancer cells. J Biol Chem 277(26):23534–23543
118. Xin X, Yang S, Kowalski J, Gerritsen ME (1999) Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis in vitro and in vivo. J Biol Chem 274(13):9116–9121

119. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR (1998) Biological action of leptin as an angiogenic factor. Science 281(5383):1683–1686
120. Goetze S, Bungenstock A, Czupalla C, Eilers F, Stawowy P, Kintscher U, Spencer-Hänsch C, Graf K, Nürnberg B, Law RE, Fleck E, Gräfe M (2002) Leptin induces endothelial cell migration through Akt, which is inhibited by PPARgamma-ligands. Hypertension 40(5):748–754
121. Freed MI, Allen A, Jorkasky DK, DiCicco RA (1999) Systemic exposure to rosiglitazone is unaltered by food. Eur J Clin Pharmacol 55(1):53–56
122. Abedin M, Lim J, Tang TB, Park D, Demer LL, Tintut Y (2006) N-3 fatty acids inhibit vascular calcification via the p38-mitogen-activated protein kinase and peroxisome proliferator-activated receptor-gamma pathways. Circ Res 98(6):727–729
123. Chinetti G, Fruchart JC, Sstaels B (2000) Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res 49(10):497–505
124. Sethi S, Eastman AY, Eaton JW (1996) Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. J Lab Clin Med 128(1):27–38
125. Pardini RS (2006) Nutritional intervention with omega-3 fatty acids enhances tumor response to antineoplastic agents. Chem Biol Interact 162(2):89–105
126. Vartak S, Robbins ME, Spector AA (1997) Polyunsaturated fatty acids increase the sensitivity of 36B10 rat astrocytoma cells to radiation-induced cell kill. Lipids 32(3):283–292
127. Colas S, Paon L, Denis F, Prat M, Louissot P, Hoinard C, LeFloch O, Ogilvie G, Bougnoux P (2004) Enhanced radiosensitivity of rat autochthonous mammary tumors by dietary docosahexaenoic acid. Int J Cancer 109(3):449–454
128. Benais-Pont G, Dupertuis YM, Kossovsky MP, Nouet P, Allal AS, Buchegger F, Pichard C (2006) Omega-3 polyunsaturated fatty acids and ionizing radiation: combined cytotoxicity on human colorectal adenocarcinoma cells. Nutrition 22(9):931–939
129. Hardman WE, Sun L, Short N, Cameron LL (2005) Dietary omega-3 fatty acids and ionizing irradiation on human breast cancer xenograft growth and angiogenesis. Cancer Cell Int 5(1):12
130. Plumb JA, Luo W, Kerr DJ (1993) Effect of polyunsaturated fatty acids on the drug sensitivity of human tumour cell lines resistant to either cisplatin or doxorubicin. Br J Cancer 67(4):728–733
131. Martin D, Meckling-Gill KA (1996) Omega-3 polyunsaturated fatty acids increase purine but not pyrimidine transport in L1210 leukaemia cells. Biochem J 315(Pt 1):329–333
132. Swamy MV, Cooma I, Patlolla JMR, Simi B, Reddy BS, Rao CV (2004) Modulation of cyclooxygenase-2 activities by the combined action of celecoxib and decosahexaenoic acid: novel strategies for colon cancer prevention and treatment. Mol Cancer Ther 3(2):215–221
133. Cha MC, Lin A, Meckling KA (2005) Low dose docosahexaenoic acid protects normal colonic epithelial cells from araC toxicity. BMC Pharmacol 5:7
134. Jordan A, Stein J (2003) Effect of an omega-3 fatty acid containing lipid emulsion alone and in combination with 5-fluorouracil (5-FU) on growth of the colon cancer cell line Caco-2. Eur J Nutr 42(6):324–331
135. Nakagawa H, Yamamoto D, Kiyozuka Y, Tsuta K, Uemura Y, Hioki K, Tsutsui Y, Tsubura A (2000) Effects of genistein and synergistic action in combination with eicosapentaenoic acid on the growth of breast cancer cell lines. J Cancer Res Clin Oncol 126(8):448–454
136. Burns CP, North JA (1986) Adriamycin transport and sensitivity in fatty acid-modified leukemia cells. Biochim Biophys Acta 888(1):10–17
137. Rudra PK, Krokan HE (2001) Cell-specific enhancement of doxorubicin toxicity in human tumour cells by docosahexaenoic acid. Anticancer Res 21(1A):29–38
138. Ikushima S, Fujiwara F, Todo S, Imashuku S (1991) Effects of polyunsaturated fatty acids on vincristine-resistance in human neuroblastoma cells.Ikushima S, Fujiwara F, Todo S, Imashuku S. Anticancer Res 11(3):1215–1220
139. Kinsella JE, Black JM (1993) Effects of polyunsaturated fatty acids on the efficacy of antineoplastic agents toward L5178Y lymphoma cells. Biochem Pharmacol 45(9):1881–1887
140. Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M et al (2005) Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst 97(12):906–916

141. Geelen A, Schouten JM, Kamphuis C, Stam BE, Burema J, Renkema JMS, Bakkers EJ (2007) van't Veer, P. Kampman, E. Fish consumption, n-3 fatty acids, and colorectal cancer: a meta-analysis of prospective cohort studies. Am J Epidemiol 166(10):1116–1125
142. Thiebaut AC, Kipnis V, Chang SC, Subar AF, Thompson FE, Rosenberg PS et al (2007) Dietary fat and postmenopausal invasive breast cancer in the National Institutes of Health-AARP Diet and Health Study cohort. J Natl Cancer Inst 99(6):451–462
143. Howe LR (2007) Inflammation and breast cancer. Cyclooxygenase/prostaglandin signaling and breast cancer. Breast Cancer Res 9(4):210
144. Dewey A, Baughan C, Dean T, Higgins B, Johnson I (2007) Eicosapentaenoic acid (EPA, an omega-3 fatty acid from fish oils) for the treatment of cancer cachexia. Cochrane Database Syst Rev 24(1):CD004597
145. Moldawer LL, Copeland EM III (1997) Proinflammatory cytokines, nutritional support, and the cachexia syndrome: interactions and therapeutic options. Cancer 79(9):1828–1839
146. Tisdale MJ (1996) Inhibition of lipolysis and muscle protein degradation by EPA in cancer cachexia. Nutrition 12(Suppl 1):S31–S33
147. Ross JA, Moses AG, Fearon KC (1999) The anti-catabolic effects of n-3 fatty acids. Curr Opin Clin Nutr Metab Care 2(3):219–226
148. Wallace FA, Neely SJ, Miles EA, Calder PC (2000) Dietary fats affect macrophage-mediated cytotoxicity towards tumour cells. Immunol Cell Biol 78(1):40–48
149. Gogos CA, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos NC, Kalfarentzos F (1998) Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy: a randomized control trial. Cancer 82(2):395–402
150. Fearon KC, von Meyendfeldt MF, Moses AGW, van Geenen R, Roy A, Gouma DJ, Giacosa A, Van Gossum A, Bauer J, Barber MD, Aaronson NK, Voss AC, Tisdale MJ (2003) Effect of a protein and energy dense N-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial. Gut 52(10):1479–1486

11 Brain Innate Immune System and Its Modulation by Diet: The Role of Polyunsaturated Fatty Acids

Sophie Layé and Anais Duffaud

Key Points

- The innate immune system of the brain is composed of microglial cells and astrocytes, which, once activated, produce proinflammatory cytokines.
- Proinflammatory cytokines act in the brain through specific receptors produced by brain cells and trigger behavioral modifications (sickness behavior) and cognitive and mood disorders.
- Aging-related neuroinflammatory processes are involved in neurodegenerative diseases.
- Polyunsaturated fatty acids (PUFAs) are essential nutrients provided by the diet that strongly regulate neuroinflammation and associated behavioral changes.

Key Words: Neuroinflammation, astrocyte, microglia, aging, omega 3, lipid, sickness behavior, mood and cognitive disorders, IL-1, NFκB.

11.1 INTRODUCTION

Inflammation is an active defense reaction against various insults which aims at neutralizing noxious agents. Although inflammation serves as a protective function in controlling infection and promoting tissue repair, it can also cause tissue damage. Inflammatory mediators include complement, adhesion molecules, products of cyclooxygenase enzymes, eicosanoids, and cytokines. Cytokines are polypeptides that are generally associated with inflammation, immune activation, and cell differentiation or death. They include interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), chemokines and growth factors. Although most of them have little or no function in healthy tissues, they are rapidly induced locally in response to tissue injury, infection,

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_11

or inflammation. Inflammatory mediators such as cytokines are not only expressed at the site of injury but also in distant organs, including the brain where they coordinate the central component of the acute phase reaction. This brain-mediated response involves, in particular, profound metabolic alterations in the form of an increased set-point for thermoregulation resulting in fever, and drastic behavioral changes commonly labeled as sickness behavior (anorexia, decreased locomotor activity; withdrawal from social contacts, etc). Brain expression of cytokines also plays a key role in the pathophysiology of immune (e.g. multiple sclerosis) and non-immune neurological disorders (e.g. brain injury, stroke, Alzheimer's disease). Study of the expression and action of proinflammatory cytokines in the brain is a rapidly growing area of experimental and clinical research. Because of the number of cytokines and the diversity of their actions, this chapter will primarily focus on the most studied cytokine in the brain: the interleukin-1 (IL-1).

In the brain, inflammatory mediators are mainly produced by endothelial cells and glial cells, including astrocytes, and microglia (1). The expression of proinflammatory cytokines in the brain is increased in response to various conditions, such as infection (bacteria, viruses,…), lesions, trauma, and oxidative stress. Central (neuroinflammation) and peripheral inflammatory responses although sustain by similar mediators, are different from a cellular and biochemical point of view.

The functional consequences of neuroinflammation include alterations in cognition, affect, and behavior, and they usually take place in the absence of neurotoxicity (2). The behavioral repertoire of humans and animals is well known to change dramatically during the course of an infection. Subjects have little motivation to eat, are listless, complain of fatigue and malaise, loose interest in social activities and have significant changes in sleep patterns. They feel sick and experience pain, display an inability to experience pleasure, and experience difficulties in attention, concentration, and memory (3). These alterations are responsible for the impaired quality of life and well-being. All these functional alterations can be reproduced in naïve individuals by peripheral or central injection of proinflammatory cytokines (4). When neuroinflammation is exacerbated or prolonged, it can lead to neuronal cell death and neurodegeneration as a consequence of the deprivation of neurons from their growth factors or the overproduction of reactive oxygen species (1, 5). As far as neurodegeneration is concerned, it is unclear if this condition is propagated through inflammation or whether, in contrast, the inflammatory response reflects an attempt to protect against further cellular injury.

There are multiple aspects of neuroinflammation, all occurring simultaneously. Following exposure to noxious stimuli, the components of neuroinflammation include activation of microglia release of cytokines, and induction of tissue repair enzymes, that all together limit cellular damage and promote repair. At the behavioral level, cytokine-induced sickness behavior is nothing other than the outward manifestation of a central motivational state that helps the body to fight infection and promote recovery (2). The extent of neuroinflammation is normally regulated by a variety of antagonist processes involving anti-inflammatory cytokines such as interleukin-10, growth factors in the form of, for instance, insulin-like growth factor 1 (IGF-1), hormones such as glucocorticoids, neuropeptides such as vasopressin, and alpha-melanotropin and endocannabinoid through their action on CB2 receptors (6–9).

Micronutrients in the diet, in the form of antioxidants and polyunsaturated fatty acids (PUFA) are also able to regulate neuroinflammation. PUFA are incorporated into cell membranes. The composition of cell membranes determines the type of inflammatory mediators that will be produced during the inflammatory response (111). It has been shown that human diet used to evolve on a ratio of n-6 to n-3 PUFA equal approximately to 1, whereas nowadays this ratio is closer to 10–20, indicating that Western diets are usually deficient in n-3 (10–12). The relative excess of n-6 fatty acids stimulates the formation of arachidonic acid (ARA), the fatty acid precursor of PGs, and other eicosanoids involved in inflammation, which accounts for their importance in chronic inflammatory disease. On the other hand, the eicosanoids derived from eicosapentaenoic acid (EPA) are less physiologically potent than mediators synthesized from ARA (13). Moreover, n-3 fatty acids, such as docosaexaenoic acid (DHA) and its derivatives, display anti-inflammatory effects and inhibit the production of proinflammatory cytokines independently of the production of eicosanoids. Since feeding animals or human subjects with diets enriched with DHA and EPA results in a decrease of the amount of ARA in glial cell membranes, there will be less substrate available for synthesis of eicosanoids from ARA (14–16). Because n-3 fatty acid are highly anti-inflammatory and are preferentially incorporated in the brain, inappropriate amounts of dietary n-6 and n-3 fatty acids could lead to neuroinflammation. Depending on which PUFA are present in the diet, neuroinflammation will therefore be kept at a minimum or exacerbated. The aim of the present chapter is to review the mechanisms of neuroinflammation, its functional consequences and its modulation by PUFA.

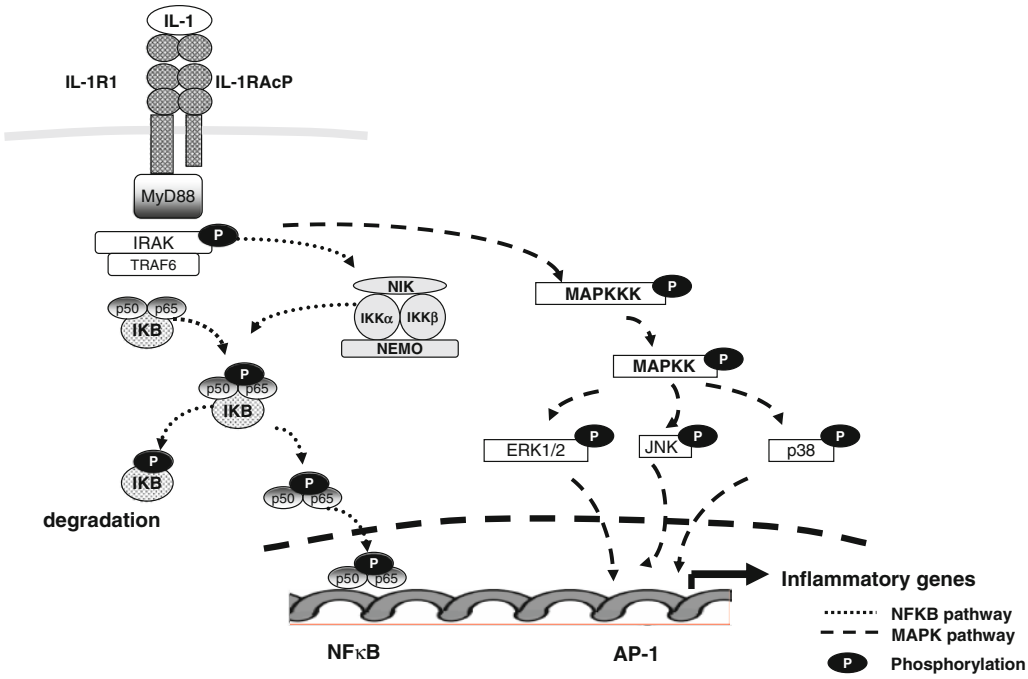
11.2 THE BRAIN INNATE IMMUNE SYSTEM

For a long time, the brain was considered to be a privileged organ from an immunological point of view, owing to its inability to mount an immune response and process antigens (17). Although this is partly true, the central nervous system (CNS) shows a well-organized innate immune reaction in response to systemic bacterial infection and cerebral injury. The hallmark of brain inflammation is the activation of glia, particularly microglia (18). Microglial cells are sensor cells in the CNS that respond to injury and brain disease (19). These cells are able to scavenge invading microorganisms and dead cells, and also to act as immune or immunoeffector cells (20). In physiologic conditions, the brain contains resting microglia, perivascular macrophages, and pericytes, as well as a few patrolling lymphocytes. The origin of pericytes, perivascular macrophages, and microglia found in the adult brain is probably represented by systemic monocytes that infiltrate the CNS during embryogenesis (19, 21). In pathological conditions, all these cells become activated and are strongly involved in the local inflammatory response (22). In particular, the resting microglia becomes activated and changes its phenotype to amoeboid microglia capable of phagocytosis. This is accompanied by a production of proinflammatory cytokines, in particular, IL-1, nitric oxide, superoxide anions, and eicosanoids (20, 23, 24).

Cell wall components of the Gram-negative or Gram-positive bacteria (lipopolysaccharide (LPS) and peptidoglycan, respectively) function as pathogen-associated molecular patterns (PAMPs) that are recognized by specific membrane receptors on innate immune cells (25). LPS and peptidoglycan bind to Toll-like receptors (TLR) (25).

While TLR2 recognizes the PAMPs produced by Gram-positive bacterial cell wall components, TLR4 is critical for the recognition of LPS. Flagellin, the principal element of bacterial flagella, is recognized by TLR5, and TLR9 is required for the inflammatory response triggered by bacterial DNA. TLR3 induces an innate immune response to double-stranded RNA (dsRNA) viruses. Microglia is the main cellular component of the innate immune system in the brain. The peripheral administration of LPS activates systemic innate immune cells, which results in the production and extracellular release of proinflammatory cytokines (26–29). Once present in the bloodstream, these cytokines are believed to mediate most of the effects of systemically injected LPS, although circulating levels of cytokines are not necessarily detectable prior to the occurrence of the early physiological responses that are induced by the endotoxin (30). The best example is fever that takes place within minutes in response to a systemic injection of LPS, even though cytokines are not yet detectable in the bloodstream (30). Because of this temporal constraint, LPS has been proposed to be a direct ligand in the brain. In accordance with this hypothesis, cytokine gene expression, in response to a peripheral LPS challenge, is first detected in the circumventricular organs (CVOs) that are devoid of a blood brain barrier (BBB), leptomeninges, and choroid plexus (ChP) (26, 29, 31). The demonstration that CD14 and TLR4 receptors are constitutively expressed in the CVOs and in parenchymal microglia reinforces this idea (18, 27, 32). Circulating LPS also causes a rapid increase in CD14 in these brain regions, and a delayed response takes place in cells located at the boundaries of the CVOs and in microglia across the brain parenchyma (33). A similar expression pattern was recently found for the gene that encodes TLR2 in the brains of mice after a single systemic injection of LPS. The signal was first detected in regions devoid of BBB and a second wave was detected in parenchymal microglial cells (33).

Interestingly, TLRs and IL-1 receptors share a cytoplasmic motif, the Toll/IL-1 receptor (TIR) domain, which is required for initiating intracellular signaling (34, 35). The TIR family of receptors uses very similar signaling mechanisms to activate downstream effector mechanisms (Fig. 11.1). While some components of the downstream signaling machinery like the adapter TNF receptor-associated factor 6 (TRAF6) are shared by other receptors of proinflammatory cytokines, one signaling module is exclusively employed by the TIR family. This consists of MyD88, interleukin-1 receptor-associated kinase (IRAK) family members, and Tollip. TRAF6 directly facilitates full activation of the Nuclear factor kappa B (NFκB) pathway and the mitogen-activated protein kinase (MAPK) signaling cascades. In unstimulated cells, the NFκB family proteins exist as heterodimers or homodimers that are sequestered in the cytoplasm by virtue of their association with a member of the Inhibitor κB (IκB) family of inhibitory proteins (Fig. 11.1). These interactions mask the nuclear localization sequence of NFκB and interfere with sequences important for DNA binding. The destruction of IκB unmasks the nuclear localization signal of NFκB, leading to its nuclear translocation and binding to the promoters of target genes. The detection of IκB induction reveals the extent and cellular location of brain-derived immune molecules in response to peripheral immune challenges. IκBα mRNA is induced in the brain after peripheral LPS injection, beginning in the cells lining the blood side of the BBB and progressing to the cells inside the brain parenchyma (36, 37). The same results were obtained after a peripheral injection of IL-1 and TNFα, but not IL-6. This spatiotemporal pattern indicates that under the effect of LPS, cells



AP-1: activating protein 1, ERK1/2: extracellular signal-regulated protein kinase ½,
IKB: inhibitor of KB, IKKα/β: IκB kinase α/β, IL-1: interleukin 1,
IL-1R: interleukin 1 receptor, IL-1RAcP: interleukin 1 receptor accessory protein,
IRAK: Interleukin-1, receptor associated kinase, JNK: cJun N-terminal kinase,
MAPK: mitogenassociated protein (MAP) kinase, MK: MAPK-activated protein kinase,
MyD88: Myeloid differentiation primary response gene (88), NEMO: NFκ Bessential modulator,
NFκB: nuclear factor kappa B, TRAF-6: TNF receptor associated factor 6.

Fig. 11.1. Signaling pathways activated by the IL-1 family and receptor complex. IL-1 family is composed of two agonists, IL-1α and IL-1β and a natural antagonist, IL-1ra. IL-1 agonists bind to the type 1 IL-1 receptor (IL-1R1) and then interact with IL-1 receptor accessory protein (IL-1RacP). They form a functional heterodimeric complex that activates downstream signaling pathways involving NFkappaB (NFκB) and the Mitogen-activated protein kinase (MAPK) family. This activation requires the formation of a complex between IRAK (IL-1 receptor associated kinase), MyD88, and Tollip. This complex activates TNF receptor-associated factor 6 (TRAF6) leading to the phosphorylation and degradation of the NFκB inhibitor, IκB, and the activation of the MAPK family.

of the BBB synthesize immune signal molecules to activate cells inside the CNS. The cerebrospinal fluid appears to be a conduit for these signal molecules. As LPS induces the expression of bioactive IL-1 in microglial cells, it is not clear whether the induction of IκB expression in the brain is because of a direct LPS effect on the brain cells or the LPS-induced IL-1 produced in the brain (37, 38). A recent study analyzed NFκB translocation and IκB expression in the brain and pituitary of rodents treated with IL-1 (39, 40). In this study, the expression of IκB mRNA did not strictly parallel NFκB nuclear translocation. This important finding indicates that peripheral IL-1 can reach the brain across the CVOs that lack a BBB and endothelial cells all over the brain and interact with its receptors to induce NFκB translocation.

IL-1 is bioactive in the brain because there are IL-1 receptors in the brain. Ligands of the IL-1 receptors (two agonists, IL-1α and IL-1β and the natural antagonist, IL-1ra)

bind to a transmembrane receptor and to soluble forms of the receptor, which are characterized by extracellular immunoglobulin (Ig)-like domains (41). The prototypes of this family are the IL-1R type 1 and an accessory protein that functions as a co-receptor molecule, the IL-1RAcP. The receptor chains contain the ligand-binding site, whereas the co-receptor IL-1RAcP is unable to bind the cytokine alone. Indeed, the deletion of IL-1R1 or IL-1RAcP, administration of antibodies to IL-1R1 or inhibition of specific MAP kinases, or NFκB abolish most actions of IL-1 *in vivo* and *in vitro*. The type 2 IL-1 receptor (IL-1R2) is a negative regulator of the IL-1 system and functions as a decoy receptor (42). Very recently, new members of the IL-1 family, named IL-1F5-10 were discovered (41). IL-1F6–F9 have proinflammatory properties, while IL-1F5 has anti-inflammatory properties. In addition, the orphan receptors IL-1R-related protein 2 (IL-1Rrp2), T1/ST2, three immunoglobulin domain-containing IL-1 receptor-related, IL-1 receptor accessory protein-like and single Ig IL-1 receptor-related molecule (SIGIRR), also called TIR8 were demonstrated to belong to the IL-1R family. In the rodent brain, IL-1R1 mRNA is diffusely spread with the highest level of binding in the granular layer of the dentate gyrus, the granule cell layer of the cerebellum, the hypothalamus and the pyramidal cell layer of the hippocampus (43–45). IL-1R1 is expressed in cells of the choroid plexus and endothelial cells of brain capillaries. Neuronal expression appears mostly in the hippocampus (46). IL-1RacP mRNA is highly expressed throughout the rat brain (47, 48). However, the presence of the IL-1RAcP in brain areas that lack type 1 IL-1 receptors indicates additional functions for this protein that are still obscure. Very recently, an isoform of the IL-1RAcP (termed AcPb) has been discovered to be exclusively expressed in the brain (49). In addition, IL-33, the IL-1-like ligand for ST2, is highly expressed in brain astrocyte (50). Interestingly, no NFκB activation is observed in the brain of IL-1R1 and IL-1RAcP knock-out mice treated with IL-1 (39). The same effect is observed in mixed glial cells *in vitro* indicating that IL-1R1 is essential for IL-1beta signaling in the brain (51). The MAPK p38, c-Jun N-terminal kinase (JNK), and the extracellular signal-regulated protein kinase (ERK1/2) are also activated in glial cells from wildtype mice, but not from IL-1R1 knock-out mice. The selective inhibition of p38 or ERK1/2 MAPKs significantly reduced IL-1β-induced IL-6 release. Whether this pathway is involved in IL-1 signaling in the brain is still unknown. This is very important since brain-produced IL-1 is a key regulator of the synthesis of other proinflammatory cytokines such as IL-6 and TNFα. Concerning the isoforms of IL-1 (IL-1F), IL-1F5 antagonizes the inflammatory effects of IL-1 and LPSin the brain (52).

11.3 CONSEQUENCE TO THE ACTIVATION OF BRAIN INNATE IMMUNE SYSTEM: FROM SICKNESS BEHAVIOR TO DEPRESSION

Proinflammatory cytokines act in the brain to induce non-specific symptoms of infection, including fever and profound psychological and behavioral changes termed "sickness behavior" (4). Sick individuals experience weakness, malaise, cognitive alterations and listlessness, hypersomnia, depressed activity, and loss of interest in social activities (2, 53). Although these symptoms are usually regarded as the result of the debilitation process that occurs during infection, they are actually part of a natural homeostatic reaction that the body uses to fight infection (53). These changes in behavior

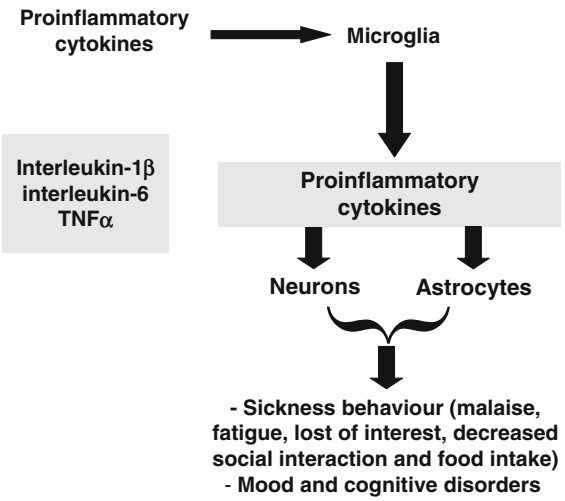


Fig. 11.2. Proinflammatory cytokines act in the brain to trigger sickness behavior and mood and cognitive disorders. Behavioral modifications are initiated by peripheral cytokines induced by infectious agents and relayed by centrally produced cytokines by microglial cells. In turn, brain cytokine acts on both neurons and glial cells and change the behavior.

have been shown to be the expression of a motivational state that resets the organism's priorities to promote resistance to pathogens and recovery from infection. By preventing the occurrence of those activities that are metabolically expensive (e.g. foraging), and favoring expression of those that decrease heat loss (e.g. rest) and increase heat production (e.g. shivering), sickness behavior positively contributes to recovery following infection (2). Sickness behavior is initiated by cytokines that are induced by infectious agents in the periphery and relayed by centrally produced cytokines (54, 55) (Fig. 11.2). Evidence of such a role of centrally produced cytokines in sickness behavior was first provided by the comparison of dose-response curves *in vivo*. In general, centrally injected cytokines induce dramatic behavioral effects at doses that are 100–1,000 times less than those needed when they are injected peripherally (56). Moreover, the behavioral effects of peripherally injected IL-1 were strongly attenuated by the central administration of the specific antagonist of IL-1 receptors, IL-1ra, at a dose that was able to inhibit the effects of centrally injected IL-1. The use of neutralizing antibodies directed against specific IL-1R subtypes strengthened these data. A monoclonal neutralizing antibody specific to IL-1R1 injected into the lateral ventricle of the brain fully abrogated the behavioral effects of centrally and peripherally injected IL-1 (57). On the other hand, the blockade of brain IL-1R2 potentiated IL-1 effect on food intake but not on body temperature, indicating that some IL-1 actions in the brain are specifically regulated by this receptor (58). The use of KO mice for IL-1R1 and IL-1RAcP reinforce the idea of a specific role of these receptors in mediating the IL-1 effect on sickness behavior (59–61). However, while the cytotoxic effect of IL-1 in traumatic brain injury was blocked by centrally injected IL-1ra, no blockade was observed in IL-1R1 KO mice (62). Furthermore, the central injection of IL-1 exacerbated ischemic brain damage but had no effect on food intake in IL-1R1 KO mice. These intriguing data indicate that

IL-1 effects in the ischemic brain are independent of IL-1R1. In other words, IL-1R1 would mediate the behavioral but not the cytotoxic effects of IL-1.

LPS-induced sickness behavior has been assessed in a strain of mice (C3H/HeJ) that is hyporesponsive to LPS. These mice have a mutation in TLR4, and it is this deficiency that leads to endotoxin hyporesponsiveness (63). The hyporesponsive C3H/HeJ mice are completely resistant to the sickness-inducing effects of LPS when injected intracerebroventricularly, but they remain fully responsive to central injections of IL-1 (64, 65). These experiments show that CNS cells derived from C3H/HeJ endotoxin hyporesponders, such as microglia, share with peripheral macrophages the inability to respond to LPS and to synthesize proinflammatory cytokines, therefore impeding the development of sickness behavior. From a practical perspective, these data show that the C3H/HeJ mouse strain is an excellent model that can be used to avoid any potential confounding effects of endotoxin contamination in the preparations of recombinant cytokines injected in the CNS.

Like at the periphery, the lack of a cytokine in the brain cytokine network can result in compensation by other cytokines that are still present in the network. For instance, peripheral or central administration of LPS still induced sickness behavior in IL-1R1 KO and IL-1RacP KO mice that did not respond any longer to IL-1 (55, 59–61). The sensitivity of IL-1R1 KO mice to LPS was due to TNFα replacing IL-1 since central administration of an antagonist of TNFα blocked LPS action in IL-1R1 KO mice but not in wild type mice (59). On the other hand, the inhibition of proinflammatory cytokine signaling pathways are associated to the improvement in neuroinflammatory processes suggesting that they are not compensated. Indeed, the inhibition of astroglial NFκB by the use of transgenic mice overexpressing a dominant negative form of the IκB super-repressor was associated with reduced neuroinflammation and ischemic damage in the retina (66).

Evidence in favor of a role of cytokines in mediating mood disorders and cognitive disturbances that develop in patients receiving cytokine immunotherapy is growing fast (67). The same mechanisms appear to be at work for the wide variety of non-specific sickness symptoms that develop in patients suffering from somatic diseases with an inflammatory component, including coronary heart disease, rheumatoid arthritis, asthma, cancer, stroke, and various neuropathologies (68–71). Many patients complain of pain, fatigue, anorexia, sleep disturbances, and cognitive and mood disorders. These non-specific neurovegetative and psychiatric symptoms are not necessarily the result of a chain of events linked to each other with more or less a direct cause (e.g. pain induces sleep disorders that impact cognition and induce fatigue and lassitude, culminating in anorexia) (68). They could actually just represent another facet of the inflammatory process. These non-specific symptoms are a major source of suffering for the patient, and often more so than the diseased organ itself. Physicians, whatever their skills, are not always well equipped to deal with these important non-specific symptoms that drastically affect the quality of life of sick patients. The challenge there is not only to bring these symptoms to the forefront of the clinician's attention, but also to be able to treat them adequately (e.g. by molecules that target cytokine production and action in the CNS).

At the experimental level, recent investigations have confirmed the depressive-like effect of cytokines in rodents. However, most of these studies have been carried out during the acute immune activation by the inflammatory inducer (cytokine itself or LPS), which can therefore bias results since sickness (including lethargy and motor impairment)

induced by immune stimulation remained maximal. Nevertheless, some studies have ruled this out by measuring the depressive-like behaviors 24 h after the immune challenge. Frenois et al. (72) demonstrated that immobility in the tail suspension test (TST) and the forced swim test (FST) – behavioral tests commonly used to assess to measure the effects of antidepressant drugs – was increased in LPS animals 24 h after treatment. Similarly, LPS-induced anhedonia, as assessed by reduced consumption of sweetened solution, was observed after dissipation of sickness behavior such as reduced food and water intakes. In a study investigating the effect of a chronic model of immune activation, Moreau et al. (73) showed the occurrence of depressive-like behavior following acute innate immune system activation by chronic inoculation of Bacillus Calmette-Guerin (BCG). That is, delayed depressive-like behaviors as assessed by increased immobility in both the FST and the TST, reduced voluntary wheel running, and decreased preference of sucrose was observed after an acute episode of sickness.

11.4 BRAIN INNATE IMMUNE SYSTEM IN THE AGING BRAIN

Microglial cell activation contributes to the onset and exacerbation of inflammation and neuronal degeneration in many brain diseases (74, 75). Nonetheless, microglial cells act also in a neuroprotective manner by eliminating excess excitotoxins in the extracellular space (74, 75). Moreover, there is accumulating evidence that microglia produce neurotrophic and/or neuroprotective molecules; in particular, it has been proposed that they promote neuronal survival in cases of brain injury. CNS inflammation occurs in myelin degenerative disorders such as multiple sclerosis (MS) and in neurodegenerative disorders such as Alzheimer's disease, HIV encephalopathy, ischemia, and traumatic brain injury (76–80). A general consequence of brain inflammation is reactive gliosis characterized by astrocyte hypertrophy and the proliferation of astrocytes and microglia (81, 82). Changes in gap junction intercellular communication as reflected by alterations in dye coupling and connection expression have been associated with numerous CNS inflammatory diseases, which may have dramatic implications on the survival of neuronal and glial populations in the context of neuroinflammation (83, 84).

IL-1 exerts a number of diverse actions in the brain, and it is currently well accepted that it contributes to experimentally induced neurodegeneration. In response to local brain injury or insult, like acute head trauma, IL-1 is overexpressed by microglia (1, 85, 86). Such acute overexpression of IL-1 has been implicated in the pathogenesis of some forms of acute brain injury. Moreover, patients with multiple sclerosis have elevated levels of IL-1 in the cerebrospinal fluid when their disease is active (87). Brain microglia may chronically overexpress IL-1 under repeated or persistent injurious stimuli, or chronic neurological conditions (Down's syndrome, HIV, epilepsy, etc). Chronic overexpression of IL-1 is also observed in a normal aging brain and in Alzheimer's disease (88–90). Recent microarray studies, assessing the gene expression of Alzheimer-related cytokines, show selective overexpression of IL-1 in Alzheimer's disease. This increase is coupled with an increase in the expression of IL-1R1 and an increased activity of IL-1 receptor-associated MAPK (91, 92). There are genetic associations between IL-1 family gene polymorphisms and Alzheimer's disease, chronic epilepsy and Parkinson's disease (93–96).

IL-1 overexpression has been implicated in both the initiation and progression of neuropathological changes (85). Overexpression of IL-1 in an Alzheimer brain is linked to

an increase in microglia activity that is frequently associated to amyloid plaques (88, 97). This specific distribution suggests a role for IL-1 in the initiation and progression of neuritic and neuronal injury in Alzheimer's disease, because of its appearance in early plaque formation and its absence in plaques that are devoid of injured neuritic elements. The brain from Tg2576 mice (a model for Alzheimer disease) showed significant increases in IL-1 expression compared to controls. Moreover, aged Tg2576 showed mounted and exacerbated cytokine response to LPS that could have amplified the degenerative processes. IL-1 administration depressed food intake more in aged mice than in adults (98). Attenuation of fever response in aged mice could be due to the lack of entry of peripheral IL-1 in the brain and not a lack of brain IL-1R functionality (99, 100). Age-induced IL-1 overproduction in the brain, and more particularly in the hippocampus, is associated with a decrease in synaptic plasticity measured by long-term potentiation (LTP) in the dentate gyrus, which could explain cognitive impairment observed in the elderly (92, 101, 102). Receptors for IL-1 are distributed with a high density in the hippocampus, where interleukin-1 exerts inhibitory effects on the release of calcium (103). There is also evidence for a role of endogenous brain IL-1 in the normal physiological regulation of hippocampal plasticity and learning processes (104, 105). Low levels of IL-1 are essential for memory and plasticity, whereas higher levels of IL-1, similar to those achieved during aging and neurodegeneration, can be detrimental (104, 105).

11.5 INFLUENCE OF POLYUNSATURATED FATTY ACID ON BRAIN INNATE IMMUNE SYSTEM

The polyunsaturated fatty acid linoleic and its n-6 derivative ARA, and α linolenic acids and its n-3 derivative, EPA and DHA, play a key role in both energy production and cell structure and are indispensable for brain development. ARA and DHA are found in large concentrations in brain lipids. In the brain, nearly 6% of the dry weight of brain is n-3 PUFA (106). They are incorporated as phospholipids and are key components of the brain cell membranes. They provide fluidity and the proper environment for active integral protein functions. Moreover, phospholipids have a role in cellular function because they are a reservoir of signaling messengers for neurotransmitters or growth factors. There are some data on PUFA contents on neurons and astrocytes (131), but nothing is known concerning microglial cells.

DHA and ARA have beneficial effects when available in moderation. As already mentioned, human beings originally consumed a diet rich in n-3 PUFA and low in saturated fatty acids because wild and free range food animals have much higher content of n-3 fatty acid than do the present day commercial livestock. PUFA were provided in the diet as a ratio 1:1 of n-6 to n-3. Nowadays the ratio is around 10:1. An excess of n-6 precursors stimulates the formation of ARA. Although some ARA is essential, the current high ratio of n-6 to n-3 may be involved in the increase in chronic inflammatory diseases (13). A high intake of n-3 PUFA such as DHA or EPA may have anti-inflammatory effects in patients with neuroinflammation. Conversely, the high dietary intake of n-6 PUFA, which lowers the intake of n-3 PUFA, may contribute to the development of the neuroinflammation. For example, ARA is the principal substrate for COX (107). Additional substrates include cannabinoids and lipoamino acids that also can be oxidized to produce PG precursors of which the pathophysiological role is poorly known (108, 109). PGs have the ability to play

either a protective or an injurious role, depending on the context and quantity produced. Therefore, the membrane levels of their precursor, ARA, are important. Moreover, the EPA contained in membrane phospholipids competes with ARA as a substrate for COX and lipooxygenase (LOX) (110). The consequences of such a competition are a decrease in the production of inflammatory metabolites such as PGE2, leucotriene A4 (LTA4) and tromboxane A2 (TXA2) and an increase in the synthesis of less inflammatory eicosanoids or even anti-inflammatory ones (110, 112). This has been demonstrated in many cells throughout the body, including glial cells (113). A 6-day LPS infusion in the brain increased phospholipase A2 activities and brain concentrations of linoleic and ARA, and of PGE2 and PGD2 (16). The occurrence of alteration in n-6 metabolism in the brain in response to LPS emphasizes once again the link between brain composition in n-3/n-6 PUFA and neuroinflammation. Interestingly, mice exposed throughout their life to a diet devoid of n-3 PUFA did not show sickness behavior in response to LPS, while IL-6 was overexpressed both at the periphery and in the hippocampus (114). However, STAT3 and STAT1 activation, a hallmark of the IL-6 signaling pathway, was poorly induced in the hippocampus of LPS-treated mice, suggesting that dietary deficiency in n-3 PUFA dysregulates neuroinflammatory events and its behavioral effect.

Numerous studies have revealed that n-3 PUFA inhibit the *in vitro* production of proinflammatory cytokines by macrophages, and their *in vivo* synthesis in healthy adults and those with autoimmune diseases. However, little is known concerning microglial cells that produce proinflammatory cytokines in the brain. Recently, DHA has been shown to be highly anti-inflammatory by targeting LPS receptor surface location, therefore reducing LPS-induced NFκB activation and proinflammatory cytokines production in microglia (115). Such an effect could be mediated by the DHA-derived neuroprotectine D1 (NPD1), which regulates Aβ peptide-induced proinflammatory cytokine expression in microglia (116–118). Interestingly, in the brain and in microglia, DHA is also converted into potent anti-inflammatory products called 17-resolvins by aspirin-induced acetylated COX-2 (119). Resolvins block the production of cytokines by microglial cells. Moreover, they protect from ischemia by blocking NFκB activation and proinflammatory cytokine production (120).

A short time n-3 supplementation attenuated the fever responses induced in rats by both i.p. and i.c.v. IL-1 without altering the thermogenic capacity of the organism (121, 122). However, Kluger's group reported that fever, lethargy, and anorexia were differentially regulated by a fish-oil diet depending on the inflammatory stimulus used (123). Turpentine is a model of local inflammation that induces a robust acute phase response consisting of fever, anorexia, cachexia, and acute phase protein production. Fish oil diet exacerbated LPS-induced lethargia and decreased temperature whereas it blocked turpentine-induced fever, lethargia, and anorexia (123). These changes were associated with a decrease in circulating LPS-induced PGE2 and an increase in LPS-induced TNFα. Because TNFα production is partially regulated by PGE2, fish oil could upregulate TNFα production by decreasing PGE2 production (124). In mice, the early hypothermic phase of fever to a high dose of LPS was exacerbated by TNFα treatment, whereas administration of the soluble TNF receptor, a blocker of TNFα activity, attenuated hypothermia (125, 126). It is questionable therefore whether ingesting high amounts of n-3 PUFA during inflammatory events is beneficial. Further studies on the role of PUFA in neuroinflammation are clearly needed.

Elderly people who eat fish or seafood, that are highly enriched in n-3 PUFA, at least once a week are at lower risk of developing dementia, including Alzheimer's disease (127–129). Because aging is associated with a decrease in membrane PUFA, including ARA, and an increase in brain IL-1 production, Lynch proposed that the age-increased in IL-1 is linked to an age-decreased membrane ARA (92, 102). Therefore IL-1, by impacting on membrane composition, would contribute to age-related impairments in neuronal function. IL-1 increased lipid peroxidation in hippocampal tissue from young but not old rats, and this effect was associated with decreased LTP (92, 102). A short-time supplementation in ARA, in combination with another long chain n-6 PUFA, gamma-linolenic acid (GLA), reversed the age-related impairment in LTP (130). EPA had a similar effect and, in this last case, there was evidence that this effect was a consequence of its ability to block the effects of IL-1, providing support for the hypothesis that EPA acts as an anti-inflammatory agent (129, 130). The anti-inflammatory effect of EPA could be due to the blockade by EPA of the IL-1 signaling pathway MAPK, and more particularly p38, in the brain (132). Interestingly, LPS-induced p38 activation in the hippocampus is accompanied by an increased activation of NFκB of which the pharmacological inhibition partially suppresses the inhibitory effects of LPS and IL-1 on LTP and sickness behavior (39, 133, 134). In addition, n-3 PUFA might protect the brain from the deleterious effects of IL-1. Irradiation induced increase in IL-1, IL-1R1 and IL-1RAcP concentration in the hippocampus. These changes were coupled with an increased activation of JNK and apoptotic cell death. Rats that had been fed a diet rich in EPA did not display any of these events. The anti-inflammatory cytokine IL-10 could explain EPA anti-inflammatory and neuroprotective effects in the brain, because EPA increased IL-10 levels and IL-10 blocked the IL-1 effect (135, 136). An EPA-supplemented diet, but not ARA, significantly attenuated centrally injected IL-1-induced anxiety behavior. Such an effect was also observed in the brain of olfactory bulbectomized mice (137). This was accompanied by a decrease in the IL-1-induced PGE2 and an increase of IL-10 or IL-4 (138–140).

11.6 CONCLUSIONS AND PERSPECTIVES

There is growing evidence that the expression and action of proinflammatory cytokines in the brain are responsible not only for the development and maintenance of sickness behavior during the host response to infection, but also for the occurrence of non-specific symptoms of sickness during chronic inflammatory disorders. In addition, neuroinflammation can have detrimental consequences on neuronal viability especially when maintained over long periods of time and transiently amplified by peripheral infectious episodes. All of this points to the interest of finding new ways of controlling inflammation in the brain. Because of their abundance in the brain and their modulatory role on inflammation and cell functions, PUFA certainly have a role to play. However, this role still needs to be better characterized by multidisciplinary studies aiming at assessing the effects of these molecules at different levels of functioning, from the molecular to the organism level.

Acknowledgements Supported by INRA and Conseil Général d'Aquitaine.

REFERENCES

1. Rothwell NJ, Luheshi GN (2000) Interleukin 1 in the brain: biology, pathology and therapeutic target. Trends Neurosci 23:618–625
2. Dantzer R (2001) Cytokine-induced sickness behavior: mechanisms and implications. Ann NY Acad Sci 933:222–234
3. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9:46–56
4. Kent S, Bluthe RM, Kelley KW, Dantzer R (1992) Sickness behavior as a new target for drug development. Trends Pharmacol Sci 13:24–28
5. Venters HD, Dantzer R, Kelley KW (2000) A new concept in neurodegeneration: TNFalpha is a silencer of survival signals. Trends Neurosci 23:175–180
6. Allan SM (2000) The role of pro- and antiinflammatory cytokines in neurodegeneration. Ann NY Acad Sci 917:84–93
7. Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI, Romero J (2008) Cannabinoid CB2 receptors in human brain inflammation. Br J Pharmacol 153:277–285
8. Lipton JM, Zhao H, Ichiyama T, Barsh GS, Catania A (1999) Mechanisms of antiinflammatory action of alpha-MSH peptides. In vivo and in vitro evidence. Ann NY Acad Sci 20:173–182
9. Strle K, Zhou JH, Shen WH, Broussard SR, Johnson RW, Freund GG et al (2001) Interleukin-10 in the brain. Crit Rev Immunol 21:427–449
10. Raper NR, Cronin FJ, Exler J (1992) Omega-3 fatty acid content of the US food supply. J Am Coll Nutr 11:304–308
11. Simopoulos AP (2001) n-3 fatty acids and human health: defining strategies for public policy. Lipids 1(Suppl):S83–S89
12. Simopoulos AP (2003) Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. World Rev Nutr Diet 92:1–22
13. Calder PC (2008) The relationship between the fatty acid composition of immune cells and their function. Prostaglandins Leukot Essent Fatty Acids 79:101–108
14. Anderson GJ, Connor WE (1994) Accretion of n-3 fatty acids in the brain and retina of chicks fed a low-linolenic acid diet supplemented with docosahexaenoic acid. Am J Clin Nutr 59: 1338–1346
15. Champeil-Potokar G, Denis I, Goustard-Langelier B, Alessandri JM, Guesnet P, Lavialle M (2004) Astrocytes in culture require docosahexaenoic acid to restore the n-3/n-6 polyunsaturated fatty acid balance in their membrane phospholipids. J Neurosci Res 75:96–106
16. Rosenberger TA, Villacreses NE, Hovda JT, Bosetti F, Weerasinghe G, Wine RN et al (2004) Rat brain arachidonic acid metabolism is increased by a 6-day intracerebral ventricular infusion of bacterial lipopolysaccharide. J Neurochem 88:1168–1178
17. Rivest S (2001) How circulating cytokines trigger the neural circuits that control the hypothalamic-pituitary-adrenal axis. Psychoneuroendocrinology 26:761–788
18. Nguyen MD, Julien JP, Rivest S (2002) Innate immunity: the missing link in neuroprotection and neurodegeneration? Nat Rev Neurosci 3:216–227
19. Guillemin GJ, Brew BJ (2004) Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. J Leukoc Biol 75:388–397
20. Kloss CU, Bohatschek M, Kreutzberg GW, Raivich G (2001) Effect of lipopolysaccharide on the morphology and integrin immunoreactivity of ramified microglia in the mouse brain and in cell culture. Exp Neurol 168:32–46
21. Theele DP, Streit WJ (1993) A chronicle of microglial ontogeny. Glia 7:5–8
22. Ransohoff RM, Perry VH (2009) Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 27:119–145
23. Nakamura Y (2002) Regulating factors for microglial activation. Biol Pharmacol Bull 25:945–953
24. Streit WJ, Walter SA, Pennell NA (1999) Reactive microgliosis. Prog Neurobiol 57:563–581
25. Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2:675–680

26. Konsman JP, Kelley K, Dantzer R (1999) Temporal and spatial relationships between lipopolysaccharide-induced expression of Fos, interleukin-1beta and inducible nitric oxide synthase in rat brain. Neuroscience 89:535–548
27. Laflamme N, Rivest S (2001) Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. FASEB J 15:155–163
28. Laye S, Parnet P, Goujon E, Dantzer R (1994) Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. Mol Brain Res 27:157–162
29. Quan N, Sundar SK, Weiss JM (1994) Induction of interleukin-1 in various brain regions after peripheral and central injections of lipopolysaccharide. J Neuroimmunol 49:125–134
30. Kluger MJ (1991) Fever: role of pyrogens and cryogens. Physiol Rev 71:93–127
31. Laflamme N, Rivest S (1999) Effects of systemic immunogenic insults and circulating proinflammatory cytokines on the transcription of the inhibitory factor kappaB alpha within specific cellular populations of the rat brain. J Neurochem 73:309–321
32. Chakravarty S, Herkenham M (2005) Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. J Neurosci 16:1788–1796
33. Laflamme N, Echchannaoui H, Landmann R, Rivest S (2003) Cooperation between toll-like receptor 2 and 4 in the brain of mice challenged with cell wall components derived from gram-negative and gram-positive bacteria. Eur J Immunol 33:1127–1138
34. Kenny EF, O'Neill LA (2008) Signaling adaptors used by Toll-like receptors: an update. Cytokine 43:342–349
35. O'Neill LA, Fitzgerald KA, Bowie AG (2003) The Toll-IL-1 receptor adaptor family grows to five members. Trends Immunol 24:286–290
36. Laflamme N, Lacroix S, Rivest S (1999) An essential role of interleukin-1beta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. J Neurosci 19:10923–10930
37. Quan N, Whiteside M, Kim L, Herkenham M (1997) Induction of inhibitory factor kappaBalpha mRNA in the central nervous system after peripheral lipopolysaccharide administration: an in situ hybridization histochemistry study in the rat. Proc Natl Acad Sci 94:10985–10990
38. Eriksson C, Nobel S, Winblad B, Schultzberg M (2000) Expression of interleukin 1 alpha and beta, and interleukin 1 receptor antagonist mRNA in the rat central nervous system after peripheral administration of lipopolysaccharides. Cytokine 12:423–431
39. Nadjar A, Combe C, Laye S, Tridon V, Dantzer R, Amedee T et al (2003) Nuclear factor kappaB nuclear translocation as a crucial marker of brain response to interleukin-1. A study in rat and interleukin-1 type I deficient mouse. J Neurochem 87:1024–1036
40. Parnet P, Pousset F, Laye S (2003) NF kappa B activation in mouse pituitary: comparison of response to interleukin-1 beta and lipopolysaccharide. J Neuroendocrinol 15:304–314
41. O'Neill LA (2008) The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 226:10–18
42. Colotta F, Dower SK, Sims JE, Mantovani A (1994) The type II decoy receptor: a novel regulatory pathway for interleukin 1. Immunol Today 15:562–566
43. Cunningham ET, Wada E, Carter DB, Tracey DE, Battey JF, De Souza EB (1991) Localization of interleukin-1 receptor messenger RNA in murine hippocampus. Endocrinology 128:2666–2668
44. Ericsson A, Liu C, Hart RP, Sawchenko PE (1995) Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. J Comp Neurol 361:681–698
45. Wong ML, Licinio J (1994) Localization of interleukin 1 type I receptor mRNA in rat brain. Neuroimmunomodulation 1:110–115
46. French RA, VanHoy RW, Chizzonite R, Zachary JF, Dantzer R, Parnet P et al (1999) Expression and localization of p80 and p68 interleukin-1 receptor proteins in the brain of adult mice. J Neuroimmunol 93:194–202
47. Ilyin SE, Gayle D, Flynn MC, Plata-Salaman CR (1998) Interleukin-1beta system (ligand, receptor type I, receptor accessory protein and receptor antagonist), TNF-alpha, TGF-beta1 and neuropeptide Y mRNAs in specific brain regions during bacterial LPS-induced anorexia. Brain Res Bull 45:507–515
48. Liu C, Chalmers D, Maki R, De Souza EB (1996) Rat homolog of mouse interleukin-1 receptor accessory protein: cloning, localization and modulation studies. J Neuroimmunol 66:41–48

49. Smith DE, Lipsky BP, Russell C, Ketchem RR, Kirchner J, Hensley K et al (2009) A central nervous system-restricted isoform of the interleukin-1 receptor accessory protein modulates neuronal responses to interleukin-1. Immunity 30:817–831
50. Hudson CA, Christophi GP, Gruber RC, Wilmore JR, Lawrence DA, Massa PT (2008) Induction of IL-33 expression and activity in central nervous system glia. J Leukoc Biol 84:631–643
51. Pinteaux E, Parker LC, Rothwell NJ, Luheshi GN (2002) Expression of interleukin-1 receptors and their role in interleukin-1 actions in murine microglial cells. J Neurochem 83:754–763
52. Costelloe C, Watson M, Murphy A, McQuillan K, Loscher C, Armstrong ME et al (2008) IL-1F5 mediates anti-inflammatory activity in the brain through induction of IL-4 following interaction with SIGIRR/TIR8. J Neurochem 105:1960–1969
53. Hart BL (1998) Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 12:123–137
54. Laye S, Bluthe RM, Kent S, Combe C, Medina C, Parnet P et al (1995) Subdiaphragmatic vagotomy blocks induction of IL-1 beta mRNA in mice brain in response to peripheral LPS. Am J Physiol 268:R1327–R1331
55. Laye S, Gheusi G, Cremona S, Combe C, Kelley K, Dantzer R et al (2000) Endogenous brain IL-1 mediates LPS-induced anorexia and hypothalamic cytokine expression. Am J Physiol 279: R93–R98
56. Kent S, Bluthe RM, Dantzer R, Hardwick AJ, Kelley KW, Rothwell NJ et al (1992) Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. Proc Natl Acad Sci 89:9117–9120
57. Cremona S, Goujon E, Kelley KW, Dantzer R, Parnet P (1998) Brain type I but not type II IL-1 receptors mediate the effects of IL-1 beta on behavior in mice. Am J Physiol 274:R735–R740
58. Cremona S, Laye S, Dantzer R, Parnet P (1998) Blockade of brain type II interleukin-1 receptors potentiates IL1beta-induced anorexia in mice. Neurosci Lett 246:101–104
59. Bluthe RM, Laye S, Michaud B, Combe C, Dantzer R, Parnet P (2000) Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behavior: a study with interleukin-1 type I receptor-deficient mice. Eur J Neurosci 12:4447–4456
60. Laye S, Liege S, Li KS, Moze E, Neveu PJ (2001) Physiological significance of the interleukin 1 receptor accessory protein. Neuroimmunomodulation 9:225–230
61. Liege S, Laye S, Li KS, Moze E, Neveu PJ (2000) Interleukin 1 receptor accessory protein (IL-1RAcP) is necessary for centrally mediated neuroendocrine and immune responses to IL-1beta. J Neuroimmunol 110:134–139
62. Touzani O, Boutin H, LeFeuvre R, Parker L, Miller A, Luheshi G et al (2002) Interleukin-1 influences ischemic brain damage in the mouse independently of the interleukin-1 type I receptor. J Neurosci 22:38–43
63. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X et al (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282:2085–2088
64. Johnson RW, Gheusi G, Segreti S, Dantzer R, Kelley KW (1997) C3H/HeJ mice are refractory to lipopolysaccharide in the brain. Brain Res 752:219–226
65. Segreti J, Gheusi G, Dantzer R, Kelley KW, Johnson RW (1997) Defect in interleukin-1beta secretion prevents sickness behavior in C3H/HeJ mice. Physiol Behav 61:873–878
66. Dvoriantchikova G, Barakat D, Brambilla R, Agudelo C, Hernandez E, Bethea JR et al (2009) Inactivation of astroglial NF-kappaB promotes survival of retinal neurons following ischemic injury. Eur J Neurosci 30:175–185
67. Capuron L, Dantzer R (2003) Cytokines and depression: the need for a new paradigm. Brain Behav Immun 17:S119–124
68. Cleeland CS, Bennett GJ, Dantzer R, Dougherty PM, Dunn AJ, Meyers CA et al (2003) Are the symptoms of cancer and cancer treatment due to a shared biologic mechanism? A cytokine-immunologic model of cancer symptoms. Cancer 97:2919–2925
69. Eikelenboom P, Bate C, Van Gool WA, Hoozemans JJ, Rozemuller JM, Veerhuis R et al (2002) Neuroinflammation in Alzheimer's disease and prion disease. Glia 40:232–239
70. Gidron Y, Gilutz H, Berger R, Huleihel M (2002) Molecular and cellular interface between behavior and acute coronary syndromes. Cardiovasc Res 56:15–21
71. Kiecolt-Glaser JK, Glaser R (2002) Depression and immune function: central pathways to morbidity and mortality. J Psychosom Res 53:873–876

72. Frenois F, Moreau M, O'Connor J, Lawson M, Micon C, Lestage J, Kelley KW, Dantzer R, Castanon N (2007) Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. Psychoneuroendocrinology 32:516–531
73. Moreau M, André C, O'Connor JC, Dumich SA, Woods JA, Kelley KW, Dantzer R, Lestage J, Castanon N (2008) Inoculation of Bacillus Calmette-Guerin to mice induces an acute episode of sickness behavior followed by chronic depressive-like behavior. Brain Behav Immun 22:1087–1095
74. Liu B, Hong JS (2003) Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. J Parmacol Exp Ther 304:1–7
75. Merrill JE, Benveniste EN (1996) Cytokines in inflammatory brain lesions: helpful and harmful. Trends Neurosci 19:331–338
76. Chopp M, Zhang RL, Chen H, Li Y, Jiang N, Rusche JR (1994) Postischemic administration of an anti-Mac-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in rats. Stroke 25:869–866
77. Dusart I, Schwab ME (1994) Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. Eur J Neurosci 6:712–724
78. Gendelman HE, Zheng J, Coulter CL, Ghorpade A, Che M, Thylin M et al (1998) Suppression of inflammatory neurotoxins by highly active antiretroviral therapy in human immunodeficiency virus-associated dementia. J Infect Dis 178:1000–1007
79. Martino G, Adorini L, Rieckmann P, Hillert J, Kallmann B, Comi G et al (2002) Inflammation in multiple sclerosis: the good, the bad, and the complex. Lancet Neurol 1:499–509
80. McGeer PL, Rogers J (1992) Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease. Neurology 42:447–449
81. Aschner M (1998) Astrocytes as mediators of immune and inflammatory responses in the CNS. Neurotoxicology 19:269–281
82. Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19:312–318
83. Kielian T, Esen N (2004) Effects of neuroinflammation on glia-glia gap junctional intercellular communication: a perspective. Neurochem Int 45:429–436
84. Nakase T, Fushiki S, Sohl G, Theis M, Willecke K, Naus CC (2003) Neuroprotective role of astrocytic gap junctions in ischemic stroke. Cell Commun Adhes 10:413–417
85. Griffin DE, Moser HW, Mendoza Q, Moench TR, O'Toole S, Moser AB (1985) Identification of the inflammatory cells in the central nervous system of patients with adrenoleukodystrophy. Ann Neurol 18:660–664
86. Hetier E, Ayala J, Denefle P, Bousseau A, Rouget P, Mallat M et al (1988) Brain macrophages synthesize interleukin-1 and interleukin-1 mRNAs in vitro. J Neurosci Res 21:391–397
87. Hauser SL, Doolittle TH, Lincoln R, Brown RH, Dinarello CA (1990) Cytokine accumulations in CSF of multiple sclerosis patients: frequent detection of interleukin-1 and tumor necrosis factor but not interleukin-6. Neurology 40:1735–1739
88. Griffin WS, Sheng JG, Roberts GW, Mrak RE (1995) Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. J Neuropathol Exp Neurol 54:276–281
89. Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ et al (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci 86:7611–7615
90. Katafuchi T, Takaki A, Take S, Kondo T, Yoshimura M (2003) Endotoxin inhibitor blocks heat exposure-induced expression of brain cytokine mRNA in aged rats. Mol Brain Res 118:24–32
91. Hacham M, Argov S, White RM, Segal S, Apte RN (2002) Different patterns of interleukin-1alpha and interleukin-1beta expression in organs of normal young and old mice. Eur Cytokine Netw 13:55–65
92. Lynch MA (1998) Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? Prog Neurobiol 56:571–589
93. Kanemoto K, Kawasaki J, Miyamoto T, Obayashi H, Nishimura M (2000) Interleukin (IL)1beta, IL-1alpha, and IL-1 receptor antagonist gene polymorphisms in patients with temporal lobe epilepsy. Ann Neurol 47:571–574
94. Mrak RE, Griffin WS (2000) Interleukin-1 and the immunogenetics of Alzheimer disease. J Neuropathol Exp Neurol 59:471–476

95. Nishimura M, Mizuta I, Mizuta E, Yamasaki S, Ohta M, Kuno S (2000) Influence of interleukin-1beta gene polymorphisms on age-at-onset of sporadic Parkinson's disease. Neurosci Lett 284:73–76
96. Chapuis J, Hot D, Hansmannel F, Kerdraon O, Ferreira S, Hubans C et al (2009) Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease. Mol Psychiatry 14(11):1004–1016
97. Sheng JG, Griffin WS, Royston MC, Mrak RE (1998) Distribution of interleukin-1-immunoreactive microglia in cerebral cortical layers: implications for neuritic plaque formation in Alzheimer's disease. Neuropathol Appl Neurobiol 24:278–283
98. Sly LM, Krzesicki RF, Brashler JR, Buhl AE, McKinley DD, Carter DB et al (2001) Endogenous brain cytokine mRNA and inflammatory responses to lipopolysaccharide are elevated in the Tg2576 transgenic mouse model of Alzheimer's disease. Brain Res Bull 56:581–588
99. McLay RN, Kastin AJ, Zadina JE (2000) Passage of interleukin-1-beta across the blood-brain barrier is reduced in aged mice: a possible mechanism for diminished fever in aging. Neuroimmunomodulation 8:148–153
100. Plata-Salaman CR, Peloso E, Satinoff E (1998) Interleukin-1beta-induced fever in young and old Long-Evans rats. Am J Physiol 275:R1633–R1638
101. Lynch AM, Lynch MA (2002) The age-related increase in IL-1 type I receptor in rat hippocampus is coupled with an increase in caspase-3 activation. Eur J Neurosci 15:1779–1788
102. Murray CA, Lynch MA (1998) Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. J Neurosci 18:2974–2981
103. Campbell VA, Segurado R, Lynch MA (1998) Regulation of intracellular Ca2+ concentration by interleukin-1beta in rat cortical synaptosomes: an age-related study. Neurobiol Aging 19:575–579
104. Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalzstein Y, Ben-Hur T et al (2007) A dual role for interleukin-1 in hippocampal-dependent memory processes. Psychoneuroendocrinology 32:1106–1115
105. Labrousse VF, Costes L, Aubert A, Darnaudery M, Ferreira G, Amedee T et al (2009) Impaired interleukin-1beta and c-Fos expression in the hippocampus is associated with a spatial memory deficit in P2X(7) receptor-deficient mice. PLoS One 4:e6006
106. Bourre JM, Dumont O, Piciotti M, Clement M, Chaudiere J, Bonneil M et al (1991) Essentiality of omega 3 fatty acids for brain structure and function. World Rev Nutr Diet 66:103–117
107. O'Banion MK (1999) Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology. Crit Rev Neurobiol 13:45–82
108. Chang L, Karin M (2001) Mammalian MAP kinase signaling cascades. Nature 410:37–40
109. Kozak KR, Prusakiewicz JJ, Rowlinson SW, Schneider C, Marnett LJ (2001) Amino acid determinants in cyclooxygenase-2 oxygenation of the endocannabinoid 2-arachidonylglycerol. J Biol Chem 276:30072–30077
110. Calder PC (2003) N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. Lipids 38:343–352
111. Calder PC, Grimble RF (2002) Polyunsaturated fatty acids, inflammation and immunity. Eur J Clin Nutr 56(Suppl 3):S14–S19
112. Zaloga GP, Marik P (2001) Lipid modulation and systemic inflammation. Crit Care Clin 17:201–217
113. Petroni A, Salami M, Blasevich M, Papini N, Galli C (1994) Inhibition by n-3 fatty acids of arachidonic acid metabolism in a primary culture of astroglial cells. Neurochem Res 19:1187–1193
114. Mingam R, Moranis A, Bluthe RM, De Smedt-Peyrusse V, Kelley KW, Guesnet P et al (2008) Uncoupling of interleukin-6 from its signaling pathway by dietary n-3-polyunsaturated fatty acid deprivation alters sickness behavior in mice. Eur J Neurosci 28:1877–1886
115. De Smedt-Peyrusse V, Sargueil F, Moranis A, Harizi H, Mongrand S, Laye S (2008) Docosahexaenoic acid prevents lipopolysaccharide-induced cytokine production in microglial cells by inhibiting lipopolysaccharide receptor presentation but not its membrane subdomain localization. J Neurochem 105:296–307
116. Bazan NG (2009) Neuroprotectin D1-mediated anti-inflammatory and survival signaling in stroke, retinal degenerations, and Alzheimer's disease. J Lipid Res 50:S400–S405
117. Bazan NG, Marcheselli VL, Cole-Edwards K (2005) Brain response to injury and neurodegeneration: endogenous neuroprotective signaling. Ann NY Acad Sci 1053:137–147

118. Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuchi A et al (2007) Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med 13:868–873
119. Serhan CN, Clish CB, Brannon J, Colgan SP, Gronert K, Chiang N (2000) Anti-microinflammatory lipid signals generated from dietary N-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and N-3 PUFA therapeutic actions. J Physiol Pharmacol 51:643–654
120. Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A et al (2003) Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. J Biol Chem 278:43807–43817
121. Cooper AL, Rothwell NJ (1993) Inhibition of the thermogenic and pyrogenic responses to interleukin-1 beta in the rat by dietary N-3 fatty acid supplementation. Prostaglandins Leukot Essent Fatty Acids 49:615–626
122. Pomposelli JJ, Mascioli EA, Bistrian BR, Lopes SM, Blackburn GL (1989) Attenuation of the febrile response in guinea pigs by fish oil enriched diets. J Parenteral Enteral Nutr 13:136–140
123. Kozak W, Soszynski D, Rudolph K, Conn CA, Kluger MJ (1997) Dietary n-3 fatty acids differentially affect sickness behavior in mice during local and systemic inflammation. Am J Physiol 272: R1298–R1307
124. Kunkel SL, Spengler M, May MA, Spengler R, Larrick J, Remick D (1988) Prostaglandin E2 regulates macrophage-derived tumor necrosis factor gene expression. J Biol Chem 263:5380–5384
125. Klir JJ, McClellan JL, Kozak W, Szelenyi Z, Wong GH, Kluger MJ (1995) Systemic but not central administration of tumor necrosis factor-alpha attenuates LPS-induced fever in rats. Am J Physiol 268:R480–R486
126. Kozak W, Conn CA, Klir JJ, Wong GH, Kluger MJ (1995) TNF soluble receptor and antiserum against TNF enhance lipopolysaccharide fever in mice. Am J Physiol 269:R23–R29
127. Barberger-Gateau P, Letenneur L, Deschamps V, Peres K, Dartigues JF, Renaud S (2002) Fish, meat, and risk of dementia: cohort study. Br Med J 325:932–933
128. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM (1997) Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 42:776–782
129. Kalmijn S, van Boxtel MP, Ocke M, Verschuren WM, Kromhout D, Launer LJ (2004) Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. Neurology 62:275–280
130. McGahon B, Murray CA, Clements MP, Lynch MA (1998) Analysis of the effect of membrane arachidonic acid concentration on modulation of glutamate release by interleukin-1: an age-related study. Exp Gerontol 33:343–354
131. Babcock T, Helton WS, Espat NJ (2000) Eicosapentaenoic acid (EPA): an antiinflammatory omega-3 fat with potential clinical applications. Nutrition 16:1116–1118
132. Martin DS, Lonergan PE, Boland B, Fogarty MP, Brady M, Horrobin DF et al (2002) Apoptotic changes in the aged brain are triggered by interleukin-1beta-induced activation of p38 and reversed by treatment with eicosapentaenoic acid. J Biol Chem 277:34239–34246
133. Kelly A, Vereker E, Nolan Y, Brady M, Barry C, Loscher CE et al (2003) Activation of p38 plays a pivotal role in the inhibitory effect of lipopolysaccharide and interleukin-1 beta on long term potentiation in rat dentate gyrus. J Biol Chem 278:19453–19462
134. Nadjar A, Bluthe RM, May MJ, Dantzer R, Parnet P (2005) Inactivation of the cerebral NFkappaB pathway inhibits interleukin-1beta-induced sickness behavior and c-Fos expression in various brain nuclei. Neuropsychopharmacology 30:1492–1499
135. Bluthe RM, Castanon N, Pousset F, Bristow A, Ball C, Lestage J et al (1999) Central injection of IL-10 antagonizes the behavioral effects of lipopolysaccharide in rats. Psychoneuroendocrinology 24:301–311
136. Lynch AM, Moore M, Craig S, Lonergan PE, Martin DS, Lynch MA (2003) Analysis of interleukin-1 beta-induced cell signaling activation in rat hippocampus following exposure to gamma irradiation. Protective effect of eicosapentaenoic acid. J Biol Chem 278:51075–51084
137. Song C, Zhang XY, Manku M (2009) Increased phospholipase A2 activity and inflammatory response but decreased nerve growth factor expression in the olfactory bulbectomized rat model of depression: effects of chronic ethyl-eicosapentaenoate treatment. J Neurosci 29:14–22

138. Kavanagh T, Lonergan PE, Lynch MA (2004) Eicosapentaenoic acid and gamma-linolenic acid increase hippocampal concentrations of IL-4 and IL-10 and abrogate lipopolysaccharide-induced inhibition of long-term potentiation. Prostaglandins Leukot Essent Fatty Acids 70:391–397
139. Song C, Horrobin D (2004) Omega-3 fatty acid ethyl-eicosapentaenoate, but not soybean oil, attenuates memory impairment induced by central IL-1beta administration. J Lipid Res 45:1112–1121
140. Song C, Li X, Leonard BE, Horrobin DF (2003) Effects of dietary n-3 or n-6 fatty acids on interleukin-1beta-induced anxiety, stress, and inflammatory responses in rats. J Lipid Res 44:1984–1991

12 Immunomodulatory Potential of Conjugated Linolenic Acid

Saji Menon, Shalini Jain, Ravinder Nagpal, Manoj Kumar, Dheeraj Mohania, Dhananjay Yadav, Fracesco Marotta, Mukesh Yadav, and Hariom Yadav

Key Points

- Conjugated linoleic acid (CLA), isomers of linoleic acid (C18:2) have many biological effects, including potential immunomodulatory potential.
- CLA is an unrecognized nutrient that significantly protects lymphoidal and nonlymphoidal tissues from lymphoid events during immune stimulation.
- This protection is through the regulation of lipid eicosanoid mediators while nonlymphoidal tissues prevent their negative feedback on the immune response.
- With lipid mediator modulation there is enhanced immunity, improved efficiency of feed use, changes in body composition, and decrease in diseases with immune response.
- With regard to the immune system, it is not clear whether individual isomers of CLA could act similarly or differently.
- In this chapter, we are trying to uncover most of the fundamental findings to explore the effects of CLA in relation to immunomodulation.

Key Words: Conjugate linolenic acid, immune system, nutrition, dietary component, functional foods.

12.1 INTRODUCTION

Lifestyle has an important influence on the development of various life threatening diseases such as cancer, autoimmunity, allergies, obesity, diabetes etc., in humans. Diet is one of the most important lifestyle factors of today's era, which has a strong impact on development/prevention in most of the above mentioned illnesses (1). Diet has various

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_12

biofunctional components other than nutritionally required ingredient, which can play an important role to enhance body strength to fight with various diseases. In mammals, a fighting system against various illnesses is called immune system, which protects the body from various direct and indirect challenges for tissue/cell damages into the body (2). A plethora of literature that supports natural components or food ingredients enhance immune system to fight against various pathogenic and nonpathogenic attacks is available (3). Among various bio-functional food ingredients conjugated linoleic acid (CLA) is one of the well-recognized functional lipid derivatives, which has been consumed in our daily meal from a long time (4). CLA has the capabilities to prevent cancer and heart disease, improve immune function, and treat obesity. CLA is the main omega-6 polyunsaturated fatty acid (PUFA) in the diet and is an important essential fatty acid (5).

CLA is found in grass-fed beef and lamb, dairy products, and most vegetable oils, such as sunflower, safflower, and flaxseed oils (Table 12.1). The most common isomer of CLA found in the diet is cis-9/trans-11. CLA contents of dairy products range from 3 to 9 mg/g fat, of which the cis-9/trans-11 CLA isomer comprises between 70 and 90% of the total CLA. CLA found in most dietary supplements is manufactured from sunflower oil or safflower oil. The human body cannot produce CLA; it can only be obtained through diet, including beef and dairy products. The amount of CLA in beef and cow's milk depends on the animals' diet, which over the years has shifted from grazing in pastures to prepared feeds to make them gain more weight and produce more milk. As a result, the CLA content in beef and dairy products has declined steadily.

Daily dietary intake of CLA by humans is rarely known, due to wide variability in the food habits among various regional human populations. Dietary CLA is approximately 0.2% by weight in vegetable oils (6). The amount of CLA is higher in partially hydrogenated soyabean oil and margarine (7). However, CLA is present in large quantities (40–80% of total fatty acids) in tung seed oil, pomegranate seed oil, catalpa seed oil, and bitter gourd seed oil (8, 9). These seed oils contain a mixture of several CLA isomers like trans9-trans11-trans13-18: 3, trans9-trans11-cis13-18: 3, cis-9-trans11-trans13-18: 3, cis9-trans11-cis13-18: 3, and trans8-trans10-cis12-18: 3. Table 12.1 presents a list of foods containing CLAs.

Table 12.1
Conjugated linoleic acid content of various foods (13, 62)

Dairy products	*mg/g*	*Meat/fish*	*mg/g*
Homogenized milk	5.5	Fresh ground beef	4.3
2% milk	4.1	Veal	2.7
Butter fat	6.1	Lamb	5.8
Condensed milk	7.0	Pork	0.6
Cultured buttermilk	5.4	Chicken	0.9
Butter	4.7	Fresh ground turkey	2.6
Sour cream	4.6	Salmon	0.3
Ice cream	3.6	Egg yolk	0.6
Custard style yogurt	4.8		
Frozen yogurt	2.8		
Plain yogurt	4.8		
Low fat yogurt	4.4		

12.2 BIOSYNTHESIS OF CLA

CLA is a derivative of positional and geometrical isomers of linoleic acid (LA, C18:2) involving a double bond at positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13 (10). Each of these positional conjugated diene isomers (Fig. 12.1) can occur in cis–trans, trans–cis, cis–cis or trans–trans geometrical configuration (10). The major contributors to the formation of CLA in the foods are due to heat treatment (11) and microbial enzymatic reactions involving long chain fatty acids mainly linoleic or linolenic acids in the rumen (12). CLA is produced in the rumen as a result of incomplete biohydrogenation of LA as well as during the commercial manufacture of dairy products (13). Dietary lipids in the rumen are rapidly hydrolyzed resulting in unsaturated free fatty acids that undergo biohydrogenation by the rumen microorganisms. When biohydrogenation is not complete, CLA can escape the rumen and be absorbed from the gastrointestinal tract, thereby providing the peripheral tissues with various isomers of CLA (14).

A first pathway is the biohydrogenation of ingested dietary unsaturated fatty acids, e.g., LA, into stearic acid by enzymes of different bacteria present in the rumen (15). Various TFA appear along this biohydrogenation pathway as intermediates, e.g., 9c11t-C18:2 (the main CLA isomer in milk) and *trans*-vaccenic acid (11t-C18:1). Kepler and Tove (16) extracted a linoleate isomerase (EC 5.2.1.5) from the rumen bacteria *Butyrivibrio fibrisolvens*, which is responsible for the isomerization of LA into 9c11t-C18:2 in the first step. In the following, the double bond in position D9 is hydrogenated to form *trans*-vaccenic acid. The last step of the bioconversion is the reduction of *trans*-vaccenic acid into stearic acid. This seems to be the rate limiting reaction. Therefore, the intermediate products 9c11t-C18:2 and *trans*-vaccenic acid are accumulated (17), and they will be absorbed in the intestine and incorporated into different tissues.

In the second pathway (Fig. 12.2), CLA is formed by D9-desaturation of *trans*-vaccenic acid in adipose tissue and in the mammary gland of the lactating cow Griinari et al. (18). The endogenous synthesis in the mammary gland was reported to be very important, as about 60% of CLA in milk fat is formed via this pathway in the lactating cow. CLAs were discovered accidentally by researchers looking for mutagens in beef.

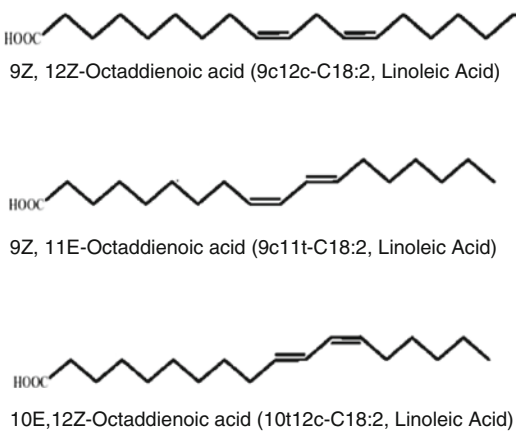


Fig. 12.1. Chemical structure of linoleic acid and conjugated linoleic acid (9c11t-/10t12c-C18:2).

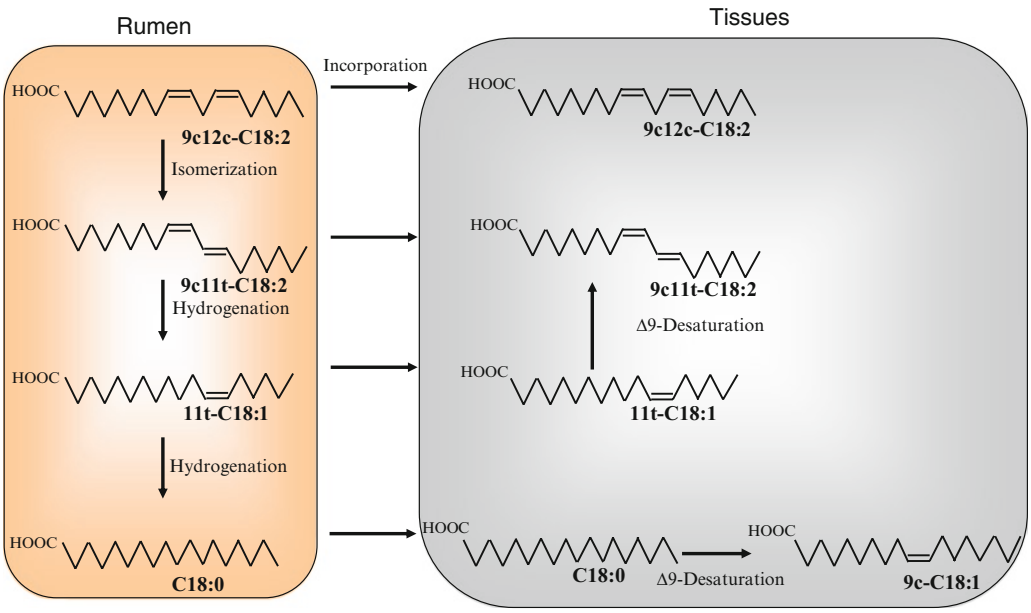


Fig. 12.2. Pathways of CLA biosynthesis (9c11t-C18:2).

In 1979, researchers from the University of Wisconsin applied a beef extract to mice skin (11). The mice were then exposed to a strong carcinogen, which was isolated from tobacco plants. When the researchers counted the number of tumors developed by the mice 16 weeks later, they found that the mice exposed to the beef extract had 20% less number of tumors. The identity of this anticarcinogen was not discovered till 1987.

12.3 IMMUNOMODULATORY POTENTIALS OF CLA

During infection pathogen and/or its end products such as endotoxins or lipopolysaccharides can extract out a series of immune responses in mammalian body (Fig. 12.3). Endotoxins initiate the secretion of cytokines (TNF-α, IL-1) which in turn cause biochemical changes in other cells (19, 20). TNF-α is a key mediator of many diseases such as cachexia (19, 21), carcinogenesis (22, 23), atherosclerosis (24), etc. Earlier research studies on rats, chicks, and mice showed that CLA feeding reduced the adverse effects of endotoxins by reducing body weight loss and improving food intake (25, 26). CLA treatment also reduced the secretion of TNF-α when cells were stimulated with lipopolysaccharides.

The mode of action of CLA that affects the immune response is through the reduction of prostaglandin E_2 which is a regulator of TNF-α. It is reported that CLA reduces arachidonic acid levels in tissues that help in the formation of prostaglandin E_2. As a result of less arachidonic acid, CLA decreased the level of prostaglandin E_2 in serum, spleen, bone, adipose tissue, keratinocytes, and macrophages (25, 27). The reduced adverse effect of endotoxin or TNF-α by CLA was not due to immune suppression because CLA also enhanced immune responses like delayed type hypersensitivity and lymphocyte blastogenesis (25, 26). The results of these studies that use animal models

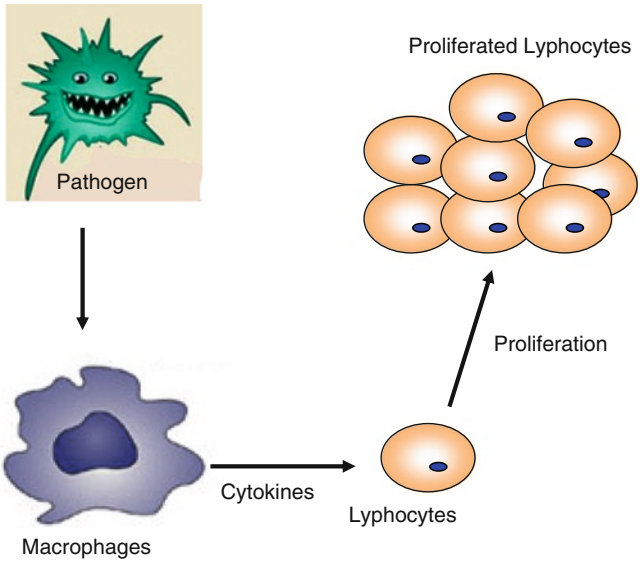


Fig. 12.3. During the immune response, cytokines are released from macrophages to stimulate lymphocytes to proliferate.

suggest that CLA enhances immune function (28–31) while ameliorating immune mediated catabolism (25, 26, 32).

Other reports state that CLA enhanced T-cell function but had no effect on delayed type hypersensitivity or B-cell or natural killer cell activity in mice (33). A research on young healthy women given a dosage of 3.9 g/day CLA for 2 months reported no difference in immune parameters tested between CLA-fed and the control groups (34). A study also stated that CLA reduced transplanted tumor growth in severe combined immunodeficient (SCID) mice, which lack both T and B lymphocytes (35, 36). All these above reported studies suggest that CLA enhance beneficial immune response and ameliorate catabolic immune responses.

In a recent study, the differences in effects of CLA on immunoglobulin subclasses has been observed, when trans-10, cis-12 CLA was fed in human subject. Lymphocytes isolated from these subjects produced more immunoglobulin A (IgA) and IgM but not IgG in comparison with controls (37). The results showed that the 50:50 mixture of fatty acids cis-9, trans-11, and cis-12 CLA increased antigen-specific antibody concentration compared with the 80:20 mixture in human subjects (38). Cis-9, trans-11 CLA isomer was also involved in the enhancement of CD8+ T cells. In this study, the 80:20 mixture significantly enhanced peripheral blood lymphocyte proliferation in response to the T-cell mitogen phytohemagglutinin, whereas the treatment with the 50:50 mixture significantly decreased concanavalin A (Con A) induced blastogenesis (39).

Suppression of the immune system could have explained the ability of dietary CLA to prevent immune-induced Cachexia. Contrary to immune suppression, dietary CLA enhanced immunological responses (40). Dietary CLA increased antibody synthesis (41), increased delayed type hypersensitivity (25), and increased lymphocyte blastogenesis (26). Hence, CLA protection against cytokine induced decreases in gain was not related to immune suppression.

To investigate CLA's role in eicosanoid regulation, an antigen sensitized guinea pig model fed with CLA was studied by Whigham et al. (42). During their experimentation, following antigen sensitization the trachea was collected and superfused with antigen. The trachea from CLA fed guinea pig has decreased antigen-induced prostaglandin E_2 and histamine release. They also checked the eicosanoid profile of antigen sensitized lung, trachea, and bladder, and found that basal mediators, prostaglandin and leukotrienes were not affected by CLA feeding. After antigen sensitization of tissues, there was a significant increase in both the leukotrienes and prostaglandins in tissues of guinea pigs fed with LA. Antigen-induced mediator release was inhibited in tissues from the CLA fed guinea pigs. Another study examined the immunomodulatory role of CLA in mice and rats (26) and results showed that CLA protected against immune stimulation across animal species. Research had shown that during the immune response, macrophages release cytokines IL-1 and TNF-α which later induce the degradation of skeletal muscle and decrease muscle synthesis (43). It was clear from study that IL-1 stimulated the production of prostaglandin E_2 in muscle (44). When prostaglandin E_2 was directly applied to muscle, it would begin a wasting process (Fig. 12.4) (45). Prostaglandin E_2 is an elongated desaturated product of LA. CLA was protecting against cytokine-induced muscle wasting by altering the eicosanoid pathway (25) but CLA decreases prostaglandin synthesis in a number of tissues (46, 47).

To determine the immune suppression activity by CLA, a study was conducted in chicks but there was no positive response. Lymphocytes were harvested from CLA fed mice to determine if it affects lymphocyte proliferation. The lymphocytes from CLA fed and lymphocytes treated in culture with CLA enhanced proliferation (26). It was also observed that CLA increased CD4 and CD8 lymphocyte synthesis increasing natural killer cell number and function (48). Hence, these studies clearly stated that CLA was not suppressing the immune response but enhances the immune response. Recent studies have suggested that CLA decreases the allergic reaction (46). The experiments conducted shows that CLA drives the immune reaction toward a TH_2 type of immune response, thereby decreasing allergies (41).

CLA and n-3 PUFAs have been proposed as important pharmaconutrients for modulating mucosal immunity and therapeutic responses in patients with inflammatory bowel disease (IBD) (49). Recent studies evaluated the ability of CLA and n-3 PUFA

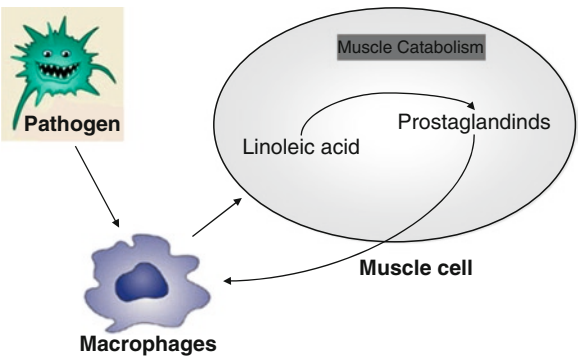


Fig. 12.4. CLA catabolism end products (prostaglandins) and their role in muscle catabolism and immune response.

alone or in combination to modulate IBD in an experimental pig model of dextran sodium sulfate (DSS)-induced colitis. The onset of IBD was delayed, colitis less severe and growth suppression attenuated in pigs fed with CLA, which correlated with induction of colonic peroxisome proliferator-activated receptor-gamma (PPAR-γ) and its responsive gene PPARγ-coactivator-1alpha (PGC-1α) and downregulation of TNF-α. However, dietary supplementation with n-3 PUFA alone or in combination with CLA resulted in an early onset of disease (i.e., day 2) and faster recovery.

CLA reduces bone inflammation (50) and has a positive role in bone formation in rats (51). CLA also improves immune response in healthy men and protects against end-stage symptoms of lupus erythematosus. Multiple studies have found CLA to increase markers of lipid peroxidation and oxidative stress in both healthy and obese humans (52, 53). Few studies supported the fact that CLA may downregulate type I hypersensitivity reactions, leading to less severe immune responses to allergens. CLA may shift the immune response from a TH-2 response (allergic reactions) in favor of a TH-1 type response (cell-mediated functions) (54).

12.4 CONCLUSIONS AND PERSPECTIVES

Several mechanisms are proposed to demonstrate the effects of CLA on the immune system. With the effects of the basal mediators of immunity, it was clearly demonstrated that CLA have modulating effects on TNF-α by altering eicosanoid signaling (55). When eicosanoid signaling is altered, it affects an array of biological functions such as cytokine synthesis, antigen presentation, and various other immunological functions. In another remarkable study, CLA was actively inhibiting prostaglandin E_2 synthesis with likely consequences on immune modulation (56). CLA supplementation in humans led to an increase in cis-9, trans-11 CLA incorporation into cell membranes of isolated peripheral mononuclear cells in comparison with the control subjects having no CLA in their diet (38). Modification of cell membrane results in eicosanoid production and various cell signaling processes. Cell to cell contact is critical during the development of T and B cell functions. This mechanism clearly demonstrates the immunomodulatory effects of CLA.

An alternative hypothesis proves that CLA interacts with peroxisome proliferators activated receptors (PPARs) that regulate the expression of genes for energy homeostasis and immune function (32). PPARs bind to PPAR receptor and suppress or induce the transcription of target genes. The change in gene expression shows effects in myriad of cellular metabolic pathways such as lipid, carbohydrate, and energy metabolism in non-immune and immune cells (57). The CLA isomers are active modulators of PPARs (58, 59). These receptors enhance immune response and regulate gene expression. In similar action to CLA, synthetic PPAR-γ agonists inhibit the proinflammatory cytokines affecting the differentiation of monocytes and macrophages (60).

Definitive molecular evidence *in vivo* on the mechanisms of CLA action is unknown. The use of tissue specific PPAR-γ deficient mice can provide an insight into the mechanism of action of CLA. The understanding of the mechanism of action of CLA that triggers immune function will aid in the development of nutritionally based therapeutic applications to enhance host resistance against infectious diseases and to treat immune imbalances, which results in inflammatory disorders, allergic reactions, and other

immunogenic reactions (61). If the potential benefits of CLA can be characterized further, it can result in increased demand for the consumption of animal derived foods such as dairy foods and a boost to the dairy industry.

REFERENCES

1. O'Keefe JH, Carter MD, Lavie CJ (2009) Primary and secondary prevention of cardiovascular diseases: a practical evidence-based approach. Mayo Clin Proc 84:741–757
2. Grumet FC (1975) Genetic control of the immune response. Am J Clin Pathol 63:646–655
3. Lomax AR, Calder PC (2009) Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. Curr Pharm Des 15:1428–1518
4. Campbell B, Kreider RB (2008) Conjugated linoleic acids. Curr Sports Med Rep 7:237–241
5. Churruca I, Fernández-Quintela A, Portillo MP (2009) Conjugated linoleic acid isomers: differences in metabolism and biological effects. Biofactors 35:105–111
6. Yurawecz MP, Molina AA, Mossoba M, Ku Y (1993) Estimation of conjugated octadecatrienes in edible fats and oils. J Am Oil Chem Soc 70:1093–1099
7. Mossaba MM, Kramer JKG, Pariza MW, Nelson GJ (eds) (1999) Advances in conjugated linoleic acid research, vol 1. AOCS press, Champaign, IL, pp 226–237
8. Takagi T, Itabashi Y (1981) Occurrence of mixtures of geometrical isomers of conjugated octadecatrienoic acids in some seed oils: analysis by open tubular gas liquid chromatography and high performance liquid chromatography. Lipids 16:546–551
9. Suzuki R, Noguchi R, Ota T, Abe M, Miyashita K, Kawada T (2001) Cytotoxic effect of conjugated trienoic fatty acids on mouse tumor and human monocytic leukaemia cells. Lipids 36:477–482
10. Eulitz K, Yurawecz MP, Sehat N, Fritsche J, Roach JA, Mossoba MM, Kramer JK, Adlof RO, Ku Y (1999) Preparation, separation, and confirmation of the eight geometrical cis/trans conjugated linoleic acid isomers 8, 10- through 11, 13-18:2. Lipids 34:873–877
11. Ha YL, Grimm NK, Pariza MW (1987) Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 8:1881–1887
12. Gurr IG (1987) Isomeric fatty acids. Biochem Soc Trans 15:336–338
13. Chin SF, Liu W, Storkson JM, Ha YL, Pariza MW (1992) Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Compost Anal 5:185–197
14. Kelly ML, Berry JR, Dwyer DA, Griinari JM, Chouinard PY, Van Amburgh ME, Bauman DE (1998) Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J Nutr 128:881–885
15. Harfoot CG, Hazelwood GP (1997) Lipid metabolism in the rumen. In: Habson PN, Strewart DS (eds) The rumen microbial ecosystem, 2nd edn. Chapman and Hall, London, UK, pp 382–426
16. Kepler CR, Tove SB (1967) Biohydrogenation of unsaturated fatty acids. 3. Purification and properties of a linoleate D12-cis, D11-trans-isomerase from Butyrivibrio fibrisolvens. J Biol Chem 242:5686–5692
17. Kemp P, White RW, Lander DJ (1975) The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including new species. J Gen Microbiol 90:100–114
18. Griinari JM, Cori BA, Lacy SH, Chouinard PY, Nurmela KVV, Bauman DE (2000) Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. J Nutr 130:2285–2291
19. Beutler B, Cerami A (1987) Cachectin: more than a tumor necrosis factor. N Engl J Med 316:379–385
20. Dinarello CA (1984) Interleukin-1 and the pathogenesis of acute phase response. N Engl J Med 311:1413–1418
21. Freeman LM, Roubenoff R (1994) The nutrition implications of cardiac cachexia. Nutr Rev 52:340–347
22. Okahara H, Yagita H, Miyake K, Okumura K (1994) Involvement of very late activation antigen 4 (VLA-4) and vascular cell adhesion molecule (VCAM-1) in tumor necrosis factor enhancement of experimental metastasis. Cancer Res 54:3233–3236
23. Suganuma M, Okabe S, Sueoka E, Iida N, Komori A, Kim SJ, Fugike H (1996) A new process of cancer prevention mediated through inhibition of tumor necrosis factor expression. Cancer Res 56:3711–3715

24. Ross R (1993) The pathogenesis of artherosclerosis: a prospective for the 1990s. Nature 362:801–809
25. Cook ME, Miller CC, Park Y, Pariza MW (1993) Immune modulation by altered nutrient metabolism: nutritional control of immune induced growth depression. Poult Sci 72:1301–1305
26. Miller CC, Park Y, Pariza MW, Cook ME (1994) Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem Biophys Res Commun 198:1107–1112
27. Park Y, Storkson JM, Liu W, Pariza MW (2000) Effects of conjugated linoleic acid on prostaglandin E2 and tumor necrosis factor in cultured RAW 264.7 cells. FASEB J 14:A238
28. Turek JJ, Li Y, Schoenlein IA, Allen KGD, Watkins BA (1998) Modulation of macrophage cytokine production by conjugated linoleic acids is influenced by the dietary n_6:n_3 fatty acid ratio. J Nutr Biochem 9:258–266
29. Wong MW, Chew BP, Wong TS, Hosick HL, Boylston TD, Shultz TD (1997) Effects of dietary conjugated linoleic acid on lymphocyte function and growth of mammary tumors in mice. Anticancer Res 17:987–993
30. Yang M, Pariza MW, Cook ME (2000) Dietary conjugated linoleic acid protects against end stage disease of systemic lupus erythematosus in the NZB/W F1 mouse. Immunopharmacol Immunotoxicol 22:433–449
31. Bassaganya-Riera J, Hontecillas R, Bregendahl K, Wannemuehler MJ, Zimmerman DR (2001) Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence. J Anim Sci 79:714–721
32. Bassaganya-Riera J, Hontecillas R, Zimmerman DR, Wannemuehler MJ (2002) Long term influence of lipid nutrition on the induction of CD8(+) responses to viral or bacterial antigens. Vaccine 20:1435–1444
33. Hayek MG, Han SN, Wu D, Watkins BA, Meydani M, Dorsey JL, Smith DE, Meydani SN (1998) Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrIBR mice. J Nutr 129:32–38
34. Kelly DS, Taylor PC, Rudolph IL, Benito P, Nelson GJ, Mackey BE, Erickson KL (2002) Dietary conjugated linoleic acid did not alter immune status in young healthy women. Lipids 35:1065–1071
35. Visonneau S, Cesano A, Teppar SA, Scimeca JA, Santoli D, Kritchevesky D (1997) Conjugated linolenic acid suppresses the growth of human breast adenocarcinoma cells in SCID mice. Anticancer Res 17:969–974
36. Cesano A, Visonneau S, Scimeca JA, Kritchevesky D, Santoli D (1998) Opposite effects of linoleic acid and conjugated linoleic acid on human prostatic cancer in SCID mice. Anticancer Res 18:833–838
37. Yamasaki M, Chujo H, Hirao A et al (2003) Immunoglobulin and cytokine production from spleen lymphocytes is modulated in C57BL/6J mice by dietary cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid. J Nutr 133:784–788
38. Albers R, vander-Wielen RPJ, Brink EJ, Hendriks HFJ, Dorovska-Taran VN, Mohede ICM (2003) Effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid (CLA) isomers on immune function in healthy men. Eur J Clin Nutr 57:595–603
39. Roche HM, Noone E, Nugent A, Gibney MJ (2001) Conjugated linoleic acid: a novel therapeutic nutrient? Nutr Res Rev 14:173–187
40. Cook ME, Drake B, Jerome D, Pariza MW (1999) The interaction of 9c, 11t/9t, 11c and 10t, 12c conjugated linoleic acid on the fat deposition in mice. FASEB J 13:A1023
41. Sugano M, Tsujita A, Yamasaki M, Noguchi M, Yamada K (1998) Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. Lipids 33:521–527
42. Whigham LD, Cook EB, Stahl JL, Saban R, Bjorling DE, Pariza MW, Cook ME (2001) Conjugated linoleic acid reduces antigen induced histamine and prostaglandin E2 release from sensitized guinea pig trachea. Am J Physiol Regul Integr Comp Physiol 280:R908–R912
43. Klasing KC, Laurin DE, Peng PK, Fry DM (1987) Immunologically mediated growth depression in chicks: influence of feed intake, corticosterone and interleukin-1. J Nutr 117:1629–1637
44. Hellerstein MK, Meydani SN, Meydani M, Wu K, Dinarello CA (1989) Interleukin-1 induced anorexia in the rat, Influence of prostaglandins. J Clin Invest 84:228–235
45. Rodemann HP, Goldberg AL (1982) Arachidonic acid, prostaglandin E2 and F2 influence rates of protein turnover in skeletal and cardiac muscle. J Biol Chem 257:1632–1638

46. Whigham LD, Cook EB, Stahl JL, Saban R, Pariza MW, Cook ME (1998) Conjugated linoleic acid reduced antigen induced prostaglandin E2 release from sensitized tracheas. FASEB J 12:A819, Abstract 223
47. Cunningham DC, Harrison LY, Shultz TD (1997) Proliferative responses of normal human mammary and MCF-7 breast cancer cell to linoleic acid, conjugated linoleic acid and eicosanoid synthesis inhibitors in culture. Anticancer Res 17:197–204
48. DeVoney D, Pariza MW, Cook ME (1997) Conjugated linoleic acid increases blood and splenic T-cell response post lipopolysaccharide injection. FASEB J 9:3355
49. Bassaganya-Riera J, Hontecillas R, Bertz D (2002) Colonic anti-inflammatory mechanisms of CLA. Clin Nutr 21:451–459
50. Watkins BA, Seifert MF (2000) Conjugated linoleic acid and bone biology. J Am Coll Nutr 19:478S–486S
51. Belury MA (2002) Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu Rev Nutr 22:505–531
52. Basu S, Riserus U, Turpeinen A, Vessby B (2000) Conjugated linoleic acid induces lipid peroxidation in men with abdominal obesity. Clin Sci (Lond) 99:511–516
53. Smedman A, Vessby B, Basu S (2004) Isomer specific effects of conjugated linoleic acid on lipid peroxidation in humans. Regulation by alpha tocopherol and cyclooxygenase-2 inhibitor. Clin Sci (Lond) 106:67–73
54. Whigham LD, Cook ME, Atkinson RL (2000) Conjugated linoleic acid: implications for human health. Pharmacol Res 42:503–510
55. Pariza MW, Park Y, Cook ME (2000) Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proc Soc Exp Biol Med 223:8–13
56. Li Y, Watkins BA (1998) Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E-2 biosynthesis in rats fed n_6 or n_3 fatty acids. Lipids 33:417–425
57. Banni S, Angioni E, Carta G (1999) Influence of dietary conjugated linoleic acid on lipid metabolism in relation to its anticarcinogenic activity. AOCS Pres, Champaign, IL, pp 307–318
58. Moya-Carnarena SY, Vanden Huevel JP, Blanchard SG, Leesnitzer LA, Belury MA (1999) Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPAR-α. J Lipid Res 40:1426–1433
59. Houseknecht KL, Vanden Heuvei JP, Moya-Camarena SY, Portocerrero CP, Peck LW, Nickel KP, Belury MA (1998) Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem Biophys Res Commun 244:678–682
60. Tontonoz P, Nagy L, Alvarez JGA, Thomazy VA, Evans RM (1998) PPAR-γ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 93:241–252
61. Nagpal R, Yadav H, Puniya AK, Singh K, Jain S, Marotta F (2007) Conjugated linoleic acid: sources, synthesis and potential health benefits: an overview. Curr Top Nutraceutical Res 5:55–66
62. Lin H, Boylston TD, Chang MJ, Luedecke LO, Shultz TD (1995) Survey of the conjugated linoleic acid content of dairy products. J Dairy Sci 78:2358–2365

13 Vitamins and Minerals: Contribution to Immune Function and Health

Silvia Maggini

Key Points

- Diet and nutrition are important in the promotion and maintenance of good health throughout the entire life.
- A common denominator playing a role in the prevention of both chronic diseases and infections and hence important for maintaining health is a good functioning immune system.
- The competence of the immune system is an excellent marker of proper nutrition status and health and several changes in immune functions have been related to longevity.
- Preservation of a functionally youthful immune system throughout the years, including ensuring adequate vitamin and mineral status, is the best way to preserve health throughout life and to gain longevity accompanied by good quality of life.

Key Words: Chronic diseases, infectious diseases, immune system, immunonutrition, vitamins, minerals, trace elements.

13.1 INTRODUCTION

Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life course. Both play a role as determinants of chronic diseases and occupy a prominent position in prevention activities (1–3). Adoption of recommended dietary behaviors was associated with lower mortality independent of other lifestyle factors (4). Nutrition has been related to 20–50% of all cancers (5–9) and in atherosclerosis dietary factors can influence inflammatory cells directly or be associated with plaque formation in coronary arteries (10, 11). The profound interactions among nutrition, infection, and health have long been recognized (12, 13). It soon became evident that the interrelationship between undernutrition and infection was synergistic with the effects of the combination being worse than what would be

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_13

predicted from either alone (14). A common denominator playing a role in the prevention of both chronic diseases and infections is a healthy and good functioning immune system. It has been demonstrated that the competence of the immune system is an excellent marker of health and several changes in immune functions have been related to longevity (15–17). The age-related impairment in immune functions, for example, is the cause of the increased vulnerability to infections, cancer, and auto-autoimmune disorders in older age (17–21). The contribution of the diet to immune function has become widely appreciated, and the influence of various dietary components on specific aspects of immune function has been extensively studied (22–27). It is now widely recognized that the general nutritional status of a person modulates his or her immunity and in fact, immunocompetence can be regarded as a measure of adequate nutrition. Next to macronutrients comprising aminoacids, fatty acids, nucleotides, gangliosides, or other dietary components such as probiotics an adequate and regular supply of essential vitamins and minerals (including trace elements) is required to maintain proper immune function (25, 28, 29), and can hence contribute to disease prevention and overall health. This chapter first describes the relationship between micronutrients and both chronic and infectious diseases, and then illustrates the role that specific micronutrients play in supporting various aspects of immune functions, thereby setting the foundation for health.

13.2 MICRONUTRIENTS, CHRONIC DISEASES, AND INFECTIONS

13.2.1 Chronic Diseases

The nutrition-transition toward higher fat and refined carbohydrate diets occurring worldwide combined with increasingly sedentary lifestyles plays a central role in the current global epidemic of obesity and the associated noncommunicable conditions such as diabetes type 2, hypertension, cardiovascular diseases, stroke, some types of cancer, and osteoarthritis. Overall, this is leading to widespread work disability and is negatively impacting the quality of life and increasing mortality (1, 30, 31). Furthermore, the currently augmented life expectancy is leading to a demographic revolution with an exponential increase of elderly individuals. As individuals age, noncommunicable diseases will become the leading causes of morbidity, disability, and mortality in all regions of the world. Chronic diseases are costly to individuals, families, and public budgets, but many of them are preventable or can be postponed (32). Nutrition is emerging as a major modifiable determinant of chronic disease, with scientific evidence increasingly supporting the view that alterations in diet have strong effects, both positive and negative, on health throughout life. Most importantly, dietary adjustments may not only influence present health, but may also determine whether or not an individual will develop such diseases as cancer, cardiovascular disease, and diabetes much later in life (1, 2, 23).

As part of a healthy diet, micronutrients such as vitamins and minerals have a role to play in the prevention of chronic diseases. The results of more than 80 studies indicate, for example, that even moderately elevated levels of homocysteine in the blood increase the risk of cardiovascular diseases (33, 34). The mechanisms by which homocysteine increases the risk of vascular disease remain the subject of a great deal of research, and they may involve adverse effects of homocysteine on blood clotting, arterial vasodilation, and thickening of arterial walls (34, 35). The amount of homocysteine in the blood

is regulated by three vitamins: folate, vitamin B12, and vitamin B6 (33, 34). Although increased homocysteine levels in the blood have been consistently associated with an increased risk of cardiovascular diseases, it is not yet clear whether lowering homocysteine levels will reduce cardiovascular disease risk. Still, most research indicates that a plasma homocysteine level of <10 µmol/l is associated with a lower risk of cardiovascular disease and is considered a reasonable level for individuals at high risk (36). A final answer on whether folate, vitamin B6, and B12 are beneficial for the prevention or treatment of heart disease or stroke is still pending depending on the outcome of ongoing clinical trials. However, it is well established that the amount of homocysteine in the blood is regulated by B vitamins. Therefore, folate, vitamin B12, and vitamin B6 (33, 34) play a role in modulating homocysteine metabolism and possibly contribute to maintaining a healthy and vital heart. Elevated homocysteine concentrations may also have indirect long-term negative effects on brain functions. It has been proposed that B-vitamins may function to preserve and protect the integrity of the central nervous system via their role in the reduction of homocysteine thus preventing vascular disease, which is in turn crucial to cognitive function (37–40). Indeed, cognitive decline in the elderly or Alzheimer's disease have been associated with inadequate nutritional status of folic acid, vitamin B12, and vitamin B6 or with elevated levels of homocysteine (37–39, 41). Furthermore, supplementation with folic acid for 3 years significantly improved domains of cognitive function that tend to decline with age in elderly with elevated homocysteine levels (42).

Antioxidants are needed by the human body for the protection of cell tissues and membranes against freeradical induced damage. Although oxidation reactions are crucial for life, they can also be damaging; for this reason, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin E, selenium as well as enzymes, such as catalase, superoxide dismutase, and various peroxidases, utilizing the trace elements copper, manganese, and selenium. Low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress which has been suggested to be involved in the etiology of a host of chronic diseases, including cancer, cardiovascular disease, cataracts, age-related maculopathies, and aging in general (43–49). Dietary antioxidants have long been considered promising for health promotion based on strong and consistent observational data linking higher intakes or status to lower risks of various chronic diseases and on a large body of experimental evidence demonstrating several mechanisms by which they antagonize pathogenic processes (43–51). Recent randomized studies largely failed to support health benefits of antioxidants for conditions such as cardiovascular disease and cancer. However, so far randomized clinical studies have largely failed to study susceptible populations; have often ignored the role of synergistic cofactors; used the incorrect type, dose and forms of antioxidants; had insufficient duration/follow-up, poor compliance, and most importantly very often lacked any determination of the antioxidant status or oxidative stress status (52–54).

The outcome of most prospective studies indicated that low or deficient intakes of vitamin C were associated with an increased risk of cardiovascular diseases and that modest dietary intakes of about 100 mg/day were sufficient for maximum reduction of cardiovascular disease risk among nonsmoking men and women (55). The results of the pooled analysis of prospective cohort studies suggested that maximum reduction of

coronary heart disease risk may require vitamin C intakes high enough to saturate plasma and circulating cells and thus the vitamin C body pool (56). It was recently shown (49) that an increase in dietary vitamin C intake was associated with less intima media thickness and may protect against the progression of carotid atherosclerosis in elderly men. The brain is also particularly vulnerable to oxidative stress, which is thought to play a role in the pathology of neurodegenerative diseases like Alzheimer's disease (51). Some studies have documented low levels of vitamin E in cerebrospinal fluid of patients with Alzheimer's disease (57). A case-control study examining risk factors for vascular dementia, another common type of dementia, in elderly men found that supplemental vitamin E and vitamin C intake was associated with a significantly decreased risk of vascular and other types of dementia but not Alzheimer's dementia (58). Recent research has identified oxidative stress as one potential feature underlying the toxic effects of air pollutants, which epidemiological studies have clearly shown to be associated with a range of negative respiratory and cardiovascular health effects and increased mortality (59, 60). Short-term randomized supplementation trials suggest that antioxidant micronutrients and n-3 polyunsaturated fatty acids might protect against the acute effect of these pollutants, particularly in vulnerable subgroups (61–63).

A vitamin which has attracted a lot of attention in the recent past is vitamin D. Vitamin D has been recognized for more than a century as essential for the normal development and mineralization of a healthy skeleton. However, the pioneering work of Holick and other researchers (64–75) has opened a wide field of investigation that has now linked vitamin D deficiency to increased risk of cancer, cardiovascular diseases, autoimmune diseases, and infectious diseases (64–78).

Inflammation is a natural process of the body's healing system, indicating increased immune activity at areas of the body which are ill or injured (79–81). As for reactive oxygen species, it is a matter of balance: too much inflammation can cause tissue damage and contribute to the development of chronic diseases. Several diseases that exist among the apparently well-nourished population have a strong immunological component, for example asthma (82–84), Crohn's disease (85), irritable bowel syndrome (86), multiple sclerosis (87–89), rheumatoid arthritis (90–93), food allergies (94, 95), atherosclerosis (96–98), and cancer (99, 100). For some of these diseases, symptoms may be caused or aggravated by an inappropriately activated immune system (101). It is now well recognized that the course of some of these diseases can be influenced by diet, including n-3 polyunsaturated fatty acids as well as various micronutrients (101). Inflammatory states promote, for instance, a decrease in the amount of systemic glutathione (reduced glutathione, GSH) levels. The functions of GSH include antioxidant defense and immune regulation (102). The vitamins B6 and B2 are important in maintaining GSH status (103). Selenium has important oxidation–reduction functions, and selenium-dependent GSH enzymes are involved in the reduction of damaging lipid and phospholipid peroxides to harmless products (104). Furthermore, any other antioxidant micronutrient – such as beta-carotene and vitamins A, C, and E – can become depleted during inflammation (105). There are, therefore, numerous examples highlighting the importance of adequate micronutrient status in order to prevent chronic diseases and to counteract some of the negative effects of excessive oxidative stress and inflammation. Some of the mechanisms involved in disease prevention are linked to immune functions.

13.2.2 Infectious Diseases

Every second of our life, during day and night, immune defenses are constantly protecting the human body not only against attack and invasion by external pathogens such as fungi, bacteria, viruses, but also against internal threats such as cancer cells. Over millions of years, the human defense arsenal has coevolved to meet various attackers and now ranges from simple physical barriers (skin, mucosa) to sophisticated cells as well as biological, chemical, and nuclear weapons (antibodies, cytokines, and free radicals, respectively) (106–109). Nevertheless, new or reemerging infectious diseases are still major health issues in the twenty-first century.

Infectious diseases are the leading cause of death worldwide and also the third leading cause of death in the United States (110). The past decades have seen the emergence of new pathogenic infectious diseases, such as acquired immunodeficiency syndrome, multidrug-resistant tuberculosis, avian (111–115) and swine (116, 117) flu, and tick-borne diseases, which represent a substantial global threat to human health (118–120). Infections are particularly prevalent in developing countries, where coinfection with opportunistic pathogens is common. The adverse impact of infectious diseases is most severe among people with poor nutritional status, no access to integrated health care, prevention tools, and medications. Infectious diseases constantly raise awareness of our global vulnerability, the need for effective preventive measures – including vaccines but also supplementation or fortification with critical micronutrients – and strong health care systems (110, 121). Between 14 and 17 million people die each year due to infectious diseases (110, 121, 122). Children are particularly vulnerable: pneumonia, diarrhea, and malaria are leading causes of death among children under age five and diseases such as cerebral malaria can cause permanent mental impairment (110, 121, 122). Studies in more than 12,000 children have demonstrated that zinc supplementation alone produced a 20% reduction in overall diarrhea incidence, a 15% reduction in overall incidence of acute lower respiratory infections, a 6% reduction in overall child mortality – the latter observed in studies with more than 200,000 children (123). Similarly, high-dose vitamin A supplementation is known to reduce morbidity and mortality in acute measles and is now the recommended therapy for measles in developing countries and in the United States (124, 125). In addition, the benefits of vitamin A supplementation in reducing the morbidity and mortality from diarrhea in preschool children in developing countries have been reviewed along with data showing reduction of morbidity and mortality from acute respiratory infections, malaria, tuberculosis, and infections in pregnant and lactating women (126). Infectious diseases are also destructive to the health of adults, causing a diminished quality of life, decreased productivity, disability or death (110, 121). People infected with one infectious disease become more susceptible to other diseases. Examples include: HIV/AIDS coinfection with tuberculosis or malaria coinfection with multiple neglected diseases (127–129).

There are various reasons why infectious diseases should not be underestimated in the developed world. By interfering with natural selection, via e.g., excessive hygiene, excessive use of antibiotics etc., the human species as a whole may have become more susceptible to new infections (120). Bacteria and viruses are where nature's immense creative power (i.e., mutation) is most directly effective, constantly generating new agents and variants to attack our defenses (130, 131). The growing human population density, cities and slums together with poverty, famine, war, etc., enable the new agents

to catch hold and reach critical epidemiological mass. This combined with the "shrinking" world means the rapid and global exposure of diverse populations facilitating the establishment of serious epidemics (118–120). Changes in the climate, such as global warming, can cause microorganisms to adapt and create new strains, which can give them an evolutionary advantage and new pathogenicity (118, 119).

While multiple factors determine whether an individual will become sick or not, the immune system remains the first line of defense against all external pathogens and noxious insults. Furthermore, infectious diseases can affect the status of several nutrients in the body, thus setting up a vicious circle of undernutrition, compromised immunity and recurrent infection. Therefore it is of utmost importance to adequately feed the immune system by providing those micronutrients – e.g. vitamins A, D, E, C, B6, B12, and folate as well as of the minerals selenium, zinc, iron, and copper (25, 28, 29) – needed to ensure proper functioning. Further examples of the roles of micronutrients in supporting immune functions and combating diseases will be given in the following sections.

13.3 THE IMMUNE SYSTEM NEEDS MICRONUTRIENTS

13.3.1 The Immune System

The immune system is a complex and sophisticated network of specialized tissues, organs, cells, proteins, and chemicals (including free radicals) which has evolved in order to protect the host from a range of dangerous agents such as bacteria, virus, fungi, parasites as well as cancer cells; foreign substances or matter as for instance organ transplants and other noxious insults (for recent reviews see (107–109)). Innate immunity ensures the first, nonspecific response of the immune system to a foreign attack. It is found in nearly all forms of life and it is present already at birth and ensures an immediate maximal response upon exposure. Examples of innate immunity include physical barriers such as skin, mucus secretions, and the acidity of the stomach, acute phase proteins and enzymes, and an array of cellular components involved in phagocytosis (monocytes, macrophages, dendritic cells, granulocytes, etc.). Adaptive immunity is the second barrier to infection taking over if the innate immunity cannot clear the infection in a short time. It is found only in jawed vertebrates and is acquired later in life, such as after an immunization or successfully fighting off an infection. It retains a memory of all the invaders it has faced, and so the second time an intruder tries to attack the body, B and T memory cells help the immune system to activate much faster. This response is pathogen and antigen specific and requires a lag time between exposure and maximal response. It consists of both cell-mediated and humoral components. Cells of adaptive immunity include B-cells and T-cells (cytotoxic, helper and regulatory T-cells). Cytotoxic T lymphocytes express the surface protein marker CD8+ and kill cells infected with viruses and cancer cells. T helper cells are characterized by the surface marker CD4+. CD4+ cells function either as T helper cells type 1 (Th1) or type 2 (Th2). Th1 and Th2-type activities are considered to be of utmost importance and guarantee an adequate and efficient immune response (132, 133). Th1 are the major promoters of cell-mediated reactions involved in effective defense against intracellular pathogens, while the Th2 response primarily activates humoral immunity and the antibodies produced are only effective against pathogens in the extracellular fluids (106). Both CD4+ and CD8+ cells produce

immunomodulatory cytokines (107). Humoral immunity refers to the branch of immunity that is mediated by secreted antibodies produced in the B-cells. Secreted antibodies bind to antigens on the surfaces of invading pathogens presenting them to macrophages for destruction. An important weapon of the immune system is generation of reactive oxygen species which can act directly to incapacitate pathogens or can act indirectly, for example, by modulating gene expression (134, 135). The oxidant-antioxidant balance is an important determinant of immune function, and immune cells are particularly sensitive to changes in this balance because of the higher percentage of polyunsaturated fatty acids in their plasma membranes (135). Oxidative damage can lead to a loss of membrane integrity, altered membrane fluidity and result in alterations in the transmission of signals both within and between different immune cells (134–139).

Immune function is influenced by a variety of different factors, including genetic as well as environmental factors. Previous exposures to a disease-causing pathogen or vaccinations also determine the immune response. Interindividual variations in many immune functions are due to genetics, age, gender, stage in the female menstrual cycle, body mass index, diet, and lifestyle (26, 27). Alcohol consumption, stress (environmental, physiological, and psychological), anxiety and depression, intensity, and duration of physical exercise are additional factors impacting immune functions (27).

13.3.2 Micronutrients for Immune Function

Diet and nutrient status are important factors contributing to immunocompetence. Recently, substantial research has focused on the role of nutrition and especially on the contribution of micronutrients to an optimum functioning of the immune system (25–29). Vitamins and minerals, including trace elements, are essential active agents required by every living cell for growth, development and all metabolic pathways and are crucial to their well-balanced coordination. They are, thus, indispensable for maintaining health and life of all living organisms, from bacteria to humans. Minerals and trace elements are inorganic substances and have to be taken up by all living organisms, whereas vitamins can be synthesized by many species. Humans, however, have lost this ability and cannot synthesize vitamins (except vitamin D) , andtherefore are dependent on a continuous exogenous supply. A regular and adequate intake of these micronutrients is required to remain healthy and to be able to function. This indispensability of regular intake is expressed in the need for official Recommended Dietary Allowances (RDAs) elaborated and published by most health authorities, especially in the developed countries for example (43, 140–142). Insufficient intake of multiple nutrients is more frequently occurring than a single deficiency due to poor nutrition, especially in developing countries, but micronutrient undernutrition is also observed in industrialized countries (25, 143, 144). Deficiencies of more than one micronutrient can arise in such situations and risk groups as weight reducing diets; insufficient and imbalanced nutrition; eating disorders; in demanding periods such as during extensive exercise or in situations of emotional and physiological stress; increased requirements, e.g., pregnancy, lactation; growth; among the elderly, in smokers and individuals with chronic alcohol abuse, in patients with certain diseases and following frequent infections (25, 143, 144).

Already marginal micronutrient deficiencies suppress immunity by affecting innate, T-cell mediated and adaptive antibody responses, leading to dysregulation of the balanced

host response. Micronutrients that have been demonstrated to be required for the immune system to function efficiently include vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, vitamin D, zinc, copper, iron, and selenium (23, 25, 27–29). These vitamins and minerals contribute to the body's natural defenses on three levels by supporting physical barriers (skin/mucosa), cellular immunity, and antibody production. Vitamins A, C, E, and the trace element zinc assist in enhancing the epithelial barrier function. The vitamins A, B6, B12, C, D, E, and folic acid and minerals iron, zinc, copper, and selenium work in synergy to support the protective activities of the immune cells. Finally, all these micronutrients, with the exception of vitamin C and iron, are essential for antibody production. Overall, inadequate intake and status of these vitamins and minerals/trace elements may lead to suppressed immunity, which predisposes to infections and aggravates malnutrition. In addition, micronutrients also have a role to play in the prevention of chronic diseases through mechanisms at least in part linked to proper immune function. Low levels of antioxidants, inhibition of the antioxidant enzymes and excessive inflammation cause oxidative stress involved in the etiology of a host of chronic diseases (20, 44, 45, 87–93, 96, 97, 99, 100). Chronic inflammation elicits changes in body composition, alters the use of various macronutrients and increases cellular consumption of important vitamins and minerals (145). Usually, the inflammation and tissue destruction that are associated with the mechanisms used to eradicate a pathogen are acceptable to the host and do not cause significant impairment of host function. However, in some cases the destruction by the activated immune system is substantial, long-lasting, and harmful. It is because of the potentially damaging effects of the immune cells on body tissues that the system is very tightly regulated. Failure of these regulatory mechanisms can result in the full might of the immune system being inappropriately directed against the body's own tissues and in the development of chronic inflammatory or autoimmune diseases. Clearly, attempts to modulate immune function by nutritional means are appropriate in these conditions. Promising micronutrients include vitamin A and D and their metabolites via their immunomodulatory properties (146–151) or vitamins and minerals involved in antioxidant defenses (145).

Several reviews have recently evaluated the role of selected vitamins and minerals in immune function (22, 23, 25–29), therefore only an overview summarizing the main outcome of these papers is shown in Tables 13.1–13.3.

The next sections focus on some examples highlighting the multifaceted roles of some selected micronutrients on different aspects of immune function and hence health.

13.3.3 Micronutrient Deficiencies and Increased Virulence

The previous sections have illustrated how proper nutrition is important from the standpoint of the host in order to support immune functions. However, the pioneering work of Melinda Beck has shown that nutrition plays a role also from the standpoint of the pathogen. On one hand, proper nutrition aids the host in withstanding and responding to a viral infection, at the same time an adequate diet may also decrease the opportunity for the pathogen to increase its fitness by mutation.

Using a mouse model of coxsackie virus-induced myocarditis, Beck and coworkers (152–156) have shown that in an infected host, deficiency in either selenium or vitamin E lead to changes in viral phenotype, such that a nonvirulent strain of the virus became

Table 13.1
Fat-soluble vitamins: functions, main roles in the immune system, consequences of deficiency

Vitamin	*Functions (43, 140, 142)*	*Main roles in the immune system (22, 23, 25–29)*	*Consequences of deficiency (22, 23, 25–29)*
Vitamin A	Vitamin A is a generic term that designates any compound having the biological activity of retinol The term retinoids includes both naturally occurring forms of vitamin A and synthetic analogs of retinol Vitamin A is essential for normal development and important for normal vision, epithelial integrity, growth and differentiation of tissues, reproductive function, growth and development of the mammalian embryo, gene expression and immune function	Important for innate, cell mediated immunity and antibody response Required for normal differentiation of epithelial tissue and gene expression Crucial in development and differentiation of Th1 and Th2 lymphocytes Retinoic acid, is essential to imprint T cells and B cells with gut-homing specificity, and thus to array T cells and IgA+ cells into intestinal tissues Supplementation reduces the morbidity and mortality from infectious diseases (especially in children) and improves antibody response to vaccinations	Decreased cellularity of lymphoid organs, circulating monocytes, lymphocytes and CD4/8 cells, lymphocyte proliferation, IL-2 and IFN-gamma production, cytotoxic T lymphocyte and NK cell activity, respiratory burst and bacterial killing, circulating immunoglobulin and antibody response, DTH response Increased susceptibility to infection (e.g. respiratory, diarrhea, severe measles) Associated with breakdown of gut barrier and skin infections Deficiency induces inflammation and potentiates existing inflammatory conditions
Vitamin D	The classical biological function of vitamin D (mediated through its metabolites) is to maintain calcium and phosphorous homeostasis by regulating the intestinal absorption of these nutrients Essential for bone formation and resorption	A potent immune system modulator when metabolized in the body to 1,25(OH)2D3 Vitamin D receptor is expressed by most cells of the immune system Involved in cell proliferation, innate and adaptive immunity Increased production of 1,25(OH)2D3 results in the synthesis of cathelicidin, a peptide capable of destroying *M. tuberculosis* and other infectious agents Polymorphisms in the vitamin D receptors seem to have an effect on the susceptibility to and outcome of some cancers	Large proportions of the population are vitamin D deficient Increased incidence of Th1 cell-mediated autoimmune diseases (e.g. multiple sclerosis, type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematous) Higher susceptibility to infections due to impaired localized innate immunity and defects in antigen specific cellular immune response

(continued)

Table 13.1
(continued)

Vitamin	*Functions (43, 140, 142)*	*Main roles in the immune system (22, 23, 25–29)*	*Consequences of deficiency (22, 23, 25–29)*
Vitamin E	A potent nonspecific chain-breaking, lipid-soluble antioxidant required for the protection of cell membrane Protects polyunsaturated fatty acids (PUFAs) within the membrane phospholipids and in plasma lipoproteins In form of α-tocopherol, inhibition of protein kinase C activity, which is involved in cell proliferation and differentiation, in e.g. monocytes	Enhances T cell-mediated functions, lymphocyte proliferation, IL-2 production, NK cell cytotoxic activity and decreases production of the immunosuppressive factor PGE2 Supplementation results in increased resistance to infection Optimizes and enhances Th1 and suppresses a Th2 response Supplementation of elderly individuals improves overall immune function	Decreased lymphocyte proliferation, natural killer (NK) cell activity, antibody production, phagocytosis, delayed type hypersensitivity (DTH) response Increased susceptibility to infection Deficiency causes viruses to undergo mutations to more virulent forms Deficiency rare in humans

Table 13.2
Water-soluble vitamins: functions, main roles in the immune system, consequences of deficiency

Vitamin	*Functions (43, 141)*	*Main roles in the immune system (22, 23, 25–29)*	*Consequences of deficiency (22, 23, 25–29)*
Vitamin C	A highly effective, water-soluble antioxidant that operates in the aqueous phases both intra- and extracellularly Can regenerate other antioxidants (e.g. vitamin E) Primarily a cofactor for reactions requiring reduced copper or iron metalloenzyme Known to be an electron donor for eight human enzymes participating in collagen hydroxylation (3 enzymes); carnitine biosynthesis (2 enzymes) and hormone and amino acid biosynthesis (3 enzymes)	Supports integrity of epithelial barrier by promoting collagen synthesis Maintains redox integrity of cells and protects against reactive oxygen species generated during respiratory burst and inflammatory response Stimulates leukocyte functions (neutrophil, monocytes movement) Regulates immune response via its antiviral and antioxidant properties Decreases duration/severity of common cold Reduces incidence of common cold and pneumonia in subjects engaged in strenuous exercise or who live in crowded situations	Decreased interferon, T lymphocyte activity, collagen production Decreased of resistance to disease. High supplemental intakes stimulate phagocytic and T-lymphocytic activity
Vitamin B6	Comprises a group of six related compounds: pyridoxal, pyridoxine, pyridoxamine and their respective 5′-phosphates The coenzyme forms are pyridoxal 5′ phosphate and pyridoxamine 5′ phosphate, and these are necessary for nearly 100 enzymatic reactions Involved in synthesis and breakdown of amino acids, the conversion of amino acids to carbohydrate or fat, metabolism of glycogen and sphingoid bases	Interferes with immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B12 and folate Adequate intake is maintaining a Th1 immune response	Decreased lymphocyte proliferation, suppression of Th1 and promotion of Th2 cytokine-mediated activity, decreased IL-2, DTH response and antibody production Overall, suppressed immune response and atrophy of lymphoid organs

(continued)

Table 13.2 (continued)

Vitamin	*Functions (43, 141)*	*Main roles in the immune system (22, 23, 25–29)*	*Consequences of deficiency (22, 23, 25–29)*
Vitamin B12	Cofactor for methionine synthase and l-methylmalonyl-CoA mutase Essential for nucleic acid metabolism, metabolism of fats and carbohydrates and synthesis of proteins Required for transport and storage of folate in cells and for conversion to its active form Needed for the formation of red blood cells, prevention of megaloblastic anemia and neurological function	Interferes with immune function through involvement in nucleic acid and protein biosynthesis in concert with vitamin B6 and folate May act as immunomodulatory for cellular immunity, especially with effects on cytotoxic cells (NK; CD8 T lymphocytes)	Suppression of NK cell activity, decreased number of lymphocytes and CD8 cells and proportion of CD4 cells leading to an abnormally high CD4/CD8 ratio Decreased neutrophil phagocytosis May lead to megaloblastic anemia (characterized by large and immature red blood cells) and defective DNA synthesis in cells
Folate/folic acid	Folate is the generic term for this water-soluble B-complex vitamin: it functions in single-carbon transfer reactions and exists in many chemical forms Folic acid is the most oxidized and stable form of folate used in vitamin supplements and in fortified food Involved in DNA synthesis, purine synthesis, generation of formate into the formate pool (and utilization of formate), and amino acid interconversions (including conversion of homocysteine to methionine) Prevention of megaloblastic anemia	Interfere with immune function through involvement in nucleic acid and protein biosynthesis in concert with vitamins B6 and B12 Maintain innate immunity (NK cell activity)	Cell-mediated immunity especially affected: decreased circulating lymphocytes, lymphocyte proliferation and cytotoxic T lymphocyte activity Overall impaired resistance to infections Atrophy of lymphoid organs

Table 13.3
Minerals: functions, main roles in the immune system, consequences of deficiency

Mineral	*Functions (43, 142)*	*Main roles in the immune system (22, 23, 25–29)*	*Consequences of deficiency (22, 23, 25–29)*
Selenium	Most selenium in animal tissues is present as selenomethionine or selenocysteine Functions largely through association with so-called selenoproteins (e.g. selenium-dependent glutathione peroxidases) Defense against oxidative stress and regulation of thyroid hormone action, and the reduction and oxidation status of vitamin C and other molecules	Essential for optimum immune response: influences both innate and acquired immunity Needed for redox regulation and antioxidant function through glutathione peroxidases by removing excess of potentially damaging radicals produced during oxidative stress Supplementation suppresses progression of HIV-1 viral burden and improves CD4 counts	Neutrophil phagocytosis may be impaired Deficiency decreases immunoglobulin titers and aspects of cell-mediated immunity. Supplementation counteracts these effects May contribute to reduced immune function, some cancers, viral diseases and overall increased susceptibility to infections Deficiency causes viruses to undergo mutations to more virulent forms
Zinc	Essential nutrient important in cellular growth and differentiation with profound effects on the immune system, in collagen synthesis and antioxidant defenses Its catalytic function is required for biological activity of more than 300 enzymes and proteins and a component of 1,000 transcription factors, including DNA-binding proteins with zinc fingers Involved in the regulation of gene expression	Acts on cellular and humoral immunity, helps to maintain skin and mucous membrane integrity Cell protection from damaging effects of reactive oxygen radicals and reactive nitrogen species produced during immune activation	Deficiency impairs phagocytosis of macrophages and neutrophils, NK cell activity, oxidative burst generation, complement activity Thymus involution, depressed lymphocytes proliferation, Th1 cytokines production, DTH skin responses and antibody response Increased susceptibility to infections, especially for children and the elderly Atrophy of lymphoid organs Adverse effects on bone marrow

(continued)

Table 13.3
(continued)

Mineral	*Functions (43, 142)*	*Main roles in the immune system (22, 23, 25–29)*	*Consequences of deficiency (22, 23, 25–29)*
Iron	Necessary component of hemoglobin for oxygen transport, of myoglobin for transporting and storing oxygen in the muscle and releasing it when needed during contractionFacilitates transfer of electrons in respiratory chain and is thus important in ATP synthesis Necessary for red blood cell formation and function Component of numerous enzymes Prevents macrocytic hypochromic anemia	Essential for cell differentiation and growth, component of enzymes critical for functioning of immune cellsRequired for T cell responses (DNA synthesis, ribonucleotide reductase) and generation of reactive oxygen specie Involved in the regulation of cytokine production and action Involved in the killing process of bacteria by neutrophils through the formation of highly toxic hydroxyl radicals	Decreased circulating monocytes, lymphocytes and CD4 cells, CD4/8 ratio, IL-2 and IFN-gamma production, cytotoxic T lymphocytes and NK cell activity, respiratory burst by neutrophilsSupplementation can reverse such alterations Increased susceptibility to infection Atrophy of lymphoid organs Changes in cellular iron homeostasis to either deficiency or overload have unfavorable functional consequences on the immune system
Copper	Involved in iron metabolism, nervous system functioning, bone health, and synthesis of proteins Essential cofactor of cytochrome *c* oxidase involved in aerobic oxidation Important for antioxidant activity and energy production	Part of Cu/Zn-superoxide dismutase, a key enzyme in the defense against reactive oxygen species Maintains intracellular antioxidant balance, suggesting an important role in inflammatory response Important role in the innate immune response (macrophages, neutrophils, monocytes), changes in homeostasis are a crucial component of respiratory burst	Abnormally low numbers of neutrophils (neutropenia), decreased mononuclear cell proliferation Increased susceptibility to infections, increased severity of infections Deficiency is linked to increased virulence (in animals) Both deficiency and oversupply detrimentally impact immune response

virulent and a virulent strain became more virulent. The change in phenotype was shown to be due to point mutations in the viral genome. Once the mutations occurred, the phenotype change was stable and could be expressed even in mice with normal nutrition (152–156). More recently (157), copper has also been linked to increased virulence of coxsackie virus strains in animals. Copper deficiency increased viral replication in the heart, increased cardiac pathology and appeared to impair specific antibody production and increase pro-inflammatory gene expression, overall leading to higher viral load and subsequently enhanced pathology (157).

Therefore, if the nutritional status of the host is poor, due to either single or multiple micronutrient deficiencies, the negative consequences are twofold. First, the host immune system is compromised, which will lead to an increased susceptibility to infectious disease. Second, the micronutrient deficiency can increase the pathogenicity of the invading viruses, further worsening the host's situation and endangering those in his/her neighborhood.

13.3.4 The Multiple Roles of Vitamin D in Immune Functions and Health

Vitamin D is produced in the skin from endogenously available 7-dehydrocholesterol, a precursor of cholesterol, by the action of sunlight or UV light. Few foods (e.g., oily fish) naturally contain or are fortified with vitamin D (73, 158, 159). Vitamin D (calciferol) itself is not known to have any biological activity as such, but has to be converted into biologically active metabolites 25-hydroxy cholecalciferol [25(OH)D3], formed in the liver, and 1,25-dihydroxy cholecalcierol [1,25(OH)2D3], produced in the kidneys. Vitamin D is historically known for its role in preventing rickets and maintaining bone health, but the subsequent discoveries of its hormonal activation and its nuclear receptor binding both possible in many tissues have opened new avenues for this "old" vitamin (160).

Serum 25(OH)D3 concentration is the functional status indicator for both vitamin D efficacy and safety. Vitamin D deficiency is defined by most experts as a 25(OH)D3 level of less than 20 ng/ml. 25(OH)D3 between 21 and 29 ng/ml indicates a relative insufficiency and a level of 30 ng/ml or greater indicates sufficient vitamin D status (73, 161, 162). Vitamin D intoxication is only observed when serum levels of 25(OH)D3 exceed 150 ng/ml (73, 163). Worldwide, it is estimated that 30–50% of the general population is vitamin D deficient (73, 164, 165). The figures are even worse for vulnerable target groups such as the elderly. According to several studies 40–100% of both the US and European elderly still living in the community (not institutionalized) are vitamin D deficient (72, 73, 162, 166–170). There is increasing consensus that current RDAs between 200 and 400 IU/day (<70 years of age) and 600 IU (>71 years of age) (140) are insufficient and experts now recommend daily intakes between 1,000 and 2,000 IU/day for adults and elderly (73, 162, 163, 171, 172).

1,25(OH)2D3 is the biologically active metabolite of vitamin D, and it regulates gene expression in target cells via binding to the vitamin D receptor. Various tissues, including brain, prostate, breast, and colon, among others, as well as immune cells carry the vitamin D receptor and respond to 1,25(OH)2D3. Directly or indirectly, 1,25(OH)2D3 controls more than 200 genes, including genes responsible for the regulation of cellular proliferation, differentiation, apoptosis, and angiogenesis (73, 173, 174). 1,25(OH)2D3 decreases cellular proliferation of both normal cells and cancer cells and induces their terminal differentiation. Recent research has confirmed that 1,25(OH)2D3 is a potent

immunomodulator (76, 175–180). Both innate and adaptive immunity are influenced by vitamin D (181) by an ever increasing array of different mechanisms (73, 149, 151, 182–187). For example, monocytes and macrophages exposed to lipopolysaccharide or to *Mycobacterium tuberculosis* upregulate the vitamin D receptor gene and the 25-hydroxyvitamin D-1α-hydroxylase gene. Increased production of 1,25(OH)2D3 results in the synthesis of cathelicidin, a peptide capable of destroying *M. tuberculosis* as well as other infectious agents (188–194). When serum levels of $25(OH)D_3$ fall below 20 ng/ml, the monocyte or macrophage is prevented from initiating this innate immune response with deleterious consequences for the immune defenses (73, 191). It has been also speculated that one of the reasons why influenza occurs in the winter time in tepid climates is because the sun is unable to produce vitamin D in the skin, and the resulting vitamin D deficiency may promote and enhance the infectivity of the influenza virus (73, 191). Vitamin D deficiency has been shown to predispose children to respiratory infections and ultraviolet radiation, either from artificial sources or from sunlight, reduced the incidence of viral respiratory infections, as did cod liver oil – a source of vitamin D (195).Volunteers inoculated with attenuated live influenza virus were more likely to develop fever and serological evidence of an immune response in the winter (195). There is promising evidence for vitamin D as an adjuvant therapy for tuberculosis, influenza, and viral upper respiratory illnesses (196).

With regard to chronic diseases, epidemiologic studies have associated vitamin D deficiency with increased risk of auto-immune diseases, including multiple sclerosis (68, 197) and rheumatoid arthritis (198–201), cardiovascular diseases (202–204), diabetes (205, 206), and cancers (207, 208) such as colon, prostate, and breast cancer. Obviously, correction of low vitamin D status does not guarantee prevention of these multifactorial disorders. However, in view of the alarming prevalence of vitamin D deficiency and the accumulating epidemiological, experimental, and interventional evidence supporting the health promoting roles of vitamin D, it is highly recommendable to adopt measures aiming at normalizing vitamin D status. A responsible and controlled sun exposure (e.g. 2–3 times a week, 5–10 min sun on face, arms, and legs as proposed by Holick (69)) or dietary supplementation with vitamin D may be easy and cost-effective measures that can improve resistance to infections, chronic diseases, and long-term health.

13.3.5 Antioxidants for Immune Functions and Longevity

From the moment we are born, our immune system continually has to face a great variety of foreign agents and has to always stay active in order to protect the organism against external and internal threats. As illustrated previously, the immune system consists of a wide arsenal of weapons to fulfill its defensive role, among them it relies on releasing toxic oxidant and inflammatory compounds. These two defense mechanisms are double-edged swords and need to be carefully balanced by adequate defense systems in order to avoid damaging the host.

Aging is accompanied by impairment of the physiological systems, including the nervous, endocrine and immune systems, as well as of the nervous-immune communication. The age-related impairment of immune function is called immunosenescence, and it causes increased susceptibility to infections, cancer, and autoimmune diseases overall leading to increased morbidity and mortality in older individuals (209, 210).

Not all immune cell types or all functions of an immune cell show a significant impairment with aging. However, a pronounced decrease in T-cell functions and age-related alterations of phagocyte cell functions have been observed (17).

A wide array of representative immune responses and several parameters of oxidative stress have been standardized at different ages in mice and humans and demonstrated to display similar changes with aging in leucocytes in both species. In a murine model of premature aging, these functions showed values characteristic of chronologically older animals, and these immunosenescent animals further had a significantly shorter life span (18–21). On the other hand, animals reaching very old age had immune parameters similar to those of younger adult animals (17, 211). These same standardized set of immune responses has been assessed in healthy centenarians, and it was found that in those individuals, the immune responses performed much better than in 70-year-old human subjects and were comparable to those observed in young adults (30 years old) (17, 212, 213). Therefore, all the above animal and human results indicate that the immune system is a marker of biological age and a predictor of longevity and health as originally postulated already some years ago (15, 16).

The causes of immunosenescence have been investigated as well. Since the oxidative theory of aging is the most widely accepted one, several parameters of oxidative stress and antioxidant defenses have been studied in animals and humans alike. Indeed, with aging, the immune cells revealed an increase in oxidant and inflammatory compounds and a decrease in antioxidant defenses, which was more evident in phagocytic cells (20, 50, 138). The immune cells of prematurely aging mice showed marked oxidative stress, whereas leucocytes from very old animals or healthy centenarians showed values of both oxidative and antioxidant compounds and defenses similar to those found in the cells of younger adult mice or humans (17, 138, 212). This chronic oxidative stress, which has among its intracellular mechanisms the activation of NF-kappa B in the leucocytes, affects all cells and especially those of regulatory systems such as the nervous, endocrine, and immune systems. Accordingly, the administration of antioxidants such as vitamins C, E, zinc, selenium, beta-carotene, polyphenols, and soy isoflavones in animals and in humans was found to improve both the nervous and immune functions, decreasing their oxidative stress, and consequently leading to a significant increase in longevity (20, 50, 138, 214–219).

These observations have led to the formulation of a new theory of aging: the so-called oxidation-inflammation theory with the immune system as a key player (17, 20). The immune system, in the context of neuroimmune communication, is pivotal in the preservation of health and longevity. If its regulation is impaired, it contributes to the chronic oxidative stress that underlies aging. An adequate regulation of this system by administration of the appropriate amounts of antioxidants can, through better neuroimmunomodulation, improve the redox condition of the cells of the involved systems and therefore organism homeostasis. Altogether this can result in a decrease in morbidity and mortality. It, therefore, seems that the preservation of a functionally youthful immune system throughout the years is the best way to gain longevity accompanied by good quality of life.

According to WHO data (110), cardiovascular diseases were the leading cause of death in the world, particularly among women, in 2004. Infectious and parasitic diseases were the next leading cause, followed by cancers, respiratory infections, and diseases.

Worldwide, the proportion of people aged 60 and over is growing faster than any other age group (32). This rise in life expectancy will inevitably be associated with a higher number of total deaths due to noncommunicable diseases over the next 25 years (110). However, the 2004 WHO data indicate that despite the progress made with vaccination campaigns and improved hygienic conditions in many parts of the world, infectious diseases continue to represent a major threat to human health. Moreover, the past decades have seen the emergence of new pathogenic infectious diseases (e.g., HIV, avian and swine flu). There are various reasons why infectious diseases should not be underestimated also in the developed world, including excessive use of antibiotics and hygiene, growing population density, poverty and war, and increased mobility. These factors will enable new pathogens to catch hold and reach critical epidemiological mass which, combined with the rapid and global exposure of diverse populations, will facilitate the establishment of serious epidemics.

A number of genetic and environmental factors as well as socioeconomic status, availability of adequate health and social services determine wellbeing, overall health and longevity. Diet and nutrition, including appropriate amounts of essential vitamins and minerals, are among the modifiable factors that can help prevent chronic disease and functional decline, thus extending longevity and enhancing quality of life. Oxidative stress and excessive inflammation, for instance, have been suggested to be involved in the etiology of a host of chronic diseases and both can be ameliorated by dietary interventions. Adequate nutrition is also required to ensure resistance to infections and the deleterious synergistic interrelationship between undernutrition and infection has been long recognized. A common denominator playing a pivotal role in the prevention of both chronic diseases and infections is a healthy and good functioning immune system. It has been demonstrated that the competence of the immune system is an excellent marker of health and several changes in immune functions have been related to longevity. In healthy centenarians, for instance, the immune responses perform much better than in 70-year-old human subjects and are comparable to those observed in young adults. Furthermore, with aging, the immune cells revealed an increase in oxidant and inflammatory compounds and a decrease in antioxidant defenses, which was more evident in phagocytic cells. Accordingly, the administration of antioxidants was found to improve immune functions, decreasing their oxidative stress, and consequently leading to a significant increase in longevity.

13.4 CONCLUSIONS AND PERSPECTIVES

It is evident that the immune system needs to be fed properly not only with energy sources, but also with essential micronutrients serving as cofactors in the development, maintenance, and expression of the immune response. Already mild deficiencies of vitamins and minerals suppress immunity by affecting innate, T-cell mediated, and antibody responses and can worsen the age-related changes in immune functions. Micronutrients that have been demonstrated to be required for the immune system to function efficiently include vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, vitamin D, zinc, copper, iron, and selenium (23, 25, 27–29). In conclusion, preservation of a functionally youthful immune system throughout the years, including ensuring adequate vitamin and mineral status, is the best way to gain longevity accompanied by good quality of life.

REFERENCES

1. World Health Organization (2003) Diet, nutrition and the prevention of chronic diseases (WHO Technical report series: 916). World Health Organization, Geneva
2. Fairfield K, Fletcher R (2002) Vitamins for chronic disease prevention in adults: scientific review. JAMA 287(23):3116–3126
3. Fletcher R, Fairfield K (2002) Vitamins for chronic disease prevention in adults: clinical applications. JAMA 287(23):3127–3129
4. Kant AK, Leitzmann MF, Park Y, Hollenbeck A, Schatzkin A (2009) Patterns of recommended dietary behaviors predict subsequent risk of mortality in a large cohort of men and women in the United States. J Nutr 139(7):1374–1380
5. Doll R, Peto R (1981) The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 66(6):1191–1308
6. Wynder EL, Gori GB (1977) Contribution of the environment to cancer incidence: an epidemiologic exercise. J Natl Cancer Inst 58(4):825–832
7. Terry P, Giovannucci E, Michels KB et al (2001) Fruit, vegetables, dietary fiber, and risk of colorectal cancer. J Natl Cancer Inst 93(7):525–533
8. Lin J, Zhang SM, Cook NR et al (2005) Dietary intakes of fruit, vegetables, and fiber, and risk of colorectal cancer in a prospective cohort of women (United States). Cancer Causes Control 16(3):225–233
9. van Duijnhoven FJ, Bueno-De-Mesquita HB, Ferrari P et al (2009) Fruit, vegetables, and colorectal cancer risk: the European prospective investigation into cancer and nutrition. Am J Clin Nutr 89(5):1441–1452
10. Amar J, Perez L, Burcelin R, Chamontin B (2006) Arteries, inflammation and insulin resistance. J Hypertens Suppl 24(5):S18–S20
11. De Caterina R, Massaro M (2005) Omega-3 fatty acids and the regulation of expression of endothelial pro-atherogenic and pro-inflammatory genes. J Membr Biol 206(2):103–116
12. Scrimshaw NS, Taylor CE, Gordon JE (1959) Interactions of nutrition and infection. Am J Med Sci 237(3):367–403
13. Scrimshaw NS (2003) Historical concepts of interactions, synergism and antagonism between nutrition and infection. J Nutr 133(1):316S–321S
14. Scrimshaw NS (2007) Prologue: historical introduction. Immunonutrition in health and disease. Br J Nutr 98(Suppl 1):S3–S4
15. Wayne SJ, Rhyne RL, Garry PJ, Goodwin JS (1990) Cell-mediated immunity as a predictor of morbidity and mortality in subjects over 60. J Gerontol 45(2):M45–M48
16. Aspinall R (2000) Longevity and the immune response. Biogerontology 1(3):273–278
17. De la Fuente M (2008) Role of neuroimmunomodulation in aging. Neuroimmunomodulation 15(4–6):213–223
18. Guayerbas N, Catalan M, Victor VM, Miquel J, De la Fuente M (2002) Relation of behaviour and macrophage function to life span in a murine model of premature immunosenescence. Behav Brain Res 134(1–2):41–48
19. Guayerbas N, De La Fuente M (2003) An impairment of phagocytic function is linked to a shorter life span in two strains of prematurely aging mice. Dev Comp Immunol 27(4):339–350
20. De la Fuente M, Hernanz A, Vallejo MC (2005) The immune system in the oxidative stress conditions of aging and hypertension: favorable effects of antioxidants and physical exercise. Antioxid Redox Signal 7(9–10):1356–1366
21. Viveros MP, Arranz L, Hernanz A, Miquel J, De la Fuente M (2007) A model of premature aging in mice based on altered stress-related behavioral response and immunosenescence. Neuroimmunomodulation 14(3–4):157–162
22. Calder PC, Jackson AA (2000) Undernutrition, infection and immune function. Nutr Res Rev 13(1):3–29
23. Field CJ, Johnson IR, Schley PD (2002) Nutrients and their role in host resistance to infection. J Leukoc Biol 71(1):16–32
24. Katona P, Katona-Apte J (2008) The interaction between nutrition and infection. Clin Infect Dis 46(10):1582–1588

25. Maggini S, Beveridge S, Sorbara PJP, Senatore G (2008) Feeding the immune system: the role of micronutrients in restoring resistance to infections. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3(No. 098)
26. Hoyles L, Vulevic J (2008) Diet, immunity and functional foods. Adv Exp Med Biol 635:79–92
27. Calder PC, Kew S (2002) The immune system: a target for functional foods? Br J Nutr 88(Suppl 2):S165–S177
28. Wintergerst ES, Maggini S, Hornig DH (2007) Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 51(4):301–323
29. Maggini S, Wintergerst ES, Beveridge S, Hornig DH (2007) Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Br J Nutr 98(Suppl 1):S29–S35
30. Popkin BM (2001) The nutrition transition and obesity in the developing world. J Nutr 131(3):871S–873S
31. Chopra M, Galbraith S, Darnton-Hill I (2002) A global response to a global problem: the epidemic of over nutrition. Bull World Health Org 80:952–958
32. World Health Organization (2002) Active aging: a policy framework. World Health Organization, Geneva
33. Gerhard GT, Duell PB (1999) Homocysteine and atherosclerosis. Curr Opin Lipidol 10(5):417–428
34. Antoniades C, Antonopoulos AS, Tousoulis D, Marinou K, Stefanadis C (2009) Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. Eur Heart J 30(1):6–15
35. Seshadri N, Robinson K (2000) Homocysteine, B vitamins, and coronary artery disease. Med Clin North Am 84(1):215–237, x
36. Malinow MR, Bostom AG, Krauss RM (1999) Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation 99(1):178–182
37. Mattson MP, Kruman II, Duan W (2002) Folic acid and homocysteine in age-related disease. Ageing Res Rev 1(1):95–111
38. Prins ND, Den Heijer T, Hofman A et al (2002) Homocysteine and cognitive function in the elderly: the Rotterdam Scan Study. Neurology 59(9):1375–1380
39. Ravaglia G, Forti P, Maioli F et al (2003) Homocysteine and cognitive function in healthy elderly community dwellers in Italy. Am J Clin Nutr 77(3):668–673
40. Seshadri S, Beiser A, Selhub J et al (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 346(7):476–483
41. Selhub J, Bagley LC, Miller J, Rosenberg IH (2000) B vitamins, homocysteine, and neurocognitive function in the elderly. Am J Clin Nutr 71(2):614S–620S
42. Durga J, van Boxtel MP, Schouten EG et al (2007) Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet 369(9557):208–216
43. Institute of Medicine (2000) DRI Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids – a report of the panel on dietary antioxidants and related compounds. National Academic Press, Washington, DC
44. Mayne ST (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 133(Suppl 3):933S–940S
45. Frei B (2004) Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. J Nutr 134(11):3196S–3198S
46. Rao AV, Rao LG (2007) Carotenoids and human health. Pharmacol Res 55(3):207–216
47. Riccioni G, Mancini B, Di Ilio E, Bucciarelli T, D'Orazio N (2008) Protective effect of lycopene in cardiovascular disease. Eur Rev Med Pharmacol Sci 12(3):183–190
48. Huang HY, Caballero B, Chang S et al (2006) Multivitamin/mineral supplements and prevention of chronic disease. Evid Rep Technol Assess (Full Rep) 139:1–117
49. Ellingsen I, Seljeflot I, Arnesen H, Tonstad S (2009) Vitamin C consumption is associated with less progression in carotid intima media thickness in elderly men: a 3-year intervention study. Nutr Metab Cardiovasc Dis 19(1):8–14
50. De la Fuente M, Hernanz A, Guayerbas N, Victor VM, Arnalich F (2008) Vitamin E ingestion improves several immune functions in elderly men and women. Free Radic Res 42(3):272–280

51. Meydani M (2001) Nutrition interventions in aging and age-associated disease. Ann NY Acad Sci 928:226–235
52. Blumberg JB (2009) The antioxidant conundrum: where do we go from here? In: Linus Pauling Institute (ed) Fifth diet and optimum health conference. Conference Binder, Portland, OR, USA
53. Blumberg JB, Frei B (2007) Clinical trials of vitamin E and cardiovascular diseases may be fatally flawed. Commentary on "The relationship between dose of vitamin E and suppression of oxidative stress in humans". Free Radic Biol Med 43(10):1374–1376
54. Roberts LJ II, Traber MG, Frei B (2009) Vitamins E and C in the prevention of cardiovascular disease and cancer in men. Free Radic Biol Med 46(11):1558
55. Carr AC, Frei B (1999) Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 69(6):1086–1107
56. Frei B (2003) To C or not to C, that is the question! J Am Coll Cardiol 42(2):253–255
57. Kontush K, Schekatolina S (2004) Vitamin E in neurodegenerative disorders: Alzheimer's disease. Ann NY Acad Sci 1031:249–262
58. Masaki KH, Losonczy KG, Izmirlian G et al (2000) Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. Neurology 54(6):1265–1272
59. Brunekreef B, Holgate ST (2002) Air pollution and health. Lancet 360(9341):1233–1242
60. Romieu I, Castro-Giner F, Kunzli N, Sunyer J (2008) Air pollution, oxidative stress and dietary supplementation: a review. Eur Respir J 31(1):179–197
61. Grievink L, Smit HA, Brunekreef B (2000) Antioxidants and air pollution in relation to indicators of asthma and COPD: a review of the current evidence. Clin Exp Allergy 30(10):1344–1354
62. Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M et al (2002) Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med 166(5):703–709
63. Romieu I, Tellez-Rojo MM, Lazo M et al (2005) Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. Am J Respir Crit Care Med 172(12):1534–1540
64. Grant WB, Holick MF (2005) Benefits and requirements of vitamin D for optimal health: a review. Altern Med Rev 10(2):94–111
65. Holick MF (1994) McCollum Award Lecture, 1994: vitamin D – new horizons for the 21st century. Am J Clin Nutr 60(4):619–630
66. Holick MF (2002) Sunlight and vitamin D: both good for cardiovascular health. J Gen Intern Med 17(9):733–735
67. Holick MF (2003) Vitamin D: a millenium perspective. J Cell Biochem 88(2):296–307
68. Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 80(Suppl 6):1678S–1688S
69. Holick MF (2004) Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 79(3):362–371
70. Holick MF (2005) Vitamin D: important for prevention of osteoporosis, cardiovascular heart disease, type 1 diabetes, autoimmune diseases, and some cancers. South Med J 98(10):1024–1027
71. Holick MF (2005) The vitamin D epidemic and its health consequences. J Nutr 135(11): 2739S–2748S
72. Holick MF (2006) High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 81(3):353–373
73. Holick MF (2007) Vitamin D deficiency. N Engl J Med 357(3):266–281
74. Holick MF (2008) The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. Mol Aspects Med 29(6):361–368
75. Holick MF (2008) Michael Holick, PhD, MD: vitamin D pioneer. Interview by Frank Lampe and Suzanne Snyder. Altern Ther Health Med 14(3):65–75
76. Mullin GE, Dobs A (2007) Vitamin D and its role in cancer and immunity: a prescription for sunlight. Nutr Clin Pract 22(3):305–322
77. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM (2001) Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358(9292):1500–1503
78. The EURODIAB Substudy 2 Study Group (1999) Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. Diabetologia 42(1):51–54

79. Feghali CA, Wright TM (1997) Cytokines in acute and chronic inflammation. Front Biosci 2:d12–d26
80. Dinarello CA (2006) Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process. Am J Clin Nutr 83(2):447S–455S
81. Kornman KS (2006) Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. Am J Clin Nutr 83(2):475S–483S
82. Lemanske RF Jr (2002) Inflammation in childhood asthma and other wheezing disorders. Pediatrics 109(Suppl 2):368–372
83. Barbato A, Turato G, Baraldo S et al (2003) Airway inflammation in childhood asthma. Am J Respir Crit Care Med 168(7):798–803
84. King TE Jr (1999) A new look at the pathophysiology of asthma. J Natl Med Assoc 91(Suppl 8):9S–15S
85. Dignass AU, Baumgart DC, Sturm A (2004) Review article: the aetiopathogenesis of inflammatory bowel disease – immunology and repair mechanisms. Aliment Pharmacol Ther 20(Suppl 4):9–17
86. De Giorgio R, Barbara G (2008) Is irritable bowel syndrome an inflammatory disorder? Curr Gastroenterol Rep 10(4):385–390
87. Bennett JL, Stuve O (2009) Update on inflammation, neurodegeneration, and immunoregulation in multiple sclerosis: therapeutic implications. Clin Neuropharmacol 32(3):121–132
88. Frischer JM, Bramow S, Dal-Bianco A et al (2009) The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 132(Pt 5):1175–1189
89. Lassmann H (2008) Mechanisms of inflammation induced tissue injury in multiple sclerosis. J Neurol Sci 274(1–2):45–47
90. Feldmann M, Maini RN (1999) The role of cytokines in the pathogenesis of rheumatoid arthritis. Rheumatology (Oxford) 38(Suppl 2):3–7
91. Lipsky PE, Davis LS, Cush JJ, Oppenheimer-Marks N (1989) The role of cytokines in the pathogenesis of rheumatoid arthritis. Springer Semin Immunopathol 11(2):123–162
92. Christodoulou C, Choy EH (2006) Joint inflammation and cytokine inhibition in rheumatoid arthritis. Clin Exp Med 6(1):13–19
93. Banning M (2005) The principles of inflammation in the development of rheumatoid arthritis. Br J Nurs 14(5):277–283
94. Nauta AJ, Engels F, Knippels LM, Garssen J, Nijkamp FP, Redegeld FA (2008) Mechanisms of allergy and asthma. Eur J Pharmacol 585(2–3):354–360
95. Bochner BS, Busse WW (2005) Allergy and asthma. J Allergy Clin Immunol 115(5):953–959
96. Eldika N, Yerra L, Chi DS, Krishnaswamy G (2004) Atherosclerosis as an inflammatory disease: implications for therapy. Front Biosci 9:2764–2777
97. Liuzzo G (2001) Atherosclerosis: an inflammatory disease. Rays 26(4):221–230
98. Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340(2):115–126
99. Ferrara AR (2008) Inflammatory breast cancer: challenges and developments. Radiol Technol 80(1):49M–60M, quiz 61M–63M
100. Mann JR, Backlund MG, DuBois RN (2005) Mechanisms of disease: inflammatory mediators and cancer prevention. Nat Clin Pract Oncol 2(4):202–210
101. Calder PC (2006) n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83(Suppl 6):1505S–1519S
102. Sen CK (1997) Nutritional biochemistry of cellular glutathione. J Nutr Biochem 8(12):660–672
103. Grimble RF (1998) Modification of inflammatory aspects of immune function by nutrients. Nutr Res Rev 18(7):1297–1317
104. Rayman M (2000) The importance of selenium to human health. Lancet 356(9225):233–241
105. Semba RD, Tang AM (1999) Micronutrients and the pathogenesis of human immunodeficiency virus infection. Br J Nutr 81(3):181–189
106. Albers R, Antoine JM, Bourdet-Sicard R et al (2005) Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr 94(3):452–481
107. Delves PJ, Roitt IM (2000) The immune system. Second of two parts. N Engl J Med 343(2):108–117
108. Delves PJ, Roitt IM (2000) The immune system. First of two parts. N Engl J Med 343(1):37–49
109. Parkin J, Cohen B (2001) An overview of the immune system. Lancet 357(9270):1777–1789

110. World Health Organization (2008) The global burden of disease: 2004 update. World Health Organization, Geneva
111. Khanna M, Kumar P, Choudhary K, Kumar B, Vijayan VK (2008) Emerging influenza virus: a global threat. J Biosci 33(4):475–482
112. Maines TR, Szretter KJ, Perrone L et al (2008) Pathogenesis of emerging avian influenza viruses in mammals and the host innate immune response. Immunol Rev 225:68–84
113. Kuiken T, Taubenberger JK (2008) Pathology of human influenza revisited. Vaccine 26(Suppl 4):D59–D66
114. Michaelis M, Doerr HW, Cinatl J Jr (2009) Of chickens and men: avian influenza in humans. Curr Mol Med 9(2):131–151
115. Pappaioanou M (2009) Highly pathogenic H5N1 avian influenza virus: cause of the next pandemic? Comp Immunol Microbiol Infect Dis 32(4):287–300
116. Chowell G, Bertozzi SM, Colchero MA et al (2009) Severe respiratory disease concurrent with the circulation of H1N1 influenza. N Engl J Med 361(7):674–679
117. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S et al (2009) Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med 361(7):680–689
118. Desselberger U (2000) Emerging and re-emerging infectious diseases. J Infect 40(1):3–15
119. Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife – threats to biodiversity and human health. Science 287(5452):443–449
120. Binder S, Levitt AM, Sacks JJ, Hughes JM (1999) Emerging infectious diseases: public health issues for the 21st century. Science 284(5418):1311–1313
121. World Health Organization (2002) WHO global burden of disease (GBD) 2002 estimates (revised). World Health Organization, Geneva
122. Breman JG, Alilio MS, Mills A (2004) Conquering the intolerable burden of malaria: what's new, what's needed: a summary. Am J Trop Med Hyg 71(Suppl 2):1–15
123. Brown KH, Peerson JM, Baker SK, Hess SY (2009) Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. Food Nutr Bull 30(Suppl 1):S12–S40
124. D'Souza RM, D'Souza R (2002) Vitamin A for the treatment of children with measles – a systematic review. J Trop Pediatr 48(6):323–327
125. American Academy of Pediatrics Committee on Infectious Diseases (1993) Vitamin A treatment of measles. Pediatrics 91(5):1014–1015
126. Semba RD (2004) Vitamin A. Humana Press, Totowa, NJ
127. World Food Programme (2004) HIV/AIDS and tuberculosis: addressing co-infection. World Food Programme, Rome
128. World Health Organization (2006) Neglected tropical diseases: hidden successes, emerging opportunities. World Health Organization, Geneva
129. Korenromp EL, Williams BG, de Vlas SJ et al (2005) Malaria attributable to the HIV-1 epidemic, sub-Saharan Africa. Emerg Infect Dis 11(9):1410–1419
130. Krause RM (1994) Dynamics of emergence. J Infect Dis 170(2):265–271
131. Vijaykrishna D, Bahl J, Riley S et al (2008) Evolutionary dynamics and emergence of panzootic H5N1 influenza viruses. PLoS Pathog 4(9):e1000161
132. Berger A (2000) Th1 and Th2 responses: what are they? BMJ 321(7258):424
133. Kidd P (2003) Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev 8(3):223–246
134. Knight JA (2000) Review: free radicals, antioxidants, and the immune system. Ann Clin Lab Sci 30(2):145–158
135. Meydani SN, Wu D, Santos MS, Hayek MG (1995) Antioxidants and immune response in aged persons: overview of present evidence. Am J Clin Nutr 62(Suppl 6):1462S–1476S
136. Meydani SN, Santos MS, Wu D, Hayek MG (1998) Antioxidant modulation of cytokines and their biologic function in the aged. Z Ernahrungswiss 37(Suppl 1):35–42
137. Knight JA (2000) The biochemistry of aging. Adv Clin Chem 35:1–62
138. De la Fuente M (2002) Effects of antioxidants on immune system ageing. Eur J Clin Nutr 56(Suppl 3):S5–S8
139. Traber MG, Atkinson J (2007) Vitamin E, antioxidant and nothing more. Free Radic Biol Med 43(1):4–15

140. Institute of Medicine (1997) DRI dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academic Press, Washington, DC
141. Institute of Medicine (1998) DRI Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B 6, folate, vitamin B 12, pantothenic acid, biotin, and choline. National Academic Press, Washington, DC
142. Institute of Medicine (2001) DRI dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academic Press, Washington, DC
143. Huskisson E, Maggini S, Ruf M (2007) The role of vitamins and minerals in energy metabolism and well-being. J Int Med Res 35(3):277–289
144. Huskisson E, Maggini S, Ruf M (2007) The influence of micronutrients on cognitive function and performance. J Int Med Res 35(1):1–19
145. Enwonwu CO, Ritchie CS (2007) Nutrition and inflammatory markers. J Am Dent Assoc 138(1):70–73
146. Blomhoff HK, Smeland EB, Erikstein B et al (1992) Vitamin A is a key regulator for cell growth, cytokine production, and differentiation in normal B cells. J Biol Chem 267(33):23988–23992
147. Dennert G, Lotan R (1978) Effects of retinoic acid on the immune system: stimulation of T killer cell induction. Eur J Immunol 8(1):23–29
148. Ertesvag A, Engedal N, Naderi S, Blomhoff HK (2002) Retinoic acid stimulates the cell cycle machinery in normal T cells: involvement of retinoic acid receptor-mediated IL-2 secretion. J Immunol 169(10):5555–5563
149. Lemire JM, Adams JS, Sakai R, Jordan SC (1984) 1 alpha, 25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J Clin Invest 74(2):657–661
150. Mora JR, Iwata M, von Andrian UH (2008) Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol 8(9):685–698
151. Rigby WF, Stacy T, Fanger MW (1984) Inhibition of T lymphocyte mitogenesis by 1, 25-dihydroxyvitamin D3 (calcitriol). J Clin Invest 74(4):1451–1455
152. Beck MA (2007) Selenium and vitamin E status: impact on viral pathogenicity. J Nutr 137(5):1338–1340
153. Beck MA, Kolbeck PC, Rohr LH, Shi Q, Morris VC, Levander OA (1994) Vitamin E deficiency intensifies the myocardial injury of coxsackievirus B3 infection of mice. J Nutr 124(3):345–358
154. Beck MA, Kolbeck PC, Rohr LH, Shi Q, Morris VC, Levander OA (1994) Benign human enterovirus becomes virulent in selenium-deficient mice. J Med Virol 43(2):166–170
155. Beck MA, Kolbeck PC, Shi Q, Rohr LH, Morris VC, Levander OA (1994) Increased virulence of a human enterovirus (coxsackievirus B3) in selenium-deficient mice. J Infect Dis 170(2):351–357
156. Beck MA, Shi Q, Morris VC, Levander OA (1995) Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. Nat Med 1(5):433–436
157. Smith AD, Botero S, Levander OA (2008) Copper deficiency increases the virulence of amyocarditic and myocarditic strains of coxsackievirus B3 in mice. J Nutr 138(5):849–855
158. Holick MF (1981) The cutaneous photosynthesis of previtamin D3: a unique photoendocrine system. J Invest Dermatol 77(1):51–58
159. Holick MF (1985) The photobiology of vitamin D and its consequences for humans. Ann NY Acad Sci 453:1–13
160. DeLuca HF (2008) Evolution of our understanding of vitamin D. Nutr Rev 66(10 Suppl 2):S73–S87
161. Cavalier E, Delanaye P, Chapelle JP, Souberbielle JC (2009) Vitamin D: current status and perspectives. Clin Chem Lab Med 47(2):120–127
162. Holick MF, Chen TC (2008) Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 87(4):1080S–1086S
163. Heaney RP (2008) Vitamin D: criteria for safety and efficacy. Nutr Rev 66(10 Suppl 2):S178–S181
164. Prentice A (2008) Vitamin D deficiency: a global perspective. Nutr Rev 66(10 Suppl 2):S153–S164
165. Bandeira F, Griz L, Dreyer P, Eufrazino C, Bandeira C, Freese E (2006) Vitamin D deficiency: a global perspective. Arq Bras Endocrinol Metabol 50(4):640–646
166. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B (2006) Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 84(1):18–28
167. Malabanan A, Veronikis IE, Holick MF (1998) Redefining vitamin D insufficiency. Lancet 351(9105):805–806

168. Thomas MK, Lloyd-Jones DM, Thadhani RI et al (1998) Hypovitaminosis D in medical inpatients. N Engl J Med 338(12):777–783
169. Chapuy MC, Preziosi P, Maamer M et al (1997) Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 7(5):439–443
170. Holick MF, Siris ES, Binkley N et al (2005) Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 90(6):3215–3224
171. Vieth R, Bischoff-Ferrari H, Boucher BJ et al (2007) The urgent need to recommend an intake of vitamin D that is effective. Am J Clin Nutr 85(3):649–650
172. Holick MF (2008) Vitamin D: a D-lightful health perspective. Nutr Rev 66(10 Suppl 2):S182–S194
173. Nagpal S, Na S, Rathnachalam R (2005) Noncalcemic actions of vitamin D receptor ligands. Endocr Rev 26(5):662–687
174. Christakos S, Dhawan P, Benn B et al (2007) Vitamin D: molecular mechanism of action. Ann NY Acad Sci 1116:340–348
175. Griffin MD, Xing N, Kumar R (2003) Vitamin D and its analogs as regulators of immune activation and antigen presentation. Annu Rev Nutr 23:117–145
176. Cantorna MT, Zhu Y, Froicu M, Wittke A (2004) Vitamin D status, 1, 25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 80(Suppl 6):1717S–1720S
177. Cantorna MT (2006) Vitamin D and its role in immunology: multiple sclerosis, and inflammatory bowel disease. Prog Biophys Mol Biol 92(1):60–64
178. Adams JS, Liu PT, Chun R, Modlin RL, Hewison M (2007) Vitamin D in defense of the human immune response. Ann NY Acad Sci 1117:94–105
179. Tavera-Mendoza LE, White JH (2007) Cell defenses and the sunshine vitamin. Sci Am 297(5):62–65, 68–70, 72
180. Hayes CE, Nashold FE, Spach KM, Pedersen LB (2003) The immunological functions of the vitamin D endocrine system. Cell Mol Biol (Noisy-le-grand) 49(2):277–300
181. Adams JS, Hewison M (2008) Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab 4(2):80–90
182. Cohen MS, Mesler DE, Snipes RG, Gray TK (1986) 1, 25-Dihydroxyvitamin D3 activates secretion of hydrogen peroxide by human monocytes. J Immunol 136(3):1049–1053
183. Roux-Lombard P, Cruchaud A, Dayer JM (1986) Effect of interferon-gamma and 1 alpha, 25-dihydroxyvitamin D3 on superoxide anion, prostaglandins E2, and mononuclear cell factor production by U937 cells. Cell Immunol 97(2):286–296
184. Fagan DL, Prehn JL, Adams JS, Jordan SC (1991) The human myelomonocytic cell line U-937 as a model for studying alterations in steroid-induced monokine gene expression: marked enhancement of lipopolysaccharide-stimulated interleukin-1 beta messenger RNA levels by 1,25-dihydroxyvitamin D3. Mol Endocrinol 5(2):179–186
185. Prehn JL, Fagan DL, Jordan SC, Adams JS (1992) Potentiation of lipopolysaccharide-induced tumor necrosis factor-alpha expression by 1, 25-dihydroxyvitamin D3. Blood 80(11):2811–2816
186. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM (1983) Specific high-affinity receptors for 1, 25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. J Clin Endocrinol Metab 57(6):1308–1310
187. Rigby WF, Noelle RJ, Krause K, Fanger MW (1985) The effects of 1, 25-dihydroxyvitamin D3 on human T lymphocyte activation and proliferation: a cell cycle analysis. J Immunol 135(4):2279–2286
188. Chocano-Bedoya P, Ronnenberg AG (2009) Vitamin D and tuberculosis. Nutr Rev 67(5):289–293
189. Wang TT, Nestel FP, Bourdeau V et al (2004) Cutting edge: 1, 25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 173(5):2909–2912
190. Gombart AF, Borregaard N, Koeffler HP (2005) Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1, 25-dihydroxyvitamin D3. FASEB J 19(9):1067–1077
191. Liu PT, Stenger S, Li H et al (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311(5768):1770–1773
192. Hiemstra PS (2007) The role of epithelial beta-defensins and cathelicidins in host defense of the lung. Exp Lung Res 33(10):537–542
193. Schutte BC, McCray PB Jr (2002) [beta]-defensins in lung host defense. Annu Rev Physiol 64:709–748

194. Beisswenger C, Bals R (2005) Antimicrobial peptides in lung inflammation. Chem Immunol Allergy 86:55–71
195. Cannell JJ, Vieth R, Umhau JC et al (2006) Epidemic influenza and vitamin D. Epidemiol Infect 134(6):1129–1140
196. Yamshchikov AV, Desai NS, Blumberg HM, Ziegler TR, Tangpricha V (2009) Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. Endocr Pract 15(5):438–449
197. Cantorna MT (2008) Vitamin D and multiple sclerosis: an update. Nutr Rev 66(10 Suppl 2):S135–S138
198. Cutolo M, Otsa K, Paolino S, Yprus M, Veldi T, Seriolo B (2009) Vitamin D involvement in rheumatoid arthritis and systemic lupus erythaematosus. Ann Rheum Dis 68(3):446–447
199. Leventis P, Patel S (2008) Clinical aspects of vitamin D in the management of rheumatoid arthritis. Rheumatology (Oxford) 47(11):1617–1621
200. Cutolo M, Otsa K, Uprus M, Paolino S, Seriolo B (2007) Vitamin D in rheumatoid arthritis. Autoimmun Rev 7(1):59–64
201. Ranganathan P (2009) Genetics of bone loss in rheumatoid arthritis – role of vitamin D receptor polymorphisms. Rheumatology (Oxford) 48(4):342–346
202. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF (2008) Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? J Am Coll Cardiol 52(24):1949–1956
203. Michos ED, Melamed ML (2008) Vitamin D and cardiovascular disease risk. Curr Opin Clin Nutr Metab Care 11(1):7–12
204. Perez-Lopez FR (2009) Vitamin D metabolism and cardiovascular risk factors in postmenopausal women. Maturitas 62(3):248–262
205. Michos ED (2009) Vitamin D deficiency and the risk of incident type 2 diabetes. Future Cardiol 5(1):15–18
206. Penckofer S, Kouba J, Wallis DE, Emanuele MA (2008) Vitamin D and diabetes: let the sunshine in. Diabetes Educ 34(6):939–940, 942,944 passim
207. Mohr SB (2009) A brief history of vitamin d and cancer prevention. Ann Epidemiol 19(2):79–83
208. Tuohimaa P (2008) Vitamin D, aging and cancer. Nutr Rev 66(10 Suppl 2):S147–S152
209. Pawelec G (2006) Immunity and ageing in man. Exp Gerontol 41(12):1239–1242
210. Pawelec G, Larbi A (2008) Immunity and ageing in man: Annual Review 2006/2007. Exp Gerontol 43(1):34–38
211. Puerto M, Guayerbas N, Alvarez P, De la Fuente M (2005) Modulation of neuropeptide Y and norepinephrine on several leucocyte functions in adult, old and very old mice. J Neuroimmunol 165(1–2):33–40
212. De la Fuente M (2008) Nutrition and immunity in the elderly. Proc Nutr Soc 67(OEC):E6
213. Alonso-Fernandez P, Puerto M, Mate I, Ribera JM, de la Fuente M (2008) Neutrophils of centenarians show function levels similar to those of young adults. J Am Geriatr Soc 56(12):2244–2251
214. Alvarado C, Alvarez P, Jimenez L, De la Fuente M (2005) Improvement of leukocyte functions in young prematurely aging mice after a 5-week ingestion of a diet supplemented with biscuits enriched in antioxidants. Antioxid Redox Signal 7(9–10):1203–1210
215. Alvarado C, Alvarez P, Jimenez L, De la Fuente M (2006) Oxidative stress in leukocytes from young prematurely aging mice is reversed by supplementation with biscuits rich in antioxidants. Dev Comp Immunol 30(12):1168–1180
216. Alvarado C, Alvarez P, Puerto M, Gausseres N, Jimenez L, De la Fuente M (2006) Dietary supplementation with antioxidants improves functions and decreases oxidative stress of leukocytes from prematurely aging mice. Nutrition 22(7–8):767–777
217. Alvarez P, Alvarado C, Mathieu F, Jimenez L, De la Fuente M (2006) Diet supplementation for 5 weeks with polyphenol-rich cereals improves several functions and the redox state of mouse leucocytes. Eur J Nutr 45(8):428–438
218. Alvarez P, Alvarado C, Puerto M, Schlumberger A, Jimenez L, De la Fuente M (2006) Improvement of leukocyte functions in prematurely aging mice after five weeks of diet supplementation with polyphenol-rich cereals. Nutrition 22(9):913–921
219. Baeza I, de Castro NM, Alvarado C et al (2007) Improvement of immune cell functions in aged mice treated for five weeks with soybean isoflavones. Ann NY Acad Sci 1100:497–504

14 Trace Elements and Immune Function

James P. McClung and Daniel G. Peterson*

Key Points

- Trace elements are essential nutrients that are required in minute quantities to support the optimal function of an organism.
- This chapter reviews the role of four trace elements, including copper, iron, selenium, and zinc in immune function.
- Each of these trace elements has a function in both the innate and acquired immune system.

Key Words: Trace elements, minerals, immune function, copper, iron, selenium, zinc.

14.1 INTRODUCTION

Trace elements are essential nutrients required in minute quantities to support the growth, development, and optimal function of an organism (1). Nutritionally essential trace elements include copper, iron, selenium, and zinc. Maintaining trace element balance is critical for the prevention of both deficiency and overload disorders. Negative trace element balance may result in distinct deficiency disorders, such as the development of anemia in iron-deficient individuals or Keshan disease during selenium deficiency. Likewise, genetic factors or dietary overconsumption of trace elements may lead to overload disorders, including chronic liver disease. Apart from the avoidance of deficiency or overload

* The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_14

disorders, maintaining trace element balance is essential for the optimal functioning of a series of physiologic systems, including those conferring immune function.

The human immune system is divided into two interactive components: innate (non-specific) immunity and adaptive (specific or acquired) immunity. Innate immunity comprises epithelial barriers, cellular components, including macrophages, natural killer, and dendritic cells, and non-cellular components, including acute phase proteins (2). Adaptive immunity can be further divided into cell-mediated immunity, associated with T lymphocytes, and humoral immunity, associated with B lymphocytes. Cell-mediated immunity functions through three types of T cells: cytotoxic T cells, which act directly to kill invading pathogens; suppressor T cells, which prevent cytotoxic T cells from becoming overly reactive; and helper T cells, which support the function of cytotoxic and suppressor cells. Humoral immunity functions through the secretion of antibodies produced by B cells.

Trace elements confer function in all aspects of the immune system. For example, immune cells require trace elements for the structure and function of metalloproteins which participate in energy production and protection from oxidant stress (3). Furthermore, the production of immune cells by the bone marrow requires trace elements, including zinc, for DNA replication and cell division (4). In another example, trace elements are required by a series of enzymes that function in the immune system, such as myeloperoxidase, which serves to generate hypochlorous acid, a potent microbiocide (3).

This chapter will focus on the role of selected trace elements with well-described functions in immunity, including copper, iron, selenium, and zinc. Specific examples of the roles of each of these trace elements in immune function will be cited, including recent discoveries, such as the effect of selenium status on viral virulence and the role of iron balance in critical interactions between the host and pathogen.

14.2 COPPER

Copper was first identified as an essential trace element in the 1960s through a study of Peruvian children who presented with anemia refractory to iron therapy (5). These children also presented with neutropenia (a diminished number of neutrophils) and bone abnormalities which were responsive to dietary copper. Since the initial discovery of the nutritional essentiality of copper, a series of proteins and enzymes that bind copper have been identified. These include cytochrome *c* oxidase, which is required for electron transport, and superoxide dismutase, an antioxidant enzyme. Studies assessing the role of copper in immune function have been limited by the lack of an accurate indicator of marginal copper status in blood; however, copper is known to confer function in both the innate and adaptive immune systems. Perhaps the most profound effect of copper deficiency on the innate immune system is the reduced number of neutrophils observed in human and animal populations. In fact, neutropenia has been utilized as a clinical indicator of copper deficiency in humans (6). The cause of neutropenia in copper deficiency is not clear; however, it is possible that neutrophil secretion from the bone marrow or redistribution of neutrophils into tissues or organs may be responsible (7). Furthermore, anti-neutrophil antibodies have been detected in the serum of copper-deficient patients, indicating another possible mechanism underlying neutropenia (8).

Beyond the diminished number of neutrophils observed in copper deficiency, the function of remaining neutrophils is impaired, as reduced superoxide anion production by both neutrophils and macrophages has been observed in animal studies (9, 10). Copper may also affect intestinal immune barriers as indicated by the similar effects of copper supplementation to sub-therapeutic antibiotic treatment in animals that is known to reduce pathogen load in the intestine (11).

Copper deficiency has a pronounced effect on the adaptive immune system. Early studies demonstrated anemia and reduced thymus weights in copper-deficient animals (12). Furthermore, spleen weights may be affected by copper deficiency, and antibody production is reduced (13). Although the definitive cause of diminished antibody production has not been elucidated, cytokines, which serve as a vehicle for communication within the immune system, appear to be involved. Diminished levels of interleukin-2 (IL-2), the cytokine responsible for the regulation of the cell cycle in T lymphocytes, have been observed in a number of animal and cell studies of copper deficiency (14). In fact, adding IL-2 to splenocyte cultures isolated from copper-deficient rats restores the ability of those cells to proliferate (12).

Human studies investigating the role of copper in immune function are limited. From the few existing studies, it has been determined that copper status may affect phagocytosis in infants (15), and that the ability of peripheral blood mononuclear cells to proliferate is reduced following consumption of a copper-deficient diet (16). Although it is clear that copper is essential for immune function, more research is required in human populations to elucidate the mechanistic role of this trace element.

14.3 IRON

Iron is an essential component of a number of proteins and enzymes, including hemoglobin and myoglobin. These heme-containing proteins function in the transport and storage of oxygen. Iron-containing enzymes, including aconitase, function in energy metabolism. Because of the role of iron-containing proteins and enzymes in oxygen transport and energy metabolism, iron deficiency and iron deficiency anemia are known to affect physical performance. Iron deficiency refers to the depletion of iron stores; iron deficiency anemia occurs when the depletion of iron affects hemoglobin levels. Despite the advanced understanding of iron metabolism, iron deficiency and iron deficiency anemia affect billions of people in both the developing and developed world. In the developing world, poor iron status typically occurs due to a lack of foods containing bioavailable iron. In the developed world, poor iron status often affects premenopausal women, due to the combination of low dietary iron intake, and the loss of iron that occurs through menstruation.

The role of iron in immune function differs based upon the iron status of the individual and the pathogens that the individual may encounter. Iron deficiency and iron deficiency anemia are known to impart a series of negative effects on components of immune function due to the role of iron in enzymes such as myeloperoxidase, which is required for the production of hydroxyl radicals by neutrophils in response to bacteria (2). One experiment with iron-deficient and iron-deficient anemic children demonstrated a reduced ability of isolated neutrophils to kill bacteria (17). Treating the

children with iron restored the bacteria killing activity of the isolated neutrophils. Other immune cells, including T cells, are affected by iron deficiency, as iron is required for the differentiation and proliferation of these cells (18). Specifically, the ratio of T lymphocytes (CD4+ to CD8+ cells) is reduced in iron deficiency, although the total number of cells does not change (2). Furthermore, the proliferation of T-cells isolated from elderly women with iron deficiency is diminished upon stimulation in the laboratory as compared to cells from elderly women with normal iron status (19).

Unlike some of the other trace elements, there appear to be circumstances where maintaining a normal or elevated iron status may not be beneficial for certain populations. Evidence that iron supplementation could have a negative impact on the immune response began with studies in malarious regions of the world. One early study demonstrated an increased rate of clinical malaria and severe lower respiratory infections in infants provided with parenteral iron supplementation in Papau New Guinea as compared to infants provided with no supplemental iron (20, 21). In that study, hospital admissions for clinical symptoms of malaria, measles, and acute otitis media were all greater in infants supplemented with iron. Subsequent studies have demonstrated an increased risk of clinical malaria in children (22) and adults (23) provided with iron supplements. Since the discovery that iron status may affect susceptibility to malarial infection, relationships between positive iron status and poor disease outcomes in both human immunodeficiency virus and tuberculosis have been described (24). Although the definitive mechanism by which iron supplementation seems to result in increased susceptibility to infection remains unknown, a number of hypotheses have been proposed. First, it has been demonstrated that iron treatment impairs the ability of cells to synthesize nitric oxide in response to cytokine stimulation (25). Reduced nitric oxide synthesis results in a reduced ability to kill parasites, including *Plasmodium falciparum*, which is one agent responsible for malarial infection. A second hypothesis indicates that iron status may interact with genetic components in affecting susceptibility to disease (24). Other hypotheses indicate that iron supplementation may promote infection due to the growth-promoting effects of iron on the pathogen itself (26–28). Accordingly, the immune system has adapted to limit iron availability during infection through increased production of iron-binding proteins such as lactoferrin and ferritin that reduce the availability of iron to both the host and invading pathogens. Iron overload in patients at risk for infection has been shown to correlate with a greater severity of infection and greater risk of systemic bacteremia (29). This iron sequestration is considered to be of particular importance to intracellular innate defense, as pathogens residing within host cells avoid detection and destruction by the cellular and complement protein components of the innate immune system (30).

When considering the potentially detrimental effects of positive iron balance in infection, it is important to consider the environment; studies in nonmalarious regions indicate that oral iron supplementation may provide protection against respiratory and diarrheal disease, with little evidence of harm due to iron treatment (26). There has been significant controversy within the scientific and medical community regarding iron requirements due to the potentially differential effects of iron status on disease incidence based upon geographic location and the type of pathogens the population may encounter; for this reason, the role of iron in immune function remains an active area of scientific research.

14.4 SELENIUM

Similar to other trace elements, selenium functions primarily through incorporation into proteins and enzymes. More than 25 selenoproteins have been identified, including glutathione peroxidase, thioredoxin reductase, and selenoprotein P. Many of the selenoproteins function as antioxidant enzymes. For example, cellular glutathione peroxidase, or GPX1, functions as an antioxidant by reducing hydrogen peroxide to water using glutathione as a reductant (31). Phospholipid hydroperoxide glutathione peroxidase, or GPX4, functions as an antioxidant enzyme within the plasma membrane by reducing fatty acid hydroperoxides present in phospholipids. It is generally believed that the role of selenium in protecting the host against oxidant stress is beneficial for immune function (32). Furthermore, antioxidant selenoproteins may protect the host from oxidant stress generated by macrophages during the immune response (2).

Roles for selenium in both the innate and adaptive immune system have been described. For example, lymphocytes harvested from selenium-deficient animals demonstrate diminished proliferation in response to mitogens as compared to lymphocytes from selenium-supplemented animals (33). Furthermore, leukotriene B4 (required for neutrophil chemotaxis) synthesis is diminished in selenium-deficient lymphocytes. Decreased pools of mature T cells and a defect in T cell-dependent antibody responses have been observed in mice genetically modified to produce T cells without selenoproteins (34). Antibody production is also affected by selenium deficiency; IgM, IgG, and IgA titers are reduced in selenium-deficient rats, and IgG and IgM titers are reduced in selenium-deficient humans (32).

Recent studies suggest that selenium status modulates viral pathogenicity. Keshan disease, a selenium deficiency disorder, is associated with cardiomyopathy in children. Early studies demonstrated that selenium supplementation of at-risk populations reduced the prevalence of Keshan disease; however, the seasonal incidence of the disease indicated an infectious cofactor. Subsequently, scientists in China discovered coxsackieviruses in the blood of patients with Keshan disease (35), which led to the development of an animal model to study the disease. In the ensuing mouse model, feeding a selenium-deficient diet followed by infection with a strain of the coxsackievirus that typically produces no cardiac pathology led to the development of severe myocarditis. This was this initial discovery that selenium deficiency could affect viral virulence (36). In fact, the genome of the coxsackievirus used to inoculate the mice had mutated in the selenium-deficient animals to cause the development of myocarditis (37), a relationship that has been attributed to the selenium-dependent expression of glutathione peroxidase (38). Of added interest, the newly mutated virus then developed the ability to cause myocarditis in selenium-adequate hosts. A schematic depicting the interaction between selenium status and viral pathogenicity appears in Fig. 14.1.

Since the initial discovery that the selenium status of the host could affect the virulence of a virus, a number of studies have further explored this relationship. In a study of acquired immunodeficiency in a coxsackievirus-resistant strain of mice, researchers found that coinfection of animals with retrovirus and coxsackievirus resulted in myocarditis (39). Selenium supplementation during retroviral infection significantly improved chances of animal survival. Mouse models also indicate that host selenium status can affect influenza infection; inoculation of selenium-deficient mice with influenza results

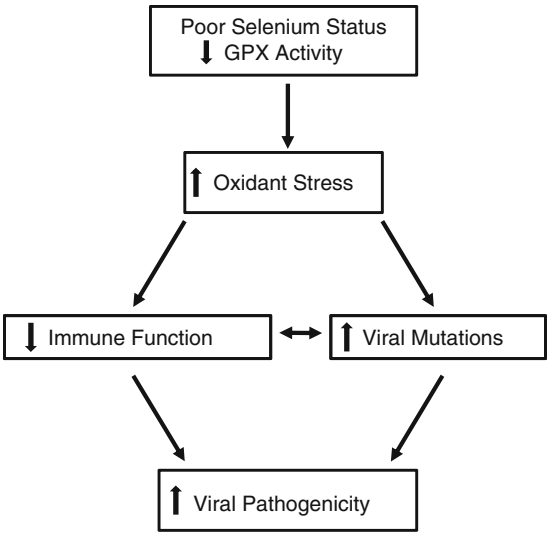


Fig. 14.1. The interaction between selenium status and viral pathogenicity. Adapted from Beck (55).

in severe pneumonitis in the selenium-deficient, but not in the selenium-adequate animals (40). Cytokine expression is also affected in selenium-deficient animals.

Human studies in the United Kingdom indicate that adults with low selenium status have a decreased immune response to live poliovirus vaccination and have an increased shedding of vaccine strain mutations, which can be attenuated with selenium supplementation (41). Other human studies indicate that selenium supplementation may benefit even those with normal selenium status, as supplementation with 200 µg/day of selenium, an amount well beyond the current recommended dietary allowance (RDA) of 55 µg/day, results in enhanced proliferation and differentiation of cytotoxic effector cells and natural killer cell activity (42, 43).

The fascinating role of selenium status in determining viral virulence and pathogenicity continues to serve as an important research area in the understanding of the role of trace elements in host-pathogen relationships. It is likely that this line of research will lead to further study of the role of other trace elements in viral virulence, and may affect public health recommendations as increased travel and industrialization facilitate viral transmission in the continuously expanding human population.

14.5 ZINC

Zinc is incorporated into over 300 proteins and enzymes, including DNA polymerase, thymidine kinase, and DNA-dependent RNA polymerase, which are enzymes required for the synthesis of nucleic acids (4). Further, zinc functions through the formation of zinc finger proteins, which bind to DNA and act as transcriptional regulators. Zinc has a profound role in immune function. In fact, the first human population with known zinc deficiency also suffered from immune dysfunctions and often died as a result of infection at an early age (44). Furthermore, patients with acrodermatits enteropathica, a

genetic disorder affecting zinc absorption, suffer from frequent bacterial, fungal, and viral infections. These patients have reduced lymphocyte proliferative responses to mitogens, a diminished number of T helper cells, and reduced thymic hormone activity – all of which can be corrected with zinc supplementation (45).

Zinc functions in both the innate and adaptive immune systems. In the innate immune system, human studies have demonstrated that diminished zinc status impairs natural killer cell activity and diminishes phagocytosis of macrophages and neutrophils (46, 47). Furthermore, natural killer cell number and activity are zinc status-dependent (48), and zinc status may affect the ability of these cells to recognize pathogenic cells for killing (47).

Of the many roles for zinc in the adaptive immune system, its role as an essential cofactor for thymulin may be the most profound. Thymulin is a hormone that is produced by the thymus, which regulates the differentiation of immature T cells in the thymus and the function of mature T cells in the periphery. Thymulin also modulates cytokine release by peripheral blood mononuclear cells, and affects the proliferation of cytotoxic T lymphocytes (47). Studies with zinc-deficient animals indicate that T cell proliferation is diminished following mitogen stimulation (49), and that zinc supplementation may reverse the effects of zinc deficiency on the thymus and peripheral cells (50). Although B cells are less sensitive to zinc status than T cells, B cells are affected by zinc deficiency. The number of B lymphocytes and their precursors is reduced during zinc deficiency, although it results in only modest changes in mature B cells (47). Zinc deficiency does impart important functional defects in B cells, as B lymphocyte antibody production is affected by zinc depletion (51).

Changes in the immune function in response to poor zinc status may affect susceptibility to infection, especially early in life. A number of studies have demonstrated the protective effects of zinc supplementation against both diarrhea and pneumonia in children (2). In one study, zinc supplementation resulted in a reduced incidence of pneumonia, reduced mortality secondary to pneumonia, and a reduced incidence of diarrhea in Bangladeshi children (52). Zinc supplementation may be beneficial for patients with sickle cell anemia, as one trial demonstrated reduced incidence of staphylococcus aureus pneumonia and urinary tract infections in zinc-supplemented individuals (53). Although evidence suggests that zinc supplementation may provide benefits at the cellular level in healthy adults, only a few studies have demonstrated a reduced incidence of infection or disease. Several studies have investigated the potential role of zinc in protection from rhinoviruses, or the common cold, although meta-analyses indicate that data may not consistently support this role for zinc in immune function (54). Carefully designed clinical trials will be required to determine the efficacy of zinc supplementation against rhinovirus infection.

14.6 CONCLUSIONS AND PERSPECTIVES

Trace elements are essential nutrients required for life. Trace elements have many functions in the body, including a role in the immune system. Many of the trace elements, including copper, iron, selenium, and zinc function in both the innate and adaptive immune systems. Recent studies have demonstrated interesting roles for iron in the maintenance of the relationship between the host and pathogens and for selenium status

in the virulence of viral infection. The role of trace elements in immune function remains an important area of scientific research that will undoubtedly continue to highlight the importance of nutrition for human health.

REFERENCES

1. Bowen HJM (1966) Trace elements in biochemistry. Academic, New York
2. Wintergerst ES, Maggini S, Hornig DH (2007) Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 51:301–323
3. Failla ML (2003) Trace elements and host defense: recent advances and continuing challenges. J Nutr 133:1443S–1447S
4. Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. Phys Rev 73:79–118
5. Cordano A, Baertl JM, Graham G (1964) Copper deficiency in humans. Annu Rev Nutr 34:324–326
6. Williams D (1983) Copper deficiency in humans. Semin Hematol 20:118–128
7. Percival SS (1995) Neutropenia caused by copper deficiency: possible mechanisms of action. Nutr Rev 53:59–66
8. Higuchi S, Higashi A, Nakamura T, Yanabe Y, Matsuda I (1991) Anti-neutrophil antibodies in patients with nutritional copper deficiency. J Pediatr 150:327–330
9. Babu U, Failla ML (1990) Respiratory burst and candidacidal activity of peritoneal macrophages are impaired in copper deficient rats. J Nutr 120:1692–1699
10. Hopkins RG, Failla ML (1995) Chronic intake of a marginally low copper diet impairs in vitro activities of lymphocytes and neutrophils from male rats despite minimal impact on conventional indicators of copper status. J Nutr 125:2658–2668
11. Arias VJ, Koutsos EA (2006) Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poult Sci 85:999–1007
12. Lukasewycz OA, Prohaska JR (1990) The immune response in copper deficiency. Ann NY Acad Sci 587:147–159
13. Koller LD, Mulhern SA, Frankel NC, Steven MG, Williams JR (1987) Immune dysfunction in rats fed a diet deficient in copper. Am J Clin Nutr 45:997–1006
14. Bonham M, O'Conner JM, Hannigan BM, Strain JJ (2002) The immune system as a physiological indicator of marginal copper status. Br J Nutr 87:393–403
15. Heresi G, Castillo-Duran C, Munoz C, Arevalo M, Schlesinger L (1985) Phagocytosis and immunoglobulin levels in hypocupremic infants. Nutr Res 5:1327–1334
16. Kelley DS, Daudu PA, Taylor PC, Mackey BE, Turnlund JR (1995) Effects of low copper diets on human immune response. Am J Clin Nutr 62:412–416
17. Chandra RK (1973) Reduced bactericidal capacity of polymorphs in iron deficiency. Arch Dis Child 48:864–866
18. Brock JH, Mulero V (2000) Cellular and molecular aspects of iron and immune function. Proc Nutr Soc 59:537–540
19. Ahluwalia N, Sun J, Krause D, Mastro A, Handte G (2004) Immune function is impaired in iron-deficient home-bound, older women. Am J Clin Nutr 79:516–521
20. Oppenheimer SJ, Gibson FD, Macfarlane SBJ, Moody JB, Harrison C, Spencer A, Bunari O (1986) Iron supplementation increases prevalence and effects of malaria. Report on clinical studies in Papua New Guinea. Trans R Soc Trop Med Hyg 80:603–612
21. Oppenheimer SJ, Macfarlane SBJ, Moody JB, Bunari O, Hendrickse RG (1986) Effect of iron prophylaxis on morbidity due to infectious disease. Report on clinical studies in Papua New Guinea. Trans R Soc Trop Med Hyg 80:596–602
22. Smith AW, Hendrickse RG, Harrison C, Hayes RJ, Greenwood BM (1989) The effects of malaria on treatment of iron-deficiency anaemia with oral iron in Gambian children. Ann Trop Paediatr 9:17–23
23. Murray MJ, Murray AB, Murray MB, Murray CJ (1978) The adverse effect of iron repletion on the course of certain infections. Br Med J 2:1113–1115
24. McDermid JM, Prentice AM (2006) Iron and infection: effects of host iron status and the iron-regulatory genes haptoglobin and NRAMPI (SLCIIAI) on host-pathogen interactions in tuberculosis and HIV. Clin Sci 110:503–524

25. Fritsche G, Larcher C, Schennach H, Weiss G (2001) Regulatory interactions between iron and nitric oxide metabolism for immune defense against *Plasmodium falciparum* infection. J Infect Dis 183:1388–1394
26. Oppenheimer SJ (2001) Iron and its relation to immunity and infectious disease. J Nutr 131:616S–635S
27. Weiss G (2002) Iron and immunity: a double-edged sword. Eur J Clin Invest 32:70–78
28. Markel TA, Crisostomo PR, Wang M, Herring CM, Meldrum KK, Lillemoe KD, Meldrum DR (2007) The struggle for iron: gastrointestinal microbes modulate the host immune response during infection. J Leukoc Biol 81:393–400
29. Pieracci FM, Barie PS (2005) Iron and the risk of infection. Surg Infect 6:S41–S46
30. Radtke AL, O'Riordan X (2006) Intracellular innate resistance to bacterial pathogens. Cell Microbiol 8:1720–1729
31. Lei XG, Cheng WH, McClung JP (2007) Metabolic regulation and function of glutathione-peroxidase-1. Annu Rev Nutr 27:41–61
32. Arthur JR, McKenzie RC, Beckett GJ (2003) Selenium in the immune system. J Nutr 133:1457S–1459S
33. Cao YZ, Maddox JF, Mastro AM, Scholz RW, Hildenbrandt G, Reddy CC (1992) Selenium deficiency alters the lipoxygenase pathway and mitogenic response in bovine lymphocytes. J Nutr 122:2121–2127
34. Shrimali RK, Irons RD, Carlson BA, Sano Y, Gladyshev VN, Park JM, Hatfield DL (2008) Selenoproteins mediate T cell immunity through an antioxidant mechanism. J Biol Chem 283:20181–20185
35. Su C, Gong C, Li Q, Chen L, Zhou D, Jin Q (1979) Preliminary results of viral etiology of Keshan disease. Chin Med J 59:466–472
36. Beck MA, Kolbeck PC, Rorh LH, Shi Q, Morris VC, Levander OA (1994) Benign human enterovirus becomes virulent in selenium-deficient mice. J Med Virol 43:166–170
37. Beck MA, Shi Q, Morris VC, Levander OA (1995) Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. Nat Med 1:433–436
38. Beck MA, Esworthy RS, Ho Y-S, Chu F-F (1998) Glutathione peroxidase protects mice from viral induced myocarditis. FASEB J 12:1143–1149
39. Sepulveda RT, Zhang J, Watson RR (2002) Selenium supplementation decreases coxsackievirus heart disease during murine AIDS. Cardiovasc Toxicol 2:53–61
40. Beck MA, Nelson HK, Shi Q, Van Dael P, Schriffin EJ, Blum S, Barclay D, Levandar OA (2001) Selenium deficiency increases the pathology of an influenza virus infection. FASEB J 15:1481–1483
41. Broome CS, McArdle F, Kyle JA, Andrews F, Lowe NM, Hart CA, Arthur JR, Jackson MJ (2004) An increase in selenium uptake improves immune function and poliovirus handling in adults with marginal selenium status. Am J Clin Nutr 80:154–162
42. Kiremidjian-Schumacher L, Roy M, Wishe HI, Cohen MW, Stotzky G (1994) Supplementation with selenium and human immune cell functions. II. Effect on cytotoxic lymphocytes and natural killer cells. Biol Trace Elem Res 41:115–127
43. Roy M, Kiremidjian-Schumacher L, Wishe HI, Cohen MW, Stotzky G (1994) Supplementation with selenium and human immune cell functions. I. Effect on lymphocyte proliferation and interleukin 2 receptor expression. Biol Trace Elem Res 46:3–14
44. Prasad AS (2008) Zinc in human health: effect of zinc on immune cells. Mol Med 14:353–357
45. Dardenne M (2002) Zinc and immune function. Eur J Clin Nutr 56:S20–S23
46. Allen JL, Perri RT, McClain CJ, Kay NE (1983) Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. J Lab Clin Med 102:577–589
47. Ibs K-H, Rink L (2003) Zinc-altered immune function. J Nutr 133:1452S–1456S
48. Ravaglia G, Fort P, Maioli F, Bastagali L, Facchini A, Erminia M, Savarino L, Sassi S, Cucinotta D, Lenaz G (2000) Effect of micronutrient status on natural killer cell immune function in healthy free-living subjects 90 y. Am J Clin Nutr 71:590–598
49. Dowd PS, Kelleher J, Guillou PJ (1986) T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. Br J Nutr 55:59–69
50. Mocchegiani E, Santarelli L, Muzzioli M, Fabris N (1995) Reversibility of thymic involution and age-related peripheral immune dysfunction by zinc supplementation in old mice. Int J Immunopharmacol 17:703–718
51. Depasquale-Jardieu P, Fraker PJ (1984) Interference in the development of a secondary immune response in mice by zinc deprivation: persistence on effects. J Nutr 114:1762–1769

52. Brooks WA, Santosham M, Naheed A, Goswami D, Waheed MA, Diener-West M, Faruque ASG, Black RE (2005) Effect of weekly zinc supplements on incidence of pneumonia and diarrhea in children younger than 2 years in an urban, low-income population in Bangladesh: randomized controlled trial. Lancet 366:999–1004
53. Prasad AS, Beck WJ, Kaplan J, Chandrasekar PH, Ortega J, Fitzgerald JT, Swerdlow P (1999) Effect of zinc supplementation on incidence of infections and hospital admissions in sickle cell disease (SCD). Am J Hematol 61:194–202
54. Marshall I (1999) Zinc for the common cold. Cochrane Database Syst Rev 2:CD001364
55. Beck MA (2007) Selenium and vitamin E status: impact on viral pathogenicity. J Nutr 137:1338–1340

15 Vitamin Supplements and Disease Resistance in HIV-Infected Women and Children

Joanne E. Arsenault and Eduardo Villamor

Key Points

- Multivitamin supplements (including vitamins B, C, and E) can improve immune parameters, pregnancy outcomes, and child growth and health among HIV-infected women and children.
- Vitamin A supplementation is beneficial against morbidity and mortality when given to children, but increases the risk of mother-to-child HIV transmission when given to pregnant, HIV-infected women.
- The majority of research pertaining to vitamins and HIV disease progression has been conducted in patients not receiving antiretrovial therapy, and so further research is urgently needed to examine the efficacy of nutritional interventions as coadjuvants of antiretrovial therapy.

Key Words: Vitammins, HIV, women, children.

15.1 INTRODUCTION

High rates of HIV infection and malnutrition coexist in the same geographic regions of the world, particularly in Sub-Saharan Africa and Southeast Asia. Approximately 2/3 of those afflicted with HIV live in Sub-Saharan Africa, and 59% of affected persons are women. In the absence of interventions, mother-to-child transmission of HIV occurs in 30–45% of cases. Young children residing in these regions suffer the highest rates of stunted growth (1) and micronutrient deficiencies (2).

The relation between nutritional status and HIV infection is bidirectional. HIV infection can lead to undernutrition through decreased food intake, malabsorption, and increased utilization and excretion of nutrients (3). Poor nutritional status affects HIV transmission and disease progression. This chapter will review the literature on vitamin

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_15

supplementation studies and immunologic and clinical outcomes among HIV-infected women and children.

15.2 VITAMINS AND DISEASE PROGRESSION IN HIV-INFECTED WOMEN

15.2.1 Observational Studies

Observational studies have examined relations between HIV disease and vitamins with the use of nutrient biomarkers (e.g., serum concentrations) or dietary intake assessment. Lower plasma levels of vitamin A were associated with increased mortality in HIV-infected drug users (4). Also, reduced serum levels of vitamins E (5) and B-12 (6) were associated with faster HIV disease progression. Lower serum vitamin D concentrations were reported in HIV-infected patients compared to uninfected controls (7–9). In a small study of HIV-infected patients, reduced vitamin D concentrations at baseline appeared to be related to a greater risk of mortality (9). Serum concentrations of vitamins may not represent true micronutrient status in HIV-infected patients. The acute phase response to infection reduces serum vitamin A concentrations (10); thus, decreased serum retinol may be a marker of advanced HIV disease stage rather than a reflection of limited stores of the vitamin. The associations between low serum concentrations of nutrients and HIV disease may also be due to reverse causality, since advanced HIV infection could decrease absorption and augment the utilization of nutrients.

The higher intake of some nutrients has been associated with slower disease progression. Daily multivitamin use was related to higher CD4+ counts and to a significant reduction in risk of progression to AIDS diagnosis in a longitudinal study (11). In another study, vitamin A intakes between 9,000 and 20,000 IU, about three to nine times the recommended daily allowance (RDA) (12), were associated with slower disease progression to AIDS (13). The daily intake of B-complex supplements in South African HIV-infected patients was associated with lower risk of disease progression (14). Higher intakes of vitamins B1, B2, B6, and niacin were associated with 40–60% reductions in risk of death during 8 years of follow-up (15). The risk reductions were only seen at levels of intake that were several times higher than the RDA. This suggested that large supplemental doses may be needed to correct profound nutrient deficiencies in HIV-infected patients.

15.2.2 Intervention Studies

A number of intervention studies have examined the effect of vitamin supplements on HIV disease progression (Table 15.1). High doses of β-carotene (180 mg/day) for 4 weeks in HIV-infected adults resulted in higher CD4+ cell counts (16), but had no effect on CD4+ or viral load in another study with the same dosage and duration (17). In a longer trial, no differences in immune cell counts were found between HIV-patients receiving β-carotene plus multivitamins for 3 months, compared to those receiving multivitamins alone (18). Two weeks of daily multimicronutrient supplementation in HIV-infected Zambian adults with persistent diarrhea had no effect on CD4+ cell counts or clinical markers of disease severity (19). In a large community-based trial in Zambia including

Table 15.1
Vitamin supplementation trials among HIV-infected women and disease progression

Reference	*Population*	*Intervention*	*Outcome*	*Treatment effect*					
US (16)	21 HIV-infected men and women	β-carotene (180 mg) daily for 4 weeks or placebo, crossover at 4 weeks for an additional 4 weeks	Absolute (Δ) and % change in:	*β-carotene*		*Placebo*		*P* value	
				Δ	%	Δ	%	for Δ	for %
			WBC	420.0	10.7	−600.0	−10.8	0.01	0.01
			Lymphocyte count	88.2	9.2	−170.6	−9.8	0.41	0.46
			B-lymphocytes	15.5	1.4	−17.4	25.0	0.41	0.98
			CD4+ count	13.4	17.0	−44.9	−17.3	0.11	0.02
			CD4+/CD8+ ratio	−0.01	21.4	−0.05	−20.4	0.36	0.02
US (18)	72 HIV-infected men and women	β-carotene (180 mg) daily for 3 months or placebo	Immune factors at 3 months	*β-carotene*		*Placebo*		*P* value	
			CD4+ ($\times 10^6$/L)	296.3		331.0		ns	
			CD4+/CD8+ ratio	0.31		0.36		ns	
			Lymphocytes ($\times 10^9$/L)	1.77		1.83		ns	
			Natural killer cells ($\times 10^6$/L)	126.9		128.2		ns	
Zambia (19)	136 HIV-infected men and women	Multimicronutrient (M) including vitamins A, C, and E, selenium, zinc for 2 weeks or placebo		*M*		*Placebo*			
			Diarrhea during 4 or 12 weeks	No effect					
			Mortality at 4 weeks	No effect					
			CD4+ count at 4 weeks	No effect					
Zambia (20)	500 HIV-infected and uninfected men and women	Multimicronutrient (M) including β-carotene, vitamins B1, B2, B6, B12, C, D, and E, niacin, folic acid, iron, zinc, copper, selenium, iodine for 2 years or placebo, crossover at 2 years for additional 1.3 years	Among HIV-positive (n = 178):	*M*		*Placebo*		*P* value	
			Diarrhea	No effect					
			Median change in CD4+ counts	−46		4		0.55	
			Mortality (no deaths)	4		12		0.03	

(continued)

Table 15.1
(continued)

Reference	*Population*	*Intervention*	*Outcome*	*Treatment effect*		
				M	*Placebo*	*P* value
Thailand (21)	481 HIV-infected men and women	Multimicronutrient (M) including β-carotene, vitamins A, D, E, K, C, B1, B2, B6, and B12, folic acid, panthothenic acid, iron, magnesium, manganese, zinc, iodine, copper, selenium, chromium, cystine for 48 weeks or placebo	Mortality overall (HR, 95% CI)	0.53 (0.22–1.25)	Ref	0.10
			Subgroup: CD4+ < 200	0.37 (0.13–1.06)	Ref	0.05
			Subgroup: CD4+ < 100	0.26 (0.07–0.97)	Ref	0.03
			CD4+ ($\times 10^6$/L) – median	200	232	ns
			Viral load – mean $\log_{10}$/ml	4.4	4.5	ns
				MV	*A*	*A and MV*
Tanzania (24–26)	1,078 HIV-infected pregnant women	Vitamin A and β-carotene (A); Multivitamins (MV) including vitamins B1, B2, B6, B12, C, and E, niacin, folic acid; both A and MV; or placebo from 12–27 weeks gestation throughout lactation and up to a median 71 months	Progression to stage 4 or death from AIDS-related causes – RR (95% CI)	0.71 (0.51, 0.98)	0.88 (0.64, 1.19)	0.80 (0.58, 1.10)
			Clinical symptom: – RR (95% CI)			
			Thrush	0.47 (0.30, 0.73)	0.69 (0.44, 1.07)	0.58 (0.38, 0.87)
			Oral ulcers	0.44 (0.28, 0.68)	0.94 (0.59, 1.48)	0.54 (0.35, 0.86)
			Difficult or painful swallowing	0.41 (0.26, 0.63)	1.25 (0.88, 1.77)	0.68 (0.46, 1.03)
			Fatigue	0.64 (0.49, 0.86)	1.04 (0.79, 1.35)	0.97 (0.73, 1.30)
			Rash	0.74 (0.57, 0.96)	0.83 (0.64, 1.06)	0.76 (0.59, 0.98)
			Acute upper respiratory infection	0.79 (0.66, 0.96)	0.96 (0.80, 1.14)	0.87 (0.73, 1.03)
			CD4+ cell count/mm^3 – mean difference from placebo (95% CI)	48 (10, 85)	−15 (−45, 14)	41 (4, 77)

			CD8+ cell count/mm^3 – mean difference from placebo (95% CI)	43 (−15, 101)	−48 (−100, 4)	12 (−44, 69)
			Viral load (log) – mean difference from placebo (95% CI)	−0.18 (−0.32 to −0.03)	−0.03 (−0.17, 0.11)	−0.07 (−0.21, 0.09)
			Wasting (first episode of midupper arm circumference < 22 cm) – RR (95% CI) compared to those not receiving the supplement	*MV (alone or with A)*	*A (alone or with MV)*	
			at 2 years	0.71 (0.54, 0.93)	1.09 (0.83, 1.42)	
			at 4 years	0.79 (0.62, 1.00)	1.14 (0.89, 1.45)	
			Hemoglobin (g/dL)– mean difference from those not receiving the supplement	*MV (alone or with A)*	*A (alone or with MV)*	
				0.33 (p = 0.07)	0.07 (p = 0.68)	
Kenya (27)	400 HIV-infected nonpregnant women	Multimicronutrients (M) including vitamins B1, B2, B6, B12, C, E, folic acid, selenium for 6 weeks or placebo	Mean difference (95% CI) from placebo	*M*	*P value*	
			CD4+ (cells/μL)	23 (3, 43)	0.03	
			CD8+ (cells/μL)	74 (23, 126)	0.005	
			Viral load ($\log_{10}$ copies/mL)	−0.01 (−0.12, 0.09)	0.8	

Abbreviations used: *HR* hazard ratio; *RR* relative risk

HIV-positive and HIV-negative individuals, multimicronutrient supplements reduced mortality among HIV-positive participants but had no effect on CD4+ cell counts (20). Inconsistencies in the results of these studies could be due to their short duration, the heterogeneity of study designs, including differences in the nutrient composition of supplements, and varying patterns of underlying nutrient deficiencies across populations.

The efficacy of daily multivitamin supplementation of HIV-infected individuals on clinical outcomes was examined in two randomized, double-blind, placebo-controlled trials from Thailand and Tanzania. In Thailand, supplements including 21 vitamins and minerals were associated with a nonstatistically significant reduction in overall mortality among HIV-infected men and women, and with a significant reduction among individuals who had low baseline CD4+ counts (21). There were no effects on CD4+ cell counts or viral load. Although these results suggested a beneficial effect of micronutrient supplements on disease progression, the study had limited statistical power due to a relatively small sample size (n = 481), and few reported deaths during the 48-week follow-up.

Further evidence in support of the benefits of vitamin supplementation in HIV-infected individuals emerged from a large, double-blind, placebo-controlled trial in Tanzania among 1,078 HIV-infected pregnant women. The women were randomized to receive daily multivitamins (vitamins B-complex, C, and E) at 2–20 times the RDA (22, 23) and/or vitamin A (preformed vitamin A and ß-carotene) at ~5 times the RDA, or placebo, using a two-by-two factorial design. The median follow-up time for morbidity outcomes was 71 months. Multivitamin supplementation resulted in a 29% reduced risk of progression to stage 4 HIV disease or AIDS-associated death, higher CD4+ and CD8+ cell counts and lower viral load, and lower rates of clinical symptoms such as oral ulcers, thrush, difficulty swallowing, fatigue, rash, and acute upper respiratory tract infections (24). Vitamins B, C, and E reduced the incidence of wasting (25) and improved hemoglobin concentrations (26). Vitamin A had no beneficial effects on these outcomes. In a smaller trial among nonpregnant, HIV-infected women in Kenya, daily supplementation with the same nutrients plus selenium significantly increased CD4+ cell counts (27). Therefore, the results in the Tanzania trial may be generalizable to nonpregnant HIV-infected women in developing countries.

15.2.3 Potential Mechanisms for Benefits of Vitamin Supplementation on Disease Progression

Improved immune function associated with vitamin supplementation could lead to improved health and reduced mortality in HIV-positive individuals. The positive effect of vitamin supplementation on CD4+ cell counts is consistent with results from *in vitro* and *in vivo* studies of vitamins and immune function. Vitamin B-6 deficiency affects cellular immunity. Depletion of vitamin B-6 in elderly subjects reduced total lymphocyte counts, lymphocyte proliferation, and IL-2 production in response to T-cell mitogens; these alterations were corrected with supplementation (28). Vitamin B-2 deficiency impairs the ability to generate humoral antibodies in response to test antigens whereas vitamin B-12 supplements are associated with enhanced antibody function and mitogenic responses (29). Vitamin C deficiency is associated with depressed cell-mediated immune response, and T- and B-lymphoctye proliferative responses increase after vitamin C supplementation in humans (30). Enhanced vitamin C status was associated with a lower

rate of respiratory infections (31). Vitamins C and E could affect viral load via their antioxidant properties. HIV replication *in vitro* is increased by oxidative stress (32). In a small randomized, placebo-controlled study of HIV-positive patients, those who received daily vitamin E (800 IU) and C (1,000 mg) for 3 month experienced a decrease in lipid peroxidation and a trend toward reduced viral load (33).

15.3 VITAMIN SUPPLEMENTATION AND CHILD HEALTH

Intervention studies have examined the effects of vitamin supplements on pregnancy outcomes, mother-to-child transmission of HIV, and child health (Table 15.2).

15.3.1 Maternal Supplementation and Pregnancy Outcomes

Vitamin supplementation of HIV-infected women during pregnancy has resulted in reduced incidence of preterm delivery and low birth weight. In South Africa, 728 HIV-infected women were randomized to receive either vitamin A (5,000 IU retinyl palmitate and 30 mg β-carotene) or placebo daily during the third trimester (34). Women receiving vitamin A were less likely to have a preterm delivery (11.4% in the vitamin A and 17.4% in the placebo group; $p = 0.03$), but vitamin A supplementation had no effect on birth weight. In Malawi, 697 HIV-infected women received daily iron and folate either alone or in combination with vitamin A (3 mg retinol equivalent or 10,000 IU) from 18 to 28 weeks gestation to delivery (35). Infants born to mothers who received vitamin A had greater birth weight ($p = 0.05$) and body weight at 6 weeks ($p = 0.03$) than those born to mothers who did not receive vitamin A. In the same study, prenatal vitamin A supplementation was also associated with significantly higher hemoglobin concentrations and lower rates of anemia in infants at 6 weeks of age. Pregnant women in Tanzania were randomized to daily high dose vitamin A (preformed vitamin A and ß-carotene) and/or multivitamins (vitamins B-complex, C and E) using a two-by-two factorial design. Multivitamin supplements, but not vitamin A/ß-carotene alone, resulted in approximately 40% reductions in the risks of fetal loss, low birth weight, and severe prematurity (36). Women on multivitamins had heavier placentas, larger increases in hemoglobin at 6 weeks postpartum (36), and greater weight gain during pregnancy (37). These findings could explain the improvements in pregnancy outcomes.

15.3.2 Maternal Supplementation and HIV Transmission to Children

Observational studies suggested that low serum vitamin A concentrations among HIV-infected women were associated with vertical transmission of HIV to their infants (38). Subsequent randomized trials have not found that vitamin A supplementation reduces risk of transmission (34, 35, 39). Studies in South Africa and Malawi found no overall effect of vitamin A supplementation during pregnancy on mother-to-child HIV transmission from 3 to 24 months of age (34, 35). By contrast, in the Tanzania trial vitamin A/ß-carotene supplementation resulted in a statistically significant increase of 38% in the risk of vertical transmission of the virus throughout the lactation period (39). Vitamin A/ß-carotene supplementation also resulted in a significant increase in lower genital viral shedding (40). A trial testing the efficacy of a single large dose of vitamin

Table 15.2
Vitamin supplementation trials among HIV-infected women and child health outcomes

Reference	*Population*	*Intervention*	*Outcome*	*Treatment effect*		
South Africa (34)	728 HIV-infected pregnant women	Vitamin A and β-carotene (A) during third trimester or placebo (P)	Pregnancy outcomes	*A*	*P*	*P value*
			Preterm birth 37 weeks (%)	11.4	17.4	0.03
			Low birth weight < 2,500 g (%)	12.2	14.1	ns
			Child HIV-infection by 3 months (%)	20.3	22.3	ns
Malawi (35)	697 HIV-infected pregnant women	Vitamin A (A) or placebo from 18–28 weeks gestation until delivery; both groups received iron and folate	Pregnancy outcomes	*A*	*P*	*P value*
			Birth weight (g)	2,895 ± 31	2,805 ± 32	0.05
			Low birth weight < 2,500 g (%)	14.0	21.1	0.03
			Infant hemoglobin by 6 weeks (g/L)	116 ± 1	112 ± 1	0.04
			Infant anemia by 6 weeks (%)	23.4	40.6	0.001
			Child HIV-infection (%), by age			
			6 weeks	26.6	27.8	0.76
			12 months	27.3	32.0	0.25
			24 months	27.7	32.8	0.21
Tanzania (36, 37, 39, 47, 48)	1,078 HIV-infected pregnant women	Vitamin A and β-carotene (A); Multivitamins (MV) including vitamins B1, B2, B6, B12, C, and E, niacin, folic acid; both A and MV; or placebo from 12 to 27 weeks gestation through lactation	Pregnancy outcomes – RR (95% CI) compared to those not receiving the supplement	*MV*		*A*
			Miscarriage	0.66 (0.32, 1.36)		0.73 (0.36, 1.50)
			Stillborns	0.58 (0.33, 1.02)		1.00 (0.58, 1.73)
			Fetal deaths	0.61 (0.39, 0.94)		0.89 (0.58, 1.36)
			Low birth weight < 2,500 g	0.56 (0.38, 0.82)		0.89 (0.61, 1.29)
			Preterm birth < 37 weeks	0.86 (0.68, 1.10)		1.06 (0.83, 1.35)
			Preterm birth < 34 weeks	0.61 (0.38, 0.96)		1.09 (0.70, 1.70)
			Placental weight – mean ± SD	526 ± 107		507 ± 104 (p = 0.02)

Weight gain during third trimester – mean difference in change (95% CI) compared to those not receiving the supplement	304 (17, 590)	−31 (−256, 319)
Child HIV-infection by 2 years – RR (95% CI) compared to those not receiving the supplement	1.04 (0.82, 1.32)	1.38 (1.09, 1.76)
Child mortality by 2 years – RR (95% CI)	0.82 (0.66, 1.02)	1.00 (0.80, 1.24)
Child health during first 2 years –RR (95% CI) compared to those not receiving the supplement		
Diarrhea	0.83 (0.71, 0.98)	0.95 (0.81, 1.12)
Cough and rapid respiration	1.22 (0.87, 1.70)	0.69 (0.49, 0.96)
CD4+ cell counts/μL – mean difference (95% CI)	151 (64, 237)	ns
Child growth by 2 years – mean difference (95% CI) from placebo		
Weight (g)	459 (35, 882)	9 (−436, 453)
Length (cm)	0.36 (−0.61, 1.34)	−0.40 (−1.40, 0.60)
Weight-for-age Z-score	0.42 (0.07, 0.77)	0.04 (−0.34, 0.41)
Weight-for-length Z-score	0.38 (0.07, 0.68)	0.11 (−0.21, 0.42)
Length-for-age Z-score	0.14 (−0.17, 0.44)	−0.10 (−0.41, 0.22)

Abbreviations used: *RR* relative risk

A given to women during the early postpartum period (200,000 IU) and/or to neonates (50,000 IU) in Zimbabwe found an increased risk of infant HIV-infection or death when vitamin A was provided to either, but not both, the mother or infant, compared to placebo (41).

The mechanisms by which vitamin A/ß-carotene could increase HIV shedding and transmission of HIV are not clear. It has been hypothesized that by increasing the multiplication and differentiation of lymphoid and myeloid cells, preformed vitamin A leads to increased density of CCR5 receptors that are expressed on these cells and which are necessary for attachment and subsequent replication of the virus (42). It is also possible that the adverse effect noted in the Tanzania study is due to the ß-carotene component of the vitamin A intervention. Although high doses of ß-carotene provided for short periods to HIV-infected individuals were apparently safe (16, 17), studies of the safety of prolonged supplementation are lacking.

In the Tanzania trial, multivitamins (B, C, and E) had no effect on the risk of vertical transmission overall; however, children born to women who were in relatively poor nutritional or immunological conditions at baseline experienced reduced risk of HIV transmission through breastfeeding in relation to multivitamin supplementation (39). Multivitamins could affect vertical transmission of HIV by enhancing the mother's immune function, or reducing clinical or virological progression (43). Micronutrients may also affect transmission through breastmilk by reducing the risk of mastitis, an inflammation of breast tissue that is associated with higher viral load and increased risk of transmission (44) or by improving the micronutrient status of the infants (45) through improved milk concentrations of nutrients (46) which may enhance integrity of the infant's intestinal mucosa.

15.3.3 Maternal Supplementation and Child Health

Vitamin supplementation of HIV-infected women during pregnancy and lactation can also have beneficial effects on child health outcomes. In Tanzania, children of women receiving multivitamin supplements (vitamins B, C, and E) had a 17% significantly lower risk of diarrhea during the first 2 years of life than those whose mothers did not receive multivitamins ($p = 0.03$) (47). Children whose mothers were in the multivitamin arm also had significantly higher CD4+ cell counts than those whose mothers did not receive multivitamins, irrespective of their own HIV status. Children whose mothers received vitamin A/β-carotene alone had a statistically significant reduction in the risk of cough with rapid respiratory rate. Multivitamin supplementation during pregnancy and lactation also resulted in greater weight gain in the children up to 24 months of age (48). Vitamins could affect child growth by increasing intrauterine growth through placental transfer of nutrients, and/or extrauterine growth through increased concentrations of nutrients in breastmilk (46). The effect of vitamins on child growth could be mediated by improvements in the immune system of the child. Maternal vitamin A supplementation improved intestinal impermeability of HIV-infected infants, which may decrease gastrointestinal morbidity (49). The effects of multivitamins on child health could also be mediated through their benefits on the mothers' health, which would enable them to take better care of their children.

15.3.4 Supplementation of Children and Health Outcomes

Evidence of the efficacy of vitamin supplementation to HIV-infected children from randomized trials is limited primarily to vitamin A, and one recent trial with vitamin D (Table 15.3). In a US trial among HIV-infected children aged 2–17 years, vitamin A administered before influenza vaccination was associated with a dampening in HIV viral load postimmunization (50). In South Africa, vitamin A supplementation of young HIV-infected children decreased diarrhea episodes by 49%, but had no effect in uninfected children who were born to HIV-infected mothers (51). In Uganda, vitamin A supplementation of HIV-infected children reduced mortality by 46% and decreased the prevalence of persistent cough and chronic diarrhea (52). A single dose of vitamin A given to HIV-negative neonates born to HIV-positive women reduced mortality by 28% in those infants who became infected by 6 weeks of age (41). In a trial in Tanzania, children hospitalized for pneumonia were randomized to receive large doses of vitamin A during hospitalization and 4 and 8 months after discharge; vitamin A resulted in a 49% reduction in mortality overall, with greater effects among HIV-infected children than among those who were uninfected (53). Vitamin A also resulted in greater increases in linear growth (54) only among the HIV-infected children. Vitamin A could affect child growth through improvements in humoral (55) and cellular (56) immunity and through protecting the integrity of the gastrointestinal epithelium (49, 57, 58), which may in turn decrease diarrheal infections.

In a recent small trial in the US, HIV-infected children aged 6–16 years were randomly assigned to receive vitamin D (100,000 IU) bimonthly with calcium daily or placebo for 1 year (59). No significant treatment effects were found on viral load or CD4+ counts. Vitamin D is an immune system regulator and the investigation of vitamin D and HIV is a promising area of research (60, 61). Randomized trials examining the effect of multivitamin supplements administered to children born to HIV-infected women are currently ongoing.

15.4 VITAMIN SUPPLEMENTATION IN HIV-INFECTED PATIENTS RECEIVING ANTIRETROVIRAL TREATMENT

Since the introduction of highly active antiretroviral therapy (HAART) as a standard for HIV treatment, a few observational and intervention trials have been conducted among patients receiving HAART. In a small study, patients receiving HAART had lower vitamin A concentrations than those not taking HAART (62). Other observational studies have found higher concentrations of α-carotene, β-carotene, and α-tocopherol (63); and folate and vitamin B-12 (64), among patients receiving HAART compared to those not receiving HAART. In a small randomized trial of Brazilian patients receiving HAART, vitamin E supplementation for 6 months had no effect on CD4+, CD8+, or viral load compared to placebo (65). In a small trial from Poland, vitamins A, C, and E for 6 months had no effect on CD4+ counts compared to placebo (66). In a trial from the US, patients receiving multiple micronutrient supplements for 12 weeks had increased CD4+ cell counts whereas those receiving placebo did not experience a change ($p = 0.01$), but supplementation had no effect on viral load (67). All these studies had relatively small sample sizes and a short duration of follow-up. Large, long-term

Table 15.3
Vitamin supplementation trials among HIV-infected children and health outcomes

Reference	*Population*	*Intervention*	*Outcome*	*Treatment effect*		
US (50)	60 HIV-infected children ages 2–17 years	Vitamin A single dose on 2 days or placebo; all children administered inactivated influenza vaccine 14 days later		*A*	*P*	*P value*
			Viral load (log_{10} copies/mL) – change 14 days after vaccination	−0.13 ± 0.09	0.14 ± 0.08	0.02
South Africa (51)	118 children born to HIV-infected women	Vitamin A in single dose at 1, 3, 6, 9, 12 and 15 months of age or placebo		*A*	*P*	*OR (95% CI)*
			Diarrhea – incidence/100 child-months	19.7	25.6	0.71 (0.47, 1.08)
			Subgroup: HIV-infected	21.6	35.1	0.51 (0.27, 0.99)
			Subgroup: Uninfected	21.1	23.1	0.89 (0.23, 3.13)
Uganda (52)	181 HIV-infected children 6 months of age	Vitamin A or placebo every 3 months from ages 15 to 36 months		*A*	*P*	*RR (95% CI)*
			Mortality during median 17.8 months (%)	20.6	32.9	0.54 (0.30, 0.98)
			Morbidity – point prevalence/ child-months			*OR (95% CI)*
			Diarrhea in last 7 days	0.061	0.054	1.13 (0.88, 1.46)
			Persistent cough > 30 days	0.004	0.010	0.47 (0.23, 0.96)
Tanzania (53, 54)	687 children 6–60 months of age admitted to hospital with pneumonia	Vitamin A or placebo on admission, on following day, and at 4 and 8 months after discharge		*A*	*P*	*RR (95% CI)*
			Mortality (%)	5.9	11.4	0.51 (0.29, 0.90)
			Subgroup: HIV-infected	21.4	56.0	0.37 (0.14, 0.95)
			Subgroup: Uninfected	4.3	7.3	0.58 (0.28, 1.19)
			Growth – at 1 year			*Difference (95% CI)*
			Weight gain (kg) – mean (SE)	2.24 (0.07)	2.27 (0.07)	0.03 (−0.16, 0.23)
			Height gain (cm) – mean (SE)	7.8 (0.22)	7.8 (0.21)	0.00 (−0.6, 0.6)
			Height gain at 4 months:			
			Subgroup: HIV-infected			2.8 (1.0, 4.6)
			Subgroup: Uninfected			−0.2 (−0.8, 0.5)

US (59)	56 HIV-infected children 6–16 years of age	Vitamin D and calcium (D) or placebo for 12 months	At 12 months – mean ± SD	*D*	*P*	*P value*
			CD4+ cell count/mL	776 ± 359	661 ± 363	0.18
			CD4+ %	30.8 ± 9.1	27.0 ± 9.9	0.09
			Viral load (log_{10} copies/mL)	2.4 ± 0.9	2.5 ± 1.1	0.66

Abbreviations used: *OR* odds ratio, *RR* relative risk

studies are needed to determine if vitamin supplementation is beneficial in HIV-infected individuals receiving HAART.

15.5 CONCLUSIONS AND PERSPECTIVES

Supplementation with vitamins B-complex, C, and E has been shown to reduce disease progression and mortality of HIV-infected women and children who were not receiving antiretrovial treatment. Additional beneficial effects of these vitamins on birthweight, prematurity, and fetal deaths have been demonstrated. Effects of maternal vitamin A supplementation on these pregnancy outcomes have been conflicting, and adverse effects regarding increased HIV transmission to children have been reported. Maternal supplementation with vitamins B, C, and E reduced child morbidity and improved growth, but had no significant effects on child mortality by 2 years. Vitamin A supplementation of children who were born to HIV-infected women or who were already HIV-infected reduced morbidity and mortality, and improved growth. In conclusion, while multivitamin supplementation appeared to be an effective intervention to improve the health and nutritional status of women and children infected with HIV in the pre-HAART era, the impact of this intervention among patients receiving highly active antiretrovial therapy is still uncertain. Given that life-saving antiretroviral therapy is becoming more widely available in the regions of the world most hardly hit by the HIV epidemic, the efficacy and safety of nutritional interventions as coadjuvants of HAART need to be urgently examined in large-scale, longitudinal studies. Prenatal vitamin A supplementation of HIV-infected women is not recommended based on evidence of increased transmission to children; however, vitamin A supplementation of HIV-infected children appears to be beneficial.

REFERENCES

1. de Onis M, Blossner M, Borghi E, Morris R, Frongillo E (2004) Methodology for estimating regional and global trends of child malnutrition. Int J Epid 33:1260–1270
2. Micronutrient Initiative, UNICEF (2004) Vitamin and mineral deficiency: a global damage assessment report. Micronutrient Initiative, Ottawa, Canada
3. Keusch G, Farthing M (1990) Nutritional aspects of AIDS. Annu Rev Nutr 10:475–501
4. Semba R, Caiaffa W, Graham N, Cohn S, Vlahov D (1995) Vitamin A deficiency and wasting as predictors of mortality in human immunodeficiency virus-infected injection drug users. J Infect Dis 171:1196–1202
5. Tang A, Graham N, Semba R, Saah A (1997) Association between serum vitamin A and E levels and HIV-1 disease progression. AIDS 11:613–620
6. Tang A, Graham N, Chandra R, Saah A (1997) Low serum vitamin B-12 concentrations are associated with faster human immunodeficiency virus type 1 (HIV-1) disease progression. J Nutr 127:345–351
7. Teichmann J, Stephan E, Discher T, Lange U, Federlin K, Stracke H, Friese G, Lohmeyer J, Bretzel R (2000) Changes in calciotropic hormones and biochemical markers of bone metabolism in patients with human immunodeficiency virus infection. Metabolism 49:1134–1139
8. Teichmann J, Stephan E, Lange U, Discher T, Friese G, Lohmeyer J, Stracke H, Bretzel R (2003) Osteopenia in HIV-infected women prior to highly active antiretroviral therapy. J Infect 46:221–227
9. Haug C, Muller F, Aukrust P, Froland S (1994) Subnormal serum concentration of 1, 25-vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. J Infect Dis 169:889–893

10. Stephensen C, Gildengorin G (2000) Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 72:1170–1178
11. Abrams B, Duncan D, Hertz-Picciotto I (1993) A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. J Acquir Immune Defic Syndr 6:949–958
12. Food and Nutrition Board, and Institute of Medicine. (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Washington, DC
13. Tang A, Graham N, Kirby A, McCall A, Willett W, Saah A (1993) Dietary micronutrient intake and risk progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. Am J Epidemiol 138:1–15
14. Kanter A, Spencer D, Steinberg M, Soltysik R, Yarnold P, Graham N (1999) Supplemental vitamin B and progression to AIDS and death in black South African patients infected with HIV. J Acquir Immune Defic Syndr 21:252–253
15. Tang A, Graham N, Saah A (1996) Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection. Am J Epidemiol 143:1244–1256
16. Coodley G, Nelson H, Loveless M, Folk C (1993) Beta-carotene in HIV infection. J Acquir Immune Defic Syndr 6:272–276
17. Nimmagadda A, Burri B, Neidlinger T, O'Brien W, Goetz M (1998) Effect of oral beta-carotene supplementation on plasma human immunodeficiency virus (HIV) RNA levels and CD4+ cell counts in HIV-infected patients. Clin Infect Dis 27:1311–1313
18. Coodley G, Coodley M, Lusk R, Green T, Bakke A, Wilson D, Wachenheim D, Sexton G, Salveson C (1996) Beta-carotene in HIV infection: an extended evaluation. AIDS 10:967–973
19. Kelly P, Musonda R, Kafwembe E, Kaetano L, Keane E, Farthing M (1999) Micronutrient supplementation in the AIDS diarrhoea-wasting syndrome in Zambia: a randomized controlled trial. AIDS 13:495–500
20. Kelly P, Katubulushi M, Todd J, Banda R, Yambayamba V, Fwoloshi M, Zulu I, Kafwebe E, Yavwa F, Sanderson I, Tomkins A (2008) Micronutrient supplementation has limited effects on intestinal infectious disease and mortality in a Zambian population of mixed HIV status: a cluster randomized trial. Am J Clin Nutr 88:1010–1017
21. Jiamton S, Pepin J, Suttent R, Filteau S, Mahakkanukrauh B, Hanshaoworakul W, Chaisilwattana P, Suthipinittharm P, Shetty P, Jaffar S (2003) A randomized trial of the impact of multiple micronutrient supplementation on mortality among HIV-infected individuals living in Bangkok. AIDS 17:2461–2469
22. Food and Nutrition Board, and Institute of Medicine. (1998) Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. National Academy Press, Washington, DC
23. Food and Nutrition Board, and Institute of Medicine. (2000) Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. National Academy Press, Washington, DC
24. Fawzi W, Msamanga G, Spiegelman D, Wei R, Kapiga S, Villamor E, Mwakagile D, Mugusi F, Hertzmark E, Essex M, Hunter D (2004) A randomized trial of multivitamin supplements and HIV disease progression and mortality. N Engl J Med 351:23–32
25. Villamor E, Saathoff E, Manji K, Msamanga G, Hunter D, Fawzi W (2005) Vitamin supplements, socioeconomic status, and morbidity events as predictors of wasting in HIV-infected women from Tanzania. Am J Clin Nutr 82:857–865
26. Fawzi W, Msamang G, Kupka R, Spiegelman D, Villamor E, Mugusi F, Wei R, Hunter D (2007) Multivitamin supplementation improves hematologic status in HIV-infected women and their children in Tanzania. Am J Clin Nutr 85:1335–1343
27. McClelland R, Baeten J, Overbaugh J, Richardson B, Mandaliya K, Emery S, Lavreys L, Ndinya-Achola J, Bankson D, Bwayo J, Kreiss J (2004) Micronutrient supplementation increases genital tract shedding of HIV-1 in women: results of a randomized trial. J Acquir Immune Defic Syndr 37:1657–1663
28. Meydani S, Ribaya-Mercado J, Russell R, Sahyoun N, Morrow F, Gershoff S (1991) Vitamin B-6 deficiency impairs interleukin 2 production and lymphocyte proliferation in elderly adults. Am J Clin Nutr 53:1275–1280

29. Bendich A, Cohen M (1988) B vitamins: effects on specific and nonspecific immune responses. In: Chandra RK (ed) Nutrition and immunology. Alan R. Liss, New York, pp 101–123
30. Bendich A, Cohen M (1988) Antioxidant vitamins and immune responses. In: Chandra RK (ed) Nutrition and immunology. Alan R. Liss, New York, pp 125–147
31. Hemila H (1997) Vitamin C and infectious diseases. In: Packer L, Fuchs J (eds) Vitamin C in health and disease. Marcel Dekker, Inc, New York
32. Schreck R, Rieber P, Baeuerle P (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kB transcription factor and HIV-1. EMBO J 10:2247–2258
33. Allard J, Aghdassi E, Chau J, Tam C, Kovacs C, Salit I, Walmsley S (1998) Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. AIDS 12:1653–1659
34. Coutsoudis A, Pillay K, Spooner E, Kuhn L, Coovadia H (1999) Randomized trial testing the effect of vitamin A supplementation on pregnancy outcomes and early mother-to-child HIV-1 transmission in Durban. South Africa. South African Vitamin A Study Group. AIDS 13:1517–1524
35. Kumwenda N, Miotti P, Taha T, Broadhead R, Biggar R, Jackson J, Melikian G, Semba R (2002) Antenatal vitamin A supplementation increases birth weight and decreases anemia among infants born to human immunodeficiency virus-infected women in Malawi. Clin Infect Dis 35:618–624
36. Fawzi W, Msamanga G, Spiegelman D, Urassa E, McGrath N, Mwakagile D, Antelman G, Mbise R, Herrera G, Kapiga S, Willett W, Hunter D (1998) Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. Lancet 351:1477–1482
37. Villamor E, Msamanga G, Spiegelman D, Antelman G, Peterson K, Hunter D, Fawzi W (2002) Effect of multivitamin and vitamin A supplements on weight gain during pregnancy among HIV-1-infected women. Am J Clin Nutr 76:1082–1090
38. Semba R, Miotti P, Chiphangwi J, Saah A, Canner J, Dallabetta G, Hoover D (1994) Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. Lancet 343:1593–1597
39. Fawzi W, Msamanga G, Hunter D, Renjifo B, Antelman G, Bang H, Manji K, Kapiga S, Mwakagile D, Essex M, Spiegelman D (2002) Randomized trial of vitamin supplements in relation to transmission of HIV-1 through breastfeeding and early child mortality. AIDS 16:1935–1944
40. Fawzi W, Msamanga G, Antelman G, Xu C, Hertzmark E, Spiegelman D, Hunter D, Anderson D (2004) Effect of prenatal vitamin supplementation on lower-genital levels of HIV type 1 and interleukin type 1 beta at 36 weeks of gestation. Clin Infect Dis 38:716–722
41. Humphrey J, Iliff P, Marinda E, Mutasa K, Moulton L, Chidawanyika H, Ward B, Nathoo K, Malaba L, Zijenah L, Zvandasara P, Ntozini R, Mzengeza F, Mahomva A, Ruff A, Mbizvo M, Zunguza C (2006) Effects of a single large dose of vitamin A, given during the postpartum period to HIV-positive women and their infants, on child HIV infection, HIV-free survival, and mortality. J Infect Dis 193:860–871
42. MacDonald K, Malonza I, Chen D, Nagelkerke N, Nasio J, Ndinya-Achola J, Bwayo J, Sitar D, Aoki F, Plummer F (2001) Vitamin A and risk of HIV-1 seroconversion among Kenyan men with genital ulcers. AIDS 15:635–639
43. Fawzi W, Hunter D (1998) Vitamins in HIV disease progression and vertical transmission. Epidemiology 9:457–466
44. Kantarci S, Koulinska I, Aboud S, Fawzi W, Villamor E (2007) Subclinical mastitis, cell-associated HIV-1 shedding in breast milk, and breast-feeding transmission of HIV-1. J Acquir Immune Defic Syndr 46:651–654
45. Baylin A, Villamor E, Rifai N, Msamanga G, Fawzi W (2005) Effect of vitamin supplementation to HIV-infected pregnant women on the micronutrient status of their infants. Eur J Clin Nutr 59:960–968
46. Webb A, Aboud S, Furtado J, Murrin C, Campos H, Fawzi W, Villamor E (2009) Effect of vitamin supplementation on breast milk concentrations of retinol, carotenoids and tocopherols in HIV-infected Tanzanian women. Eur J Clin Nutr 63:332–339
47. Fawzi W, Msamanga G, Wei R, Spiegelman D, Antelman G, Villamor E, Manji K, Hunter D (2003) Effect of providing vitamin supplements to human immunodeficiency virus-infected, lactating mothers on the child's morbidity and CD4+ cell counts. Clin Infect Dis 36:1053–1062

48. Villamor E, Saathoff E, Bosch R, Hertzmark E, Baylin A, Manji K, Msamanga G, Hunter D, Fawzi W (2005) Vitamin supplementation of HIV-infected women improves postnatal child growth. Am J Clin Nutr 81:880–888
49. Filteau S, Rollins N, Coutsoudis A, Sullivan K, Willumsen J, Tomkins A (2001) The effect of antenatal vitamin A and beta-carotene supplementation on gut integrity of infants of HIV-infected South African women. J Pediat Gastroenterol Nutr 32:464–470
50. Hanekom W, Yogev R, Heald L, Edwards K, Hussey G, Chadwick E (2000) Effect of vitamin A therapy on serologic responses and viral load changes after influenza vaccination in children infected with the human immunodeficiency virus. J Pediatr 136:550–552
51. Coutsoudis A, Bobat R, Coovadia H, Kuhn L, Tsai W, Stein Z (1995) The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. Am J Public Health 85:1076–1081
52. Semba R, Ndugwa C, Perry R, Clark T, Jackson J, Melikian G, Tielsch J, Mmiro F (2005) Effect of periodic vitamin A supplementation on mortality and morbidity of human immunodeficiency virus-infected children in Uganda: a controlled clinical trial. Nutrition 21:25–31
53. Fawzi W, Mbise R, Hertzmark E, Fataki M, Herrera M, Ndossi G, Spiegelman D (1999) A randomized trial of vitamin A supplements in relation to mortality among human immunodeficiency virus-infected and uninfected children in Tanzania. Pediatr Infect Dis J 18:127–133
54. Villamor E, Mbise R, Spiegelman D, Hertzmark E, Fataki M, Peterson K, Ndossi G, Fawzi W (2002) Vitamin A supplements ameliorate the adverse effect of HIV-1, malaria, and diarrheal infections on child growth. Pediatrics 109:E6
55. Semba R, Muhilal SA, Natadisastra G, Wirasasmita S, Mele L, Ridwan E, West K, Sommer A (1992) Depressed immune response to tetanus in children with vitamin A deficiency. J Nutr 122:101–107
56. Coutsoudis A, Kiefpiela P, Coovadia H, Broughton M (1992) Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. Pediatr Infect Dis J 11:203–209
57. Chandra RK, Wadhwa M (1989) Nutritional modulation of intestinal mucosal immunity. Immunol Invest 18:119–126
58. Thurnham D, Northrop-Clewes C, McCullough F, Das B, Lunn P (2000) Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants. J Infect Dis 182(Suppl 1):S23–S28
59. Arpadi S, McMahon D, Abrams E, Bamji M, Purswani M, Engelson E, Morlick M, Shane E (2009) Effect of bimonthly supplementation with oral cholecalciferol on serum 25-hydroxyvitamin D concentrations in HIV-infected children and adolescents. Pediatrics 123:e121–e126
60. Cantorna M, Zhu Y, Froicu M, Wittke A (2004) Vitamin D status, 1, 25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 80(6 suppl):1717S–1720S
61. Villamor E (2006) A potential role for vitamin D on HIV infection? Nutr Rev 64:226–233
62. Toma E, Devost D, Chow LN, Bhat P (2001) HIV-protease inhibitors alter retinoic acid synthesis. AIDS 15:1979–1984
63. Tang A, Smit E, Semba R, Shah N, Lyles C, Li D, Vlahov D (2000) Improved antioxidant status among HIV-infected injecting drug users on potent antiretroviral therapy. J Acquir Immune Defic Syndr 23:321–326
64. Remacha A, Cadafalch J, Sarda P, Barcelo M, Fuster M (2003) Vitamin B-12 metabolism in HIV-infected patients in the age of highly active antiretroviral therapy: role of homocysteine in assessing vitamin B-12 status. Am J Clin Nutr 77:420–424
65. de Souza Júnior O, Treitinger A, Baggio GL, Michelon C, Verdi JC, Cunha J, Ferreira SI, Spada C (2005) Alpha-tocopherol as an antiretroviral therapy supplement for HIV-1-infected patients for increased lymphocyte viability. Clin Chem Lab Med 43:376–382
66. Jaruga P, Jaruga B, Gackowski D, Olczak A, Halota W, Pawlowka M, Olinski R (2002) Supplementation with antioxidant vitamins prevents oxidative modification of DNA in lymphocytes of HIV-infected patients. Free Radic Biol Med 32:414–420
67. Kaiser J, Campa A, Ondercin J, Leoung G, Pless R, Baum M (2006) Micronutrient supplementation increases CD4 count in HIV-infected individuals on highly active antiretroviral therapy: a prospective, double-blinded, placebo-controlled trial. J Acquir Immune Defic Syndr 42:523–528

Section C
Nutriceuticals and Immune-mediated Cancer Therapy

16 Fruit, Vegetables, and Extracts: Role in Cancer Prevention

Susan S. Percival

Key Points

- Gamma delta T cells are cytotoxic lymphocyte cells that kill malignant cells.
- They also kill macrophages when the immune response is completed and no longer needed.
- These cells are modified by dietary bioactive compounds in what is known as nonantigen priming. These cells respond faster and to a greater extent after priming.
- Modification of these cells by diet is hypothesized to prevent cancer because they cannot only kill malignant cells but they can also reduce the inflammation that has been associated with an increased incidence of cancer.

Key Words: Gamma/delta T cell, nonantigen priming, pathogen-associated molecular pattern, inflammation, catechin.

16.1 INTRODUCTION

This chapter will discuss the effect that fruits and vegetables and their extracts have in preventing cancer. In the overall broad look at cancer (Fig. 16.1), the role of immune cells as cytotoxic cells that kill malignant cells will be examined. The immune cell's role in inflammation that has been associated with the risk of cancer will also be discussed. The chapter will specifically address γδ T cells, their ability to destroy malignant cells, their anti-inflammatory activity, and the capability of fruits and vegetables to modify these abilities. Modifications to these cells may occur by mechanisms involving differentiation, by altering the response capacity of the mature cell, or, lastly, by helping to reduce the immune system's capacity for chronic inflammation.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_16

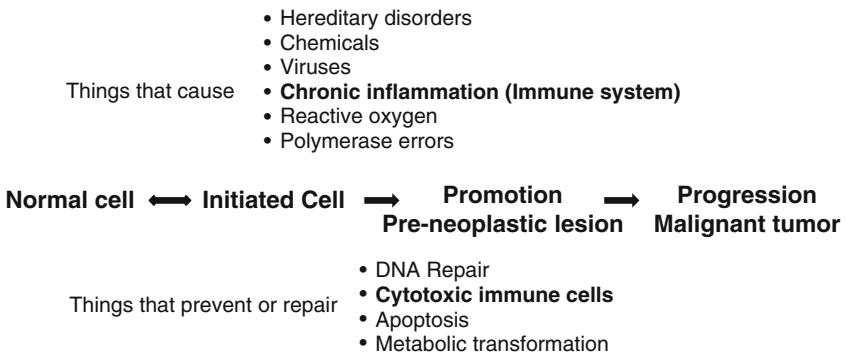


Fig. 16.1. Overview of mechanisms by which cancer becomes a malignancy. Immunological mechanisms that are marked in bold are the focus of the chapter.

16.2 EXPERIMENTAL MODELS USED TO STUDY PHYTOCHEMICALS AND IMMUNITY

Research to understand fruit and vegetable influences on immunity and, subsequently, on the risk of cancer have been performed in cell culture models, in animals, and indirectly in humans. Investigations have utilized the whole food, dried whole foods in capsule form, extracts, and individual compounds. Some scientists estimate that the number of plant compounds exceeds thousands, many of them in minute amounts or with obscure structures. Just which ones are active in modifying immune function is not known, nor do we know the additive, synergistic, or antagonistic interactions among them. Laboratory evidence, although not related to immune function, has suggested that whole food has more activity than the sum of the parts (1). Hence, this chapter will focus on studies using whole foods or extracts of foods, rather than on single compounds. For nomenclature, fruit and vegetable compounds are used interchangeably with plant bioactive compounds and represent the thousands of polyphenols and flavonoid families of flavonols, flavan-3-ols, flavones, anthocyanins, etc.

The activity of fruit and vegetable compounds is studied in cell culture using either cancer cell lines or cells derived from the blood, usually peripheral blood mononuclear cells (PBMC). PBMC are largely T lymphocytes, B lymphocytes, and monocytes. Cancer cell lines are useful because of their perpetual life span, while PBMC survive only a short time in culture. There are many limitations to cell culture studies. The compounds may be used in culture in greater quantity than what might be found in the blood after being ingested. Moreover, while there is little modification to plant bioactive compounds in the mouth, there may be many changes occurring due to the action of stomach acid (instability), and change of pH in the lumen of the small intestine (oxidation) (2). During absorption of the compounds across the enterocyte and the first pass through the liver, the bioactive compounds are de- and reglycosylated, methylated, sulfated, and glucuronidated resulting in a pattern of photochemicals that are drastically altered from the pattern that was consumed. Our knowledge of the compounds formed from the parent compound is incomplete, which may result in an underestimate in the

amount absorbed *in vivo*. Estimates of flavan-3-ol absorption have been suggested to be 10–20% of that which is consumed (2). Following that, what is not absorbed is modified by the microbiota and subsequent colon absorption of compounds modified by demethylation, decarboxylation, dehydroxylation, ring cleavage, and hydrolysis. Compounds resulting from the action of colonic microbes have been found in the urine 7–14 h after the parent compounds were consumed. It has been estimated that microbial action and subsequent absorption in the colon account for 6–39% of the ingested flavan-3-ol (2). Cell culture studies do not take into account digestion, absorption, or microbial action. Because of these limitations, this chapter will largely focus on research where the foods or extracts have been consumed in experimental animal or human models.

16.3 EPIDEMIOLOGY OF CANCER AND FRUITS AND VEGETABLES

It was recognized many years ago that there were components of plants that were not essential to life, but provided health benefits beyond the basic vitamins and minerals. One benefit suggested by research was the reduction in the incidence of cancer (3). Epidemiological studies, however, have shown somewhat conflicting information regarding the consumption of fruits and vegetables and the risk of cancer. For example, low fruit and vegetable consumption is inversely associated with cancer, but interventions using vitamin and/or carotenoid supplements did not reduce the risk of cancer (4–6). Some types of cancer have a stronger inverse relationship with fruit and vegetable intake than others. For example, the risk of prostate cancer was not associated with fruit and vegetable intake (7), but was associated with cruciferous vegetables (8) and with high vitamin C intake (9). Fruit and vegetable consumption was associated with a reduced risk of colon and rectal cancer in men, but not in women (10), while another study found a modest reduction in colon cancer but didn't separate genders (11). An additional study found that fruit and vegetable consumption lowered risk of the distal colon but not colon cancer incidence overall (12). Fruit and vegetable consumption reduced the risk of bladder cancer (13, 14) and gastric and oral cancers (15, 16), while another study found that only vegetables, but not fruit, reduced the risk of gastric and esophageal cancers (17). Factors that may influence the results of epidemiological studies include the time at which the cancer was initiated, the location of the cancer, the timing of the supplement, if used, and the life-time intake of fruits and vegetables, making these types of studies difficult to interpret. However, numerous biochemical and physiological studies, as well as biomarker studies, strongly suggest a benefit of fruit and vegetable compounds in health related to cancer-risk reduction.

16.4 GENERAL IMMUNITY

Immunity is complex and redundant and uses several different cells to carry out the function of surveillance and response. Some of these cells are cytotoxic and have the potential to kill malignant cells. These include cytotoxic CD8+ T cells, NK cells, NK-T cells, and γδ T cells. A healthy immune system is dependent upon all nutrients, both macro and micro. A deficiency in any one nutrient or energy source will impair the functions of both surveillance and response, including cytotoxic activity. Without

cytotoxic activity, malignant cells may proliferate. Thus, a well-nourished immune system is vital to reducing the risk of cancer.

In general, the immune system is categorized into two systems, the innate and the acquired. The innate system, sometime referred to as the first line of defense, includes neutrophils and macrophages, as well as the skin and mucosal membrane barriers. The cells of the innate immune system identify a foreign pathogen using a toll-like receptor (TLR) that can recognize the pattern that is unique to the pathogen, that is, a pathogen-associated molecular pattern (PAMP). These cell types kill pathogens by ingestion (phagocytosis) and then by bombardment with free radicals and other damaging chemicals. The action of these cells is responsible for driving the inflammatory response.

The acquired immune system comprises lymphocytes, T cells, and B cells, and is somewhat slower to respond than the innate system. Each of these cells encodes a unique protein receptor on its surface that is highly specific for the antigen. These cells are responsible for self/nonself recognition and develop into memory cells. The B cells proliferate and differentiate into plasma cells that secrete antibody during the response. The T cells respond by proliferating after antigen is presented to them by antigen-presenting cells. The majority of T cells in the circulation express a T-Cell Receptor (TCR) having αβ chains. A small number of T cells in the circulation express a TCR having γδ chains. Most γδ T cells are found in the epithelial linings of the lung, the gut, and the urinary and reproductive tracts. γδ T cells have characteristics of both the innate and the acquired immune systems. Their phenotype is that of a lymphocyte, yet, during pathogen invasion, γδ T cells are not activated in the same way as the αβ cells, but are activated more like cells of the innate immune system, by recognition of PAMP (18, 19). Jutila et al. showed multiple PAMP receptors on bovine, human and mouse γδ T cells (20). Prenyl phosphate is a molecule having a pattern that is recognized by receptors of the γδ T cells. The interaction of prenyl phosphate with the γδ T-cell receptor resulted in γδ T-cell proliferation as well as synthesis and secretion of cytokines necessary for communicating with other parts of the immune system (21, 22). Other γδ T-cell PAMP include alkylamines (22, 23), heat shock proteins (24, 25), and intermediates of the mevalonate pathway (26, 27). Because of their location in the epithelia, γδ T cells are on the front-line to respond to pathogens that we breathe in or consume. Their nonspecific but rapid interactions with pathogens pave the way for the rest of the immune system to participate in the elimination of the foreign invader.

16.5 γδ T CELLS AND CANCER

γδ T cells, known cytotoxic cells, have two pathways to draw on to kill tumor cells. A small fraction of tumor cells, such as Burkitt's lymphoma and multiple myeloid cells, are killed in a TCR-dependent manner (28), while others are killed by a mechanism similar to the NK pathway that uses perforin, NKG2D, FAS/FAS ligand, and TRAIL to induce apoptosis (29–32). There is active immuno-therapy research taking place to enhance the activity of γδ T cells in cancer patients, using a family of nitrogen-containing bisphosphonates (N-BP) to enlarge the population (33–36). Dieli et al. gave an I.V. infusion of N-BP along with IL-2 to metastatic hormone-insensitive prostate cancer patients and showed a significant improvement in γδ T-cell activation, production

of interferon-γ (IFN-γ), and expression of perforin. The patients also had increased serum levels of the apoptosis factor, TRAIL. Moreover, enhancing the activation of γδ T cells was associated with reduced prostate specific antigen levels, partial remission in three patients, and stable disease in five patients. As a side note, the number of patients who received the N-BP without IL-2 progressively declined. These results, and others, imply that the strengthening of γδ T cells might be an important tool in the arsenal needed to prevent cancer. A strong γδ T cell population would, perhaps, be able to kill malignant cells before they replicated to disease conditions.

As noted previously, γδ T cells are one of several kinds of cancer-eliminating immune cells. What makes these cells unique to our discussion in this chapter is that they can be modified by dietary compounds.

16.6 γδ T CELLS AND DIFFERENTIATION: IMPACT OF FRUITS AND VEGETABLES

Information regarding improved differentiation of γδ T cells by compounds found in fruits and vegetables is scarce. In sum, the vitamins associated with differentiation, vitamin A and D, have been shown to play a role in γδ T-cell differentiation (37). These γδ T cells have been shown to have the vitamin D receptor that is upregulated via a mechanism involving protein kinase C (33). However, adequate vitamin A and D intake is necessary for the differentiation and function of all immune cells and is not unique to the γδ T cell.

16.7 γδ T CELLS AND PRIMING: IMPACT OF FRUITS AND VEGETABLES

What appears to be distinctive about the γδ T cell, compared to other immune cells, is its ability to be modified by dietary compounds. In a mouse study investigating dietary lipids, the relative amount of γδ T cells was proportional to the concentration of 18:2(n-6), and inversely proportional to the concentration of long chain (n-3) polyenes in the diet (38). This study was one of the first to establish an association between diet and γδ T cells (Table 16.1). In an animal model of allergy, ovalbumin-sensitized mice were fed unripe apple polyphenols. The apple polyphenols inhibited the development of sensitization by reducing levels of IgE, IgG1, and IgG2a. These results corresponded with an increase in γδ T cells in the intestinal intraepithelial lymphocyte population (39). In an *in vitro* model, a fairly low concentration – 20–40 μg/ml – of apple polyphenols upregulated CD11b on γδ T cells. Removing the proanthocyanidins from the apple polyphenol preparation by column chromatography removed the activity that caused the upregulation of cytokines (40). The authors suggest that this is a form of nonantigen priming of the γδ T cell (20).

Kamath et al. (41) compared γδ T-cell proliferation and IFN-γ secretion in people who drank five to six cups of black tea per day for 4 weeks compared to people who drank the same amount of coffee. Both beverages contained caffeine, but only the tea contained catechins and another γδ T-cell antigen, l-theanine. ELISPOT analysis showed a two- to threefold increase in IFN-γ spots from PBMC of the tea drinkers.

Table 16.1
Summary of the effects of bioactive compounds on γδ T cell

Model	*Bioactive under study*	*Length of time*	*Biochemical and/or physiological measures*	*Clinical measures*	*References*
In vivo mouse study	Lipids	5 months	Increased number in intestine	NA	(62)
In vivo mouse	Unripe apple polyphenols	9 weeks	Increased number in intestine	NA	(39)
In vitro PBMC culture	Apple polyphenols	24 h	Increased CD11b surface expression	NA	(40)
In vivo human	Tea versus coffee	8 weeks	Increased IFN-γ secretion	NA	(41)
In vivo human	Fruit and vegetable juice concentrate	11 weeks	Increased circulating γδ T cells	Fewer cold and flu symptoms	(42)
In vivo human	L-theanine + catechins	3 weeks	Increased IFN-γ secretion; increased proliferation of γδ T cell	Fewer incidences of cold and flu	(43)

A summary table of the studies using plant bioactive compounds to impact γδ T cells

The PBMC from people who drank tea were also able to secrete more IFN-γ in response to exposure to two different bacterial preparations *ex vivo*.

In another human study, 30 people consumed a powdered concentrate of fruits and vegetables for 11 weeks (42). The study participants were recruited from first and second year law students as a model of stress. Circulating γδ T-cell numbers increased by about 30% in those consuming the fruit and vegetable concentrate, while γδ T-cell numbers in those taking the placebo did not change. Antioxidant activity in the serum increased as well as serum carotenoids in fruit and vegetable concentrate consumers compared to those consuming the placebo. Fruit and vegetable capsule consumption was associated with a trend in less severe cold and flu symptoms, suggesting modest changes to immunity that resulted in less illness.

An *in vivo* human study was performed with a capsule containing two components found in tea: the family of catechins and l-theanine (43). The catechins were enriched for epigallocatechin gallate (EGCG) to about 60% and the l-theanine was highly (99%) purified. As such, the tea formula preparation was standardized and well defined. Tea, as a beverage, is not well defined in the literature. Not only are there differences in the climate and geography of the plant, but there are differences in the beverage due to brewing time and temperature. The literature is replete with papers that discuss "a cup" of tea without defining "a cup"; or distinguishing between a small, four-ounce Asian cup, a six-ounce tea cup or a literal eight-ounce cup.

Subjects consumed this supplement or placebo capsules for 3 months. PBMC from both the baseline blood draw and the postconsumption blood draw were cultured for

10 days with ethylamine, the compound derived from l-theanine metabolism *in vivo* (44–46). The cells derived from people who consumed the tea formula capsules secreted a greater amount of IFN-γ and their γδ T-cell population proliferated to a greater extent than the cells from people consuming the placebo. In addition, subjects consuming the tea formula capsules had fewer incidences of cold and flu. Both tea formula compounds, the catechins and the l-theanine, are hypothesized to interact with γδ T cells in such a way as to prime them without overtly activating them.

This was further substantiated when it was shown that the changes in the γδ T-cell cultures were due to a change in the cell itself as opposed to changes occurring due to the presence of a compound or metabolite in the serum. Incubating the primed γδ T cell, that is, the γδ T cell after consumption of l-theanine, with the serum derived from the baseline blood draw or with the serum after consumption of the l-theanine showed no difference in the ability of the cell population to proliferate (Fig. 16.2). This indicated that the cell was able to proliferate regardless of the presence (or absence) of compounds in the serum.

Some have argued that many of the bioactive compounds consumed in the diet may not be absorbed or are absorbed only minimally. How can these bioactive compounds influence the immunity of the entire body? First, vitamins and minerals and other bioactive compounds have been shown to be absorbed and therefore directly support the growth and maintenance of blood or bone marrow immune cells. Second, many compounds that are not absorbed still interact directly with the immune cells residing in the intestine, in the Peyer's patches and the intraepithelial cells lining the microvilli. Many of the intraepithelial T cells have the gamma delta TCR. Since gut immune cells migrate in and out of tissues, through the circulation and through the lymph system (47, 48), functional changes in the blood-borne γδ T cells can be measured regardless of whether the bioactive compound is absorbed or not. Even so, there is evidence that the catechins (2, 49–52) and l-theanine (46) are absorbed. Studies that measure changes in the blood cells do not know the origin of the cells, only that they have been modified by the diet. Third, the compounds that are not absorbed have been shown to be modified by colonic microbiota. Metabolites of the parent compounds have been shown to be absorbed from the colon. Catechins, for example, may be degraded by the microbiota and the metabolites absorbed in the colon. Valerolactones, a family of microbial products derived from catechins were found in the urine 7–14 h after the consumption of catechins (53). The metabolites formed from the action of the microbiota could possibly influence the blood cells of the systemic immune system; however, this has not been proven. In summary, dietary bioactive compounds do not have to be absorbed to alter immune cells. The cells obtained from the blood are indicative of what has happened regardless of where the interaction, that is, priming, actually took place. Thus, in human studies, changes in the blood cells are observed whether the immune cell was directly modified in the blood, or gut, or, indirectly, by metabolites formed by microbial action.

Several *in vivo* and *in vitro* studies show that there are certain food components that modify γδ T cells and that this modification is consistent with priming. The bioactive compounds found in some fruits and vegetables, as well as in tea, do not interact with the TLR with enough affinity to cause the cell to respond. However, when primed cells encounter a malignant cell, they can react faster and with greater intensity. Further research is needed to define and measure exactly what the priming of γδ T cell involves.

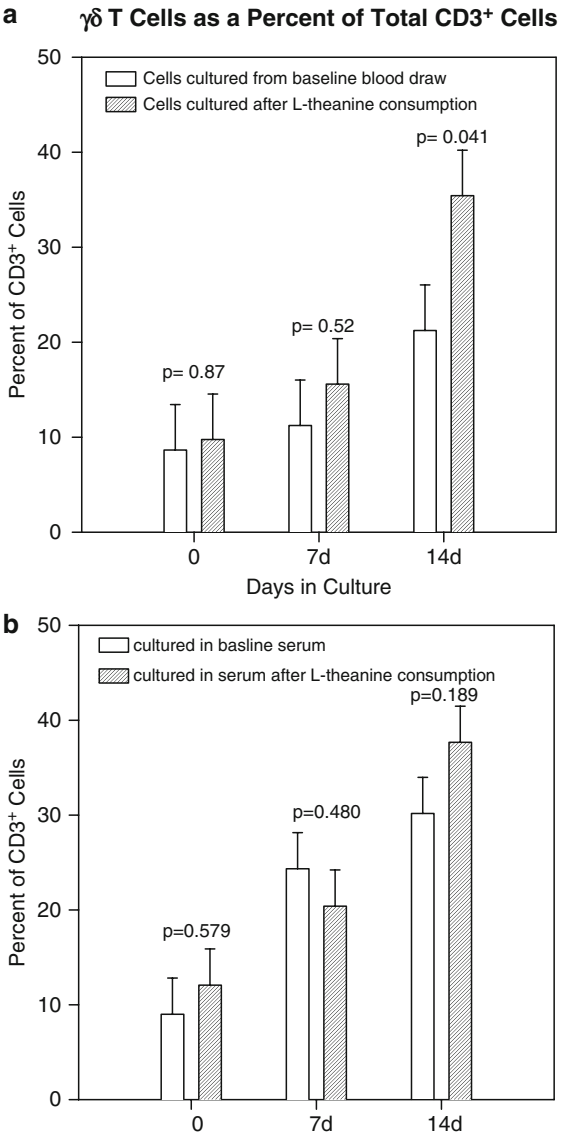


Fig. 16.2. Human subjects (n = 12) donated a sample of blood, then consumed capsules containing 500 mg L-theanine for 7 days after which they donated a second sample of blood. Serum and peripheral blood mononuclear cells were prepared from both blood draws. *Panel A*: Cells derived from the baseline blood draw and post-L-theanine consumption were cultured for 7 and 14 days with ethylamine. Cells from subjects who consumed the l-theanine (*hatched bars*) proliferated to a greater extent than those from the baseline blood draw (*open bars*). *Panel B*: Cells derived after consuming L-theanine were cultured in serum from the baseline blood draw (*open blood draw*) or the post-L-theanine blood draw (*hatched bars*). The source of serum had no effect on the proliferation, suggesting that the changes to proliferation was from the cell itself and not from something in the serum.

If results support the hypothesis that certain dietary compounds can prime γδ T cells, then this would identify a mechanism by which fruit and vegetable consumption can reduce the risk of cancer.

16.8 INFLAMMATION'S ROLE IN CARCINOGENESIS

Numerous studies and reviews discuss the role of inflammation in the risk for cancer (see below). It appears clear that inflammation and the accompanying free radicals are a major source of damage to cell membranes, enzymes, DNA, and lipid moieties. This damage can accumulate, may not be repairable, and may ultimately initiate a cell allowing for progression to a cancerous state (Fig. 16.1).

Inflammation is initiated by foreign pathogens, such as would occur in a normal immune response, or by self-antigens produced by damage to tissues (heat shock proteins). Toxins or xenobiotics may also play a role. Responding to antigens, toxins, or xenobiotics is a normal response of the immune system and is necessary to fight infection or to enhance wound healing. However, after the pathogen has been dispatched, it is critical that the inflammatory process be stopped. Disease is associated with the persistence of inflammation. Numerous examples of inflammatory biomarkers such as cytokines (54–56), transcription factors (57, 58), and cells (59) have been implicated in the initiation and progression of cancer. This is by no means an exhaustive citation list, but rather a few recent reviews that illustrate the pervasiveness of inflammation to the incidence of cancer.

16.9 γδ T CELLS AND INFLAMMATION

Another function of the γδ T cells is their capacity to end the inflammatory response. Genetically altered mice, with their γδ TCR knocked out, were able to resolve an infection of *Listeria monocytogenes*, but developed tissue necrosis at the site of infection (60). Compared to wild type mice, γδ knockout mice had an abnormal accumulation of neutrophils and macrophages in the liver and spleen, which was ultimately responsible for the tissue necrosis. Macrophages isolated from the peritoneum of the infected knockout mice were less apoptotic, more viable, and more active than those from the infected wild type mice. Further experiments showed that in the wild-type mouse, macrophages were killed by apoptosis via cell-to-cell contact with γδ T cells. Knockout mice were not able to kill macrophages, resulting in an inability of the animal to resolve inflammation. Their findings provide evidence that γδ T cell-mediated cytotoxicity is a means of terminating macrophage activity after the inflammatory response is no longer needed (60, 61). Priming γδ T cells with bioactive compounds may help resolve inflammation, thereby reducing cell damage that could lead to cancer.

16.10 CONCLUSIONS AND PERSPECTIVES

It has been shown in various clinical studies that fruit and vegetable consumption impacts γδ T cell proliferation and cytokine secretion. Others have shown fruit and vegetable compounds prime γδ T cells for a quicker, more productive response. Immunotherapy trials showed that the γδ T cell activity was associated with improved survival and quality of life. Thus, maintaining γδ T-cell activity is important to cancer prevention. In addition, their regulatory effect that resolves inflammation may be enhanced by fruit and vegetable consumption and therefore might be associated with providing yet another possible way to reduce the risk of cancer.

Acknowledgments This chapter would not be possible without the hard work and dedication of my laboratory staff, Meri Nantz and Cheryl A. Rowe, Ph.D. and my collaborators, Jack Bukowski and Susanne and Steve Talcott.

REFERENCES

1. Percival SS, Talcott ST, Chin ST, Mallak AC, Lounds-Singleton A, Pettit-Moore J (2006) Neoplastic transformation of BALB/3T3 cells and cell cycle of HL-60 cells are inhibited by mango (*Mangifera indica* L.) juice and mango juice extracts. J Nutr 136:1300–1304
2. Spencer JP, Schroeter H, Rechner AR, Rice-Evans C (2001) Bioavailability of flavan-3-ols and procyanidins: gastrointestinal tract influences and their relevance to bioactive forms in vivo. Antioxid Redox Signal 3:1023–1039
3. Palmer S (1985) Diet, nutrition, and cancer. Prog Food Nutr Sci 9:283–341
4. Navarro Silvera SA, Mayne ST, Risch H, Gammon MD, Vaughan TL, Chow WH, Dubrow R, Schoenberg JB, Stanford JL, West AB, Rotterdam H, Blot WJ, Fraumeni JF Jr (2008) Food group intake and risk of subtypes of esophageal and gastric cancer. Int J Cancer 123:852–860
5. Briviba K, Bub A, Moseneder J, Schwerdtle T, Hartwig A, Kulling S, Watzl B (2008) No differences in DNA damage and antioxidant capacity between intervention groups of healthy, nonsmoking men receiving 2, 5, or 8 servings/day of vegetables and fruit. Nutr Cancer 60:164–170
6. Plummer M, Vivas J, Lopez G, Bravo JC, Peraza S, Carillo E, Cano E, Castro D, Andrade O, Sanchez V, Garcia R, Buiatti E, Aebischer C, Franceschi S, Oliver W, Munoz N (2007) Chemoprevention of precancerous gastric lesions with antioxidant vitamin supplementation: a randomized trial in a high-risk population. J Natl Cancer Inst 99:137–146
7. Suzuki R, Allen NE, Key TJ, Appleby PN, Tjonneland A, Johnsen NF, Jensen MK, Overvad K, Boeing H, Pischon T, Kaaks R, Rohrmann S, Trichopoulou A, Misirli G, Trichopoulos D, Bueno-de-Mesquita HB, van DF, Sacerdote C, Pala V, Palli D, Tumino R, Ardanaz E, Quiros JR, Larranaga N, Sanchez MJ, Tormo MJ, Jakszyn P, Johansson I, Stattin P, Berglund G, Manjer J, Bingham S, Khaw KT, Egevad L, Ferrari P, Jenab M, Riboli E (2009) A prospective analysis of the association between dietary fiber intake and prostate cancer risk in EPIC. Int J Cancer 124:245–249
8. Kirsh VA, Peters U, Mayne ST, Subar AF, Chatterjee N, Johnson CC, Hayes RB (2007) Prospective study of fruit and vegetable intake and risk of prostate cancer. J Natl Cancer Inst 99:1200–1209
9. Ambrosini GL, de Klerk NH, Fritschi L, Mackerras D, Musk B (2008) Fruit, vegetable, vitamin A intakes, and prostate cancer risk. Prostate Cancer Prostatic Dis 11:61–66
10. Nomura AM, Wilkens LR, Murphy SP, Hankin JH, Henderson BE, Pike MC, Kolonel LN (2008) Association of vegetable, fruit, and grain intakes with colorectal cancer: the Multiethnic Cohort study. Am J Clin Nutr 88:730–737
11. Millen AE, Subar AF, Graubard BI, Peters U, Hayes RB, Weissfeld JL, Yokochi LA, Ziegler RG (2007) Fruit and vegetable intake and prevalence of colorectal adenoma in a cancer screening trial. Am J Clin Nutr 86:1754–1764
12. Koushik A, Hunter DJ, Spiegelman D, Beeson WL, van Den Brandt PA, Buring JE, Calle EE, Cho E, Fraser GE, Freudenheim JL, Fuchs CS, Giovannucci EL, Goldbohm RA, Harnack L, Jacobs DR Jr, Kato I, Krogh V, Larsson SC, Leitzmann MF, Marshall JR, McCullough ML, Miller AB, Pietinen P, Rohan TE, Schatzkin A, Sieri S, Virtanen MJ, Wolk A, Zeleniuch-Jacquotte A, Zhang SM, Smith-Warner SA (2007) Fruits, vegetables, and colon cancer risk in a pooled analysis of 14 cohort studies. J Natl Cancer Inst 99:1471–1483
13. Garcia-Closas R, Garcia-Closas M, Kogevinas M, Malats N, Silverman D, Serra C, Tardon A, Carrato A, Castano-Vinyals G, Dosemeci M, Moore L, Rothman N, Sinha R (2007) Food, nutrient and heterocyclic amine intake and the risk of bladder cancer. Eur J Cancer 43:1731–1740
14. Sacerdote C, Matullo G, Polidoro S, Gamberini S, Piazza A, Karagas MR, Rolle L, De SP, Casetta G, Morabito F, Vineis P, Guarrera S (2007) Intake of fruits and vegetables and polymorphisms in DNA repair genes in bladder cancer. Mutagenesis 22:281–285
15. Lunet N, Valbuena C, Vieira AL, Lopes C, Lopes C, David L, Carneiro F, Barros H (2007) Fruit and vegetable consumption and gastric cancer by location and histological type: case-control and meta-analysis. Eur J Cancer Prev 16:312–327

16. Pavia M, Pileggi C, Nobile CG, Angelillo IF (2006) Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. Am J Clin Nutr 83:1126–1134
17. Gonzalez CA, Pera G, Agudo A, Bueno-de-Mesquita HB, Ceroti M, Boeing H, Schulz M, Del GG, Plebani M, Carneiro F, Berrino F, Sacerdote C, Tumino R, Panico S, Berglund G, Siman H, Hallmans G, Stenling R, Martinez C, Dorronsoro M, Barricarte A, Navarro C, Quiros JR, Allen N, Key TJ, Bingham S, Day NE, Linseisen J, Nagel G, Overvad K, Jensen MK, Olsen A, Tjonneland A, Buchner FL, Peeters PH, Numans ME, Clavel-Chapelon F, Boutron-Ruault MC, Roukos D, Trichopoulou A, Psaltopoulou T, Lund E, Casagrande C, Slimani N, Jenab M, Riboli E (2006) Fruit and vegetable intake and the risk of stomach and oesophagus adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). Int J Cancer 118:2559–2566
18. Hayday A, Tigelaar R (2003) Immunoregulation in the tissues by gammadelta T cells. Nat Rev Immunol 3:233–242
19. Hedges JF, Lubick KJ, Jutila MA (2005) Gamma delta T cells respond directly to pathogen-associated molecular patterns. J Immunol 174:6045–6053
20. Jutila MA, Holderness J, Graff JC, Hedges JF (2008) Antigen-independent priming: a transitional response of bovine gammadelta T-cells to infection. Anim Health Res Rev 9:47–57
21. Morita CT, Lee HK, Leslie DS, Tanaka Y, Bukowski JF, Marker-Hermann E (1999) Recognition of nonpeptide prenyl pyrophosphate antigens by human gammadelta T cells. Microbes Infect 1:175–186
22. Morita CT, Jin C, Sarikonda G, Wang H (2007) Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vgamma2Vdelta2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. Immunol Rev 215:59–76
23. Bukowski JF, Morita CT, Brenner MB (1999) Human γδ T cells recognize alkylamines derived from microbes, edible plants, and tea: implications for innate immunity. Immunity 11:57–65
24. Hirsh MI, Junger WG (2008) Roles of heat shock proteins and {gamma}{delta}T cells in inflammation. Am J Respir Cell Mol Biol 39:509–513
25. Hirsh MI, Hashiguchi N, Chen Y, Yip L, Junger WG (2006) Surface expression of HSP72 by LPS-stimulated neutrophils facilitates gammadeltaT cell-mediated killing. Eur J Immunol 36:712–721
26. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G (2003) Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. J Exp Med 197:163–168
27. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR (1995) Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. Nature 375:155–158
28. Tanaka Y (2006) Human gamma delta T cells and tumor immunotherapy. J Clin Exp Hematop 46:11–23
29. Bilgi O, Karagoz B, Turken O, Kandemir EG, Ozturk A, Gumus M, Yaylaci M (2008) Peripheral blood gamma-delta T cells in advanced-stage cancer patients. Adv Ther 25:218–224
30. Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, Hobby P, Sutton B, Tigelaar RE, Hayday AC (2001) Regulation of cutaneous malignancy by gamma delta T cells. Science 294:605–609
31. Lilienfeld-Toal M, Nattermann J, Feldmann G, Sievers E, Frank S, Strehl J, Schmidt-Wolf IG (2006) Activated gammadelta T cells express the natural cytotoxicity receptor natural killer p 44 and show cytotoxic activity against myeloma cells. Clin Exp Immunol 144:528–533
32. Oppenheim D, Girardi M, Steele C, Lewis J, Fuller R, Gluscac E, Hobby P, Sutton B, Tigelaar R, Hayday A (2001) Rae-1 recognition by NKG2d: a molecular mechanism for gamma delta T-cell mediated surveillance of cutaneous malignancy in the mouse. Cancer Gene Ther 8:915
33. Beetz S, Marischen L, Kabelitz D, Wesch D (2007) Human gamma delta T cells: candidates for the development of immunotherapeutic strategies. Immunol Res 37:97–111
34. Galluzzo S, Santini D, Vincenzi B, Caccamo N, Meraviglia F, Salerno A, Dieli F, Tonini G (2007) Immunomodulating role of bisphosphonates on human gamma delta T cells: an intriguing and promising aspect of their antitumour activity. Expert Opin Ther Targets 11:941–954
35. Goto S, Noguchi A, Jinguji H, Takahara M (2006) The therapeutic potential of immuno-cell therapy of cancer in combination with aminobisphosphonates. Anticancer Res 26:3989–3995
36. Dieli F, Vermijlen D, Fulfaro F, Caccamo N, Meraviglia S, Cicero G, Roberts A, Buccheri S, D'Asaro M, Gebbia N, Salerno A, Eberl M, Hayday AC (2007) Targeting human {gamma}{delta} T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. Cancer Res 67:7450–7457

37. Gombart AF, Luong QT, Koeffler HP (2006) Vitamin D compounds: activity against microbes and cancer. Anticancer Res 26:2531–2542
38. Jameson JM, Sharp LL, Witherden DA, Havran WL (2004) Regulation of skin cell homeostasis by gamma delta T cells. Front Biosci 9:2640–2651
39. Akiyama H, Sato Y, Watanabe T, Nagaoka MH, Yoshioka Y, Shoji T, Kanda T, Yamada K, Totsuka M, Teshima R, Sawada J, Goda Y, Maitani T (2005) Dietary unripe apple polyphenol inhibits the development of food allergies in murine models. FEBS Lett 579:4485–4491
40. Graff JC, Jutila MA (2007) Differential regulation of CD11b on gammadelta T cells and monocytes in response to unripe apple polyphenols. J Leukoc Biol 82:603–607
41. Kamath AB, Wang L, Das H, Li L, Reinhold VN, Bukowski JF (2003) Antigens in tea-beverage prime human Vgamma 2Vdelta 2 T cells in vitro and in vivo for memory and nonmemory antibacterial cytokine responses. Proc Natl Acad Sci USA 100:6009–6014
42. Nantz MP, Rowe CA, Nieves C Jr, Percival SS (2006) Immunity and antioxidant capacity in humans is enhanced by consumption of a dried, encapsulated fruit and vegetable juice concentrate. J Nutr 136:2606–2610
43. Rowe CA, Nantz MP, Bukowski JF, Percival SS (2007) Specific formulation of *Camellia sinensis* prevents cold and flu symptoms and enhances γδ T cell function: a randomized, double-blind, placebo-controlled study. J Am Coll Nutr 26:445–452
44. Unno T, Suzuki Y, Kakuda T, Hayakawa T, Tsuge H (1999) Metabolism of theanine, gamma-glutamylethylamide, in rats. J Agric Food Chem 47:1593–1596
45. Terashima T, Takido J, Yokogoshi H (1999) Time-dependent changes of amino acids in the serum, liver, brain and urine of rats administered with theanine. Biosci Biotechnol Biochem 63:615–618
46. Tsuge H, Sano S, Hayakawa T, Kakuda T, Unno T (2003) Theanine, gamma-glutamylethylamide, is metabolized by renal phosphate-independent glutaminase. Biochim Biophys Acta 1620:47–53
47. Brandes M, Willimann K, Lang AB, Nam KH, Jin C, Brenner MB, Morita CT, Moser B (2003) Flexible migration program regulates gamma delta T-cell involvement in humoral immunity. Blood 102:3693–3701
48. Brandes M, Willimann K, Lang AB, Nam KH, Jin C, Brenner MB, Morita CT, Moser B (2003) Flexible migration program regulates gamma delta T-cell involvement in humoral immunity. Blood 102:3693–3701
49. Henning SM, Niu Y, Lee NH, Thames GD, Minutti RR, Wang H, Go VL, Heber D (2004) Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. Am J Clin Nutr 80:1558–1564
50. Hollman PC, Katan MB (1998) Bioavailability and health effects of dietary flavonols in man. Arch Toxicol Suppl 20:237–248
51. Manach C, Texier O, Morand C, Crespy V, Regerat F, Demigne C, Remesy C (1999) Comparison of the bioavailability of quercetin and catechin in rats. Free Radic Biol Med 27:1259–1266
52. Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130:2073S–2085S
53. Li C, Lee MJ, Sheng S, Meng X, Prabhu S, Winnik B, Huang B, Chung JY, Yan S, Ho CT, Yang CS (2000) Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. Chem Res Toxicol 13:177–184
54. Sethi G, Sung B, Aggarwal BB (2008) TNF: a master switch for inflammation to cancer. Front Biosci 13:5094–5107
55. Fantini MC, Pallone F (2008) Cytokines: from gut inflammation to colorectal cancer. Curr Drug Targets 9:375–380
56. Naugler WE, Karin M (2008) The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 14:109–119
57. Khor TO, Yu S, Kong AN (2008) Dietary cancer chemopreventive agents – targeting inflammation and Nrf2 signaling pathway. Planta Med 74:1540–1547
58. Fraser CC (2008) G protein-coupled receptor connectivity to NF-kappaB in inflammation and cancer. Int Rev Immunol 27:320–350
59. Ostrand-Rosenberg S, Sinha P (2009) Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol 182:4499–4506

60. Egan PJ, Carding SR (2000) Downmodulation of the inflammatory response to bacterial infection by gammadelta T cells cytotoxic for activated macrophages. J Exp Med 191:2145–2158
61. Carding SR, Egan PJ (2000) The importance of gamma delta T cells in the resolution of pathogen-induced inflammatory immune responses. Immunol Rev 173:98–108
62. Berger A, German JB, Chiang BL, Ansari AA, Keen CL, Fletcher MP, Gershwin ME (1993) Influence of feeding unsaturated fats on growth and immune status of mice. J Nutr 123:225–233

17 Lactoferrin in Immune Function, Cancer and Disease Resistance

Ann M. Mulder and Carol A. Morris

Key Points

- Lactoferrin (Lf) is a glycoprotein found in milk and other body fluids. Supplements of Lf are isolated from cow's milk.
- Lf possesses immune-modulating, antioxidant and anti-inflammatory properties which together support its anticancer activity.
- Lf also possesses strong antimicrobial activity against a diverse range of bacteria, fungi, parasites and viruses.

Key Words: Antibacterial, anticancer, antifungal, antiparasitic, antiviral, immunomodulation, lactoferrin.

17.1 ENDOGENOUS LACTOFERRIN

Lactoferrin (Lf) is a protein, found in milk and a number of other body fluids. Milk proteins are divided into either casein or whey fractions; Lf is a whey protein and while accounting for <0.1% of total bovine milk protein, it possesses a disproportionate amount of biological activity (1). Milk is the specific diet of young mammals, and colostrum, milk produced in the early days of the neonate, is especially rich in growth factors, immunoglobulins and antimicrobial polypeptides such as Lf. Breast milk, in particular, is well recognized as providing superior resistance to pathogens by maternal immunoglobulins and other protective factors such as Lf, and supporting maturation of the immune system (2). Evidence continues to accumulate, as discussed in this chapter, supporting the premise that milk-derived proteins, in particular Lf, provide a variety of health-promoting benefits including immunomodulation, anti-inflammatory, anticancer, and antimicrobial properties.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_17

17.1.1 Structure of Lf

Structurally Lf is an 80 kDa iron-binding glycoprotein first identified in bovine milk in 1939 (3). After isolation and purification from bovine and human milk in the 1960's, it was named "lactotransferrin," suggesting a variant of transferrin. The determination of the human Lf's amino acid sequence and three-dimensional structure revealed a single polypeptide chain (~690 amino acid residues) folded into two homologous globular lobes, linked by an α-helix residue, with each lobe binding to one molecule of iron. This reversible iron-binding property creates two forms: an iron-bound (holo) and an iron-free (apo) state. The highly stable holo state induces a "closed" structure whereby each lobe encloses the bound Fe^{3+} ion and is conformationally rigid. In contrast, the apo state is less stable, less compact, and more flexible (4).

Studies have established Lf as a member of the transferrin (Tf) family of non-heme iron-binding proteins; however, unlike the other members of the Tf proteins, Lf also exhibits a variety of biological activities unrelated to its iron-binding activity such as antibacterial, antiviral, antitumor, and immune modulation (5); activities, which rely on an ability to bind to biological molecules and cells. Furthermore, studies reveal that the surface of Lf, unlike other members of the Tf family, is highly cationic, a property which supports its ability to bind to many anionic molecules and cell surfaces and proposed as a major factor in Lf's additional activities.

17.1.2 Distribution of Lf

Lf is widely distributed throughout body fluids. It is produced from surface epithelial cells, secreted in the iron-free form, and apart from high levels in milk, is also found in exocrine fluids such as tears, semen, mucosal secretions and saliva, and fluids of the digestive tract, including bile, pancreatic juice and small intestine secretions. These secretions, which interface with the external environment, play an important role in the first line of host defense. The concentration of Lf in body fluids is variable; in tears, it is as high as 2 mg/mL, whereas in blood, it can be as low as 1 μg/mL (6). Milk, in particular, colostrum, is the most abundant source of Lf. Concentration in humans may vary from 5.9 mg/mL in colostrum, 2.9 mg/mL in transitional milk and 2.5 mg/mL in mature milk (7). Apart from body fluids, Lf is produced in great amounts in neutrophils (8). Here Lf is stored in the iron-free form in the cytoplasmic secondary granules of neutrophils and released during inflammation to play an important role in the feedback mechanism of the inflammatory response. Under normal conditions plasma Lf concentration is around 0.4–2.0 μg/mL, increasing to 200 μg/mL during inflammation (6). Lf synthesis is also reported to occur in the human kidney. It is believed to support immune defense through the reduction of free iron in the urine, to contribute to the antioxidant defense system, and to play a role in iron metabolism by recovering free iron in the urine to make it available for metabolic functions (9).

The response of Lf to a variety of physiological, pathological and environmental challenges is well documented. Increased levels are found in human secretions associated with chronic bronchitis (10), inflammatory bowel disease feces (11), and in IgE-mediated allergic skin conditions (12).

17.2 LACTOFERRIN SUPPLEMENTS

Isolation and purification of Lf from bovine milk commenced in 1985 in the anticipation of effective supplementation of infant formula (13); recent evidence supports improved iron status and reduced infections in Lf-supplemented infant formulae (14). *In vitro* studies demonstrating Lf's diverse range of biological activities have led to the purification, isolation, and sale of bovine Lf (bLf) as a neutraceutical. A range of oral supplements of Lf have been used for *in vivo* studies and clinical trials, including bovine Lf (bLf), recombinant human Lf (rhLf), and the Lf fragments lactoferricin (Lfcin) and lactoferrampin (Lfampin). BLf is isolated from cow's milk while recombinant human Lf (rhLf) is produced in microorganisms and plants. At present, only natural ~15% iron-saturated bLf is commercially available as a dietary supplement for human consumption.

17.2.1 Production of bLf Supplements

Concentrations of Lf in bovine milk vary from 0.1 – 0.3 mg/mL in mature milk, to 2–5 mg/mL in colostrum (15). A variety of purification methods are available for the isolation of bLf; however, for large-scale production, a cation-exchange chromatography system is generally used (16). Currently, the worldwide production of bLf is estimated to be over 60 tons/year, and presently it is produced in Germany, the Netherlands, Belgium, New Zealand, Australia and France (17).

Since early research on Lf suggested its importance in breast milk, the first major application for bLf was the supplementation of infant milk formulae, commencing in 1986 in Japan (17). Many benefits of bLf supplementation in infant formulae were reported, including enhanced microbial flora (18, 19), enhanced serum ferritin levels (20), and enhanced haematocrit levels along with reduced lower respiratory tract infections (14). Bovine Lf-supplemented infant formulae are currently marketed in Indonesia, Korea and Japan (17). In addition, bLf is now added to cosmetics, pet care supplements and immune-enhancing neutraceuticals, which include drinks, fermented milks and chewing gum (16).

17.2.2 Absorption and Bioavailability of Lf Supplements

The fate of ingested Lf is yet to be elucidated. In terms of exogenously administered Lf, either as a supplement or through mature breast milk, there appears to be no conclusive evidence that ingested Lf, or its fragments, are absorbed in the healthy gut. *In vivo* studies on rats concluded that neither orally administered bLf nor its large molecular fragments were absorbed by normal adult rat intestines (21) or detected in portal blood (22). Furthermore, in adult human serum, Lf levels did not increase after oral administration of rhLf (23). Instead, studies suggest that undigested Lf and its digested fragments such as lactoferricin or lactoferrampin may bind to gastrointestinal epithelial cells, modulating mucosal immunity, thereby resulting in the enhancement of systemic immunity (21, 24).

A small number of studies, however, indicate the presence of ingested Lf in the blood and urine of newborn and gut-inflamed animals and humans. A study of the urine of

human preterm infants detected the presence of significant levels of nearly intact maternal hLf molecules (25). BLf orally administered to newborn piglets was found in bile (26) and cerebrospinal fluid (27), and fragments were detected in the blood of rats exhibiting colitis (28). It is suspected that in all these cases, the barrier function of the gastrointestinal tract is either less fully developed or damaged, allowing the passage of large molecules (21).

17.2.3 Bioactive Fragments of Lactoferrin Supplements

Orally administered bLf and hLf are unusually resistant to proteolytic degradation. About 60% of the orally administered bLf has been shown to pass through the human stomach undigested and reach the small intestine (29), and substantial quantities of lactoferrin are found in the stool of breastfed infants (30); however, some breakdown does occur during gastric processing and on exposure to intestinal bacteria-derived and digestive enzymes (31). This proteolytic breakdown generates peptides or amino acid residues which also possess biological properties, often more potent than the parent Lf molecule. Fragments from Lf identified to date include lactoferricin (Lfcin) and lactoferrampin (Lfampin).

17.2.3.1 Lactoferricin

Lactoferricins (Lfcin) are peptides produced by pepsin cleavage of lactoferrin. They were first identified in 1991 and while they appear to possess no iron-binding capacity, they exhibit greater antibacterial activity than the parent Lf (32). Lf in milk, which is catabolised in the digestive tract, is believed to produce these Lfcins. The activity of bovine Lfcin (bLfcin) is proposed to occur at the surface of bacterial membranes. It has been demonstrated that bLfcin produces blisters on the outer bacterial membrane (33), resulting in autolysis and disruption of cell-membrane permeability (34). Lfcin binds to lipopolysaccharide (LPS) and techoic acid (35). *In vitro* studies show it to be highly antibacterial against a range of bacteria and like the parent Lf, the beneficial bacteria *B. bifidum* strains are highly resistant to Lfcin (36). In addition, Lfcin has also been shown to inhibit and inactivate the fungi *Candida albicans* (37), to possess antiviral properties against cytomegalovirus (38), and possess potential anticancer activity by inducing apoptosis in human leukemia and carcinoma cell lines (39). *In vivo* studies reveal Lfcin-supported clinical improvement of skin lesions after infection by the fungi *Trichophyton mentagrophytes* (tinea corporis) in guinea pigs (40), while demonstrating anti-angiogenic properties in mice (41).

17.2.3.2 Lactoferrampin

In 2004, another bioactive fragment of Lf, lactoferrampin (Lfampin) was identified. It is situated in close proximity to Lfcin and exhibits at this early stage of research, antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *C. albicans* (42).

17.2.4 Safety of Lf Supplements

Both animal and human studies support the notion that bLf supplements are well tolerated. No serious adverse events have been reported both on healthy participants

and patients exhibiting pathology. These include healthy male participants who were administered 200 mg/day (43), female athletes at 1.8 g/day (44), patients exhibiting chronic hepatitis C virus (HCV) infection taking 1.8, 3.6 or 7.2 g/day (45–47), young children (12–36 months) ingesting 1 g/day for 9 months (48) and pregnant women administering 200 mg/day (49).

17.3 BIOLOGICAL PROPERTIES OF LACTOFERRIN

Both *in vitro* and *in vivo* studies (animal and human) reveal that Lf has a diversity of physiological properties all of which could potentially and significantly impact the development of cancer and disease. This chapter will primarily focus on those properties that are likely to affect immunomodulation and the development of cancer. A brief overview of antimicrobial properties including antibacterial, antifungal, antiparasitic and antiviral activity is included, due to their potential impact on cancer development.

17.3.1 Immunomodulatory Activity

The first suggestion that Lf may play a part in the regulation of the immune system was in 1980, when a patient who suffered recurrent infections, was found to have normal Lf content in glandular secretions in contrast to a total absence in neutrophils (50). Supporting this hypothesis are *in vivo* studies using transgenic and knockout mice (51, 52), and evidence of increased antimicrobial activity in mice (53) and immunomodulation in humans after oral administration of bLf (43, 54).

Endogenous Lf belongs to the innate non-specific immune system; however, evidence indicates that it may also contribute to acquired immunity and protect against the damaging effects of inflammation. As discussed earlier, it appears that oral bLf and its digested fragments act initially on the intestinal immune system and subsequently promote systemic immunity as a secondary effect (21). As a powerful modulator of inflammatory and immune responses, Lf supports protection against both microbial infections and inflammatory disorders (5, 55, 56). The modulating effects by Lf are related to its ability to interact with both specific cell receptors on a broad range of epithelial and immune cells (57), and pro-inflammatory bacterial components such as lipopolysaccharide (LPS) (58, 59). This interaction gives protection against both the excesses of bacterial inflammation such as septic shock, and inflammatory diseases such as allergy, arthritis, chronic hepatitis, neurodegenerative disorders and cancer (60).

17.3.1.1 Immune Changes and Lactoferrin

At the molecular level, the modulatory properties of Lf involve iron binding, and interactions with a multitude of compounds, either soluble or membrane bound such as lipopolysaccharide. At the cellular level, Lf modulates the migration, maturation, differentiation, activation, proliferation and function of immune cells. It is not clear how exactly Lf triggers signals in cells although a number of mechanisms have been postulated, which include the modulation of nuclear factor-kappa B (NF-κB) and MAP kinase signaling (55).

Many *in vitro* studies have indicated the ability of lactoferrin to activate and support the immune process. Lf has been shown to enhance the accumulation of neutrophils to sites of injury (61), promote cell-to-cell interaction by promoting “stickiness” (62), promote activation of, and phagocytosis by, polymorphonuclear leukocytes (PMNs) and

monocyte/macrophages (63, 64), promote motility and superoxide production (65), decrease the release of pro-inflammatory cytokines (discussed below), increase the number and activity of NK cells (66, 67) and support the maturation of lymphocytes (68). *In vivo* oral administration of bLf resulted in increased NK cell activity, and upregulation of CD4+ and CD8+ T-cells in tumor-bearing mice (69–71), and increased CD3+ and CD4+ T-cells in immunocompromised mice (72).

Clinical studies report increases in phagocytosis by, and differentiation of, lymphocytes (73) while data on CD4+ counts is inconsistent. One recent study on children with HIV reported mild increases (74), although most studies report no increases (73, 75–77). Similarly, we recently reported no changes in lymphocyte subset numbers such as CD4+ after bLf supplementation in healthy males; however, there was a significant increase in total (CD3+), helper (CD4+) and cytotoxic (CD8+) T-cell activation (43), suggesting an immune regulatory role for Lf, dependent on the host's immune status. Activation of CD4+ T-cells stimulates the production of plasma B cells, memory B cells and antibodies resulting in an increased surveillance and the tagging of bacteria and fungi as demonstrated in numerous *in vivo* studies (78–81). In addition, CD4+ activation enhances the function of macrophages, stimulating release of cytokines, both pro-inflammatory and anti-inflammatory depending on the immune status (82). Further activation of CD4+ T-cells stimulates the production of cytotoxic CD8+ T-cells, which attack and destroy virus-invaded cells, cancer cells, intracellular bacteria, intracellular parasites and foreign cells as indicated by *in vitro* studies on the herpes simplex virus (83), hepatitis C virus (77) and experimentally induced cancers (84, 85).

17.3.1.2 Anti-inflammatory Activity of Lf

A variety of studies confirm the anti-inflammatory activity of Lf. *In vivo* studies have shown that oral Lf administration reduces gut mucosal injury caused by lipopolysaccharide (LPS) (86), *E. coli* infection (78) and gastritis caused by *Helicobacter* (87). The protective effect is most likely due to a decreased production of pro-inflammatory cytokines such as IFN-γ, TNF-α, IL-1β, IL-6 and granulocyte macrophage colony-stimulating factor (GMCSF) (88–92), while enhancing anti-inflammatory cytokine production such as IL-10 (93, 94). In contrast some *in vivo* studies have revealed enhanced production of pro-inflammatory cytokines accompanying a decrease in anti-inflammatory cytokines, suggesting a modulatory role of Lf. Some examples include (1) an increase in IFN-γ and IL-12 after herpes simplex virus infection (24); (2) ex vivo upregulation of TNF-α and IFN-γ accompanying a decrease in IL-5 and IL-10 upon stimulation with the exotoxin toxic shock syndrome toxin-1 (51); and (3) increased IL-12 production and reduced IL-10 production in HIV-infected children (73). Together these results indicate that Lf affects the Th1/Th2 cytokine balance in a manner dependent on the host immune status. Thus, Lf can enhance Th1 cytokines in diseases requiring an increased ability to control infection or alternatively may reduce the Th1 cytokines to limit excessive inflammatory response (82).

One mechanism behind the ability of Lf to inhibit pro-inflammatory cytokines is its ability to neutralize pro-inflammatory molecules such as LPS (59). *In vivo* studies reveal that orally administered bLf protected against a lethal dose of LPS in rats and mice (78, 95). In addition, the intravenous administration of bLf 24 h pre-surgery eased thymectomy- and splenectomy-stimulated TNF-α and IL-6 production, suggesting that

lactoferrin may have therapeutic application in cases of clinically induced shock (96). Similarly, down-regulation of TNF-α, IL-1β and IL-6 and upregulation of anti-inflammatory cytokines, in particular IL-4 and IL-10, was found after the oral administration of bLf in rats with experimentally induced colitis (28), after induced human skin inflammation (97) and in stimulated arthritis in rats (98). In addition, a small clinical trial on healthy individuals showed that after supplementation with either 10 or 50 mg of lactoferrin per day, the ability of peripheral blood cells to spontaneously produce pro-inflammatory IL-6 and TNF-α was also significantly reduced (54).

17.3.1.3 Clinical Trials

A small number of clinical trials support the upregulation of the immune system by Lf. A study on healthy males (n = 10) examined the effects of bLf on the activation of immune competent cells. After the oral administration of 2 g bLf/day for 4 weeks, there was increased phagocytic activity of PMNs in three participants and of these, two co-expressed increased CD16+ T cell counts. Superoxide production activity also increased in seven men after 2 weeks. An increase in the percentage of NK cells also appears probable as the percentage of CD11b+ and CD56+ in the T-cell population increased in four participants, and of these three also exhibited increased CD16+ counts (99). Another study investigated the immune effects of a commercially available nutritional product, *Nutrifemme* which contained bLf along with several other micronutrients. The oral administration of 40 mg bLf equivalent/day for 10 days in healthy participants (n = 17) resulted in an increased percentage of lymphocytes and immature cell forms, accompanying a decreased percentage of neutrophils, eosinophils and monocytes; changes, however, depended on the initial blood status. Additionally, TNF-α levels decreased, while changes in IL-6 were not significant. All of these effects may be attributed to Lf; however, the potential contribution of antioxidants and other micronutrients in the product cannot be discounted (100).

In order to confirm these immune changes, the same group investigated the effects of bLf only, on immune parameters. The oral administration of either placebo, 2, 10 or 50 mg of bLf daily, for 7 days in healthy participants (n = 28) revealed a significant, though transient increase in the number of immature neutrophils, and a significant decrease in the spontaneous production of IL-6 and TNF-α by cultures of peripheral blood cells. Interestingly, the dosage with the most pronounced effect was 10 mg (54).

Kimber et al. (101), investigated the effects of bLf on cytokine-mediated immune response in the skin; specifically, the migration of epidermal Langerhans cells. Initially, a bLf-containing cream was topically applied followed by stimulation with an allergen. Results indicated a reduction in Langerhans cell migration with prior application of lactoferrin. In a second experiment, the bLf-containing cream was applied, followed by intradermal administration of TNF-α. Results, however, indicated no reduction in Langerhans cell migration with prior application of lactoferrin. Together these results provide indirect evidence that the inhibition of Langerhans cell migration by lactoferrin is secondary to compromised production of TNF-α. The authors concluded that a function of lactoferrin may be to modulate inflammatory reactions through the regulation of cytokine production (101).

Ishikado et al. (76) investigated the effects of liposomal Lf vs. non-liposomal Lf; liposomalization of insulin reportedly provided a more intensive hypoglycemic effect

than naked insulin via oral route (102). Initially, five healthy males orally administered 300 mg liposomal Lf/day for 7 days. Results indicated a significant increase of IFN-α in all participants, whilst NK cell activity was unchanged. In a second experiment, ten males orally administered 320 mg/day of either liposomal or non-liposomal Lf for 4 weeks. Liposomal Lf significantly increased IFN-α production during the intake period while production from non-liposomal Lf remained unchanged. NK cell activity increased significantly in both groups after 1 week; however, this was restored to baseline levels at 3 weeks post intake. The authors concluded that liposomal lactoferrin increases IFN-α production in contrast to the ineffectiveness of non-liposomal lactoferrin (76).

17.3.2 Anticancer Activity

Epidemiological evidence indicates that the risk of cancer may decline by reducing negative lifestyle factors and/or increasing exposure to chemopreventive agents, particularly for individuals at high risk of developing cancer (84). Since carcinogenic mechanisms are complex, agents possessing multiple mechanisms of action would be most promising. There are ten suggested mechanisms underlying chemopreventive potential, including the antioxidant, anti-inflammatory, immune-enhancing, anti-hormone effects, modification of phase-1 drug-metabolizing enzymes, oncogene modification, regulation of cell growth, regulation of cell differentiation, promotion of apoptosis, and inhibition of angiogenesis (84).

The first suggestion that Lf may possess anticancer activity was in 1995 when whey concentrate containing Lf was reported to deplete tumor cells of glutathione, rendering them more vulnerable to chemotherapy (103). Since then *in vitro* studies have revealed anticancer properties of Lf against a variety of cancer cell lines including breast (66, 104), pancreatic (105), colon (71), and oral squamous cell (106). Mechanisms include increased NK cell cytotoxicity and the inhibition of cell growth and metastatic colony formation.

In addition, numerous *in vivo* studies support the use of bLf in the chemoprevention, treatment and metastasis of tumors of the colon, peritoneum, lung, esophagus, mouth and neck. Rats were orally administered 0.2 or 2% bLf for 36 weeks after treatment with the carcinogen azoxymethane. The incidence of large intestine adenocarcinomas was significantly decreased in both bLf groups compared to controls (107). Mice with subcutaneous implants of highly metastatic colon carcinoma 26, were orally administered bLf, bLf hydrolysate and bLfcin. Both bLF and the bLF hydrolysate demonstrated a significant inhibition of lung metastatic colony formation while bLfcin displayed a tendency (71). Rats treated with carcinogens were then orally administered bLf. All major organs were examined for tumors. A reduction in tumors compared to controls was found in the esophagus and lung indicating a chemopreventive effect for bLf in these organs (108).

Additionally, orally administered rhLF has been shown to inhibit tumor growth. Mice with subcutaneous and intraperitoneal transplants of cancer cells which resulted in tumor formation and carcinomatous peritonitis, showed delayed the growth of tumors if treated with rhLf (105). Mice exhibiting both squamous cell carcinoma and fibrosarcoma of the floor of the mouth were administered intra-tumoral injection of human and

murine recombinant Lf. Results revealed the growth inhibition of ~50% compared with controls for both human and murine tumor cells in immunodeficient and immunocompetent mice. There was a more dramatic effect in immunocompetent mice suggesting immunomodulation as an important mechanism of action (109). Mice implanted with head and neck squamous cell carcinoma tumors were orally administered rhLf. Results revealed a tumor growth inhibition of 75% accompanying a 20-fold increase in lymphocytes within treated animals compared with controls. When mice were depleted of CD3+ cells, all rhLf-induced tumor inhibition was abrogated, again supporting an immunomodulatory mechanism (88).

A recent interesting and significant study by Kanwar et al. (110) examined the effects of iron-saturated bLf on the augmentation of chemotherapy. Different forms and doses of bLf (apo-bLf: 4% iron-saturated (i-s); natural-bLf: 15%i-s; 50%i-s and 100%i-s) were supplemented into the diet of mice that were subsequently challenged subcutaneously with tumor cells and treated with chemotherapy. Results showed that chemotherapy eradicated large lymphomas only in mice fed 100%i-s bLf for at least 2 weeks prior to chemotherapy (ideally 6 weeks), but not in mice fed lesser saturated forms of bLf or control mice fed no bLf. While most of the study was performed with Lf at 28 g per 2.4 kg of diet, Lf was nevertheless effective in augmenting chemotherapy at the lowest dose tested (1 g per 2.4 kg of diet), which equates to a 70 kg person ingesting 3 g of Lf/day. In addition, 100%i-s bLf reduced angiogenesis, increased apoptosis and supported immunomodulation, by increasing production of both Th1 (TNF-α, IFN-γ and IL-18) and Th2 (IL-4, IL-5, IL-6, IL-10) cytokines, both of which are required for maximal anti-tumor vigilance. Significantly, 100%i-s bLf also restored both red and white blood cell numbers depleted by chemotherapy. The study concluded that 100%i-s bLf was a potent natural adjuvant supporting cancer chemotherapy, and may be a therapeutic option in humans to enhance the efficacy of chemotherapy, while simultaneously reducing its damaging side effects, including anemia and immune impairment (110).

However, the ability of orally administered Lf to exert a protective effect at sites distant from the gastrointestinal tract is poorly understood (111).

17.3.3 Evidence for Chemopreventive Potential

17.3.3.1 AntiOxidant Activity

Since oxidative stress is a critical pathophysiological mechanism in carcinogenesis (112), antioxidants should exhibit a potential protective role in the development of cancer. Lf has been shown to sequester free ferric ions, a function particularly evident at sites of inflammation and infection where free iron is likely to be present. Lf can therefore reduce free radical production, potentially acting as an antioxidant (113). Supporting this proposal, we recently reported a clinical study that revealed significantly increased serum antioxidant levels in healthy males who orally administered bLf supplements. We suggest that since Lf is not absorbed through the gut wall, the antioxidant increase is not due to its iron-sequestering ability but rather that digested fragments such as Lfcin, bind to gastro-epithelial cells and modulate intra-cellular antioxidant production (43).

Possibly only in the gastrointestinal tract does Lf act as an iron-sequestering antioxidant, as revealed in *in vitro* studies (114, 115) in the GI tract.

17.3.3.2 Anti-Inflammatory Activity

It is estimated that chronic infections and resultant inflammation contribute to approximately one-third of the world's cancer. For example, Hepatitis B and C viruses lead to chronic liver inflammation and cancer, *Schistosoma japonicum* causes colon cancer, *S. haematobium* causes bladder cancer, *Helicobacter pylori* may cause stomach cancer, and asbestos exposure leading to chronic inflammation is a significant risk factor for cancer of the lung (116). Inflammatory states appear to predispose to cancer development in any part of the body. The metabolic pathways switched on under these inflammatory conditions would appear to be natural targets for chemoprevention. As already discussed, Lf possesses potent modulatory properties in this regard, essentially reducing pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β, IL-6) while upregulating anti-inflammatory cytokines (IL-10).

17.3.3.3 Immune Enhancing

As already discussed, Lf also possesses immune-modulating properties. *In vivo* studies on the oral administration of bLf in mice revealed increased levels of NK cells, CD4+ and CD8+ cells and IFNγ+ cells, in both the mucosal layer of the small intestine and the peripheral cells (69–71). In addition, NK cell cytotoxicity is promoted both *in vitro* and *in vivo* (66, 117, 118). In humans CD3+, CD4+, and CD8+ T-cell activation has also been observed (43) as cited earlier.

17.3.3.4 Modification of Phase-1 Drug-Metabolizing Enzymes

In vivo studies indicate that the oral administration of both bLf and carcinogens to rodents results in decreased production of enzymes promoting carcinogenesis. In contrast Lf activated antioxidant- and carcinogen-detoxification enzyme activities, blocking cancer development (119, 120).

17.3.3.5 Regulation of Cell Differentiation

Lf downregulates cancer cell growth via cell cycle arrest at the G1/S transition. *In vitro* studies report arrested cell growth in a range of cell lines via a variety of pathways. These include breast cancer cell lines arresting in G1 via the MAPK pathway (121), head and neck cancer cells via the p27/cyclin E-dependent pathway (122), and HeLa cervical cell lines via the NF-κB pathway (123). In addition, Lf induces over-expression of the retinoblastoma protein, resulting in the arrest of cell growth in leukemia, lung and breast cancer cell lines (124). Similarly, the growth of transplanted tumors and the prevention of metastasis in rodents were inhibited after the ingestion or injection of bLf and/or recombinant hLf (71, 117, 125).

17.3.3.6 Promotion of Apoptosis

Lf promotes apoptosis in cancer cells. One proposed mechanism involves activation of caspase-3 and caspase-8 which are central to a variety of apoptosis cascades. *In vitro* (126) and *in vivo* studies (127) reveal that Lf enhances procaspase-3 maturation. In addition, the activation of caspases 3 and 8 have been reported in *in vivo* studies (128, 129). Importantly, it also appears that hLf can promote or inhibit apoptosis in a

dose-dependent manner. *In vitro* studies reveal that high doses of hLf result in activation of caspases 3 and 8, leading to decreases in phosphorylated ERK1/2 and Bcl-2 and increased apoptosis. In contrast, low doses upregulate phosphorylated ERK1/2 and Bcl-2 protecting cells from apoptosis (130). Bovine Lf peptides, natural or synthetic, also promote apoptosis in both human leukemia and carcinoma cell lines through mechanisms including caspase-3 activation, G1 arrest and the production of reactive oxygen species triggering apoptosis (39, 131).

17.3.3.7 Inhibition of Angiogenesis

Orally administered bLf was found to inhibit angiogenesis in rats (132) and tumor-induced angiogenesis in mice (133). In contrast, hLf promotes angiogenesis (134). Proposed mechanisms of bLf angiogenesis inhibition involve a reported enhancement of IL-18 production (67, 69, 70, 135). The ability of IL-18 to act as an anti-angiogenic compound is suggested to partly explain bLf inhibition of angiogenesis. In addition, increased levels of IL-18 are reported to elevate mucosal and systemic immune responses through cytokine production and the activation of NK cells (136). A complete understanding of this mechanism remains unknown. However, since Lf decreases pro-inflammatory cytokines such as IL-6 and IL-1β which are potent angiogenic stimulators, Lf action may partly involve the suppression of angiogenic cytokine production (137).

17.3.3.8 Antimicrobial Activity

In vitro studies reveal significant antimicrobial activity of Lf against a variety of bacteria, fungi, parasites and viruses. Lf appears highly antibacterial against a diverse range of bacteria, including *E. coli, Klebsiella pneumoniae, S. aureus*, and *Streptococcus* spp. (15), and utilizes a number of different antibacterial mechanisms including bacteriostatic, bactericidal, anti-invasive, anti-adhesive, and inhibition of bacterial biofilm development. In addition, *in vitro* studies report potent Lf activity against the fungi *C. albicans* (138) and *Trichophyton* (40) and the parasites *Plasmodium falciparum* (139), *Giardia lamblia* (140) and *Toxoplasma gondii* (141).

Lf also appears capable of inhibiting the replication of an extensive range of both naked and enveloped viruses including, but not limited to, human immunodeficiency virus (HIV) (142), hepatitis C virus (HCV) (143) and the herpes simplex virus (HSV) I (144)and II (145). Though the specific antiviral mechanisms of Lf are still unclear, a number of antiviral mechanisms have been proposed, and most studies conclude that both hLf and bLf affect the adsorption and internalization of a virus, preventing infection of the host cell, rather than inhibiting viral replication after the host has become infected (146–148).

Furthermore, both *in vivo* and clinical trials support the use of bLf as an effective adjunct in the treatment of a number of pathogenic bacteria, fungi, parasites and viruses (Table 17.1). Briefly these include the bacteria *H. pylori*, Group A *Streptococci*, *Clostridium* spp. and *E. coli*, the fungi *C. albicans* and *Trichophyton* spp. (tinea corporis and pedis) and the parasite *G. lamblia.* In addition, *in vivo* studies support the use of bLf as a useful adjunct in decreasing the severity of the influenza virus, HSV, and cytomegalovirus, and clinical trials support the use of bLf as an effective adjunct to standard drug therapy in the treatment of both HIV and HCV.

Table 17.1
Antimicrobial activity of orally administered bLf

Microbe	*Antimicrobial activity*
Helicobacter pylori	Increase in H. pylori eradication after concurrent administration of standard triple therapy (STT) with 400 mg bLf compared with STT only (149–153); no significant difference in H. pylori eradication after oral administration of 400 mg bLf in two studies (154, 155)
Group A *Streptococci* (GAS)	Decrease in numbers of intracellular GAS in tonsil specimens after 100 mg bLF gargles and erythromycin, compared with only erythromycin for 15 days preceding tonsillectomy (156)
Clostridium spp.	Suppressed proliferation of *Clostridium* spp in the fecal microflora of mice (157) and dogs (158)
Escherichia coli	Suppressed proliferation of *E. coli* in the fecal microflora of mice (159) and dogs (158)
Candida albicans	Complete resolution of oral candidiasis in a HIV-infected patient after using a mouth wash containing bLf and lysozyme combined with an antifungal drug (160)
Trichophyton (tinea corporis, tinea pedis)	Supported improvement of skin lesions in guinea pigs after the peak of the symptoms of tinea corporis (40), and improved dermatological symptoms of tinea pedis (161)
Giardia	A lower prevalence of colonisation with *Giardia* and better growth for young children after 1 g bLf/day (48)
Herpes simplex virus (HSV)	Inhibition of the appearance of HSV-1 skin lesions in mice (24)
Cytomegalovirus (CMV)	Protection of mice from death due to infection by CMV (162)
Human immunodeficiency virus (HIV)	Oral administration of 3 g/day bLf with anti-retroviral therapy in HIV-positive children, decreased viral load and increased CD4+ counts (74), whilst bLf alone supported immune modulation (73)
Hepatitis C virus (HCV)	Reduction in the level of HCV, serum alanine transaminase (ALT) and HCV RNA concentrations in chronic hepatitis C (CHC) patients with low levels of HCV after 3.6 g/day (45), and HCV RNA concentrations in CHC patients with high levels of HCV (46, 47, 163). No virologic or biochemical response was observed after 600 mg/day (77), or 1.8 g/day (75)
Influenza virus	Attenuation of pneumonia in influenza virus-infected mice (164)

17.4 CONCLUSIONS AND PERSPECTIVES

Lactoferrin, specifically oral administration of bovine lactoferrin supplements, appears to possess enormous potential as an excellent adjunct to standard therapy both in the treatment of cancer and disease resistance. Its extensive range of properties, including antibacterial, antifungi, antiparasitic, antiviral, antioxidant and the recently discovered immuno-modulatory properties, suggest that bLf and, in the future, recombinant human lactoferrin will be of vital importance as an emerging nutritional supplement.

REFERENCES

1. Zimecki M, Kruzel M (2007) Milk-derived proteins and peptides of potential therapeutic and nutritive value. J Exp Ther Oncol 6:89–106
2. Kelly D, Coutts A (2000) Early nutrition and the development of immune function in the neonate. Proc Nutr Soc 59:177–185
3. Sørensen M, Sørensen S (1939) The proteins in whey C. R Trav Lab Carlsberg Chim 23:55–99
4. Baker E, Baker H (2009) A structural framework for understanding the multifunctional character of lactoferrin. Biochimie 91:3–10
5. Ward P, Paz E, Conneely O (2005) Multifunctional roles of lactoferrin: a critical overview. Cell Mol Life Sci 62:2540–2548
6. Farnaud S, Evans R (2003) Lactoferrin – a multifunctional protein with antimicrobial properties. Mol Immunol 40:395–405
7. Cuillière M, Montagne P, Molé C, Béné M, Faure G (1997) Microparticle-enhanced nephelometric immunoassay of lactoferrin in human milk. J Clin Lab Anal 11:239–243
8. Masson P, Heremans J, Schonne E (1969) Lactoferrin, an iron-binding protein in neutrophilic leukocytes. J Exp Med 130:643–658
9. Abrink M, Larsson E, Gobl A, Hellman L (2000) Expression of lactoferrin in the kidney: implications for innate immunity and iron metabolism. Kidney Int 57:2004–2010
10. Thompson A, Bohling T, Payvandi F, Rennard S (1990) Lower respiratory tract lactoferrin and lysozyme arise primarily in the airways and are elevated in association with chronic bronchitis. J Lab Clin Med 115:148–158
11. Uchida K, Matsuse R, Tomita S, Sugi K, Saitoh O, Ohshiba S (1994) Immunochemical detection of human lactoferrin in feces as a new marker for inflammatory gastrointestinal disorders and colon cancer. Clin Biochem 27:259–264
12. Zweiman B, Kucich U, Shalit M, Von Allmen C, Moskovitz A, Weinbaum G, Atkins P (1990) Release of lactoferrin and elastase in human allergic skin reactions. J Immunol 144:3953–3960
13. Fairweather Tait S, Balmer S, Scott P, Ninski M (1987) Lactoferrin and iron absorption in newborn infants. Pediatr Res 22:651–654
14. King JJ, Cummings G, Guo N, Trivedi L, Readmond B, Keane V, Feigelman S, de Waard R (2007) A double-blind, placebo controlled, pilot study of bovine lactoferrin supplementation in bottle fed infants. J Pediatr Gastroenterol Nutr 44:245–251
15. Nonnecke B, Smith K (1984) Inhibition of mastitic bacteria by bovine milk apo-lactoferrin evaluated by in vitro microassay of bacterial growth. J Dairy Sci 67:606–613
16. Wakabayashi H, Yamauchi K, Takase M (2006) Lactoferrin research, technology and applications. Int Dairy J 16:1241–1251
17. Tomita M, Wakabayashi H, Shin K, Yamauchi K, Yaeshima T, Iwatsuki K (2009) Twenty-five years of research on bovine lactoferrin applications. Biochimie 91:52–57
18. Kawaguchi S, Hayashi T, Masano J, Okuyama K, Suzuki T, Kawase K (1989) A study concerning the effect of lactoferrin-enriched infant formula on low birth weight infants. Perinat Med 19:557–562
19. Roberts A, Chierici R, Sawatzki G, Hill M, Volpato S, Vigi V (1992) Supplementation of an adapted formula with bovine lactoferrin: 1. Effect on the infant faecal flora. Acta Paediatr 81:119–124

20. Chierici R, Sawatzki G, Tamisari L, Volpato S, Vigi V (1992) Supplementation of an adapted formula with bovine lactoferrin: 2. Effects on serum iron, ferritin and zinc levels. Acta Paediatr 81:475–479
21. Teraguchi S, Wakabayashi H, Kuwata H, Yamauchi K, Tamura Y (2004) Protection against infections by oral lactoferrin: evaluation in animal models. Biometals 17:231–234
22. Wakabayashi H, Kuwata H, Yamauchi K, Teraguchi S, Tamura Y (2004) No detectable transfer of dietary lactoferrin or its functional fragments to portal blood in healthy adult rats. Biosci Biotechnol Biochem 68:853–860
23. Hayes T, Falchook G, Varadhachary G et al (2006) Phase 1 trial of oral talactoferrin alpha in refractory solid tumors. Invest New Drugs 24(3):233–240
24. Wakabayashi H, Kurokawa M, Shin K, Teraguchi S, Tamura Y, Shiraki K (2004) Oral lactoferrin prevents body weight loss and increases cytokine responses during herpes simplex virus type 1 infection in mice. Biosci Biotechnol Biochem 68:537–544
25. Hutchens T, Henry J, Yip T et al (1991) Origin of intact lactoferrin and its DNA-binding fragments found in th urine of human milk-fed preterm infants. Evaluation by stable isotopic enrichment. Pediatr Res 29:243–250
26. Harada E, Itoh Y, Sitizyo K, Takeuchi T, Araki Y, Kitagawa H (1999) Characteristic transport of lactoferrin from the intestinal lumen into the bile via the blood in piglets. Comp Biochem Physiol 124:321–327
27. Harada E, Araki Y, Furumura E, Takeuchi T, Sitizyo K, Yajima T, Kuwata T (2002) Characteristic transfer of colostrum-derived biologically active substances into cerebrospinal fluid via blood in natural suckling neonatal pigs. Am J Vet Med 49:358–364
28. Togawa J, Nagase H, Tanaka K, Inamori M, Nakajima A, Ueno N, Saito T, Sekihara H (2002) Oral administration of lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. J Gastroenterol Hepatol 17:1291–1298
29. Troost F, Steijns J, Saris W, Bruumer R (2001) Gastric digestion of bovine lactoferrin in vivo in adults. J Nutr 131:2101–2104
30. Davidson L, Lonnerdal B (1987) Persistence of human milk proteins in the breastfed infant. Acta Paediatr Scand 76:733–740
31. Kuwata H, Yip T, Tomita M, Hutchens T (1998) Direct evidence of the generation in human stomach of an antimicrobial peptide domain (lactoferricin) from ingested lactoferrin. Biochim Biophys Acta 1429:129–141
32. Tomita M, Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K (1991) Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin. J Dairy Sci 74:4137–4142
33. Yamauchi K, Tomita M, Giehl T, Ellison R (1993) Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. Infect Immun 61:719–728
34. Bellamy W, Wakabayashi H, Takase M, Kawase K, Shimamura S, Tomita M (1993) Role of cell-binding in the antibacterial mechanism of lactoferricin B. J Appl Bacteriol 75:478–484
35. Vorland L (1999) Lactoferrin: a multifunctional glycoprotein. APMIS 107:971–981
36. Bellamy W, Takase M, Wakabayashi H, Kawase K, Tomita M (1992) Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. J Appl Bacteriol 73:472–479
37. Bellamy W, Wakabayashi H, Takase M, Kawase K, Shimamura S, Tomita M (1993) Killing of Candida albicans by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. Med Microbiol Immunol 182:97–105
38. Andersen J, Osbakk S, Vorland L, Traavik T, Gutteberg T (2001) Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. Antiviral Res 51:141–149
39. Mader J, Salsman J, Conrad D, Hoskin D (2005) Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines. Mol Cancer Ther 4:612–624
40. Wakabayashi H, Uchida K, Yamauchi K, Teraguchi S, Hayasawa H, Yamaguchi H (2000) Lactoferrin given in food facilitates dermatophytosis cure in guinea pig models. J Antimicrob Chemother 46:595–602
41. Mader J, Smyth D, Marshall J, Hoskin D (2006) Bovine lactoferricin inhibits basic fibroblast growth factor- and vascular endothelial growth factor165-induced angiogenesis by competing for heparin-like binding sites on endothelial cells. Am J Pathol 169:1753–1766

42. van der Kraan M, Groenink J, Nazmi K, Veerman E, Bolscher J, Nieuw Amerongen A (2004) Lactoferrampin: a novel antimicrobial peptide in the N1-domain of bovine lactoferrin. Peptides 25:177–183
43. Mulder A, Connellan P, Oliver C, Morris C, Stevenson L (2008) Bovine lactoferrin supplementation supports immune and antioxidant status in healthy human males. Nutr Res 28:583–589
44. Koikawa N, Nagaoka I, Yamaguchi M, Hamano H, Yamauchi K, Sawaki K (2008) Preventive effect of lactoferrin intake on anemia in female long distance runners. Biosci Biotechnol Biochem 72:931–935
45. Tanaka K, Ikeda M, Nozaki A, Kato N, Tsuda H, Saito S, Sekihara H (1999) Lactoferrin inhibits hepatitis C virus viremia in patients with chronic hepatitis C: a pilot study Japanese. J Cancer Res 90:367–371
46. Okada S, Tanaka K, Sato T, Ueno H, Saito S, Okusaka T, Sato K, Yamamoto S, Kakizoe T (2002) Dose-response trial of lactoferrin in patients with chronic hepatitis C Japanese. J Cancer Res 93:1063–1069
47. Kaito M, Iwasa M, Fujita N, Kobayashi Y, Kojima Y, Ikoma J, Imoto I, Adachi Y, Hamano H, Yamauchi K (2007) Effect of lactoferrin in patients with chronic hepatitis C: combination therapy with interferon and ribavirin. J Gastroenterol Hepatol 22:1894–1897
48. Ochoa T, Chea-Woo E, Campos M, Pecho I, Prada A, McMahon R, Cleary T (2008) Impact of lactoferrin supplementation on growth and prevalence of Giardia colonization in children. Clin Infect Dis 46:1881–1883
49. Paesano R, Torcia F, Berlutti F, Pacifici E, Ebano V, Moscarini M, Valenti P (2006) Oral administration of lactoferrin increases hemoglobin and total serum iron in pregnant women. Biochem Cell Biol 84:377–380
50. Breton-Gorius J, Mason D, Buriot D, Vilde J, Griscelli C (1980) Lactoferrin deficiency as a consequence of a lack of specific granules in neutrophils from a patient with recurrent infections. Detection by immunoperoxidase staining for lactoferrin and cytochemical electron microscopy. Am J Pathol 99:413–428
51. Guillén C, McInnes I, Vaughan D, Kommajosyula S, Van Berkel P, Leung B, Aguila A, Brock J (2002) Enhanced Th1 response to Staphylococcus aureus infection in human lactoferrin-transgenic mice. J Immunol 168:3950–3957
52. Schaible U, Collins H, Priem F, Kaufmann S (2002) Correction of the iron overload defect in beta-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis. J Exp Med 196:1507–1513
53. Takakura N, Wakabayashi H, Ishibashi H, Teraguchi S, Tamura Y, Yamaguchi H, Abe S (2003) Oral lactoferrin treatment of experimental oral candidiasis in mice. Antimicrob Agents Chemother 47:2619–2623
54. Zimecki M, Spiegel K, Wlaszczyk A, Kubler A, Kruzel M (1999) Lactoferrin increases the output of neutrophil precursors and attenuates the spontaneous production of TNF-alpha and IL-6 by peripheral blood cells. Arch Immunol Ther Exp (Warsz) 47:113–118
55. Legrand D, Elass E, Carpentier M, Mazurier J (2005) Lactoferrin: a modulator of immune and inflammatory responses. Cell Mol Life Sci 62:2549–2559
56. Yamauchi K, Wakabayashi H, Shin K, Takase M (2006) Bovine lactoferrin: benefits and mechanisms of action against infections. Biochem Cell Biol 84:291–296
57. Suzuki Y, Lopez V, Lonnerdal B (2005) Mammalian lactoferrin receptors: structure and function. Cell Mol Life Sci 62:2560–2575
58. Na Y, Han S, Kang J, Yoon Y, Park S, Kim H, Yang K, Joe C (2004) Lactoferrin works as a new LPS-binding protein in inflammatory activation of macrophages. Int Immunopharmacol 4:1187–1199
59. Elass-Rochard E, Roseanu A, Legrand D, Trif M, Salmon V, Motas C, Montreuil J, Spik G (1995) Lactoferrin-lipopolysaccharide interaction: involvement of the 28-34 loop region of human lactoferrin in the high-affinity binding to Escherichia coli 055B5 lipopolysaccharide. Biochem J 312:839–845
60. Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, Mazurier J (2008) Lactoferrin structure and functions. Adv Exp Med Biol 606:163–194
61. Birgens H (1984) The biological significance of lactoferrin in haematology. Scand J Haematol 33:22–30

62. Boxer L, Haak R, Yang H, Wolach J, Whitcomb J, Butterick C, Baehner R (1982) Membrane-bound lactoferrin alters the surface properties of polymorphonuclear leukocytes. J Clin Invest 70:1049–1057
63. Szuster-Ciesielska A, Kaminska T, Kandefer-Szerszen M (1995) Phagocytosis-enhancing effect of lactoferrin on bovine peripheral blood monocytes in vitro and in vivo. Arch Vet Pol 35:63–71
64. Kai K, Komine K, Komine Y, Kuroishi T, Kozutsumi T, Kobayashi J, Ohta M, Kitamura H, Kumagai K (2002) Lactoferrin stimulates a staphylococcus aureus killing activity of bovine phagocytes in the mammary gland. Microbiol Immunol 46:187–194
65. Gahr M, Speer C, Damerau B, Sawatzki G (1991) Influence of lactoferrin on the function of human polymorphonuclear leukocytes and monocytes. J Leukoc Biol 49:427–433
66. Damiens E, Mazurier J, El Yazidi I, Masson M, Duthillie I, Spik G, Boilly-Marer Y (1998) Effects of human lactoferrin on NK cell cytotoxicity against haematopoietic and epithelial tumor cells. Biochim Biophys Acta 1402:277–287
67. Kuhara T, Yamauchi K, Tamura Y, Okamura H (2006) Oral administration of lactoferrin increases NK cell activity in mice via increased production of IL-18 and type 1 IFN in the small intestine. J Interferon Cytokine Res 26:489–499
68. Zimecki M, Mazurier J, Machnicki M, Wieczorek Z, Montreuil J, Spik G (1991) Immunostimulatory activity of lactotransferrin and maturation of CD4- CD8- murine thymocytes. Immunol Lett 30:119–123
69. Kuhara T, Iigo M, Itoh T, Ushida Y, Sekine K, Terada N, Okamura H, Tsuda H (2000) Orally administered lactoferrin exerts an antimetastatic effect and enhances production of IL-18 in the intestinal epithelium. Nutr Cancer 38:192–199
70. Wang W, Iigo M, Sato J, Sekine K, Adachi I, Tsuda H (2000) Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. Jpn J Cancer Res 91:1022–1027
71. Iigo M, Kuhara T, Ushida Y, Sekine K, Moore M, Tsuda H (1999) Inhibitory effects of bovine lactoferrin on colon carcinoma 26 lung metastasis in mice. Clin Exp Metastasis 17:35–40
72. Artym J, Zimecki M, Paprocka M, Kruzel M (2003) Orally administered lactoferrin restores humoral immune response in immunocompromised mice. Immunol Lett 89:9–15
73. Zuccotti G, Vigano A, Borelli M, Saresella M, Giacomet V, Clerici M (2007) Modulation of innate and adaptive immunity by lactoferrin in human immunodeficiency virus (HIV)-infected, antiretroviral therapy-naïve children. Int J AntimicrobAgents 29:353–355
74. Zuccotti G, Salvini F, Riva E, Agostoni C (2006) Oral lactoferrin in HIV-1 vertically infected children: an observational follow-up of plasma viral load and immune parameters. J Int Med Res 34:88–94
75. Ueno H, Sato T, Yamamoto S, Tanaka K, Ohkawa S, Takagi H, Yokosuka O, Furuse J, Saito H, Sawaki A, Kasugai H, Osaki Y, Fujiyama S, Sato K, Wakabayashi K, Okusaka T (2006) Randomised, double-blind, placebo-controlled trial of bovine lactoferrin in patients with chronic hepatitis C. Cancer Sci 97:1105–1110
76. Ishikado A, Imanaka H, Kotani M, Fujita A, Mitsuishi Y, Kanemitsu T, Tamura Y, Makino T (2004) Liposomal lactoferrin induced significant increase of the interferon-alpha (IFN-alpha) producibility in healthy volunteers. Biofactors 21:69–72
77. Ishii K, Takamura N, Shinohara M, Wakui N, Shin H, Sumino Y, Ohmoto Y, Teraguchi S, Yamauchi K (2003) Long term follow-up of chronic hepatitis C patients treated with oral lactoferrin for 12 months. Hepatol Res 25:226–233
78. Zagulski T, Lipinski P, Zagulska A, Broniek S, Jarzabek Z (1989) Lactoferrin can protect mice against a lethal dose of Escherichia coli in experimental infection in vivo British. J Exp Pathol 70:697–704
79. Lee WJ, Farmer JL, Hilty M, Kim YB (1998) The protective effects of lactoferrin feeding against endotoxin lethal shock in germfree piglets. Infect Immunol 66:1421–1426
80. Wada T, Aiba Y, Shimizu K, Takagi A, Miwa T, Koga Y (1999) The therapeutic effect of bovine lactoferrin in the host infected with Helicobacter pylori. Scand J Gastroenterol 34:238–243
81. Takakura N, Wakabayashi H, Ishibashi H, Yamauchi K, Teraguchi S, Tamura Y, Yamaguchi H, Abe S (2004) Effect of orally administered bovine lactoferrin on the immune response in the oral candidiasis murine model. J Med Microbiol 53:495–500
82. Fischer R, Debbabi H, Dubarry M, Boyaka P, Tomé D (2006) Regulation of physiological and pathological Th1 and Th2 responses by lactoferrin. Biochem Cell Biol 84:303–311
83. Marchetti M, Trybala E, Superti F, Johansson M, Bergström T (2004) Inhibition of herpes simplex virus infection by lactoferrin is dependent on interference with the virus binding to glycosaminoglycans. Virology 318:405–413

84. Tsuda H, Ohshima Y, Nomoto H, Fujita K, Matsuda E, Iigo M, Takasuka N, Moore M (2004) Cancer prevention by natural compounds. Drug Metab Pharmacokinet 19:245–263
85. Yoo YC, Watanabe S, Watanabe R, Hata K, Shimazaki K, Azuma I (1998) Bovine lactoferrin and lactoferricin inhibit tumor metastasis in mice. Adv Exp Med Biol 443:285–291
86. Kruzel M, Harari Y, Chen C, Castro G (2000) Lactoferrin protects gut mucosal integrity during endotoxemia induced by lipopolysaccharide in mice. Inflammation 24:33–44
87. Dial EJ, Lichtenberger LM (2002) Effect of lactoferrin on Helicobacter felis induced gastritis. Biochem Cell Biol 80:113–117
88. Wolf J, Li G, Varadhachary A, Petrak K, Schneyer M, Li D, Ongkasuwan J, Zhang X, Taylor R, Strome S, O'Malley BJ (2007) Oral lactoferrin results in T cell-dependent tumor inhibition of head and neck squamous cell carcinoma in vivo. Clin Cancer Res 13:1601–1610
89. Hartog A, Leenders I, van der Kraan P, Garssen J (2007) Anti-inflammatory effects of orally ingested lactoferrin and glycine in different zymosan-induced inflammation models: evidence for synergistic activity. Int Immunopharmacol 7:1784–1792
90. Spagnuolo P, Bird R, Hoffman-Goetz L (2007) Effect of short-term dietary intake of bovine lactoferrin on intestinal lymphocyte apoptosis in healthy mice. Nutr Cancer 23:812–817
91. Lee H, Park J, Seok S, Baek M, Kim D, Lee B, Kang P, Kim Y, Park J (2005) Potential antimicrobial effects of human lactoferrin against oral infection with Listeria monocytogenes in mice. J Med Microbiol 54:1049–1054
92. Zimecki M, Artym J, Chodaczek G, Kocieba M, Kruzel M (2004) Protective effects of lactoferrin in Escherichia coli-induced bacteremia in mice: relationship to reduced serum TNF alpha level and increased turnover of neutrophils. Inflamm Res 53:292–296
93. Lupetti A, Brouwer C, Bogaards S, Welling M, de Heer E, Campa M, van Dissel J, Friesen R, Nibbering P (2007) Human lactoferrin-derived peptide's antifungal activities against disseminated Candida albicans infection. J Infect Dis 196:1416–1424
94. Takakura N, Wakabayashi H, Yamauchi K, Takase M (2006) Influences of orally administered lactoferrin on IFN-gamma and IL-10 production by intestinal intraepithelial lymphocytes and mesenteric lymph-node cells. Biochem Cell Biol 84:363–368
95. Dial EJ, Dohrman A, Romero J, Lichtenberger LM (2005) Recombinant human lactoferrin prevents NSAID-induced intestinal bleeding in rodents. J Pharm Pharmacol 57:93–99
96. Zimecki M, Wlaszczyk A, Zagulski T, Kubler A (1998) Lactoferrin lowers serum interleukin 6 and tumor necrosis factor alpha levels in mice subjected to surgery. Arch Immunol Ther Exp (Warsz) 46:97–104
97. Cumberbatch M, Bhushan M, Dearman R, Kimber I, Griffiths C (2003) IL-1β induced Langerhans' cell migration and TNF-α production in human skin: regulation by lactoferrin. Clin Exp Immunol 132:352–359
98. Hayashida K, Kaneko T, Takeuchi T, Shimizu H, Ando K, Harada E (2004) Oral administration of lactoferrin inhibits inflammation and nociception in rat adjuvant-induced arthritis. J Vet Med Sci 66:149–154
99. Yamauchi K, Wakabayashi H, Hashimoto S, Teraguchi S, Hayasawa H, Tomita M (1998) Effects of orally administered bovine lactoferrin on the immune system of healthy volunteers. Adv Exp Med Biol 443:261–265
100. Zimecki M, Właszczyk A, Cheneau P, Brunel A, Mazurier J, Spik G, Kübler A (1998) Immunoregulatory effects of a nutritional preparation containing bovine lactoferrin taken orally by healthy individuals. Arch Immunol Ther Exp (Warsz) 46:231–240
101. Kimber I, Cumberbatch M, Dearman R, Headon D, Bhushan M, Griffiths CE (2002) Lactoferrin: influences on Langerhans cells, epidermal cytokines, and cutaneous inflammation. Biochem Cell Biol 80:103–107
102. Rogers J, Anderson K (1998) The potential of liposomes in oral drug delivery. Crit Rev Ther Drug Carrier Syst 15:421–480
103. Kennedy R, Konok G, Bounous G, Baruchel S, Lee T (1995) The use of a whey protein concentrate in the treatment of patients with metastatic carcinoma: a phase I-II clinical study. Anticancer Res 15:2643–2649
104. Damiens E, El Yazidi I, Mazurier J, Elass-Rochard E, Duthille I, Spik G, Boilly-Marer Y (1998) Role of heparan sulphate proteoglycans in the regulation of human lactoferrin binding and activity in the MDA-MB-231 breast cancer cell line. Eur J Cell Biol 77:344–351

105. Sakamoto N (1998) Antitumor effect of human lactoferrin against newly established human pancreatic cancer cell line SPA. Cancer Chemother 25:1557–1563
106. McKeown S, Lundy F, Nelson J, Lockhart D, Irwin C, Cowan C, Marley J (2006) The cytotoxic effects of human neutrophil peptide-1 (HNP1) and lactoferrin on oral squamous cell carcinoma (OSCC) in vitro. Oral Oncol 42:685–690
107. Sekine K, Watanabe E, Nakamura J, Takasuka N, Kim D, Asamoto M, Krutovskikh V, Baba-Toriyama H, Ota T, Moore M, Masuda M, Sugimoto H, Nishino H, Kakizoe T, Tsuda H (1997) Inhibition of azoxymethane-initiated colon tumor by bovine lactoferrin administration in F344 rats. Jpn J Cancer Res 88:523–526
108. Ushida Y, Sekine K, Kuhara T, Takasuka N, Iigo M, Maeda M, Tsuda H (1999) Possible chemopreventive effects of bovine lactoferrin on esophagus and lung carcinogenesis in the rat. Jpn J Cancer Res 90:262–267
109. Wolf J, Li D, Taylor R, O'Malley BJ (2003) Lactoferrin inhibits growth of malignant tumors of the head and neck. ORL J Otorhinolaryngol Relat Spec 65:245–249
110. Kanwar J, Palmano K, Sun X, Kanwar R, Gupta R, Haggarty N, Rowan A, Ram S, Krissansen G (2008) Iron-saturated lactoferrin is a potent natural adjuvant for augmenting cancer chemotherapy. Immunol Cell Biol 86:277–288
111. Rodrigues L, Teixeira J, Schmitt F, Paulsson M, Månsson H (2009) Lactoferrin and cancer disease prevention. Crit Rev Food Sci Nutr 49:203–217
112. Mena S, Ortega A, Estrela J (2009) Oxidative stress in environmental-induced carcinogenesis. Mutat Res 674:36–44
113. Larkins N (2005) Potential implications of lactoferrin as a therapeutic agent. Am J Vet Res 66:739–742
114. Shoji H, Oguchi S, Shinohara K, Shimizu T, Yamashiro Y (2007) Effects of iron-unsaturated human lactoferrin on hydrogen peroxide-induced oxidative damage in intestinal epithelial cells. Pediatr Res 61:89–92
115. Letchoumy P, Mohan K, Stegeman J, Gelboin H, Hara Y, Nagini S (2008) In vitro antioxidative potential of lactoferrin and black tea polyphenols and protective effects in vivo on carcinogen activation. DNA damage, proliferation, invasion, and angiogenesis during experimental oral carcinogenesis. Oncol Res 17:193–203
116. Ames B, Gold L, Willett W (1995) The causes and prevention of cancer. Proc Natl Acad Sci USA 92:5258–5265
117. Bezault J, Bhimani R, Wiprovnick J, Furmanski P (1994) Human lactoferrin inhibits growth of solid tumors and development of experimental metastases in mice. Cancer Res 54:2310–2312
118. Sekine K, Ushida Y, Kuhara T, Iigo M, Baba-Toriyama H, Moore M, Murakoshi M, Satomi Y, Nishino H, Kakizoe T, Tsuda H (1997) Inhibition of initiation and early stage development of aberrant crypt foci and enhanced natural killer activity in male rats administered bovine lactoferrin concomitantly with azoxymethane. Cancer Lett 121:211–216
119. Tsuda H, Sekine K, Fujita K, Ligo M (2002) Cancer prevention by bovine lactoferrin and underlying mechanisms – a review of experimental and clinical studies. Biochem Cell Biol 80:131–136
120. Fujita K, Ohnishi T, Sekine K, Iigo M, Tsuda H (2002) Down-regulation of 2-amino-3, 8-dimethylimidazo[4, 5-f]quinoxaline (MeIQx)-induced CYP1A2 expression is associated with bovine lactoferrin inhibition of MeIQx-induced liver and colon carcinogenesis in rats. Jpn J Cancer Res 93:616–625
121. Damiens E, El Yazidi I, Mazurier J, Duthillie I, Spik G, Boilly-Marer Y (1999) Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells. J Cell Biochem 74:486–498
122. Xiao Y, Monitto C, Minhas K, Sidransky D (2004) Lactoferrin down-regulates G1 cyclin-dependent kinases during growth arrest of head and neck cancer cells. Clin Cancer Res 10:8683–8686
123. Oh S, Pyo C, Kim Y, Choi S (2004) Neutrophil lactoferrin upregulates the human p53 gene through induction of NF-κB activation cascade. Oncogene 23:8282–8291
124. Son H, Lee S, Choi S (2006) Human lactoferrin controls the level of retinoblastoma protein and its activity. Biochem Cell Biol 84:345–350
125. Varadhachary A, Wolf J, Petrak K, O'Malley BJ, Spadaro M, Curcio C, Forni G, Pericle F (2004) Oral lactoferrin inhibits growth of established tumors and potentiates conventional chemotherapy. Int J Cancer 111:398–403

126. Katunuma N, Murata E, Le Q, Hayashi Y, Ohashi A (2004) New apoptosis cascade mediated by lysosomal enzyme and its protection by epigallo-catechin gallate. Adv Enzyme Regul 44:1–10
127. Katunuma N, Le Q, Murata E, Matsui A, Majima E, Ishimaru N, Hayashi Y, Ohashi A (2006) A novel apoptosis cascade mediated by lysosomal lactoferrin and its participation in hepatocyte apoptosis induced by D-galactosamine. FEBS Lett 580:3699–3705
128. Fujita K, Matsuda E, Sekine K, Iigo M, Tsuda H (2004) Lactoferrin modifies apoptosis-related gene expression in the colon of the azoxymethane-treated rat. Cancer Lett 213:21–29
129. Chandra Mohan K, Devaraj H, Prathiba D, Haru Y, Nagini S (2006) Antiproliferative and apoptosis inducing effect of lactoferrin and black tea polyphenol combination on hamster buccal pouch carcinogenesis. Biochim Biophys Acta 1760:1536–1544
130. Lin T, Chiou S, Chen M, Kuo C (2005) Human lactoferrin exerts bidirectional actions on PC12 cell survival via ERK1/2 pathway. Biochem Biophys Res Commun 337:330–336
131. Yoo YC, Watanabe R, Koike Y, Mitobe M, Shimazaki K, Watanabe S, Azuma I (1997) Apoptosis in human leukemic cells induced by lactoferricin, a bovine milk protein-derived peptide: involvement of reactive oxygen species. Biochem Biophys Res Commun 237:624–628
132. Norrby K, Mattsby-Baltzer I, Innocenti M, Tuneberg S (2001) Orally administered bovine lactoferrin systemically inhibits VEGF(165)-mediated angiogenesis in the rat. Int J Cancer 91:236–240
133. Shimamura M, Yamamoto Y, Ashino H, Oikawa T, Hazato T, Tsuda H, Iigo M (2004) Bovine lactoferrin inhibits tumor-induced angiogenesis. Int J Cancer 111:111–116
134. Norrby K (2004) Human apo-lactoferrin enhances angiogenesis mediated by vascular endothelial growth factor a in vivo. J Vasc Res 41:293–304
135. Iigo M, Shimamura M, Matsuda E, Fujita K, Nomoto H, Satoh J, Kojima S, Alexander D, Moore M, Tsuda H (2004) Orally administered bovine lactoferrin induces caspase-1 and interleukin-18 in the mouse intestinal mucosa: a possible explanation for inhibition of carcinogenesis and metastasis. Cytokine 25:36–44
136. Pinchuk G (2002) Schaum's outline of theory and problems of immunology. McGraw-Hill, New York
137. Haversen L, Ohlsson B, Hahn-Zoric M, Hanson L, Mattsby-Baltzer I (2002) Lactoferrin down-regulates the LPS-induced cytokine production in monocytic cells via NF-κB. Cell Immunol 220:83–95
138. Xu Y, Samaranayake Y, Samaranayake L, Nikawa H (1999) In vitro susceptibility of Candida species to lactoferrin. Med Mycol 37:35–41
139. Fritsch G, Sawatzki G, Treumer J, Jung A, Spira D (1987) Plasmodium falciparum: Inhibition in vitro with lactoferrin, desferriferrithiocin, and desferricrocin. Exp Parasitol 63:1–9
140. Turchany J, Aley S, Gillin F (1995) Giardicidal activity of lactoferrin and N-terminal peptides. Infect Immun 63:4550–4552
141. Tanaka T, Omata Y, Saito A, Shimazaki K, Igarashi I, Suzuki N (1996) Growth inhibitory effects of bovine lactoferrin to Toxoplasma gondii parasites in murine somatic cells. J Vet Med Sci 58:61–65
142. Harmsen M, Swart P, de Béthune M, Pauwels R, De Clercq E, The T, Meijer D (1995) Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication in vitro. J Infect Dis 172:380–388
143. Ikeda M, Sugiyama K, Tanaka T, Tanaka K, Sekihara H, Shimotohno K, Kato N (1998) Lactoferrin markedly inhibits hepatitis C virus infection in cultured human hepatocytes. Biochem Biophys Res Commun 245:549–553
144. Hasegawa K, Motsuchi W, Tanaka S, Dosako S (1994) Inhibition with lactoferrin of in vitro infection with human herpes virus. Jpn J Med Sci Biol 47:73–85
145. Marchetti M, Pisani S, Antonini G, Valenti P, Seganti L, Orsi N (1998) Metal complexes of bovine lactoferrin inhibit in vitro replication of herpes simplex virus type 1 and 2. Biometals 11:89–94
146. Marchetti M, Superti F, Ammendolia M, Rossi P, Valenti P, Seganti L (1999) Inhibition of poliovirus type 1 infection by iron-, manganese- and zinc-saturated lactoferrin. Med Microbiol Immunol 187:199–204
147. Lin T, Chu C, Chiu C (2002) Lactoferrin inhibits enterovirus 71 infection of human embryonal rhabdomyosarcoma cells in vitro. J Infect Dis 186:1161–1164
148. Drobni P, Näslund J, Evander M (2004) Lactoferrin inhibits human papillomavirus binding and uptake in vitro. Antiviral Res 64:63–68

149. Di Mario F, Aragona G, Bò N, Ingegnoli A, Cavestro G, Moussa A, Iori V, Leandro G, Pilotto A, Franzè A (2003) Use of lactoferrin for Helicobacter pylori eradication. Preliminary results. J Clin Gastroenterol 36:396–398
150. Di Mario F, Aragona G, Dal Bó N, Cavallaro L, Marcon V, Olivieri P, Benedetti E, Orzès N, Marin R, Tafner G, Chilovi F, De Bastiani R, Fedrizzi F, Franceschi M, Salvat M, Monica F, Piazzi L, Valiante F, Vecchiati U, Cavestro G, Comparato G, Iori V, Maino M, Leandro G, Pilotto A, Rugge M, Franzè A (2006) Bovine lactoferrin for Helicobacter pylori eradication: an open, randomized, multicentre study. Aliment Pharmacol Ther 23:1235–1240
151. de Bortoli N, Leonardi G, Ciancia E, Merlo A, Bellini M, Costa F, Mumolo M, Ricchiuti A, Cristiani F, Santi S, Rossi M, Marchi S (2007) Helicobacter pylori eradication: a randomised prospective study of triple therapy versus triple therapy plus lactoferrin and probiotics. Am J Gastroenterol 102:951–956
152. Tursi A, Elisei W, Brandimarte G, Giorgetti G, Modeo M, Aiello F (2007) Effect of lactoferrin supplementation on the effectiveness and tolerability of a 7-day quadruple therapy after failure of a first attempt to cure Helicobacter pylori infection. Med Sci Monit 13:CR187–CR190
153. Okuda M, Nakazawa T, Yamauchi K, Miyashiro E, Koizumi R, Booka M, Teraguchi S, Tamura Y, Yoshikawa N, Adachi Y, Imoto I (2005) Bovine lactoferrin is effective to suppress Helicobacter pylori colonization in the human stomach: a randomized, double-blind, placebo-controlled study. J Infect Chemother 11:265–269
154. Zullo A, De Francesco V, Scaccianoce G, Hassan C, Panarese A, Piglionica D, Panella C, Morini S, Ierardi E (2005) Quadruple therapy with lactoferrin for Helicobacter pylori eradication: a randomised, multicentre study. Dig Liver Dis 37:496–500
155. Zullo A, De Francesco V, Scaccianoce G, Manes G, Efrati C, Hassan C, Maconi G, Piglionica D, Cannaviello C, Panella C, Morini S, Ierardi E (2007) Helicobacter pylori eradication with either quadruple regimen with lactoferrin or levofloxacin-based triple therapy: a multicentre study. Dig Liver Dis 39:806–810
156. Ajello M, Greco R, Giansanti F, Massucci M, Antonini G, Valenti P (2002) Anti-invasive activity of bovine lactoferrin towards group a streptococci. Biochem Cell Biol 80:119–124
157. Teraguchi S, Shin K, Ozawa K, Nakamura S, Fukuwatari Y, Tsuyuki S, Namihira H, Shimamura S (1995) Bacteriostatic effect of orally administered bovine lactoferrin on proliferation of Clostridium species in the gut of mice fed bovine milk. Appl Environ Microbiol 61:501–506
158. Hellweg P, Krammer-Lukas S, Strasser A, Zentek J (2008) Effects of bovine lactoferrin on the immune system and the intestinal microflora of adult dogs. Arch Anim Nutr 62:152–161
159. Kawasaki Y, Tazume S, Shimizu K, Matsuzawa H, Dosako S, Isoda H, Tsukiji M, Fujimura R, Muranaka Y, Isihida H (2000) Inhibitory effects of bovine lactoferrin on the adherence of enterotoxigenic Escherichia coli to host cells. Biosci Biotechnol Biochem 64:348–354
160. Masci J (2000) Complete response of severe, refractory oral candidiasis to mouthwash containing lactoferrin and lysozyme. AIDS 14:2403–2404
161. Yamauchi K, Hiruma M, Yamazaki N, Wakabayashi H, Kuwata H, Teraguchi S, Hayasawa H, Suegara N, Yamaguchi H (2000) Oral administration of bovine lactoferrin for treatment of tinea pedis. a placebo-controlled, double-blind study. Mycoses 43:197–202
162. Shimizu K, Matsuzawa H, Okada K, Tazume S, Dosako S, Kawasaki Y, Hashimoto K, Koga Y (1996) Lactoferrin-mediated protection of the host from murine cytomegalovirus infection by a T-cell dependent augmentation of natural killer cell activity. Arch Virol 141:1875–1889
163. Iwasa M, Kaito M, Ikoma J (2002) Lactoferrin inhibits hepatitus C virus viremia in chronic hepatitis C patients with high viral loads and HCV genotype 1b. Am J Gastroenterol 97:766–767
164. Shin K, Wakabayashi H, Yamauchi K, Teraguchi S, Tamura Y, Kurokawa M, Shiraki K (2005) Effects of orally administered bovine lactoferrin and lactoperoxidase on influenza virus infection in mice. J Med Microbiol 54:717–723

18 Plant-Derived Anticancer Agents Used in Western and Oriental Medicine

Ah-Reum Han, Ye Deng, Yulin Ren, Li Pan, and A. Douglas Kinghorn

Key Points

- Cancer chemotherapeutic agents derived from higher plants are used in Western medicine.
- Secondary metabolites from plants are used in oriental medicine are utilized in anticancer therapy.
- Immunomodulatory small organic molecules from plant species are employed in Chinese traditional medicine are renewed.

Key Words: Higher plants, western medicine, oriental medicine, traditional chinese medicine, anticancer agents, immunomodulatory agents, secondary metabolites.

18.1 INTRODUCTION

Plant-derived cancer chemotherapeutic agents have been used in Western medicine for about 50 years as single chemical entities, and their number of clinical applications is continuing to increase. In recent years, there has been an effort to modernize traditional systems of medicine used in East Asian countries, by purifying, structurally characterizing, and biologically testing the active principles of herbs used as anticancer drugs. Another approach toward treating cancer in oriental medicine is to use small-molecule immunomodulatory agents of plant origin in patient therapy. In this chapter, the authors provide updated information on antitumor agents from plants currently approved for use in the United States, and provide a comparison with analogous agents utilized in East Asia. A summary is provided on compounds with immunomodulatory effects from selected plants used in Traditional Chinese Medicine (TCM). The small-molecule

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_18

plant-derived natural products discussed in this chapter are of diverse structural types, and many additional examples of such compounds with antitumor and immunomodulatory activity may be expected to be elucidated in the years to come.

18.2 ANTICANCER AGENTS IN WESTERN MEDICINE

Natural products have played an important role in anticancer drug development for many decades. A recent review analyzing clinically approved anticancer drugs in North America, Europe, and Japan during the 25 year period from 1981 to 2006 showed that under the class of "non-biologicals/vaccines", 63 of 81 (77.8%) anticancer drugs were either natural products or their derivatives, or synthesized molecules based on natural product pharmacophores (1). Currently, there are about ten approved plant-derived anticancer drugs used clinically in the United States, which are classified into four groups: the podophyllotoxin derivatives, the vinca alkaloids, the camptothecin derivatives, and the taxanes (1, 2). The importance of plant natural products and derivatives in cancer therapy can be demonstrated by their overall major market share. For example, in the year 2002, derivatives of paclitaxel and camptothecin represented nearly one-third of all anticancer agents available in terms of sales volume (3). The history, structures (Fig. 18.1), applications, and mechanisms of action of these drugs as used in western medicine will be discussed briefly in the following paragraphs.

Podophyllotoxin (**1**) is a lignan first isolated by Podwyssotzki in 1880 from *Podophyllum peltatum* L. (Berberidaceae), a plant commonly known as American mayapple or mandrake, but it was structurally characterized much later, in 1951 (4). An impure extractive from *P. peltatum*, commonly known as "podophyllin", has been used topically in the treatment of genital warts and hairy leukoplakia. Mayapple extract was listed in the first edition of the U.S. Pharmacopoeia in 1820, but was withdrawn in 1840 due to its associated toxicity. The plant regained the interest of the pharmaceutical industry when a therapeutic effect for condylomata acuminata was demonstrated in the 1940s (5). Presently, plant extracts containing arylnaphthalene-type lignans are prepared from *P. peltatum* and a second species, *Podophyllum hexandrum* Royle. The latter species is known as "Indian mayapple" or "Himalayan mayapple", with a higher content of lignans than the American mayapple. Two drugs derived from the parent lignan, podophyllotoxin (**1**), namely, SP-G (Proresid oral®), the condensation product of *P. peltatum* glucoside fraction with benzaldehyde, and SP-I (Proresid i.v.®), podophyllinic acid ethyl hydrazide, were first commercialized in 1963 for the treatment of systematic cancers by Sandoz Pharma A.G. (Basel, Switzerland) (6). Further studies of SP-G by Sandoz in the 1960s led to the development of etoposide (**2**, VePesid®) and teniposide (**3**, Vumon®), which were produced by condensation with acetaldehyde and thiophene, respectively. Even though Sandoz had commercialized both drugs in certain countries, etoposide and teniposide were licensed to Bristol-Myers in 1978 for commercialization in the U.S. market. Etoposide (Vepesid®, VP-16®) was first approved in 1983 by the United States Food and Drug Administration (FDA) for the treatment of refractory testicular tumors, and then in 1996 for small-cell lung cancer (SCLC) therapy. The etoposide phosphate salt, etopophos (**4**, Eposin®, Etopophos®) was developed as a prodrug to resolve the water solubility limitation encountered in drug formulation and administration of etoposide. Studies have

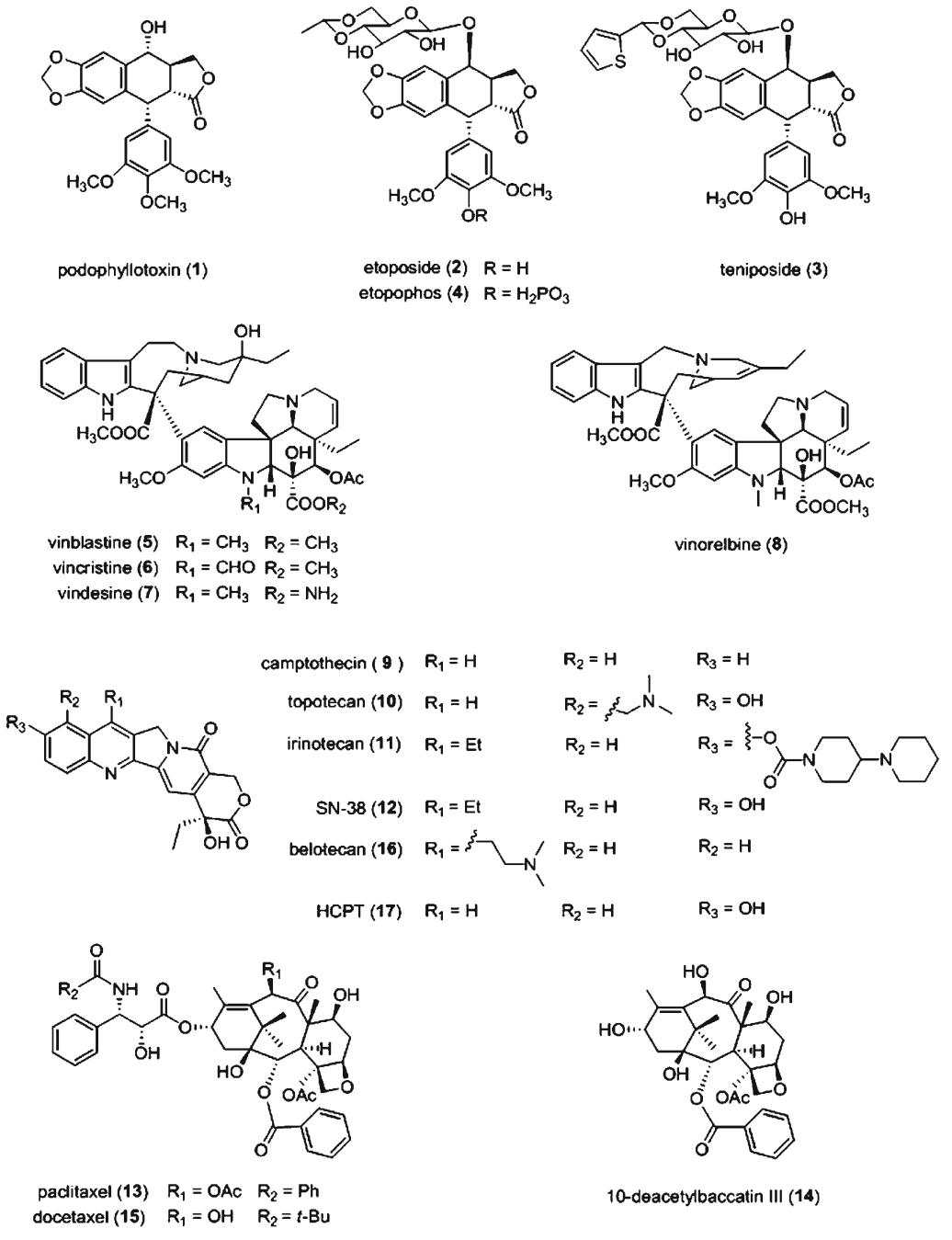


Fig. 18.1. Structures of plant-derived anticancer agents used in western medicine and some derivatives.

shown that etoposide phosphate salt is less toxic and more active than etoposide, with increased bioavailability to over 50%, from only 0.04% for the parent compound. The FDA approved the intravenous use of etopophos for SCLC in 1996 (7). Teniposide was

approved by the FDA in 1992 for the treatment of childhood acute lymphoblastic leukemia. In contrast to the mechanism of action of podophyllotoxin, which binds to tubulin and inhibits the assembly of microtubules to form mitotic spindles in mitosis, etoposide and teniposide are topoisomerase II inhibitors. They stabilize the covalent DNA-enzyme cleavable complex, and induce topoisomerase II-mediated DNA breakage, leading to arrest of the cell cycle in the metaphase (8).

Vinblastine (**5**, VLB, formerly known as vincaleukoblastine) and vincristine (**6**, VCR, formerly known as leurocristine) are bis-indole alkaloids found in the plant *Catharanthus roseus* (L.) G. Don (Apocynaceae), previously classified taxonomically as *Vinca rosea* L. This plant is cultivated as an ornamental, under the common names, "Madagascar periwinkle" and "rosy periwinkle". The first vinca alkaloid, vinblastine, was isolated independently by Beer and colleagues at the University of Western Ontario and by researchers at Eli Lilly Co. in Indianapolis, in the late 1950s. Vincristine was isolated later by Svoboda and colleagues (9). Vincristine (Oncovin®) and vinblastine (Velban®) were approved by the United States Food and Drug Administration (U.S. FDA) in 1963 and 1965, respectively. Clinically, vinblastine is used mainly to treat Hodgkin's disease and vincristine is employed for the treatment of non-Hodgkin's lymphoma, Hodgkin's lymphoma, acute lymphoblastic leukemia, and nephroblastoma. Vindesine (**7**, Eldisine®) is a semisynthetic drug derived from vinblastine, approved for clinical use in France, the UK, and several other countries, but not in the USA. The drug is utilized clinically for the treatment of several different types of cancer, including acute lymphocytic leukemia, breast cancer, chronic myelocytic leukemia, colorectal cancer, non-SCLC, and renal cell cancer (10). Vinorelbine (**8**, Navelbine®) is a semisynthetic anhydro derivative of 8 -nor-vinblastine, obtained from anhydrovinblastine. The drug was first approved to treat bronchial cancer in France in 1989, and was later approved for the therapy of non-SCLC in 1991. In Europe, vinorelbine is also approved for breast cancer and prostate cancer treatment. Vinorelbine received approval from the U.S. FDA in 1994 for the treatment of advanced non-SCLC (11). The vinca alkaloids bind to the microtubulin "vinca domain" site in the β-subunit, and interrupt the formation of microtubules in the metaphase during mitosis, leading to the apoptosis of cancer cells (12).

Camptothecin (**9**) is a quinoline alkaloid isolated from the bark and stems of *Camptotheca acuminata* Decne (Cornaceae; formerly Nyssaceae), a tree native to mainland China that was introduced to the USA in 1911 as an ornamental. Camptothecin was discovered in the mid-1960s by Monroe E. Wall and Mansukh C. Wani, in a systematic screening of anticancer natural products carried out at the Research Triangle Institute in North Carolina, with support from the U.S. National Cancer Institute (NCI), Bethesda, MD (13, 14). Although a Phase II study of camptothecin sodium salt in treating gastrointestinal cancer was suspended in 1972 due to poor efficacy and toxic side effects, interest in camptothecin was regained after its unique mechanism of action was elucidated (3). Studies have shown that camptothecin arrests the cell cycle at the S-phase by binding to topoisomerase I, leading to the inhibition of DNA replication and transcription (15, 16). Among numerous semisynthetic camptothecin derivatives aimed at improving the solubility and efficacy of the parent compound, as well as decreasing its side effects, two analogs, topotecan (**10**, Hycamtin®, GlaxoSmithKline) and irinotecan (**11**, Camptosar®, Pfizer), have been launched successfully onto the cancer chemotherapy market. The substitution of a *N,N*-dimethylaminomethyl group at the C-9 position and the insertion of a hydroxy group at the C-10 position as in topotecan significantly

increases water solubility and bioavailability, when compared to camptothecin. Topotecan was approved by the FDA for treating ovarian cancer in 1996, for the therapy of stage IVB recurrent or persistent cervical carcinoma in 2006, and also for relapsed SCLC in 2007. This is the only oral single-agent cancer chemotherapic approved to manage SCLC after the failure of first-line therapy. Irinotecan is a prodrug of the 7-ethyl-10-hydroxycamptothecin analog, SN-38 (**12**), with the substitution of a bis-piperidine functionality at the C-10 position. The drug was developed initially in Japan by Yakult Pharmaceutical Industries. In the human body, the prodrug irinotecan is hydrolyzed by liver carboxylesterases into a metabolite, SN-38 (17). Irinotecan was approved by the FDA in 1996 for the treatment of metastatic colorectal cancer, in combination with 5-fluorouracil and leucovorin (5-FU/LV).

Paclitaxel (**13**) is a nitrogen-containing diterpenoid discovered from the bark of the Pacific yew tree, *Taxus brevifolia* Nutt. (Taxaceae), and was reported structurally in 1971 by Wall and Wani (Research Triangle Institute) and Andrew McPhail (Duke University), in a study sponsored by NCI. The compound was originally given the trivial name "taxol", but its generic name was adopted as "paclitaxel" when Bristol-Myers Squibb (B-MS) acquired the trade-marked name "Taxol" from a French company, who had used the name for an unrelated laxative product several years earlier (18). Paclitaxel was approved in 1992 by the FDA for the treatment of patients with refractory metastatic carcinoma of the ovary after the failure of first-line or subsequent chemotherapy. The drug is currently used to treat patients with lung, ovarian, breast, and head and neck cancer, and advanced forms of Kaposi's sarcoma. With the rapid application of the drug in the early 1990s, the increasing clinical demands for paclitaxel presented a strain on the resources of the Pacific yew tree, for which extraction of the bark was the only commercial production method of the drug at the time. This costly and environmentally challenging method was replaced by a semisynthetic process using 10-deacetylbaccatin III (**14**), which is found abundantly in the leaves of a European yew tree, *Taxus baccata* L., a renewable resource. Presently, paclitaxel is also produced commercially by B-MS by plant tissue culture on a large scale (19). Abraxane® is an albumin-covered paclitaxel injectable suspension, using "nanoparticle albumin bound" (*nab*™) technology to facilitate drug delivery. Abraxane was approved by the FDA in 2005 for the treatment of breast cancer. Docetaxel (**15**, Taxotere®) is a semisynthetic paclitaxel analog produced from 10-deacetylbaccatin III, where a *N*-*tert*-butoxycarbonyl group is substituted for the *N*-benzoyl group in the side chain of **13** and the C-10 hydroxy group is free. Docetaxel was approved in 1996 by the FDA, and is presently used for the treatment of breast, non-small cell lung, gastric, prostate, as well as head and neck cancers (20). In contrast to the mechanism of action of vinca alkaloids, which disrupt the assembly of microtubules in mitosis, paclitaxel and docetaxel stabilize microtubules by binding to the taxane site, and, as a result, interfere with the normal breakdown of microtubules during cell division (19).

18.3 ANTICANCER AGENTS IN ORIENTAL MEDICINE

The value of medicinal plants for healing has been established for at least 2,000 years in oriental medicine (21). In particular, the species used in TCM are representative of a valuable resource in the search for new anticancer drugs employing modern scientific methods. Thorough pharmacological and clinical experimentation on plants and their

constituents used in traditional medicine is progressing in order to establish effectiveness and safety (22, 23). A summary of some important oriental medicinal plant-derived anticancer agents and their modified forms is provided in the paragraphs below, and their structures are shown in Fig. 18.2.

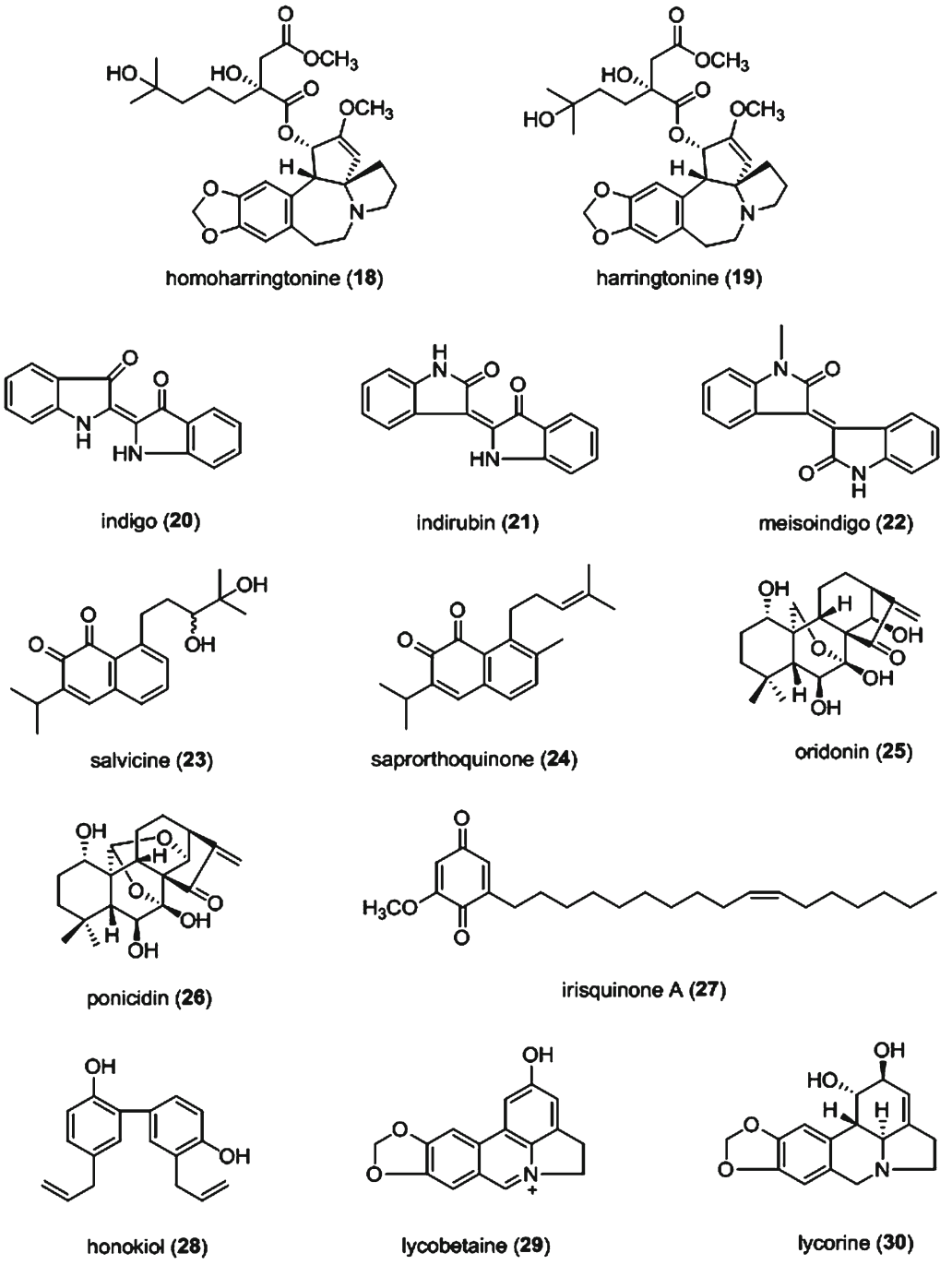


Fig. 18.2. Structures of anticancer agents used in oriental medicine and some derivatives.

Camptothecin (**9**) is a quinoline-based alkaloid mentioned earlier in this chapter. The plant of origin, *C. acuminata* is distributed only in Tibet and western mainland China, but **9** was also reported to be produced by other plants such as *Ervatamia heyneana* Cooke (Apocynaceae) (24), *Merrilliodendron megacarpum* (Hemsl.) Sleumer (Icacinaceae) (25), *Nothapodytes foetida* (Wight) Sleumer (Icacinaceae) (24), *Ophiorrhiza mungos* L. (Rubiaceae) (24), and *Pyrenacantha klaineana* Pierre ex Exell & Mendonça (Icacinaceae) (26). The development of novel analogs that optimize and exploit important structural features and expand the therapeutic potential of camptothecin, is being carried out. A new semisynthetic camptothecin analog currently under evaluation, belotecan (**16**, Camtobell®, Chong Keun Dang) with the structure 7-[2-{(1-methylethyl)amino}ethyl]camptothecin, is presently in phase III clinical trials for SCLC in South Korea (27). 10-Hydroxycamptothecin (**17**, HCPT) is undergoing clinical studies for non-small cell lung and advanced gastric cancers in the People's Republic China (28, 29).

Homoharringtonine (**18**, Omacetaxine®, ChemGenex Pharmaceuticals) and related compounds were discovered from coniferous *Cephalotaxus* species indigenous to eastern Asia (30, 31). A mixture of compound **18** and harringtonine (**19**) has been used for the treatment of acute myeloid leukemia (AML) clinically in mainland China, since 1974 (32). However, compound **18** has remained as the most promising antileukemic agent of its structural class, showing *in vivo* effects on L1210 leukemia cells implanted in mice in the dose range of 0.5 to 4.0 mg/kg (i.p.) as well as on murine leukemia P388 cells resistant to vincristine, adriamycin, and 1-β-D-arabinofuranosylcytosine, given i.p. at 1.3 mg/kg/day (33), and in a RPMI8226 myeloma xenograft model (i.p., 3 and 15 mg/kg) (34). Many other structurally related alkaloids have been isolated from the genus *Cephalotaxus* and numerous semisynthetic derivatives have been prepared. However, research on **18** has predominated since its content in the plant of origin and activity are much higher than those of the related alkaloids. It is interesting to note that a phase I trial using the purified alkaloid **18** was performed in the U.S. on patients with advanced solid tumors and leukemias (35). Compound **18** has been investigated also in phase II/III clinical trials against chronic myelogenous leukemia (CML), supported by the U.S. NCI and ChemGenex Pharmaceuticals, Geelong, Australia (36).

Since the 1960's, "Danggui Longhui Wan", a mixed product containing 11 ingredients, has been used in TCM for CML (37). The antileukemic activity was found to be associated with one component, Indigo Naturalis (Qing Dai), which is a dark-blue powder from the leaves or leaves and stems of *Baphicacanthus cusia* (Nees) Bremek. (Acanthaceae), the leaves of *Indigofera suffruticosa* Mill. (Fabaceae), the leaves or roots of *Isatis tinctoria* L. (Brassicaceae), or the leaves of *Polygonum tinctorium* Ait. (Polygonaceae) (38). Of the chemical constituents of Indigo Naturalis, a blue dye, indigo (**20**) and a red-colored isomer, indirubin (**21**) were found, but the antileukemic activity was attributed to indirubin, which has been reported to have *in vivo* effects in rat carcinosarcoma W256 and mouse leukemia L7212 models that were given 200 mg/kg/day (i.p.) (39), and this compound exhibits cyclin-dependent kinase (CDK) inhibitory potential and broad-spectrum antitumor activity (40). However, several derivatives have been designed and synthesized to produce new agents with an indirubin-like structure but showing better efficacy and lower toxicity, since **21** shows poor solubility in water and has exhibited side effects such as gastrointestinal toxicity (41). Among them, meisoindigo (**22**), *N*-methylisoindigotin, has been reported to significantly inhibit tumor growth

in HT-29 colon cancer xenografts at a dose of 100 mg/kg (i.p.) (42). This compound has been investigated in the People's Republic of China in phase II clinical studies in about 400 cases of CML, at a dose of 75–150 mg/mouse/day, administrated i.p. (43).

Salvicine (**23**) is a diterpenoid quinone obtained by the structural modification of a natural product lead, saprorthoquinone (**24**), isolated from the Chinese herb *Salvia prionitis* Hance (Lamiaceae). An *in vivo* effect of **23** was found in four subcutaneously transplanted tumor murine models, S-180 sarcoma, Lewis lung, A-549 lung, and LAX-83 lung adenocarcinoma xenografts, in a dose range of 7.5–30 mg/kg (i.p.) (44). A mechanism of action study on **23** has shown that it inhibits topoisomerase II (45). More recently, compound **23** has been found to have antimetastatic activity as shown in a human breast cancer MDA-MB-435 orthotopic xenograft system at doses of 6, 12, and 24 mg/kg (i.p.) (46). Salvicine has been in phase II clinical trials for cancer therapy in mainland China and is a promising multi-drug resistant tumor treatment candidate (47).

Rabdosia species (Lamiaceae) are used in TCM as antitumor and anti-inflammatory agents. *ent*-Kaurane diterpenoids such as oridonin (**25**) and ponicidin (**26**) have been found to be the cytotoxic principles of *Rabdosia rubescens* (Hemsl.) Hara (48). Compound **25** was reported to be active *in vivo* against Ehrlich ascites carcinoma in mice at doses of 20 and 30 mg/kg (i.p.) (49). The *in vivo* efficacy of **25** in AML has been found in C57 mice bearing AEtr-expressing leukemic cells at doses of 7.5 and 15 mg/kg (i.p.) (50). In an investigation of the antiproliferative effect of **25** against HT29 human colorectal carcinoma cells implanted *in vivo*, the inhibition of these solid tumors was observed at 10, 15, and 20 mg/kg/day (i.p.) (51). Compounds **25** and **26** have been tested in clinical trials for the treatment of esophageal cancer in mainland China (52). PC-SPES, an eight-herb formulation, including *R. rubescens*, has been evaluated for the treatment of prostate cancer and significantly decreased serum prostate specific antigen (PAS) of patients (53). Oridonin (**25**) and ponicidin (**26**), two constituents of PC-SPES, were considered responsible for its perceived antiangiogenic activity (54).

Irisquinone A (**27**), 2-(*cis*-10-heptadecenyl)-6-methoxy-*p*-benzoquinone, was isolated from the seeds of *Iris lactea* Pall. var. *chinensis* (Fisch.) Koidz (Iridaceae), which has been used in Chinese folk medicine as a fertility-regulating agent and for the treatment of malignant diseases (55). This compound was found to be antitumorigenic against lymphosarcoma, cervical cancer U_{14}, as well as hepatic and Ehrlich carcinoma in murine experimental systems, with 50% inhibition at 25.4 mg/kg (i.p.) and 2.8 g/kg (p.o.) (56). The radiosensitizing effects of **27** were also found against Ma 7373 breast cancer cells implanted in mice and human intestinal mucoadenocarcinoma cells in nude mice. The mechanism of action of **27** is considered to be the inhibition of oxygen consumption and deletion of glutathione in tumor cells (57). Recently, compound **27** has been found to inhibit metastasis on H22 lung cancer cell-bearing mice, improving immune function and reducing the expression of VEGF and MVD in the tumors (58).

Honokiol (**28**) is an active compound purified from various species of the genus *Magnolia* used in the traditional Chinese and Japanese systems of medicine. Compound **28** inhibited tumor growth and/or prolonged the life span in numerous *in vivo* models. The administration of **28** (3 mg/mouse/day i.p.) to SVR angiosarcoma-bearing mice resulted in the inhibition of tumor growth (59). The combination of **28** (100 mg/kg i.p.) and low-dose docetaxel (5 mg/kg i.p.) was shown to inhibit potently prostate cancer growth and bone metastasis in mouse bone bearing C4-2 prostate tumor xenografts (60).

In a study of the *in vivo* activity of **28** against human breast cancer, the administration of this compound (100 mg/kg) led to the inhibition of MDA-MB-231 breast cancer tumor growth in nude mice (61). In SKOV3 ovarian cancer tumor-bearing mice treated with 1 mg liposome-encapsuled honokiol (40%) (i.p.), microvessel density and tumor volumes were decreased (62). Recently, liposomal honokiol has been reported to exhibit its therapeutic effect by inhibiting tumor growth in the A549 lung cancer xenograft model (25 mg/kg/day i.p.) (63) and by inhibiting lymphangiogenesis and metastasis via a vascular endothelial growth factor receptor-3 (VEGFR-3) pathway in a VEGF-D Lewis lung carcinoma cell xenograft model (12.5–50 mg/kg/day i.p.) (64). In terms of the preclinical efficacy of **28**, the induction of caspase-dependent or -independent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells from patients (65) and multiple myeloma cells from patients with relapsed refractory multiple myeloma (66) were observed.

Lycobetaine (**29**, ungeramin, AT-1840) is a phenanthridine alkaloid partially synthesized from lycorine (**30**), a major constituent of plants in the family Amaryllidaceae from mainland China, such as *Lycoris radiata* Herb. (67). Compound **29** has exhibited inhibitory effects on mice and rats inoculated with Ehrlich ascites carcinoma, ascites hepatoma, leukemia L-1210, leukemia P-388, Lewis lung carcinoma, or Yoshida ascites sarcoma, at a dose of 72 mg/kg (i.p.) (68). In an examination of the interaction of lycobetaine with DNA, it interacted by intercalation, preferentially on G-C base pairs, but did not bind to DNA covalently and did not cause DNA alkylation (69, 70). The calculated interaction energy of **29** with a double-stranded polynucleotide was correlated with its anticancer potency (71). Recently, compound **29** was found to inhibit tumor growth in GXF251 xenografts at a dose of 480 mg/kg (i.p.), and acts as a selective topoisomerase IIβ poison (72). In clinical trials in the People's Republic of China, lycobetaine was effective for patients with ovarian and gastric carcinomas (73).

18.4 IMMUNOMODULATORY AGENTS IN TCM

Immunomodulators include immunosuppressants and immunostimulants, and may be used as agents to treat immune-mediated diseases. Immunosuppressants suppress the immune system to protect transplanted organs or to treat immunological diseases, such as rheumatic arthritis, systemic lupus erythematosus, hepatitis, and cancer (74). Immunostimulants may be either specific and nonspecific in their action. Specific immunostimulants provide antigenic specificity, and nonspecific immunostimulants act irrespective of antigenic specificity. The nonspecific immune response fights against the effects of microbial pathogens by increasing the secretion of cytokines or by the activation of B- or T-lymphocytes, and the nonspecific immunostimulants are used widely to treat chronic infections, immunodeficiency, autoimmunity, and neoplastic diseases (75–78).

Chinese herbs have been used to treat immune-mediated disorders for thousands of years. Shen Nong is widely regarded as the father of TCM, who investigated hundreds of Chinese herbs and recorded their effects around 4,000 years ago. Up to the present time, around 40 medicinal herbs have been used clinically as immunomodulators in TCM (79). These herbs are processed into different preparations to be used as immunomodulators either directly to treat human diseases, or to serve as adjuvants for therapeutic purposes. The seven such plants most frequently encountered for immunomodulation,

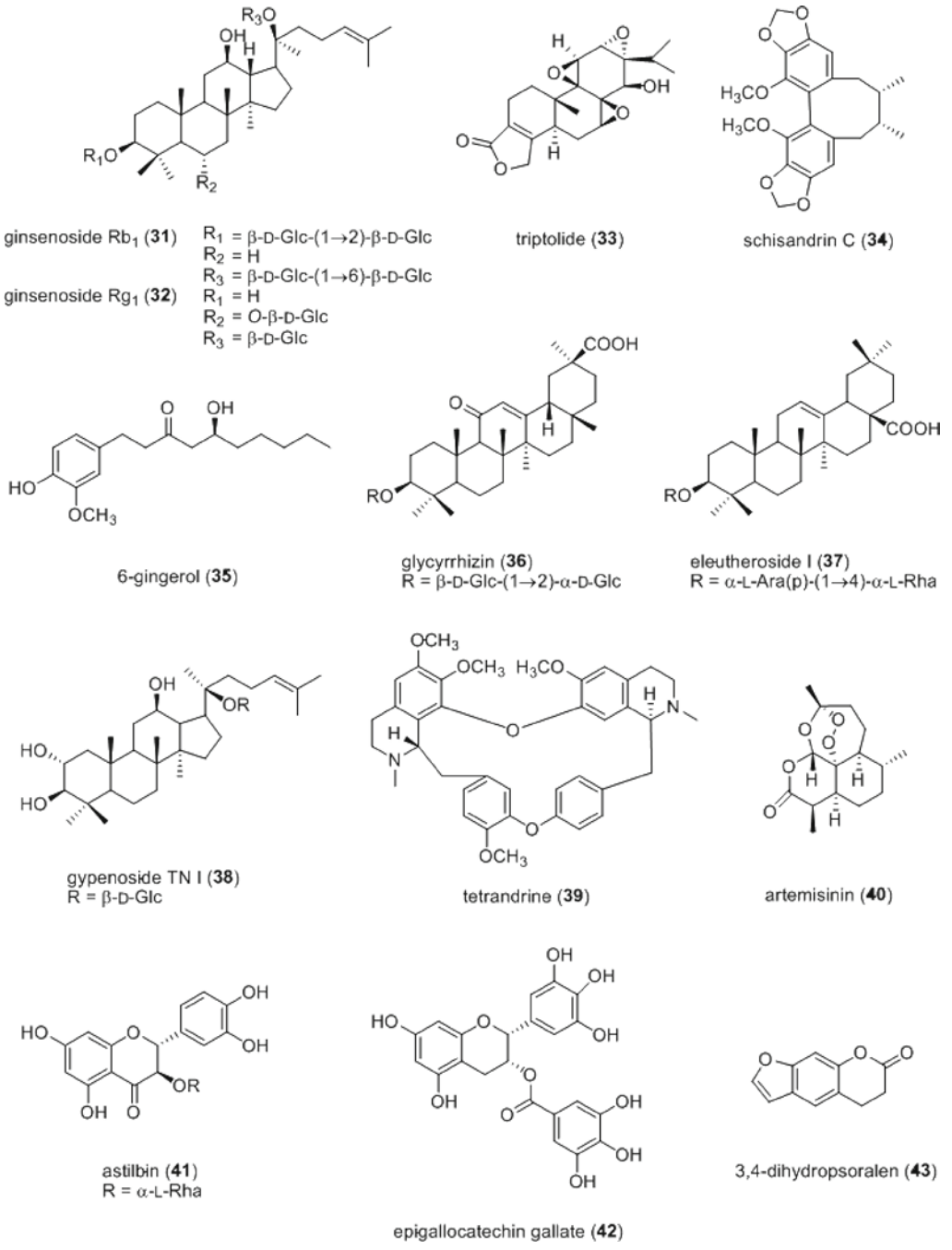


Fig. 18.3. Structures of immunomodulatory agents used in Traditional Chinese Medicine (TCM).

including for each their name, active constituent(s) and structure(s) (Fig. 18.3), organism of origin, biological effects, and mechanism of action, are described briefly in the paragraphs below.

Panax ginseng C.A. Mey. (Araliaceae), is a perennial herb and popular herbal medicine used to treat cardiovascular and immune-mediated disorders (80). After being grown for more than 5 years, *P. ginseng* is harvested in September and October. The aerial parts and hairy roots are removed, and the remaining roots are usually pretreated by steaming, boiling, or dipping in a saturated sugar solution before use. The total extract of *P. ginseng* modulates peripheral blood mononuclear cells (PBMC) and results in an elevated IL-12 production, thus inducing a stronger T helper 1 (Th1) cell response to protect other cells against infection (81). This plant product also modulates pro-inflammatory cytokine production by increasing macrophage toll-like receptor 4 expression (82). Bioactivity-guided identification has shown that the ethanol-water extract of *P. ginseng* roots significantly inhibited the transcription and secretion of CXCL-10 cells following TNF-α stimulation (83), and it targets different levels of TNF-α to exhibit an anti-inflammatory property (80, 84). The main active components have been identified as ginsenosides, primarily ginsenosides Rb1 (**31**) and Rg1 (**32**) (83).

Tripterygium wilfordii Hook.f. (Celastraceae), known as "Thunder God Vine", is a perennial woody vine and a product prepared from the water-soluble extract of the roots of this plant is one of the most commonly recognized and prescribed antirheumatic drugs in the People's Republic of China. Triptolide (**33**) and its analogs are the main active principles with antirheumatic effects from *T. wilfordii* roots (85–87). Triptolide inhibits the expression of IL-18 and its receptor to exhibit immunomodulatory activity (87).

Schisandra chinensis K. Koch (Schisandraceae), is a small woody shrub. The fruits of this plant afford a widely prescribed antihepatitis injection used in mainland China (80). This water-soluble extract of *S. chinensis* fruits can activate the xenobiotic orphan nuclear receptor, the pregnane X receptor (PXR), and induce detoxifying enzymes (88). This extract also decreases malondialdehyde concentrations in the serum, and elevates glutathione reductase activity to improve antibody titers against Newcastle disease virus and lymphocyte proliferation in broilers (89). Schisandrin C (**34**) and related compounds are the main active constituents of *S. chinensis* fruits. The anti-inflammatory properties of the schisandrins result from the inhibition of nitric oxide (NO) production, prostaglandin E_2 (PGE_2) release, and cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression (90).

Ginger, the rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae), originates from Asia, and is now consumed as a food and dietary supplement worldwide. This species has a long history of use to treat rheumatoid arthritis and other diseases (80, 91). 6-Gingerol (**35**) and its analogs are the main pungent principles of *Z. officinale* rhizomes, and suppress NO production in murine macrophages by partially inhibiting iNOS enzymatic activity and reducing iNOS protein production (92).

Glycyrrhiza uralensis Fisch. ex DC. (Leguminosae), a perennial herb and a herbal medicine used to treat infections and immune-mediated disorders, is prepared from this plant and other two species in the same genus, *Glycyrrhiza inflata* Batalin and *Glycyrrhiza glabra* L. (80). A preparation from *G. uralensis* roots and rhizomes is utilized to treat coughs in mainland China, with glycyrrhizin (**36**) and its analogs considered as the active components (80). These triterpene saponins enhance Con A-, LPS-, and OVA-induced splenocyte proliferation in OVA-immunized mice, and they

also augment OVA-specific IgG, IgG1, and IgG2b antibody titers in the serum (93). Furthermore, they inhibit nitric oxide production by downregulating iNOS, and modulate COX-2 expression in LPS-stimulated RAW264.7 macrophages, at both the mRNA and protein level (94).

Acanthopanax senticosus Harms (Araliaceae) is a small woody shrub, and a product is used to treat immune-mediated diseases. This is prepared from the water-soluble extract of the roots of this plant (80). This product is an imunomodulator, of which the major components are polysaccharides and eleutheroside I (**37**) and its analogs (80). An eleutheroside-containing extract including mainly eleutherosides B and D was reported to show cytostatic activity in a mice *in vivo* model (i.p. 18 mg/day), inducing the production of interleukin-2 and γ-interferon, and stimulating macrophagal T-cell and B-cell immunity (95). The polysaccharides of *A. senticosus* are known to inhibit transplanted tumor growth and suppress human TB propagation in experimental animals (97).

Gynostemma pentaphylla (Thunb.) Makino (Cucurbitaceae), a vine, and a popular herbal remedy used to treat immune-mediated diseases, is prepared from this plant (80). This plant is harvested from 10 cm above the ground, twice a year in June and November, cleaned, and dried. The main immunomodulatory components of *G. pentaphylla* are triterpene saponins, including gypenoside TN I (**38**) (80). These triterpene saponins enhance Con A-, LPS- and OVA-induced splenocyte proliferation in OVA-immunized mice, and enhance OVA-specific IgG, IgG1, and IgG2b antibody levels in the serum (97).

18.5 CONCLUSIONS AND PERSPECTIVES

Not only herbal remedies but also some pure phytochemicals are used as immunomodulators in TCM. For example, tetrandrine (**39**), a bisbenzylisoquinoline alkaloid isolated from *Stephania tetrandra* S. Moore (Menispermaceae), is one of the most widely prescribed antirheumatic drugs (74), which downregulates the secretion of Th1 and Th2 cytokines and NF-κB DNA-binding, inhibits MAP kinase and NF-κB transcription, and blocks IκBα degradation through the inhibition of IKKα and IKKβ (74). Artimisinin (**40**), an antimalarial drug discovered from *Artemisia annua* L. (Asteraceae), has been utilized recently to treat cancer for its immunosuppressive activity by the inhibition of the calmodulin-mediated activation of phosphodiesterase (98). Astilbin (**41**), a compound derived from the rhizomes of *Smilax glabra* Roxb. (Smilacaceae), inhibits delayed-type hypersensitivity by upregulating IL-10 (99). Epigallocatechin gallate (**42**), a major flavonoid from *Camellia sinensis* Kuntze. (Theaceae) (green tea), inhibits T cell-mediated inflammation by binding to CD11b in $CD8^+$ T cells and by activating caspases in monocytes to induce apoptosis (99). 3,4-Dihydropsoralen (**43**), a dihydrocoumarin constituent of *Psoralea corylifolia* L. (Leguminosae), is an immunomodulator with the lymphocyte potassium channel Kvl 3 being a molecular target (100).

Herbal remedies and their active constituents play a key role in the management of immunological diseases. Further study of the human immune system in the context of drug discovery should provide additional novel drug candidates to help resolve human health problems including cancer.

REFERENCES

1. Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. J Nat Prod 70:461–477
2. Chin Y-W, Balunas MJ, Chai HB, Kinghorn AD (2006) Drug discovery from natural sources. AAPS J 8:E239–E253
3. Oberlies NH, Kroll DJ (2004) Camptothecin and taxol, historic achievements in natural products research. J Nat Prod 67:129–135
4. Hartwell JL, Schrecker AW (2005) Components of podophyllin. V. The constitution of podophyllotoxin. J Am Chem Soc 73:2909–2916
5. Canel C, Moraes RM, Dayan FE, Ferreira D (2000) Molecules of interest: podophyllotoxin. Phytochemistry 54:115–120
6. Stahelin HF, von Wartburg A (1991) The chemical and biological route from podophyllotoxin glucoside to etoposide. Cancer Res 51:5–15
7. Bohlin L, Rosen B (1996) Podophyllotoxin derivatives: drug discovery and development. Drug Discov Today 1:343–351
8. Hande KR (1998) Etoposide: four decades of development of a topoisomerase II inhibitor. Eur J Cancer 34:1514–1521
9. Noble RL (1990) The discovery of the vinca alkaloids – chemotherapeutic agents against cancer. Biochem Cell Biol 68:1344–1351
10. Dancey J, Steward WP (1995) The role of vindesine in oncology – recommendations after 10 years' experience. Anticancer Drugs 6:625–636
11. Gregory RK, Smith IE (2000) Vinorelbine: a clinical review. Br J Cancer 82:1907–1913
12. Jordan MA, Wilson L (2004) Microtubules as a target for anticancer drugs. Nat Rev 4:253–265
13. Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AI, Sim GA (1966) Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88:3888–3890
14. Wall ME (1994) Wani MC Camptothecin and analogs: from discovery to clinic. In: Potmesil M, Pinedo H (eds) Camptothecins: new anticancer agents. CRC Press, Boca Raton, FL, pp 21–42
15. Hsiang Y-H, Hertzberg R, Hecht S, Liu LF (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 260:14873–14878
16. Hsiang Y-H, Lihou MG, Liu LF (1989) Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complex as a mechanism of cell killing by camptothecin. Cancer Res 49:5077–5082
17. Ma MK, McLeod HL (2003) Lessons learned from the irinotecan metabolic pathway. Curr Med Chem 10:41–49
18. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93:2325–2327
19. Kingston DGI (2009) Tubulin-interactive natural products as anticancer agents. J Nat Prod 72:507–515
20. Montero A, Fossella F, Hortobagyi G, Valero V (2005) Docetaxel for treatment of solid tumours: a systematic review of clinical data. Lancet Oncol 6:229–239
21. Huang KC (2000) History of Chinese medicine. In: The pharmacology of Chinese herbs. CRC Press, Boca Raton, FL, pp 9–17.
22. Qicheng F (1980) Some current study and research approaches relating to the use of plants in the traditional Chinese medicine. J Ethnopharmacol 2:57–63
23. Han J (1988) Traditional Chinese medicine and the search for new antineoplastic drugs. J Ethnopharmacol 24:1–17
24. Ku K-Y, Tang T-C (1980) Several botanical sources of camptothecin – an antitumor alkaloid. Zhong Cao Yao 11:476–479
25. Arisawa M, Gunasekera SP, Cordell GA, Farnsworth NR (1981) Plant anticancer agents. XXI. Constituents of *Merrilliodendron megacarpum*. Planta Med 43:404–407
26. Zhou B-N, Hoch JM, Johnson RK, Mattern MR, Eng W-K, Ma J, Hecht SM, Newman DJ, Kingston DGI (2000) Use of COMPARE analysis to discover new natural product drugs: isolation of camptothecin and 9-methoxycamptothecin from a new source. J Nat Prod 63:1273–1276

27. Lee DH, Kim S-W, Suh C, Lee J-S, Lee JH, Lee S-J, Ryoo BY, Park K, Kim JS, Heo DS, Kim NK (2008) Belotecan, new camptothecin analogue, is active in patients with small-cell lung cancer: results of a multicenter early phase II study. Ann Oncol 19:123–127
28. Shi J-H, Zhu Z-Z, Hou S, Liu S-X, Han Z-Q, Sun C-X (2007) The clinical study of HC regimen chemotherapy consisted of hydroxy camptothecin and carboplatin for the senile patients with advanced non-small cell lung cancer. Linchuang Zhongliuxue Zazhi 12:498–500
29. Wu Z-H (2007) Clinical research of HCPT in combination with LV+5Fu-DDP regimen in treatment of advanced gastric cancer. Hainan Yixue 18:18–19
30. Paudler WW, Kerley GI, McKay J (1963) Alkaloids of *Cephalotaxus drupacea* and *Cephalotaxus fortunei*. J Org Chem 28:2194–2197
31. Powell RG, Weisleder D, Smith CR Jr, Rohwedder WK (1970) Structures of harringtonine, isoharringtonine, and homoharringtonine. Tetrahedron Lett 11:815–818
32. Kantarjian HM, Talpaz M, Santini V, Murgo A, Cheson B, O'Brien SM (2001) Homoharringtonine: history, current research, and future direction. Cancer 92:1591–1605
33. Itokawa H, Wang X, Lee K-H (2005) Homoharringtonine and related compounds. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products. CRC Press, Boca Raton, FL, pp 47–70
34. Meng H, Yang C, Jin J, Zhou Y, Qian W (2008) Homoharringtonine inhibits the AKT pathway and induces in vitro and in vivo cytotoxicity in human multiple myeloma cells. Leuk Lymphoma 49:1954–1962
35. Stewart JA, Krakoff IH (1985) Homoharringtonine: a phase I evaluation. Invest New Drugs 3:279–286
36. Quintas-Cardama A, Cortes J (2008) Homoharringtonine for the treatment of chronic myelogenous leukemia. Expert Opin Pharmacother 9:1029–1037
37. Xiao Z, Hao Y, Liu B, Qian L (2002) Indirubin and meisoindigo in the treatment of chronic myelogenous leukemia in China. Leuk Lymphoma 43:1763–1768
38. Deng B (1986) Direct colorimetric method for determination of indigo and indirubin in Qingdai. Zhong Cao Yao 17:163–164
39. Du D, Ceng Q (1981) Effect of indirubin on the incorporation of isotope labeled precursors into nucleic acid and protein of tumor tissues. Zhong Cao Yao 12:406–409
40. Eisenbrand G, Hippe F, Jakobs S, Muehlbeyer S (2004) Molecular mechanisms of indirubin and its derivatives: novel anticancer molecules with their origin in traditional Chinese phytomedicine. J Cancer Res Clin Oncol 130:627–635
41. Jautelat R, Brumby T, Schaefer M, Briem H, Eisenbrand G, Schwahn S, Krueger M, Luecking U, Prien O, Siemeister G (2005) From the insoluble dye indirubin towards highly active, soluble CDK2-inhibitors. Chembiochem 6:531–540
42. Zuo M, Li Y, Wang H, Zhou J, Li H, Liu H, Liu H, Xin H, Zhang S, Chen X (2008) The antitumor activity of meisoindigo against human colorectal cancer HT-29 cells in vitro and in vivo. J Chemother 20:728–733
43. Cooperative Study Group of Phase III Clinical Trial on Meisoindigo, Tianjin, People's Republic of China (1997) Phase II clinical trial on meisoindigo in the treatment of chronic myelogenous leukemia. Zhonghua Xue Ye Xue Za Zhi 18:69–72
44. Zhang J-S, Ding J, Tang Q-M, Li M, Zhao M, Lu L-J, Chen L-J, Yuan S-T (1999) Synthesis and antitumor activity of novel diterpenequinone salvicine and the analogs. Bioorg Med Chem Lett 9:2731–2736
45. Meng LH, Ding J (2001) Induction of bulk and c-myc P2 promoter-specific DNA damage by an antitopoisomerase II agent salvicine is an early event leading to apoptosis in HL-60 cells. FEBS Lett 501:59–64
46. Lang J-Y, Chen H, Zhou J, Zhang Y-X, Zhang X-W, Li M-H, Lin L-P, Zhang J-S, Waalkes MP, Ding J (2005) Antimetastatic effect of salvicine on human breast cancer MDA-MB-435 orthotopic xenograft is closely related to Rho-dependent pathway. Clin Cancer Res 11:3455–3464
47. Cai Y, Lu J, Miao Z, Lin L, Ding J (2007) Reactive oxygen species contribute to cell killing and P-glycoprotein downregulation by salvicine in multidrug resistant K562/A02 cells. Cancer Biol Ther 6:1794–1799

48. Sun H-D, Lin Z-W, Qin C-Q, Chao J-H, Zhao Q-Z (1981) Studies on the chemical constituents of antitumor plant *Rabdosia rubescens* (Hemsl.) Hara. Yunnan Zhi Wu Yan Jiu 3:95–100
49. Fujita T, Takeda Y, Sun HD, Minami Y, Marunaka T, Takeda S, Yamada Y, Togo T (1988) Cytotoxic and antitumor activities of *Rabdosia* diterpenoids. Planta Med 54:414–417
50. Zhou G-B, Kang H, Wang L, Gao L, Liu P, Xie J, Zhang F-X, Weng X-Q, Shen Z-X, Chen J, Gu L-J, Yan M, Zhang D-E, Chen S-J, Wang Z-Y, Chen Z (2007) Oridonin, a diterpenoid extracted from medicinal herbs, targets AML1-ETO fusion protein and shows potent antitumor activity with low adverse effects on t(8;21) leukemia in vitro and in vivo. Blood 109:3441–3450
51. Zhu Y, Xie L, Chen G, Wang H, Zhang R (2007) Effects of oridonin on proliferation of HT29 human colon carcinoma cell lines both in vitro and in vivo in mice. Pharmazie 62:439–444
52. Wang RL (1993) A report of 40 cases of esophageal carcinoma surviving for more than 5 years after treatment with drugs. Zhonghua Zhong Liu Za Zhi 15:300–302
53. Marks LS, DiPaola RS, Nelson P, Chen S, Heber D, Belldegrun AS, Lowe FC, Fan J, Leaders FE Jr, Pantuck AJ, Tyler VE (2002) PC-SPES: herbal formulation for prostate cancer. Urology 60:369–375
54. Meade-Tollin LC, Wijeratne EMK, Cooper D, Guild M, Jon E, Fritz A, Zhou G-X, Whitesell L, Liang J-Y, Gunatilaka AAL (2004) Ponicidin and oridonin are responsible for the antiangiogenic activity of *Rabdosia rubescens*, a constituent of the herbal supplement PC SPES. J Nat Prod 67:2–4
55. Xia GC, Zang JY, Lu XM, Xiao PG (1985) Resource utilization and herbal study of "malinzi" (*Iris lactea* Pall. var. *chinensis*). Yao Xue Xue Bao 20:316–319
56. Li D-H, Hao X-G, Zhang S-K, Wang S-X, Liu R-Y, Ma K-S, Yu S-P, Jiang H, Guan J-F (1981) Antitumor effect and toxicity of irisquinone. Zhongguo Yao Li Xue Bao 2:131–134
57. Wang X-W (1999) Irisquinone: antineoplastic, radiosensitizer. Drugs Future 24:613–617
58. Zhu W, Sun W, Yu Y, Wang F, Wang S, Wang L (2008) The impact of radiosensitizer irisquinone on lung metastasis in H22-bearing mice. Jiang Su Yi Yao 34:176–178
59. Bai X, Cerimele F, Ushio-Fukai M, Waqas M, Campbell PM, Govindarajan B, Der CJ, Battle T, Frank DA, Ye K, Murad E, Dubiel W, Soff G, Arbiser JL (2003) Honokiol, a small molecular weight natural product, inhibits angiogenesis in vitro and tumor growth in vivo. J Biol Chem 278:35501–35507
60. Shigemura K, Arbiser JL, Sun S-Y, Zayzafoon M, Johnstone PAS, Fujisawa M, Gotoh A, Weksler B, Zhau HE, Chung LWK (2007) Honokiol, a natural plant product, inhibits the bone metastatic growth of human prostate cancer cells. Cancer 109:1279–1289
61. Wolf I, O'Kelly J, Wakimoto N, Nguyen A, Amblard F, Karlan BY, Arbiser JL, Koeffler HP (2007) Honokiol, a natural biphenyl, inhibits in vitro and in vivo growth of breast cancer through induction of apoptosis and cell cycle arrest. Int J Oncol 30:1529–1537
62. Li Z, Liu Y, Zhao X, Pan X, Yin R, Huang C, Chen L, Wei Y (2008) Honokiol, a natural therapeutic candidate, induces apoptosis and inhibits angiogenesis of ovarian tumor cells. Eur J Obstet Gynecol Reprod Biol 140:95–102
63. Jiang Q-Q, Fan L-Y, Yang G-L, Guo W-H, Hou W-L, Chen L-J, Wei Y-Q (2008) Improved therapeutic effectiveness by combining liposomal honokiol with cisplatin in lung cancer model. BMC Cancer 8:242
64. Wen J, Fu A-F, Chen L-J, Xie X-J, Yang G-L, Chen X-C, Wang Y-S, Li J, Chen P, Tang M-H, Shao XM, Lu Y, Zhao X, Wei Y-Q (2009) Liposomal honokiol inhibits VEGF-D-induced lymphangiogenesis and metastasis in xenograft tumor model. Int J Cancer 124:2709–2718
65. Battle TE, Arbiser J, Frank DA (2005) The natural product honokiol induces caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells. Blood 106:690–697
66. Ishitsuka K, Hideshima T, Hamasaki M, Raje N, Kumar S, Hideshima H, Shiraishi N, Yasui H, Roccaro AM, Richardson P, Podar K, Le Gouill S, Chauhan D, Tamura K, Arbiser J, Anderson KC (2005) Honokiol overcomes conventional drug resistance in human multiple myeloma by induction of caspase-dependent and -independent apoptosis. Blood 106:1794–1800
67. Owen T-Y, Wang S-Y, Chang S-Y, Lu FL, Hsu B (1976) A new antitumor substance – lycobetaine (AT-1840) *Kexue Tongbao* **21**, 285–287
68. Zhang S-Y, Lu F-L, Yang J-L, Wang L-J, Xu B (1981) Effect on animal tumors and toxicity of lycobetaine acetate. Zhongguo Yao Li Xue Bao 2:41–45
69. Liu J, Yang S, Xu B (1989) Characteristics of the interaction of lycobetaine with DNA. Zhongguo Yao Li Xue Bao 10:437–442

70. Liu J, Yang S, Xu B (1990) Effects of lycobetaine on chromatin structure and activity of murine hepatoma cells. Sci China B 33:1459–1465
71. Chen J, Chen K, Jiang H, Lin M, Ji R (1997) Theoretical investigation on interaction binding of analogs of AT-1840 to double-stranded polynucleotide. Prog Nat Sci 7:329–335
72. Barthelmes HU, Niederberger E, Roth T, Schulte K, Tang WC, Boege F, Fiebig H-H, Eisenbrand G, Marko D (2001) Lycobetaine acts as a selective topoisomerase II poison and inhibits the growth of human tumour cells. Br J Cancer 85:1585–1591
73. Xu B (1991) Recent advances in pharmacologic study of natural anticancer agents in China. Mem Inst Oswaldo Cruz 86:51–54
74. Ho LJ, Lai JH (2004) Chinese herbs as immunomodulators and potential disease-modifying antirheumatic drugs in autoimmune disorders. Curr Drug Metab 5:181–192
75. Eichler F, Krueger GR (1994) Effects of non-specific immunostimulants (echinacin, isoprinosine and thymus factors) on the infection and antigen expression in herpes virus-6 exposed human lymphoid cells. In Vivo 8:565–575
76. Wynn JL, Neu J, Moldawe LL, Levy O (2009) Potential of immunomodulatory agents for prevention and treatment of neonatal sepsis. J Perinatol 29:79–88
77. Broide DH (2009) Immunomodulation of allergic disease. Annu Rev Med 60:279–291
78. Sicherer SH, Sampson HA (2009) Food allergy: recent advances in pathophysiology and treatment. Annu Rev Med 60:261–277
79. Chen P, Lu YB, Lin CC (1999) Concepts and theories of Traditional Chinese Medicine advanced TCM series, advanced TCM series, vol 2. Press of Science, Beijing, pp 50–150
80. Xu GJ, He HX, Xu LS, Jing RY (1996) Chinese Materia Medica. The Press of Chinese Medical Technology, Beijing, pp 250–310, 1038, 1605
81. Larsen MW, Moser C, Hoiby N, Song ZJ, Kharazmi A (2004) Ginseng modulates the immune response by induction of interleukin-12 production. APMIS 112:369–373
82. Pannacci M, Lucini V, Colleoni F, Martucci C, Grosso S, Sacerdote P, Scaglione F (2006) *Panax ginseng* C.A. Mayer G115 modulates pro-inflammatory cytokine production in mice throughout the increase of macrophage toll-like receptor 4 expression during physical stress. Brain Behav Immun 20:546–551
83. Lee DCW, Yang CLH, Chik SCC, Li JCB, Rong JH, Chan GCF, Lau ASY (2009) Bioactivity-guided identification and cell signaling technology to delineate the immunomodulatory effects of *Panax ginseng* on human promonocytic U937 cells. J Transl Med 7:34
84. Sun WJ, Shen JF (1996) Brief handbook of naturally bioactive components. The Press of Chinese Medical Technology, Beijing, pp 200–380
85. Tang W, Yang Y, Zhang F, Li YC, Zhou R, Wang JX, Zhu YN, Li XY, Yang YF, Zuo JP (2005) Prevention of graft-versus-host disease by a novel immunosuppressant, (5*R*)-5-hydroxytriptolide (LLDT-8), through expansion of regulatory T cells. Int Immunopharmacol 5:1904–1913
86. Ma J, Dey M, Yang H, Poulev A, Pouleva R, Dorn R, Lipsky PE, Kennelly EJ, Raskin I (2007) Anti-inflammatory and immunosuppressive compounds from *Tripterygium wilfordii*. Phytochemistry 68:1172–1178
87. Lu Y, Wang WJ, Leng JH, Cheng LF, Feng L, Yao HP (2008) Inhibitory effect of triptolide on interleukin-18 and its receptor in rheumatoid arthritis synovial fibroblasts. Inflamm Res 57:260–265
88. Mu Y, Zhang JN, Zhang SM, Zhou HH, Toma D, Ren SR, Huang L, Yaramus M, Baum A, Venkataramanan R, Xie W (2006) Traditional Chinese medicines Wu Wei Zi (*Schisandra chinensis* Baill.) and Gancao (*Glycyrrhiza uralensis* Fisch.) activate pregnane X receptor and increase warfarin clearance in rats. J Pharmacol Exp Ther 316:1369–1377
89. Ma DY, Li QD, Du J, Liu YQ, Liu SW, Shan AS (2006) Influence of mannan oligosaccharide, *Ligustrum lucidum* and *Schisandra chinensis* on parameters of antioxidative and immunological status of broilers. Arch Anim Nutr 60:467–476
90. Guo LY, Hung TM, Bae KH, Shin EM, Zhou HY, Hong YN, Kang SS, Kim HP, Kim YS (2008) Anti-inflammatory effects of schisandrin isolated from the fruit of *Schisandra chinensis* Baill. Eur J Pharmacol 591:293–299
91. Srivastava KC, Mustafa TG (1989) Ginger (*Zingiber officinale*) and rheumatic disorders. Med Hypotheses 29:25–28

92. Aktan F, Henness S, Tran VH, Duke CC, Roufogalis BD, Ammit AJ (2006) Gingerol metabolite and a synthetic analogue capsarol inhibit macrophage NF-κB-mediated iNOS gene expression and enzyme activity. Planta Med 72:727–734
93. Sun HX, Pan HJ (2006) Immunological adjuvant effect of *Glycyrrhiza uralensis* saponins on the immune responses to ovalbumin in mice. Vaccine 24:1914–1920
94. Shin EM, Zhou HY, Guo LY, Kim JA, Lee SH, Merfort I, Kang SS, Kim HS, Kim S, Kim YS (2008) Anti-inflammatory effects of glycyrol isolated from *Glycyrrhiza uralensis* in LPS-stimulated RAW264.7 macrophages. Int Immunopharmacol 8:1524–1532
95. Barenboim GM, Sterlina AG, Bebyakova NV, Ribokas AA, Fuks BB (1986) Investigation of the pharmacokinetics and mechanism of action of *Eleutherococcus* glycosides. VIII. Investigation of natural killer activation by the *Eleutherococcus* extract. Khim Farm Zh 20:914–917
96. Shen ML, Zhai SK, Chen HL, Luo YD, Tu GR, Ou DW (1991) Immunopharmacological effects of polysaccharides from *Acanthopanax senticosus* on experimental animals. Int J Immunopharmacol 13:549–554
97. Sun HX, Zheng QF (2005) Haemolytic activities and adjuvant effect of *Gynostemma pentaphyllum* saponins on the immune responses to ovalbumin in mice. Phytother Res 19:895–900
98. Noori S, Hassan ZM, Rezaei B, Rustaiyan A, Habibi Z, Fallahian F (2008) Artemisinin can inhibit the calmodulin-mediated activation of phosphodiesterase in comparison with cyclosporin A. Int Immunopharmacol 8:1744–1747
99. Chinen J, Finkelman F, Shearer T (2006) Advances in basic and clinical immunology. J Allergy Clin Immunol 118:489–495
100. Vennekamp J, Wulff H, Beeton C, Calabresi PA, Grissmer S, Hänsel W, Chandy KG (2004) Kvl 3-blocking 5-phenylalkoxypsoralens, a new class of immunomodulators. Mol Pharmacol 65:1364–1374

19 The Immunological Modulation of Fuzheng TCM Herbs in Cancer Treatment

Hongsheng Lin, Jie Li, and Ying Zhang

Key Points

- Reinforcing healthy Qi and replenishing archaeus is an important principle of immunoregulation therapy in cancer treatment by TCM.
- Immune regulation of single Chinese herbs of cancer patients is discussed.
- The immunomodulatory effects of FZ Chinese Patent Medicines in cancer treatment is included.
- The immunomodulatory effects of other TCM compound prescriptions are also included.

Key Words: Traditional Chinese Medicine (TCM), immune regulation, cancer.

19.1 INTRODUCTION

Traditional Chinese Medicine (TCM) is the important feature of cancer treatment in China. Especially in the last 10 years, the effect of TCM in cancer treatment has been of high concern and has been accepted by experts and patients internal and overseas. On the basis of the review of the clinical practices and basic researches these years, it has been found that immunoregulation is the most remarkable advantage and characteristic of TCM in cancer treatment, especially Fuzheng (FZ) TCM medicines (the function of this medicine is Reinforcing healthy Qi and replenishing archaeus). The body's immune function is concerned with the occurrence and development of tumor; therefore, it is the key research point in recent years. From the widely used immune function index T-lymphocyte subpopulation and NK cells to recently research hotspot dentritic cells (DC), regulatory T cell (Treg cells), Th17 cells as well as the latest index myeloid-derived suppressor cells (MDSC) in the 2 years, the progressions not only establish the

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_19

recognition of the relationship between cancer and immune function, but also add some new scientific meaning to the immunoregulation effect of TCM in cancer treatment.

19.2 REINFORCING HEALTHY QI AND REPLENISHING ARCHAEUS IS AN IMPORTANT PRINCIPLE OF IMMUNOREGULATION THERAPY IN CANCER TREATMENT BY TCM

In TCM, the occurrence of tumor is concerned with weakness, poison and blood stasis, Accordingly, there are therapeutic principles and methods like invigorating spleen and replenishing Qi, activating blood and resolving stasis, cleaning heat and removing toxin, among others. It has been proved by a number of clinical practices that reinforcing healthy Qi and replenishing archaeus is the vital principle of cancer therapy and can be applied in the whole treatment process, the main mechanism is through immunoregulation effect on tumor-bearing body.

Different from the western medicine therapies like operation, radiotherapy, and chemotherapy, TCM is based on the holism and treats cancer by methods like tonifying Qi, blood, yin and yang of the Zangfu, and adjusting the imbalance of the body, thereby reestablishing harmony, improving constitution condition and disease resistance of patients. The feature of TCM is not to clear the tumor directly but improve the life quality and extend the survival time with premise of tumor stabile. The principle of reinforcing healthy Qi and replenishing archaeus is the best one to represent this feature, so it has been used in cancer treatment most widely.

The method includes two aspects which make it different from other supportive therapies. One is "strengthening" to reinforce healthy Qi, the other is "regulating" to harmonize Qi, blood, yin and yang to balance. The ultimate purpose is to recover the status that Yin is at peace and Yang is sound. The principle of reinforcing healthy Qi and replenishing archaeus has profound connotations and includes many different methods.

19.2.1 Common Therapeutic Methods

19.2.1.1 Invigorating Spleen and Replenishing Qi

Because the patients with malignant cancer always present symptoms of deficiency on the whole and excess at the part, therefore, treatment by TCM is based on the holistic concept with the principles of harmonizing spleen and stomach to establish the middle Qi. This method may not be a rapid effective therapy, but it can reinforce healthy Qi gradually and always prolong the survival time for patients with tumor. Especially for patients in advanced stages, it is vital to protect stomach Qi carefully. The common used herbs include *Radix Pseudostellatiae, Rhizoma Atractylodis Macrocephalae, Poria, Radix Astragali seu Hedysari, Pericarpium Citri Reticulatae, Semen Coicis*, et al., which are ingredients of formulas Four Gentlemen decoction and Center-Supplementing and Qi-Boosting Decoction. It has been proved by clinical practices that these formulas have good effects on improving the quality of life and prolonging survival time. The results of pharmacology research showed such formulas can improve immune function significantly as well as kill the cancer cells directly or indirectly.

19.2.1.2 Nourishing Yin and Promoting Fluid Production

This method is mainly suitable for the patients whose yin fluid has been ruined after radiotherapy or chemotherapy, or the patients in late stage who present tumor toxin exuberance. The syndromes could be dry mouth or thirst without desire to drink, constipation with blood-stranguria and insomnia. Frequently used herbs include: *Radix Rehmanniae, Radix Glehniae, Radix Ophiopogonis, Herba Dendrobii, Rhizoma Polygonati Odorati, Rhizoma Polygonati, Radix Scophulariae, Rhizoma Dioscoreae, Fructus Lycii, Radix Trichosanthis, Radix Rehmanniae Praeparata, Rhizoma anemarrhenae, Carapax Trionycis, Fructus Schisandrae* and so on. Modern research has found that immunodeficiency maybe the essence of Yin deficiency syndromes. The above herbs could prolong the lifetime of an immune antibody, adjust sympathetic nervous system and endocrine system, relieve hypermetabolism, maintain the equilibrium, promote phagocytic power of monocyte and proliferation of myeloid cells, and reduce proteolysis. Over-nourishing herbs should be avoided or accompanied by herbs with the function of invigorating spleen and regulating Qi, especially for the patients who have symptoms like weakness of the spleen and stomach, the stagnation of phlegm-damp, abdominal distention with loose stool.

19.2.1.3 Tonifying Kidney and Warming Yang

On the basis of the TCM theory, the kidney is the innate foundation, governing the bone marrow and yang Qi of the whole body. This opinion is in coincidence with the results from immunology and endocrinology research. The occurrence and development of cancer is intimately concerned with immunodeficiency caused by kidney deficiency. Herbs warming and tonifying the kidney can activate the immune system and excite the pituitary-adrenal cortex system, and therefore restrain the occurrence and development of cancer.

This method is mainly applicable to patients in late stage, especially for old patients or women who have castration operation because of mastocarcinoma with symptoms like cold body and limb, mental fatigue and lack of strength, sore waist and lumbago, frequent and clear urination, loose stools. Commonly used herbs include *Fructus Psoraleae, Herba Cistanchis, Herba Epimedii, Rhizoma Curculiginis, Radix Morindae Offcinalis, Radix Aconiti Lateralis Praeparata, Cordyceps, Cortex Eucommiae, Radix Dipsaci*, Kidney Qi Pills and Right-restoring Pill. Modern pharmacological researches have proved that herbs tonifying kidney yang can adjust the immune system, improve phagocytic function of phagocyte, enhance the synthesis of protein and nucleic acid, regulate metabolism of cells, improve formation of anti-body and increase the level of corticosterone and estradiol in blood. Moreover, it can increase the level of cAMP in rat cell, which may cure yang deficiency, promote lymphocyte transformation, recover marrow haemopoiesis and have antitumor effects. Application with this method should avoid the state of warm-dryness. One should be very cautious when using it on patients with yin deficiency syndromes or accompanied by other herbs in the formula, in order to avoid inducing heat in the body and damaging yin.

19.2.1.4 Replenishing Qi and Nourishing Blood

Because of the presence of a malignant tumor and the side effects of radiotherapy and chemotherapy, patients always present hemogram restrain, accompanied by symptoms like dizziness and tinnitus, palpitation and shortness of breath, fatigue and lack of strength, shallow yellow complexion, pale tongue with thin coating, thready and weak pulse and so on. Ordinarily used herbs: *Radix Astragali seu Hedysari, Radix Angelicae Sinensis, Radix Paeoniae Alba, Radix Polygoni Multiflori, Radix Rehmanniae*

Praeparata, Arillus Longan, Ziziphus Jujube, Caulis Spatholobi, Placenta Hominis, Fructus Lycii, Chinese Angelica Blood-Supplementing Decoction and four Gentlemen Decoction. Modern pharmacological researches have proved that herbs replenishing Qi and nourishing blood could elevate hemogram, improve recovering haemopoiesis, surmounting the unsteady hemogram due to hematopoietic treatment.

19.3 THE REVIEW OF THE IMMUNE REGULATION OF FZ CHINESE MEDICINE IN CANCER TREATMENT

19.3.1 Regulation of Single Chinese Herb on the Immune Function of Cancer Patients

19.3.1.1 Radix Ginseng

Radix Ginseng is a perennial herb from the Araliaceae family with the main active ingredient of ginsenosides and ginseng polysaccharides. Studies have shown that it has a strong activity in cancer prevention and treatment. With the development of studies, the antitumor effect of *Radix Ginseng* and its mechanism are found to be extensive, but the most important ones are immune function regulation, body resistance strengthening, and thereby tumor growth inhibition.

Cellular Immunity. Ginsenosides and ginseng polysaccharides have an effect on the regulation of specific cellular immunity. For example, Kim et al. (1) found that *Radix Ginseng* could improve the phagocytic function of macrophages, induce the expression of interleukin-2 (IL-2), interferon-γ (IFN-γ) mRNA, and increase the activity of lymphokine-activated killer (LAK), NK, and CTL cells. Liu (2) demonstrated that Ginsenosides induced the release of IL-2 with the mechanism of having an effect on the body microenvironment of cAMP and/or cGMP. Wu et al. (3) observed that Rg3 increased the percentage of $CD4^+$ cells of significantly decreased lymphocytes of intestinal mucosal lamina propria, and had no significant effect on the percentage of $CD8^+$ cells on tumor-bearing mice with cyclophosphamide. Ginsenoside Rg3 was thought to obviously improve the intestinal mucosal immune function suppressed by tumor-bearing and cyclophosphamide.

Humoral Immunity. Ginseng polysaccharides can enhance the macrophage function of mice, accelerate antibody production, and improve the function of body immune surveillance. Ginsenoside significantly increased the number of plague-forming cells induced by spleen cells and antibody-formed cells, and improved first antibody responses after antigen immune with low dosages. Extensive researches showed that Ginsenosides and ginseng polysaccharides increased the components of body serum complements and the lysozyme level.

Dendritic Cells. DC, the most powerful professional antigen-presenting cells (APC) at present, play an important role in the suppression of tumorigenesis and tumor development. Recent studies showed that ginsenosides Rg1 and Rh1 had an effect on the function of the proliferation of T cell stimulated by dendritic cells and the antitumor activity of LPAK in the peripheral blood of a normal person (4). Wang et al. studied the effect of ginsenoside Rg1 on the function of the proliferation of T cell stimulated by

dendritic cells and antitumor activity of LPAK activated by PHA and IL-2, and found that Rg1 could promote the ability of DC on stimulating the proliferation of T cells and cytotoxicity of DC-LPAK (4).

19.3.1.2 Radix Astragali

Radix Astragali, first recorded in *Shennong's Herbal,* which is sweet tasting, slightly warm in nature, and relating to spleen and lung meridians, has effects on invigorating spleen and strengthening the middle warmer, raising spleen-Qi and yang, strengthening defensive and superficial, inducing diuresis, expelling toxin, and promoting tissue regeneration. Modern researches show that *Radix Astragali* contains a variety of glycosides, polysaccharides, flavonoids, amino acids, trace elements, and other substances with pharmacological anti-tumor, immune regulation, anti-virus, anti-aging, antioxidant, anti-radiation, and anti-stress effects among others. In recent years, there have been more researches about the mechanism of anti-tumor immunity on astragalus polysaccharides. Present results show that astragalus polysaccharides have an antitumor effect by regulating the body immune system: (1) regulating body cellular immune function; (2) increasing the secretion of anticancer cytokines. For example, Shan et al. (5) identified that APS for injection significantly improved chemotherapy and induced the decrease of CD4, CD8, ratios of CD4/CD8, and NK cells. Zheng et al. (6) discovered that *Radix Astragali* could regulate lung cancer cell lines and PBMCs Th1/Th2 immune function of lung cancer patients, and reverse immune state to Th1. Dong et al. (7) found that Huangqi injection promoted the immune response of tumor-bearing hosts and enhanced anti-tumor metastases of dendritic cells and the activity of immunocytes. Cho et al. (8) studied that AI from *Radix Astragali* could induce the activity of lymphokine-activated killer cells cultured with spleen cells. Xu et al. (9) investigated that the Huangqi granule could strengthen both nonspecific and specific immunity of mice with low immune function induced by cyclophosphamide. Wang et al. (10) found that the extract *Radix Astragali* could promote the proliferation of peripheral blood mononuclear cells, enhance the killing activity of tumor cells by CTL, improve the function of tumor cell phagocytosis and cytokine production, and promote peripheral blood B cell induced IgG.

19.3.1.3 Cordyceps

Cordyceps has been a TCM. *Radix Ginseng*, *Cornu Cervi Pantotrichum* and *Cordyceps* are known as three major tonics in China. The muscardine cadaver part of Cordyceps is named as worm, and the stroma of head part is named as grass, which explains the origin of the name. Chinese caterpillar fungus is known as a medicine of treating and preventing disease, and enhancing the health with a history of more than 1,200 years. It is sweet and warm in nature, relates to lung and kidney meridians with the effects of reinforcing kidney to replenish essence, enriching yin and boosting Qi. Clinical and experimental studies showed that Chinese caterpillar fungus played an important role in antitumor effect, which was mainly presented by improving immune function of multiple malignant tumors, enhancing antigen–antibody response, and reinforcing immune surveillance and killing the cleaning effects of mutant cells. It has a significant role in preventing carcinogenesis. As to formed cancer cells, *Cordyceps* increased the killing and phagocytosis function of NK cells, LAK cells, and mononuclear phagocytes, and prevented the growth and metastasis of tumor cells (11–13). Qiu et al. (14) found that the eous extract of

Cordyceps could improve the function of NK cells in peripheral blood. Zhang et al. (15) proved that Chinese caterpillar fungus had immune regulation, anti-virus, anti-tumor effects through the TNF-α production by stimulation. Lin et al. studied that polysaccharides from *Cordyceps* induced the TNF-α production of mice peritoneal macrophage *in vitro* (16). *Cordyceps* extracts could increase the expression of major histocompatibility complex class II MHC and has a function of immune surveillance on tumor cells with decreased MHC-II antigen expression (17).

19.3.1.4 Lentinan

Lentinus edodes belongs to oyster mushroom of Basidiomycotina and Bas' diomycetes, fungus family. Traditional Chinese medical theory considers it sweet in flavor, and neutral in nature; its effects include supplementing the spleen and Qi, strengthening healthy Qi to eliminate pathogens, harmonizing yin and yang, and it is used as both food and medicine. Modern pharmacological studies detected that *L. edodes* could decrease cholesterol, soften blood vessels, strengthen body immunity, prevent and treat disease, and so on. In the aspect of treating cancer, it activated anti-tumor immune response of host to control and kill tumor cells by specific activation and enhancing body immune function. It is considered as an ancillary drug with the combination of dietotherapy, operation, chemotherapy, radiotherapy, and other conventional therapies. Lentinan has anti-tumor effect because of the CTL, M Φ, NK, ADCC, LAK cells. Wang et al. (18) demonstrated that lentinan could significantly increase the phagocytosis function of peritoneal macrophages of S180-bearing mice, and improve the transformation function of S180-bearing mice and the activity of NK cells. Li et al. (19) studied that lentinan had an effect of increasing killing activity of TIL and secretion of TNF-α, INF-γ. Rong et al. (20) confirmed that lentinan played an antitumor role by regulating immune organ function (increasing immune organ weight), improved the immune function of dendritic cells of tumor-bearing mice, and decreased the proliferation activity of tumor cells.

19.3.1.5 Ganoderma Lucidum

Ganoderma Lucidum belongs to Bas'diomycetes, and polypore, family fungus. *Ganoderma Lucidum* applied as a medicine has been more than 2,000 years of history. It is generally agreed that *Ganoderma Lucidum* has effects of supporting the healthy energy, strengthening with tonics, treating many disease. Modern medical researches show that *Ganoderma Lucidum* and its extracts have many pharmacological functions, such as: anti-tumor, strengthening immune functions, lowering blood glucose, protecting liver, anti-aging, anti-inflammatory, anticoagulated blood, and so on. It was reported that *Ganoderma Lucidum* polysaccharides were the basis of anti-tumor and immune regulation (21). Lin (22) found that the serum of mice treated with *Ganoderma Lucidum* polysaccharides contained redundant IL-2, TNF-α, and r-IFN, which demonstrated that *Ganoderma Lucidum* polysaccharides had an effect of suppressing tumor growth by regulating tumor immune factors. Chen et al. (23) found that *Ganoderma Lucidum* polysaccharides significantly improved the immune function of tumor patients through the elevation of the number of T4 lymphocytes. Zhou et al. discovered that *Ganoderma Lucidum* polysaccharides could distinctly stimulate the activity of NK cells and increase the production of IL-2 on Lewis-bearing mice (24). *Ganoderma Lucidum* polysaccharides were identified to elevate the activity of NK cells and TNF-α, IL-2 level in serum evidently on tumor-bearing mice (25).

19.3.1.6 Tremella Fuciformis Berk

Tremella Fuciformis Berk is a fungus which belongs to Bas'diomycetes, family tremella. *Tremella Fuciformis Berk* is known as the gracious tonic and an important herb with a long history. Pharmacological researches showed that Tremella polysaccharides (TP) had extensive physiological activities, such as immune strengthening (26), antitumor (27), anti-radiation (28), anti-aging (29) effects, etc.

TP and tremella spore polysaccharides (TSP) could overall improve body immunity by strengthening the functions of the mononuclear phagocytic system, reinforcing humoral immunity and cellular immunity, and elevating the immune organ weight (30). In recent years, the mechanism of immunological enhancement has been further studied. Mali (31) identified that TP could promote the production of IL-2 on spleen cells of both normal and senior mice, relieve the suppression of IL-2 production on mice spleen cells induced by hydrocortisone and cyclosporin A. Lin (32) discovered that *Tremella Fuciformis Berk* solution improved proliferative responses of T lymphocytes and the activity of IL-2. Xu et al. (33) found that TSP improved the killing activity of spleen NK cells and transformation efficiency of lymphocytes on tumor-bearing mice, obviously elevated the weight of normal mice spleen and thymic and phagocytosis effects of the endothelial system. It is believed that TSP has certain antagonism on transplantation tumor, and the anti-tumor effect relates to body immune enhancement.

19.3.1.7 Poria

Poria is from the drying sclerate of Polyporaceae and eumycete poria and a traditional herb with effects of promoting diureses and anchoring mind. *Poria* polysaccharides are one of the main effective components of *Poria*. Since *Poria* polysaccharides were discovered to have the activity of anti-tumor, many kinds of *Poria* polysaccharides modified by chemicals have been used in the application of tumor treatment. The antitumor effect of *Poria* polysaccharide is one of the hot spots in the anti-tumor research of polysaccharide (34). The mechanism of the anti-tumor effect of *Poria* polysaccharide has two aspects: one is direct cytotoxicity, another is to stimulate immune surveillance by strengthening body immunity and then suppress tumor growth. Immune enhancement of *Poria* polysaccharide is implemented in two ways: cellular immunity and humoral immunity. In humoral immunity, *Poria* polysaccharide elevated IgG level; while in the cellular immunity, *Poria* polysaccharide activated Mφ, strengthened phagocytosis of phagocytes, stimulated T cell transformation, and induced the production of cytokines such as IL-1, IL-2 and so on (35). Chen (36) confirmed that carboxymethylpachymaran (CMP) obviously strengthened phagocytosis of peritoneal macfophage on tumor-bearing mice, increased the number of spleen PFC and SRFC, enhanced bovine serum albumin (BSA), induced delayed type hypersensitivity (DTH), and promoted the growth of T cell growth factor (TCGF) on mice, which is one of the mechanisms of enhancing immunologic responses and suppressing tumor ratios.

There are many herbal tonics with antitumor effects that regulate the body immune functions. Chinese date, is one such common herbal tonic with functions of invigorating the spleen and supplementing Qi, nourishing the blood and calming the mind, giving astringents, and strengthening with tonics. Lang et al. (37) discovered that crude polycose of *Fructrs Jujubae* had effects of anti-complementary activity and promoting the proliferation of mice spleen cells. Polysaccharide from *Fructrs Jujubae* (purification

polysaccharide, TDP-N) activated macrophage and promoted the cytotoxicity to induce the secretion of IL-1, tumor necrosis factor (TNF), nitrogen monoxidum (NO) (38). *Radix Notoginseng* belongs to Araliaceae family. Recent studies showed that saponins from *Radix Notoginseng* had anti-tumor mechanisms by directly suppressing tumor cell growth and metastasis, inducing apoptosis and differentiation of tumor cells, enhancing and stimulating the body immunity (39). Li et al. (40) detected that the effective anti-tumor components of *Radix Notoginseng* were saponin Rh2 and Rg3 from *Radix Notoginseng*, which markedly elevated the TNF-α, IL-2 level in peripheral blood and the immune organ weight of tumor-bearing mice. In conclusion, single Chinese herbal tonics have great research potentials and clinical application value in the immune regulation of cancer treatment.

19.3.2 The Immunomodulatory Effects of FZ Chinese Patent Medicines in Cancer Treatment

More and more attention has been focused in cancer treatment with TCM. Its characteristic clinical efficiency is gaining more approval in modern medicine and patients. So far, there are plenty of Chinese Patent Medicines being applied in the anticancer practice. They are beneficial to enrich the methods and increase the clinical efficiency of TCM therapy. Here I would like to introduce the immunomodulatory effects of some FZ Chinese Patent Medicines.

19.3.2.1 ZhenQiFuZheng Capsule (Granula, Injection)

ZhenQiFuZheng Capsule (Granula, Injection) is composed of *Radix Astragali, Fructus Ligustri Lucidi,* etc. Animal experiments show that *Radix Astragali* has the ability to strengthen immune function; it can significantly increase the quantity of white blood cells (WBC) and polycyte cells in peripheral blood of patients. *Fructus Ligustri Lucidi* has the ability to increase the quantity of WBC, especially approve the leukopenia induced by chemotherapy and radiotherapy (41).Otherwise, *Radix Astragali* can promote cellular immune function of organisms, and decrease or eliminate the activity of suppressor T cells (42). So, ZhenQiFuZheng Capsule is suitable to the cancer patients who possess obvious consumptive symptoms, especially the patients with hemogram and immune function descending after chemotherapy and radiotherapy. Liu et al. (43) found that ZhenQiFuZheng injection could significantly promote the activity of anti-tumor efficiency of CTX on H22 bearing mice, enhance the thymus index number and spleen index number, prevent leukopenia induced by chemotherapy and radiotherapy. This indicates that ZhenQiFuZheng can not only promote the activity of anti-tumor efficiency of CTX, but also regulate the immune function both in cytoimmunity and humoral immunity function. He et al. (44) verified that ZhenQiFuZheng Oral-liquid can significantly improve the immune-suppression induced by chemotherapy. Indicate that ZhenQiFuZheng Oral-liquid can assist chemotherapy and radiotherapy by strengthening immune function.

19.3.2.2 ShenQiFuZheng Injection

ShenQiFuZheng injection is composed of *Radix Codonopsis and Radix Astragali,* which are traditional Chinese herbs of reinforcing Qi. The main function of this injection is nourishing the Qi to invigorate spleen. Modern researches show that *Radix Codonopsis* can increase the quantity of RBC, Hemoglobin (HB), and WBC, therefore

improve the immune function. *Radix Astragali* has the ability to strengthen immune function, including activated T cells and NK cells, etc. So the injection of the extracts of these two herbs should have the ability to improve the immune function. For example, abdominal cavity heat irrigated chemotherapy combined with ShenQiFuZheng injection can protect Myeloid Hematopoiesis, shorten the time of myelosuppression, and, at the same time, enhance the immune function of lymphocyte and the activity of anti-tumor ability (45). Chen et al. (46) found that a ShenQiFuZheng injection can increase the number of Ag-NORs, this may be the possible mechanism of ShenQiFuZheng injection activating T lymphocyte. Liu et al. (47) treated patients who were suffering from hematological system cancer with ShenQiFuZheng injection 14 day to. They found that the numbers of RBC-C3bRR and TRR were all increased, and RBC-ECRs were decreased significantly. It indicates that ShenQiFuZheng injection can also improve the immune function of RBC.

19.3.2.3 AiDi injection

AiDi injection is composed of *Ginseng, Radix Astragali, Radix Acanthopanacis Senticosi, and Mylabris*. The main effects of AiDi injection are strengthening the healthy Qi to eliminate pathogens, anti-tumor and immunological regulation. It is a common used Chinese Patent Medicine in clinical practice. Tang et al. (48) found that AiDi injection peritoneal injected can significantly improve the immune function of tumor bearing mice, and indicate that the antagonistic effect of IL-2, TNF-α produce decreasing was the important mechanism. Hou et al. (49) indicated that the AiDi injection could not only promote the Cellular immune function, but also humoral immune function; the evidence is that it could increase the activity of NK cells, the expression of IL-2R, and decrease the expression of sIL-2R and TNF-α. Liu et al. (50) observed the change in NK cells and T cell of colorectal cancer patient who were treated with the AiDi injection. And they found that the activity of NK cells, the number of $CD3^+$ T cells , $CD4^+$ T cells, and the value of $CD4^+/CD8^+$ were all increased compared to the patients who did not receive AiDi injection therapy. This is a great evidence to prove that AiDi injection can enhance the cellular immune function. Chen (51) considered that AiDi injection can also improve the RBC immune function. He confirmed his theory finally by doing some experiments.

19.3.2.4 Liuwei Dihuang Boluses

The formula of Liuwei Dihuang Boluses was formed in Song dynasty. It is composed of *prepared rhizome of rehmannia, Fructus Corni, Rhizoma Dioscoreae, the root bark of the peony tree, Poria, and Rhizoma Alismatis*. It is a famous formula of invigorating the kidney. Clinical practice proves that Liuwei Dihuang Boluses is an Immune Adjusting Reagent. For example, Jiang et al. (52) observed the nitrosamine-induced tumor model mice which were treated with this formula, and found that the function of mononuclear phagocyte system was strengthened remarkably, and the proliferation of the marrow stem cells and lymphocyte tissue were be activated too. The results were that the tumorigenesis decreased significantly. Li (53) found that this formula can counteract the following immune changes of the mice induced by CTX, such as the weight ease of thymus and spleen, the decrease of serological specific antibody and lymphocyte transformation. Xu et al. (54) reported that this formula protected the activity of NK cell and the lymphocyte transformation of T cells and B cells during chemotherapy.

Gong et al. (55) found that this formula can enhance the ADCC function of peritoneal macrophage cells of normal and Yin asthenia rats. This may be one mechanism of the immune function strengthening the effect of Liuwei Dihuang Boluses.

19.3.2.5 Kanglaite Injection

Coicis semen is the seed of adlay, it is a common TCM herb. Its main function is invigorating the spleen and damp elimination, removing heat to eliminate pura. It is applied in most anticancer TCM formulas. Now the extracts of coicis semen have been made into Kanglaite (KLT) injection, and KLT injection is one of the generally used medicines in cancer therapy of China. The pharmaco-activities of KLT injection are all focused on the anti-tumor and immunological regulation function. For example, the research of Yonsei University of Korea (56) indicated that the immune function of mesenterium was significantly enhanced when coicis semen was added in rat food. The research of Japan (57) found that after coicis semen treatment, the percent of $CD3^+$, $CD6^+$, $CD16^+$, and $CD57^+$ T cell were increased, indicating that coicis semen could improve the immune function by increasing the quantity of cytotoxic lymphocyte. The researchers of Shanghai (58) found that the oral preparation of KLT could promote the proliferation of lymphocyte, activity of NK cells, and the secretion of IL-2 in spleen of mice. Hou et al. (59) found that KLT can enhance the quantity of NK cells of peripheral Blood. Zhang et al. (60) reported that at the same time of killing tumor cells, KLT could protect immune organs and the their immune function too. Wu et al. (61) reported that KLT could make Tumor Infiltrative Cells (TIL) survive for a long time *in vitro* (>17 days), and the activity of proliferated TIL cells were significantly amplified just like the effect of recombined IL-2 (rIL-2).

Chinese Patent Medicines have more advantages in antitumor and preventing tumor. So the research and development of Chinese Patent Medicines have become a hot research subject in the world. Even though the anti-tumor mechanisms of many herbs and their extracts are still unknown, the immunological regulation is an undoubted pathway. Along with the development of immunology, molecular biology, TCM pharmacology and the extract technique of Chinese herbs, the FZ Chinese Patent Medicine would play a great role in the immunological regulation of cancer therapy.

19.3.3 The Immunomodulatory Effects of Other TCM Compound Prescriptions

Besides former mentioned single herbs and Chinese patent medicines, there are plenty of effective FZ formulas which gradually formed anti-tumor progressing in the past 50 years. And these formulas are all efficient in regulating the immune function of tumor-bearing organism, both in basic and clinical research.

19.3.3.1 The immunomodulatory Effects in Immune Cells and Cytokines

T Lymphocyte. T cell-mediated immune reaction is important in the immune response of Strong antigenicity tumor cells. And activated T cells can secrete many cytokines to kill tumor cells. Li (62) reported that when 55 case patients after radical resection were treated with adjuvant chemotherapy plus TCM Compound Prescriptions (consisting of *Radix Astragali, Radix Codonopsis, Radix Pseudostellariae, Polyporus, Poria, Fructus*

Ligustri Lucidi, Fructus Lycii, Semen Coicis, Radix Polygoni Multiflori), the results showed that immune function (CD3, CD4, CD4/CD8) were enhanced significantly compared with the group of adjuvant chemotherapy alone. Zhang et al. (63) tested $CD28^+$ T cells, and the expression of IL-2, TNF-γ of the patients who received BaoYuanDan treatment. And they found that compared with control group, BaoYuanDan group could promote the proliferation of T cells, increase the percent of $CD28^+$ cell in T cells, and raise the expression of IL-2 and TNF in the peripheral blood of patients.

NK Cells and Immune Cytokine. NK cell is a subpopulation of lymphocyte. There are almost 5–10% NK cells in peripheral blood. Its nonactivated style can kill some tumor cell efficiency. When it was been activated by some cytokine, its anti-tumor efficiency and anti tumor spectrum would be increased significantly. Li et al. (64) found that NK cells in peripheral blood of lung-Qi insufficiency Nonsmall Cell Lung Cancer (NSCLC) patients and lung-Yin insufficiency NSCLC patients were lower than normal persons. But after treatment with the Chinese herb compound – ZhengDeKang (consisting of *Radix Astragali, root of straight ladybell, Fructus Ligustri Lucidi*) , not only NK cells but IL-2, CD3, CD4, CD4/CD8 were all increased (65). There were reports (66) that Chinese Herb FZ Mixtura – another Chinese herb compound (consisting of *Radix Astragali, Radix Angelicae Sinensis, Rhizoma Polygonati, Fructus Ligustri Lucidi, Radix Codonopsis*) could influence the contents of IL-2, TNF-α, IL-6 in tumor tissue and peripheral blood, the result was IL-6 decreasing, TNF-α and IL-2 increasing. The same results were observed in the patients treated with empirical FZ formula (consisting of Radix Notoginseng, Radix Glycytthizae, Radix Astragali, Radix Scutellariae, Radix Salviae Miltiorrhiae, Radix Angelicae Sinensis) (67).

Macrophage Cells. Macrophage cells are the important constituent cells of innate immune system; it is an important APC too. So it plays an important role in inducing and regulating the Antigen-Specific Immune Response. Some FZ TCM Compound Prescriptions can improve the function of macrophagocyte. For example, No. 2 Capsule of FZ and Anti-Tumor (consisting of Radix Astragali, Radix Pseudostellariae, Fructus Corni, Rhizoma Atractylodis Macrocephalae, Radix Angelicae Sinensis, Herba epimedii, Fructus Psoraleae, medlar, Gold Theragran, Fructus Mori, and Poria) can increase the immune organs weight (thymus and spleen), improve the cytophagic index of Macrophage (68). Li et al. (69) found that YiQiBuShen formula (consisting of JinKuiShenQi formula plus *Radix Astragali and Ginseng*) can significantly increase the activation of macrophagocyte and the expression of IL-12 in spleen cells of tumor bearing hypoimmunity mice induced with cyclophosphamide (CTX). Wang et al. (70) considered that ZhenQi Casein Compound peptide could promote the expression of antigen LA on the macrophagocyte, and then activated the immune response, strengthening the ADCC effect of macrophage cells. Bi et al. (71) applied the purified liquid of Liver Cancer No. 1 formula (consisting of *Poria, Radix Astragali, Rhizoma Atractylodis Macrocephalae, Herba Artemisiae Scopariae*) to treat Wistar rats, and found that the tumor control rate of macrophage cells in treated rats were increased in a dose-dependent manner. And at the same time the mRNA level of TNF-α, NF-kB and iNOS were increased too. So they thought that Liver Cancer No. 1 formula may be increasing the killing ability of macrophage by inducing the expression of TNF-α, NF-kB and iNOS.

Tumor Infiltrative Cells and Lymphokine-Activated Killer Cells. Reome et al. (72) found that CD44 expression increased in most of CD4 and CD8 TIL cells, and these cells also express the cell-activated marker CD25, CD69, and CD95, etc. It indicates that TIL cells play an important role in anti-tumor immune response. And TIL cells can make LAK cells re-activated by secreting IL-6 against TGF-1 which is secreted by tumor (73). TCM Compound Prescriptions can also regulate the function of TIL cells and LAK cells. Huang et al. (74) found that YiQiPingXuanYin can significantly increase the anti-autologous tumor cells (ATC) ability of TIL cells. Zhang et al. (75) found that No. 1 and No. 2 of TiaoHeng formulas could enhance the activity of LAK cells and the level of TNF in peripheral blood of S180 and H22 tumor-bearing mice.

Red Cell Immune. Red Cell Immune system was raised firstly by the American scholar Siegel in 1981; it is the important ingredient of immune system. Li et al. (76) found it can correct the Red Cell Immune functional disorder of esophageal carcinoma patients during radiotherapy that applied Chinese herb compound FuZhengYiLiu granula. Ren (77) found that QiRuiFuZheng capsule can enhance the activity of erythrocyte CR1 and serum complement C3, thus to increase the immune adherence of red blood cells.

The Effect on Multi-Immunocytes and Cytokines. XiaoLiuTang (consist of *Radix Astragali, Radix Glehniae, Fructus Ligustri Lucidi, Radix Pseudostellariae, eclipta, toad's skin, Rhizoma Paridis, house lizard, and Radix Sophorae Tonkinensis*) can enhance lymphocyte transformation efficiency of tumor-bearing mice, the activity of NK cells and the content of TNF-α in PB. In addition, it can accelerate the apoptosis of Vitro tumor cells too (78). Li et al. (79) found that ShenXi capsules (consist of *Radix Astragali seu Hedysari , Radix et Caulis Acanthopanacis Senticosi, Radix Salviae Miltiorrhizae,* and Se) increased the activity of NK cells and the quantity of CD3 cells, CD4 cells, and decreased the quantity of CD8 cells obviously. And the ratio of CD4/CD8 was ascended. Sun et al. found that the activity of NK, IL-2 and the amount of CD3 cells, CD4 cells, and the value of CD4/CD8 raised distinctly while taking QiRuiFuZhengPulvis (consist of *Radix Astragali seu Hedysari, Fructus Ligustri Lucidi, C. Eucommiae, C. Spatholobi, and Fructus Corni, etc.*)-accompanied chemotherapy (80).

19.3.3.2 The Regulated Effects in Immune Escape

The immune system can be activated to eradicate tumor cells by immune response, but sometimes the immune system is not able to control the tumorigenesis and the tumor progression in nature. So it is very important to study the mechanism of immune escape of tumor cells. It is absolutely complex; one of the most important mechanisms is Fas/FasL counterattack-induced apoptosis of CTL cells. Sung-Hyung et al. (81) consider that Fas/FasL-mediated killing pathway is the key anticancer effect mechanism of CTL cell and NK cell in immune response. So regulating the efficiency of Fas/FasL may control immune escape effectively. Zhao et al. (82) study the protein expression of apoptosis-associated Fas/FasL of H22 tumor-bearing mice which are treated with FuZhengYiLiu granula. The result is the expression of Fas increased and FasL decreased in FuZhengYiLiu granula group compared the with control group. It indicates that FuZhengYiLiu granula can inhibit the apoptosis of T cell of H22-bearing mice, and it is important in immune escape. He et al. (83) observed the level of TGF-β1 and VEGF

of H22 tumor-bearing mice using the ELISA method. They found that *Lycium barbarum* polysaccharide (LBP) could downregulate the level of TGF-β1 and VEGF, thus the anti-tumor roles of LBP are related to the effects of restraining the secretion of TGF-β1 and VEGF and immune escape.

19.4 CONCLUSIONS AND PERSPECTIVES

From the literature and reports published in recent years, we can see that the reports of FZ anticancer TCM herbs are all focused on the immune response, including regulation of T cells, NK cells, mononuclear macrophages, TIL, LAK and IL-2, IL6, TNF-α, etc. In domestic reports, it is a common opinion that FZ anticancer TCM herbs can also strengthen red cell immunity. FZ anticancer TCM herbs also gradually show its importance during its study on intervening tumor immune escape (for instance, Fas/FasL and restrain tumor cells to secrete immunosuppressive factor, etc). However, at present, the mechanism study of FZ anticancer TCM herbs increasing the proliferation of lymphocytes and cytokines as well as the comparative study of the theory of FZ anticancer TCM herbs and tumor immunology are relatively weak and need further discussion. In conclusion, the incessant new production of FZ anticancer TCM medicines on immune regulation provide more and more theory for applying formula of FZ Chinese formula of anticancer therapy flexibly and widely.

REFERENCES

1. Kim KH, Lee YS, Jung IS et al (1998) Acidic Polysaccharide from Panax ginseng, ginsan, induces Thl cell and macphage cytokinesand generates LAK cells in synergy with rIL22. Planta Med 64(2):110–115
2. Liu M, Zhang J (1996) Mechanism of immunoregulative funcion of gesinoside Rg1 in aged mice. Acta Pharmaceutica Sinica 31(2):95–100
3. Wu H, Lin H, Pei Y et al (2006) Influence of gesinoside Rg3 on mucous membrane immunity in tumor-bearing mice and CTX-treated mice. Bull Chin Cancer 15(6):369–371
4. Wang Y, Hao Y, Qiu J et al (2003) Influence of gesinoside Rg1, Rgh1 on T lymphocyte propagation stimulation and LPAK cell antitumor activity in dendritic cell. Chin J Immunol 19(4):248–252
5. Shan G, Ye X (2007) Clinical observation of astragalus polysaccharide injection combined with chemotherapy in 84 patients with advanced malignant tumors. Chin J Clin Oncol 34(6):355–356
6. Zheng C, Xiao W (2002) Reactive state of PBMC Th1/Th2 in lung cancer patients and the regulative function of astragalus. Chin J Immunol 18(7):502–504
7. Dong JC, Dong XH (2005) Comparative study on effect of astragulus injection and interleukin-2 in enhancing anti-tumor metastasis action of dendritic cells. Chin J Integrated Tradit West Med 25(3):236–239
8. Cho WC, Leung KN (2007) In vitro and in vivo anti-tumor effects of Astragalus membranaceus. Cancer Lett 252(1):43–54
9. Xu P, Sun J, Gao F et al (2007) Experimental study of huangqikeli on immune function of mice. Chin J Inf Tradit Chin Med 14(4):27–28
10. Wang RT, Shan BE, Li QX et al (2002) Extracorporeal experimental study on immuno-modulatory activity of astragalus memhranaceus extract. Chin J Integrated Tradit West Med 22(6):453–456
11. Luo H, Luo D (1999) Immunology of Traditional Chinese Medicine. Beijing Medical University and Chinese Peking Union Medical College Affiliated Publications, Beijing, pp 130–133
12. Ji Y (1999) Pharmacology and application of antitumor Traditional Chinese Medicine. HarbinScience and Techonology Press, Harbin, pp 494–501

13. Liu M, Ma S, An T et al (1999) Effects of cordyceps militaris on immunological function in mice sarcoma S180 Model. Chin J Tradit Chin Med Pharm 14(1):25–27
14. Qiu Z, Lin Z, Qian L et al (1994) The influence of Aweto on NK and LAK cells activity in leukemia patients. Shanghai J Immunol 14(1):30–31
15. Zhang J, Wei X, Bao L et al (1997) Effect of Aweto Polysaccharide on TNF-α, IL2 and the soluble receptor. Chin J Pract Intern Med 17(12):727–728
16. Lin X, Zheng Y, Chen J, Huang J (2004) Effect of crude polysaccharide from cordyceps sinensis strains on induction of macrophage from abdominal cavity to produce TNF-α in vitro. J Microbiol 524(3):22–23
17. Chiu JH, Ju CH, Wu LH et al (1998) Cordyceps sinensis increases the expression of major histocompatibility complex class II antigens on human hepatoma cell line HA22T/VGH cells. Am J Chin Med 26(2):159–170
18. Wang H, Xia M, Xia D et al (2006) Study on lentinan in enhancing antitumor effect and its mechanism Zhejiang. J Integrated Tradit Chin West Med 16(5):2911
19. Li G, Kang B, Guo B (2000) The study of lentinan to increaseantitumor activity and secretion of cytokines of Tumor Infiltrating Lymphocyte Chinese. J Modern Appl Pharm 17(5):3541
20. Rong W, Jing H, Liu C (2006) Effect of lentinan on the function of dendritic cell in tumor-bearing mice. Chin Archiv Tradit Chin Med 24(4):681
21. Dai Z, Dong C (2005) Progress of biological activity of polysaccharide from *Ganoderma Lucidum*. J Anhui Tradit Chin Med Coll 24(5):60–62
22. Lin Z (2007) Modern research of *Ganoderma Lucidum*. Shanghai Science and Technology Literature Publishing House, Shanghai, p 8.
23. Chen G, Chen H (2006) *Ganoderma Lucidum* and tumor. Shanghai Science and Technology Literature Publishing House, Shanghai, pp 69, 70, 97, 99, 133.
24. Zhou C, Tang Q, Yang Y et al (2004) Antitumor effect of ganoedric acid from *Ganoderma Lucidum*. Mycosystema 23(2):275–279
25. Ning A, Cao J, Huang M et al (2004) The antitumor action and influence of immune system of ganoderma polysaccharides in mice. Chin J Microecol 16(1):13–14
26. Deng W (1984) Immunopharmacology of tremella polysaccharides. Chin Tradit Herb Drugs 15(9):23
27. Ukai S (1972) Antitumor activity on sacoma 180 of polysaccha-ride from tremella fucifomis berk. Chem Pharm Bull 20:2293
28. Zhao T (1982) Effect of tremella fuciformis berk on acute radiation sickness in dogs. Acta Academiae Medicinae Sinicae 4(1):20
29. Chen H (1989) Anti-aging functon of black fungus polysaccharides and tremella polysaccharides. J China Pharm Univ 20(5):303
30. Zheng S (1993) The progress of the chemical composition and pharmacology of Tremella. Chin J Pharm 28(5):264
31. Mali Ma L (1992) Effection of tremella fuciformis polysaccharide on the production of IL22 in mice spleen cells. Acta Pharmaceutica Sinica 27(1): 1.
32. Lin X (1995) Effects of *Tremella* and *Tuckahoe* on immunoregulation in mice. Immunol J 11(1):65
33. Xu H, Yu X, Qu S (2008) Effect of tremella fuciformis spores polysaccharides on transplanated tumor and Immunological function in mice. Chin J Mod Appl Pharm 25(2):93–95
34. Jiang Y, Yin B (2006) Current situation and strategies to the therapy of malignant tumors in China. Med Philos (Clinical Decision-making Forum Edition) 27(11):323
35. Yu J, Jiang Z, Yan H et al (2004) Effect of pachyman on cell-medicated immunity activity and antitumor function in chicken infected with vMDV. Chin J Vet Sci Technol 34(11):70
36. Chen C (2001) The antitumor activity and immune effect of carboxymethylpachymaran. Acta Edulis Fungi 8(3):39–44
37. Lang X, Li M, Jia B (1991) Experimental study of polysaccharidefrom spina date seed and flesh on enhancing immunity and anti-radiation injury in mice. Chin J Chin Materia Medica 16(6):366–368
38. Zhang Q, Lei L, Sun L et al (1998) Effects of crude polysaccharide from fructus zizyphi sativae on anti-complement activity and enhancing the propagation of mice Spleen Lymphocyte in vitro. Pharmacol Clin Chin Materia Medica 14(5):19–21
39. Wakabayashi C, Murakami K, Hasegawa H et al (1998) An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. Bio Chem Biophys Res Commun 246:725–730

40. Li Y, Ma C, Chen X (2008) Antitumor effect and immune mechanism of active compositions from Panax notoginseng. J Beijing Univ Tradit Chin Med 15(1):17–19
41. Wu B (1982) Pharmacology of chinese materia medica, 1st edn. People's Medical Publishing House, Beijing, pp 50–201
42. Yin J (1993) Modern research and clinical application of traditional Chinese medicine, First Edition, Beijing Academic Press, Beijing, p 595
43. Liu H, Chen X, Su S (2008) Zhenqifuzheng injection on the synergy and attenuation of Cyclophosphamidum. Pharmacol Clin Chin Materia Medica 24(4):50–52
44. He J, Wu X, Yang H, He X, Feng Y (2007) Treatment of deficiency of kidney and spleen of malignant tumor after radiotherapy and chemotherapy with Zhenqifuzheng oral liquid. Shandong J Tradit Chin Med 26(5):299–300
45. Ni B, Luo Z, Zhang Z et al (2008) Shenqifuzheng injectioncombined with hyperthermic intraperitoneal chemoperfusion treating advanced peritoneal Tumor. Chin J Med 4(31):50–52
46. Chen X, Yuan J, Liu C et al (2006) Observation of Shenqifuzheng injection combined with chemotherapy on improving health status in aged gastrointestinal cancer patients. Shaanxi J Tradit Chin Med 27(3):287–288
47. Liu L, Shan B, Diao L et al (2003) Effect of Shenqifuzheng injection on the immune of erythrocytes in patients with hematological malignancy. Zhejiang J Tradit Chin Med 38(12):542
48. Tang L, Zeng F, Wu M, Lin J (2006) Effects of Aidi injection on the immunefunction in mice bearing H22. Herald Medicine 25(2):104–106
49. Hou B, Shu X, Zhou S, Fang S, Li X (2008) Effects of Aidi injection on immunologic function in in postoperative patients with colorectal cancer. J Fourth Mil Med Univ 29(10):933–935
50. Liu T, Zheng H (2003) Effects of Aidi injection on celluar immunologic function in colorectal cancer patients. Liaoning J Tradit Chin Med 30(11):161–162
51. Chen D, Pan L (2004) Clinical study of Aidi injection on the immune of erythrocytes in postoperative patients with esophageal cancer. J Southeast Univ (Medical Science Edition) 23(2):117–119
52. Jiang T, Shen J, Li X et al (1980) Inhibitory effect of liuweidihuang decoction on ethyl p-aminobenzoate inducing pulmenary adenoma. J Tradit Chin Med 21(7):72
53. Li P, Shi X, Wang F (1987) Immunopharmacology research of liuweidihuang pill and rehmannia. Chin J Immunol 3(5):296
54. Xu J, Zhou Z, Yang F et al (1992) Study of liuweidihuang oral liquid on resisting the side effect of chemotherapy. Chin J Integrated Tradit West Med 12(12):734
55. Gong J, Ma J, Fan Q et al (1997) Effect of Liuweidihuang decoction and its disassembled prescriptions on the activity of ADCC of macrophages in rats. Chin J Exp Tradit Med Formulae 3(6):24
56. Park JY, Yang MZ, Jun HS et al (2003) Effect of raw brown rice and Job's tear supplemented diet on seem and hepatic lipid concentrations, antioxidative system, and immune function of rats. J Korean Soc Food Sci Nutr 32(2):197–206
57. Hidaka Y, Kaneda T, Amino N et al (1992) Chinese medicine, Coix seeds increase peripheral cytotoxic T and NK cells. Biotherapy 5(3):201–203
58. Yao Y, Chen X, Li B et al (2002) Study of Kanglaite soft capsule on enhancing immunity in mice. Tradit Chin Drug Res Clin Pharmacol 13(4):233–235
59. Hou B, Limian, Liu H et al (1999) Effect of Kanglaite injection on the NK-Activity, SIL-2R, TNF-2 in advanced colorectal cancer patients. J Pract Oncol 14(6):382–383
60. Zhang A, Sun Z, Ma S (2008) Effect of Kanglaite injection on VEGF-C protein and mRNA expression in Lewis lung cancer mice. Pract Clin J Integrated Tradit Chin West Med 8(1):61–64
61. Wu L, Chen L, Li Q et al (2004) Effect of Kanglaite on the in vitro propagation and antineoplasm function of tumor infiltrating lymphocyte. Chin J Tradit Chin Med Pharm 19(11):656–658
62. Li D (2005) Effect of Traditional Chinese Medicine on immune function in patients with adjuvant chemotherapy. Hubei J Tradit Chin Med 27(9):44
63. Zhang Y, Guo J (2005) Effect of baoyuandan on T lymphocyte of lung cancer patients after chemotherapy. J Fourth Mil Med Univ 26(13):1219–1220
64. Li Z, Zhang J, Pengbo et al (2005) Analysis on expression of NK cells in peripheral blood in patients of lung-qi deficiency or lung-yin deficiency. J Tradit Chin Med 46(7):533–534
65. Zhao L, Xu W (2004) Clinical observation of Zhengdekang on strengthening health function in non-small cell lung cancer patients. Pract Clin J Integrated Tradit Chin West Med 4(4):23, 32

66. Liu H, Dai X (2004) Research of inhibitory effect and mechanism of Fuzheng mistura in LA795 mice with metastatic lung cancer. Jiangsu J Tradit Chin Med 25(10):56–57
67. Feng L, Shou C, Feng Y et al (2006) Function of Traditional Chinese Medicine Fuzheng prescription in treating alimentary tract tumor. Zhejiang J Integrated Tradit Chin West Med 16(5):272–274
68. Zhu X, Wang A, Wang S et al (2003) Immunologic effection of Fuzhengkangai capsule 2 on mice with tumor. Chin J Clin Oncol Rehab 10(6):517–518
69. Li J, Song S, Han L et al (2004) The influence of tonifying kidney plus strengthening vital energy recipe on immunity function in tumor transplanted mice. Acta Laboratorium Animalis Scientia Sinica 12(3):152–155
70. Wang Y, Zhang Y, Li G et al (2006) Regulational function of ZhenQi casein peptide complex on macrophage function in rats. Tradit Chin Med Res 19(3):22–24
71. Bi M, Liu Z, Wang H et al (2006) Effect of gan'ai-I on competence of murine macrophage in killing hepatic carcinoma cells and its mechanism. World J Gastroenterol 14(5):526–529
72. Reome JB, Hylind JC, Dutton RW et al (2004) Type 1 and type 2 tumor infiltrating effector cell subpopulations in progressive breast cancer. Clin Immunol 111(1):69–81
73. Hsiao YW, Liao KW, Hung SW et al (2004) Tumor-infiltrating lymphocyte secretion of IL-6 antagonizes tumor-derived TGF-beta 1 and restores the lymphokine-activated killing activity. J Immunol 172(3):1508–1514
74. Huang L, Wu C, Wang Y et al (2004) Yiqipingxuanyin extract on the activity of tumor infiltrating lymphocyte of pleural effusion in lung cancer patients. Chin Arch Chin Med 22(2):230–232
75. Zhang H, Ma Z, Xu B et al (2005) Effect of Zhongyaotiaohengfang on TNF and LAK celluar activity in S180 bearing mice and H22 bearing mice. Shanxi J Tradit Chin Med 26(9):983–985
76. Li X, Zhao J (2004) Effect of Fuzhengyiliu granule on the immune of erythrocytes in postradiotherapy patients with esophageal tumor. Shanghai J Tradit Chin Med 38(7):51–52
77. Ren W, Wang Y (2004) Effect of Qiruifuzheng capsule on the immune of erythrocytes in immunosupressed Mice. Shandong J Tradit Chin Med 23(1):36–38
78. Fu Y, Xia T (2004) Experimental study of xiaoliu decoction treating tumorand modulating immune fuction. Chin J Integrated Tradit West Med 24(12):1114–1117
79. Li Y, Zhang Y (2004) Influence of Shenxi capsule on celluar immune function of tumor patients. Forum Tradit Chin Med 19(2):21–22
80. Sun T, Zheng Y, Wang X (2004) Influence of Qiruifuzheng capsule on the activity of IL-2 NK cell activity and T lymphocyte subsets in lung cancer patients. Chin Arch Tradit Chin Med 22(12):2229–2230
81. Sung-Hyung L, Erez BH, Arthur M et al (2004) In vivo rejection of tumor cells dependent on CD8 cells that kill independently of perforin and FasL. Cancer Gene Ther 11:237–248
82. Zhao J, Zhu Y, Shen S et al (2006) Effect of Fuzhengyiliu granule on Fas/FasL protein expression of T lymphocytes in H22-Bearing mice. Chin J Tradit Med Sci Technol 3(1):14–15
83. He Y, Ying Y, Luo R et al (2005) Effect of lycium barbarum polysaccharide on immunosuppressive factors VEGF, TGF-β1 in H-22 bearing mice. Tradit Chin Drug Res Clin Pharmacol 16(3):172–174

Section D
Dietary Components: Allergy and Asthma

20 Role of Dietary Components in the Epidemic of Allergic Disease

Susan L. Prescott, Sarah Jennings, David Martino, Nina D'Vaz, and Henning Johannsen

Key Points

- As the source of all nutrients, diet is arguably one the most important environmental exposures during early development.
- Maternal dietary exposures *in utero* have implications for most aspects of fetal development and there is growing evidence that this includes immune pathways that have the capacity to influence the risk of allergic disease.
- In the postnatal period, oral exposures, including a broad range of immunomodulatory dietary nutrients, potential allergens and colonizing bacteria, play a major role in the maturation of the mucosal immune system.
- Many of these factors have the capacity to influence the success or failure of subsequent tolerance, including extensive "modernization" which has led to many of these enteric exposures causing changes in colonization patterns and dietary composition.
- It is likely that the recent epidemic rise of allergic disease is multifactorial and that changes in at least some of these factors play a contributing role.
- It is also evident that a wide range of function genetic polymorphisms may influence the relative effects of these environmental exposures on the developing phenotype.
- A better understanding of these complex interactions may ultimately lead to individualized early interventions that are tailored and targeted according to genetic predisposition. Early dietary exposures are likely to play an important role in this.

Key Words: Probiotics, prebiotics, antioxidants, fatty acids, folate, allergy prevention, atopic dermatitis, eczema, infants, food allergy.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_20

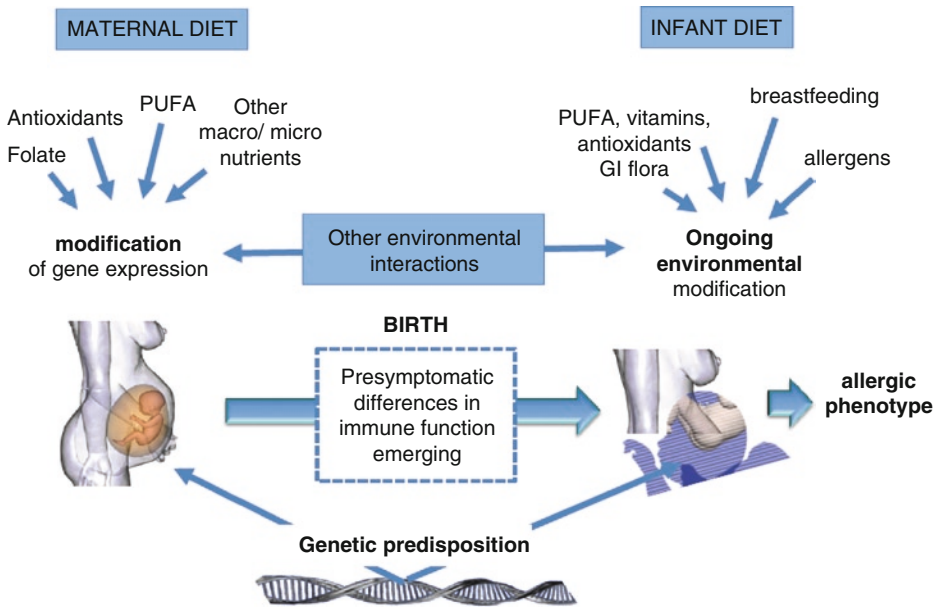


Fig. 20.1. Dietary influences play an important role in both the prenatal and the postnatal periods, with the potential to modify disease risk and the resulting allergic phenotype.

20.1 INTRODUCTION

While there is compelling evidence for a hereditary component in the pathogenesis of allergic diseases, the dramatic recent increase in disease also indicates a clear role of the modern environment. Dietary changes are among the many complex environmental changes implicated in the allergy epidemic. Is it also evident that the environmental influences driving this increased disease predisposition occur very early during development. In the antenatal period, maternal diet is a major exposure with the capacity to influence many aspects of fetal immune development, including the developing immune system (Fig. 20.1). There is also growing evidence that the predisposing patterns of immune dysfunction are already evident at birth and that maternal diet (1), along with other antenatal exposures (2, 3) can modify neonatal immune responses. In the postnatal period, when the gut is the largest and the most important site for the development of immune tolerance, a range of dietary exposures are highly likely to influence the success of these processes, including breast milk, dietary allergens, colonizing bacteria and many immunomodulatory dietary nutrients. The role of these various factors is discussed here, along with the potential role in immune immunomodulation and the prevention of allergic disease.

20.2 EVENTS LEADING TO IMMUNE TOLERANCE

First, it is important to consider the events that lead to immune tolerance, and how this fails in allergic disease. While the culminating events in the development of oral tolerance occur in the postnatal period, it is increasingly likely that antenatal events may also influence susceptibility to immune dysregulation.

20.2.1 The Importance of Antenatal Events

While the clinical symptoms of allergic disease are not evident until the postnatal period, there is mounting evidence of pre-symptomatic differences in immune function in neonates who go on to develop disease. The most consistent and well recognized of these is a relatively deficient production of the type 1 T helper (Th1) cell cytokine interferon (IFN)γ (4, 5), normally important for suppressing pro-allergic Th2 responses. More recent evidence suggests that perinatal immaturities of allergic predisposition extend to include differences in regulatory T cell (Treg) function (6) and innate immune function (7). As discussed further below, a number of maternal dietary factors have been associated with variations in perinatal and/or postnatal immune function, including polyunsaturated fatty acids (PUFA) (8), folate (9) and antioxidants (10) (Fig. 20.1). This is also consistent with the well-recognized immunomodulatory effects of these and other dietary nutrients.

It is now clear that early environmental exposures may influence development by altering gene expression through "epigenetic modification" (reviewed in ref. (11)). This can lead to heritable changes in gene expression, and disease propensity, without any change in gene sequence. The main epigenetic processes that regulate gene expression include DNA and histone methylation, histone acetylation and patterns of chromatin structure, which thereby determine the degree of DNA compaction and accessibility for gene transcription. These pathways are highly sensitive to environmental exposures. Many elegant models show how environmental changes at critical times during development can profoundly alter the phenotype of genetically identical animals, and alter subsequent disease predisposition (11). While there are well-established models from other disease processes, epigenetic models of allergic disease are only now beginning to emerge. Notably, one recent study demonstrated that maternal dietary supplementation with folate (a methyl donor) in pregnancy induced changes in gene methylation and associated allergic airways disease in the offspring. Specifically, they identified more than 80 genes that were differentially methylated after *in utero* supplementation, with decreased transcriptional activity (silencing) and reduced expression of genes normally important for inhibiting the allergic phenotype. Moreover, this effect was shown to be heritable across generations (9). To our knowledge, this is the first study to show that dietary factors can modify the risk of allergic airway disease through epigenetic mechanisms in the antenatal period. It is likely that further investigation of other immunomodulatory dietary factors may reveal additional epigenetic effects on immune development in the future. Thus, antenatal events appear to "set the scene" and influence the outcome of subsequent postnatal interactions, the success of tolerance and the risk of disease. The frequent expression of allergic disease within months of birth is further evidence that the predisposing events occur during this very early period of development.

20.2.2 Evolving Immune Tolerance in the Early Postnatal Period: A Central Role of the Gut

The gastrointestinal tract is arguably the most important site for the development of immune tolerance. In the immediate postnatal period, the gut undergoes rapid colonization and this induces maturation of the associated mucosal immune networks that comprise more than 70% of the total immune system. During this time, the infant is exposed to a vast array of new environmental antigens. Most of this foreign antigenic load is

derived from colonizing commensal bacteria and food components. To prevent inflammatory responses to these largely harmless antigens, the gastrointestinal associated lymphoid tissue (GALT) has evolved complex mechanisms to promote tolerance as a default response (reviewed recently by ref. (12)). Early microbial colonization is essential for the establishment and homeostasis of this tolerogenic microenvironment. Animal studies in germ-free conditions demonstrate that oral tolerance cannot be induced in the absence of gut microbiota (13, 14). Although the restoration of full immune function and oral tolerance can be achieved by colonization, this is age-dependent and cannot be achieved in mature animals (14), highlighting the importance of events during this window of time.

Tolerance has been described to occur in the "two phases" (12). In the first instance, there is the establishment of an immunosuppressive milieu, which prevents unwanted local inflammation in the gut. Second, this provides optimal conditions for the development of highly regulated systemic immune responses. Dendritic cells (DC) play a central role in both of these processes. They produce immunomodulatory cytokines (IL-10 and TGFβ), which suppress local inflammation in an antigen-nonspecific manner. DC also promote the differentiation of antigen-specific Treg, which are essential for systemic immune surveillance and tolerance. The type of regulatory T cell populations induced depend on the specific patterns of surface marker expression and cytokine production, and include IL-10 producing-Treg (Tr1), TGF β-producing Treg (Th3) and CD4+CD25+FOXP3+ Treg. There is growing speculation that other dietary immunomodulatory factors can promote tolerogenic conditions in the gut, including PUFA, which influence DC function (15), vitamin A (retanoic acid) (16) and vitamin D (16) which also promote Treg differentiation.

Thus, the postnatal events that lead to immune tolerance are centered on the gut. Exposure to most allergens and antigens through this route leads to tolerance of those proteins. The success of oral tolerance appears to depend on a number of oral exposures, including optimal colonization and exposure to other dietary immunomodulatory factors. With the rising rates of food allergies and other allergic disease, it is clear that modern environmental exposures are not optimal for the development of tolerance. There is consequently strong interest in understanding the role of specific factors in this process and how they may be optimally modified to prevent allergic disease.

20.3 EFFECTS OF SPECIFIC DIETARY FACTORS IN THE DEVELOPMENT OF TOLERANCE, IMMUNE REGULATION AND THE PREVENTION OF ALLERGIC DISEASE

The epidemic rise of allergic disease demonstrates that immune tolerance pathways are highly susceptible to environmental change and successful tolerance is therefore likely to depend also on a complement of additional exposures, including favorable gut colonization (14), breast milk (17) and/or other immunomodulatory factors, including postnatal and *in utero* dietary exposures. It has been common practice to assess the effects of individual nutrients in both observational and intervention studies, which may be over-simplistic. Another approach has been to investigate the effects of common dietary "patterns", such as the "Mediterranean diet" on disease outcomes. Several recent studies have suggested that the "Mediterranean diet" may protect against wheezing in

early childhood (18, 19), and that this effect was largely dependent on dietary patterns in pregnancy (19). In a much larger population-based UK birth cohort, some dietary patterns were associated with eczema, IgE, lung function and asthma; however, none had any effect after allowing for other environmental confounders (20). Thus, although the effects of specific factors will be discussed separately here, it is important to recognize that there are complex inter-relationships and correlations between individual nutrients.

20.3.1 Breast Milk as a Source of Immunomodulatory Factors

Breast milk is ideally the first nutrition source in the postnatal period. The tolerogenic and immunoprotective properties of breast milk are well recognized. It contains a complex array of immunologically active compounds that may both protect from infection and promote oral tolerance (including immunoglobulins, lactoferrin, lysozymes, oligosaccharides, long chain fatty acids, cytokines, nucleotides, cytokines, hormones, antioxidants and maternal immune cells). Breast milk is also the first oral source of food allergens ingested by the mother (21). At least some of the tolerogenic properties of maternal milk appear to depend on the presence of cytokines such as TGFβ, which induce regulatory T cells in the infant gut (22). The addition of TGFβ to formula milk also increases tolerance in animal models (23) and this is now being explored in humans.

Breastfeeding is strongly encouraged for many reasons. However, the relationship with allergic disease has been conflicting in population studies, and it is not possible to perform randomized controlled studies. There is some evidence that continued breastfeeding during the introduction of complementary foods promotes tolerance (17) and it seems logical to encourage continued breastfeeding in conjunction with "solid" food introduction. Ongoing studies of the tolerogenic properties of breast milk and its components may reveal further avenues for allergy prevention.

20.3.2 Role of Dietary Allergens in the Development and Prevention of Allergic Disease

Dietary allergens have been of long-standing interest in the development of allergic disease and many earlier prevention strategies centered around the avoidance of allergenic foods in early life. While certain foods are clearly more "allergenic" than others (including cow's milk, eggs, nuts and seafood), it is now increasingly evident that early exposure to these allergens is not the primary cause of the rising rates of food allergy. Furthermore, the avoidance of allergenic foods has been largely unsuccessful in preventing disease (reviewed in ref. (24)).

Although delayed introduction of infant complementary feeding (beyond 6 months of age) and continued avoidance of "allergenic" foods became common practice until recently, there was no strong evidence to support this (25). Rather, there has been growing evidence that these practices may actually increase the risk of allergic disease (26–28). Delayed introduction of wheat (26), cow's milk (29), egg (28) and fish (30) have all been associated with increased development of food allergy and atopic dermatitis/eczema. Thus, although there is a recognized need for more research, based on the

current evidence, it is difficult to justify continued restrictive feeding practices and most expert panels now recommend the introduction of complementary feeding somewhere between 4 and 6 months of age, with no specific avoidance of allergenic foods (24, 31–33). There is also no clear evidence to support the practice of excluding allergenic foods including cow's milk, eggs and peanuts from the maternal diet during pregnancy and lactation for allergy prevention (as reviewed by ref. (32)).

As indicated above, focus has instead shifted to dietary factors that may influence the ability to develop immune tolerance. With regard to food as a source of allergens, there is a mounting body of evidence to indicate that oral tolerance, an antigen-driven process (34, 35), is induced by exposure to dietary allergens during critical early stages of development (reviewed in ref. (24)). The timing, dosage, interval and regularity of allergen exposure can all affect the development of oral tolerance (34, 35)with evidence that under optimal conditions, early, regular oral exposure to food allergens will induce tolerance rather than sensitisation, and may be critical to prevent the development of food allergy (26, 36).

Animal models suggest that the exposure to food proteins during a "critical early window" of development may be essential to promote the induction of oral tolerance (34, 35). In humans, this has not been proven. There is some evidence that giving food allergens "too early", that is, before 3–4 months of age, has been shown to increase the risk of allergic disease (39, 40). This is presumably because local immune networks are still immature and the optimal microbial colonization, which is essential for tolerance has not yet been fully established (41). Similarly, delayed exposure to allergenic foods beyond 6 months of age, including peanuts (37, 38), wheat (26) and fish (36) may also lead to increase in allergic disease, providing indirect evidence of a possible "optimal window" for first complementary feeding between the 4th and 6th month of age (reviewed in ref. (24)). This remains to be confirmed.

There are currently a number of randomized controlled trials to directly investigate if early introduction of specific allergenic foods will actually promote oral tolerance to these foods and reduce the risk of specific allergies. Approaches are aimed at earlier oral exposure to induce tolerance before sensitisation occurs through cutaneous or respiratory routes (reviewed by ref. (42)). The results of these studies are awaited with interest. Thus, allergy prevention approaches using food allergens are shifting from "avoidance" to possibly earlier exposure to these foods, although this should still be approached with caution until the findings are clear. It remains important to ensure that other environmental conditions are also optimized to promote tolerance, as without this early allergen exposure may still result in sensitisation, as suggested by recent trends in disease.

20.3.3 Role of Dietary Polyunsaturated Fatty Acids in Allergic Disease

Another significant dietary change that has been implicated in the rise in allergic disease has been a progressive decline in the consumption of omega-3 (n-3) PUFA in favor of omega-6 (n-6) PUFA. This could have plausible effects on immune development and the development of allergic disease, particularly as the metabolic products of n-6 PUFA are significantly more inflammatory than those of n-3 PUFA (as reviewed by ref. (43)). Logically, this has led to interest in the role of n-3PUFA supplementation (using fish oil) in the treatment and prevention of allergic diseases and other inflammatory diseases.

The proportional composition of n-3/n-6 PUFA in cell membranes has a number of recognized effects on immune function (as reviewed in ref. (44)), which are related to metabolic properties, structural properties (i.e. effects on membrane fluidity) and effects on gene expression (signal transduction).

20.3.3.1 Effects of PUFA on Eicosanoid Mediators

PUFA are the major substrates for the production of eicosanoid mediators such as the prostaglandins and leukotrienes. Higher levels of n-6 PUFA favor the production of the 2-series prostaglandins (such as PGE2) and the 4-series leukotrienes (LTB4), both of which are highly inflammatory. In contrast, n-3PUFA-derived products (PGE3 and LTB5) are significantly less inflammatory. Furthermore, other n-3 metabolites, including resolvins, can also actively assist in suppressing inflammation (reviewed in ref. (45)). Thus, an alteration in the n-3/n-6 PUFA composition can alter the propensity for local tissue inflammation.

20.3.3.2 Effects of PUFA on Antigen-Presenting Cells

The level of APC activation and receptor expression determines the degree and pattern of T cell response. Increasing the n-3PUFA content of APC cell membranes has been shown to inhibit the surface expression of MHC class II and co-stimulatory molecules, thereby reducing antigen presentation and T cell activation (46, 47). One study has shown that at least some of the immunomodulatory activities of n-3 PUFA are mediated through the modulation of microbial pattern recognition receptors (toll-like receptors (TLR)) on APC (15). In animal models, docosahexanoic acid (DHA), which is more highly concentrated in cord blood, has been shown to inhibit MHC class II and immune activation in APC (47), which may have relevance for APC function in the early postnatal period. The n-3PUFA also have suppressive effects on the production of inflammatory cytokines IL-1, IL-6 and TNFα as well as on NFκB pathways, possibly through an interaction with nuclear lipid-activated transcription factors, peroxisome proliferator-activated receptors (PPARs), which regulate cellular responses including inflammation.

20.3.3.3 Effects of PUFA on T Cell Function

The anti-inflammatory effects of n-3 PUFA on T cell function have been extensively studied, with well documented suppression of lymphoproliferation and cytokine production following dietary fish oil supplementation (48–50). We have also demonstrated that increasing maternal n-3PUFA status in pregnancy (with fish oil) is associated with altered allergen-specific immune responses in the offspring (1). We also observed effects on T cell signaling, with down regulation of most protein kinase C (PKC) isozymes in the fish oil group compared to the placebo group, with the exception of PKCz, which was upregulated in the supplementation group. These changes in T cell signaling were associated with protection from subsequent allergic disease (51).

20.3.3.4 Effects of PUFA on T Regulatory Cell Function

The effects of n-3PUFA on specific populations of regulatory T cells (Treg) are not yet well understood. This may be mediated through recognized effects on Treg receptors (such as TLR (15)) or by metabolic products, such as PGE2 which can induce Treg (52). At this stage, this is not clear. The fact that n-3PUFA can suppress specific inflam-

matory responses, without the adverse effects of a global immunosuppression (such as increased risk of infection), suggests specific immunomodulatory effects.

The potential protective role of n-3PUFA in allergic disease was based on a number of observations studies that the consumption of oily fish in childhood (higher n-3/n-6 ratio) was associated with less wheeze and asthma (53, 54). Some more recent observational studies have supported this (55), but others have not (56). This led to the first postnatal intervention study using fish oil supplementation for allergy (asthma) prevention. The supplements (tuna oil or a placebo) were given from around 6 months of age. Although there was a reduction in wheezing at 18 months of age (57), there were no long-term benefits for the reduction of allergic sensitisation or any diagnosed allergic disease, as assessed at 5 years of age (58). As the increased expression of Th2 allergic responses is frequently already established in subsequently allergic children by 6 months of age (albeit asymptomatic) (59) it is possible that earlier intervention could be more effective. There has subsequently been more interest in PUFA status in pregnancy, when developmental programming is arguably more critical. Observational studies have again suggested that maternal fish oil consumption in pregnancy has protective effects on the development of allergic sensitisation (60), eczema (61), and asthma (62) although this has not been seen in all studies (63). There are now a series of randomized controlled trials of fish oil in pregnancy studies still in progress to assess this more definitively. A recent 16-year follow-up of a cohort of children involved in a pregnancy fish oil supplementation study (originally performed to assess pregnancy outcomes such as gestational length (64)) showed a reduction in subsequent asthma (65). Our initial study, which showed neonatal immunomodulatory effects of maternal fish oil (1), also provided preliminary evidence of reduced risk of sensitisation and other allergic outcomes (1). Although the study was not powered to examine these clinical outcomes, children in the fish oil group were also three times less likely to have a positive skin prick test to egg at 1 year of age with a relative risk reduction of 54.6%. Another more recent study (66) has now also shown a significant reduction both in food allergy and IgE-associated eczema in fish oil supplemented children (compared with a placebo group). Other larger studies are also underway in an attempt to verify this.

In summary, there is clear evidence of the anti-inflammatory properties of n-3 PUFA together with epidemiological data to suggest that these may have a role in disease prevention. So far, limited data on intervention studies suggest that benefits may be greatest in early development (pregnancy) before the immune propensity for allergic disease becomes established. However, at this stage, no specific recommendations can be made regarding the use of fish oil in pregnancy, and the results of larger studies are awaited with interest.

20.3.4 Role of Antioxidants and Other Vitamins in Allergic Disease

Another group of dietary components that may have declined with "western" dietary changes include antioxidants (such as vitamin C, vitamin E, beta-carotene, zinc and selenium). There have been some epidemiological associations between lower intakes of antioxidant rich foods (such as fresh fruits and vegetables) with reduced pulmonary function (67), and increased risk of wheeze in both adults (68) and children (69, 70). It has been proposed that these associations may reflect a protective effect of antioxidant

rich diets in the development of allergic diseases such as asthma (71, 72). At least some of the immunological properties of antioxidants have supported this hypothesis. Antioxidant status can modulate a number of cell processes implicated in immune programming and regulation. In the presence of vitamin C and E antioxidants, APC (specifically DC) have been shown to promote the development of regulatory T cells *in vitro* (73). By favorably altering the "redox" status of cells, antioxidants can also enhance IL-12 production by APC and favor Th1 differentiation (74). Although it is not clear whether this can be extrapolated to the *in vivo* setting, this could theoretically favor the development of Th1 responses and inhibit the development of allergic Th2 responses. However, there has also been conjecture that oxidative stress, which increases the production of reactive oxygen species (ROS) by macrophages, could also favor Th1 immune differentiation. This alternative hypothesis proposes a theoretical concern that antioxidant supplementation could increase the probability of Th2 differentiation (by inhibiting oxidative stress) and favor the development of asthma and allergic disease (75).

So far, the relationship between antioxidant status in pregnancy and subsequent allergic disease has been limited to observational studies. Maternal intake of vitamin E *in utero* has been associated with an increased risk of developing childhood wheeze, asthma and eczema by 2 and 5 years of age (71, 76). These studies followed earlier observations, by the same UK group, that that higher maternal vitamin E intake during pregnancy was associated with reduced cord blood mononuclear cell proliferative responses to allergens (10). They have also shown that maternal vitamin D intake during pregnancy may have the potential to reduce early childhood wheezing (77). A Boston study has also reported that maternal intakes of vitamin E and zinc were negatively associated with wheezing at 2 years of age, although they did not observe any correlations between the risk of eczema and antioxidant intake in the same children (78). Relationships between allergic disease and other antioxidant levels, such as vitamin C, have been inconsistent. Some studies have suggested negative associations (increased risk of wheezing) with increased vitamin C intake (76) while others have demonstrated beneficial decreases in the risk of wheeze (69, 79).

In summary, it is likely that maternal antioxidant intake during pregnancy does influence the antioxidant status of the developing fetus, and it is also possible (but not yet confirmed) that this modulates the risk and development of childhood atopy (10, 80, 81). To our knowledge, there have been no intervention studies in pregnancy or early childhood to specifically examine the effects of antioxidant supplementation on immune development or allergy prevention. Such studies should be approached with caution as there have been concerns that this could have adverse effects (75). In the meantime, it is best to advocate a healthy balanced diet rather than specific vitamin supplementation. Importantly, it is also likely that fresh foods (such as fruits and vegetables) may have additional "protective" properties that cannot be replicated by specific vitamin supplements (82).

20.3.5 Emerging Relationships Between Dietary Folate and Allergic Disease

As noted above, one of the most interesting developments in the allergy field has been the recent animal model demonstrating the capacity for dietary folate to alter fetal immune development and promote a subsequent asthma phenotype through effects on

gene methylation (9). This is the first study to show that that dietary modification in pregnancy can alter allergic risk through epigenetic effects. It is often difficult to extrapolate from animal studies, and at this stage, the implications for humans are not known. It is also not clear if (at all) the common practice of folic acid supplementation to prevent neural tube defects, has contributed to the rise in allergic disease (83). Human studies are still limited in this context. There is preliminary evidence that folate supplementation in pregnancy is associated with increased childhood wheezing (84), but other studies have shown that higher serum folate levels are associated with a lower risk of atopy and wheeze (85). It is not clear how variation in folate metabolism conferred by genetic polymorphisms (i.e. the MTHFR (C677T genotype) affects these relationships, as the relationships with allergic disease have been conflicting (86, 87). There has been an urgent call for more human studies to address these issues.

20.3.6 Role of Dietary Probiotics and Prebiotics in the Prevention of Allergic Disease

With the recognized importance of gut microflora in the development of immune tolerance, there has been enormous interest in dietary supplements which promote colonization, including (a) health-promoting apathogenic bacteria (probiotics), (b) the fermentable oligosaccharide substrates (prebiotics) which promote their growth, (c) and the byproducts of fermentation (postbiotics). Of these, the role of probiotics have been most studied for allergy prevention (reviewed in ref. (88)) although there has also been some early promise with prebiotics in this context (89, 90).

The rationale for using these products for allergy prevention is based firmly on the hygiene hypothesis and on the immunoregulatory properties of enteric microflora (reviewed in ref. (91)). This has been further supported by apparent differences in perinatal colonization patterns with both the level of industrialization and subsequent allergic outcomes (92, 93).

At present, there are at least 13 published randomized controlled trials of maternal and/or infant supplementation with probiotics for the prevention of allergic disease (as of July 2009), and at least four others that are still in progress, as recently summarized (88). While the initial Cochrane systematic review found a small benefit of probiotic supplementation in the reduction of eczema (94), this was performed before the completion of the majority of the current studies and is currently being revized. Currently, just over half of the now reported studies have shown a reduction in eczema ((95–100), but many have not (101–106). Most studies have not shown any reduction in sensitisation, food allergy or allergic disease in general. However, while some have shown no overall benefits of supplementation, they may have noted a reduction in allergic outcomes in subgroups, such as those born by cesarean section (107) or those with maternal sensitisation (102, 105). Long-term outcomes such as allergic rhinitis or asthma have not yet been satisfactorily assessed in most of these cohorts, although one study has reported a trend for higher rates of asthma and allergic rhinitis in the probiotic group (108) and another has reported no effects (107).

There has been considerable heterogeneity in virtually all aspects of these studies, including the strains used, the timing and method of administration, the duration of

administration, the populations studied and the clinical outcomes measured. This may account for the conflicting outcomes between studies (91). It will also continue to make meta-analyses both difficult to perform and to interpret. There are likely to be differences in the clinical effects between stains as demonstrated by a New Zealand study, which found reduced eczema with one strain (*Lactobacillus rhamnosus HN001*) but not another (*Bifidobacterium lactis HN019*) (98). *Lactobacillus rhamnosus* strains have been among the most commonly used in prevention studies, again with some studies showing eczema reduction (95, 97, 98, 100) but others not (103–105, 107, 109). The duration and timing of treatment has also varied quite substantially with postnatal supplementation varying from 1 month (96) to 2 years (98). Most studies have included a prenatal treatment period, typically for 4–6 weeks, and there has been speculation (102) that studies which included supplementation in pregnancy (95, 97–99) may have been more likely to show a benefit than those that did not (101, 104). However, some studies which included antenatal supplementation have not shown *any* benefit (103, 105). One other study did not start probiotic supplementation (*Lactobacillus F19*) until the weaning period (4–13 months of age) and found a 50% reduction in eczema (110). Thus, while there has been some evidence of beneficial effects of probiotics in the prevention of allergic disease, this has been largely limited to eczema and appears to vary with the properties of the strain and a range of other host and environmental factors. Further research is needed before any specific recommendations can be made.

The use of prebiotics also holds some promise. These non-digestible, fermentable oligosaccharides selectively favor colonization with favorable microflora, particularly *bifidobacteria* (89, 111). This could theoretically have more global effects on colonization than supplementing with single strains. Commercial preparations include polydextrose, fructo-oligosaccharides, galacto-oligosaccharides and mixtures of these. The first study to assess prebiotics in allergy prevention (89, 90) added a mixture of 90% short chain galacto-oligosaccharides (GOS) and 10% long chain fructo-oligosaccharides (FOS) or a placebo to infant formula that was started around 11 days if mothers decided not to breastfeed. There was a significant increase in bifidobacteria and a significant decrease in the cumulative incidence of eczema at 6 months of age in the GOS/FOS supplementation group compared with the placebo group (89). At 2 years of age, there was a significant reduction in the cumulative incidence of eczema, recurrent wheezing and allergic urticaria compared with the control group, although a high proportion were lost to follow-up (90). Again, further studies are needed before the place of prebiotics is clear in allergy prevention.

In summary, while there is no doubt that colonization is essential for the development of immune tolerance, the optimal pattern of colonization and how to achieve this are far from clear. At this stage, prebiotics, probiotics and combinations of these (synbiotics) are still logical avenues to investigate further but there is insufficient data to recommend these in clinical practice for allergy prevention. Considerable research is still required to understand the inherent complexities of the vast gut microbiome. The effects are also likely to vary with individual genetic factors including genetic polymorphisms in host microbial recognition pathways, and many other pathways involved in gene–environmental interactions (as discussed further below).

20.4 GENETIC AND ENVIRONMENTAL INTERACTIONS: IMPLICATIONS FOR "INDIVIDUALIZED" INTERVENTIONS

It is now clear that some specific environmental exposures may only contribute to disease in individuals with functional genetic polymorphisms in relevant pathways. Similarly, the effects of genetic polymorphisms may only be relevant in certain environments. For example, it has been shown that functional genetic polymorphisms in microbial recognition pathways (TLR2) can confer protection from allergic disease but *only* in the context of high bacterial exposure (112). This may explain apparent inconsistencies between studies conducted under different environmental conditions. Genetic differences between individuals (and populations) may also explain some of the differences in the effectiveness of microbial interventions (such as probiotics) between studies.

Functional polymorphisms have been identified in the metabolic pathways of other dietary candidates including folate metabolism (86) and fatty acid metabolism (113, 114). These genetic polymorphisms could potentially alter the relationship between the environmental exposure and biological effects. At this stage, the relationship between these interactions and allergic disease too is not clear. Genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster are associated with variations in the PUFA in cell membranes (113); however, at this stage, the relationship with allergic disease needs to be further investigated. Similarly, the effect of variations in folate metabolism conferred by the MTHFR (C677T) genotype are not clear, with one study suggesting an association with allergic disease (86) and another unable to confirm this (87).

Failure to examine the relative influences and interactions between genetic or environmental factors could obscure potentially important causal pathways and could account for the many inconsistencies between studies. As these issues are understood better it is possible that optimal prevention strategies (for many possible exposures) may need to be individualized according to genetic propensity.

20.5 CONCLUSIONS AND PERSPECTIVES

As the source of all nutrients, diet is arguably one the most important environmental exposures in both the prenatal and postnatal periods. Maternal dietary exposures *in utero* have implications for most aspects of fetal development and further research is required to consolidate the preliminary evidence of effects on the developing immune system. In the postnatal period, the gastrointestinal tract is the most critical interface between the infant and its new environment, providing the largest exposure to microbial products, potential allergens and a broad range of dietary nutrients with immunomodulatory properties. These enteric exposures play a major role in the maturation of the mucosal immune system and have major implications for the success or failure of subsequent tolerance. Extensive "modernization" has had effects of many of these enteric exposures, including changes in colonization patterns and dietary composition. Just as no single gene is responsible for disease predisposition, it is equally unlikely that a single environmental change is responsible for the rise in disease. Future research strategies should attempt to account for complex multifactorial genetic and environmental interactions. We are now beginning to recognize that this may ultimately lead to

individualized early interventions that are tailored and targeted according to genetic predisposition, but this is still a distant horizon. In the meantime, diet should remain a research priority. It is highly likely that dietary strategies will play a major role in future strategies to prevent many diseases.

REFERENCES

1. Dunstan J, Mori TA, Barden A, Beilin LJ, Taylor A, Holt PG et al (2003) Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized controlled trial. J Allergy Clin Immunol 112:1178–1184
2. Noakes PS, Holt PG, Prescott SL (2003) Maternal smoking in pregnancy alters neonatal cytokine responses. Allergy 58:1053–1058
3. Matsuoka T, Matsubara T, Katayama K, Takeda K, Koga M, Furukawa S (2001) Increase of cord blood cytokine-producing T cells in intrauterine infection. Pediatr Int 43:453–457
4. Tang MLK, Kemp AS, Thorburn J, Hill D (1994) Reduced interferon gamma secretion in neonates and subsequent atopy. Lancet 344:983–985
5. Prescott SL, Holt PG (1998) Abnormalities in cord blood mononuclear cytokine production as a predictor of later atopic disease in childhood. Clin Exp Allergy 28:1313–1316
6. Smith M, Tourigny MR, Noakes P, Thornton CA, Tulic MK, Prescott SL (2008) Children with egg allergy have evidence of reduced neonatal CD4(+)CD25(+)CD127(lo/-) regulatory T cell function. J Allergy Clin Immunol 121:1460–1466, 1466.e1–1466.e7
7. Prescott SL, Noakes P, Chow B, Breckler L, Thornton CA, Hollams EM et al (2008) Presymptomatic differences in toll-like receptor function in infants who develop allergy. J Allergy Clin Immunol 122(2):391–399
8. Calder PC (1998) Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. Braz J Med Biol Res 31:467–490
9. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J et al (2008) In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest 118:3462–3469
10. Devereux G, Barker RN, Seaton A (2002) Antenatal determinants of neonatal immune responses to allergens. Clin Exp Allergy 32:43–50
11. Waterland RA, Michels KB (2007) Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr 27:363–388
12. Tsuji NM, Kosaka A (2008) Oral tolerance: intestinal homeostasis and antigen-specific regulatory T cells. Trends Immunol 29:532–540
13. Crabbe PA, Nash DR, Bazin H, Eyssen H, Heremans JF (1970) Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. Lab Invest 22:448–457
14. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y (1997) The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 159:1739–1745
15. Weatherill AR, Lee JY, Zhao L, Lemay DG, Youn HS, Hwang DH (2005) Saturated and polyunsaturated fatty acids reciprocally modulate dendritic cell functions mediated through TLR4. J Immunol 174:5390–5397
16. Shreffler WG, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson HA (2009) Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. J Allergy Clin Immunol 123:43.e7–52.e7
17. Ivarsson A, Hernell O, Stenlund H, Persson LA (2002) Breast-feeding protects against celiac disease. Am J Clin Nutr 75(5):914–921
18. Castro-Rodriguez JA, Garcia-Marcos L, Alfonseda Rojas JD, Valverde-Molina J, Sanchez-Solis M (2008) Mediterranean diet as a protective factor for wheezing in preschool children. J Pediatr 152:823–828, 828.e1–828.e2

19. Chatzi L, Torrent M, Romieu I, Garcia-Esteban R, Ferrer C, Vioque J et al (2008) Mediterranean diet in pregnancy is protective for wheeze and atopy in childhood. Thorax 63:507–513
20. Shaheen SO, Northstone K, Newson RB, Emmett P, Sherriff A, Henderson J (2009) Dietary patterns in pregnancy and respiratory and atopic outcomes in childhood. Thorax 64(5):411–417
21. Palmer DJ, Gold MS, Makrides M (2008) Effect of maternal egg consumption on breast milk ovalbumin concentration. Clin Exp Allergy 38:1186–1191
22. Verhasselt V, Milcent V, Cazareth J, Kanda A, Fleury S, Dombrowicz D et al (2008) Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. Nat Med 14(2):170–175
23. Penttila I (2006) Effects of transforming growth factor-beta and formula feeding on systemic immune responses to dietary beta-lactoglobulin in allergy-prone rats. Pediatr Res 59:650–655
24. Prescott SL, Smith P, Tang MLK, Palmer DJ, Sinn J, Huntley SJ et al (2008) The importance of early complementary feeding in the development of oral tolerance: concerns and controversies. Pediatr Allergy Immunol 19(5):375–380
25. Tarini BA, Carroll AE, Sox CM, Christakis DA (2006) Systematic review of the relationship between early introduction of solid foods to infants and the development of allergic disease. Arch Pediatr Adolesc Med 160:502–507
26. Poole JA, Barriga K, Leung DYM, Hoffman DR, Eisenbarth G, Rewers M et al (2006) Timing of initial exposure to cereal grains and the risk of wheat allergy. Pediatrics 117(6):2175–2182
27. Filipiak B, Zutavern A, Koletzko S, von Berg A, Brockow I, Grubl A et al (2007) Solid food introduction in relation to eczema: results from a four-year prospective birth cohort study. J Pediatr 151:352–358
28. Zutavern A, von Mutius E, Harris J, Mills P, Moffat S, White C et al (2004) The introduction of solids in relation to asthma and eczema. Arch Dis Child 89:303–308
29. Snijders BE, Thijs C, van Ree R, van den Brandt PA (2008) Age at first introduction of cow milk products and other food products in relation to infant atopic manifestations in the first 2 years of life: the KOALA Birth Cohort Study. Pediatrics 122:e115–e122
30. Alm B, Aberg N, Erdes L, Mollborg P, Pettersson R, Norvenius SG et al (2009) Early introduction of fish decreases the risk of eczema in infants. Arch Dis Child 94:11–15
31. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B et al (2008) Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 46:99–110
32. Greer FR, Sicherer SH, Burks AW (2008) Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 121:183–191
33. Host A, Halken S, Muraro A, Dreborg S, Niggemann B, Aalberse R et al (2008) Dietary prevention of allergic diseases in infants and small children. Pediatr Allergy Immunol 19:1–4
34. Smith KM, Eaton AD, Finlayson LM, Garside P (2000) Oral tolerance. Am J Respir Crit Care Med 162:S175–S178
35. Chehade M, Mayer L (2005) Oral tolerance and its relation to food hypersensitivities. J Allergy Clin Immunol 115:3–12, quiz 3
36. Kull I, Bergstrom A, Lilja G, Pershagen G, Wickman M (2006) Fish consumption during the first year of life and development of allergic diseases during childhood. Allergy 61:1009–1015
37. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR et al (2008) Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol 122:984–991
38. Fox AT, Sasieni P, du Toit G, Syed H, Lack G (2009) Household peanut consumption as a risk factor for the development of peanut allergy. J Allergy Clin Immunol 123:417–423
39. Zutavern A, Brockow I, Schaaf B, Bolte G, von Berg A, Diez U et al (2006) Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. Pediatrics 117:401–411
40. Fergusson DM, Horwood LJ, Shannon FT (1982) Risk factors in childhood eczema. J Epidemiol Community Health 36:118–122
41. Halken S, Host A (1996) Prevention of allergic disease. Exposure to food allergens and dietetic intervention. Pediatr Allergy Immunol 7:102–107

42. Lack G (2008) Epidemiologic risks for food allergy. J Allergy Clin Immunol 121:1331–1336
43. Calder PC (2003) N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. Lipids 38:343–352
44. Prescott SL, Dunstan JA (2007) Prenatal fatty acid status and immune development: the pathways and the evidence. Lipids 42:801
45. Calder PC (2009) Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie 91:791–795
46. Hughes DA, Southon S, Pinder AC (1996) (n-3) Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes in vitro. J Nutr 126:603–610
47. Khair-el-Din TA, Sicher SC, Vazquez MA, Wright WJ, Lu CY (1995) Docosahexaenoic acid, a major constituent of fetal serum and fish oil diets, inhibits IFN gamma-induced Ia-expression by murine macrophages in vitro. J Immunol 154:1296–1306
48. Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC (2001) Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. J Nutr 131:1918–1927
49. Endres S, Meydani SN, Ghorbani R, Schindler R, Dinarello CA (1993) Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. J Leukoc Biol 54:599–603
50. Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrill-Labrode A et al (1991) Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J Nutr 121:547–555
51. Prescott SL, Irvine J, Dunstan JA, Hii C, Ferrante A (2007) Protein kinase-C zeta: a novel "protective" neonatal T cell marker that can be up-regulated by allergy prevention strategies. J Allergy Clin Immunol 120:200–206
52. Baratelli F, Lin Y, Zhu L, Yang SC, Heuze-Vourc'h N, Zeng G et al (2005) Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. J Immunol 175:1483–1490
53. Peat J, Salome C, Woolcock A (1992) Factors associated with bronchial hyper-responsiveness in Australian adults and children. Eur Respir J 5:921–929
54. Hodge L, Salome C, Peat J, Haby M, Xuan W, Woolcock A (1996) Consumption of oily fish and childhood asthma risk. Med J Aust 164:137–140
55. Oddy WH, de Klerk NH, Kendall GE, Mihrshahi S, Peat JK (2004) Ratio of omega-6 to omega-3 fatty acids and childhood asthma. J Asthma 41:319–326
56. Takemura Y, Sakurai Y, Honjo S, Tokimatsu A, Gibo M, Hara T et al (2002) The Relationship between Fish Intake and the Prevalence of Asthma: The Tokorozawa Childhood Asthma and Pollinosis Study. Prev Med 34:221
57. Mihrshahi S, Peat JK, Marks GB, Mellis CM, Tovey ER, Webb K et al (2003) Eighteen-month outcomes of house dust mite avoidance and dietary fatty acid modification in the Childhood Asthma Prevention Study (CAPS). J Allergy Clin Immunol 111:162–168
58. Marks GB, Mihrshahi S, Kemp AS, Tovey ER, Webb K, Almqvist C et al (2006) Prevention of asthma during the first 5 years of life: a randomized controlled trial. J Allergy Clin Immunol 118:53–61
59. Prescott SL, Wiltschut J, Taylor A, Westcott L, Jung W, Currie H et al (2008) Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. Allergy 63:1481–1490
60. Calvani M, Alessandri C, Sopo SM, Panetta V, Pingitore G, Tripodi S et al (2006) Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. Pediatr Allergy Immunol 17:94–102
61. Sausenthaler S, Koletzko S, Schaaf B, Lehmann I, Borte M, Herbarth O et al (2007) Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. Am J Clin Nutr 85:530
62. Salam MT, Li YF, Langholz B, Gilliland FD (2005) Maternal fish consumption during pregnancy and risk of early childhood asthma. J Asthma 42:513–518
63. Newson RB, Shaheen SO, Henderson AJ, Emmett PM, Sherriff A, Calder PC (2004) Umbilical cord and maternal blood red cell fatty acids and early childhood wheezing and eczema. J Allergy Clin Immunol 114:531–537

64. Olsen S, Sorenson J, Secher N, Hedegaard M, Henriksen T (1992) Randomized controlled trial of effect of fish oil supplementation on pregnancy duration. Lancet 339:1003–1007
65. Olsen SF, Osterdal ML, Salvig JD, Mortensen LM, Rytter D, Secher NJ et al (2008) Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. Am J Clin Nutr 88:167–175
66. Furuhjelm C, Warstedt K, Larsson J, Fredriksson M, Bottcher MF, Falth-Magnusson K et al (2009) Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. Acta Paediatr 98(9):1461–1467
67. Gilliland FD, Berhane KT, Li Y-F, Gauderman WJ, McConnell R, Peters J (2003) Children's lung function and antioxidant vitamin, fruit, juice, and vegetable intake. Am J Epidemiol 158:576–584
68. Bodner C, Godden D, Brown K, Little J, Ross S, Seaton A (1999) Antioxidant intake and adults onset wheeze: a case control study. Eur Respir J 13:22–30
69. Forastiere F, Pistelli R, Sestini P, Fortes C, Renzoni E, Rusconi F et al (2000) Consumption of fresh fruit rich in vitamin C and wheezing symptoms in children. SIDRIA Collaborative Group, Italy (Italian Studies on Respiratory Disorders in Children and the Environment). Thorax 55:283–288
70. Okoko B, Burney P, Newson R, Potts J, Shaheen S (2007) Childhood asthma and fruit consumption in South London. Eur Respir J 29(6):1161–1168
71. Devereux G, Turner SW, Craig LCA, McNeill G, Martindale S, Harbour PJ et al (2006) Low maternal vitamin E intake during pregnancy is associated with asthma in 5-year-old children. Am J Respir Crit Care Med 174:499–507
72. Nja F, Nystad W, LodrupCarlsen KC, Hetlevik O, Carlsen K-H (2005) Effects of early intake of fruit or vegetables in relation to later asthma and allergic sensitization in school-age children. Acta Paediatr 94:147–154
73. Tan PH, Sagoo P, Chan C, Yates JB, Campbell J, Beutelspacher SC et al (2005) Inhibition of NF-kappa B and oxidative pathways in human dendritic cells by antioxidative vitamins generates regulatory T cells. J Immunol 174:7633–7644
74. Utsugi M, Dobashi K, Ishizuka T, Endou K, Hamuro J, Murata Y et al (2003) c-Jun N-terminal kinase negatively regulates lipopolysaccharide-induced IL-12 production in human macrophages: role of mitogen-activated protein kinase in glutathione redox regulation of IL-12 production. J Immunol 171(2):628–635
75. Murr C, Schroecksnadel K, Winkler C, Ledochowski M, Fuchs D (2005) Antioxidants may increase the probability of developing allergic disease and asthma. Med Hypotheses 64:973–977
76. Martindale S, McNeill G, Devereux G, Campbell D, Russell G, Seaton A (2005) Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life. Am J Respir Crit Care Med 171:121–128
77. Devereux G, Litonjua AA, Turner SW, Craig LC, McNeill G, Martindale S et al (2007) Maternal vitamin D intake during pregnancy and early childhood wheezing. Am J Clin Nutr 85:853–859
78. Litonjua AA, Rifas-Shiman SL, Ly NP, Tantisira KG, Rich-Edwards JW, Camargo CA Jr et al (2006) Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. Am J Clin Nutr 84:903–911
79. Farchi S, Forastiere F, Agabiti N, Corbo G, Pistelli R, Fortes C et al (2003) Dietary factors associated with wheezing and allergic rhinitis in children [see comment]. Eur Respir J 22:772–780
80. Lee BE, Hong YC, Lee KH, Kim YJ, Kim WK, Chang NS et al (2004) Influence of maternal serum levels of vitamins C and E during the second trimester on birth weight and length. Eur J Clin Nutr 58:1365–1371
81. Scaife AR, McNeill G, Campbell DM, Martindale S, Devereux G, Seaton A (2006) Maternal intake of antioxidant vitamins in pregnancy in relation to maternal and fetal plasma levels at delivery. Br J Nutr 95:771–778
82. Stone WL, LeClair I, Ponder T, Baggs G, Reis BB (2003) Infants discriminate between natural and synthetic vitamin E. Am J Clin Nutr 77:899–906
83. Miller RL, Ho SM (2008) Environmental epigenetics and asthma: current concepts and call for studies. Am J Respir Crit Care Med 177:567–573
84. Haberg SE, London SJD, Stigum H, Nafstad P, Nystad W (2009) Folic acid supplements in pregnancy and early childhood respiratory health. Arch Dis Child 94(3):180–184

85. Matsui EC, Matsui W (2009) Higher serum folate levels are associated with a lower risk of atopy and wheeze. J Allergy Clin Immunol 123:1253e.2–1259.e2
86. Husemoen LL, Toft U, Fenger M, Jorgensen T, Johansen N, Linneberg A (2006) The association between atopy and factors influencing folate metabolism: is low folate status causally related to the development of atopy? Int J Epidemiol 35:954–961
87. Granell R, Heron J, Lewis S (2008) Davey Smith G, Sterne JA, Henderson J. The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort. Clin Exp Allergy 38:320–328
88. Johannsen H, Prescott SL (2009) Practical prebiotics, probiotics and synbiotics for allergists: how useful are they? Clin Exp Allergy 39(12):1801–1814
89. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G (2006) A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Arch Dis Child 91:814–819
90. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G (2008) Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. J Nutr 138((6):1091–1095
91. Prescott SL, Björkstén B (2007) Probiotics for the prevention or treatment of allergic diseases. J Allergy Clin Immunol 120:255–262
92. Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M (2001) Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol 108:516–520
93. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E (2001) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J Allergy Clin Immunol 107:129–134
94. Osborn D, Sinn J (2007) Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database Syst Rev 4:CD006475
95. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E (2001) Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial. Lancet 357:1076–1079
96. Kocourková I, Zádníkováa R, Zižkab J, Rosovác V (2007) Effect of oral application of a probiotic E. coli strain on the intestinal microflora of children of allergic mothers during the first year of life. Folia Microbiol 52(2):189–193
97. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T et al (2007) Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: A randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol 119:192–198
98. Wickens K, Black PN, Stanley TV, Mitchell E, Fitzharris P, Tannock GW et al (2008) A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol 122:788–794
99. Niers L, Martín R, Rijkers G, Sengers F, Timmerman H, van Uden N et al (2009) The effects of selected probiotic strains on the development of eczema (The PandA study). Allergy 64(9):1349–1358
100. Dotterud C, Oien T, Storro O, Johnsen R (2009) Probiotic supplementation given to mothers in primary prevention of allergic diseases in early childhood – a randomized controlled trial in a non-selected population. Allergy 64(suppl 90):64
101. Taylor AL, Dunstan JA, Prescott SL (2007) Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. J Allergy Clin Immunol 119:184–191
102. Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, Bjorksten B et al (2007) Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol 119:1174–1180
103. Kopp MV, Hennemuth I, Heinzmann A, Urbanek R (2008) Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. Pediatrics 121:e850–e856
104. Soh SE, Aw M, Gerez I, Chong YS, Rauff M, Ng YP et al (2009) Probiotic supplementation in the first 6 months of life in at risk Asian infants – effects on eczema and atopic sensitization at the age of 1 year. Clin Exp Allergy 39(4):571–578

105. Huurre A, Laitinen K, Rautava S, Korkeamaki M, Isolauri E (2008) Impact of maternal atopy and probiotic supplementation during pregnancy on infant sensitization: a double-blind placebo-controlled study. Clin Exp Allergy 38:1342–1348
106. Rautava S, Kalliomaki M, Isolauri E (2002) Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. J Allergy Clin Immunol 109:119–121
107. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T et al (2009) Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. J Allergy Clin Immunol 123:335–341
108. Kalliomaki M, Salminen S, Poussa T, Isolauri E (2007) Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. J Allergy Clin Immunol 119:1019–1021
109. Rautava S, Arvilommi H, Isolauri E (2006) Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. Pediatr Res 60:221–224
110. West CE, Hammarstrom ML, Hernell O (2009) Probiotics during weaning reduce the incidence of eczema. Pediatr Allergy Immunol 20(5):430–437
111. Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B et al (2002) Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. Arch Dis Child Fetal Neonatal Ed 86:F178–F181
112. Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C et al (2004) Toll-like receptor 2 as a major gene for asthma in children of European farmers. J Allergy Clin Immunol 113:482–488
113. Rzehak P, Heinrich J, Klopp N, Schaeffer L, Hoff S, Wolfram G et al (2009) Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. Br J Nutr 101:20–26
114. Xie L, Innis SM (2008) Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. J Nutr 138:2222–2228

21 Feeding in the First Month of Life and Prevention of Allergy

J. P. Chouraqui

Key Points

- Allergic diseases depend on a complex interaction between genetic factors, environmental exposure to allergens and gut microbiota.
- Allergy results from immune reactions triggered by allergens in the digestive or in the respiratory tract.
- The frequency and severity of allergy are increasing.
- Preventive measures derive from mechanisms implicated in the development of allergy. The easiest intervention process is the reduction of the allergenic load.
- Following birth, the primary prevention strategy relies first on the detection of at-risk newborns, that is, with allergic first degree relatives.
- In this targeted population, as well as for the general population, exclusive breastfeeding is recommended for 4–6 months.
- In the absence of breastfeeding, an hydrolyzed formula is suggested for which foods without allergenicity can be used for feeding at-risk newborns.
- Complementary feeding should not be started before the age of 4–6 months, which seems partially efficacious on early allergy.
- Probiotics, prebiotics as well as n-3 fatty acids have not yet demonstrated any definitive protective effect.

Key Words: Prevention, allergy, diet, breast milk, formula, infant, probiotics.

Allergies are a common and increasing health problem in Western countries as well as in developing ones. Allergy can be defined as a detrimental immune-mediated hypersensitivity response to common environmental substances, almost always proteins, present in foods, mites, pollens, hairs or feathers of animals and certain medications. However, despite the large extent of dietary antigenic exposure, only a small percentage

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_21

of individuals will develop allergy, and more particularly food allergy (FA), which is considered as the first manifestation of childhood allergic disease leading to the "allergic march" (1). The development of food allergy depends on several factors, including genetic factors and early exposure to allergenic proteins in the diet, food protein uptake and handling and the development of tolerance. Oral tolerance refers to a state of active inhibition of immune responses to an antigen by means of prior exposure to that antigen through the oral route (2). Several factors can influence tolerance induction. Some are antigen-related, and others are inherent to the host and his environment such as the intestinal microbiota in early infancy. It is becoming necessary to define the causal pathways and develop strategies to avert the process that leads to allergy.

The prevalence of FA in children is considered to be presently at 4–6% (3, 4). The clinical manifestations mainly concern the gastrointestinal tract, the skin and/or the respiratory tract, which are at the interface between the internal milieu and the environment. Approximately 90% of all allergic reactions to foods are caused by eight foods: milk, egg, peanuts, tree nuts, fish, soya, wheat and shellfish. The first months of life appear to be a critical period during which exogenous stimuli prime the immune system.

Due to the well-known clinical course of FA in early childhood (the allergy march), the greatest possible impact of primary dietary allergy prevention may be on its development, particularly for cow's milk allergy (CMA), and atopic dermatitis (AD). Although there has been much conjecture on how to influence the infantile immune response to reduce the likelihood of allergen sensitization and subsequent allergic disease, effective specific preventive modalities are still matter of debate. Studies on this field are difficult as they need to take into consideration the prevalence of allergy in the community, relatives' allergy status, maternal diet, exposure to environmental allergens, parental smoking and other potentially confounding variables.

The role of primary prevention of allergic diseases has nevertheless been reviewed by several groups of experts as position papers giving guidelines for developing strategies (5–7). The following review aims at summarizing the recent research contributing to the current understanding of dietary interventions for preventing or delaying atopic diseases (target population, breastfeeding, hypoallergenic infant formula, introduction of solid foods, or specific complementary foods).

21.1 GENETICS PLUS ENVIRONMENT CONTRIBUTE TO ALLERGY

There is a major genetic contribution to allergy and a number of candidate genes have been identified for both eczema and asthma (8). Infants with at least one first-degree relative (parent or sibling) with documented allergic disease are more likely to develop allergic diseases before the age of 7 years (20–40% risk) compared with those with no family history of atopy (12% risk) (9). The risk appears to be higher if both parents are allergic (40–70%), and the risk is also higher if the mother (rather than the father) has allergic disease. The analysis of family history of atopy identifies the "high risk infants". It is easier to perform and seems more efficient than the search for biological markers, such as elevated IgE in the umbilical cord (5–13). Moreover, a protective effect of breastfeeding on allergic diseases (AD and asthma) has only been established for infants

with a family history of allergy (see further on). This way it is possible to define the target population likely to benefit from preventive measures, even if approximately 50% of the children suffering from an allergic disease do not have any family history of atopy (14). Genetics alone cannot account for the significant increase in the prevalence of allergic diseases during the past decades. A series of complex gene–environment interactions occurring either during pregnancy or during the first few months of life also plays a crucial role in determining the time of onset, maintenance, and severity of allergies. Healthy infants typically adopt a type 1 T helper cell (Th1) cytokine profile at birth, while atopic infants maintain the type 2 T helper cell (Th2) skewed environment normally associated with pregnancy.

21.2 MATERNAL DIET DURING PREGNANCY

Some dietary protein can be detected in the fetal circulation and amniotic fluid (15). The fetus seems able to mount an immunologic response to foods and other allergens. Occasionally, specific IgE responses to foods and frequently T-cell responses to milk and egg proteins and aeroallergens are found in blood samples from fetus and newborn, as well as in some amniotic fluids (16–18) Positive skin tests to peanuts or eggs were observed at birth before any direct contact with food (19). These biological activities are not always related to the subsequent development of clinical food allergy. Nevertheless, very early manifestations of allergy to cow's milk protein have been reported in the form of colitis,which occurs before the third day of life (20). Attempts to prevent allergy with maternal allergenic foods avoidance during the third trimester failed to reduce food allergy or any other atopic disorder or sensitization from birth through 5 years (21–24). In addition, maternal weight gain during pregnancy was compromised by such a maternal exclusion diet (25). Although some of the previous guidelines (11–13) have suggested that pregnant women should avoid peanuts, a more recent study has reported that there is no association between the maternal consumption of peanuts during pregnancy and childhood peanut allergy (26). Therefore, the general consensus is that there is no reason to follow a specific diet during pregnancy (5–7).

Data about the benefit of an adequate intake of vitamin D during pregnancy are inconsistent. Two recent studies reported reduced early childhood wheezing in offspring to mothers who had higher compared with lower vitamin D intakes (27, 28) while another was suggestive of a greater prevalence of early eczema and asthma in offspring to mothers of higher 25-hydroxyvitamin D concentration during pregnancy (28). These data as others obtained in adults could suggest that there may be a threshold effect with both low and high vitamin D levels associated with elevated IgE concentration (29).

Maternal diets high in omega-3 Long Chain Poly-Unsaturated Fatty Acids (LCPUFAs) during pregnancy have been thought to have a protective effect against the development of allergies in the newborn (31). A randomized clinical trial of maternal fish oil supplementation during pregnancy demonstrated a significant decrease in cord blood concentrations of Th-2 cytokines (IL-4 and IL-13) as well as increased levels of oral tolerance-inducing TGF-β (32). Recent studies (33–35) showed a protective effect of maternal fish intake during pregnancy against eczema and asthma but not on food allergy in high-risk infants.

21.3 ROLE OF HUMAN MILK AND BREASTFEEDING ON THE DEVELOPMENT OF ATOPIC DISEASE

21.3.1 Protective Factors

Breast milk provides the most appropriate source of nutrition for the young infant as it contains a species-specific mixture of nutrients, but also growth factors and protective maternal antibodies. It could be speculated that the presence of food-specific secretory immunoglobulin A (IgA) antibodies in breast milk may modulate, perhaps in relation to their level, the host immune response to ingested food antigens (36). Such a protective effect was demonstrated with mature milk in some (37), but not confirmed by others (38, 39). The presence of specific IgA in breast milk should otherwise play a role in developing oral tolerance (37). Other immune-modulating breast-milk compounds like polyamines, cytokines and TGF-β are also suspected to mediate a protective effect by stimulating IgA production, reducing the production of IgE and inducing the mechanisms of oral tolerance (39–43). The concentration of TGF-β in milk from atopic mothers is lower than in that from non-atopic ones (43). The level of TGF-β2 in milk from mothers who were given a probiotic (*Lactobacillus* GG) during pregnancy and breastfeeding was higher than in milk from those who received placebo (44). At 2 years, the risk of eczema in the offspring was 15% in the probiotic group versus 47%. Furthermore, the *Bifidobacterium*-rich microbiota of breast-fed infants stimulate T-regulatory responses via the innate immune system and Toll-like receptors (45).

Breast-milk is also rich in LCPUFAs based upon the composition of the mother diet. A high omega 3/omega 6 LCPUFAs ratio would favor a Th1 response, while a weak ratio a Th2 one (46). Breast milk is usually rich in omega 6, but allergic mothers would have a lower level (47). Thirteen children undergoing an AD were consuming milk higher in saturated fatty acids and lower in omega 3 fatty acids than 21 healthy children (48).

Breast milk is a complex food, the composition of which varies from one woman to another but also according to the duration of breastfeeding. These variations probably explain, at least partially, the contradictory clinical results. On the other hand, it must be kept in mind that, as known since over 25 years, most of the food antigens ingested by a mother can be detected in her milk and cause clinical problems (49–56).

21.3.2 Breastfeeding

The effectiveness of breastfeeding on the development of atopic disease is controversial because of methodological bias in relating studies with many confounding variables (57, 58). In general, owing to ethical considerations these have been nonrandomized, retrospective, or observational in design with varying duration of exclusive breastfeeding, and diagnostic criteria for atopic outcomes. Moreover the choice of breastfeeding is more common in families with allergic risk, which can lead to a reverse causation.

An early study, published in 1930 (59) enrolling a cohort of 20,000 children, showed that the prevalence of eczema at the age of 9 months was seven times lower in breastfed children than in those fed cow's milk. Several studies followed, resumed in two more recent meta-analyses (60, 61) reporting a protective effect of exclusive breast feeding for 4–6 months on the risk of allergic disease (eczema and asthma) in early childhood, mainly in high-risk infants. A review of the literature (62) concludes that more than half

of the studies demonstrate a protective role of breastfeeding. This effect is influenced by the duration of breastfeeding and maternal allergy status (63). But some studies do not confirm the benefits of prolonged breastfeeding (3, 64, 65) and indeed, some even suggest that breastfeeding might increase the risk of allergy (66–69). In fact, the higher incidence of atopic dermatitis in the breastfed group seems to be the result of a reverse causal relationship, thus, mothers whom infants already developed atopic dermatitis or were at risk of developing allergy were more likely not only to breastfeed but also to breastfeed for a longer period of time. Anyway there is strong evidence that breastfeeding for at least 4 months, compared with infant formula designed with intact cow's milk protein, prevents or delays the occurrence of atopic dermatitis, wheezing and CMA.

21.3.2.1 Atopic Dermatitis

A 2001 meta-analysis of 18 prospective studies among 208 listed ones compared the incidence of atopic dermatitis in breastfed infants versus formula-fed infants (60). Overall, there was a protective effect of 3 months exclusive breastfeeding (odds ratio (OR): 0.68; 95% confidence interval (CI): 0.52–0.88). The stronger effect has been shown for infants with a family history of allergy (OR: 0.58; 95% CI: 0.4–0.92), whereas no protective effect was seen in children without the risk of developing allergy (OR: 1.43; 95% CI: 0.72–2.86). Two more recent Swedish studies were contradicting: the first one (70) found no effect of exclusive breastfeeding for 4 months on the incidence of atopic dermatitis in the first year of life in an infant with or without a family history of atopic disease, whereas the second showed that exclusive breastfeeding for more than 4 months reduced the risk of atopic dermatitis at 4 years of age (OR: 0.78; 95% CI: 0.63–0.96) (71). The report from the German Infant Nutritional Intervention (GINI) Program (72) also demonstrated that exclusive breastfeeding for 4 months in high-risk infants reduces the incidence of atopic dermatitis, when compared with breastfeeding with supplemental cow's milk formula or cow's milk formula (CMF). The advantages of breastfeeding are less clear for infants who are not selected for high risk of developing atopic disease, as shown in the noninterventional arm of the GINI Program.

21.3.2.2 Asthma

The protective effects of human milk on the development of asthma are also debated. As for DA, a 2001 meta-analysis of 12 prospective studies found that exclusive breastfeeding for at least 3 months decrease the incidence of asthma between 2 and 5 years of age (OR: 0.70; 95% CI: 0.60–0.81) (61). It was a more clear-cut effect when the analysis was limited to at-risk children (OR: 0.52; 95% CI: 0.35–0.79). No benefit was obtained in children from families without a history of atopic disease (OR: 0.99; 95% CI: 0.48–2.03). Conversely, the Cochrane review did not find any benefit of exclusive breastfeeding beyond 3 months on the incidence of asthma in families not preselected for a history of atopic disease (73). In the Tucson Children's Respiratory Study (74), a cohort of 1,246 children was followed from birth to 13 years of age. During the first 2 years of life, exclusive breastfeeding was associated with significantly lower rates of recurrent wheezing of infancy (OR: 0.45; 95% CI: 0.2–0.9). The risk of developing asthma between 6 and 13 years of age was, however, increased in exclusively breastfed infants with atopic disease whose mothers had asthma (OR: 5.7; 95% CI: 2.3–14.1). Infants whose mothers had asthma were at greatest risk of developing asthma by 13 years

of age if they had been breastfed exclusively for 4 months (OR: 8.7; 95% CI: 3.4–22.2). There was no increased risk of developing asthma in breastfed children of mothers without asthma. In a long-term longitudinal study from New Zealand, 1,037 children from a general population (not selected for risk of allergic disease) were followed from 3 to 26 years of age (67). Breastfeeding for more than 4 weeks significantly increased the risk of developing asthma at 9 years (OR: 2.40; 95% CI: 1.36–4.6) and at 21 years (OR: 1.83; 95% CI: 1.35–2.47), without any relationship to the presence of maternal atopic disease. The Canadian Childhood Asthma Primary Prevention Study gave more relevant results showing a significant lower prevalence of asthma in a cohort of high-risk children as a result of a multifaceted intervention that has included encouragement of breastfeeding and avoidance of house dust, pets and tobacco smoke (12.9% vs. 25.0% in the control group; adjusted risk ratio, 0.39; 95% CI: 0.22–0.71) (75).

In summary, breastfeeding seems to decrease the wheezing episode seen in younger children (<4 years of age) that are often associated with respiratory infections. At the present time, it is not possible to conclude that exclusive breastfeeding does protect young infants who are at risk of atopic disease from developing asthma in the long term (>6 years of age).

21.3.2.3 Food Allergy

Food allergy, similar to atopic dermatitis and asthma which are more closely associated with the development of food allergy, is more likely to occur in infants with a family history of atopic disease. It is difficult to subsequently sort out the effect of breastfeeding on the development of food allergy. Investigations of the role of breastfeeding on the outcomes

of allergies to specific foods have been rare, and the results may have been influenced by the length and degree of breastfeeding exclusivity, the age of introduction of solid foods and the presence of maternal atopic disease. A review of the available studies concluded, in 2004, that exclusive breastfeeding for at least 4 months in infants who are at risk of developing atopic diseases was associated with a lower cumulative incidence of CMA until 18 months of age (76). A previous Cochrane review including only one study concluded that at least 4 months of exclusive breastfeeding did not protect against food allergy at 1 year of age (73). Overall, firm conclusions about the role of breastfeeding in either preventing or delaying the onset of specific food allergies will need further well conducted studies.

21.3.3 Maternal Avoidance of Allergenic Foods During Lactation

Dietary food allergens, including cow milk proteins, peanuts, and egg, at a concentration of few ng/l, can be detected 1–6 h after being ingested in breast milk (49–56). This secretion does not depend on the presence of maternal atopic disease. Up to 6% of high risk breastfed infants can develop an IgE sensitisation (77, 78) while food allergy concern 0.004–0.5% of the overall breastfed infants (79–82). In an attempt to reduce the incidence of allergy in breastfed infants, it has been thought that maternal dietary avoidance could reduce the secretion of intact food allergens into breast milk (83). Many studies have evaluated the effect of maternal food allergen avoidance diets during lactation for preventing atopic disease in high-risk infants and noted significant reductions in eczema in the maternal diet groups by 3, 6 and 18 months (84, 85), but not at

10 years (86). A relatively recent Cochrane review concluded there was insufficient evidence that antigen avoidance during lactation was beneficial in preventing atopic disease in the breastfed infant, with the exception of atopic dermatitis (25). Considering the uncertainty of the effect of lactation diets for primary prevention, it might be feasible to implement maternal lactation avoidance diets only after individual evaluation of each family's atopic risk and circumstances. In such a case it should be recommended that mothers take supplemental calcium (up to 1,000 mg daily) and vitamin D during restricted lactation diets.

In summary, although the size of the protective effect of exclusive breastfeeding against food allergy is still controversial, breastfeeding for 4–6 months is recommended by most experts panel not only because of its likely protective effects against allergic disease in early life and but also because of its nutritional and anti-infective properties (5–7). This recommendation is consistent with that of the World Health Organization (WHO), which advocates exclusive breastfeeding for all infants for at least 6 months. Further studies on the late effects of exclusive breastfeeding on allergic diseases are required.

21.4 EFFICIENCY OF HYDROLYZED FORMULA ON PREVENTING THE DEVELOPMENT OF ATOPIC DISEASE

The dogma that allergen avoidance would be the main strategy for food allergy prevention leads to the use of hypoallergenic (HA) formula. These formulas are termed protein hydrolysates which are either partial or extensive depending on the extent of hydrolysis and ultra filtration to which they are subjected. It has previously been assumed that allergenicity lessens as hydrolysis and filtration become more extensive (87). Partially hydrolyzed formula (phF) contains reduced oligopeptides that have a molecular weight of generally less than 5,000 Da, whereas extensively hydrolyzed formula (ehF) contains only peptides that have a molecular weight of less than 3,000 kDa (88–90). Significant clinical hypoallergenicity would occur when whey and casein antigenic equivalents are reduced approximately 105–106 (91). On the basis of current knowledge, *in vitro* characterization of the size of peptides (molecular weight) or biological determination of allergenicity cannot predict the immunogenicity or allergenicity of these products, thereby ESPGHAN's and ESPACI's panel of experts ask for appropriate preclinical testing and above all prospective controlled, randomized, double-blind trials (12). As emphasized by expert groups, the evaluation of hypoallergenic formula should be made per brand of hydrolyzed formula and not by source of protein (casein or whey) because the amount of residual protein but also the results of clinical trials may vary considerably between different hydrolyzed products (5, 92). The data from the GINI studies were surprising leading to the conclusion that the degree of hydrolysis, the resulting molecular weight, and the protein source have little predictive value for the immunogenic or allergenic effect or the capacity to prevent the onset of allergies suggesting the possibility of a tolerogenic effect of milk peptides in phF on early immune competence (93–95).

Most of the more than 60 studies concerning the efficiency of phF and ehF for the prevention of atopic disease done over the last 20 years have been conducted on infants at high risk of developing allergy. Using very strict criteria, a 2006 Cochrane review selected only 14 randomized or quasirandomized trials in term infants in which the use

of phF or ehF were compared with the use of human milk or an adapted cow milk formula (96). No significant difference in infant allergy or childhood CMA was reported in the trials comparing early, short-term (during the stay in the maternity yard), HA formula to CMF feeding. No long-term studies have compared hydrolyzed formula to exclusive breastfeeding; thereby there is no evidence that the use of such formulas is any better than human milk in the prevention of atopic disease. Meta-analysis of ten eligible studies comparing prolonged feeding with HA formula in high-risk infants found a significant reduction in infant allergy (seven studies, Relative Risk RR: 0.79, 95% CI: 0.66–0.94) but not in childhood. No differences were observed regarding the occurrence of eczema, asthma or rhinitis. Three more studies examined prolonged feeding of ehF compared with phF in 411 infants at a high risk of developing allergy and found no difference in the incidence of atopic dermatitis between the two feeding groups. Although associated with a similar protective effect as phF, ehF is more expensive, worse tasting and therefore mainly considered a treatment formula for infants with established CMA.

The GINI studies are to date the largest randomized trial comparing the effects of phF, ehF and cow's milk formula (CMF) (93–95). They have so far provided the most convincing data for a protective effect of phF and ehF on atopic dermatitis, as compared with CMF. In the interventional arm of this study, 945 newborn infants were identified as being at high risk of developing atopic disease and were enrolled in a longitudinal, prospective study through 6 years of age. After initial breastfeeding lasting less than 4 weeks, infants were randomly assigned to receive either a ph-whey-based formula, or an eh-whey-based formula, or an eh-casein-based formula or CMF. The incidence of atopic dermatitis was significantly reduced by more than 40% at the age of 1 year and 3 years and by 30% at 6 years in those using eh-casein-based formula (at 1 year, RR: 0.42, 95% CI: 0.22–0.79; at 3 years, RR = 0.53, 95% CI: 0.32–0,88; at 6 years, RR: 0.79, 95% CI: 0.64–0.97) and the ph-whey-based formula (at 1 year, RR: 0.56, 95% CI: 0.32–0.99; at 3 years RR = 0.60, 95% CI: 0.37–0.97; at 6 years, RR: 0.71, 95% CI: 0.58–0.88) but not the eh-whey-based formula, compared with the incidence in those in the CMF group. No significant effect on other allergic manifestations was found. These data confirm a long-term allergy-preventive effect of the tested phF on DA. More well-designed studies are needed to determine if any of the available hydrolyzed formulas on the market have any effect on the incidence of atopic disease later in childhood and adolescence and whether the relatively modest effects of the use of ehF or phF in early childhood can be confirmed and are sustained. Additional studies are also needed among unselected infants or infants at low risk.

The preventive effects of amino acid-based formula have never been assessed. The high cost of this kind of formula prohibits moreover its widespread use in allergy prevention.

Soy formulas, on the other hand, have a long history of use for treatment of atopic disease in infants but also with the aim to prevent it (59). A recent Cochrane review based on three randomized clinical trials showed no significant preventive effect of soy formula on the development of allergic disease (97). Moreover, over recent years, there has been a growing concern about the safety of exclusive soy feeding in infants under 6 months, as reflected in several position papers which do not recommend its use for the purpose of allergy prevention (5–7, 98, 99).

21.5 ROLE OF IMMUNE-MODULATING MICRONUTRIENTS

Several immune-modulating micronutrients seem to be relevant for the prevention of food allergy, including omega-3 long-chain polyunsaturated fatty acids(LCPUFA), several antioxidants and vitamin D.

21.5.1 Omega 3 LCPUFA

A high proportion of omega 6 PUFA in the diet and low intakes of omega 3 PUFA have been suggested as risk factors for allergic diseases. Fish oils are particularly rich in omega 3 fatty acids, and the potential anti-inflammatory active ingredient in these is eicosapentaenoic acid, which may reduce inflammation and favor a Th1 profile. Studies reviewed by Heine (100) or Anadan et al. (101) have shown that omega 3 dietary supplementation during pregnancy can modify immune responses in infants and may reduce subsequent infant allergy as discussed above. High levels of omega 3 fatty acids in the diet have found to be associated with a decreased risk of allergic sensitization and allergic rhinitis and regular fish consumption before the age of 12 months appears to be associated with a reduced risk of allergic sensitization to food and inhalant allergens during the first 4 years of life. But contrary to these evidences a recent meta-analysis failed to identify any consistent or clear benefits associated with use of omega 3 (atopic eczema: RR = 1.10 (95% CI: 0.78–1.54); asthma: RR = 0.81 (95% CI: 0.53–1.25); allergic rhinitis: RR = 0.80 (95% CI: 0.34–1.89) or food allergy RR = 0.51 (95% CI: 0.10–2.55)) or omega 6 oils (atopic eczema: RR = 0.80 (95% CI: 0.56–1.16)) for the prevention of clinical disease (101).

21.5.2 Antioxidants

The role of antioxidants in the prevention of allergies has recently been reviewed (100). Maternal vitamin A intake during pregnancy and the early neonatal period in mice reduces the risk of food sensitization. Mediterranean diets, rich in fruits, vegetables, olive oil, nuts and subsequently antioxidants, may have positive effects on allergy prevention. A study in school children (aged 7–18 years) in rural Crete suggested a reduced risk for asthma and rhinitis, whereas no protective effects were found for food allergy. No data are available for infants.

21.5.3 Vitamin D

Vitamin D3 is thought to inhibit the maturation of dendritic cells and may impede the development of Th1 responses. Data on this topic are inconsistent (27–30). Some have proposed an increased occurrence of allergies by high dose of vitamin D supplementation early in life while others suggest a reduced risk of wheezing in offspring to mothers with higher vitamin intake. Methodological limitations in these studies (loss to follow-up and residual confounding) might explain these discrepancies; it is also possible that the inconsistency reflects dual influences of vitamin D on allergic conditions as suggested by the U-shaped relation reported in a recent study (31).

21.6 ROLE OF INTRODUCTION OF COMPLEMENTARY FOODS ON ATOPIC DISEASE

The age when solid foods are introduced to infants has varied greatly during the past century. Few studies have examined the timing of the introduction of complementary foods as an independent risk factor for atopic disease in breastfed or formula-fed infants. In 2001, the World Health Organization issued a revised global recommendation that mothers exclusively breastfeed until 6 months of age. A very important determinant of the appropriate age for weaning is the physiological maturity of gastrointestinal and renal function. There are concerns, firstly that the high permeability of the young infant's digestive tract may permit large foreign proteins to penetrate and provoke immune sensitization. Systematic review of the available evidence suggests that early solid feeding before 4 months of age may increase the risk of eczema but there are little data supporting an association between early solid feeding and other allergic conditions (102). A large German birth cohort study (103) found that a delayed introduction of solids (past 4 or 6 months) was not associated with decreased odds for asthma, allergic rhinitis, or sensitization against food or inhalant allergens at 6 years of age. The relationship between the timing of solid food introduction and eczema was not clear. There was no protective effect of a late introduction of solids or a less diverse diet within the first 4 months of life. However, when children without early skin or allergic symptoms were considered, eczema was significantly more frequent in children who received a more diverse diet within the first 4 months. Thus although solid foods should not be introduced before 4–6 months of age, there is no current convincing evidence that delaying their introduction beyond this period has a significant protective effect on the development of atopic disease regardless of whether infants are fed cow milk protein formula or human milk. This includes delaying the introduction of foods that are considered to be highly allergic, such as fish, eggs, and foods containing peanut protein. The more recent findings may support the concept of a "window period" for tolerance induction between 4 and 6 months of age.

21.7 ROLE OF INTESTINAL MICROFLORA IN INFANCY AND RATIONALE FOR THE USE OF PROBIOTIC BACTERIA IN PREVENTION OF ALLERGIC DISEASES

The gut mucosa constitutes not only the largest internal interface of the body with the exterior world but also the largest immune organ of the human body, containing approximately 80% of all of the body's antibody-producing cells. The gut is also home to a total of more than 10^{14} bacteria. This intestinal ecosystem is the result of the interrelationship between the intestinal microbiota and the host and plays a decisive role in the imprinting of the immune system. During gestation, the fetus develops in a sterile environment. Within hours after birth, as the infant is exposed to bacteria from the mother herself and from his/her environment, the colonization process begins. Several factors can considerably influence the bacterial colonization of the gut. They include birth by cesarean section, preterm birth, measures of hygiene, intensive care, antibiotics and lack of breastfeeding. Given the effects gut microbial populations have on gut immunity, it has been expected that "alterations" in resident intestinal flora

might be associated with conditions resulting from altered immune responses. For example, increased fecal Bacteroides and Clostridia and lower and atypical Bifidobacteria counts have been associated with infants who develop atopic disease compared with normal controls. The "hygiene hypothesis" suggests that a lower exposure in early childhood to bacterial and other antigens in industrialized societies has led to inadequate development and maturation of immune responses and appears responsible for the growing epidemic of asthma and allergies due to inadequate defensive and immune-modulating gut immune diseases. Infants are born with a predominance of Th2 lymphocyte activity, which predisposes them to an exaggerated response to allergens, with increased IgE production. Exposure to intestinal bacteria, on the other hand, stimulates Th1 activity. Microbial-gut interactions can improve the integrity of the gut barrier by decreasing intestinal permeability, reducing both adherence of potential antigens and their systemic effect, and by modulating immune response toward antigen tolerance, thus providing a rationale for the use of probiotic bacteria in pediatric populations (104). The expected effects could be mediated via the innate immune system (Toll-like receptors), resulting in the promotion of Th1 differentiation, production of regulatory cytokines (IL-10 and TGF-b) and enhanced intestinal IgA responses, as shown in infants receiving cow's milk formula supplemented with probiotics (*Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis* Bb-12) (105). Perinatal administration of probiotics to mothers in the last weeks of pregnancy and to infants in the first few months of life is associated with a significant reduction in atopic eczema (100, 106). Nevertheless, results are varied, depending on the probiotic strain, dose, timing and food matrix used. Much remains to be learned, and further studies are required to maintain the role of probiotics in allergy prevention. Data on the effect of probiotics are only that obtained with a FOS/GOS-supplemented hydrolyzed formula. Even if the FOS/GOS group demonstrates significantly lower rates of eczema than the placebo group, it is unclear to what extent the eczema rate was influenced by provision of a hydrolyzed formula.

21.8 CONCLUSIONS AND PERSPECTIVES

Exclusive breastfeeding for 4–6 months, the use of hydrolyzed formulae when breastfeeding is not possible or sufficient, and the delayed introduction of complementary feeding from 4 to 6 months remain the main strategies in primary prevention of dietary allergy. Probiotics may prevent food sensitization in the newborn but further studies are needed. Additional studies are also needed to document the effect LCPUFA supplementation and the long-term effect of dietary interventions in infancy.

REFERENCES

1. Almqvist C, Li Q, Britton WJ, Kemp AS, Xuan W, Tovey ER, Marks GB, CAPS team (2007) Early predictors for developing allergic disease and asthma: examining separate steps in the "allergic march". Clin Exp Allergy 37:1296–1302
2. Chehade M, Mayer L (2005) Oral tolerance and its relation to food hypersensitivities. J Allergy Clin Immunol 115:3–12
3. Zeiger RS (1999) Prevention of food allergy in infants and children. Immunol Allergy Clin North Am 19:619–646

4. Leung DYM, Sampson HA, Gefa RS, Szefler SJ (2003) Pediatric allergy, principles and practice. Mosby, St. Louis, MO, 688p
5. Chouraqui JP, Dupont C, Bocquet A, Bresson JL, Briend A, Darmaun D, Frelut ML, Ghisolfi J, Girardet JP, Goulet O, Putet G, Rieu D, Rigo J, Turck D, Vidailhet M, The Committee on Nutrition of the French Society of Pediatrics (2008) Feeding during the first months of life and prevention of allergy. Arch Pediatr 15:431–442
6. Høst A, Halken S, Muraro A, Dreborg S, Niggemann B, Aalberse R, Arshad SH, von Berg A, Carlsen KH, Duschén K, Eigenmann PA, Hill D, Jones C, Mellon M, Oldeus G, Oranje A, Pascual C, Prescott S, Sampson H, Svartengren M, Wahn U, Warner JA, Warner JO, Vandenplas Y, Wickman M, Zeiger RS, EAACI section on pediatrics (2008) Dietary prevention of allergic diseases in infants and small children. Pediatr Allergy Immunol 19:1–4
7. Greer FR, Sicherer SH, Burks AW, The Committee on nutrition and section on allergy and immunology (2008) Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods and hydrolyzed formulas. Pediatrics 121:182–191
8. Zeiger RS, Heller S (1995) The development and prediction of atopy in high-risk children: follow-up at age 7 years in a prospective randomized study of combined maternal and infant food allergen avoidance. J Allergy Clin Immunol 95:1179–1190
9. Kjellman NIM (1977) Atopic disease in seven-year-old children. Incidence in relation to family history. Acta Paediatr Scand 66:465–471
10. Björksten B, Kjellman NIM, Zeiger RS (1998) Development and prevention of allergic disease in childhood. In: Middleton E Jr, Reed CE, Ellis EE, Adkinson NF Jr, Yunginger JW, Busse WW (eds) Allergy, principles and practice, 5th edn. Mosby, St. Louis, MO, pp 816–837
11. American Academy of Pediatrics, Committee on Nutrition (2000) Hypoallergenic infant formulas. Pediatrics 106:346–349
12. Høst A, Koletzko B, Dreborg S, Muraro A, Wahn U, Agett P, Bresson JL, Hernell O, Lafeber H, Michaelsen KF, Micheli JL, Rigo J, Weaver L, Heymans H, Strobel S, Vandenplas Y (1999) Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. Dietary products used in infants for treatment and prevention of food allergy. Arch Dis Child 81:80–84
13. Prescott SL, Tang MLK (2005) The Australian society of clinical immunology and allergy position statement: summary of allergy prevention in children. Med J Aust 182:464–467
14. Halken S (2004) Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. Pediatr Allergy Immunol 15(Suppl 16):4–5, 9–32
15. Szepfalusi Z, Pichler J, Elsasser S, van Duren K, Ebner C, Bernaschek G, Urbanek R (2000) Transplacental priming of the human immune system with environmental allergens can occur early in gestation. J Allergy Clin Immunol 106:530–536
16. Jones CA, Warner JO (1999) Umbilical cord blood transplantation. Arch Dis Child 81:282
17. Michel FB, Bousquet J, Greillier P, Robinet-Levy M, Coulomb Y (1980) Comparison of cord blood immunoglobulin E and maternal allergy for the prediction of atopic disease infancy. J Allergy Clin Immunol 65:422–430
18. Warner JA, Miles EZ, Jones AC, Quint DJ, Colwell BM, Warner JO (1994) Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? Clin Exp Allergy 24:423–430
19. Caffarelli C, Cavagni G, Giordano S, Stapane I, Rossi C (1995) Relationship between oral challenges with previously uningested egg and egg-specific IgE antibodies and skin prick tests in infants with food allergy. J Allergy Clin Immunol 95:1215–1220
20. Chouraqui JP, Barbier M, Joannard A, Rambaud P, Bost M (1994) Cow's milk protein-induced colitis of early infancy. Acta Endoscopica 24:461–468
21. Falth-Magnusson K, Kjellman NIM (1987) Development of atopic disease in babies whose mothers were receiving exclusion diet during pregnancy: a randomized study. J Allergy Clin Immunol 80:868–875
22. Lilja G, Dannaeus A, Foucard T, Graff-Lonnevig V, Johansson SGO, Oman H (1989) Effects of maternal diet during late pregnancy and lactation on the development of atopic diseases in infants up to 18 months of age: in vivo results. Clin Exp Allergy 19:473–479

23. Falth-Magnusson K (1989) Breast milk antibodies to foods in relation to maternal diet, maternal atopy and the development of atopic disease in the baby. Int Arch Allergy Appl Immunol 90:297–300
24. Herrmann ME, Dannemann A, Grüters A, Radisch B, Dudenhausen JW, Bergmann R, Coumbos A, Weitzel HK, Wahn U (1996) Prospective study on the atopy preventive effect of maternal avoidance of milk and eggs during pregnancy and lactation. Eur J Pediatr 155:770–774
25. Kramer MS, Kakuma R (2006) Maternal dietary antigen avoidance during pregnancy and/or lactation for preventing or treating atopic disease in the child. Cochrane Database Syst Rev 3:CD000133
26. Lack G, Fox D, Northstone K, Golding J (2003) Factors associated with the development of peanut allergy in childhood. N Engl J Med 348:977–985
27. Camargo CA, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW (2007) Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. Am J Clin Nutr 85:788–795
28. Devereux G, Litonjua AA, Turner SW, Craig LC, Mc Neil G, Martindale S, Helms PJ, Seaton A, Weiss ST (2007) Maternal vitamin D intake during pregnancy and early childhood wheezing. Am J Clin Nutr 85:853–859
29. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C, Princess Anne Hospital Study Group (2008) Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 62:68–77
30. Hyppönen E, Berry DJ, Wjst M, Power C (2009) Serum 25-hydroxyvitamin D and IgE – A significant but nonlinear relationship. Allergy 64:613–620
31. Prescott SL, Dunstan JA (2007) Prenatal fatty acid status and immune development: the pathways and the evidence. Lipids 42:801–810
32. Krauss-Etschmann S, Hartl D, Rzehak P, Heinrich J, Shadid R, Del Carmen Ramírez-Tortosa M, Campoy C, Pardillo S, Schendel DJ, Decsi T, Demmelmair H, Koletzko BV, Nutraceuticals for Healthier Life Study Group (2008) Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women. J Allergy Clin Immunol 121:464–470
33. Dunstan JA, Mori TA, Barden A, Beilin JT, Taylor AL, Holt PG, Prescott SL (2003) Fish oil supplementation in pregnancy modifies neonatal allergen specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. J Allergy Clin Immunol 112:1178–1184
34. Calvani M, Alessandri C, Sopo SM, Panetta V, Pingitore G, Tripodi S, Zappalà D, Zicari AM, Lazio Association of Pediatric Allergology (APAL) Study Group (2006) Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. Pediatr Allergy Immunol 17:94–102
35. Romieu I, Torrent M, Garcia-Esteban R, Ferrer C, Ribas-Fitó N, Antó JM, Sunyer J (2007) Maternal fish intake during pregnancy and atopy and asthma in infancy. Clin Exp Allergy 37:518–525
36. Savilahti E, Tainio VM, Salmenperä L, Arjomaa P, Kallio M, Perheentupa J, Siimes MA (1991) Low colostral IgA associated with cow's milk allergy. Acta Paediatr Scand 80:1207–1213
37. Järvinen K-M, Laine ST, Järvenpää A-L, Suomalainen HK (2000) Does low IgA in human milk predispose the infant to development of cow's milk allergy? Pediatr Res 48:457–462
38. Duchen K, Casas R, Fageras-Bottcher M, Yu G, Bjorksten B (2000) Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. Pediatr Allergy Immunol 11:29–39
39. Böttcher MF, Jenmalm MC, Bjorksten B (2003) Cytokine, chemokine and secretory IgA levels in human milk in relation to atopic disease and IgA production in infants. Pediatr Allergy Immunol 14:35–41
40. Larqué E, Sabater-Molina M, Zamora S (2007) Biological significance of dietary polyamines. Nutrition 23:87–95
41. Kalliomäki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E (1999) Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. J Allergy Clin Immunol 104:1251–1257
42. Saarinen KM, Vaarala O, Klemetti P, Savilahti E (1999) Transforming growth factor-beta1 in mothers' colostrum and immune responses to cows' milk proteins in infants with cows' milk allergy. J Allergy Clin Immunol 104:1093–1098
43. Laiho K, Lampi AM, Hamalainen M, Moilanen E, Piironen V, Arvola T, Syrjanen S, Isolauri E (2003) Breast milk fatty acids, eicosanoids, and cytokines in mothers with and without allergic disease. Pediatr Res 53:642–647

44. Rautava S, Kalliomäki M, Isolauri E (2002) Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. J Allergy Clin Immunol 109:119–121
45. Kalliomäki M, Isolauri E (2003) Role of intestinal flora in the development of allergy. Curr Opin Allergy Clin Immunol 3:15–20
46. Das UN (2004) Breast-feeding, atopy, and asthma. J Allergy Clin Immunol 113:1002
47. Kankaanpää P, Nurmela K, Erkkilä A, Kalliomäki M, Holmberg-Marttila D, Salminen S, Isolauri E (2001) Polyunsaturated fatty acids in maternal diet, breast milk, and serum lipid fatty acids of infants in relation to atopy. Allergy 56:633–638
48. Hoppu U, Rinne M, Lampi AM, Isolauri E (2005) Breast milk Fatty acid composition is associated with development of atopic dermatitis in the infant. J Pediatr Gastroenterol Nutr 41:335–338
49. Kilshaw PJ, Cant AJ (1984) The passage of maternal dietary proteins into human breast milk. Int Arch Allergy Appl Immunol 75:8–15
50. Stuart CA, Twiselton R, Nicholas MF, Hide DW (1984) Passage of cow's milk protein in breast milk. Clin Allergy 14:533–535
51. Cant AJ, Narsden RA, Kilshaw PJ (1985) Egg and cow's milk hypersensitivity in exclusively breast-fed infants with eczema, and detection of egg protein in breast milk. Br Med J 291:932–935
52. Axelsson I, Jakobsson I, Lindberg T, Benediktsson B (1986) Bovine beta-lactoglobulin in the human milk. A longitudinal study during the whole lactation period. Acta Paediatr Scand 75:702–707
53. Troncone R, Scarcella A, Donatiello A, Cannataro P, Tarabuso A, Auricchio S (1987) Passage of gliadin into human breast milk. Acta Paediatr Scand 76:453–456
54. Clyne PS, Kulczycki A Jr (1991) Human breast milk contains bovine IgG. Relationship to infant colic? Pediatrics 87:439–444
55. Jakobsson I (1991) Food antigens in human milk. Eur J Clin Nutr 45(Suppl 1):29–33
56. Vadas P, Wai Y, Burks W, Perelman B (2001) Detection of peanut allergens in breast milk of lactating women. JAMA 285:1746–1748
57. Heine RG, Hill DJ, Hosking CS (2004) Primary prevention of atopic dermatitis in breast-fed infants: what is the evidence? J Pediatr 144:564–567
58. Brand PL, Vlieg-Boerstra BJ, Dubois AE (2007) Dietary prevention of allergic disease in children: are current recommendations really based on good evidence? Pediatr Allergy Immunol 18:475–479
59. Grulee CG, Sanford HN (1930) The influence of breast and artificial feeding on infantile eczema. J Pediatr 9:223–225
60. Gdalevich M, Mimouni D, David M, Mimouni M (2001) Breast-feeding and the onset of atopic dermatitis in childhood: a systematic review and meta-analysis of prospective studies. J Am Acad Dermatol 45:520–527
61. Gdalevich M, Mimouni D, Mimouni M (2001) Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. J Pediatr 139:261–266
62. Van Odijk J, Kull I, Borres MP, Brandtzaeg P, Edberg U, Hanson LA, Høst A, Kuitunen M, Olsen SF, Skerfving S, Sundell J, Wille S (2003) Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. Allergy 58:833–843
63. Snijders BE, Thijs C, Dagnelie PC, Stelma FF, Mommers M, Kummeling I, Penders J, van Ree R, van den Brandt PA (2007) Breast-feeding duration and infant atopic manifestations, by maternal allergic status, in the first 2 years of life (KOALA study). J Pediatr 151:347–351
64. Kramer MS, Matush L, Vanilovich I, Platt R, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Mazer B, Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group (2007) Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. Br Med J 335:815
65. Mihrshahi S, Ampon R, Webb K, Almqvist C, Kemp AS, Hector D, Marks GB, Team CAPS (2007) The association between infant feeding practices and subsequent atopy among children with a family history of asthma. Clin Exp Allergy 37:671–679
66. Wetzig H, Schulz R, Diez U, Herbarth O, Viehweg B, Borte M (2000) Associations between duration of breast-feeding, sensitization to hens' eggs and eczema infantum in one and two year old children at high risk of atopy. Int J Hyg Environ Health 203:17–21

67. Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Poulton R (2002) Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. Lancet 21:901–907
68. Bergmann RL, Diepgen TL, Kuss KE, Bergmann KE, Kujat J, Dudenhausen JW, Wahn U, MAS-study group (2002) Breast-feeding duration is a risk factor for atopic eczema. Clin Exp Allergy 32:205–209
69. Pesonen M, Kalio MU, Ranki A, Siimes MA (2006) Prolonged exclusive breastfeeding is associated with increased atopic dermatitis: a prospective follow-up study of unselected healthy newborns from birth to age 20 years. Clin Exp Allergy 36:1011–1018
70. Ludvigsson JF, Mostrom M, Ludvigsson J, Duchen K (2005) Exclusive breastfeeding and risk of atopic dermatitis in some 8300 infants. Pediatr Allergy Immunol 16:201–208
71. Kull I, Bohme M, Wahlgren CF, Nordvall L, Pershagen G, Wickman M (2005) Breast-feeding reduces the risk for childhood eczema. J Allergy Clin Immunol 116:657–661
72. Laubereau B, Brockow I, Zirngibl A, Koletzko S, Gruebl A, Von Berg A, Filipiak-Pittroff B, Berdel D, Bauer CP, Reinhardt D, Heinrich J, Wichmann HE, GINI Study Group (2004) Effect of breast-feeding on the development of atopic dermatitis during the first 3 years of life – results from the GINI-birth cohort study. J Pediatr 144:602–607
73. Kramer MS, Kakuma R (2002) Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev 1:CD003517
74. Wright AL, Holberg CJ, Taussig LM, Martinez FD (2001) Factors influencing the relation of infant feeding to asthma and recurrent wheeze in childhood. Thorax 56:192–197
75. Chan-Yeung M, Ferguson A, Watson W, Dimich-Ward H, Rousseau R, Lilley M, Dybuncio A, Becker A (2005) The Canadian Childhood Asthma Primary Prevention Study: outcomes at 7 years of age. J Allergy Clin Immunol 116:49–55
76. Muraro A, Dreborg S, Halken S, Høst A, Niggemann B, Aalberse R, Arshad SH, von Berg A, Carlsen KH, Duschén K, Eigenmann P, Hill D, Jones C, Mellon M, Oldeus G, Oranje A, Pascual C, Prescott S, Sampson H, Svartengren M, Vandenplas Y, Wahn U, Warner JA, Warner JO, Wickman M, Zeiger RS (2004) Dietary prevention of allergic diseases in infants and small children. Part III: critical review of published peer-reviewed observational and interventional studies and final recommendations. Pediatr Allergy Immunol 15:291–307
77. Van Asperen PP, Kemp SS, Mellis CM (1983) Immediate food hypersensitivity reactions on the first known exposure to the food. Arch Dis Child 58:253–256
78. Hattevig G, Kjellman B, Johansson SGO, Bjorksten B (1984) Clinical symptoms and IgE responses to common food problems in atopic and healthy children. Clin Allergy 14:551–559
79. Host A, Husby S, Osterballe O (1988) A prospective study of cow's milk allergy in exclusively breast-fed infants. Acta Paediatr Scand 77:663–670
80. Cantani A, Ragno V, Businco L (1992) Natural history of IgE-mediated food allergy in fully breast-fed babies. Pediatr Allergy Immunol 2:131–134
81. Saarinen KM, Juntunen-Backman K, Järvenpää AL, Klemetti P, Kuitunen P, Lope L, Renlund M, Siivola M, Vaarala O, Savilahti E (2000) Breast-feeding and the development of cow's milk protein allergy. Adv Exp Med Biol 478:121–130
82. Jakobsson I, Lindberg T (1983) Cow's milk proteins cause infantile colic in breast-fed infants: a double-blind crossover study. Pediatrics 71:268–271
83. Palmer DJ, Makrides M (2006) Diet of lactating women and allergic reactions in their infants. Curr Opin Clin Nutr Metab Care 9:284–288
84. Hattevig G, Kjellman B, Sigurs N, Bjorksten B, Kjellman NIM (1989) Effect of maternal avoidance of eggs, cow's milk and fish during lactation upon allergic manifestations in infants. Clin Exp Allergy 19:27–32
85. Lovegrove JA, Hampton SM, Morgan JB (1994) The immunologic and long-term atopic outcome of infants born to women following a milk-free diet during pregnancy and lactation: a pilot study. Br J Nutr 71:223–238
86. Hattevig G, Sigurs N, Kjellman B (1999) Effects of maternal dietary avoidance during lactation on allergy in children at 10 years of age. Acta Paediatr 88:7–12
87. Oldaeus G, Bradley CK, Bjorksten B, Kjellman NIM (1992) Allergenicity screening of "hypoallergenic" milk-based formulas. J Allergy Clin Immunol 90:133–135

88. European Society of Pediatric Gastroenterology and Nutrition Committee on Nutrition (1993) Comment on antigen-reduced infant formulae. Acta Paediatr 82:314–319
89. European Society of Pediatric Allergy and Clinical immunology. Hydro1ysed cow's milk formulae (1993) Allergenicity and use in treatment and prevention. An ESPACI position paper. Pediatr Allergy lmmunol 4:101–111
90. Comité de Nutrition de la Société Française de Pédiatrie (2000) Utilisation des formules à charge antigénique réduite. Arch Pediatr 7:302–306
91. Oldaeus G, Bjorksten B, Einarsson R, Kjellman NIM (1991) Antigenicity of cow's milk hydrolysates intended for infant feeding. Pediatr Allergy Immunol 4:156–164
92. Muraro A, Dreborg S, Halken S, Høst A, Niggemann B, Aalberse R, Arshad SH, von Berg A, Carlsen K, Duschén K, Eigenmann P, Hill D, Jones C, Mellon M, Oldeus G, Oranje A, Pascual C, Prescott S, Sampson H, Svartengren M, Vandenplas Y, Wahn U, Warner JA, Warner JO, Wickman M, Zeiger RS (2004) Dietary prevention of allergic diseases in infants and small children. Part I: immunologic background and criteria for hypoallergenicity. Pediatr Allergy Immunol 15:103–111
93. Von Berg A, Koletzko S, Grübl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, Reinhardt D, Berdel D, German Infant Nutritional Intervention Study Group (2003) The effect of hydrolysed cow's milk formula for allergy prevention in the first year of life: The German Infant Nutritional Intervention Study, a randomized double-blind trial. Allergy Clin Immunol 111:533–540
94. Von Berg A, Koletzko S, Filipiak-Pitroff B, Laubereau B, Grübl A, Wichmann HE, Bauer CP, Reinhardt D, Berdel D, German Infant Nutritional Intervention Study Group (2007) Certain hydrolyzed formulas reduce the incidence of atopic dermatitis but not that of asthma: Three year results of the German Infant Nutritional Intervention Study. J Allergy Clin Immunol 119:718–725
95. Von Berg A, Filipiak-Pittroff B, Krämer U, Link E, Bollrath C, Brockow I, Koletzko S, Grübl A, Heinrich J, Wichmann HE, Bauer CP, Reinhardt D, Berdel D, GINIplus study group (2008) Preventive effect of hydrolyzed infant formulas persists until age 6 years: long-term results from the German Infant Nutritional Intervention Study (GINI). J Allergy Clin Immunol 121:1442–1447
96. Osborn DA, Sinn J (2006) Formulas containing hydrolysed protein for prevention of allergy and food intolerance in infants. Cochrane Database Syst Rev (4):CD003664. DOI: 0.1002/14651858.CD003664.pub3
97. Osborn DA, Sinn J (2006) Soy formula for prevention of allergy and food intolerance in infants. Cochrane Database Syst Rev (4):CD003741. DOI/10, 1002/146518CD003741.pub4
98. Rieu D, Bocquet A, Bresson JL, Briend A, Chouraqui JP, Darmaun D, Dupont C, Frelut ML, Ghisolfi J, Girardet JP, Goulet O, Putet G, Rigo J, Turck D, Vidailhet M, Comité de Nutrition de la Société Française de Pédiatrie (2006) Phytoestrogens and soy foods in infants and children: caution is needed. Arch Pediatr 13:1091–1093
99. Agostoni C, Axelsson I, Goulet O, Koletzko B, Michaelsen KF, Puntis J, Rieu D, Rigo J, Shamir R, Szajewska H, Turck D, ESPGHAN Committee on Nutrition (2006) Soy protein infant formulae and follow-on formulae: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 42:352–361
100. Heine RG, Tang MLK (2008) Dietary approaches to the prevention of food allergy. Curr Opin Clin Nutr Metab Care 11:320–328
101. Anandan C, Nurmatov U, Sheikh A (2009) Omega 3 and 6 oils for primary prevention of allergic disease: systematic review and meta-analysis. Allergy 64:840–848
102. Tarini BA, Carroll AE, Sox CM, Christakis DA (2006) Systematic review of the relationship between early introduction of solid foods to infants and the development of allergic disease. Arch Pediatr Adolesc Med 160:502–507
103. Zutavern A, Brockow I, Schaaf B, Von Berg A, Diez U, Borte M, Kraemer U, Herbarth O, Behrendt H, Wichmann H-E, Heinrich J, LISA Study Group (2008) Timing of solid food introduction in relation to eczema, asthma, allergic rhinitis, and food and inhalant sensitization at the age of 6 years: Results from the prospective birth cohort study LISA. Pediatrics 121:e44–e52
104. Isolauri E, Salminen S (2008) Probiotics: Use in allergic disorders. A nutrition, allergy, mucosal immunology, and intestinal microbiota (NAMI) Research Group Report. J Clin Gastroenterol 42:S91–S96
105. Rautava S, Arvilommi H, Isolauri E (2006) Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. Pediatr Res 60:221–224
106. Michail SK, Stolfi A, Johnson T, Onady GM (2008) Efficacy of probiotics in the treatment of pediatric atopic dermatitis: a meta-analysis of randomized controlled trials. Ann Allergy Asthma Immunol 101:508–516

Section E
Botanical Extracts and Bioactive Foods

22 *In Vitro* and *In Vivo* Immunomodulatory and Anti-allergic Effects of *Agaricus blazei* Murill

Masashi Mizuno

Key Points

- *Agaricus blazei* Murill extract appears to have an effect on the changing pattern of splenic lymphocyte subsets, particularly cytotoxic or natural killer cells.
- *A. blazei* Murill extract contains potent Th1 cytokine producing constituents.
- Oral administration of *A. blazei* Murill extract down-regulates serum IgE levels by enhancing Th1 response.

Key Words: *Agaricus blazei* Murill, anti-allergy, immunomodulatory activity, interferon-γ (IFN-γ), interleukin (IL), Th1/Th2 balance, β-glucan.

22.1 INTRODUCTION

Interest in the medicinal characters of natural products has increased due to their popular use in traditional medicine. Mushrooms have recently become attractive as a functional food and a source for the development of drugs. Many investigators have isolated and identified antitumor polysaccharides from mushrooms (1–5). The antitumor activity of these polysaccharides is caused by the potentiation of the immune response, which involves lymphocyte activation (2, 6). Hamuro et al. (7, 8) have reported that lentinan containing in *Lentinula edodes*, a β (1→3) glucan, mediates the augmentation of alloreactive murine cytotoxic T-lymphocytes and increases the generation of cytotoxic T-lymphocytes in spleen cells. Sakagami et al. (9) have also reported that schizophyllan, a β (1→6) branched β (1→3) glucan, activates macrophages, followed by the potentiation of killer T-cells through cytokine mediation. It, therefore, seems that the antitumor polysaccharides commonly show activity by stimulating T-cell subsets in spleen cells.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_22

Agaricus blazei Murill (Agaricomycetideae) (ABM) is well known among these mushrooms. It occurs naturally in a mountain region near Sao Paulo in Brazil and has become popularly known as "Himematsutake" in Japan. Mizuno et al. (4, 5) have reported that polysaccharides from ABM have antitumor activity against Sarcoma 180-bearing mice. The structure of the polysaccharides includes β-1,6-glucopyranosyl residues as a backbone (3). Ito et al. (10) have reported that macrophage activation and an alteration of the third component of complement is necessary for the induction of an antitumor effect when polysaccharides from ABM are injected intraperitoneally into a mouse-implanted tumor. In addition, it has been reported that the percentages of splenic Thy1.2-, L3T4-, and asialo GM1-positive cells in the T-cell subsets were significantly higher than those of tumor-bearing mice treated with saline (11). Moreover, the expression of interleukin (IL)-12 (a key role in Th1 differentiation) and IL-18 (a proinflammatory cytokine in enhancing Th1 immune response) mRNA in macrophage-like cell line, RAW264.7, stimulated with a polysaccharide purified in ABM were investigated by RT-PCR *in vitro* (12). Polysaccharides from ABM changed the percentage of splenic Thy 1.2- and L3T4 (CD4)-positive cells in the T-cell subsets in ABM-treated mice (13). These results were presumed that this mushroom possessed the ability of differentiation of naive T-cells into T-helper type1 (Th1), resulting an antiallergic activity.

Allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis, and food allergies are steadily increasing especially in industrialized countries. Key factors driving these rising trends are increased exposure to sensitizing allergens and reduced stimulation of the immune system during critical periods of development. Allergic responses involving IgE-dependent mast cell degranulation and eosinophil accumulation in the sites of inflammation are considered due to the development and activation of Th2 cells (14).

The production of two distinct cytokine patterns recognized in subsets of Th is especially important. The set designated as Th1 is characterized by interleukin (IL)-12, interferon (IFN)-γ and IL-2 production, and activates macrophages. The Th1 cytokines augment cell-mediated immunity. The other set, designated as Th2, is characterized by IL-4, -5, -6, -10, and -13 syntheses and Th2 cytokines promote humoral immunity (15). The representative cytokines of Th2 cells are IL-4 and IL-5. IL-4 is the major inducer of class switching to IgE biosynthesis in B-lymphocytes. IL-5 is the principal eosinophil-activating factor. On the other hand, IFN-γ, which is a representative cytokine of Th1 cells, is known to suppress the development of Th2 cells (16). Since it is suggested that Th1 and Th2 types of reactions are reciprocally regulated *in vivo*, the modulation of Th1/Th2 balance, namely, shifting the balance from Th2 to Th1 dominance, should be a strategy for the therapy of allergic diseases involving Th2 cells.

Allergic reactions, especially immediate type allergy, are genetically determined disorders characterized by an increased ability of B-lymphocytes to synthesize IgE antibodies toward ubiquitous antigens (allergens) able to activate the immune system after inhalation or ingestion and after penetration through the skin. IgE antibodies are able to bind to high affinity Fcε receptors (FcεRI) present on the surface of mast cells/basophils (15). The mast cells, which are constituents of virtually all organs and tissue, are thought to play a major role in the development of many physiologic changes during allergic responses. Among the preformed and newly synthesized inflammatory substances released on degranulation of mast cells, histamine remains the bestcharacterized and most potent vasoactive mediator implicated in the acute phase of immediate type

allergy (17). Compound 48/80 has been used as a direct and convenient reagent to study the mechanism of the anaphylactic reaction (18).

In this chapter, immunomodulating effect on immunocompetent cells in oral feeding of hot water extract from ABM to mice will be discussed with respect to anti-allergic effects.

22.2 EFFECTS OF ABM EXTRACT ON POPULATION OF LYMPHOCYTE T-CELL SUBSETS IN C3H/HE

To examine the immunomodulating effect of AMB extract, ABM extract was injected directly into the stomach of C3H/He mice. The population of T-cell subsets in lymphocytes was analyzed by flow cytometry after an oral administration of ABM extract into C3H/He mice (19). The percentages of Thy1.2-, L3T4 (CD4)-, and Lyt2 (CD8)-positive cells were greater than those of the controls, with increases of 37.1, 28.9, and 38.8%, respectively (Fig. 22.1). However, ABM extract did not show any mitogenic activity by the MTT method using spleen cells or any changes of the population in spleen cells, suggesting that ABM extract did not have any effects on the proliferation of cells. The L3T4/Lyt2 ratio decreased slightly, and the Lyt2/Thy1.2 ratio showed a tendency to increase. These results also suggest an increase especially in Lyt2 (CD8)-positive cells, which are considered to be markers of cytotoxic T-cells. We have also found that an intraperitoneal injection of ABM extract increases the number of asialo GM1-positive cells, which are considered to be markers of natural killer T-cells. Thus, ABM extract appears to have an effect on the changing pattern of splenic lymphocyte subsets, particularly cytotoxic or natural killer cells.

Polysaccharides from the mushrooms have been the focus of much research because they have shown antitumor activity against mice-implanted tumors. Also, it was clear that ABM extract contained many kinds of polysaccharides. Recently, Mizuno et al. (4) have reported that polysaccharides from ABM have antitumor activity and that one of

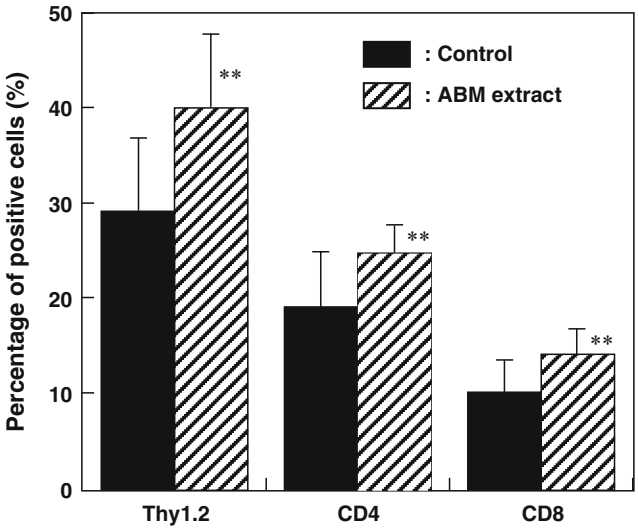


Fig. 22.1. Cell population of splenic T-lymphochte subsets after oral administration of ABM extraction. The asterisk indicated significant different from the control group ($p < 0.01$).

these polysaccharides has a 1,3- and 1,6-β-glucan complex as the main component. It has also been reported that the percentages of splenic Thy1.2-, L3T4-, and asialo GM1-positive cells were significantly higher than that in tumor-bearing mice treated with saline when polysaccharides from ABM (β-1,6-glucan) were administered (11). As the number of cytotoxic and natural killer T-cells are particularly increased in the presence of this polysaccharide, there may be a stronger possibility not only that the cancer cells are attacked by these lymphocytes, but also that naïve T-cells are differentiated into Th1.

22.3 INHIBITION EFFECTS OF ABM EXTRACT ON COMPOUND 48/80-INDUCED SYSTEMIC ANAPHYLACTIC REACTION

Compound 48/80 has been used as a direct and convenient reagent to cause the anaphylactic reaction (18). Oral administration of ABM extract vastly inhibited the compound 48/80 induced systemic anaphylaxis-like reaction. The cumulative number of scratching behavior for 30 min in saline-treated mice (control) was approximately 2,300 after intradermal injection of compound 48/80, whereas those in ABM extract decreased to be approximately 960 (20). As ABM extract administration suppressed the cumulative number of scratching behavior, histamine, which remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate type allergy, was measured in plasma. ABM extract treatment suppressed histamine release to be 36% as compared with control. Moreover, to determine the effects of ABM extract on degranulation in mast cell, skin was stained with toluidine blue. The degranulated mast cells around skin injected compound 48/80 were observed, whereas ABM extract treatment suppressed mast cell degranulation. The release of histamine from mast cells by degranulation is a prominent feature of acute inflammatory processes such as the immediate-type anaphylaxis (21). These results suggested that ABM extract may inhibit the anaphylaxis reaction by blocking histamine release from mast cells.

It has been reported that ABM extract contained the immunomodulatory polysaccharide which could elicit the production of IL-12 and 18 from macrophages (12). As IL-12 and 18 are a key role in Th1 differentiation and a proinflammatory cytokine in enhancing Th1 immune response in macrophage, ABM extract might possess the ability of differentiation of naïve T-cells into Th1. Moreover, polysaccharides from ABM extract changed the percentage of splenic CD4-positive cells in the T-cell subsets (13) and activated macrophage to produce tumor necrosis factor-α through toll-like receptor (TLR) 4 (22). TLR 4 is one of the very important receptors for innate immunity (23). These evidences suggested that ABM extract affected Th1 and Th2 balance.

To investigate the immunoregulatory effect of ABSW on ICR mice, cytokine production from splenocyte stimulated with or without Concanavalin A (Con A) *in vitro* was examined. INF-γ production significantly enhanced in the splenocytes stimulated with Con A only when mice administered with ABM extract were treated by compound 48/80 (Fig. 22.2). However, in the case of control, INF-γ production did not show any difference. INF-γ is the characteristic Th1-type cytokine and inhibits Th2 response (16). In conclusion, it was ascertained that ABM extract inhibited the degranulation and histamine release from mast cells *in vivo*, and enhanced INF-γ production from splenocytes *in vitro*. These findings suggested that ABSW contained potent Th1 cytokine producing constituents.

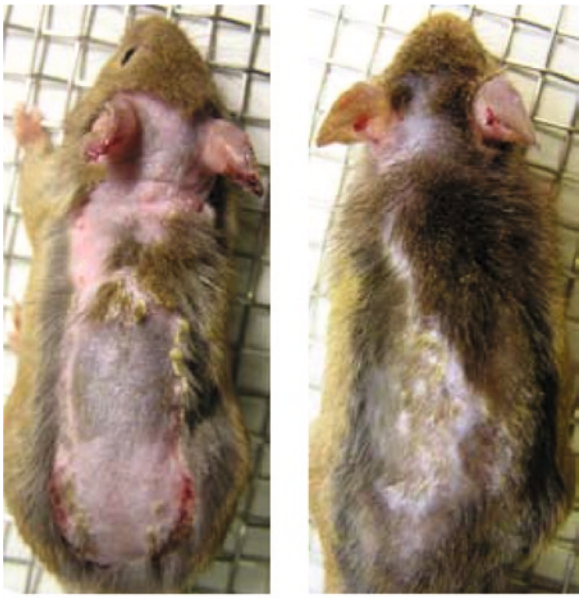


Fig. 22.2. Effect of ABM extract on atopic dermatitis score in NC/Nga. Typical pictures of criteria of dermatitis score in NC/Nga after the eighth PiCl challenge.

22.4 INHIBITION EFFECTS OF ABM EXTRACT ON DEVELOPMENT OF ATOPIC DERMATITIS-LIKE SKIN LESION INDUCED BY PICRYL CHLORIDE (PICL) IN NC/NGA

NC/Nga develops atopic dermatitis (AD)-like skin lesions when the mice are repeatedly treated with PiCl (24–26). As shown in Fig. 22.3, AD scores in the control group and ABM group after the eighth challenge of PiCl shows drastic differences to be 6.6 ± 0.7 and 5.0 ± 0.5, respectively. It was ascertained that ABM extract inhibited its development. It has been reported that oral administration of *Rumex japonicus* Houtt., one of the herbs used in Eastern countries, inhibited the development of AD-like symptoms and down-regulates serum IgE levels by suppressing the Th 2 cell response in NC/Nga (27). It has also been reported that oral administration of persimmon leaf extract to NC/Nga leads to a suppression of the development of dermatitis and serum IgE elevation by suppressing the Th 2 cell response (28). As shown in Table 22.1, oral administration of ABM extract to the PiCl-treated NC/Nga similarly down-regulated IgE levels (29).

22.5 CONCLUSIONS AND PRESPECTIVES

IgE synthesis by B cells is primarily regulated by cytokines. Th1 cells secrete IFN-γ, whereas Th2 cells produce IL-4. IFN-γ is a strong inhibitor of IgE synthesis and Th2 cell proliferation, and induces differentiation to Th1 from Th0 cells (30, 31). IL-4 plays a key role in the hyperproduction of IgE and induces differentiation to Th2 cells (32– 34). Therefore, it is believed that an increase of IFN-γ shifts predominantly the Th1/Th2

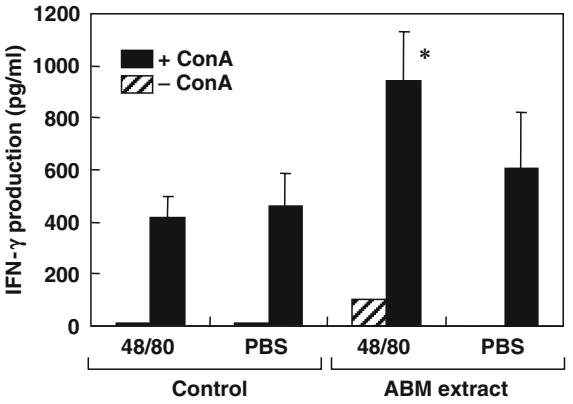


Fig. 22.3. Effects of ABM extract on the production of INF-γ from splenocytes. The asterisk indicated significant difference from the control group ($p < 0.05$).

Table 22.1
Change of serum IgE levels in each group of NC/Nga mice at 47th day after sensitization with PiCl

	IgE contents (μg/ml)
Control	40.8 ± 7.0
ABM extract	24.1 ± 5.1*

*$p < 0.05$, significantly different from the mean value of the control group

balance to Th1, and an increase of IL-4 shifts predominantly the balance to Th2. To examine the immunoregulatory effects of ABM extract *in vivo*, IFN-γ and IL-4 levels in serum were determined. IFN-γ levels in the control group and ABM group were 147.2 ± 52.5 and 292.0 ± 31.4 pg/ml, respectively. However, IL-4 contents were not detected. Since IFN-γ levels in serum increased in ABM group, it might be expected that Th1/Th2 balance of spleen cells shifted to Th1. Therefore, it was postulated that ABSW extract down-regulated serum IgE levels by enhancing Th1 response. In summary, it was demonstrated that oral administration of ABM extract down-regulated serum IgE levels by enhancing Th1 response, and suppresses the development of AD-like skin lesions.

REFERENCES

1. Nanba H, Hamaguchi A, Kuroda H (1987) The chemical structure of an antitumor polysaccharide in fruit bodies of *Grifola frondosa* (maitake). Chem Pharm Bull 35:1162–8
2. Takeyama T, Suzuki I, Ohno N, Oikawa S, Sato K, Ohsawa M, Yadomae T (1987) Host-mediated antitumor effect of grifolan NMF-5N, a polysaccharide obtained from *Grifola frondosa*. J Pharmacobiodyn 10:644–51
3. Kawagishi H, Inagaki R, Kanao T, Mizuno T, Shimura K, Ito H, Hagiwara T, Nakamura T (1989) Fractionation and antitumor activity of the water-insoluble residue of *Agaricus blazei* fruiting bodies. Carbohydr Res 186:267–73

4. Mizuno T, Hagiwara T, Nakamura T, Ito H, Shimura K, Sumiya T, Asakura A (1990) Antitumor activity and some properties of water-soluble polysaccharides from "Himematsutake" the fruiting body of *Agaricus blazei* Murill. Agric Biol Chem 54:2889–96
5. Mizuno T, Ando M, Sugie R, Ito H, Shimura K, Sumiya T, Matsuura A (1992) Antitumor activity of some polysaccharides isolated from an edible mushroom. Ningyotake, the fruiting body and the cultured mycelium of *Polyporous confluens*. Biosci Biotechnol Biochem 56:34–41
6. Suzuki I, Tanaka H, Kinoshita A, Oikawa S, Osawa M, Yadomae T (1990) Effect of orally administered β-glucan on macrophage function in mice. Int J Immunopharmacol 12:675–84
7. Hamuro J, Röllinghoff M, Wagner H (1978) β(1→3) Glucan-mediated augmentation of alloreactive murine cytotoxic T-lymphocytes in vivo. Cancer Res 38:3080–5
8. Hamuro J, Röllinghoff M, Wagner H (1978) Induction of cytotoxic peritoneal exudate cells by T-cell immune adjuvants of the β(1→3) glucan-type lentinan and its analogues. Immunology 39:551–9
9. Sakagami Y, Mizoguchi Y, Seki T, Kobayashi K, Morisawa S, Yamamoto S (1988) Release of peptide leukotrienes from rat Kupffer cells. Biochem Biophy Res Commun 155:650–5
10. Ito H, Shimura K, Itoh H, Kawade M (1997) Antitumor effects of a new polysaccharide-protein complex (ATOM) prepared from *Agaricus blazei* (Iwade strain 101) "Himematsutake" and its mechanisms in tumor-bearing mice. Anticancer Res 17:277–84
11. Itoh H, Ito H, Amano H, Noda H (1994) Inhibitory action of a (1→6)-β-D-glucan-protein complex (FIII-2-b) isolated from *Agaricus blazei* Murill ("himematsutake") on Meth A fibrosarcoma-bearing mice and its antitumor mechanism. Jpn J Pharmacol 66:265–71
12. Mizuno M, Kawakami S, Sakamoto Y, Fujitake N (2003) Macrophages stimulated by polysaccharide purified from *Agaricus brasiliensis* S. Wasser et al. (Agaricomycetideae) enhance mRNA expression of Th1 cytokine including IL-12 and 22. Int J Med Mushrooms 5:397–403
13. Mizuno M, Morimoto M, Minato K, Tsuchida H (1998) Polysaccharides from *Agaricus blazei* stimulate lymphocyte T-cell subsets in mice. Biosci Biotechnol Biochem 62:434–7
14. Holgate ST (1999) The epidemic of allergy and asthma. Nature 402:B2–B4
15. Maggi E (1998) The TH1/TH2 paradigm in allergy. Immunotechnology 3:233–44
16. Abbas AK, Murphy KM, Sher A (1996) Functional diversity of helper T lymphocytes. Nature 383:787–93
17. Petersen LJ, Mosbech H, Skov PS (1996) Allergen-induced histamine release in intact human skin in vivo assessed by skin microdialysis technique: characterization of factors influencing histamine releasability. J Allergy Clin Immunol 97:672–9
18. Inagaki N, Nakamura N, Nagao M, Musoh K, Kawasaki H, Nagai H (1999) Participation of histamine H1 and H2 receptors in passive cutaneous anaphylaxis-induced scratching behavior in ICR mice. Eur J Pharmacol 367:361–371
19. Mizuno M, Yamada J, Terai H, Kozukue N, Lee YS, Tsuchida H (1994) Differences in immunomodulating effects between wild and cultured Panax ginseng. Biochem Biophys Res Commun 200:1672–8
20. Mizuno M (2008) Immunomodulatory activities of beta-glucan in mushroom. In: Shibamoto T, Kanazawa K, Shahidi F, Ho C-T (eds) Functional Food and Health. American Chemical Society, Washington, DC, pp 399–407
21. Li GZ, Chai OH, Lee MS, Han EH, Kim HT, Song CH (2005) Inhibitory effects of *Houttuynia cordata* water extracts on anaphylactic reaction and mast cell activation. Biol Pharm Bull 28:1864–8
22. Mizuno M, Kawakami S (2006) An immunomodulating polysaccharide in *Agaricus brasiliensis* S. Wasser et al. (Agaricomucetideae) activates macrophages through Toll-like receptor 4. Int J Med Mushrooms 8:223–9
23. Takeda K, Akira S (2004) TLR signaling pathways. Semin Immunol 16:3–9
24. Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, Matsumoto M, Ushio H, Saito S, Askenase PW, Ra C (1997) Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int Immunol 9:461–6
25. Taniguchi Y, Kohno K, Inoue S, Koya-Miyata S, Okamoto I, Arai N, Iwaki K, Ikeda M, Kurimoto M (2003) Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int Immunopharmacol 3:1313–24
26. Vestergaard C, Yoneyama H, Matsushima K (2000) The NC/Nga mouse: a model for atopic dermatitis. Mol Med Today 6:209–10

27. Lee HS, Kim SK, Han JB, Choi HM, Park JH, Kim EC, Choi MS, An HJ, Um JY, Kim HM, Min BI (2006) Inhibitory effects of *Rumex japonicus* Houtt on the development of atopic dermatitis-like skin lesions in NC/Nga mice. Br J Dermatol 15:33–38
28. Kotani M, Matsumoto M, Fujita A, Higa S, Wang W, Suemura M, Kishimoto T, Tanaka T (2000) Persimmon leaf extract and astragalin inhibit development of dermatitis and IgE elevation in NC/Nga mice. J Allergy Clin Immunol 106:159–166
29. Morimoto T, Takagi M, Mizuno M (2008) Oral administration of *Agaricus brasiliensis* S. Wasser et al. extract down-regulates serum immunoglobulin E levels by enhancing Th1 response. Int J Med Mushrooms 10:15–24
30. McKnight AJ, Zimmer GJ, Fogelman I, Wolf SF, Abbas AK (1994) Effects of IL-12 on helper T cell-dependent immune responses in vivo. J Immunol 152:2172–9
31. Leung DY, Bieber T (2003) Atopic dermatitis. Lancet 361:151–160
32. Gieni RS, Yang X, HayGlass KT (1993) Allergen-specific modulation of cytokine synthesis patterns and IgE responses in vivo with chemically modified allergen. J Immunol 150:302–10
33. Pene J, Rousset F, Briere F, Chretien I, Paliard X, Banchereau J, Spits H, De Vries JE (1988) IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. J Immunol 141:1218–24
34. Swain SL, Weinberg AD, English M, Huston G (1990) IL-4 directs the development of Th2-like helper effectors. J Immunol 145:3796–3806

23 Resveratrol and Bioactive Flavonoids in Immune Function

Nasiruddin Khan, Rafael Llorach, Mireia Urpi-Sarda, Raul Zamora-Ros, Antonio Cherubini, and Cristina Andres-Lacueva

Key Points

- Resveratrol and other bioactive flavonoids play an important role in our health and immune system and could also have beneficial effects against many diseases.
- Biological effects of resveratrol and bioactive compounds include those affecting immunity.
- Resveratrol and bioactive flavonoids in human immune function are related to chemokines, macrophages, and caspases.

Key Words: Bioactive compounds, resveratrol, flavonoids, immune system.

23.1 INTRODUCTION

Food consists of a complex mixture of a wide variety of components, many of which are biologically active. Some components, identified some time ago, have been classified as nutrients and these are essential for growth, maintenance, and repair of the body. More recently, scientists have identified biologically active substances in plants that have been proven to have potential beneficial effects on health (e.g., the cholesterol-lowering effects of phytosterols).

Polyphenols are phytochemical constituents from the secondary metabolism of plants that typically occur in small quantities in foods; specifically they can be defined as food components that influence physiological or cellular activities and affect health as a result. Polyphenols may have activities such as antioxidant activities, wherein multiple

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_23

compounds may perform the same function or have similar activities. These compounds vary widely in chemical structure and function and are grouped accordingly.

Polyphenolic compounds occur widely in the plant kingdom and are present in many edible plants. Over the past few years, there has been a growing interest in the research field of polyphenols because of the recognition of their antioxidant properties, their great abundance in our diet, and their possible role in the prevention of various diseases. Moreover, polyphenols, as active substances found in many medicinal plants, modulate the activity of a wide range of enzymes and cell receptors (1).

Polyphenols are classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. They can be divided into flavonoids and non-flavonoids.

23.1.1 Flavonoids

These comprise the most common group of plant polyphenols in various fruits and vegetables, with various subclasses.

Flavonols, including mainly quercetin and kaempferol, can be found in such sources as onions, curly kale, leeks, broccoli, blueberries, red wine, and tea (2–4). Flavones, mainly as glycosides of luteolin and apigenin, are found in edible sources like parsley and celery (2, 3).

Isoflavones, structurally similar to estrogens, include genistein, daidzein, and glycitein and are present exclusively in leguminous plants (5). Flavanones, represented primarily by naringenin, eriodictyol, and hesperetin, are present in high concentrations only in citrus fruit (6). Anthocyanidins, which are color-imparting pigments in flowers and fruits, include mainly cyanidins and are chiefly present in the skin and flesh of sources. Flavanols (catechins and proanthocyanidins) exist in both the monomer form (catechins) and the polymer form (proanthocyanidins). Catechin and epicatechin are the main flavanols in fruit, whereas gallocatechin, epigallocatechin, and epigallocatechin gallate (EGCG) are found in certain seeds of leguminous plants, in grapes, and more significantly in tea (7, 8). In contrast to other classes of flavonoids, flavanols are not glycosylated in foods.

Proanthocyanidins, which are also known as condensed tannins, are dimers, oligomers, and polymers of catechins that are responsible for the astringent character of fruit and beverages.

23.1.2 Non-Flavonoids

This group generally includes simple phenols (tyrosol), phenolic acids and aldehydes (derivatives of benzoic and cinnamic acids), hydrolysable tannins (gallotannins and ellagitannins), phenylacetic acid/acetophenone, hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, and sinapic acids), coumarins (scopoletin, esculin), benzophenones (maclurin), xanthones, chalcones (chalconaringenin), lignans (secoisolariciresinol), and secoiridoids (oleuropein).

Stilbenes also belong to a group of non-flavonoids. The most important and studied compound of stilbenes is resveratrol (3,5,4′-trihydroxystilbene), which is present in low quantities in the human diet and exists in its glucosilated form and in *cis* and *trans* forms. It is predominantly found in grapes and grape products like red wine (9–11). Other natural sources of resveratrol include peanuts, mulberries, blueberries, cranberries, bilberries, turmeric, and hops (12–14) Table 23.1.

Table 23.1
Effects of resveratrol on immune functions in *in vitro* and *in vivo* studies

Cell type/specie	*Compound*	*Dose (day)*	*Effect*	*Reference*
In vitro and ex vivo assays				
Murine splenocytes from mice C-57	Resveratrol	0.01, 0.1, 1 μM	↓ IFNγ and ↑ IL-10↓ IFNγ/IL-10 ratio	(90)
Tumor cells from C3H/HeN and C3M/MeJ mice	Resveratrol	0, 25, 50, 75, 100, 150 μM (25 weeks of age)	↑ IFN-γ and IL-12 through TLR4 (toll-like receptor)	(91)
Lymphocytic leukemia L1210 cells from mice	Resveratrol	0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 μM	Inhibits proliferation Induces apoptosis Influences cell cycle of L1210 cells in dose- and time-dependent manner	(92)
T and B cells and macrophages from inbred female BALB/c mice (8–10 weeks old)	Resveratrol	1, 5, 10, 20 μM	↓ Expression of CD28 and CD80,↑ Production IL-10	(93)
Blood cells from male Sprague–Dawley rats	Resveratrol	0, 0.01, 0.1, 1, 10 μM	↓ TNF-alpha, IL-1beta and IL-6 cytokines in a concentration-dependent manner	(94)
Spleen cells from male C3H (H-2k) mice (8–10 weeks old)	Resveratrol	6.25, 12.5, 25, 50 μM	> 90% suppression of the mitogen/antigen-induced T cell proliferation and development of allo-antigen specific CTLs	(95)
Spleen cells from male C3H (H-2k) and C57BL/6 (H-2b) mice (8–10 weeks old)	Resveratrol	6.25, 12.5, 25, 50 μM	↓ Cell proliferation, cell-mediated cytotoxicity, and cytokine production, ↓ NF-κB activation	(96)
In vivo assays				
Female C3H/HeN and C3H/HeJ mice (6–8 weeks of age)	Resveratrol	10 μM/mouse topical application	↑ Contact hypersensivity response to DMBA with functional TLR4	(91)
Female C57BL/6 and BALB/c mice (6–10 weeks old)	Resveratrol	4 mg/kg ip (intraperitoneal)	↓ $CD4^+CD25^+$ cell and TGF-β↑ IFN-γ expression in $CD8^+$ T cells	(97)

(continued)

Table 23.1
(continued)

Cell type/specie	*Compound*	*Dose (day)*	*Effect*	*Reference*
Female BALB/c mice (6–10 weeks old)	Resveratrol complex (RC)	100 µg ip injected	↑ IL-6, IL-1 and TNF-α↑ Expression $CD4^+$ lymphocytes ↑ NF-kB2, Cdc42 and Bcl-2genes	(98)
Male BALB/c mice (6–8 weeks old)	Resveratrol	12.5, 25, 50 mg/kg	Normalization of CD4/CD8 ↑ Lymphocyte proliferation, NK cell activity, and anti-SRBC titers Interleukin-6 cellular content and release are suppressed by resveratrol as well as mRNA expression	(92)
Male C3H (H-2k) mice (8–10 weeks old)	Resveratrol	80 mg/kg day, 5 days/ week for 4 weeks	↓ alloantigen-induced T cell proliferation and the generation of cytotoxic T lymphocytes in the draining lymph nodes	(95)
Inbred BALB/c mice (6–8 weeks of age)	Resveratrol	4 mg/kg ig + 16% (w/v) ethanol	Reverse the suppressive effect of ethanol both on macrophage % and on macrophage MHC-II molecule expression	(99)

The purpose of this section is mainly to deal with the effect of resveratrol and other bioactive compounds such as flavonoids on the immune system, and to provide a close look at the recent studies and scientific evidences that support their differing biological and health effects on our biological system, specifically the immune system Table 23.2**.**

23.2 IMMUNOMODULATING FUNCTIONS OF RESVERATROL

Resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin produced naturally by plants including red grapes, peanuts, and various berries with anti-inflammatory and antioxidant activities. Many studies have suggested that resveratrol can have a potent effect on the human immune system and this is supported by the following evidences.

Resveratrol inhibits the expression of vascular endothelial growth factor (VEGF), matrix matalloproteinases (MMP-3, MMP-9) and cyclooxygenase-2(COX-2) in human articular chondrocytes stimulated by the pro-inflammatory cytokine interleukine (IL-1β) through its actions on the nuclear factor-κB (NF-κB) pathway in human chondrocytes (15). According to an *in vitro* study, resveratrol seems to be an effective anti-inflammatory agent and has a chondroprotective capacity through suppression of (a) IL-1β (b) reactive oxygen species (ROS) and (c) tumor suppressor protein p53-production (16).

Cultures of human chondrocytes treated with 100 μM of resveratrol reduced the IL-1β-induced inhibition of the expression of cartilage-specific collagen type II and signal transduction receptor beta1-integrin in a time-dependent manner and abolished the activation of caspase-3 and poly(ADP-ribose) polymerase (PARP) cleavage (17).

Resveratrol has the ability to reduce superoxide anion production by phagocytic leukocytes in response to many chemotactic peptide formylpeptide receptors (FPR), and A beta42, an Alzheimer's disease-associated peptide and a ligand for the FPR variant (FPRL1), and reduces the phosphorylation of extracellular signal-regulated kinase (ERK1/2) and the activation of NF-κB induced by FPR agonists (18).

When the concentration of resveratrol used is over 2.5 mg/L, especially 10 mg/L, the proliferation and transformation rate of human peripheral blood T lymphocytes (hPBTCs) decreased significantly, and its combination at a given concentration with cyclosporine A can enhance immune suppression (19).

Using immune response models, one *in vitro* study suggested that resveratrol produces a biphasic effect on the anti-CD3/anti-CD28-induced development of both Interferon-γ (IFN-γ), IL2- and IL4-producing CD8+ and CD4+ T cells, with stimulation at low and suppression at high resveratrol concentrations, with a similar trend for both cytotoxic T lymphocytes (CTL) and natural killer (NK) cell cytotoxic activity, thus participating in several immune cell functions (20).

Resveratrol at 10^{-4} M inhibited (69%) the PHA-stimulated Peripheral Blood Mononuclear Cell (PBMC) proliferation. At 10^{-4} M, resveratrol strongly inhibited PHA-stimulated IFN-γ and tumor necrosis factor-α (TNF-α) release from PBMC, but it did not cause inhibition at 10^{-5} or 10^{-7} M. Resveratrol, typically present in red wine at about 10^{-5} M, is unlikely to cause inhibitory immune effects, but low concentrations of resveratrol are capable of stimulating the immune system (21).

Table 23.2
Effects of flavonoids on immune functions in *in vitro* and *in vivo* studies

Cell type/specie	*Subclass*	*Compound*	*Dose (day)*	*Effect*	*Reference*
In vitro assays					
Spleen cells from Female C57BL/6 (H-2b), (C57BL/6_DBA/2)F1, BDF1 (H-2b/d), BALB/c (H-2b), and DBA/2 (H-2d) mice	Flavonol	Kaempferol	2.18, 4.37, 8.73, 17.47 μM	↓ IFNγ and IL-2 and shifted the Th1/Th2 balance into the Th2 phenotype, ↓ $CD8^-$ T cells and allospecific CTL activity	(100)
T helper cells from wild type C57BL6, T-bet-deficient mice and T-bet transgenic/ deficient mice	Flavonol	Quercetin	20, 40 μM	↓ IFNγ and IL-2 cytokine production	(101)
Human PBMC	Flavonol	Shikimic acid and Quercetin	10, 100 nM	↑ IL-8 and IL-6	(102)
Spleen and thymus cells from male Wistar rats	Flavonol	Rutin	0.01, 1, 10 μM	↓ IFN-γ and ↑ IL-10	(103)
Dendritic cells from male C57BL/6 (H-2Kb and I-Ab) and BALB/c (H-2Kd and I-Ad) mice (8–12 weeksold)	Flavone	Apigenin	5, 10, 20 μM	↓ CD80, CD86, and MHC class I and II molecules	(104)
Glial cells from breeding pairs of BALB/c mice	Flavone	Apigenin and luteolin	10, 20, 30, 40, 50 μM	↓ IFN-γ-induced CD40 expression and TNF-α and IL-6 production	(105)
Mouse alveolar macrophage and peripheral macrophage RAW 264.7 cells	Flavone	Luteolin	5, 10, 25 μM	Block NF-κB and AP-1 activation	(106)
Intestinal epithelial cells and bone-marrow derived dendritic cells from BALB/c and C57BL/6 mice	Flavone	Luteolin	10, 25, 50 μM	Blocks LPS-induced NF-κB signaling and proinflammatory gene expression through the inhibition of IKK activity	(107)
Murine dendritic cells from male C57BL/6 mice	Flavanol	EGCG	25, 50 μM	↓ IDO expression↓ STAT1 activation and COX-2 expression	(108)
RINm5F pancreatic beta-cell line	Flavanol	EGCG	20, 50, 100, 200 μM	Effectively protected IL-1 and IFN-γ mediated cytotoxicity	(109)
$CD8^+$ T cells from female SKH-1 hairless mice (6–7 weeks old)	Flavanol	EGCG	1 mg/cm^2 skin area topical application	↓ Proliferating cell nuclear antigen in UV-B-induced tumors and higher numbers of cytotoxic T lymphocytes ($CD8^+$ T cells)	(110)

Murine splenocytes from mice C-57	Isoflavones	Genistein	0.01, 0.1, 1 μM	↓ IFNγ and ↑ IL-10 ↓ IFNγ/IL-10 ratio	(90)
MonoMac6 cells from the human monocytic cell	Isoflavones	Daidzein	11.8, 39.3, 295 μM	↓ IL-6 and IL-8 production by TLR-2, and TLR4-stimulated monocytes in a dose-dependent manner	(111)
Mesenteric lymph nodes cells from C57BL/6 female mice (8–12 weeks old)	Isoflavones	Daidzein	Mice were fed 100 mg/kg	↓ IFN-γ, IL-12p40, IL-6, IL-10, ↓ $CD11b^+CD80^+$, $CD11b^+CD86^+$ cells in a dose-dependent manner	(111)
In vivo assays					
Male MRL/lpr mice (26 weeks old)	Isoflavones	Genistein	30 mg/kg	↓ DTH response	(112)
BALB/c mice	Isoflavones	Genistein	0, 4, 20 mg/kg	↓ OVA-specific IFN-γ production ↓ immunoglobulin (Ig) G1, IgG2a, IgG2b	(113)
Female C57BL/6 mice	Isoflavones	Genistein	8,20,80 mg/kg	↓ Anti-collagen II Ab↓ DTH response	(114)
Female DO11.10 transgenic mice	Isoflavones	Genistein	4, 20 mg/kg	↑ IFN-γ and IL-4	(115)
Female ICR-CD1 mice	Isoflavones	Genistein, Diadzein, Glycitein	1 mg/kg	↑ Lymphoproliferative response of T cells	(116)
Swiss mice	Isoflavones	Daidzein	10, 20, 40 mg/kg	↑ Phagocytic activity↑ Ag-specific IgM Ab	(117)
Adult female B6C3F1 mice	Isoflavones	Genistein	2,6,20 mg/kg	↑ Host resistance↑ Activity of cytotoxic T cells and NK cells	(118)
Pregnant young adult Sprague–Dawley rats	Isoflavones	Genistein	300, 800 mg/kg	↓ $CD4^-$ $CD8^+$ T cells↓ $CD4^-$ $CD8^+$ T cells	(119)
Female C57BL/6 mice	Isoflavones	Genistein	2, 8, 20, 80, 200 mg/kg per day	↑ Thymocyte apoptosis ↓ Peripheral lymphocytes, ↓ Ag-specific Ab titer	(120)
NC mice (7 weeksold)	Isoflavones	Genistein	4, 20 mg/kg	↓ Inflammatory dermatitis in NC mice, ↓ IFN-γ,↑ IL-4	(121)
Male Hartley guinea pigs	Isoflavones	Genistein	15 mg/kg	↓ Ag-induced asthma	(122)

(continued)

Table 23.2
(continued)

Cell type/specie	*Subclass*	*Compound*	*Dose (day)*	*Effect*	*Reference*
Dams C57BL/6 mice	Isoflavones	Genistein	25, 250, 1, 250 mg/kg	↑ Activity of NK cells, ↑ Splenic T cells and NK cells	(123)
F1 male C57BL/6 mice	Isoflavones	Genistein	25, 250, 1,250 mg/kg	↑ $CD4^+$ $CD8^+$ and $CD4^-$ $CD8^+$ thymocytes ↑ Activity of NK cells ↑ Splenic T cells and NK cells ↑ Anti-CD3-mediated splenocyte proliferation	(123)
F1 female C57BL/6 mice	Isoflavones	Genistein	25, 250, 1, 250 mg/kg	↓ Splenic NK cells ↓ Activity of NK cells ↑ $CD4^-CD8^+$ and $CD4^-CD8^-$ thymocytes ↑ $CD4^+CD8^-$ and $CD4^+CD8^+$ splenocytes ↓ $CD4^+CD8^+$ thymocytes	(123)
Female BALB/c mice (8–16 weeks old)	Flavonol	Kaempferol	2.18, 4.37, 8.73, 17.47 mg/kg	↓ Th1 cytokine production and modulate the Th1/Th2 balance	(100)
BALB/c mice	Flavonol	Rutin	6, 12 mg/kg	↑ Macrophage phagocytosis in cells and ↓ spleen leukemia tumor growth	(124)
Male BALB/c mice (6 weeks old)	Flavonone	Hesperidin	200 mg/kg oral administration	↓ LPS induced expression of TNF-α, IL-1β, IL-6, MIP-2, MCP-1, IL-12 ↓ Total leukocyte count, nitric oxide, iNOS	(133)
Male C57BL/6N mice (8 weeks old)	Flavone	Apigenin	Diet containing 0.025% apigenin	↓ IgE and inflammatory cytokines such as RANTES and sTNFRI (tumor necrosis factor receptor I)	(125)
Male BALB/c mice (8 weeks old)	Flavone	Chrysin	0.005 mg/kg for 3 weeks	↓ IgE level, 6 IgE biosynthesis through the suppression of Th2 cytokines expression	(126)
Male BALB/c mice (8 weeks old)	Flavone	Apigenin.	0.005 mg/kg for 3 weeks	↓ IgE level, ↓ IgE biosynthesis through the suppression of Th2 cytokines expression	(126)

Female C57BL/6 mice	Flavanol	EGCG	Drinking water containing 0.1, 0.5, 2.5 mg/mL	↑ E-7-specific $CD8^+$ T cells immune response↑ IFN-γ	(127)
Male C57BL/6 mice	Flavanol	EGCG	5 mg/kg	Regulate immune-mediated liver injury, ↓ Inflammatory mediators	(128)
Strain male albino Wistar rats	Flavanol	EGCG	200 mg/kg	Modulates both the expression of glycoconjugates and immunological	(129)
C3H/HeN mice (6–7 weeks old)	Proanthocyanidin	Grape seed proanthocyanidins	Diet containing 0.2, 0.5 and 1.0%	↓ UVB induced inflammatory reaction and modulated UVB-induced immunosuppression	(130)
Female BALB/c mice (6–8 weeks old)	Proanthocyanidin	Proanthocyanidin	10 mg/kg	Partial enhancement of lymphocyte proliferation, NK cell cytotoxicity, $CD4^+$/$CD8^+$ ratio, IL-2 and IFN-γ productions	(131)
Female C57BL/6 mice (8–12 weeks old)	Isoflavones	Daidzein	100/kg	↓ Severity and extent of inflammation, and crypt damage	(111)
Female Sprague–Dawley rats (5 weeks old)	Isoflavones	Genistein	20 mg/kg	↓ IFN-γ, ↑ IL-4 production and maintain the Th1/Th2 balance	(132)

OVA ovalbumin, *IFN* interferon, *Ig* immunoglobulin, *NK* natural killer, *Ag-specific Ab* Antigen specific antibody, DTH response- Delayed type hypersensitivity, *IL* interleukin, *ARDS* acute respiratory distress syndrome, *DC* dendritic cells, *MHC* major histocompatibilty complex, *NF-κB* nuclear factor kappa B, AP-1, activator-protein 1, *IKK* IkappaB kinase, *IDO* Indoleamine 2,3-dioxygenase, *STAT-1* signal transducers and activators of transcription, *COX* cyclooxygenase, *RANTES* regulated on activation, normal T expressed and secreted, *sTNFRI* soluble tumor necrosis factor receptor I, *DMBA* 7,12-dimethylbenz (a) anthracene, *TLR* toll-like receptor, *SRBC* sheep red blood cells, *EGCG* Epigallocatechin gallate, *GTP* green tea polyphenol, *GSPs* grape seed proanthocyanidins, *PA* proanthocyanidin, *DRIA* daidzein-rich isoflavone aglycones

23.3 FLAVONOIDS AS IMMUNOMODULATORS

As mentioned earlier, flavonoids are plant chemicals with potent antioxidant properties. In autoimmune diseases, flavonoids are known to reduce inflammation and oxidative stress, promote healing of nerves and blood vessels and repair skin damage. Flavonoids have been referred to as "nature's biological response modifiers" or immunomodulators because of their ability to modify the immune system's reaction. Immunomodulators are used to strengthen the immune system and help it function properly (22).

23.3.1 Flavonols

Generally, humoral (Th-1)-derived cytokines such as IL-2, IFNγ, and IL-12 promote cellular immunity while cellular (Th-2)-derived cytokines such as IL-4, IL-5, IL-6 exert negative effects on cellular immunity while upregulating humoral immunity. In this context, mast cells that participate in allergies also have an effect on immunity and inflammation by secreting proinflammatory cytokines. Flavonols confer beneficial effects on these complications. These compounds, in association with other therapeutical molecules, could be a possible treatment for neurological diseases mediated by mast cell degranulation or mast cell-derived allergic inflammatory diseases.

Kempuraj et al. (23) proposed that quercetin, kaempferol, myricetin, and morin (0.01, 0.1, 1, 10 or 100 μM) showed inhibition of IL-6, IL-8, and TNF-α by 82–93% at 100 μM quercetin and kaempferol, and 31–70% by myricetin and morin through the suppression of intracellular calcium ion elevations and protein kinase C-θ (PKC-θ) signaling in a dose-response manner in human umbilical cord blood-derived cultured mast cells (hCB-MCs). Another study suggested that quercetin (10–50 μM) could dramatically inhibit mast cell tryptase and IL-6 release and HDC (Histidine decarboxylase) mRNA transcription by HMC-1 (human mast cell) cell line (24). Moreover, quercetin decreased the gene expression and production of TNF-α, IL-1β, IL-6, and IL-8 in HMC-1 cell and attenuated activation of NFκB and p38 mitogen-activated protein kinase (25).

Mast cell Fc immunoglobulin E receptors (FcvarepsilonRI) linkage by multivalent antigen triggers the secretion of granule-stored mediators and cytokines, including IL-6. Quercetin (1–100 μM) inhibited IL-1-induced IL-6 secretion, p38, and PKC-θ phosphorylation in a dose-dependent manner and also blocked both IL-6 secretion and two key signal transduction steps involved in this mechanism (26, 27).

The toxicity of chemotherapeutic drugs toward normal cells is a serious side effect of cancer treatment. It has been shown that quercetin, while incapable of inducing apoptosis in normal cells under several conditions, could interfere with effector T cell function (27).

It is well known that Granulocyte-macrophage colony-stimulating factor (GM-CSF) activates the host immune system. In the PC-3 cell, kaempferol and quercetin could result in the recruitment of dendritic cells (DCs) to the tumor site by stimulating GM-CSF production without affecting its mRNA levels (28).

Quercetin significantly induces the gene expression as well as the production of Th-1-derived IFN-γ and downregulates Th-2-derived IL-4 by normal PBMC. Also quercetin could increase the phenotypic expression of IFN-γ cells and decrease IL-4 positive cells (29).

The inhibitory effects of quercetin on leukotriene synthesis (5-LO effect) and of catechins on prostaglandin synthesis (CO effect) may account for the anti-inflammatory

effects of flavonoids. In addition, flavonoids also inhibit cyclic nucleotide phosphodiesterases, but are more selectively inhibitory of cyclic guanosine monophosphate (cGMP) breakdown than that of cyclic adenosine monophosphate (cAMP). Higher levels of cGMP are known to increase lymphocyte proliferation and function, whereas high levels of cAMP decrease proliferation and function.

Quercetin 0.5–50 μmol/L markedly increased the amount of cGMP in the human umbilical vein endothelial cell (HUVEC) stimulated by thrombin and activated platelets. Quercetin also inhibited the release of ET (endothelin) from unstimulated HUVEC, but increased the production of cGMP from unstimulated EC (30).

Quercetin in excess of 100 μM inhibited the phorbol-induced respiratory burst with decreased production of superoxide anion and H_2O_2 (31, 32). Presumably, quercetin, by inhibiting myeloperoxidase activity, affects its hypochlorous acid scavenging activity (32). Moreover, the production of oxidants by neutrophils treated with γ-interferon, IL-1, or tumor necrosis factor was also inhibited by flavonoids in a dose-dependent manner (31). In addition, quercetin inhibited the phosphorylation of neutrophil proteins (33), degranulation, and the release of lysosomal enzymes in zymosan-activated neutrophils (34). In another study, quercetin tended to decrease the antigenic stimulation of CTL (35), alloantigenic specific T lymphocytes (36) and also inhibited NK cell-mediated cytolysis (37).

One recent study in PBMC showed that quercetin reduced, in a dose-dependent manner, the proliferation of PBMC and modulated the level of IL-1β and TNF-α released by PBMC in the culture supernatants. It also reduced the MMP-9/TIMP-1 ratio (38) (tissue inhibitors of MMPs).

Other flavonols also have anti-inflammatory properties by inhibiting the production of TNF-α and nitric oxide along with other important proinflammatory factors. Treatment with 10 μM of kaempferol significantly inhibited activation of NF-kB p65 elicited by ultra violetB (UVB) irradiation in normal human epidermal keratinocytes (NHEKs). It decreased expression patterns in protein-encoding genes involved in inflammatory and immune responses, such as CD68 antigen, interferon (α, β, ω) receptor 1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor beta, interferon alpha-inducible protein (clone IFI-15 K), interferon-induced protein with tetratricopeptide repeats 3, Toll-like receptor adaptor molecule 1 and signal transducer and activator of transcription 3 (acute phase response factor) (39). Kaempferol blocked TNFα-induced translocation of the NF-κB subunit p65 from the cytoplasm to the nucleus in mouse primary calvarial osteoblasts (40) and down-regulated iNOS and TNFα expression via NF-κB inactivation in aged rat gingival tissues (41).

Kaempferol exhibits strong radical-scavenging activity (42) and inhibits the formation of superoxide anion radicals by xanthine oxidase (43). Chemical stress-induced ROS production is decreased by kaempferol in various cell types (44, 45). Kaempferol has also been shown to have cytoprotective and anti-apoptotic activities (44, 45) and to inhibit damage to DNA by certain hormones (46) and H_2O_2 (47).

23.3.2 Flavanones

Like flavonols, flavanones participate as cytotoxic, chemopreventive agents and are well-established bioactive compounds.

Naringenin, one of the most abundant flavonoids in citrus fruits, has been shown to inhibit *in vitro* growth of human cancer cells. In human leukemia THP-1 cells, naringenin treatment inhibited THP-1 cells accompanied by mitochondria dysfunctions, activation of caspases, inactivation of the phosphatidylinositol 3-kinase (PI3K/Akt) pathway, inducing apoptosis, downregulation of B-cell lymphoma (Bcl-2), upregulation of Bcl-2-associated X protein (Bax), and growth PARP cleavages in a concentration-dependent manner (48).

Hesperidin, a citrus flavanone, possesses chemopreventive efficacies that could suppress the production of prostaglandin E2 (PGE2), nitrogen dioxide (NO_2), and the expression of iNOS protein and COX-2 inhibitor, which might be related to anti-inflammatory and anti-tumorigenic efficacies (49).

As promising bioactive compounds against hyperlipidemia and lipid biosynthesis, aglycones such as naringenin and hesperetin exhibited the growth arrest of human preadipocyte cell line acute myeloid leukemia (AML-1) cells, anti-apoptotic proteins such as p-Akt, NF-κB, and Bcl-2 were decreased, and the pro-apoptotic protein Bad was accumulated, while expression of Fatty Acid Synthase (FAS) and Peroxisome Proliferator Activated Receptor (PPAR)-γ was enhanced in naringenin-treated cells (50).

23.3.3 Flavones

Flavones can modulate immune responses and inflammatory reactions by controlling the production of nitric oxide (51).

According to Nicholas et al. (52), apigenin inhibits *in vivo* lipopolysaccharide (LPS)-induced TNF and also modulates the immune response. In addition, apigenin inhibits the production of proinflammatory cytokines IL-1β, IL-8, and TNF in LPS-stimulated human monocytes and mouse macrophages by inhibiting the transcriptional activity of NF-κB. In another study, apigenin inhibited the production of nitric oxide and prostaglandin E(2) by suppressing the expression of inducible nitric oxide synthase and cyclooxygenase-2 protein, respectively, and also suppressed p38 MAPK, c-JNK phosphorylation (53).

Chrysin, in an *in vitro* study (54), shut down the Stem Cell Factor (SCF/c-Kit) complex-induced signaling cascade that confers hematopoiesis, melanogenesis, and gametogenesis.

Likewise, luteolin at 25, 50, or 100 μM inhibited LPS-induced COX-2 protein expression. Both luteolin and chrysin suppressed LPS-induced PGE2 formation. Luteolin also inhibited xanthine/xanthine oxidase-generated superoxide formation at 100 μM (55). Luteolin inhibited LPS-induced PGE synthesis, and moreover, mRNA and protein expression of COX-2 and prostaglandin E synthases (mPGES-1) in RAW264.7 cells were also decreased by luteolin (56). Luteolin, fisetin and apigenin were found to ameliorate allergic symptoms by being the strongest inhibitors of both IL-4 and IL-13 production by basophils but they did not affect leukotriene C4 synthesis. At higher concentrations, these flavonoids suppressed IL-4 production by T cells (57). Luteolin and quercetin, as scavengers of H_2O_2 and inhibitors of O_2, were as potent as genistein, having a moderate effect. Quercetin and luteolin were potent in inhibiting

lipid peroxidation induced by $FeCl_2$, while genistein had a very weak inhibitory effect. The magnitude of the quenching effect on 8-hydroxy-2 -deoxyguanosine (8-OHdG) formation induced by UV light irradiation was in the order of genistein > luteolin > quercetin (58).

Tangeretin and nobiletin in human breast cancer cell lines inhibited proliferation in a dose- and time-dependent manner, and proved to be cytostatic by suppressing cell cycle arrest at G1 without apoptosis. Such an agent could be expected to spare normal tissues from toxic side effects (59). The results of a study in human carcinoma cells revealed that the tangeretin inhibition of IL-1β-induced COX-2 expression, at least in part, was mediated through the suppression of NF-κB transcription factor as well as through suppression of the signaling proteins of mitogen-activated protein kinases (p38 MAPK), c-Jun amino-terminal kinases (JNK), and Phosphoinositide 3-kinases (PI3K) (60).

Baicalein suppressed hypoxia-inducible factor-1 (HIF-1) alpha protein and activation as well as the expression of hypoxia-responsive genes by inhibiting the ROS and PI 3-kinase/Akt pathway (61).

23.3.4 Flavanols

Because of its anti-inflammatory and immunomodulatory activities, EGCG, a component of green tea catechin, has attracted attention in recent years. It is well known that dendritic cells (DCs) are professional antigen-presenting cells that play key roles in the activation of T-cell-mediated immune responses.

EGCG induced immunosuppressive alterations on human monocyte-derived dendritic cells (MoDCs) both by suppression of cell surface molecules like CD83, CD80, CD11c, and the major histocompatibilty complex (MHC class II) and by the induction of apoptosis (62).

As described above, mast cells are multipotent effector cells of the immune system. In a study of HMC-1 cell line, EGCG treatment reduced expression of integrins like α5 and β3 and a chemokine, monocyte chemoattractant protein-1 (MCP-1), resulting in a lower adhesion of mast cells and hence decreased potential to favor monocyte recruitment (63).

Supporting the above statement, another *in vitro* study with human peripheral blood cells revealed that the mechanism by which catechin, especially EGCG, exerted its anti-inflammatory effects was due to the downregulating CD11b expression on CD8+ T cells and, in consequence, the inhibiting infiltration of these cells into the sites of inflammation (64). Another important study indicated that the pretreatment or co-treatment of human PBMC or murine lymphnode cells with EGCG significantly reduced SEB-induced proliferation and IL-2, IFN-γ, and TNF-α production, suggesting its role as a useful addition to current treatments for enteric immune disorders and T cell-driven immunopathologies (65).

The homeostatis of monocytes is crucial for immune responses, as they are the main effector cells of the immune system. In this context, EGCG and epicatechin gallate-induced apoptosis of monocytes and also apoptotic caspases 3, 8, and 9 were dose-dependently activated by EGCG treatment, making them valuable as an anti-inflammatory agent (66).

EGCG pretreatment (20–80 μM) of normal human bronchial epithelial (NHBE) cells suppressed the cigarette smoke condensate (CSC)-induced phosphorylation of IκBα, and activation and nuclear translocation of NF-κB/p65, along with downregulation of NF-κB-regulated proteins cyclin D1, MMP-9, IL-8 and iNOS. Furthermore, EGCG also inhibited the CSC-induced phosphorylation of ERK1/2, JNK and p38 MAPKs (67).

In studies on nonspecific immunological responses, Busse et al. (34) demonstrated that the release of oxidants by phagocytosizing neutrophils was suppressed by flavonoids such as quercetin and catechin. Similarly, Daniel et al. (68) demonstrated that catechin inhibited CO activity and PGE2 secretion in macrophages (35, 69), thus equating macrophage activity with the release of oxidants and PGE2 synthesis. The cGMP level in macrophages increased at low levels of catechin; but with further increases in the catechin concentration, the level of cGMP peaked and then declined precipitously. A concurrent increase in IL-1 secretion was observed at high cGMP levels (35).

Melanin, an endogenous pigment which scatters and absorbs UV light, protects human skin against UV radiation. Upon sun exposure, pigmentation is enhanced by stimulated synthesis of melanin in the epidermal melanocytes (suntan), and a cascade of photo-induced chemical and biological reactions takes place in the target tissue. The chemical reaction cascade leads to cellular biochemical responses including modified gene expression, impact on kinase-dependent signaling pathways, immune and inflammatory events, or induction of apoptosis. Polyphenols attracted attention as protective agents against UV-induced damage. EGCG has been shown to exert anti-inflammatory effects on inflammatory skin conditions. The topical application of EGCG may improve atopic dermatitis (AD)-like skin lesions by suppressing migration inhibitory factor (MIF) and T helper 1 cytokines (TNF-alpha, IFN-gamma, IL-2 and IL-12). It is suggested that EGCG may be a potential therapeutic modality for AD (70). In another study, topical application of EGCG (3 mg/mouse/3 cm^2 of skin area) caused the prevention of UV-B-induced infiltrating leukocytes, specifically the CD11b + cell type, myeloperoxidase activity, a marker of tissue infiltration of leukocytes, antigen-presenting cells, and oxidative stress by UV-B-induced immunosuppression and photocarcinogenesis (71).

Oligomers are potent stimulators of both the innate immune system and early events in adaptive immunity. In a recent *in vitro* study with human PBMC treated with 0.7 g of semi-purified cocoa extract, it was found that long-chain flavanol fraction (LCFF) and short-chain flavanol fraction (SCFF), in the absence of LPS, stimulated the production of GM-CSF. In addition, LCFF and SCFF increased the expression of the B cell markers CD69 and CD8 (72). In another study, PBMC from 14 healthy subjects were treated to individual CFP fractions for 72 h. The intermediate-sized CFP fractions (tetramers through octamers) were the most active on resting cells, causing a three to fourfold increase in TNF-α while the monomers and dimers were the least stimulatory, displaying a 42% and 31% increase, respectively, whereas the trimers, nonamers and decamers showed an intermediate stimulation of this cytokine as compared to baseline control. In the presence of PHA, the intermediate-sized CFP fractions enhanced TNF-alpha secretion in the range of 48–128% relative to the PHA control, lending support to the concept that CFP can be immunomodulatory (73). A study with 13 healthy subjects having low baseline levels of transforming growth

factor beta(1) (TGF-beta(1)), when treated with individual FP fractions (25 μg/ml), enhanced TGF-beta(1) release in the range of 15–66% over baseline (monomer, dimer, and tetramer). Pentamers were more effective at augmenting TGF-beta(1) secretion than hexamers, with the monomers and dimers inducing the greatest increases (66% and 68%, respectively) (74).

In an *in vitro* study, normal human peripheral blood lymphocytes (PBL) treated with cacao liquor polyphenol (CLP 25–100 μg/ml) expressed an inhibition of both hydrogen peroxide and superoxide anion and also inhibited the mitogen-induced proliferation of T cells and polyclonal Ig production by B cells in a dose-dependent manner. CLP treatment inhibited both IL-2 mRNA expression of and IL-2 secretion by T cells (75). According to one study, epicatechin conjugates derived from grape polymeric flavanols showed antioxidant and immunopharmacological activity. In this study, (−)-epicatechin and its related compounds inhibited the production of IL-1β and IL-6 in whole blood incubated in the presence of Escherichia coli LPS (76).

Pretreatment of red wine on human healthy mononuclear cells promoted the *in vitro* release of regulatory IL-12, proinflammatory (IL-1β and IL-6), and anti-inflammatory (IL-10) cytokines as well as that of immunoglobulins IgA and IgG from B cells (77).

The zeta chain-associated 70-kDa zinc finger antiviral protein (ZAP-70) of tyrosine kinase played a critical role in T cell receptor-mediated signal transduction and the immune response. A study proposed that ZAP-70 activity was inhibited specifically by EGCG, which contributed to suppressing the CD3-mediated T cell-induced pathways in leukemia cells (78).

23.3.5 Anthocyanins

Anthocyanidins are present in many pigmented fruits and vegetables, and possess antioxidant, anti-inflammatory, and antiangiogenic properties.

Delphinidin treatment of different human cell lines resulted in a dose-dependent induction of apoptosis and the arrest of cells in G(2)-M phase and also dose-dependent decrease in (a) phosphorylation of IκB kinase gamma (NEMO), (b) phosphorylation of NF-κB inhibitory protein IκBα, (c) phosphorylation of NF-κB/p65 at Ser(536) and NF-κB/p50 at Ser(529), (d) NF-κB/p65 nuclear translocation, and (e) NF-κB DNA binding activity (79). In another *in vitro* study, delphinidin inhibited serum and vascular endothelium growth factor-induced bovine aortic endothelial cells (BAECs) proliferation triggered by ERK-1/-2 activation, through the suppression of cell progression by blocking the cell cycle in G(0)/G(1) phase (80).

Chang liver cells represent nonmalignant human cells of epithelial origin. Grape seed proanthocyanidin extract (GSPE-25 μg/ml) showed an increased expression of Bcl-2 in the chang liver cells; however, there was a significant decrease in the expression of other cell cycle-related genes such as p53 and c-myc in these cells following treatment with GSPE (81). As mentioned earlier, solar ultraviolet radiation-induced oxidative stress has been implicated in various skin diseases. Treatment of NHEK with GSPs inhibited UVB-induced hydrogen peroxide (H_2O_2), lipid peroxidation, protein oxidation, and DNA damage and scavenged hydroxyl radicals and superoxide anions. The antioxidant defense components, such as glutathione peroxidase, catalase, superoxide dismutase, and glutathione, were also inhibited by GSPs along with

the inhibition of UVB-induced phosphorylation of ERK1/2, JNK, and p38 and UVB-induced activation of NF-κB/p65 (82).

23.3.6 Isoflavones

Estrogen, as an immune-modulating hormone, could play a role in postmenopausal condition and aging. Isoflavone (71.6 mg isoflavones derived from 706 mL soymilk/d plus a placebo supplement or 70 mg isoflavones in a supplement plus 706 mL cow milk/d) intervention in postmenopausal women resulted in higher B cell populations and lower plasma concentrations of 8-hydroxy-2-deoxy-guanosine, an oxidative marker of DNA damage (83).

Monocytes or whole blood from postmenopausal women, treated with soy isoflavones genistein and daidzein (10–1,000 nM), tamoxifen (10–1,000 nM), or 17β-estradiol (0.1–10 nM) inhibited LPS-stimulated TNF-α production up to 55.8%. Serum levels of TNF-α decreased by 25.1% (27.2 ± 10.3 pg/ml) and 66.7% (11.6 ± 5.3 pg/ml) after 2- and 10-weeks soy consumption, respectively. A decrease of 56.6% and 14.4% in serum IL-1α and the mean percentage of blood monocytes was also reported, indicating the immune modulatory properties of isoflavones (84).

Isoflavones, such as daidzein, are thought to possess vasculoprotective properties, perhaps through a mechanism similar to estrogen. Daidzein (0.2 mg/kg per day sc) and 17β-estradiol (0.1 mg/kg per day sc) enhanced endothelium-dependent relaxation through an increase in eNOS activity associated with the decreased expression of caveolin-1 and an increased expression of calmodulin in endothelial cells (85).

Formononetin, a phytoestrogen, and its metabolites, daidzein and equol, significantly increased IL-4 production by the increased activation of AP-1 through the PI3-K/PKC/p38 MAPK signaling pathway from both CD4+ T cells and EL4 cells in a dose-dependent manner (86).

Adding more evidence for phytoestrogens, including isoflavones and lignans as an immunomodulator, one study showed that genistein, daidzein, and its metabolite equol were potent inhibitors of leukocyte functions. Genistein (10 μM) had the capability to decrease proliferation, lytic activity of NK cells, and cytokine secretions and could act as a sensitive marker of immune functions (87).

An epidemiologic study also supported the immune modulating role of soy isoflavones. Infants who were fed a soy protein isolate-based formula have immunization responses similar to breast-fed infants. Newborn term infants assigned randomly to soy formula groups with and without added nucleotides ($n = 94$, $n = 92$) demonstrated immune cell status similar to human milk/formula-fed infants, consistent with normal immune system development. The addition of nucleotides to soy formula tended to increase numbers and percentages of T cells and decreased numbers and percentages of NK cells (88).

23.3.7 Other Flavonoids

Amentoflavone, a biflavonoid from *Biophytum sensitivum*, in an *in vitro* study elevated the production of interleukin-2 and interferon-gamma and enhanced NK cell activity in normal (42.8% cell lysis) and tumor-bearing animals (48.2% cell lysis) (89).

23.4 CONCLUSIONS AND PERSPECTIVES

From the above discussion and scientific evidences, it is clear that resveratrol and other bioactive flavonoids could play an important role in our health and immune system and could also have beneficial effects against many diseases. But there is also evidence that much research needs to be done in order to identify the biology of each important bioactive compound and its effect on health. With the advent of contemporary and powerful methodologies for conducting applied and basic science, rapid progress can be made in understanding the functional importance of each bioactive compound. Determining the biological effects of these compounds requires more *in vitro* or *in vivo* experiments that must be correlated to a health outcome. Acute and chronic effects, and direct and indirect effects, need to be studied and the mechanisms of action must be delineated. Moreover, it will be important to assess possible interactive effects with other dietary nutrients/dietary constituents that may potentiate or antagonize functions of these bioactive compounds. To accomplish this task, research integrating various scientific disciplines is required, culminating in well-designed large intervention trials with the compound(s) of interest.

REFERENCES

1. Middleton E Jr, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751
2. Herrmann K (1958) Flavonols & phenols in onions (Allium cepa L.). Arch Pharm Ber Dtsch Pharm Ges 291(63):238–247
3. Justesen U, Knuthsen P, Leth T (1998) Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. J Chromatogr A 799:101–110
4. Crozier A, Burns J, Aziz AA, Stewart AJ, Rabiasz HS, Jenkins GI, Edwards CA, Lean ME (2000) Antioxidant flavonols from fruits, vegetables and beverages: measurements and bioavailability. Biol Res 33:79–88
5. Franke AA, Hankin JH, Yu MC, Maskarinec G, Low SH, Custer LJ (1999) Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. J Agric Food Chem 47:977–986
6. Mouly P, Gaydou EM, Estienne J (1993) Column liquid chromatographic determination of flavanone glycosides in Citrus. Application to grapefruit and sour orange juice adulterations. J Chromatogr 634:129–134
7. Arts IC, de van PB, Hollman PC (2000) Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. J Agric Food Chem 48:1746–1751
8. Arts IC, de van PB, Hollman PC (2000) Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. J Agric Food Chem 48:1752–1757
9. Burns J, Yokota T, Ashihara H, Lean ME, Crozier A (2002) Plant foods and herbal sources of resveratrol. J Agric Food Chem 50:3337–3340
10. Sobolev VS, Cole RJ (1999) Trans-resveratrol content in commercial peanuts and peanut products. J Agric Food Chem 47:1435–1439
11. Wang Y, Catana F, Yang Y, Roderick R, van Breemen RB (2002) An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and in wine. J Agric Food Chem 50:431–435
12. Sanders TH, McMichael RW Jr, Hendrix KW (2000) Occurrence of resveratrol in edible peanuts. J Agric Food Chem 48:1243–1246
13. Lyons MM, Yu C, Toma RB, Cho SY, Reiboldt W, Lee J, van Breemen RB (2003) Resveratrol in raw and baked blueberries and bilberries. J Agric Food Chem 51:5867–5870

14. Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5:493–506
15. Shakibaei M, Csaki C, Nebrich S, Mobasheri A (2008) Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochem Pharmacol 76:1426–1439
16. Csaki C, Keshishzadeh N, Fischer K, Shakibaei M (2008) Regulation of inflammation signalling by resveratrol in human chondrocytes in vitro. Biochem Pharmacol 75:677–687
17. Shakibaei M, John T, Seifarth C, Mobasheri A (2007) Resveratrol inhibits IL-1 beta-induced stimulation of caspase-3 and cleavage of PARP in human articular chondrocytes in vitro. Ann NY Acad Sci 1095:554–563
18. Tao HY, Wu CF, Zhou Y, Gong WH, Zhang X, Iribarren P, Zhao YQ, Le YY, Wang JM (2004) The grape component resveratrol interferes with the function of chemoattractant receptors on phagocytic leukocytes. Cell Mol Immunol 1:50–56
19. Yu L, Wu SL, Zhang M, Pan CE (2003) Effect of resveratrol alone and its combination with cyclosporin A on the immune function of human peripheral blood T lymphocytes. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 19:549–551
20. Falchetti R, Fuggetta MP, Lanzilli G, Tricarico M, Ravagnan G (2001) Effects of resveratrol on human immune cell function. Life Sci 70:81–96
21. Boscolo P, del Signore A, Sabbioni E, Di Gioacchino M, Di Giampaolo L, Reale M, Conti P, Paganelli R, Giaccio M (2003) Effects of resveratrol on lymphocyte proliferation and cytokine release. Ann Clin Lab Sci 33:226–231
22. Comalada M, Ballester I, Bailon E, Sierra S, Xaus J, Galvez J, de Medina FS, Zarzuelo A (2006) Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. Biochem Pharmacol 72:1010–1021
23. Kempuraj D, Madhappan B, Christodoulou S, Boucher W, Cao J, Papadopoulou N, Cetrulo CL, Theoharides TC (2005) Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. Br J Pharmacol 145:934–944
24. Kempuraj D, Castellani ML, Petrarca C, Frydas S, Conti P, Theoharides TC, Vecchiet J (2006) Inhibitory effect of quercetin on tryptase and interleukin-6 release, and histidine decarboxylase mRNA transcription by human mast cell-1 cell line. Clin Exp Med 6:150–156
25. Min YD, Choi CH, Bark H, Son HY, Park HH, Lee S, Park JW, Park EK, Shin HI, Kim SH (2007) Quercetin inhibits expression of inflammatory cytokines through attenuation of NF-kappaB and p38 MAPK in HMC-1 human mast cell line. Inflamm Res 56:210–215
26. Kandere-Grzybowska K, Kempuraj D, Cao J, Cetrulo CL, Theoharides TC (2006) Regulation of IL-1-induced selective IL-6 release from human mast cells and inhibition by quercetin. Br J Pharmacol 148:208–215
27. Lugli E, Ferraresi R, Roat E, Troiano L, Pinti M, Nasi M, Nemes E, Bertoncelli L, Gibellini L, Salomoni P, Cooper EL, Cossarizza A (2009) Quercetin inhibits lymphocyte activation and proliferation without inducing apoptosis in peripheral mononuclear cells. Leuk Res 33:140–150
28. Bandyopadhyay S, Romero JR, Chattopadhyay N (2008) Kaempferol and quercetin stimulate granulocyte-macrophage colony-stimulating factor secretion in human prostate cancer cells. Mol Cell Endocrinol 287:57–64
29. Nair MP, Kandaswami C, Mahajan S, Chadha KC, Chawda R, Nair H, Kumar N, Nair RE, Schwartz SA (2002) The flavonoid, quercetin, differentially regulates Th-1 (IFNgamma) and Th-2 (IL4) cytokine gene expression by normal peripheral blood mononuclear cells. Biochim Biophys Acta 1593:29–36
30. Zhao XY, Gu ZL (1996) Effects of quercetin on production and release of endothelin and cGMP from cultured endothelial cells. Zhongguo Yao Li Xue Bao 17:442–444
31. Pagonis C, Tauber AI, Pavlotsky N, Simons ER (1986) Flavonoid impairment of neutrophil response. Biochem Pharmacol 35:237–245
32. Pincemail J, Deby C, Thirion A, Bruyn-Dister M, Goutier R (1988) Human myeloperoxidase activity is inhibited in vitro by quercetin. Comparison with three related compounds. Experientia 44:450–453

33. Blackburn WD Jr, Heck LW, Wallace RW (1987) The bioflavonoid quercetin inhibits neutrophil degranulation, superoxide production, and the phosphorylation of specific neutrophil proteins. Biochem Biophys Res Commun 144:1229–1236
34. Busse WW, Kopp DE, Middleton E Jr (1984) Flavonoid modulation of human neutrophil function. J Allergy Clin Immunol 73:801–809
35. Berg PA, Daniel PT (1988) Effects of flavonoid compounds on the immune response. Prog Clin Biol Res 280:157–171
36. Schwartz A, Middleton E Jr (1984) Comparison of the effects of quercetin with those of other flavonoids on the generation and effector function of cytotoxic T lymphocytes. Immunopharmacology 7:115–126
37. Leung KH, Ip MM (1986) Regulation of rat natural killing. II. Inhibition of cytolysis and activation by inhibitors of lipoxygenase: possible role of leukotrienes. Cell Immunol 100:474–484
38. Sternberg Z, Chadha K, Lieberman A, Hojnacki D, Drake A, Zamboni P, Rocco P, Grazioli E, Weinstock-Guttman B, Munschauer F (2008) Quercetin and interferon-beta modulate immune response(s) in peripheral blood mononuclear cells isolated from multiple sclerosis patients. J Neuroimmunol 205:142–147
39. Kang BY, Kim S, Lee KH, Lee YS, Hong I, Lee MO, Min D, Chang I, Hwang JS, Park JS, Kim DH, Kim BG (2008) Transcriptional profiling in human HaCaT keratinocytes in response to kaempferol and identification of potential transcription factors for regulating differential gene expression. Exp Mol Med 40:208–219
40. Pang JL, Ricupero DA, Huang S, Fatma N, Singh DP, Romero JR, Chattopadhyay N (2006) Differential activity of kaempferol and quercetin in attenuating tumor necrosis factor receptor family signaling in bone cells. Biochem Pharmacol 71:818–826
41. Kim HK, Park HR, Lee JS, Chung TS, Chung HY, Chung J (2007) Down-regulation of iNOS and TNF-alpha expression by kaempferol via NF-kappaB inactivation in aged rat gingival tissues. Biogerontology 8:399–408
42. Murota K, Shimizu S, Miyamoto S, Izumi T, Obata A, Kikuchi M, Terao J (2002) Unique uptake and transport of isoflavone aglycones by human intestinal caco-2 cells: comparison of isoflavonoids and flavonoids. J Nutr 132:1956–1961
43. Selloum L, Djelili H, Sebihi L, Arnhold J (2004) Scavenger effect of flavonols on HOCl-induced luminol chemiluminescence. Luminescence 19:199–204
44. Wang IK, Lin-Shiau SY, Lin JK (1999) Induction of apoptosis by apigenin and related flavonoids through cytochrome c release and activation of caspase-9 and caspase-3 in leukaemia HL-60 cells. Eur J Cancer 35:1517–1525
45. Samhan-Arias AK, Martin-Romero FJ, Gutierrez-Merino C (2004) Kaempferol blocks oxidative stress in cerebellar granule cells and reveals a key role for reactive oxygen species production at the plasma membrane in the commitment to apoptosis. Free Radic Biol Med 37:48–61
46. Dobrzynska MM, Baumgartner A, Anderson D (2004) Antioxidants modulate thyroid hormone- and noradrenaline-induced DNA damage in human sperm. Mutagenesis 19:325–330
47. Noroozi M, Angerson WJ, Lean ME (1998) Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. Am J Clin Nutr 67:1210–1218
48. Park JH, Jin CY, Lee BK, Kim GY, Choi YH, Jeong YK (2008) Naringenin induces apoptosis through downregulation of Akt and caspase-3 activation in human leukemia THP-1 cells. Food Chem Toxicol 46:3684–3690
49. Sakata K, Hirose Y, Qiao Z, Tanaka T, Mori H (2003) Inhibition of inducible isoforms of cyclooxygenase and nitric oxide synthase by flavonoid hesperidin in mouse macrophage cell line. Cancer Lett 199:139–145
50. Morikawa K, Nonaka M, Mochizuki H, Handa K, Hanada H, Hirota K (2008) Naringenin and hesperetin induce growth arrest, apoptosis, and cytoplasmic fat deposit in human preadipocytes. J Agric Food Chem 56:11030–11037
51. Krol W, Czuba ZP, Threadgill MD, Cunningham BD, Pietsz G (1995) Inhibition of nitric oxide (NO) production in murine macrophages by flavones. Biochem Pharmacol 50:1031–1035
52. Nicholas C, Batra S, Vargo MA, Voss OH, Gavrilin MA, Wewers MD, Guttridge DC, Grotewold E, Doseff AI (2007) Apigenin blocks lipopolysaccharide-induced lethality in vivo and proinflammatory cytokines expression by inactivating NF-kappaB through the suppression of p65 phosphorylation. J Immunol 179:7121–7127

53. Ha SK, Lee P, Park JA, Oh HR, Lee SY, Park JH, Lee EH, Ryu JH, Lee KR, Kim SY (2008) Apigenin inhibits the production of NO and PGE2 in microglia and inhibits neuronal cell death in a middle cerebral artery occlusion-induced focal ischemia mice model. Neurochem Int 52:878–886
54. Lee SJ, Yoon JH, Song KS (2007) Chrysin inhibited stem cell factor (SCF)/c-Kit complex-induced cell proliferation in human myeloid leukemia cells. Biochem Pharmacol 74:215–225
55. Harris GK, Qian Y, Leonard SS, Sbarra DC, Shi X (2006) Luteolin and chrysin differentially inhibit cyclooxygenase-2 expression and scavenge reactive oxygen species but similarly inhibit prostaglandin-E2 formation in RAW 264.7 cells. J Nutr 136:1517–1521
56. Wang XG, Chen GY, Chen MP (2007) Effect of luteolin on COX-2 and mPGES-1 expression in LPS-induced RAW264.7 cells. Zhong Yao Cai 30:1263–1266
57. Hirano T, Higa S, Arimitsu J, Naka T, Shima Y, Ohshima S, Fujimoto M, Yamadori T, Kawase I, Tanaka T (2004) Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. Int Arch Allergy Immunol 134:135–140
58. Cai Q, Rahn RO, Zhang R (1997) Dietary flavonoids, quercetin, luteolin and genistein, reduce oxidative DNA damage and lipid peroxidation and quench free radicals. Cancer Lett 119:99–107
59. Morley KL, Ferguson PJ, Koropatnick J (2007) Tangeretin and nobiletin induce G1 cell cycle arrest but not apoptosis in human breast and colon cancer cells. Cancer Lett 251:168–178
60. Chen KH, Weng MS, Lin JK (2007) Tangeretin suppresses IL-1beta-induced cyclooxygenase (COX)-2 expression through inhibition of p38 MAPK, JNK, and AKT activation in human lung carcinoma cells. Biochem Pharmacol 73:215–227
61. Hwang KY, Oh YT, Yoon H, Lee J, Kim H, Choe W, Kang I (2008) Baicalein suppresses hypoxia-induced HIF-1alpha protein accumulation and activation through inhibition of reactive oxygen species and PI 3-kinase/Akt pathway in BV2 murine microglial cells. Neurosci Lett 444:264–269
62. Yoneyama S, Kawai K, Tsuno NH, Okaji Y, Asakage M, Tsuchiya T, Yamada J, Sunami E, Osada T, Kitayama J, Takahashi K, Nagawa H (2008) Epigallocatechin gallate affects human dendritic cell differentiation and maturation. J Allergy Clin Immunol 121:209–214
63. Melgarejo E, Medina MA, Sanchez-Jimenez F, Botana LM, Dominguez M, Escribano L, Orfao A, Urdiales JL (2007) (−)-Epigallocatechin-3-gallate interferes with mast cell adhesiveness, migration and its potential to recruit monocytes. Cell Mol Life Sci 64:2690–2701
64. Kawai K, Tsuno NH, Kitayama J, Okaji Y, Yazawa K, Asakage M, Hori N, Watanabe T, Takahashi K, Nagawa H (2004) Epigallocatechin gallate attenuates adhesion and migration of CD8+ T cells by binding to CD11b. J Allergy Clin Immunol 113:1211–1217
65. Watson JL, Vicario M, Wang A, Moreto M, McKay DM (2005) Immune cell activation and subsequent epithelial dysfunction by Staphylococcus enterotoxin B is attenuated by the green tea polyphenol (−)-epigallocatechin gallate. Cell Immunol 237:7–16
66. Kawai K, Tsuno NH, Kitayama J, Okaji Y, Yazawa K, Asakage M, Sasaki S, Watanabe T, Takahashi K, Nagawa H (2005) Epigallocatechin gallate induces apoptosis of monocytes. J Allergy Clin Immunol 115:186–191
67. Syed DN, Afaq F, Kweon MH, Hadi N, Bhatia N, Spiegelman VS, Mukhtar H (2007) Green tea polyphenol EGCG suppresses cigarette smoke condensate-induced NF-kappaB activation in normal human bronchial epithelial cells. Oncogene 26:673–682
68. Daniel PT, Holzschuh J, Diao GJ, Berg PA (1988) Interference of the flavonoid compound cianidanol with macrophage function and lymphocyte activating mechanisms. Prog Clin Biol Res 280:205–209
69. Daniel PT, Falcioni F, Berg AU, Berg PA (1986) Influence of cianidanol on specific and non-specific immune mechanisms. Methods Find Exp Clin Pharmacol 8:139–145
70. Noh SU, Cho EA, Kim HO, Park YM (2008) Epigallocatechin-3-gallate improves dermatophagoides pteronissinus extract-induced atopic dermatitis-like skin lesions in NC/Nga mice by suppressing macrophage migration inhibitory factor. Int Immunopharmacol 8:1172–1182
71. Katiyar SK, Mukhtar H (2001) Green tea polyphenol (−)-epigallocatechin-3-gallate treatment to mouse skin prevents UVB-induced infiltration of leukocytes, depletion of antigen-presenting cells, and oxidative stress. J Leukoc Biol 69:719–726
72. Kenny TP, Keen CL, Schmitz HH, Gershwin ME (2007) Immune effects of cocoa procyanidin oligomers on peripheral blood mononuclear cells. Exp Biol Med (Maywood) 232:293–300

73. Mao TK, van de WJ, Keen CL, Schmitz HH, Gershwin ME (2002) Modulation of TNF-alpha secretion in peripheral blood mononuclear cells by cocoa flavanols and procyanidins. Dev Immunol 9:135–141
74. Mao TK, van de WJ, Keen CL, Schmitz HH, Gershwin ME (2003) Cocoa flavonols and procyanidins promote transforming growth factor-beta1 homeostasis in peripheral blood mononuclear cells. Exp Biol Med (Maywood) 228:93–99
75. Sanbongi C, Suzuki N, Sakane T (1997) Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans in vitro. Cell Immunol 177:129–136
76. Mitjans M, del Campo J, Abajo C, Martinez V, Selga A, Lozano C, Torres JL, Vinardell MP (2004) Immunomodulatory activity of a new family of antioxidants obtained from grape polyphenols. J Agric Food Chem 52:7297–7299
77. Magrone T, Tafaro A, Jirillo F, Amati L, Jirillo E, Covelli V (2008) Elicitation of immune responsiveness against antigenic challenge in age-related diseases: effects of red wine polyphenols. Curr Pharm Des 14:2749–2757
78. Shim JH, Choi HS, Pugliese A, Lee SY, Chae JI, Choi BY, Bode AM, Dong Z (2008) (−)-Epigallocatechin gallate regulates CD3-mediated T cell receptor signaling in leukemia through the inhibition of ZAP-70 kinase. J Biol Chem 283:28370–28379
79. Hafeez BB, Siddiqui IA, Asim M, Malik A, Afaq F, Adhami VM, Saleem M, Din M, Mukhtar H (2008) A dietary anthocyanidin delphinidin induces apoptosis of human prostate cancer PC3 cells in vitro and in vivo: involvement of nuclear factor-kappaB signaling. Cancer Res 68:8564–8572
80. Martin S, Favot L, Matz R, Lugnier C, Andriantsitohaina R (2003) Delphinidin inhibits endothelial cell proliferation and cell cycle progression through a transient activation of ERK-1/-2. Biochem Pharmacol 65:669–675
81. Joshi SS, Kuszynski CA, Bagchi M, Bagchi D (2000) Chemopreventive effects of grape seed proanthocyanidin extract on Chang liver cells. Toxicology 155:83–90
82. Mantena SK, Katiyar SK (2006) Grape seed proanthocyanidins inhibit UV-radiation-induced oxidative stress and activation of MAPK and NF-kappaB signaling in human epidermal keratinocytes. Free Radic Biol Med 40:1603–1614
83. Ryan-Borchers TA, Park JS, Chew BP, McGuire MK, Fournier LR, Beerman KA (2006) Soy isoflavones modulate immune function in healthy postmenopausal women. Am J Clin Nutr 83:1118–1125
84. Huang Y, Cao S, Nagamani M, Anderson KE, Grady JJ, Lu LJ (2005) Decreased circulating levels of tumor necrosis factor-alpha in postmenopausal women during consumption of soy-containing isoflavones. J Clin Endocrinol Metab 90:3956–3962
85. Woodman OL, Missen MA, Boujaoude M (2004) Daidzein and 17 beta-estradiol enhance nitric oxide synthase activity associated with an increase in calmodulin and a decrease in caveolin-1. J Cardiovasc Pharmacol 44:155–163
86. Park J, Kim SH, Cho D, Kim TS (2005) Formononetin, a phyto-oestrogen, and its metabolites up-regulate interleukin-4 production in activated T cells via increased AP-1 DNA binding activity. Immunology 116:71–81
87. Gredel S, Grad C, Rechkemmer G, Watzl B (2008) Phytoestrogens and phytoestrogen metabolites differentially modulate immune parameters in human leukocytes. Food Chem Toxicol 46:3691–3696
88. Cordle CT, Winship TR, Schaller JP, Thomas DJ, Buck RH, Ostrom KM, Jacobs JR, Blatter MM, Cho S, Gooch WM III, Pickering LK (2002) Immune status of infants fed soy-based formulas with or without added nucleotides for 1 year: part 2: immune cell populations. J Pediatr Gastroenterol Nutr 34:145–153
89. Guruvayoorappan C, Kuttan G (2007) Amentoflavone, a biflavonoid from Biophytum sensitivum augments lymphocyte proliferation, natural killer cell and antibody dependent cellular cytotoxicity through enhanced production of IL-2 and IFN-gamma and restrains serum sialic acid and gamma glutamyl transpeptidase production in tumor – bearing animals. J Exp Ther Oncol 6:285–295
90. Rachon D, Rimoldi G, Wuttke W (2006) In vitro effects of genistein and resveratrol on the production of interferon-gamma (IFNgamma) and interleukin-10 (IL-10) by stimulated murine splenocytes. Phytomedicine 13:419–424
91. Yusuf N, Nasti TH, Meleth S, Elmets CA (2009) Resveratrol enhances cell-mediated immune response to DMBA through TLR4 and prevents DMBA induced cutaneous carcinogenesis. Mol Carcinog 48(8):713–723

92. Li T, Fan GX, Wang W, Li T, Yuan YK (2007) Resveratrol induces apoptosis, influences IL-6 and exerts immunomodulatory effect on mouse lymphocytic leukemia both in vitro and in vivo. Int Immunopharmacol 7:1221–1231
93. Sharma S, Chopra K, Kulkarni SK, Agrewala JN (2007) Resveratrol and curcumin suppress immune response through CD28/CTLA-4 and CD80 co-stimulatory pathway. Clin Exp Immunol 147:155–163
94. Marier JF, Chen K, Prince P, Scott G, del Castillo JR, Vachon P (2005) Production of ex vivo lipopolysaccharide-induced tumor necrosis factor-alpha, interleukin-1beta, and interleukin-6 is suppressed by trans-resveratrol in a concentration-dependent manner. Can J Vet Res 69:151–154
95. Gao X, Deeb D, Media J, Divine G, Jiang H, Chapman RA, Gautam SC (2003) Immunomodulatory activity of resveratrol: discrepant in vitro and in vivo immunological effects. Biochem Pharmacol 66:2427–2435
96. Gao X, Xu YX, Janakiraman N, Chapman RA, Gautam SC (2001) Immunomodulatory activity of resveratrol: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production. Biochem Pharmacol 62:1299–1308
97. Yang Y, Paik JH, Cho D, Cho JA, Kim CW (2008) Resveratrol induces the suppression of tumor-derived CD4 + CD25+ regulatory T cells. Int Immunopharmacol 8:542–547
98. Vetvicka V, Volny T, Saraswat-Ohri S, Vashishta A, Vancikova Z, Vetvickova J (2007) Glucan and resveratrol complex-possible synergistic effects on immune system. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 151:41–46
99. Feng YH, Zhou WL, Wu QL, Li XY, Zhao WM, Zou JP (2002) Low dose of resveratrol enhanced immune response of mice. Acta Pharmacol Sin 23:893–897
100. Okamoto I, Iwaki K, Koya-Miyata S, Tanimoto T, Kohno K, Ikeda M, Kurimoto M (2002) The flavonoid Kaempferol suppresses the graft-versus-host reaction by inhibiting type 1 cytokine production and CD8+ T cell engraftment. Clin Immunol 103:132–144
101. Yu ES, Min HJ, An SY, Won HY, Hong JH, Hwang ES (2008) Regulatory mechanisms of IL-2 and IFNgamma suppression by quercetin in T helper cells. Biochem Pharmacol 76:70–78
102. Bertelli AA, Mannari C, Santi S, Filippi C, Migliori M, Giovannini L (2008) Immunomodulatory activity of shikimic acid and quercitin in comparison with oseltamivir (Tamiflu) in an in vitro model. J Med Virol 80:741–745
103. Roseghini R, Rocha DS, Clarencio J, Costa SL, Costa MF, Tardy M, Nascimento R, Schaer R, Velozo E, Meyer R, Freire S (2007) Flavonoid rutin alters the viability and function of mitogen-stimulated splenocytes and thymocytes compared with non stimulated cells. Immunopharmacol Immunotoxicol 29:271–285
104. Yoon MS, Lee JS, Choi BM, Jeong YI, Lee CM, Park JH, Moon Y, Sung SC, Lee SK, Chang YH, Chung HY, Park YM (2006) Apigenin inhibits immunostimulatory function of dendritic cells: Implication of immunotherapeutic adjuvant. Mol Pharmacol 70:1033–1044
105. Rezai-Zadeh K, Ehrhart J, Bai Y, Sanberg PR, Bickford P, Tan J, Shytle RD (2008) Apigenin and luteolin modulate microglial activation via inhibition of STAT1-induced CD40 expression. J Neuroinflammation 5:41
106. Chen CY, Peng WH, Tsai KD, Hsu SL (2007) Luteolin suppresses inflammation-associated gene expression by blocking NF-kappaB and AP-1 activation pathway in mouse alveolar macrophages. Life Sci 81:1602–1614
107. Kim JS, Jobin C (2005) The flavonoid luteolin prevents lipopolysaccharide-induced NF-kappaB signalling and gene expression by blocking IkappaB kinase activity in intestinal epithelial cells and bone-marrow derived dendritic cells. Immunology 115:375–387
108. Jeong YI, Jung ID, Lee JS, Lee CM, Lee JD, Park YM (2007) (−)-Epigallocatechin gallate suppresses indoleamine 2, 3-dioxygenase expression in murine dendritic cells: evidences for the COX-2 and STAT1 as potential targets. Biochem Biophys Res Commun 354:1004–1009
109. Han MK (2003) Epigallocatechin gallate, a constituent of green tea, suppresses cytokine-induced pancreatic beta-cell damage. Exp Mol Med 35:136–139
110. Mantena SK, Roy AM, Katiyar SK (2005) Epigallocatechin-3-gallate inhibits photocarcinogenesis through inhibition of angiogenic factors and activation of CD8+ T cells in tumors. Photochem Photobiol 81:1174–1179

111. Morimoto M, Watanabe T, Yamori M, Takebe M, Wakatsuki Y (2009) Isoflavones regulate innate immunity and inhibit experimental colitis. J Gastroenterol Hepatol 24(6):1123–1129
112. Verdrengh M, Jonsson IM, Holmdahl R, Tarkowski A (2003) Genistein as an anti-inflammatory agent. Inflamm Res 52:341–346
113. Kogiso M, Sakai T, Mitsuya K, Komatsu T, Yamamoto S (2006) Genistein suppresses antigen-specific immune responses through competition with 17beta-estradiol for estrogen receptors in ovalbumin-immunized BALB/c mice. Nutrition 22:802–809
114. Yellayi S, Zakroczymski MA, Selvaraj V, Valli VE, Ghanta V, Helferich WG, Cooke PS (2003) The phytoestrogen genistein suppresses cell-mediated immunity in mice. J Endocrinol 176:267–274
115. Sakai T, Kogiso M, Mitsuya K, Komatsu T, Yamamoto S (2006) Genistein enhances antigen-specific cytokine production in female DO11.10 transgenic mice. J Nutr Sci Vitaminol (Tokyo) 52:327–332
116. Baeza I, de Castro NM, Alvarado C, Alvarez P, Arranz L, Bayon J, de la FM (2007) Improvement of immune cell functions in aged mice treated for five weeks with soybean isoflavones. Ann NY Acad Sci 1100:497–504
117. Zhang R, Li Y, Wang W (1997) Enhancement of immune function in mice fed high doses of soy daidzein. Nutr Cancer 29:24–28
118. Guo TL, McCay JA, Zhang LX, Brown RD, You L, Karrow NA, Germolec DR, White KL Jr (2001) Genis∞tein modulates immune responses and increases host resistance to B16F10 tumor in adult female B6C3F1 mice. J Nutr 131:3251–3258
119. Guo TL, Zhang XL, Bartolucci E, McCay JA, White KL Jr, You L (2002) Genistein and methoxychlor modulate the activity of natural killer cells and the expression of phenotypic markers by thymocytes and splenocytes in F0 and F1 generations of Sprague-Dawley rats. Toxicology 172:205–215
120. Yellayi S, Naaz A, Szewczykowski MA, Sato T, Woods JA, Chang J, Segre M, Allred CD, Helferich WG, Cooke PS (2002) The phytoestrogen genistein induces thymic and immune changes: a human health concern? Proc Natl Acad Sci USA 99:7616–7621
121. Sakai T, Kogiso M, Mitsuya K, Komatsu T, Yamamoto S (2006) Genistein suppresses development of spontaneous atopic-like dermatitis in NC/Nga mice. J Nutr Sci Vitaminol (Tokyo) 52:293–296
122. Duan W, Kuo IC, Selvarajan S, Chua KY, Bay BH, Wong WS (2003) Antiinflammatory effects of genistein, a tyrosine kinase inhibitor, on a guinea pig model of asthma. Am J Respir Crit Care Med 167:185–192
123. Guo TL, Chi RP, Zhang XL, Musgrove DL, Weis C, Germolec DR, White KL Jr (2006) Modulation of immune response following dietary genistein exposure in F0 and F1 generations of C57BL/6 mice: evidence of thymic regulation. Food Chem Toxicol 44:316–325
124. Lin JP, Yang JS, Lu CC, Chiang JH, Wu CL, Lin JJ, Lin HL, Yang MD, Liu KC, Chiu TH, Chung JG (2009) Rutin inhibits the proliferation of murine leukemia WEHI-3 cells in vivo and promotes immune response in vivo. Leuk Res 33:823–828
125. Yano S, Umeda D, Maeda N, Fujimura Y, Yamada K, Tachibana H (2006) Dietary apigenin suppresses IgE and inflammatory cytokines production in C57BL/6N mice. J Agric Food Chem 54:5203–5207
126. Yano S, Umeda D, Yamashita T, Ninomiya Y, Sumida M, Fujimura Y, Yamada K, Tachibana H (2007) Dietary flavones suppresses IgE and Th2 cytokines in OVA-immunized BALB/c mice. Eur J Nutr 46:257–263
127. Kang TH, Lee JH, Song CK, Han HD, Shin BC, Pai SI, Hung CF, Trimble C, Lim JS, Kim TW, Wu TC (2007) Epigallocatechin-3-gallate enhances CD8+ T cell-mediated antitumor immunity induced by DNA vaccination. Cancer Res 67:802–811
128. Wang Y, Mei Y, Feng D, Xu L (2006) (−)-Epigallocatechin-3-gallate protects mice from concanavalin A-induced hepatitis through suppressing immune-mediated liver injury. Clin Exp Immunol 145:485–492
129. Srinivasan P, Sabitha KE, Shyamaladevi CS (2006) Modulatory efficacy of green tea polyphenols on glycoconjugates and immunological markers in 4-Nitroquinoline 1-oxide-induced oral carcinogenesis – a therapeutic approach. Chem Biol Interact 162:149–156
130. Sharma SD, Katiyar SK (2006) Dietary grape-seed proanthocyanidin inhibition of ultraviolet B-induced immune suppression is associated with induction of IL-12. Carcinogenesis 27:95–102
131. Zhang XY, Li WG, Wu YJ, Zheng TZ, Li W, Qu SY, Liu NF (2005) Proanthocyanidin from grape seeds potentiates anti-tumor activity of doxorubicin via immunomodulatory mechanism. Int Immunopharmacol 5:1247–1257

132. Wang J, Zhang Q, Jin S, He D, Zhao S, Liu S (2008) Genistein modulate immune responses in collagen-induced rheumatoid arthritis model. Maturitas 59:405–412
133. Yeh CC, Kao SJ, Lin CC, Liu CJ, Kao ST (2007) The immunomodulation of endotoxin-induced acute lung injury by hesperidin in vivo and in vitro. Life Sci 20:1821–1831

24 Antiviral Activity of Phytochemicals: A Current Perspective

Rajesh Naithani, Rajendra G. Mehta, Deepak Shukla, Susantha N. Chandersekera, and Robert M. Moriarty

Key Points

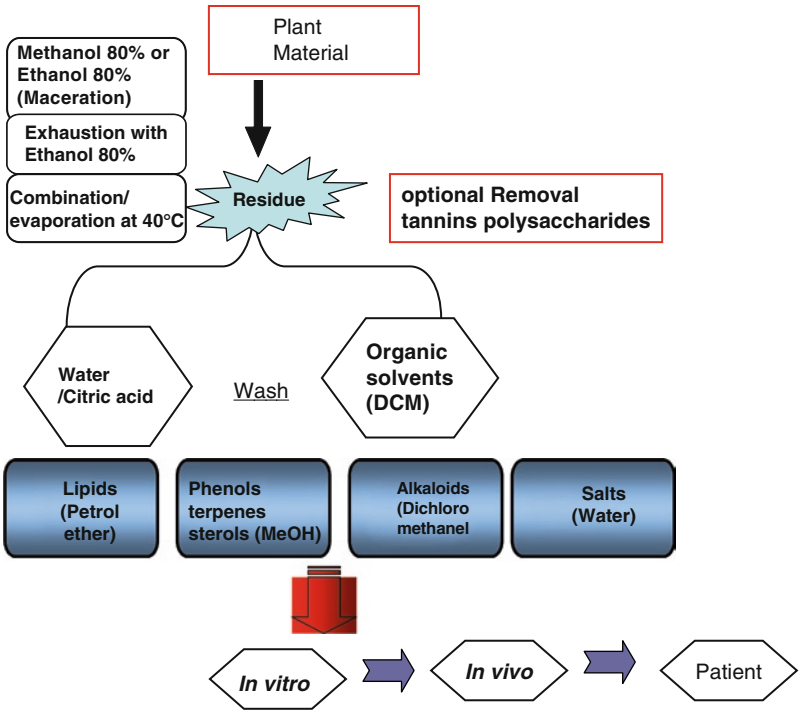
- A wide variety of active phytochemicals have been found to have therapeutic applications against genetically and functionally diverse viruses.
- The antiviral mechanism of these agents may be explained on the basis of their antioxidant activities, scavenging capacities, the inhibition of DNA, RNA synthesis, or the blocking of viral reproduction, etc.
- Numerous epidemiological and experimental studies have revealed that a large number of phytochemicals have promising antiviral activities. Especially in the last decade, a number of promising leads have been identified by a combination of *in vitro* and *in vivo* studies using diverse biological assays.

Key Words: Antiviral, phytochemical, infection, replication, flavonoids, clinical trials, mechanism.

24.1 INTRODUCTION

Throughout the human history, man has been dependent on plant sources for his very basic needs (1). The use of medicinally active plants predates modern history. As per a World Health Organization estimate more than 80% of the world's population is dependant on traditional plants to meet their health requirements (2). A large number of plants used in the traditional medicine have now become a part of the modern world health care system because of their unique ability to synthesize a wide array of compounds with diverse health-related benefits (3, 4). Most recently, the introduction of plant-based products in the form of nutraceuticals and dietary supplements have made

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_24



Scheme 24.1. Plants provide compounds possessing broad range of activities such as antimicrobial, antiviral, antioxidative, immunomodulatory and antitumor properties.

a major impact in the drug industry market (5, 6). The isolation, structural elucidation, and evaluation of all major constituents of the plant-based products must occur efficiently in order to determine its pharmacological properties. A general isolation and evaluation procedure of plants-based active ingredients is depicted in Scheme 24.1. Plants provide compounds possessing a broad range of activities such as antimicrobial, antiviral, antioxidative, immunomodulatory and antitumor properties (7, 8). Because of the rapid advancement in modern day biology, drug discovery from natural sources has evolved into a highly multidisciplinary field utilizing various sophisticated methods of isolation, analysis, and evaluation. Over the last few decades, natural products have been studied for anti-infective and more specifically, antiviral activities. Basic researches in experimental models using various biological systems strongly suggest the protective role of plant-derived natural compounds against different viral infections (9, 10). Despite the substantial amount of progress made in the treatment and therapeutic strategies, the incidence, morbidity, and mortality of viral infections remain a major global challenge. The conditions are more complicated in the developing world due to the unavailability of relatively expensive medicines and widespread drug resistance (11). Unfortunately, many antiviral compounds presently in clinical use have a relatively narrow spectrum of activity, limited therapeutic usefulness, and variable toxicity. Whether natural antivirals can be developed as a viable alternative medicine or a synergistic combination therapy with pre-existing antiviral therapy will entirely depend on identifying broad-spectrum plant-based antivirals combined with a method of delivery and keeping in mind its stability and bioavailability. In addition, the development of a

suitable *in vitro* pharmacodynamic screening technique could contribute to the rapid identification of potential bioactive plants and also to the standardization and/or pharmacokinetic–pharmacodynamic profiling of the bioactive components.

Originating from nucleosides or closely related carbon structure, the wellknown drugs face an emerging problem of development of resistant viral strains (12, 13). In addition, the emergence of many new and perhaps more deadly viruses such as *Ebola and Marburg* viruses and the possible threat of their use as arsenal for bioterrorism have enhanced our urgency to find new and potent antivirals as soon as possible. This need is further aggravated by the fact that viral infections are now recognized as the second most important known cause of human cancer (14). Viruses absolutely require host cell environment for survival. Besides the genetic variation, divergent invasion strategies pose a major challenge. Since medicinal plants have an endless variety of chemical constituents, it could be utilized to counter genetic and invasion divergence and thus inhibit the replication of both DNA and RNA virus. In fact, the world of ethnopharmacological knowledge increases the success probability of finding a new drug candidate (15, 16). In this article, we examine current developments on various naturally occurring antiviral compounds. The major advances in the field of virus growth inhibition have been summarized. In addition, the origin, mechanistic action, and phase trials of various plant-derived antiviral agents have been included in this chapter.

24.1.1 Antiviral Assays

In the process of drug discovery, the selection of an appropriate bioassay and its validation are the important steps to determine the activity of the products and extracts. The determination of antiviral efficacy is relatively complex because it is not possible to design a single assay for different viruses as they require different cell systems. The fact that so many so-called "exciting molecules" do not graduate to the next stage is primarily due to major flaws in the screening methodologies. Some of the major challenges include using high assays dosages, using improper test controls, and the wrong selection of targets and endpoints. In spite of the progress made, little attention has been given to the influence of various reaction parameters (17). The lack of standardization in methodologies produces highly inconsistent results posing a major obstacle in developing a novel entity as a drug molecule. More commonly, the evaluation of the antiviral is based on the capability to replicate in a particular cell system.

The efficiency of the plant extract can be evaluated by large number of methods. At a preliminary level, the *in vitro* efficacy is detected using markers such as cytopathic effect, plaque formation, or proliferative effects on diverse cell lines (Table 24.1). The detection of viral RNA and DNA do provide information about the viral replication. Although a number of assays have been developed there is still a need for more standardized assays to provide consistent results. The complexity in evaluation of viral inhibition is attributed to its efficient replication coupled with its genetic variation and diverse invasion strategy. Confluent monolayers of the cells are infected with virus in combination with a varied concentration of the plant extract and incubated followed by the calorimetric determination of viable cells. Radioactive-labeled viruses are employed to determine the mode of antiviral activity (18). Determination of the values of EC_{50} (reciprocal dilution required to prevent virus-induced cytolysis by 50%) and $TCID_{50}$ (reduction of viral titer) are used as a measure to determine viral activity.

Table 24.1
Invitro efficacy assay

	Details about the virus	*Assay type*	*Assay methods*
1	Plaque formation capability	Plaque inhibition Plaque reduction	Titer determination in the presence of non-toxic dose of compound
2	Cytopathic effect inducement capability	Inhibition of the viral-induced CPE Virus yield reduction Endpoint titer determination	CPE determination with limited dose of virus Virus yield under treatment with a given amount of virus Virus titer reduction determination after dilution
3	Negative plaques formation cytopathic effect capability	Specific function determination	Hemagglutination test Hemadsorption test Inhibition of cell transformation Immunological tests: detection of antiviral antigens in cell culture (HSV, CMV, EBV, HIV)
4	Miscellaneous tests	Nucleic acid/ polypeptide inhibition Radioisotope uptake study Genome number determination	Reduction or inhibition of viral specific nucleic acid /polypeptides synthesis in infected culture Determination of uptake of radioisotope labeled precursor Viral genome copy with single compounds or with mixtures

24.2 CLASSIFICATION OF ANTIVIRAL PHYTOCHEMICALS

24.2.1 Flavonoids

The flavonoid structure, basically a polyphenol consisting of 15-carbon atoms skeleton (C_6–C_3–C_6 system) (**1**), constitutes the largest source of antiviral agents in the entire plant kingdom. In some compounds, the C_2 carbon atom is directly linked to the oxygen as a result of which furan type molecule is formed called aurone (**2**). The further subclassification of flavonoids is based upon the oxidation and the substitution pattern of the ring C. The biochemical effects of flavonoids are attributed to their ability to inhibit the number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca^{+2}-ATPase, lipoxygenase, cycloxygenase, etc., besides the regulatory role on different hormones like estrogens, androgens, and thyroid hormone (19, 20). Evaluating flavonoids for activity against herpes simplex virus (HSV), Thomas et al. reported that flavonols are more active than flavones (galangin > kaempferol > quercetin) (21). Flavonoid-based polymer (MW 2100 Daltons) has displayed substantial activity against HSV type-1 and type-2 strains (22). On the basis of the evaluation of a flavonoid subset, Gerdin et al. found that flavan-3-o1 was more effective in selective inhibition of human immunodeficiency virus (HIV)-1, -2, and similar immunodeficiency virus infections (23).

Chalcones, having general formula ArCH=CHC(=O)Ar forms the central core for a variety of important biological compounds. Considered as precursors of flavonoids and isoflavonoids, these compounds are abundant in edible plants, and display a diverse array of pharmacological activities. Deng et al. have reported excellent antiviral activity of chalcones **3** and **4** utilizing pharmacophore models to identify chemical signatures considered important for the antiviral activity (24, 25). Dihydrochalcones (**5**) (obtained by double bond reduction of chalcone) derived from *Millettia leucantha KURZ* (*Leguminosae*) showed anti-herpes simplex virus (HSV) activity (26). Flavones, structurally characterized by 2-phenylchromen-4-one backbone, are found in *Lamiaceae*, *Apiaceous*, and *Astraea* families. Likhitwitayawuid et al. described the isolation and anti-HSV activities of a series of phenolic compounds identified from the heartwood of *Artocarpus gomezianus*, including the new antiherpetic flavone artogomezianone (**6**) (27). Prendergast et al. have used 3′,4′-diacetoxy-5,6,7-trimethoxyflavone or naringin (**7**) in the treatment of viral (e.g., HCV, HIV, a picornavirus genus virus or a respiratory virus) or parasite (e.g., toxoplasmosis) infections (28). On the basis of molecular electrostatic potential (MEP) maps, Mishra et al. proposed that the anti-picornavirus activities of the flavones are related with negative MEP values in two regions, one near the 3-methoxy group and another in a diagonally opposite region near the substituent attached to the C_7 atom of the molecules (29). We have synthesized and confirmed the antiviral activity of several novel analogs of flavanone Abyssinone II (**8**), a naturally occurring prenylated flavanone, in HeLa cells using a recombinant β-galactosidase expressing strain of HSV-1(Herpes simplex virus Type 1) (30). Characterized by hydroxyl group at position C_3 of the flavonone molecule (**9**), flavanol mixture is applied for treating and preventing hepatitis B, mycotic infection, liver protection, inflammation disease, and autoimmune disease (31). The effect of several naturally occurring dietary flavonoids including quercetin (**10**) on the infectivity and replication of herpes simplex virus type 1 (HSV-I), polio-virus type 1, Para influenza virus type 3 (Pf-3), and respiratory syncytial virus (RSV) were studied in cell culture monolayers employing the technique of viral plaque reduction. Quercetin caused a concentration-dependent reduction in the infectivity of each virus. In addition, it reduced intracellular replication of each virus when monolayers were infected and subsequently cultured in a medium containing quercetin (10). Myricetin (**11**), a bioflavonoid whose occurrence in nature is widespread among plants showed excellent antiviral effect against hepatitis B virus, influenza virus, and/or coronavirus (32). Anthocyanidin (**12**) is an important group of plant pigments having free OH group which can co-ordinate with metal ions like Ca^{2+} and Mg^{2+} under alkali conditions. This coordination ability is one the major reasons for the bioactivity of molecule. Anderson et al. have reported the therapeutic effect of anthocyanidin in the treatment of diseases caused by viruses (33). Isoflavonoids is an important class of flavonoids with impressive biological activities formed as a result of migration of phenyl group from 2 to 3 as shown in **13** (Fig. 24.1). In contrast to most other flavonoids, isoflavones (**14**) have a rather limited taxonomic distribution and occur mainly within the *Leguminosae* family. Antiviral activity on Newcastle disease virus was examined and rotenone (**15**) showed significant inhibitory effects on the viral growth in cultured cells as determined by the plate and tube assay methods (34). Isoflavanones bear the same relationship to isoflavones as flavanones do to flavones. And, as in the case of flavanones, isoflavanones have a chiral center (C_3 in isoflavanones). PMZ-1, a prenylated isofla-

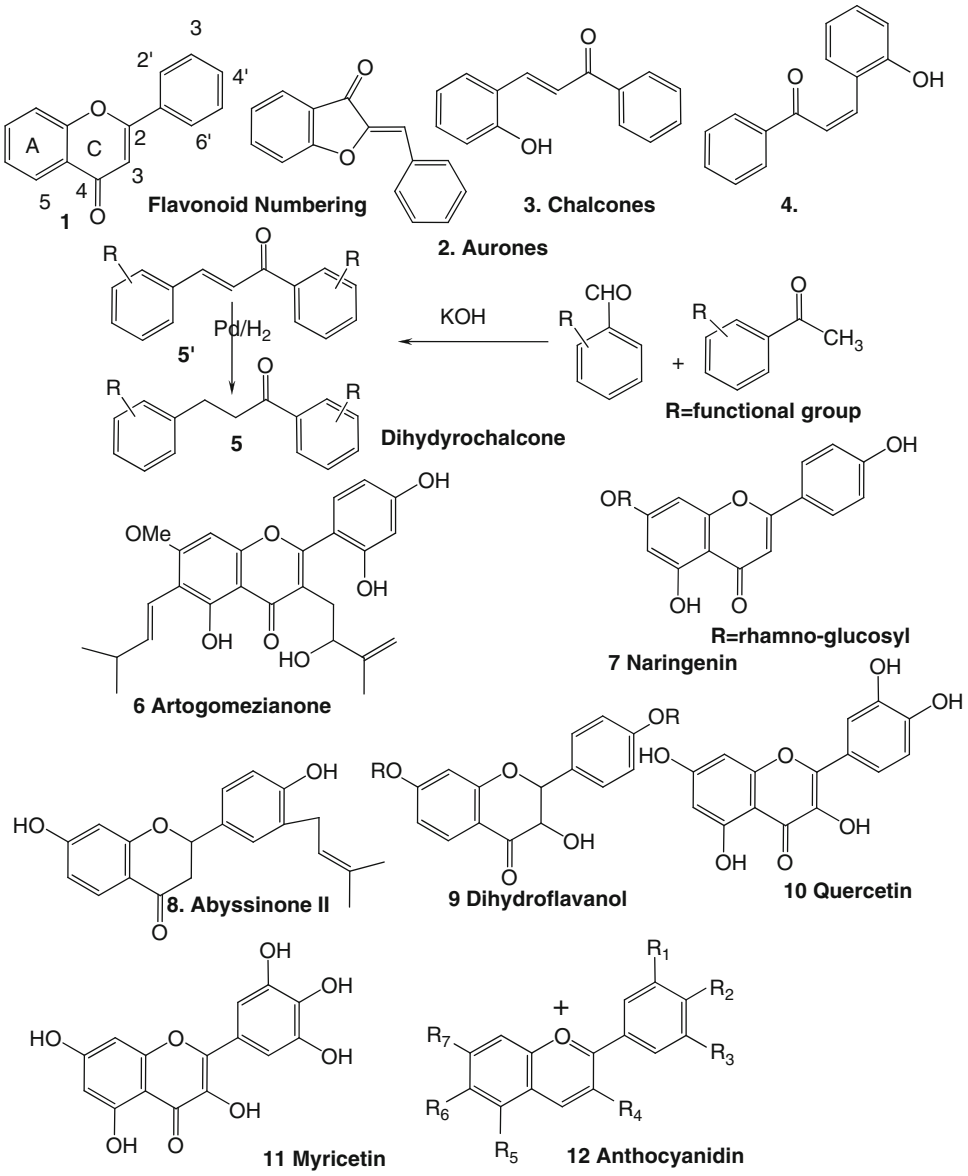


Fig. 24.1.

vonone (**16**), isolated *Bolusanthus speciosus* (Bolus Harms) has exhibited excellent activity against HIV–having a broad therapeutic index (TI > 300) (35).

Structurally, one of the simplest members of these subclasses, the isoflavans, is characterized by the fact that they do not have the carbonyl group at C_4 carbon, for example, 7,4′-dihydroxyisoflavan (**17**, equol). The effect of substituted isoflavans (**18**) (R and R1 = H, Cl, or Br) and isoflavenes (**19**) on human rhinovirus (HRV) 1B infection of HeLa cells was examined by Conti and coworkers who found that these compounds inhibited virus plaque formation in cell cultures with isoflavans being more effective than isoflavenes (36). It was found that the cells pretreated with compounds before challenge

with HRV-1B exhibited resistance to the virus-induced cytopathic effect. Arylcoumarins related to flavonoids biogenetically are characterized by the presence of a carbonyl function at C_2 and may or may not have oxygenation at C_4. A large number of coumarins has been studied for antiviral activities (37). Calanolide A (**20**) first isolated from a tropical tree (*Calophyllum lanigerum*) in Malaysia is one of the novel non-nucleoside reverse transcriptase inhibitor (NNRTI) with potent activity against HIV-1 (38).

Compounds belonging to the flavans class are normally devoid of carbonyl group at position 2. Although this class of compounds contains some common and comparatively simple compounds, catechin and epicatechin, in particular, the overall structural complexity of the group is impressive. Two new antiviral flavan derivatives were isolated from a methanol extract of leaves of *Pithecellobium clypearia* as guided by antiviral assays (7-*O*-galloyltricetifavan (**21a**) and 7,4-di-*O*-galloyltricetifavan (**21b**) (39). Neoflavonoids constitute a group of flavonoid derivatives that have their aryl group attached to C_4 as opposed in flavonoids and C_3 in isoflavonoids. A series of inophyllums **22**–**25** were isolated from the Malaysian tree *Calophyllum inophyllum* and evaluated for inhibitory activity against HIV-1 reverse transcriptase (RT). Among them, the most active compounds, inophyllum B and inophyllum P showed IC50 values against RT of 0.038 and 0.130 mM, respectively (40) (Fig. 24.2).

24.2.2 Alkaloids

Synthesized by plants from amino acids, alkaloids contain nitrogen in a heterocyclic ring. Some of the major nuclei found in various alkaloids have been shown in **26**, **27,** and **28**. Thirty-six alkaloids isolated either from *Catharanthus roseus* or *C. lanceus* were evaluated for *in vitro* activity against vaccinia and polio type III viruses. Nine of these alkaloids were effective as antiviral agents, with pericalline (**29**) being the most effective (41). In an attempt to obtain SAR data, Houghton et al. tested several naturally occurring chromone alkaloids (derived from the rootbark of *Schumanniophyton magnificum*) for the inhibition of HIV and HSV infections in C8166 and Vero cells, respectively. The authors also synthesized acyl and methyl derivatives for screening. It was found that the presence of a piperidine ring and free hydroxyl groups on the molecules seems to favor the anti-HIV activity. Irreversible binding to gp 120 was considered to be responsible for the anti-HIV activity (42).

24.2.3 Terpenoids

The terpenoids, sometimes referred to as isoprenoids, are a large and diverse class of naturally occurring phytochemicals derived from five-carbon isoprene units, which are assembled and modified in thousands of ways. Numerous phytocompounds that were evaluated for activity against anti-severe acute respiratory syndrome-associated coronavirus (SARS-CoV) activities using a cell-based assay measuring the SARS-CoV-induced cytopathogenic effect on Vero E6 cells and compounds (**30–32**) showed excellent activities (43). More than 220 phytocompounds (including ten diterpenoids, two sesquiterpenoids, and two triterpenoids) were screened for activity against anti-SARS-CoV activities utilizing a cell-based assay measuring SARS-CoV-induced cytopathogenic effect on Vero E6 cells. The bioactive compounds with anti-SARS-CoV activity in the μM range included abietane-type and labdane-type diterpenes sesquiterpenes and lupane-type triterpenes.

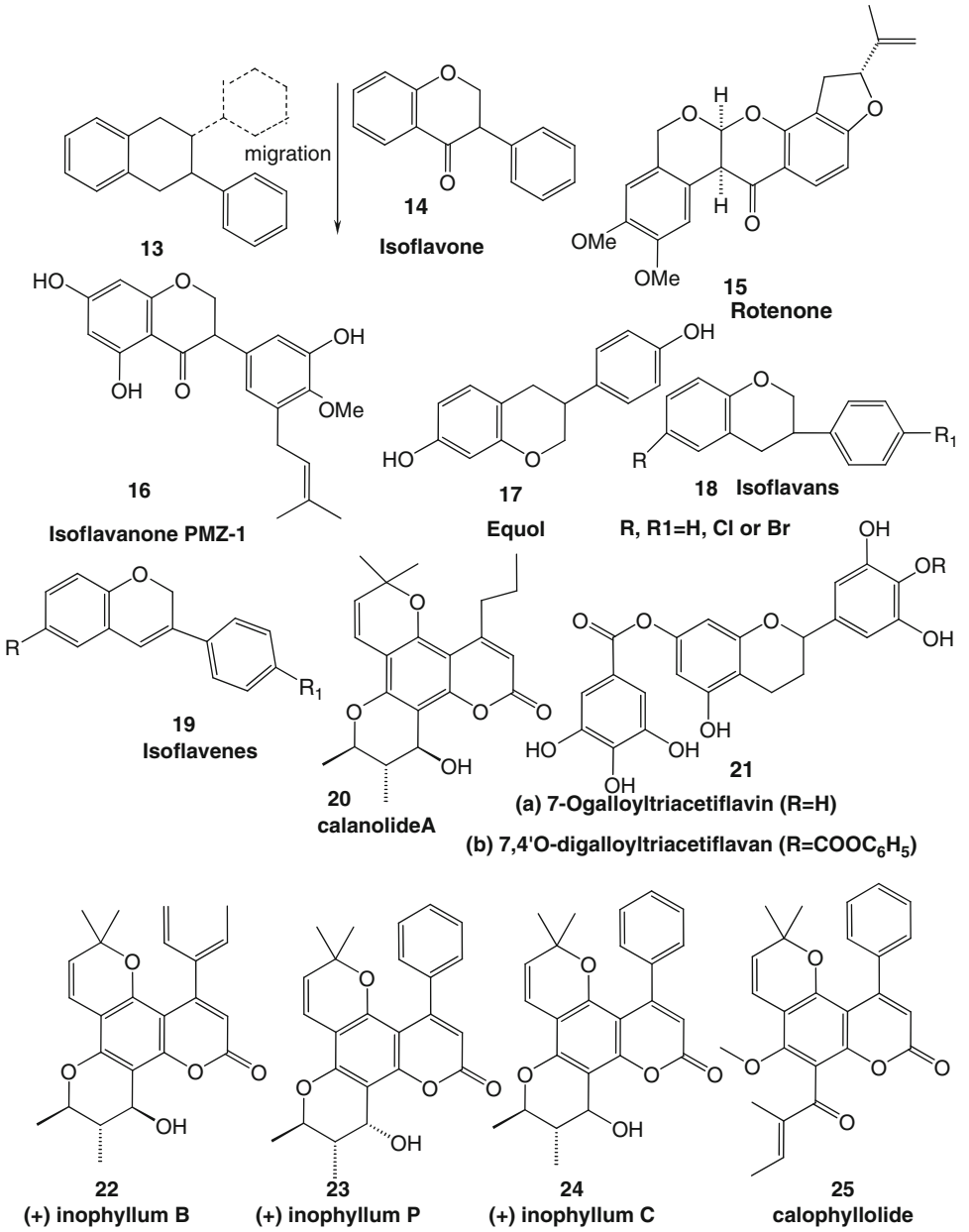


Fig. 24.2.

24.2.4 Carotenoids

Carotenoids considered as the structural backbone of compound belong to the category of tetraterpenoids (hydrocarbons resulting from the association of several isoprene units). Majority of the carotenoids are derived from the 40 carbon polyene chain which is sometimes terminated by rings. Carotenoids can be Xanthophylls (molecules containing oxygen) such as lutein and zeaxanthin and carotenes (the unoxygenated or oxygen free carotenoids). The concentrations of plasma carotenoids (α-carotene (**33**), β-carotene

(**34**), lutein/zeaxanthin (**35**) and lycopene (**36**) have been associated with the increased risk of death during HIV infection among infants in Uganda (44).

24.2.5 Organosulfur Compounds

Sulfur-containing compounds are present in all *Brasicaceae* family vegetables. In addition, plants belonging to *the Allium* family constitute an important class of antiviral agents (45). There a are number of representative examples of organosulfurs antivirals (**37–40**) that is, cauliflower, cabbage, kale, bok choy, brussels sprouts, radish mustard, and water garden cress that constitute the rich source of organosulfur compounds. Several unsymmetrical aralkyl disulfides, were synthesized and oxidized to study the relatively unexplored class of thiolsulfinate (46). The pungent odor, and chemical instability of these compounds make animal studies difficult; hence, structural modifications have been carried out. We have synthesized and screened several sulformates (based upon brassinin and sulfuraphane structures) derivatives for their HSV activities (47) (Fig. 24.3).

24.2.6 Vitamins

It has been shown that vitamin C (**41**) can increase the host immune response, and this may provide protection against infectious diseases (48). Vitamin E supplementation might be effective in the treatment of chronic hepatitis B (49). The name vitamin E covers a collection of eight fat soluble compounds, tocopherols (**42**) (methyl derivatives of tocopherol) and tocotrienols (**43**).

24.2.7 Selenium Compounds

A significant number of studies has indicated the importance of selenium compounds (**44–46**) as potent antiviral agents. The data generated from experimentation on various animal models and *in vitro* models demonstrate significant beneficial effects of selenium on different viral infections. Cermelli et al. studied the antiviral effects of three selenium compounds on the replication of Coxsackie virus B_5 replication (50). Selenite was shown to reduce viral replication in Coxsackie virus B_5 replication, but selenate and selenomethionine did not exhibit any substantial antiviral activity. Waotowicz et al. synthesized and tested different analogs of ebselen for their activity in *in vitro* antiviral assay. Some of the analogs tested had an appreciable inhibition of cytopathic activity of HSV-1 and encephalomyocarditis virus—EMCV (10) (Fig. 24.4).

24.2.8 Miscellaneous

Curcumin (**47**) derived from turmeric and a key constituent of food in the Indian subcontinent has shown potent activity against HIV-1 integrase (51). Chlorophyllin (CHLN) (**48**, **49**), a synthetic derivative of chlorophyll has been assayed for its capacity to prevent nuclear fragmentation (NF) in HEp-2 cells infected with poliovirus (52). Carboxymethyl chitin, a polysaccharides polymer containing partially deacetylated aminosugar showed a significant inhibition of Friend murine leukemia helper virus (F-MuLV) and HSV (53). Seven ellagitannins isolated from *Phyllanthus myrtifolius* and *P. urinaria*

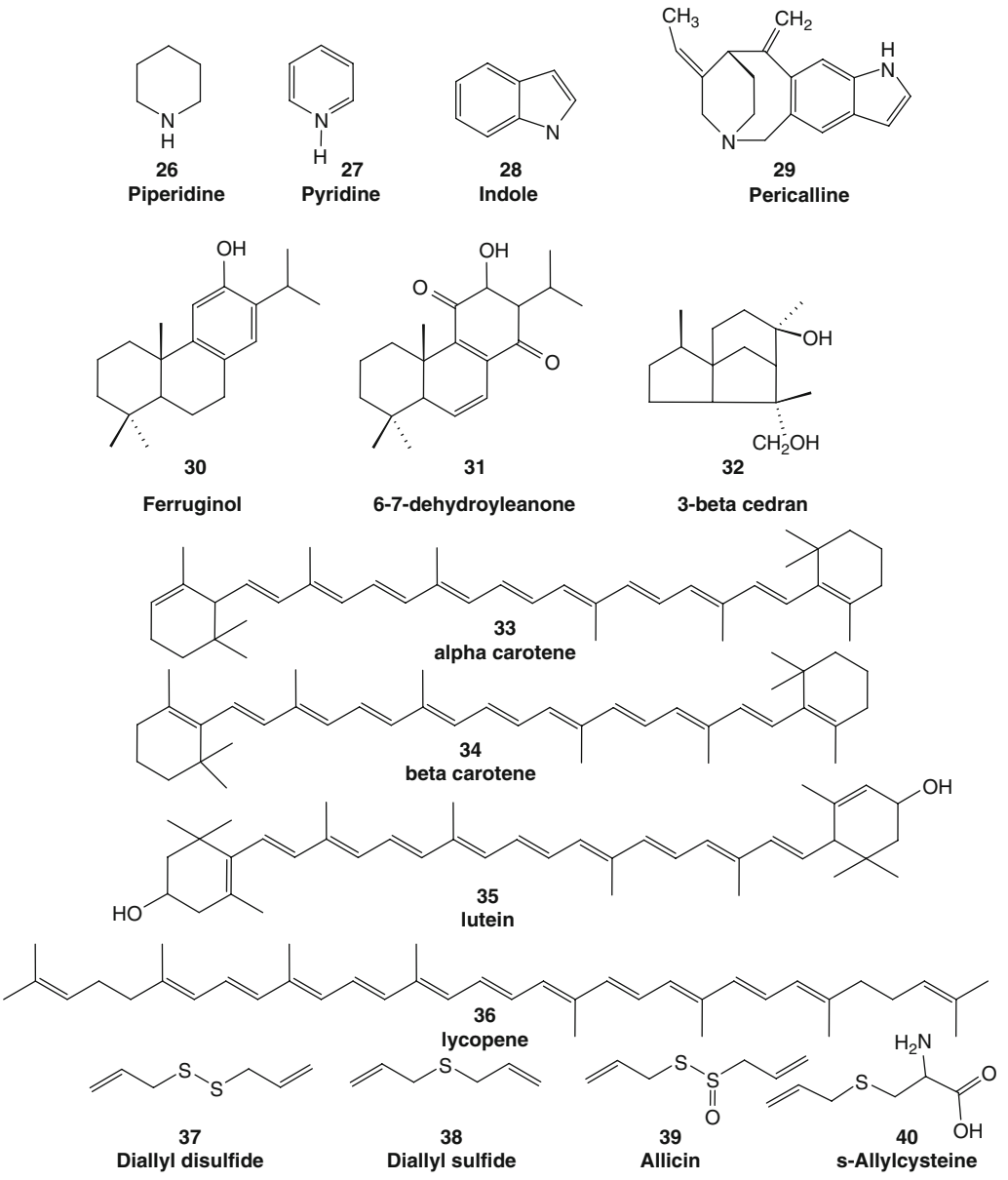


Fig. 24.3.

(*Euphorbiaceae*) have shown to be active against Epstein–Barr virus DNA polymerase (EBV-DP) (54). Polyacetylenes (**51**, **52**) are hydrocarbons that strongly absorb long-wave UV light. The medicinal activity of these compounds is altered upon exposure to light (photoactivation). The principal constituent in the leaf of *Bidens pilosa*, phenylheptatriyne (PHT), is one of the polyacetylenes that has been widely studied for its antiviral effects that is augmented by UV light exposure (55). The polyacetylenes are one of the few natural substances reported to inhibit CMV, a type of herpes virus that causes disease

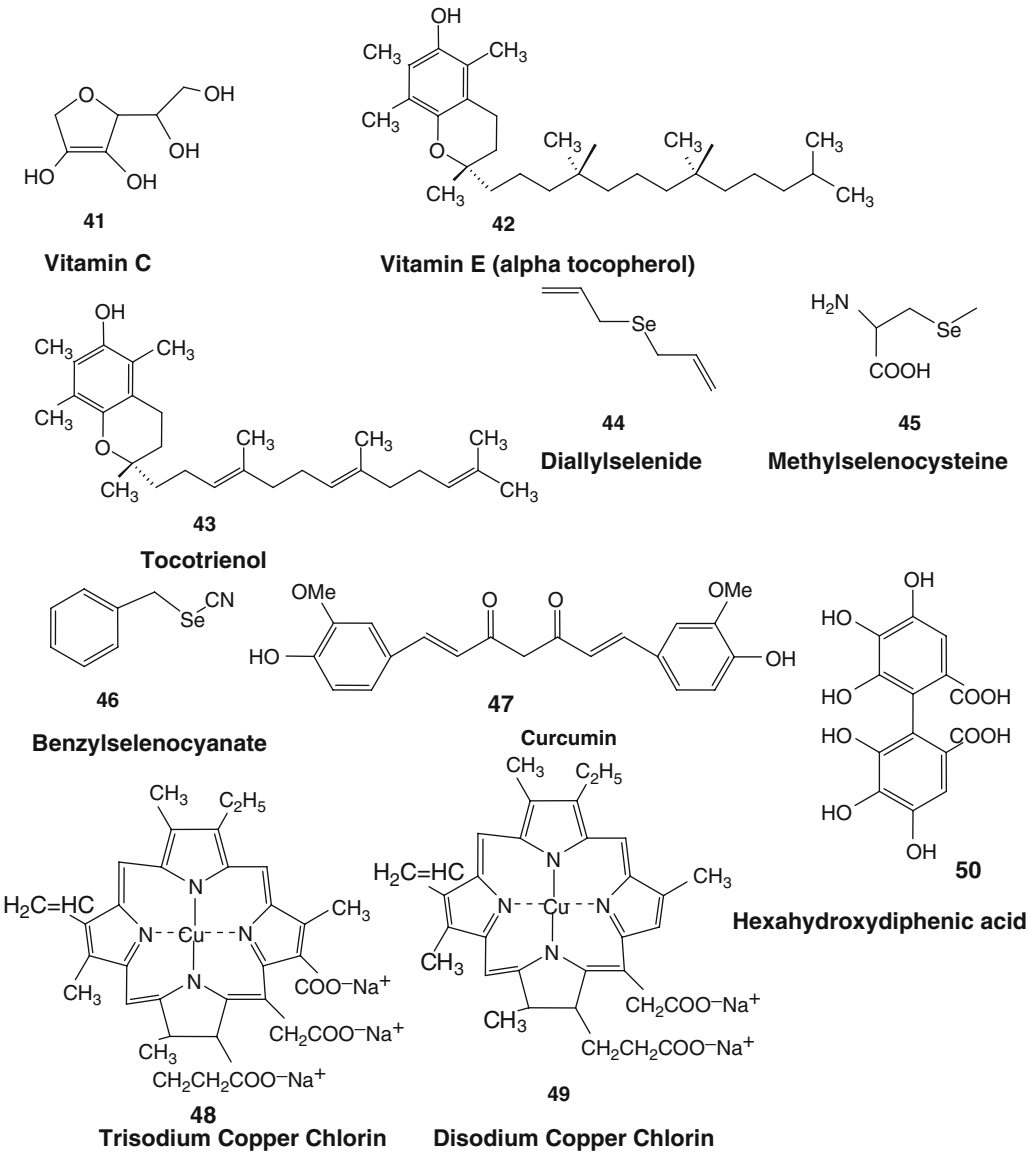


Fig. 24.4.

in immune-compromised individuals. Importantly, these polyacetylenes do not cause DNA changes (as do other herbal photoactivated substances, such as furanocoumarins found in the *Umbelliferae* plants), and the action appears to be mediated by cell surface activities, this implies a higher level of safety for their use (56).

Highly sulfated red algal polysaccharides ($C_n(H_2O)_n$ (**53**)) extracted from *Gelidium cartilagineum* afforded protection against animal virus, influenza B, and mumps viruses (57). The cell-wall sulfated polysaccharide of the red microalga *Porphyridium* sp. has impressive antiviral activity against *Herpes simplex* viruses types 1 and 2 (HSV1,2) and

varicella–zoster virus (VZV) (58, 59). Lignans are one of the major classes of phytoestrogens which are estrogen-like chemicals and also act as antioxidants (60). Nordihydroguaiaretic acid (NDGA) (**54**), a lignan present in the perspired resin of leaves of *Larrea divaricata* has displayed significant *in vitro* inhibition against several viruses, including HIV, HSV-1 and -2, and human papilloma (61). The pharmacokinetics and metabolism of retrojusticidin B, an anti-HIV reverse transcriptase agent isolated from *Phyllanthus myrtifolius*, have been studied in rats. Chrysophanic acid (**56**) (1,8-dihydroxy-3-methyl anthraquinone (**55**)), isolated from the Australian aboriginal medicinal plant *Dianella longifolia*, has been found to inhibit the replication of poliovirus types 2 and 3 (*in vitro* SARS-CoV spike (S) protein, a type I membrane-bound protein, is essential for the viral attachment to the host cell receptor angiotensin-converting enzyme 2 (ACE2) (62). Emodin (**57**), derived from genus *Rheum* and *Polygonum*, was shown to significantly block the S protein and ACE2 interaction in a dose-dependent manner. It also inhibited the infectivity of S protein-pseudotyped retrovirus to Vero E6 cells. These findings suggested that emodin may be considered as a potential lead therapeutic agent in the treatment of SARS (63). Gingerols (**58**) (derived from ginger, a typical south Asian spice) has traditionally been used to cure common colds and throat infections and form an important constituent of Ayurvedic formulations. There have been numerous studies on the efficacy of these compounds as antiviral agents (64). Salicylic acid ((**59**) $C_6H_4(OH)CO_2H$) can stimulate the inhibition of all three main stages in virus infection: replication, cell-to-cell movement, and long-distance movement. There is evidence that SA may stimulate a downstream pathway, leading to the induction of mechanism of resistance based on RNA interference (65) (Fig. 24.5).

24.2.9 Activity of Extracts/Mixtures Preparation

Traditional medicine system (Egyptian, Ayurvedic, Chinese, Unani) have utilized the plant extracts/mixtures to cure infections. The underlying idea is to achieve the synergistic or combination benefits of the formulation. In addition, herbals offer a less toxic alternative to conventional therapies thereby encouraging patients to opt for this treatment. Semple and colleagues reported that Chrysophanic acid (1,8-dihydroxy-3-methyl-anthraquinone) (isolated from the Australian Aboriginal medicinal plant Dianella longifolia) inhibits the replication of poliovirus types 2 and 3 (Picornaviridae) *in vitro* (66). Terpenes and phenol esters from *Plectranthus strigosus* were screened against herpes viruses. The bioactivity study revealed herpetic inhibitory properties for ent-16-Kauren-19-ol ent-16-kauren-19-oic acid. The compound inhibited poliovirus-induced cytopathic effects in BGM (Buffalo green monkey) kidney cells at a 50% effective concentration of 0.21 and 0.02 g/mL for poliovirus types 2 and 3, respectively. *Phellodendron amurense* bark extracts were examined and substantial antiviral activity was reported against HSV-1 utilizing the plaque inhibition assay (67). Propolis, a crude extract of the balsam which contains terpenoids, flavonoids, benzoic acids, esters, phenolics, has been found to inhibit the hemagglutination activity of inflenza virus, acyclovir resistant HSV-1, adenovirus-2 VSV, and poliovirus (68). In a study, the *Dryopteris crassirhizoma* extract was used to inhibit the reverse transcriptase associated DNA polymerase and RNAse H activity (69). An extract derived from *Asimina triloba*, has been used for the treatment of oral herpes (HSV-1) (70). Sixty-five

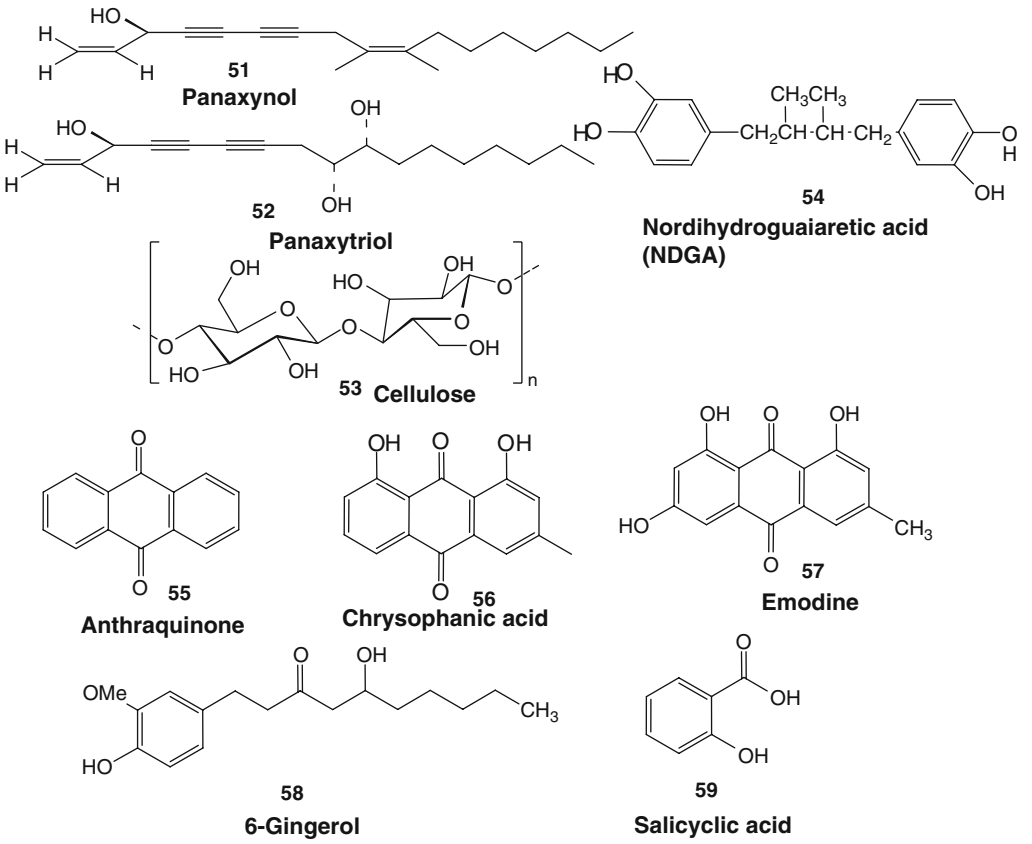


Fig. 24.5.

crude extracts from 51 selected endophytic fungi isolated from *Garcinia* species were tested for various bioactivities. Eighty percent of the fungal extracts from fermentation broths and mycelia displayed antiviral activity (71). In a related study, organosulfur compounds derived from garlic extract protected CD4 cells from HIV attack (72). Tertagalloyl glucopyranose obtained from *Juglans mandshurica* inhibited reverse transcriptase and RNAse H activity while extracts of *Centella asiataca* and magniferin of *Magnifera indica* have shown promising anti-herpes HSV activity (73). The crude extract of the roots from the Australian medicinal plant *Dianella callicarpa* (Liliaceae) displayed significant antimicrobial and antiviral activities (74). Meliacine (a partially purified extract (meliacine) from the leaves of *Melia azedarach L*) exhibits a potent antiviral effect against several viruses without displaying cytotoxicity (75). The *in vitro* antiviral activity of the Cuban-endemic plant *Phyllanthus orbicularis* against HSV-1 and -2 was confirmed and it was found that the drug acted at early stages of herpesvirus replication cycle (76). The concurrent use of natural health products (NHPs) with antiretroviral drugs (ARVs) is widespread among HIV-infected patients; however, extreme caution should be exercised since some NHPs are complex mixtures and are likely to contain organic compounds that may induce and/or inhibit drug metabolizing enzymes and drug transporters.

It has been observed that *St. John's wort* clearly induces cytochrome P450 3A4 and P-glycoprotein. This reduces protease inhibitor and non nucleoside reverse-transcriptase inhibitor concentrations, thereby increasing the likelihood of therapeutic failure (77).

24.2.10 Antiviral Mechanistic Aspects of Phytochemicals

One of the major steps in drug discovery is to identify and validate specific molecular targets. The advance of modern day biology has enabled us to identify microbial enzymes, receptors and molecular processes that facilitate drug action against a particular kind of virus. Studies have indicated that the antiviral action of plant-derived products may be attributed to a number of well-defined mechanisms (Table 24.2). It is possible that the antiviral effect of the compound may be explained on the basis of more than one mechanism and in some cases the action of mechanism may be unknown. Understanding the mechanistic pathways may help us to progress rapidly with more rational drug design and screening procedures.

24.2.11 Viral Studies

There have been numerous *in vitro* studies supporting the antiviral activity of phytochemicals. In order to further evaluate the modulation of several of these plant-derived compounds by components of tissue and body fluids, several *in vivo* studies have been carried out. However, the relative proportion of these studies is less for obvious reasons. There is tremendous amount of literature available regarding antiviral potential of phytochemicals. For the sake of clarity, the discussion has been classified into different sections with a focus on viral diseases.

24.2.12 AIDS

HIV is a retrovirus that can lead to acquired immunodeficiency syndrome (AIDS), a condition that is characterized by the failure of the immune system. According to a report by the World Health Organization, it has been estimated that 0.6% of the world's population is infected with AIDS. Until the year 2006, AIDS has killed more than 25 million people, since it was first recognized in 1981 (107). With the recent advances in understanding the biology of HIV, there has been increased focus on the usage of phytochemicals as antivirals against HIV. Owing to the vast array of chemical entities in nature, effective therapies for HIV infection are being sought in the natural world. The scope of studies of anti-HIV plant extracts is too extensive. Owing to the size limitations of the present review, we have summarized some of the major studies in Table 24.3.

24.2.13 Poliomyelitis

Poliomyelitis, caused by a human enterovirus, damages the nervous system and causes paralysis. The disease is normally prevalent in less developed Asian and African countries where polio immunization for children is not very common in spite of the massive immunization by the governments and non-governmental organizations. A large number of plant-derived products have been evaluated for their activity against

Table 24.2
Phytochemicals and antiviral activity

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
1	Flavone (4′, 5-dihydroxy 3,3′,7-trimethoxy flavone)	Effect on replication	Human: Picornaviruses Rhinoviruses Coxsackieviruses	Replication inhibition, selective inhibition of viral RNA	Ishitsuka et al. (78)
2	Polyphenolic complex (PC) containing: Catechins Flavonoids Kaempferol Myricetin Monne Quercetin Ramnasin Retusin	Effect of PC on the expression of viral proteins haemagglutin (HA), neurominidase (NA) and nucleoprotein (NP)		Inhibition of protein synthesis and synthesis of viral proteins	Serkedjieva (79)
3	Quercetin Quercetin 3-methyl ether Quercetin 7-methyl ether Quercetin 3,7,3′4′-tetramethyl ether Galangin 3-methyl ether Morin Robinin Quercetin 3,7,4′-trimethyl ether Quercetin 7,4′-dimethyl ether 7,4′-di-*O*-benzolquercetin 7-hydroxy-3,4′-dimethyl flavone 6,3′-dihydroxy-4′-methyl aurone Fisetin 4′-methyl ether	Effect on tomato ringspot nepovirus (TomRSV), infectivity in *Chenopodium quinoa*	Tomato Ringspot Nepovirus (TomRSV)	Proposed interference with an early event in the virus life cycle	Malhotra et al. (80)

(continued)

Table 24.2
(continued)

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
4	BCA, BA	Elucidation of mechanism of the antiviral effect of BA	HIV-1	Inhibition of HIV-1 infection (viral entry). Similar inhibition by Baicalein (BCA)	Li et al. (81)
5	BCA, Genistein	Investigation of antiviral activity of baicalin and genistein against human cytomegalovirus	HCMV	Blockage of HCMV infection at entry while the primary mechanism of action for genistein may be to block HCMV immediate-early protein 6 functioning	Eversa et al. (82)
6	3-Methylquercetin	Effect on methylquercetin on poliovirus replication	Poliovirus	Blocks viral replication, selective inhibition of poliovirus RNA	Castrillo and Carrasco (83)
7	Miscellaneous phenolic compounds: Anthraquinone Chrysophanic acid Caffic acid Eugenin Hypericin Tannins (condensed polymers) Proanthocyanidins Salicylates Quinones Naphthoquinones Naphthoquinones Anthraquinones, in particular *Aloe emodin*	Effect of polyphenolics on viral inhibition		Viral RNA and DNA replication cycle interference	Takechi and Tanaka (84) Sydiskis et al. (85) Kurokawa et al. (86) Liu et al. (54)

8	Quercetin (Q) Luteolin (LU) 3-*O*-methylquercetin (3MQ)	Effect on the viral replication cycle of HSV-1	HSV-1	Interference with the events HSV-1 which includes transcription and translation of viral proteins	Bettega et al. (87)
9	Amentoflavone Scutellarein Quercetin	Study of effect on DNA synthesis	AMV (RAV-2) MMLV	Inhibit three reverse transcriptases (RT): AMV RT RAV-2 RT MMLV RT	Spedding et al. (88)
10	3(2H)-isoflavene	Action of the antiviral compound 3(2H)-isoflavene against Sabin type 2 poliovirus	Sabin Type 2 Poliovirus	3(2H)-isoflavene acts as a potent inhibitor of PV2 uncoating and targets the VP1 protein	Salvati et al. (89)
11	(−)EGCG (−) ECG (−) EGC	Effect on viral synthesis	Influenza virus	Suppression of viral RNA with EGCG and ECG whereas EGC failed to show similar effect	Song et al. (90)
12	Flavonoids complex:Amentoflavone Theaflavin Iridoids Phenylpropanoid glycosides Agathisflavone Robustaflavone Rhusflavanone Succedaneflavanone Chrysosplenol C	Effect on viral replication	HIV	Blockage of RNA synthesis exhibited HIV-inhibitory activity	Lin et al. (91) Semple et al. (92) Yu et al. (93)

(continued)

Table 24.2
(continued)

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
	Morin Coumarins Galangin (3,5,7-trihydroxyflavone) Baicalin Quercetin Isoquercetin				
13	Terpenoids:Parthenolide Sesquiterpene Triterpenoids Moronic acid Ursolic acid Maslinic acid Saponin	Effect on HCV replication in a subgenomic RNA replicon assay system	HCV	Potentiates the interferon α-exerted anti-HCV effect	Hwang et al. (94)
14	Polysaccharides carrageenan	Effect of polysaccharides on viral replication	HSV-1	Inhibition of viral replication subsequent to viral internalization	González et al. (95)
15	Algal polysaccharide	Effect on the production of retroviruses (murine leukemia virus – MuLV) and cell transformation by murine sarcoma virus (MuSV-124) in cell culture	Murine leukemia virus – MuLV Murine sarcoma virus (MuSV-124)	Action against the subsequent secondary infection cycle	Talyshinsky et al. (96)

16	Alkaloids	Mechanism of action of michellamine B Effects of Amaryllidaceae alkaloids/derivatives upon herpes simplex virus (type 1)	HIV Herpes simplex virus (type 1)	Michellamine B inhibits RT (early HIV life cycle) inhibiting cellular fusion and syncytium formation later Blocking of viral DNA polymerase activity	McMahon et al. (97) Renard-Nozaki et al. (98)
17	Lignans Nordihydroguaiaretic acid (NDGA (a lignan present in the perspired resin of leaves of *Larrea divaricata*)) Podophyllotoxin and related lignans (cyclolignanolides) such as the peltatins Dibenzocyclooctadiene lignans such as Schizarin B and Taiwanschirin D Rhinacanthin E and Rhinacanthin F	Effect on HBV, Influenza virus type A infection	HBV, influenza virus type A	Viral replication cycle inhibition	Konigheim et al. (61)
18	Olomoucine and roscovitine	Potential applications of CKIs are being studied presently in viral diseases	Cytomegalovirus and herpes simplex virus	Cyclin dependant kinase inhibitor inhibits viral replication	Bresnahan et al. (99)Schang et al. (100)
19	Psoralen compounds 4′-hydroxymethyltrioxsalen 4′-aminomethyltrioxsalen	Effect on infectivity of DNA and RNA virus (radioimmunoassay immunofluorescence)	Herpes simplex virus	Binds to nucleic acids when irradiated with long wavelength of UV light	Redfield et al. (101)

(continued)

Table 24.2
(continued)

	Name of the phytochemicals/class	*Details of Study*	*Virus*	*Mechanism*	*References*
20	DNJ	Action mechanism against bovine viral diarrhea virus	Bovine viral diarrhea virus	Reduction of viral secretion due to an impairment of viral morphogenesis (ER-glucosidase inhibition)	Durantel et al. (102)
21	Naturally occurring thiophene Alpha-terthienyl (1) 15 synthetic analogs	Photoactivated antiviral and cytotoxic activities against murine cytomegalovirus and sindbis virus, and murine mastocytoma cells	Murine cytomegalovirus sindbis virus	After irradiation with near UV light, alpha-terthienyl and most of its analogs had significant toxicity, with minimum inhibitory concentrations in the range of 0.02–40 μM	Marle et al. (103)
22	Gingerol	Common cold throat infection	Common cold virus	Improvement of NK cell lysing activity positive effect on immune system	Chrubasik et al. (74)
23	Capsaicin	HSV infections	HSV	Interference with intraneuronal transport of virus	Stanberry et al. (104)
24	Curcumin	Coxsackievirus infection	Coxsackievirus	Dysregulation of the UPS	Xiaoning et al. (105)
25	Lutein/zeaxanthin	HIV	HIV	Lowers oxidative stress/ metabolism restoration	Dikici et al. (106)

Table 24.3
Anti HIV activity of phytochemicals

Compounds	*Experiment details*	*Mechanism*	*Reference*
Carnosic acid	Displayed the strongest inhibitory effect (IC 90 = 0.08 μg/mL)	Protease inhibitors	Paris et al. (108)
Isoobtusitin	Effect on HIV replication	Interferes replication of the HIV virus	Chang et al. (109)
Tannins	Potent inhibitory activity	interferes HIV-1 replication, HIV-1-mediated cell fusion, and the gp41 six-helix bundle formation	Fortin et al. (110)
Compounds from Mulberry juice	Anti HIV	Anti-stress and anti-HIV activity evaluation in different fractions of the juice	Sakagami et al. (111)
HIV alkaloid drymaritin	Anti HIV	Anti-HIV effect in H9 lymphocytes with EC50 value of 0.699 μg/mL	Hsieh et al. (112)
7-*O*-β-d-(4″-caffeoyl) glucuronide	Anti-HIV activity in a cell culture assay (EC 50 = 41.86 ± 1.43 μg/mL)	Integrase inhibitory activity (IC50 = 7.2 ± 3.4 μg/mL)	Lee et al. (113)
Ferulic acid, gallic, caffeic, furulate, gallate, curcumin	Effect on replication	Replication inhibition	Olivero-Verbal and Pacheco-Londono (114)
Scutellarein and 6-hydroxyluteolin	Antiviral effect	Displayed strong HIV reverse transcriptase inhibition	Nishibe et al. (115)
Glycoside gallate ester	Antiviral effect		Kim et al. (116)
Chrysin, apigenin (**63**) and acacetin (**64**)	Antiviral effect	Interferes with HIV activation	Critchfield et al. (117)
Flavones and flavanones	HIV-1, HIV-2 or simian immunodeficiency virus	Binding of sCD4 and antibody to gp120	Mahmood et al. (118)
1,2,5,8-tetrahydroanthraquinone and hypericin	Antiviral effect	Found to inhibit HIV-1 reverse transcriptase	Schinazi et al. (119)

polio virus. Isoobtusitin (**61**), a prenylated coumarin showed substantial *in vitro* inhibitory activity against poliovirus (IC50 = 2.9 μM) (119). Isokaempferide (5,7,4′-trihydroxy 3-methoxyflavone) derived from *Psiadia* species was found to be an inhibitor of poliovirus type 2 replication (120). Tuli and colleagues examined the antiviral action of 3-methyleneoxindole (MO), a plant metabolite, in HeLa cells infected with poliovirus. On the basis of the experiments, authors suggested that the ability of MO to bind to ribosomes of HeLa cells may underlie the antiviral affect. Experiments showed that the poliovirus messenger RNA would not attach to those ribosomes that are already bound to MO. This resulted in the nonrecovery of virus-specific polysomes from infected cells treated with antiviral concentrations of MO (121) (Fig. 24.6).

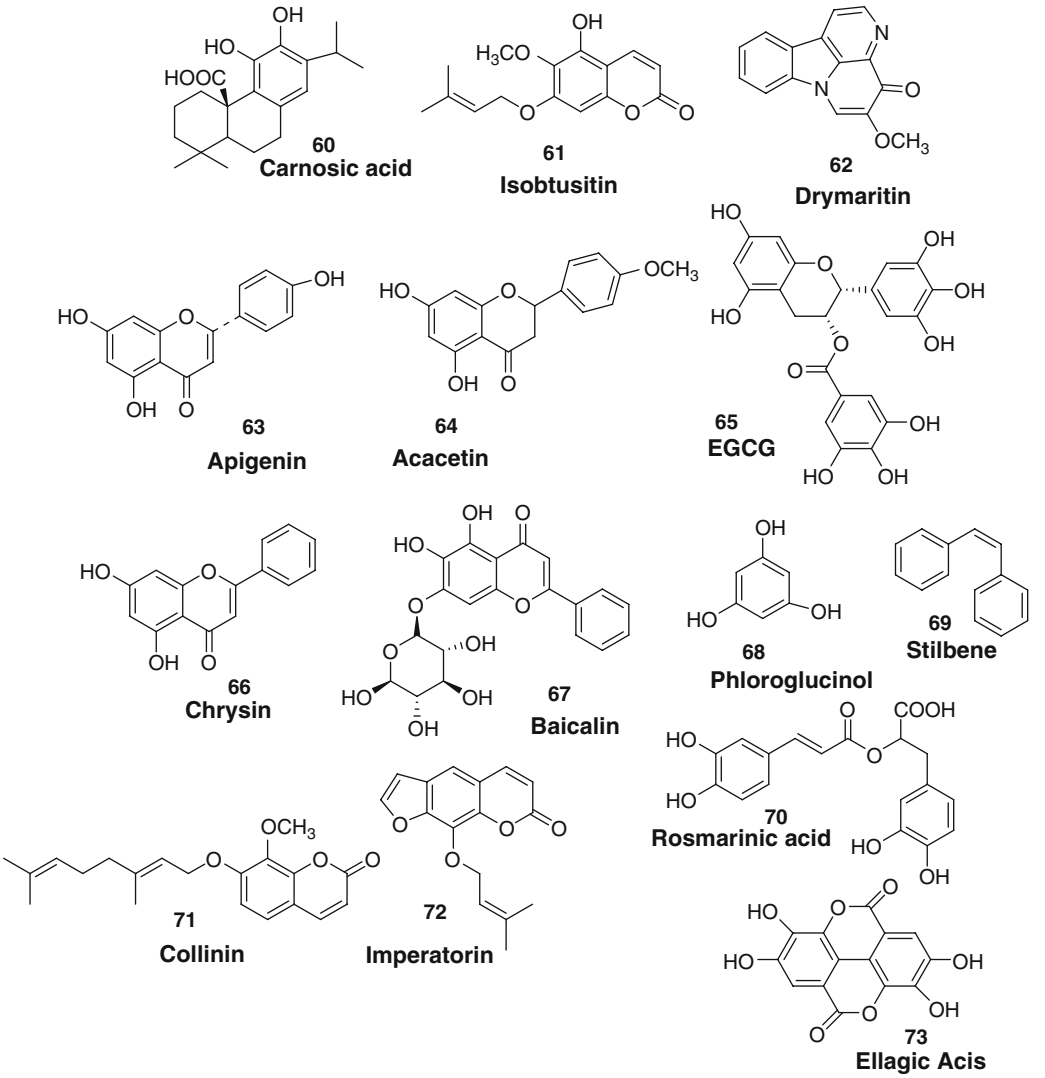


Fig. 24.6.

24.2.14 Herpes

Herpes is caused by HSV-1 and -2. It is a painful infection mainly affecting skin, eyes, mouth, and genitals. There is no permanent cure for herpes but the treatment can certainly reduce the viral shedding. There have been efforts all around the globe to identify plant-based treatment for this infection. Lyu et al. performed anti-herpetic assays on 18 flavonoids in five classes and a virus-induced cytopathic effect (CPE) inhibitory assay, plaque reduction assay, along with yield reduction assay (122). EC, ECG, galangin, and kaempferol exhibited strong antiviral activity whereas catechin, EGC, EGCG (**65**), chrysin (**66**), BA (**67**) showed moderate activity against HSV-1. Among all the flavanols, it was found that EC and ECG displayed a high level of CPE inhibitory activity (2.5 μM [0.725 μg/mL]) and 5 μM (2.21 μg/mL), respectively), while among the flavanones naringenin expressed a strong inhibitory effect (5 μM [1.36 μg/mL]) against HSV-1. Similarly, among the flavonols, quercetin exhibited a high CPE inhibitory activity (5 μM [1.69 μg/mL]), and genistein which is an isoflavone also showed an inhibitory effect (5 μM [1.35 μg/mL]). Two dibenzocyclooctane lignans, Kadsulignan L, and Neokadsuranin were tested for their anti-HBV activities *in vitro*. These compounds at 0.1 mg/mL, exhibited moderate antiviral activities, inhibiting HBsAg and HBeAg secretions by 32.6 and 36.5%, and by 14.5, and 20.2%, respectively. From a structure-activity point of view, it was found that the introduction of an a-orientated AcO group enhances the antiviral activity (123). Chattopadhyay and colleagues reported substantial anti-HSV activity of *Ophirrhiza nicobarica* extract at 300 μg/mL. The alkaloid, flavonoid, and β-sitosterol isolated from bioactive parts had a dose-dependent therapeutic efficacy, justifying their use (124). Eugenol (4-allyl-1-hydroxy-2-methoxybenzene) was screened for efficacy against HSV-1 and HSV-2 viruses. The *in vitro* experiments revealed that the replication of HSV viruses was inhibited by eugenol. The inhibitory concentration 50% values for the anti-HSV effects of eugenol were 25.6 μg/mL and 16.2 μg/mL for HSV-1 and HSV-2, respectively, with 250 μg/mL being the maximum dose at which cytotoxicity was tested. In addition, it's worth mentioning that eugenol showed no cytotoxicity at the concentrations tested. Furthermore, the eugenol–acyclovir combinations have synergistically inhibited herpesvirus replication *in vitro* (125). Nineteen compounds isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus* were tested for inhibitory effects on hepatitis B virus (HBV) and HSV-1. The experiments revealed that apigenin 4′-*O*-α-rhamnopyranoside, apigenin 7-*O*-β-glucopyranosyl-4′-*O*-α-rhamnopyranoside, tricin 7-*O*-β-glucopyranoside, tricin, and isoscopoletin (**18**) possessed excellent antiviral activity against HBV replication. In addition, protocatechuyl aldehyde (**19**) also displayed substantial inhibiting activity on HSV-1 replication (126). Likhitwitayawuid et al. tested flavonoids, coumarins, phloroglucinol (**68**), and stilbenes (**69**) derivatives derived from *Mallotus pallidus*, *Artocarpus gomezianin,* and *Triphasia trifolia*. It was concluded that bis hydroxyphenyl structures are promising candidates for anti-HSV and anti-HIV drug development (127). The *in vitro* antiviral activity of galangin (3,5,7-trihydroxyflavone), the major antimicrobial compound isolated from the shoots of *Helichrysum aureonitens*, was investigated against herpes simplex virus type 1. The compound showed significant antiviral activity against HSV-1 (an enveloped double-stranded DNA virus) and Cox B1 (an un enveloped single-stranded RNA virus) at concentrations varying from 12 to 47 μg/mL (128).

Epigallocatechin 3-*O*-gallate, samarangenin B derived from the roots of *Limonium sinense* had higher inhibitory activity than the positive control acyclovir. All of these were examined for inhibitory effect against the replication of HSV-1 virus in Vero cells (129). Du et al. isolated flavonoid leachianone from the root bark of *Morus alba* showing potent antiviral activity. A flavonoid moralbanone, having characteristic prenyl chain, along with seven other known compounds, was isolated from the root bark of *Morus alba* L. Among all the isolated compounds, Leachianone G showed potent antiviral activity (IC50 = 1.6 μg/mL) (130). Three new flavonol glycosides, namely, isorhamnetin 3-*O*-(6″-*O*-(Z)-p-coumaroyl)-β-d-glucopyranoside, quercetin 3-*O*-α-l-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl(1-2)-α-L-rhamnopyranoside, and quercetin 3-*O*-α-L-arabinopyranosyl(1-2)-α-L-rhamnopyranoside, were isolated from the stems of *Alphitonia philippinensis* collected from Hainan Island, China. Some of the isolated triterpenoids and flavonoid glycosides showed cytotoxicity against human PC-3 cells and hepatoma HA22T cells, and the inhibition of replication on HSV-1 (131). Viral diseases, especially of skin, can be treated with a virucide encapsulated in multilamellar phospholipid liposomes. Rosmarinic acid (**70**), incorporated in phospholipid mixture demonstrated effectiveness in humans afflicted with HSV (132). Flavonol glycosides (from quercetin and isorhamnetin) derived from the stems of *Alphitonia philippinensis* have been reported to inhibit the replication of HSV-1. Isodihydrosyringetin, a new (2R,3S)-3,5,7,4′-tetrahydroxy-3′,5′-dimethoxyflavanone was extracted from the root of *Limonium sinense* (Girard) along together with nine other known compounds. Out of all the compounds examined for their inhibitory effects on HSV-1, replication in vero cells, epigallocatechin 3-*O*-gallate and samarangenin B exhibited potent inhibitory activities on HSV-1 replication. Comparison of the IC50 values indicated that these both compounds had higher inhibitory activities than the positive control acyclovir (38.6 ± 2.6 vs. 55.4 ± 5.3 μM, $P < 0.001$; 11.4 ± 0.9 vs.55.4 ± 5.3 μM, $P < 0.0005$) (129). *Cedrus libani*, widely used as traditional medicine in the middle east for the treatment of different infections was studied for its antiviral potential. The phytochemical components isolated himachalol (22.50%), β-himachalene (21.90%), and α-himachalene (10.50%) showed promising results against herpes simplex virus type 1 (HSV-1) (133). Harden et al. evaluated the antiviral activity of extracts from *Undaria pinnatifida*, *Splachnidium rugosum*, *Gigartina atropurpurea*, and *Plocamium cartilagineum* against HSV-1 and HSV-2. Different assays showed that the compounds had potent virucidal activity and were active at very low concentrations (134). There are already reports in literature regarding excellent anti-HSV activity of *Maclura cochinchinensis* in several *in vitro* experiments. The authors have carried out biologically-guided separation of the active component(s). Ethyl acetate and methanol extracts exhibited anti-HSV-2 activity at EC50 values of 38.5 μg/mL and 50.8 μg/mL, respectively. Biologically-guided chromatographic separation of the ethyl acetate extract yielded compound A, identified as morin using a spectroscopic method. Morin exhibited anti-HSV-2 activity at an EC50 value of 53.5 μg/mL. In order to test the activity of acetate derivative, morin penta acetate was synthesized; however, the compound did not show any activity. It was concluded that free hydroxyl groups were required for anti-HSV-activity, as demonstrated previously by other workers for the antiviral activity of other flavonoids (135).

24.2.15 Hepatitis

Hepatitis derives its name from the Greek words *hepato* and *itis* which literally stands for liver inflammation. There are several types of viral Hepatitis such as Hepatitis A, B,C,D,E,F,G. Hepatitis is also caused by mumps virus, rubella virus, and cytomegalovirus. A large number of herbal products have been screened to measure their efficacy as anti-hepatitis drugs. One of the coumarin derivative geranyloxy-8-methoxycoumarin, best known as collinin (**71**) obtained from *Zanthoxylum schinifolium* was shown to significantly inhibit the replication of hepatitis B virus DNA (IC50 = 17.1 µg/mL (109). Seven plant extracts from six different families were found to have antiviral activity against HSV-1, at a concentration non toxic to the cell line (Vero) used. It was shown that most of these extracts have partial activity at the low concentration used. The methanol extracts of the aerial parts of *Hypericum mysorense* and *Hypericum hookerianum*, exhibited detectable antiviral effect towards HSV-1 with an inhibitory concentration for 50% (IC^sub 50^) of 100 and 50 µg/mL respectively (135). The administration of concanavalin A (Con A) to mice induces cytokine-dependent hepatitis. Okamoto et al. examined the effect of glycyrrhizin on Con A-induced hepatitis and showed that glycyrrhizin inhibited Con A-induced hepatitis without affecting cytokine expression (136) (Fig. 24.7).

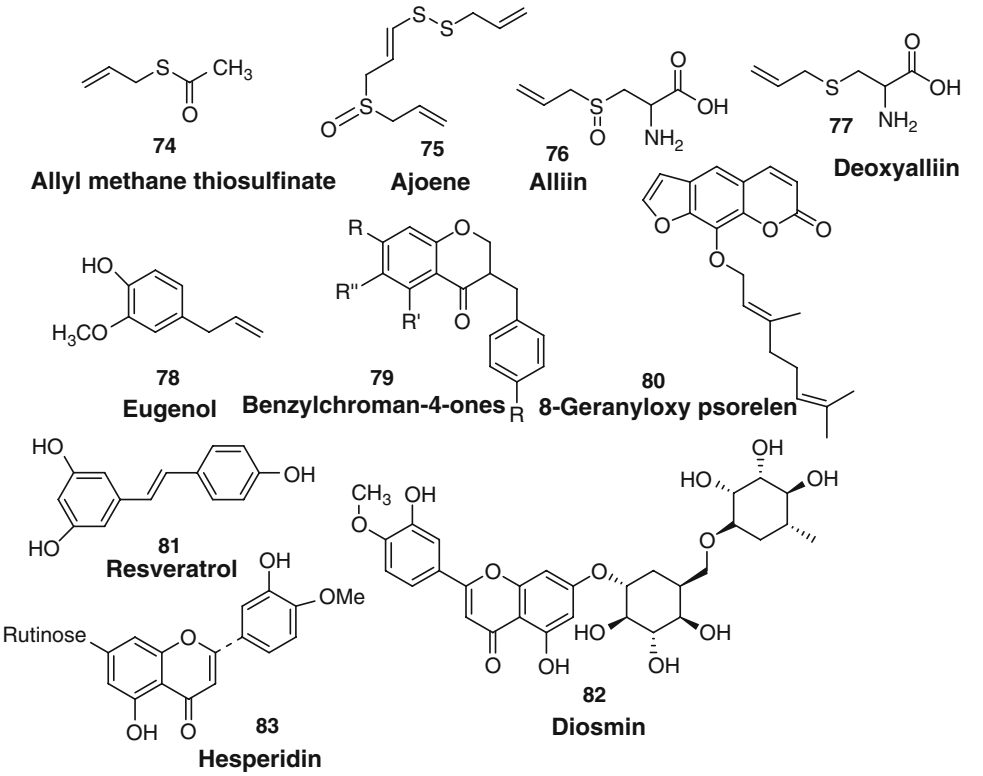


Fig. 24.7.

Constituents isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus* were tested for inhibitory effects on hepatitis B virus (HBV) and HSV-1. It was shown that apigenin 4′-*O*-α-rhamnopyranoside, apigenin 7-*O*-β-glucopyranosyl-4′-*O*-α-rhamnopyranoside, tricin 7-*O*-β-glucopyranoside, tricin, and isoscopoletin possessed substantial inhibitory activity against HBV replication (126). Ellagic acid (**73**), isolated from *Phyllanthus urinaria,* has exhibited the blockage of HBeAg secretion in HepG2 2.2.15 cells. Since HBeAg is involved in immune tolerance during HBV infection, ellagic acid may be a new therapeutic candidate against immune tolerance in HBV-infected individuals (137).

24.2.16 Influenza

Influenza virus (an RNA virus belonging to the *Orthomyxoviridae* family) is the causative organism for influenza commonly known as viral flu. There are three types of viruses known to cause influenza: influenza virus A, B and C. As an integral part of traditional therapy in India and China, plant extracts have been routinely used to cure flu since old times. A number of active biological compounds have been found to possess excellent antiviral activity against the influenza virus. Antiviral flavonoid 2″-*O*-(2‴-methylbutanoyl) isoswertisin obtained from the flower of *Trollius chinensis* was found to be moderately active toward influenza virus A. Two new flavonoid-type C-glycosides, trollisin I (=(1S)-1,5-anhydro-1-[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-4H-[1]benzopyran-8-yl]-2-*O*-(2-methylbutanoyl)-d-glucitol) and its 2-*O*-benzoyl congener trollisin II, were isolated from *Trollius chinensis*, together with the two known compounds 2″-*O*-(2‴-methylbutanoyl) isoswertisin and vitexin galactoside. In antiviral assays, the compounds were found to be moderately active towards influenza virus A (138). The inhibiting effects of isoscutellarein-8-methylether (5,7,4′-trihydroxy-8-methoxyflavone, F36) obtained from *Scutellaria baicalensis* on the single-cycle replication of mouse-adapted influenza viruses A/Guizhou/54/89 (H3N2 subtype) and B/Ibaraki/2/85 was evaluated and it was reported that the flavone significantly suppressed the replication of these viruses in a dose-dependent manner. It was noticed that the agents suppressed the replication of these viruses from 6 to 12 h after incubation in a dose-dependent manner by 50% at 20 μM and 90% at 40 μM, respectively. Remarkably 5,7,4′-trihydroxy-8-methoxyflavone, at the concentration of (50 μM) reduced the release of B/Ibaraki virus in the medium by 90–93% when it was added to the MDCK cells at 0–4 h after incubation (139). In a series of experiments, the phenolic biopolymer SP-303 was tested for its efficacy against experimentally induced influenza A (H1N1) virus infections in mice. It was found that when 30, 10, or 3 mg/kg/day of SP-303 was administered intraperitoneally once daily for 8 days, beginning either 48 h before or 4 h after virus exposure, only lung consolidation was significantly reduced (140).

24.2.17 Common Cold

Common cold is caused by Rhinovirus, (derived from the Greek word rhin- denoting nose) belonging to the *Picornaviridae* family. Traditional forms of medicines have relied on plant preparations to cure common cold, especially in the Indian subcontinent. Employing a plaque reduction assay, several homo-isoflavonoids and chloro-substituted

rac-3-benzylchroman-4-ones were evaluated for *in vitro* activity against selected picornaviruses. All homo isoflavonoids that were tested exhibited an inhibitory effect on rhinovirus replication with an activity depending on virus serotype and compound (141). Douglas and colleagues have reported antiviral activity of Vitamin C against rhinovirus (142). In another report, plants derived from the *Echinacea* family (family *Asteraceae*) have been shown useful for preventing and treating the common cold (143). The antiviral activity of different 2-styrylchromones was evaluated and almost all of them displayed activity against serotypes of human rhinovirus, 1B in a plaque reduction assay in HeLa cell cultures. Mechanistically, the compounds were found to interfere with HRV 1B replication. The antiviral activity of 2-styrylchromones and 3-hydroxy-1-(2-hydroxyphenyl)-5-phenyl-2,4-pentadien-1-ones, which are intermediates in the synthesis have been evaluated against two selected serotypes of human rhinovirus, 1B and 14, by a plaque reduction assay in HeLa cell cultures. It was found that al most all the compounds interfered with HRV 1B replication, with the exception of 3-hydroxy-1-(2-hydroxyphenyl)-5-(4-methoxyphenyl)-2,4-pentadien-1-one which did not show any significant activity. It is worth mentioning that the majority of derivatives were found to be effective against serotype 14, often with a higher potency (144, 145).

24.2.18 Multiple Targets

A considerably large number of studies has reported the activity of various phytochemicals against multiple targets. Weber et al. used direct pre-infection incubation assays to determine the *in vitro* virucidal effects of fresh garlic extract, its polar fraction, and other garlic-associated compounds, that is, diallyl disulfide (**37**), diallyl thiosulfinate (**39**) (allicin), allyl Me thiosulfinate (**74**), ajoene (**75**), alliin (**76**), deoxyalliin (**77**), and diallyl trisulfide (146).

In an effort to determine the mechanistic action of garlic compounds to explain their antiviral action, direct pre-infection incubation assays were used to determine the *in vitro* virucidal effects against selected viruses including, HSV-1, HSV-2, Para influenza virus type 3, vaccinia virus, vesicular steatites virus, and human rhinovirus type 2. These results indicate that virucidal activity and cytotoxicity may have depended upon the viral envelope and cell membrane, respectively. However, activity against non-enveloped virus may have been due to the inhibition of viral adsorption or penetration. The order for virucidal activity generally was: ajoene (**66**) > allicin (**39**) > allyl Me thiosulfinate (**74**). Tait et al. showed marked antiviral activity of homoisoflavonoids against coxsackie virus B1, B3, B4, A9, and echovirus 30. The inhibition of viral replication was monitored on BGM cells. Out of the various tested compounds, 3-benzyl chroman-4-ones (**79**) have displayed substantial antiviral effect towards PI-3 (parainfluenza-3) in the range of 8–32 µg/mL of inhibitory concentration for cytopathogenic effect (CPE) in Madin–Darby bovine kidney and vero cell lines (146) Eugenol, (**78**) a traditional medicine has also been used against multiple viral targets (125). Singh et al. have investigated the interaction between chemokine receptor CXCR4 and flavonoids using in silico docking studies. On the basis of their studies, the authors concluded that flavonoids may also be useful as topical agents to inactivate virus, or may act as adjuvant with other antiviral drugs. Interaction network formed by disulfide bonds, hydrogen bonds, van der Waals force, and salt bridges between extracellular segments helped in maintaining the conformation

of the docked complex (147). The moderate antiviral activity of the mixture of quercetin 3-*O*-β-glucoside and quercetin 3-*O*-β-galactoside derived from *Chamaesyce thymifolia* against HSV-1 and BVDV viruses was also reported (148). A number of substituted homo-isoflavonoids were synthesized in order to study their *in vitro* anti-picornavirus activity. Experiments were performed to determine the ability of non-cytotoxic concentrations to interfere with plaque formation by HRV 1B and 14 and poliovirus (PV) 2. Experiments suggested that serotype 1B was much more sensitive than 14 to the action of the compounds, and the presence of one or more chlorine atoms increased the antiviral effect in all homo isoflavonoids tested, confirming the positive influence of this substituent on activity (149). In an attempt to search for novel active agents from plant source pure flavonoids and aqueous extracts of *Caesalpinia pulcherrima* Swartz were screened to test their influence on a series of viruses, namely HSV-1, HSV-2, and adenoviruses (ADV-3, ADV-8, ADV-11). Results showed that the aqueous extracts of *C. pulcherrima* and its related quercetin possessed a broad-spectrum antiviral activity. The experiments have shown that fruit and seed extract showed the best activity (EC50 = 41.2 mg/L, SI = 83.2) as compared to stem and leaf (EC50 = 61.8 mg/L, SI = 52.1) and flower (EC50 = 177.9 mg/L, SI = 15.5). Quercetin derived from the plant possessed the strongest anti-ADV-3 activity (EC50 = 24.3 mg/L, SI = 20.4) (150). In the last decade, there has been a lot of focus on the amino sugar glucosidase inhibitors have selective antiviral activity against certain enveloped, mammalian viruses (151). It has been shown that deoxynojirimycins (DNJs) modified by reductive amination to attach a long chain to N atom (their N-DNJ derivative) were shown to be, for example, at least 20 times more potent than the non-alkylated DNJ in inhibiting hepatitis B virus (HBV) and bovine viral diarrhea virus (BVDV) in cell based assays. These data suggested that the modification of the alkyl side chain could influence antiviral activity (152). De Almeida et al. reported strong inhibition of an infusion of *Persea americana* leaves against HSV-1, Aujeszky's disease virus (ADV) and adenovirus type 3 (AD3) in cell cultures. An extract of *Persea americana* leaves (Lauraceae) strongly inhibited herpes simplex virus type 1 (HSV-1), Aujeszky's disease virus (ADV) and adenovirus type 3 (AD3) in cell cultures. Its fractionation, guided by anti-HSV-1 and ADV assays, allowed the isolation and identification of two new flavonol monoglycosides, kaempferol and quercetin 3-*O*-α-d-arabinopyranosides, along with the known kaempferol 3-*O*-α-l-rhamnopyranoside (afzelin), quercetin 3-*O*-α-l-rhamnopyranoside (quercitrin), quercetin 3-*O*-β-glucopyranoside and quercetin. In the extract, the known quercetin 3-*O*-β-galactopyranoside was also identified. The authors have reported that afzelin and quercetin 3-*O*-α-d-arabinopyranoside showed higher activity against acyclovir-resistant HSV-1. Chlorogenic acid significantly inhibited the HSV-1 replication without any cytotoxicity. However, all the substances tested were less active than the infusion or fractions (153). A summary of major classes of antiviral phytochemicals along with their source and viral targets has been provided in Table 24.4.

24.2.19 Miscellaneous

There exists a huge volume of literature regarding the evaluation of plant-derived compounds against several other viral targets apart from the ones listed above. Substantial antiviral activity of 8-geranyloxypsoralen (**80**) (isolated in low yields from

Table 24.4
Major classes of antiviral phytochemicals

Compound class	*Virus type*	*Name of the plants*	*References*
Polyphenols	HSV-1	*Agrimonia pilosa*, *Punica granatum*, *Moringa oleifera*, *Aglaia odorata*, *Ventilago enticulata*	Li et al. (154)
Torvanol		*Solanum torvum*	Lipipun et al. (155)
Flavonoids		*Morus alba*	Arthan et al. (156)
Saponins		*Maesa lanceolata*	Du et al. (130)
Anthraquinone, flavone		*Rheum officinale*, *Aloe barbadensis*, *Cassia augustifolia*	Aspers et al (157)
Essential oil		*Santalum album*, *lemon grass*	Sydiskis et al. (158)
Essential oil		*Artemisia douglasiana*, *Eupatorium patens*, *Tessaria absinthioides*	Jassim and Naji (159)
Meliacine (peptide)		*Melia azedarach*	Garcia et al. (160)
Saponin		*Bupleurum rigidum*	Alche et al. (161)
Rosmarinic acid		*Aloe emodin*	Baermejo et al. (162)
Oils		*Minthostachys verticillata*	Sydiskis et al. (158)
			Primo et al. (163)
Essential oil	HSV-1 and HSV-2	*Eupatorium patens*	Garcia et al. (160)
Eugeniin (tannin)		*Geum japonicum*	Khan et al. (164)
Ursolic acid		*Alstonia macrophylla*	Chattopadhyay et al. (165)
Morin (triterpene)	HSV-2	*Rus javanica*	Khan et al. (164)
Casuarinin (tannin)		*Terminalia arjuna*	Cheng et al. (166)
Oils		*Melissa officinalis*	Allahveridev et al. (167)
Tannins	Measles	*Bambusa vulgaris*	Ojo et al. (168)
Phenolic compounds	Yellow fever Polio	*Aframomum melegueta*	
Rosmarinic chlorogenic caffeic acids (phenolics)	VZV influenza, PRV	*Aloe emodin*, *Aloe barbadensis*	Sydiskis et al. (158)
Essential oil	HSV, ADV 8	*Boussingaultia gracilis*, *Serissa japonica*	Chiang et al. (169)
Essential oil	Dengue-2	*Artemisia douglasiana*, *Eupatorium patens*	Garcia et al. (160)

(continued)

Table 24.4
(continued)

Compound class	*Virus type*	*Name of the plants*	*References*
Oils	Psuedorabies	*Minthostachys verticillata*	Primo et al. (163)
Flavonoids	Influenza A	*Barleria prionitisBlumea laciniata*, *Markhamia lutea*,	Choi et al. (170)
Polyphenols	RSV	*Elephantos scaber*, *Mussaenda pubescens*, *Scutellaria indica*	Kernan et al. (171) Li et al. (81)
Flavonoids	RSV, influenza	*Aesculus chinensis*	Wei et al. (172)
Tannins polyphenols	Influenza	*Bergenia ligulataGeranium sanguineum*	Rajbhandri et al. (173) Sokmen et al. (174)
Diterpenoid	Para influenza 3	*Caesalpinia minax*	Jassim and Naji (159)
Alkaloid	Measles	*Zanthoxylum chalybeum*	Olila et al. (175)
Essential oil	HBV	*Rheum palmatumPhyllanthus niruri*,	Jassim and Naji (159)
Chebulic acid (tannin)		*Phyllanthus urinaria*	Thyagarajan et al. (176)
Niruriside		*Phyllanthus* spp.	Thyagarajan et al. (177)
		Sophorae flavescentis	Liu et al. (178)
Flavonoids	HCV	*Amebia*, *euchroma*, *Thalaspi arvens*, *Poncirus trifoliata*	Ho et al. (179)
Flavonoids	HCV	*Glycyrrhizae radix*	Sekine et al (180)
Usnic acid	HIV	Lichen *Ramalina farinacea*	Esimone et al. (181)
Triterpenoid		*Brazilian propolis*	Manfredi et al. (182)
Flavonoid		*Glycyrrhiza lepidota*, *G. glabra*	Manfredi et al. (182)
Saponin		*Maesa lanceolata*	Apers et al. (157)
Flavone		*Desmos* spp.	Wu et al. (183)
Flavonoids		*Alianthus altissima*	Chang et al. (184)
Flavonoids		*Begonia nantoensis*	Wei et al. (172)
Specific lectin		*Momordica charantia* L.	Cos et al. (72)
Proteins (RIP), mannose specific lectins		*Cymbidium* spp.	Balzarini et al. (185)
		Urtica dioca	Yogeeswari and Sriram (186)
GAP-31 lectins			Jay et al. (187)
Lectin			

Saponin	HIV entry	*Tieghemella heckelii*	Gosse et al. (188)
Alkaloid	HIV-1	*Stephania cepharantha*	Ma et al. (189)
Coumarin		*Prangos tschimganica*	Shikishima et al. (190)
Triterpenoids		*Vatica cinerea*	Zhang et al. (191)
Alkaloids		*Leucojum vernum*	Szlavik et al. (192)
Flavonoid	HIV replication	*Scutellaria baicalensis*	Li et al. (81)
Polypeptides	HIV-1 RT	*Phaseolus vulgaris* and *Phaseolus coccineus*	Ye and Ng (193)
Coumarins		*Callophyllum lanigerum*	Cos et al. (72)
Flavonoids		*Dryopteris crasissirhizoma*	Min et al. (194)
Polypeptides		*Momordica charantia*	Jiratchariyakul et al. (195)
Protein		*Panax notoginseng*	Ye and Ng (193)
Tannin		*Shepherdia argentia*	Notka et al. (196)
Gallotannins		*Phyllanthus* spp.	Notka et al. (196)
Ursolic acid (triterpene)	HIV-1 protease	*Geum japonicum*	Park et al. (197)
Camelliatannin (tannin)		*Camellia japonica*	
Polyphenol	HIV fusion	*Prunella vulgaris*	Liu et al. (198)
Tannin		*Rhizoma cibolte*	
Curcumin (phenolics)	HIV integrase and protease	*Curcuma linga* L., *Larrea tridentata* L.	Cos et al. (70)
Lignan			
Phorbol ester	HIV replication	*Homolanthus mutans*	Yogeeswari and Sriram (186)
Phorbol ester	HIV gene expression	*Euphorbia poissonii*	Yogeeswari and Sriram (186)
Epigallocatechin-3-gallate	Epstein–Barr virus	*Green tea*	Choi et al. (199)
Kaempferol	Poliovirus 2,3	*Dianella longifolia*, *Pterocaulus sphaedatum*	Andres et al. (200)
Anthraquinone		*Psiadia dentata*	Semple et al. (92)
Kaempferol			Robin et al. (201)
Oils	Junin virus	*Lippia junelliana*, *Lippia turbinata*, *Heterotheca latifolia*, *Tessaria absinthides*	Garcia et al. (160)
Tannin	Epstein–Barr virus	*Syzygium aromaticum*	Jassim and Naji (159)
Oligophenols	HCV protease	*Stylogne cauliflora*	Hegde et al. (202)
Theaflavin catechin flavonoid	Rotavirus, coronavirus	*Camellia sinensis*, *Eleutherococcus senticosus*	Clark et al. (203) Turan et al. (204)

Citrus limon) was reported against tumor promoter TPA-induced Epstein–Barr virus activation (10 μM, the inhibitory activity was 79.3%) (111). Well-studied polyphenol Resveratrol (**81**) was found to inhibit varicella–zoster virus (VZV) replication in a dose-dependent and reversible manner. RT-PCR studies showed that protein and mRNA levels of IE62, an essential early viral protein, were reduced when compared to controls (205). Baicalin (BA) derived from *Scutellaria baicalensis* has shown substantial antiviral activities. Mechanistically, it was shown that BA inhibited the binding of a number of chemokines to human leukocytes or cells transfected to express specific chemokine receptors (206).

Antiviral activities of seven compounds belonging to kaempferol family were evaluated against human HCMV and it was confirmed that the presence of acyl group is important for the activity (207). A freshly prepared extract of *Chelidonium majus* was tested *in vivo* for anti-retroviral activity using highly susceptible C57Bl/6 strain in a mouse. The mice were infected intraperitoneally with 0.2 mL of the stock virus pool of defective murine leukemia retroviruses (MuLVs) LP-BM5. The animals were sacrificed (after 4 months) and a significant reduction in the weight of spleen and cervical lymph nodes was noticed in chronically infected mice treated with freshly prepared crude extract of *Chelidonium majus* ($P = 0.0057$ and $P < 0.001$) (208). In an effort to elucidate the action mechanism of 3-methyl quercetagetin, it was reported that the significant activity of the compound against tomato bushy stunt virus was attributed to the interference during the virus infection initiation (209). Sanchez and colleagues evaluated the possible antiviral effect of flavonoids obtained from *Tephrosia madrensis*, *Tephrosia viridiflora,* and *Tephrosia crassifolia* on dengue viruses and concluded that glabranine and 7-*O*-methyl-glabranine presented 70% inhibition on the dengue virus (210). 4-hydroxypanduratin A and panduratin chalcone derivatives derived from *Boesenbergia rotunda* displayed substantial inhibitory activities toward dengue 2 virus NS3 protease (Ki values of 21 and 25 μM, respectively) (211). The inhibitory effects of diosmin (**82**) and hesperidin (**83**) on the infectivity of rotavirus causing sporadic diarrhea in infants was evaluated and it was shown that both compounds were effective against rotavirus infection (212). Some of the phytochemicals have graduated to the clinical trials. Owing to the space constraints only the major clinical trials relating to the antiviral activities of phytochemicals have been summarized in Table 24.5.

24.3 CONCLUSIONS AND PERSPECTIVES

Numerous epidemiological and experimental studies have revealed that a large number of the phytochemicals have promising antiviral activities. However, as discussed earlier, the development of new and better antiviral agents from plants pose a formidable challenge. One of the major challenges has been the relatively fewer number of *in vivo* studies coupled with inconsistency in results due to a lack of uniformity in the assays. Further, the data on the absorption metabolism and the excretion of phytochemicals in humans is contradictory and scarce. A highly interdisciplinary approach with meticulous planning and design needs to be followed for conducting the *in vivo* studies in a highly standardized environment. Consequently, the properly designed and rigorously executed clinical trial can help us to establish the efficacy and safety of the potential drug. In order to apply plant-based agents as an effective strategy, it is of

Table 24.5
Clinical trials

	Study details/condition treated	*Agent*	*Results*	*References*
1	Chronic hepatitis B	*Phyllanthus amarus*	Substantially cleared hepatitis B surface antigen	Thyagarajan et al. (176)
2	Chronic hepatitis B	*Phyllanthus amarus*	Significantly cleared hepatitis B surface antigen	Thyagarajan et al. (177)
3	Chronic hepatitis B	*Phyllanthus amarus*	No significant changes in levels of HbsAg	Thamlikitkul et al. (213)
4	Chronic hepatitis B	*Phyllanthus amarus*	No changes in levels of HBsAg, HBeAg, HBV DN	Berk et al. (214)
5	Chronic hepatitis B	*Phyllanthus amarus*	Significant reduction in HBeAg in treated group	Zhang et al. (215)
6	Chronic hepatitis B	*Phyllanthus amarus*	No significant changes in levels of HBsAg, HbeAg	Miln et al. (216)
7	Upper Respiratory Tract Infections (URTIs)	Chinese Herbal Medicine	Relieves external symptoms and effectively clear up the pathogenic cold	(217)
8	Chronic hepatitis B	*Phyllanthus amarus*	Significant reduction in HBeAg in treated group	Huang et al. (218)
9	Chronic hepatitis B	*Phyllanthus urinaria*	Significant reduction in HBeAg in treated group	Zhu et al. (219)
10	Chronic hepatitis B	*Phyllanthus urinaria*	Changes in levels of HBsAg, HBeAg, HBV DNA, HBV DNAP, significant reduction in HBsAg, HBV DNA, HBV DNAP in treated group	Cao et al. (220)
11	Chronic hepatitis B	*Phyllanthus urinaria*	Significant reduction in HBV DNA in treated group, improvement in liver function	Huang et al. (218)
12	Chronic hepatitis B	*Phyllanthus amarus*, *Phyllanthus niruri*, *Phyllanthus urinaria*	Improvement in patient condition *Phyllanthus urinaria* specifically effective, no side effect	Wang et al. (221)
13	Chronic hepatitis B Acute hepatitis B	*Glycyrrhiza glabra* *Glycyrrhiza glabra*	Changes in HBsAg, HBeAg, liver function, IgA, Ig G, IgM 50% acute and chronic cases cleared HBsAg and HBeAg in treated group vs. none of controls	Su et al. (222)
14	Chronic hepatitis B and C	*Glycyrrhiza glabra*	ALT levels in Group A significantly improved over levels in Group B ($P = 0.005$)	Iino et al. (223)

(continued)

Table 24.5
(continued)

	Study details/condition treated	*Agent*	*Results*	*References*
15	Hepatitis B virus	*Phyllanthus amarus*	Failed to inhibit B surface antigen in patient with hepatitis B virus	Doshi et al. (224)
16	Chronic hepatitis C	*Iscador* (*Viscum album extract*)	Substantial decrease in HCV production	Tusenius et al. (225)
17	Chronic hepatitis C	*Glycyrrhiza glabra*	Mean decrease in ALT levels 26% in treated group, 6% in placebo	van Rossum et al. (226)
18	Chronic hepatitis C	*Glycyrrhiza glabra*	Significantly greater reduction in all three parameters AST, ALT, GGT in Group B than in Group A	Tsubota et al. (227)
19	Hepatitis C	*Glycyrrhiza glabra*	Mean change in ALT levels 47%	van Rossum et al. (228)
20	Hepatitis C	*Phyllanthus niriri*	Healing complete by day 8 in 96% (natural recovery usually 10–14 days)	Mehrotra et al. (229)
21	Hepatitis C patients	Phlogenzym, a combination of hydrolytic enzymes with the flavonoid rutosid	Phlogenzym superior to ribavirin and interferon (established medication). The tolerance of the oral enzymes was excellent	Stauder and Kabil (230)
22	Chronic hepatitis	*Glycyrrhiza glabra*	Overall improvement in clinical markers and in some liver function tests	Suzuki et al. (231)
23	Chronic viral hepatitis	*Glycyrrhiza glabra*	Improvement ALT levels in Group A than Group B ($P = 0.0002$)	Miyake et al. (232)
24	Alcoholic or non-alcoholic chronic hepatitis	Silybin/phosphatidylcholine complex	Statistically significant drop ($P < 0.01$–0.001) in ALT and GGT occurred at doses of 240 mg or more	Vailaii et al. (233)
25	Jaundice in HBV persons	*Phyllanthus amarus*	No significant intergroup differences	Narendranathan et al. (234)
26	Liver cirrhosis	*Silymarin*	Silymarin has no effect on survival	Peres et al. (235)

27	Liver cirrhosis	*Silymarin*	Indicated effectiveness in patients with alcoholic cirrhosis	Ferenci et al. (236)
28	Influenza A	*Sambucus nigra* L.	Disappeared 4 days earlier in treated group	Thom et al. (237)
29	Influenza B	*Sambucus nigra* L.	Significantly faster recovery(treated group)	Zakay-Rones et al. (238)
30	Common cold	*Andrographis paniculata*	Visual analog scale, assessment of symptoms, days sick leave significant reduction in symptoms and in days sick leave in treated group	Melchior et al. (239)
31	Common cold	*Andrographis paniculata*	Significant reduction in intensity of symptoms in treated group	Caceres et al. (240)
32	Common cold	*Andrographis paniculata*	Reduced symptoms and faster recovery in treated group	Hancke et al. (241)
33	Common cold (prevention and treatment)	*Allium sativum* L.	Significantly fewer colds of shorter duration in treated group	Josling (242)
34	Rhinovirus infection	*Dichloroflavan* was given orally	Administration of dichloroflavan in the oral formulation tested is not of value in the treatment of human rhinovirus infection	Phillpotts et al. (243)
35	Chronic HCV patients	*Silymarin* capsules	Well tolerated patients improved over time	Tanamly et al. (244)
36	Chronic HCV	*Silymarin*	No visible effect	Gordon et al. (245)
37	Chronic HCV	*Silymarin* antioxidants and vitamins herbals	Well tolerated 48% patients positive response	Melham et al. (246)
38	HCV patients	*Silymarin*	No adverse effect but no effect on outcome	Strickland et al. (247)
39	Patients with detectable HCV RNA	*Silymarin* extract	Non interferon based standard therapy better than silymarin	El-Zayadi et al. (248)
40	HSV viral infection	*Melissa officinalis*	Treatment effective without any side cytotoxic side effects	Koytchev et al. (249)
41	Herpes simplex infection <72 h duration	*Melissa officinalis* L.	Significant reduction in symptom score in treated group on day 2	Wöbling et al. (250)

(continued)

Table 24.5
(continued)

	Study details/condition treated	*Agent*	*Results*	*References*
42	Genital herpes	*Aloe vera*	Mean days to healing, number of patients cured Significantly shorter mean time to healing in group (a) cured patient numbers greater in group (a) than group (b) or placebo	Syed et al. (251)
43	Genital herpes	*Aloe vera*	Mean days to healing, number of patients cured, shorter mean time to healing and cured patients at 2 weeks in treated group	Syed et al. (252)
44	Genital herpes	*Clinacanthus nutans*	*Phyllanthus niruri*, has anti-HBsAg activity	Jayavasu et al. (253)
45	Recurrent herpes labialis	*Melaleuca alternifolia*	Time to lesion healing No significant intergroup differences	Carson et al. (254)
46	Herpes labialis <24 h	*Salvia officinalis*	No significant intergroup differences	Saller et al. (255)
47	Herpes zoster	*Clinacanthus nutans*	Lesion healing significantly faster in treated group	Sangkitporn et al. (256)
48	Herpes zoster	*Clinacanthus nutans*	Complete healing, lesion healing significantly faster in treated group	Charuwichitra et al. (257)
49	HIV	*Andrographis paniculata*	Changes in levels of CD4 HIV-1 RNA Significant rise in CD4 Levels after 10 mg/kg, trial interrupted at 6 weeks due to adverse events	Calabrese et al. (258)
50	HIV	*Buxus sempervirens*	Significant delay of progression to disease	Durant et al. (259)
51	HIV	*Glycyrrhiza glabra*	Some improvement in asymptomatic carriers, none in AIDS patients	Gotoh et al. (260)

extreme importance to understand the molecular and cellular mechanism of the compounds with proper understanding of metabolite retention process of the system. A greater emphasis on the use of combination of micro array and proteomics techniques is needed to define the molecular targets for various micronutrients. Various techniques such as the serial expression of gene expression, protein arrays, and the evaluation of the mechanism will not only enhance our understanding of antiviral action at molecular level, but also help in finding the most effective strategy. Rational synthesis of the diverse derivatives with a more favorable profile activity can be of immense value along with the development of agent-selective endpoint markers. There is immediate need for crafting and executing an aggressive strategy involving nongovernmental organizations, chemists, microbiologists, clinicians, and experts with indigenous knowledge, failing which there are high chances of losing several untapped resources due to the extinction of plants. Combination studies/synergism is another area that has remained neglected. Further detailed studies to specify the minimum quantity of the phytochemicals to be consumed since the dosage of pure compounds effective in animals may not stay realistic when extrapolated to human system. By covering all the above gaps, it would be possible to strike a balance between the toxicity and the activity of a particular agent, which is essential for developing a new drug.

REFERENCES

1. Solecki RS (1975) Shanidar IV, a Neanderthal flower burial in northern Iraq. Science 190:880–881
2. International Symposium on Medicinal Plants, April 19–21, 1993, in Philadelphia, USA. The Morris Arboretum and the World Health Organization (WHO)
3. Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 109(Suppl 1):69–75
4. Clardy J, Walsh C (2004) Lessons from natural molecules. Nature 432:829–837
5. Cardellina JH (2002) Challenges and opportunities confronting the botanical dietary supplement industry. J Nat Prod 65:1073–1084
6. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, O'Neal JM, Cornwell T, Pastor I, Fridlender B (2002) Plants and human health in the twenty-first century. Trends Biotechnol 20:522–531
7. Pietta P, Gardana C, Pietta A (2003) Flavonoids in health. In: Rice-Evans C, Packer L (eds) Flavonoids in health and disease, 2nd edn. Marcel Dekker, New York, NY, p 43
8. Selway JWT (1986) Antiviral activity of flavones and flavans. In: Cody V, Middleton E, Harborne JB (eds) Plant flavonoids in biology and medicine: biochemical, pharmacological, and structure–activity relationships. Liss, A.R, New York, NY
9. Martin KW, Ernst E (2003) Antiviral agents from plants and herbs: a systematic review. Antivir Ther 8(2):77–90
10. Naithani R, Huma L, Holland LE Shukla D, Mccormick DL, Mehta RM, Moriarty RM (2008) Antiviral activity of phytochemicals: a comprehensive review. Mini Rev Med Chem 8(11):1106
11. Okeke IN, Laxmaninarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP (2005) Antimicrobial resistance in developing countries. Part 1: recent trends and current status. Lancet Infect Dis 5:481–493
12. Rose RE, Gong YF, Greytok JA, Bechtold CM, Terry BJ, Robinson BS, Alam M, Colonno RJ, Lin PF (1996) Human immunodeficiency virus type 1 viral background plays a major role in development of resistance to protease inhibitors. Proc Natl Acad Sci USA 93:1648
13. Balfour HH (1999) Antiviral drugs. N Engl J Med 340:1255
14. Kuper H, Adami HO, Trichopoulos D (2000) Infections as a major preventable cause of human cancer. J Int Med 48:171

15. Patwardhan B (2005) Ethnopharmacology and drug discovery. J Ethnopharm 100:50–52
16. Cordell GA, Colvard MD (2005) Some thoughts on the future of ethnopharmacology. J Ethnopharm 100:5–14
17. Hudson JB, Graham EA, Towers GHN (1994) Antiviral assays on phytochemicals: the influence of reaction parameters. Planta Med 60:329–332
18. Jensik SC, Northrop RL (1971) Incorporation of radioactive seleno-(^{75}Se)-methionine into mumps virus. Appl Microbiol 21(3):451–455
19. Ververidis F, Trantas E, Douglas C, Vollmer G, Kretzschmar G, Panopoulos N (2007) Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: chemical diversity, impacts on plant biology and human health. Biotechnol J 2(10):1214
20. Spencer JP (2008) Flavonoids: modulators of brain function? Br J Nutr 99:ES60–ES77
21. Thomas PRS, Nash GB, Dormandly JA (1988) White cells accumulation in dependent legs of patients with venous hypertension: A possible mechanism for trophic changes in the skin. Br Med J 296:1673
22. Loewenstein WR (1979) Junctional intercellular communication and the control of growth. Biochem Biophys Acta 560:1
23. Gerdin B, Srensso E (1983) Inhibitory effect of flavonoids on increased microvascular permeability induced by various agents in rat skin. Int J Microcir Clin Exp 2:39
24. Deng J, Kelley JA, Barchi JJ, Sanchez T, Dayam R, Pommier Y, Neamati N (2006) Mining the NCI antiviral compounds for HIV-1 integrase inhibitors. Bioorg Med Chem 14:3785
25. Nowakowska Z (2007) A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem 42:125
26. Phrutivorapongkul A, Lipipun V, Ruangrungsi N, Kirtikara K, Nishikawa K, Maruyama S, Watanabe T, Ishikawa T (2003) Studies on the chemical constituents of stem bark of *Millettia leucantha*: isolation of new chalcones with cytotoxic, anti-herpes simplex virus and anti-inflammatory activities. Chem Pharm Bull 51:187
27. Likhitwitayawuid K, Chaiwiriyaa S, Sritularaka B, Lipipunb V (2006) Antiherpetic flavones from the heartwood of *Artocarpus gomezianus*. Chem Biodivers 3:1138
28. Prendergast PT (2003) US Patent No 6555523
29. Santhosh C, Mishra PC (1996) Molecular electrostatic potential mapping and structure-activity relationship for 3-methoxy flavones. Indian J Biochem Biophys 33(6):458
30. Moriarty RM, Surve BC, Naithani R, Chandersekera SN, Tiwari V, Shukla D (2007) Synthesis and antiviral activity of Abyssinone II analogs. In: Abstracts of papers, 233rd ACS National Meeting, Chicago, IL, 25–29 March 2007
31. Zhao Yu, Wang F, Liu W, Bai H. (2007) Method for preparation and application dihydroflavanonol compounds. Faming Zhuanli Shenqing Gongkai Shuomingshu 34
32. Ren Q, Song X (2005) Use of a composition containing dihydromyricetin and myricetin in preparation of antiviral medicines. Faming Zhuanli Shenqing Gongkai Shuomingshu 20
33. Andersen, O. M, Helland, D. E, Andersen, K. J. (1997) Anthocyanidin and anthocyanidin derivatives, and their isolation, for treatment of cancer, diseases caused by lesions in connective tissues, and diseases caused by viruses. PCT Int Appl 121
34. Takatsuki A, Tamura G, Arima K (1969) Antiviral and antitumor antibiotics. XIV. Effects of ascochlorin and other respiration inhibitors on multiplication of Newcastle disease virus in cultured cells. App Microbiol 17:825
35. Mashava P (1996) PCT Int Appl 38
36. Conti C, Orsi N, Stein ML (1988) Effect of isoflavans and isoflavenes on rhinovirus 1B and its replication in HeLa cells. Antiviral Res 10:117
37. Yu D, Suzuki M, Xie L, Morris-Natschke SL, Lee K-H (2003) Recent progress in the development of coumarin derivatives as potent anti-HIV agents. Med Res Rev 23:322
38. Creagh T, Ruckle JL, Tolbert DT (2001) Safety and pharmacokinetics of single doses of (+)-calanolide a, a novel, naturally occurring nonnucleoside reverse transcriptase inhibitor, in healthy, human immunodeficiency virus-negative human subjects. Antimicrob Agents Chem 45:1379
39. Li Y, Leung KT, Yao F, Ooi LSM, Ooi VEC (2006) Antiviral flavans from the leaves of *Pithecellobium clypearia*. J Nat Prod 69:833

40. Charles L, Laure F, Raharivelomanana P, Bianchini JP (2005) Sheath liquid interface for the coupling of normal-phase liquid chromatography with electrospray mass spectrometry and its application to the analysis of neoflavonoids. J Mass Spectrom 40:75
41. Farnsworth NR (2006) Biological and phytochemical screening of plants. J Pharm Sci 55(3):225
42. Houghton PJ, Woldemariam TZ, Khan AI, Burke A, Mahmood N (1994) Antiviral activity of natural and semi-synthetic chromosome alkaloids. Antiviral Res 25(3–4):235
43. Wen CC, Kuo YH, Jan JT, Liang PH, Wang SY, Liu HG, Lee CK, Chang ST, Kuo CJ, Lee SS, Hou CC, Hsiao PW, Chien SC, Shyur LF, Yang NS (2007) Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus. J Med Chem 50:4087
44. Melikian G, Mmiro F, Ndugwa C, Perry R, Jackson JB, Garrett E, Tielsch J, Semba RD (2001) Relation of vitamin A and carotenoid status to growth failure and mortality among Ugandan infants with human immunodeficiency virus. Nutrition 17:567
45. Heldreth B, Turos E (2005) N-Thiolated beta-lactam antibacterials: effects of the N-organothio substituent on anti-MRSA activity. Curr Med Chem 4:295
46. Hunter R, Caira M, Stellenboom N (2005) Nat Prod Mol Ther 6:234
47. Moriarty RM, Naithani R, Surve BC, Tiwari V, Shukla D (2006) Synthesis and antiviral activity of Sulforamate derivatives. In: Abstracts-233rd ACS National Meeting Chicago, IL, 25–29 March 2006
48. Jariwalla R, Harakeh S (1996) Antiviral and immunomodulatory activities of ascorbic acid. In: Harris JR (ed) Subcellular biochemistry: ascorbic acid: biochemistry and biomedical cell biology, vol 25. Plenum, New York, p 215
49. Andreone P, Fiorino S, Cursaro C, Gramenzi A, Margotti M, Di Giammarino L, Biselli M, Miniero R, Gasbarrini G, Bernardi M (2001) Vitamin E as treatment for chronic hepatitis B: results of a randomized controlled pilot trial. Antiviral Res 49:75
50. Cermelli C, Vinceti M, Scaltriti E, Bazzani E, Beretti F, Vivoli G, Portolani M (2002) Selenite inhibition of Coxsackie virus B5 replication: implications on the etiology of Keshan disease. J Trace Elem Med Biol 16:41
51. La Colla P, Tramontano E, Musiu C, Marongiu ME, Novellino E, Greco G, Massa S, Di Santo R, Costi R, Marino A (1998) In vitro and in vivo antiproliferative activity of IPCAP, a new pyrazole. Antiviral Res 7:A57
52. Botelho MV, Orlandi JM, de Melo FL, Mantovani MS, Linhares RE, Nozawa C (2004) Chlorophyllin protects HEp-2 cells from nuclear fragmentation induced by poliovirus. Lett Appl Microbiol 39:174
53. Ishihara C, Yoshimatsu K, Tsuji M, Arikawa J, Saiki I, Tokura S, Azuma I (1993) Antiviral activity of sulfated chitin. Vaccine 11:670
54. Liu KC, Lin MT, Lee SS, Chiou JF, Ren S, Lien EJ (1999) Antiviral tannins from two Phyllanthus species. Planta Med 65:43
55. Hudson JB (1990) Antiviral compounds from plants. CRC, Boca Raton, FL
56. Wat CK, Biswas RK, Graham EA, Bohm L, Towers GH, Waygood ER (1979) Ultraviolet-mediated cytotoxic activity of phenylheptatriyne from *Bidens pilosa*. J Nat Prod 42:103
57. Neushul M (1990) Antiviral carbohydrates from marine red algae. Hydrobiologia 205:99
58. Gerber P, Dutcher JD, Adams EV, Sherman JH (1958) Protective effect of seaweed extracts for chicken embryos infected with influenza B or mumps virus. Proc Soc Exp Biol Med 99:590
59. Witvrouw M, DeClerq E (1997) Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. Gen Pharmacol 29:497
60. Huleihel M, Ishanu V, Tal J, Arad SM (2001) Antiviral effect of red microalgal polysaccharide on Herpes simplex and Varicella zoster viruses. J Appl Phycol 13:127
61. Konigheim BS, Goleniowski ME, Contigiani MS (2005) Citotoxicity and antiviral activity of a lignan extracted from *Larrea divaricata*. Drug Des Rev 2:81
62. Wang CY, Sun SW, Lee SS (2004) Pharmacokinetic and metabolic studies of retrojusticidin B, a potential antiviral lignan, in rats. Planta Med 70:1161
63. Ho TY, Wu SL, Chen JC, Li CC, Hsiang CY (2007) Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction. Antiviral Res 74:92
64. Chrubasik S, Pittler MH, Roufogalis BD (2005) Zingiberis rhizome: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine 12:684

65. Singh DP, Moore CA, Gilliland A, Carr JP (2004) Activation of multiple antiviral defence mechanisms by salicylic acid. Mol Plant Pathol 5:57
66. Semple SJ, Pyke GD, Reynolds SM, Flower RLP (2001) In vitro antiviral activity of the anthraquinone chrysophanic acid against poliovirus. Antiviral Res 49:169
67. Wang W, Zu Y, Fu Y, Reichling J, Suschke U, Nokemper S, Zhang Y (2009) In vitro antioxidant, antimicrobial and anti-herpes simplex virus type 1 activity of *Phellodendron amurense* China. Am J Chin Med 37(1):195–203
68. Amoros M, Simoes CMO, Girre L (1992) Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. J Nat Prod 155:1732–1740
69. Min BS, Tomiyama M, Ma CM, Nakamura N, Hattori M (2001) Kaempferol acetylrhamnosides from the rhizome of *Dryopteris crassirhizoma* and their inhibitory effects on three different activities of human immunodeficiency virus-1 reverse transcriptase. Chem Pharm Bull 49:546–550
70. McLaughlin JL (2008) Paw paw and cancer: annonaceous acetogenins from discovery to commercial products. J Nat Prod 71(7):1311–1321
71. Phongpaichit S, Nikom J, Rungjindamai N, Sakayaroj J, Hutadilok-Towatana N, Rukachaisirikul V, Kirtikara K (2007) Biological activities of extracts from endophytic fungi isolated from Garcinia plants. FEMS Immunol Med Microbiol 51(3):517–525
72. Cos P, Maes L, Vander Berghe D, Hermen N, Pieters L, Vlietinck A (2004) Plant substances as Anti-HIV agents selected according to their putative mechanism of action. J Nat Prod 67:284–293
73. Yoosook C, Bunyapraphatsara N, Boonakiat Y, Kantasuk C (2000) Anti-herpes simplex virus activities of crude water extract of Thai medicinal plants. Phytomedicine 6:411–419
74. Dias DA, Silva CA, Urban S (2009) Naphthalene aglycones and glycosides from Australian medicinal plant, *Dianella callicarpa*. Planta Med 75((13)):1442–1447
75. Petrera E, Coto CE (2009) Therapeutic effect of meliacine, an antiviral derived from *Melia azedarach* L, in mice genital herpetic infection. Phytother Res 23(12):1771–1777
76. Alvarez AL, Del Barrio G, Kourí V, Martínez PA, Suárez B, Parra F (2009) In vitro anti-herpetic activity of an aqueous extract from the plant *Phyllanthus orbicularis*. Phytomedicine 16(10):960–966
77. Lee LS, Andrade AS, Flexner C (2006) Interactions between natural health products and antiretroviral drugs: pharmacokinetic and pharmacodynamic effects. Clin Infect Dis 43(8):1052
78. Ishitsuka H, Ohsawa C, Ohiwa T, Umeda I, Suhara Y (1982) Antipicornavirus flavone Ro 09-0179. Antimicrob Agents Chemother 22:611
79. Serkedjieva J (1995) Inhibition of influenza virus protein synthesis by a plant preparation from *Geranium sanguineum* L. Acta Virol 39(1):5–10
80. Malhotra B, Onyilagha JC, Bohm BA, Towers GHN, James D, Harborne JB, French CJ (1996) Inhibition of tomato ringspot virus by flavonoids. Phytochemistry 43:1271
81. Li BQ, Fu T, Dongyan Y, Mikovits JA, Ruscetti FW, Wang JM (2000) Flavonoid baicalin inhibits HIV infection at the level of viral entry. Biochem Biophysical Res Comm 276:534
82. Eversa DL, Chaoa CF, Wanga X, Zhanga Z, Huonga SM, Huang ES (2005) Antiviral Res 68:124
83. Castrillo JL, Carrasco L (1987) Action of 3-methylquercetin on poliovirus RNA replication. J Virol 61:3319
84. Takechi M, Tanaka Y (1981) Purification and characterization of antiviral substance from the bud of *Syzygium aromatica*. Planta Med 42:69
85. Sydiskis RJ, Owen DG, Lohr JL, Rosler KH, Blomster RN (1991) Inactivation of enveloped viruses by anthraquinones extracted from plants. Antimicrob Agents Chemother 35:2463
86. Kurokawa M, Hozumi T, Basnet P, Nakano M, Kadota S, Namba T, Kawana T, Shiraki K (1998) Purification and characterization of eugeniin as an anti-herpes virus compounds from *Geum japonicum* and *Syzygium aromaticum*. J Pharmacol Exp Ther 284:728
87. Bettega JMR, Teixeira H, Bassani VL, Barardi CRM, Simões MO (2004) Evaluation of the antiherpetic activity of standardized extracts of *Achyrocline satureioides*. Phytother Res 18:819
88. Spedding G, Ratty A, Middleton E (1989) Inhibition of reverse transcriptases by flavonoids. Antiviral Res 12:99
89. Salvati AL, De Dominicis A, Tait S, Canitan A, Lahm A, Fiore L (2004) Mechanism of action at the molecular level of the antiviral drug 3(2H)-isoflavene against type 2 poliovirus. Antimicrob Agents Chemother 48:2233

90. Song JM, Lee KH, Seong BL (2005) Antiviral effect of catechins in green tea on influenza virus. Antiviral Res 68:66
91. Lin YM, Flavin MT, Schure R, Chen FC, Sidwell R, Barnard DL, Huffman JH, Kern ER (1999) Antiviral activities of biflavonoids. Planta Med 65:120
92. Semple SJ, Nobbs SF, Pyke SM, Reynolds GD, Flower RL (1999) Antiviral flavonoid from *Pterocaulon sphacelatum*, an Australian Aboriginal medicine. J Ethnopharmacol 68:283
93. Yu YB, Miyashiro H, Nakamura N, Hattori M, Cheol PJ (2007) Effects of triterpenoids and flavonoids isolated from Alnus firma on HIV-1 viral enzymes. Arch Pharm Res 30:820
94. Hwanga DR, Wua YS, Changa CW, Liena TW, Chena WC, Tanb UK, Hsua JTA, Hsieh HP (2006) Synthesis and antiviral activity of a series of sesquiterpene lactones and analogues in the subgenomic HCV replicon system. Bioorg Med Chem 14:83
95. González ME, Alarcón B, Carrasco L (1987) Polysaccharides as antiviral agents: antiviral activity of carrageenan. Antimicrob Agents Chemother 31:1388
96. Talyshinsky MM, Souprun YY, Huleihe MM (2002) Antiviral activity of red microalgal polysaccharides against retroviruses. Cancer Cell Int 2:8
97. McMahon JB, Currens MJ, Gulakowski RJ, Buckheit RW Jr, Lackman-Smith C, Hallock YF, Boyd MR (1995) Michellamine B, a novel plant alkaloid, inhibits human immunodeficiency virus-induced cell killing by at least two distinct mechanisms. Antimicrob Agents Chemother 39:484
98. Renard-Nozaki J, Kim T, Imakura Y, Kihara M, Kobayashi S (1989) Effect of alkaloids isolated from Amaryllidaceae on herpes simplex virus. Res Virol 140:115
99. Bresnahan WA, Boldogh I, Chi P, Thompson EA, Albrecht T (1997) Inhibition of cellular Cdk2 activity blocks human cytomegalovirus replication. Virology 231:239
100. Schang LM, Phillips J, Schaffer PA (1998) Roscovitine, a specific inhibitor of cellular cyclin-dependent kinases, inhibits herpes simplex virus DNA synthesis in the presence of viral early proteins. J Virol 72:5626
101. Redfield DC, Oxman RDD, MN KLH (2001) Rapid serological technique for typing herpes simplex viruses. Infect Immun 32:1216
102. Durantel D, Branza-Nichita N, Carrouée-Durantel S, Butters TD, Dwek RA, Zitzmann N (2001) Study of the mechanism of antiviral action of iminosugar derivatives against bovine viral diarrhea virus. J Virol 75:8987
103. Marles RJ, Hudson JB, Graham EA, Soucy-Breau C, Morand P, Compadre RL, Compadre CM, Towers GH, Arnason JT (1992) Structure-activity studies of photoactivated antiviral and cytotoxic tricyclic thiophenes. Photochem Photobiol 56:479
104. Stanberry LR, Bourne N, Bravo FJ, Bernstein DI (1992) Capsaicin-sensitive peptidergic neurons are involved in the zosteriform spread of herpes simplex virus infections. J Med Virol 38:142
105. Si X, Wang Y, Wong J, Zhang J, McManus BM, Luo H (2007) Dysregulation of the ubiquitin-proteasome system by curcumin suppresses coxsackievirus B3 replication. J Virol 81:3142
106. Dikici I, Mehmetoglu I, Dikici N, Bitirgen M, Kurban S (2005) Investigation of oxidative stress and some antioxidants in patients with acute and chronic viral hepatitis B and the effect of interferon-alpha treatment. Clin Biochem 38:1141
107. Joint United Nations Programme on HIV/AIDS (2006) Overview of the global AIDS epidemic. 2006 Report on the global AIDS epidemic, UNAIDS, Geneva, Switzerland
108. Paris A, Strukelj B, Renko M, Turk V, Pukl M, Umek A, Korant BD (1993) Inhibitory effect of carnosolic acid on HIV-1 protease in cell-free assays. J Nat Prod 56:1426
109. Chang CT, Doong SL, Tsai IL, Chen IS (1997) Coumarins and anti-HBV constituents from *Zanthoxylum schinifolium*. Phytochemistry 45:1419
110. Fortin H, Tomasi S, Jaccard P, Robin V, Boustie J (2001) A prenyloxycoumarin from *Psiadia dentata*. Chem Pharm Bull 49:619
111. Sakagami H, Asano K, Satoh K, Takahashi K, Kobayashi M, Koga N, Takahashi H, Tachikawa R, Tashiro T, Hasegawa A, Kurihara K, Ikarashi T, Kanamoto T, Terakubo S, Nakashima H, Watanabe S, Nakamura W (2007) Anti-stress, anti-HIV and vitamin C-synergized radical scavenging activity of mulberry juice fractions. In Vivo 21:499
112. Hsieh P-W, Chang F-R, Lee K-H, Hwang T-L, Chang SM, Wu YC (2004) A new anti-HIV alkaloid, drymaritin, and a new C-glycoside flavonoid, diandraflavone, from *Drymaria diandra*. J Nat Prod 67:1175

113. Lee JS, Kim HJ, Lee YS (2003) A new anti-HIV flavonoid glucuronide from *Chrysanthemum morifolium*. Planta Med 69:859
114. Olivero-Verbel J, Pacheco-Londono L (2002) Structure activity relationships for the anti-HIV activity of flavonoids. J Chem Inf Comput Sci 42:1241
115. Nishibe S, Ono K, Nakane H, Kawamura T, Noro Y, Tanaka T (1997) Inhibitory effects of flavonoids from Plantago species on HIV reverse transcriptase activity. Nat Med 51:547
116. Kim HJ, Woo ER, Shin CG, Park H (1998) A new flavol glycoside gallate ester from Acer okamotoanum and its inhibitory activity against human immunodeficiency virus (HIV-1) integrase. J Nat Prod 61:145
117. Critchfield JW, Butera ST, Folks TM (1996) Inhibition of HIV activation in latently infected cells by flavonoid compounds. AIDS Res Hum Retroviruses 12:39
118. Mahmood N, Pizza C, Aquino R, De Tommasi N, Piacente S, Colman S, Burke A, Hay AJ (1993) Inhibition of HIV infection by flavanoids. Antiviral Res 22:189
119. Schinazi RF, Chu CK, Babu JRB, Oswald V, Saalman DL, Erickson BF (1990) Anthraquinones as a new class of antiviral agents against AIDS. Antiviral Res 13:265
120. Robin V, Boustie J, Amoros M, Girre L (1998) In-vitro antiviral activity of seven Psiadia species. Pharmacol Commun 4:61
121. Tuli V (1974) Mechanism of the antiviral action of 3-methyleneoxindole. Antimicrob Agents Chemother 5(5):485
122. Lyu S-Y, Rhim JY, Park WB, Lyu SY, Rhim JY, Park WB (2005) Antiherpetic activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). Arch Pharm Res 28:1293
123. Ma W, Ma X, Huang H, Zhou P, Chen D (2007) Lignans and triterpenoids from the stems of *Kadsura induta*. Chem Biodivers 4:966
124. Chattopadhyay D (2007) In: Ahmad I, Aqil F, Owais M (eds) Ethnomedicinal antivirals: scope and opportunity modern phytomedicine, p 313
125. Benencia F, Courrèges MC (2000) In vitro and in vivo activity of eugenol on human herpesvirus. Phytother Res 14:495
126. Li H, Zhou CX, Pan Y, Gao X, Wu X, Bai H, Zhou L, Chen Z, Zhang S, Shi S, Luo J, Xu J, Chen L, Zheng X, Zhao Y (2005) Evaluation of antiviral activity of compounds isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus*. Planta Med 71:1128
127. Likhitwitayawuid K, Supudompol B, Sritularak B, Lipipun V, Rapp K, Schinazi RF (2005) Phenolics with anti-HSV and anti-HIV activities from *Artocarpus gomezianus*, *Mallotus pallidus* and *Triphasia trifolia*. Pharm Biol 43:651
128. El Sohly HN, El-Feraly FS, Joshi AS, Walker LA (1997) Antiviral flavonoids from *Alkanna orientalis*. Planta Med 63:384
129. Lin LC, Kuo YC, Chou CJ (2000) Anti-herpes simplex virus type-1 flavonoids and a new flavanone from the root of *Limonium sinense*. Planta Med 66:333
130. Du J, He ZD, Jiang RW, Ye WC, Xu HX, But PP (2003) Antiviral flavonoids from the root bark of *Morus alba* L. Phytochemistry 62:1235–1238
131. Jou S-J, Chen C-H, Guh J-H, Lee C-N, Lee SS (2004) Flavonol glycosides and cytotoxic triterpenoids from *Alphitonia philippinensis*. J Chin Chem Soc (Taipei, Taiwan) 51:827
132. Atta-ur-Rahman (ed) (1995) Studies in natural products chemistry, structure and chemistry, vol 17, Part D. Elsevier, Amsterdam
133. Loizzo M, Saab A, Tundis R, Statti G, Lampronti I, Menichini F, Gambari R, Cinatl J, Doerr H (2008) Phytochemical analysis and in vitro evaluation of the biological activity against herpes simplex virus type 1 (HSV-1) of *Cedrus libani* A. Phytomedicine 15:79–83
134. Harden EA, Falshaw R, Carnachan SM, Kern ER, Prichard MN (2009) Virucidal activity of polysaccharide extracts from four algal species against herpes simplex virus. Antiviral Res 83(3):282–289
135. Bunyapraphatsara N, Dechsree S, Yoosook C, Herunsalee A, Panpisutchai Y (2000) Anti-herpes simplex virus component isolated from *Maclura cochinchinensis*. Phytomedicine 6:421
136. Okamoto T, Kanda T (1999) Glycyrrhizin protects mice from concanavalin A-induced hepatitis without affecting cytokine expression. Int J Mol Med 4:149–152
137. Shin MS, Kang EH, Lee YI (2005) A flavonoid from medicinal plants blocks hepatitis B virus-e antigen secretion in HBV-infected hepatocytes. Antiviral Res 67:163

138. Cai SQ, Wang R, Yang X, Shang M, Ma C, Shoyama Y (2006) Antiviral flavonoid-type C-glycosides from the flowers of *Trollius chinensis*. Chem Biodivers 3:343
139. Nagai T, Suzuki Y, Tomimori T, Yamada H (1995) Antiviral activity of plant flavonoid, 5, 7, 4′-trihydroxy-8-methoxyflavone, from roots of *Scutellaria baicalensis* against influenza A (H3N2) and B viruses. Biol Pharm Bull 18:95
140. Sidwell RW, Huffman JH, Moscon BJ, Warren RP (1994) In vitro and in vivo sensitivity of a non-mouse-adapted influenza A (Beijing) virus infection to amantadine and ribavirin. Chemotherapy 40:42
141. Quaglia MG, Desideri N, Bossu E, Sgro R, Conti C (1999) Enantioseparation and anti-rhinovirus activity of 3-benzylchroman-4-ones. Chirality 11(5–6):495–500
142. Douglas RM, Chalker EB, Treacy B (2000) Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev 2:CD000980
143. Melchart D, Linde K, Fischer P, Kaesmayr J (2000) Echinacea for preventing and treating the common cold [Review]. Cochrane Database Syst Rev (2):CD000530
144. Desideri N, Conti C, Mastromarino P, Mastropaolo F (2000) Synthesis and anti-rhinovirus activity of 2-styrylchromones. Antivir Chem Chemother 11:373
145. Weber ND, Andersen DO, North JA, Murray BK, Lawson LD, Hughes BG (1992) In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Med 58:417
146. Tait S, Salvati AL, Desideri N, Fiore L (2006) Antiviral activity of substituted homoisoflavonoids on enteroviruses. Antiviral Res 72:252
147. Singh S, Malik BK, Sharma DK (2007) Targeting HIV-1 through molecular modeling and docking studies of CXCR4: leads for therapeutic development. Chem Biol Drug Des 69:191
148. Amaral ACF, Kuster RM, Goncalves JLS, Wigg MD (1999) Phytochemical investigation of an antiviral fraction of *Chamaesyce thymifolia*. Fitoterapia 70:293
149. Desideri N, Oliveri S, Stein ML, Sgro R, Orsi N, Conti C (1997) Synthesis and anti-rhinovirus activity of 2-styrylchromones. Antivir Chem Chemother 8:545
150. Chiang LC, Chiang W, Liu MC, Lin CC (2003) In vitro antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids. J Antimicrob Chemother 52:194
151. Moriarty RM, Naithani R, Hirtopeanu A, Gao K, Kazerani S, Badlani R, Staszewski JP, Zitzmann, N, Dwek, RA, Jacob, GS, Picker D, Mehta A, Block TM. N-Alkyl deoxynojirimycins as antiviral compounds. In: Abstracts of papers, 224th ACS National Meeting, Boston, MA, 18-22 August 2002
152. Mehta A, Ouzounov S, Jordan R, Simsek E, Lu X, Moriarty RM, Jacob G, Dwek RA, Block TM (2002) Imino sugars that are less toxic but more potent as antivirals, in vitro, compared with N-n-nonyl DNJ. Antivir Chem Chemother 13:299
153. De Almeida AP, Miranda MMFS, Simoni IC, Wigg MD, Lagrota MHC, Costa SS (1998) Flavonol monoglycosides isolated from the antiviral fractions of *Persea americana* (Lauraceae) leaf infusion. Phytotherapy Res 12:562
154. Li Y, Ooi LS, Wang H, But PP, Ooi VE (2004) Antiviral activities of medicinal herbs traditionally used in southern mainland China. Phytother Res 18:718–722
155. Lipipun V, Kurokawa M, Suttisri R, Taweechotipatr P, Pramyothin P, Hattori M, Shiraki K (2003) Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection in vitro and in vivo. Antiviral Res 60:175–180
156. Arthan D, Svasti J, Kitaakoop P, Pittayakhachonwut D, Toem M, Thebtaranonth Y (2002) Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochem 59:459–463
157. Aspers S, Baronikova S, Sindambiwe JB, Witvrouwm M, De Clercqm E, Vanden Berghe D, Van Maeck E (2001) Antiviral, haemolytic and molluscicidal activities of triterpenoid saponins from *Maesa lanceolata*: establishment of structure-activity relationships. Planta Med 67:521
158. Sydiskis RJ, Owen DG, Lohr JL, Rosler KH, Blomster N (1999) Inactivation of enveloped viruses by anthraquinones extracted from plants. Antimicrob Agents Chemother 35:2463–2666
159. Jassim SA, Naji MA (2003) Novel antiviral agents: A medicinal plant perspective. J Appl Microbiol 95:412–417
160. Garcia CC, Talarico L, Almeida N, Colombres S, Duschatzky C, Damonte EB (2003) Virucidal activity of essential oils from aromatic plants of San Luis, Argentina. Phytother Res 17:1073–1075

161. Alche LE, Berra A, Veloso MJ, Coto CE (2000) Treatment with meliacine, a plant derived antiviral, prevents the development of herpetic stromal keratitis in mice. J Med Virol 61:474–480
162. Baermejo P, Abad MJ, Diaz AM, Ferenandez L, Santos JD, Sachez S, Vallaescusa L, Carrasco L, Irurzun A (2002) Antiviral activity of seven iridoids, three saikosaponins and one phenylpropanoid glycoside extracted from *Bupleurum rigidum* and *Scrophularia scorodonia*. Planta Med 68:106–110
163. Primo V, Rovera M, Zanon S, Oliva M, Demo M, Daghero J, Sabini L (2001) Determination of the antibacterial and antiviral activity of the essential oil from *Minthostachys verticillata* (Griseb.). Rev Argent Microbiol 33:113–117
164. Khan MT, Ather A, Thompson KD, Gambari R (2005) Extracts and molecules from medicinal plants against herpes simplex viruses. Antiviral Res 67:107–119
165. Chattopadhyay D, Arunachalam G, Mandal AB, Bhattacharya SK (2006) Dose-dependent therapeutic antiinfectives from ethnomedicines of bay islands. Chemotherapy 52(3):151–157
166. Cheng HY, Lin CC, Lin TC (2002) Antiherpes simplex virus type 2 activity of casuarinin from the bark of *Terminalia arjuna* Linn. Antiviral Res 55:447–455
167. Allahveridev A, Duran N, Ozguven M, Koltas S (2004) Investigation of the anticancerogenic effect of the essential oil of *Melissa officinalis* L. Phytomedicine 11:657
168. Ojo OO, Oluyege JO, Famurewa O (2009) Antiviral properties of two Nigerian plants African. J Plant Sci 3(7):157–159
169. Chiang LC, Cheng HY, Liu MC, Chiang W, Lin CC (2003) In vitro anti-herpes simplex viruses and anti-adenoviruses activity of twelve traditionally used medicinal plants in Taiwan. Biol Pharm Bull 26:1600–1604
170. Choi HJ, Song HJ, Park KS, Kwon DH (2009) Inhibitory effects of quercetin 3-rhamnoside on influenza A virus replication. Eur J Pharm Sci 3–4:329–333
171. Kernan MT, Amarquaye A, Chen JL, Chan J, Sesin DF, Parkinson N, Ye Z, Barrett M (1998) Antiviral phenylpropanoid glycosides from the medicinal plant *Markhamia lutea*. J Nat Prod 61:564–570
172. Wei F, Ma SC, Ma LY, But PP, Lin RC, Khan IA (2004) Antiviral flavonoids from the seeds of *Aesculus chinensis*. J Nat Prod 67:650–653
173. Rajbhandari M, Wegner U, Schopke T, Lindequist U, Mentel R (2003) Inhibitory effect of *Bergenia ligulata* on influenza virus. Pharmazie 58:268–271
174. Sokmen M, Angeova M, Krumova E, Peshova S, Ivancheva S, Sokmen A, Serkedijieva J (2005) In vitro antioxidant activity of polyphenol extracts with antiviral properties from *Geranium sanguineum*. Life Sci 76:2981–2993
175. Olila D, Olwa-Odyejk A, Opudo A (2002) Screening extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis* for activity against measles virus (Swartz and Edmonston strains) in vitro. J Afr Health Sci 2:2–10
176. Thyagarajan SP, Subramanian S, Thirunalasundari TN, Venkateswaran PS, Blumberg BS (1998) Effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. Lancet 2(8614):764–766
177. Thyagarajan SP, Jayaram S, Valiammai TT (1990) *Phyllanthus amarus* and hepatitis B. Lancet 336:949–950
178. Liu J, Zhu M, Shi R, Yang M (2003) Radix *Sophorae flavescentis* for chronic hepatitis B: a systematic review of randomized trials. Am J Chin Med 31:337–354
179. Ho TY, Wu SL, Lai IL, Cheng KS, Kao ST, Hsiang CY (2003) An in vitro system combined with an in-house quantitation assay for screening hepatitis C virus inhibitors. Antiviral Res 58:199
180. Sekine-Osajima Y, Sakamoto N, Nakagawa M, Itsui Y, Tasaka M, Nishimura-Sakurai Y, Chen CH, Suda G, Mishima K, Onuki Y, Yamamoto M, Maekawa S, Enomoto N, Kanai T, Tsuchiya K, Watanabe M (2009) Two flavonoids extracts from *Glycyrrhizae radix* inhibit in vitro hepatitis C virus replication. Hepatol Res 39(1):60–69
181. Esimone CO, Grunwald T, Nworu CS, Kuate S, Proksch P, Uberla K, Uberla K (2009) Broad spectrum antiviral fractions from the lichen *Ramalina farinacea* (L.) Ach. Chemotherapy 55:119–126
182. Manfredi KP, Vallurupalli V, Demidova M, Kindscher K, Pannell LK (2001) Isolation of anti-HIV diprenylated bibenzyl from *Glycyrrhiza lepidota*. Phytochemistry 58:153–157
183. Wu JH, Wang XH, Yi YH, Lee KH (2003) A potent anti-HIV chalcone and flavonoids from genus Desmos. Bioorg Med Chem Lett 13:1813–1815
184. Chang YS (2003) Woo ER (2003) Korean medicinal plants inhibiting to human immunodeficiency virus type 1 (HIV-1) fusion. Phytother Res 17:426–429

185. Balzarini JC, Daelemans BD, Meertens M, Cornier EG, Reinus JF, Peumans WJ, Van Damme EJM, Oki YIT, Schols D, Dragic T (2007) Carbohydrate-binding agents (CBAs) selectively target the glycoproteins of the HCV and HIV envelope to prevent viral entry. Antiviral Res 74:A43
186. Yogeeswari P, Sriram D (2005) Betulinic acid and its derivatives: a review on their biological properties. Curr Med Chem 12:657–666
187. Jay J, Lai B, Kiser P (2009) Multivalent synthetic lectin polymers against HIV. Antiviral Res 82:A63–A64
188. Gosse B, Gnabre J, Bates RB, Dicus CW, Nakkiew P, Huangg RC (2002) Antiviral saponins from *Tieghemella heckelii*. J Nat Prod 65:1942–1944
189. Ma CM, Nakamura N, Miyashiro H, Hattori M, Komatsu K, Kawahata T, Otake T (2002) Screening of Chinese and Mongolian herbal drugs for anti-human immunodeficiency virus type 1 (HIV-1) activity. Phytother Res 16:186–189
190. Shikishima Y, Takaishi Y, Honda G, Ito M, Takfda Y, Kodzhimatov OK, Ashurmetov O, Lee KH (2001) Chemical constituents of *Prangos tschimganica*; structure elucidation and absolute configuration of coumarin and furanocoumarin derivatives with anti-HIV activity. Chem Pharm Bull 49:877–880
191. Zhang HJ, Tan GT, Honag VD, Hung NV, Cuong NM, Soejarto DD, Pezzuto JM, Fong HH (2003) Natural anti-HIV agents. Part IV. Anti-HIV constituents from *Vatica cinerea*. J Nat Prod 65:1942–1944
192. Szlavik L, Gyuris A, Minarotvis J, Forgo P, Molnar J, Hohmann J (2004) Alkaloids from *Leucojum vernum* and antiretroviral activity of *Amaryllidaceae* alkaloids. J Planta Med 70:71–873
193. Ye XY, Ng TB (2001) Peptides from pinto bean and red bean with sequence homology to cowpea 10-kDa protein precursor exhibit antifungal, mitogenic, and HIV-1 reverse transcriptase-inhibitory activities. Biochem Biophys Res Commun 285:424–429
194. Min BS, Tomiyamam CM, Nakamura N, Hattori M (2001) Kaempferol acetylrhamnosides from the rhizome of *Dryopteris crassirhizoma* and their inhibitory effects on three different activities of human immunodeficiency Virus-I reverse transcriptase. Chem Pharm Bull 49:546–550
195. Jiratchariyakul W, Wiwat C, Vonsakul M, Somanabandhu A, Leelamanit W, Fujii I, Suwannoroj N, Ebizuka W (2001) HIV inhibitor from Thai bitter gourd. Planta Med 67:350–353
196. Notka F, Meier GR, Wagner R (2003) Immunodeficiency virus and reverse transcriptase inhibitor-resistant variants by *Phyllanthus amarus*. Antiviral Res 58:175–186
197. Park JC, Hur JM, Park JG, Hatano T, Yoshida T, Miyashiro H, Min BS, Hattori M (2002) Inhibitory effects of Korean medicinal plants and camelliatannin H from *Camellia japonica* on human immunodeficiency virus type 1. Phytother Res 16:422–426
198. Liu S, Jiang S, Wu Z, Lv L, Zhabg J, Zhu Z, Wu S (2002) Identification of an inhibitor of the HIV-1 gp41 six-helix bundle formation from extracts of Chinese medical herbs *Prunella vulgaris* and *Rhizoma Cibotte*. Life Sci 71:1779–1791
199. Choi KC, Jung MG, Lee YH, Yoon JC, Kwon SH, Kang HB, Kim MJ, Cha JH, Kim YJ, Jun WJ, Lee JM, Yoon HG (2009) Epigallocatechin-3-gallate, a histone acetyltransferase inhibitor, inhibits EBV-induced B lymphocyte transformation via suppression of RelA acetylation. Cancer Res 69(2):583–592
200. Andres A, Donovan SM, Kuhlenschmidt MS (2009) Isoflavones and viral infections. J Nutr Biochem 20:563–569
201. Robin V, Irurzun A, Amoros M, Boustie J, Carrasco L (2001) Antipoliovirus flavonoids from *Psiadia dentata*. Antivir Chem Chemother 12:283–291
202. Hegde VH, Pu H, Patel M, Das PR, Butkiewicz N, Arreaza G, Gullo VP, Chan TM (2003) Two antiviral compounds from the plant *Stylogne cauliflora* as inhibitors of HCV NS3 protease. Bioorg Med Chem Lett 13:2925
203. Clark KJ, Grnat PG, Sarr AB, Belakere JR, Swaggerty CL, Phillips TD, Woode GN (1998) An in vitro study of theaflavins extracted from black tea to neutralize bovine rotavirus and bovine coronavirus infections. Vet Microbiol 63:147–157
204. Turan K, Nagata K, Kuru A (1996) Antiviral effect of Sanicula europaea L. leaves extract on influenza virus-infected cells. Biochem Biophys Res Commun 225:22–26
205. Docherty JJ, Sweet TJ, Bailey E, Faith SA, Booth T (2006) Resveratrol inhibition of Varicella-Zoster Virus replication in vitro. Antiviral Res 72:171

206. Li BQ, Fu T, Gong WH, Dunlop N, Kung HF, Yan Y, Kang J, Wang JM (2000) The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. Immunopharmacology 49:295
207. Mitrocosta D, Mitaku S, Axarlis S, Harvala C, Malamas M (2000) Evaluation of the antiviral activity of kaempferol and its glycosides against human cytomegalovirus. Planta Med 66:377
208. Gerencer M, Turecek PL, Kistner O, Mitterer A, Savidis-Dacho H, Barrett NP (2006) In vitro and in vivo anti-retroviral activity of the substance purified from the aqueous extract of *Chelidonium majus* L. Antiviral Res 72:153
209. Rusak G, Krajacic M, Plese N (1997) Inhibition of tomato bushy stunt virus infection using a quercetagetin flavonoid isolated from *Centaurea rupestris* L. Antiviral Res 36:125
210. Sanchez I, Gomez-Garibay F, Taboada J, Ruiz BH (2000) Antiviral effect of flavonoids on the Dengue virus. Phytother Res 14:89
211. Kiat TS, Pippen R, Yusof R, Ibrahim H, Khalid N, Rahman NA (2006) Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease. Bioorg Med Chem Lett 16:3337
212. Bae EA, Han MJ, Lee M, Kim DH (2000) In vitro inhibitory effect of some flavonoids on rotavirus infectivity. Biol Pharm Bull 23:1122
213. Thamlikitkul V, Wasuwat S, Kanchanapee P (1991) Efficacy of *Phyllanthus amarus* for eradication of hepatitis B virus in chronic carriers. J Med Assoc Thai 74:381
214. Berk L, de Man RA, Schalm SW, Labadie RP, Heijtink RA (1991) Beneficial effects of *Phyllanthus amarus* for chronic hepatitis B, not confirmed. J Hepatol 12:405
215. Zhang JL, He WN, Ye P (1992) Clinical observation on *Phyllanthus amarus* for treating chronic hepatitis B virus infection 1992. Chin J Integr Tradit West Med Liver Dis 2:8
216. Milne A, Hopkirk N, Lucas CR, Waldon J, Foo Y (1994) A two-stage clinical trial of *Phyllanthus amarus* in hepatitis B carriers: failure to eradicate the surface antigen. N Z Med J 107:253
217. The University of Hong Kong Trial of Chinese Herbal Medicine in the Treatment of Upper Respiratory Tract Infections (URTIs) The Research Fund for the Control of Infectious Diseases of the Food and Health Bureau, the Government of the Hong Kong SAR 2009
218. Huang ZR, Zhong JP, Zhu GL, Chen YR, Wang GQ (1993) Therapeutic observation on *Phyllanthus amarus* for hepatitis B. Chin J Clin Hepatol 9:108
219. Zhu FM, Zhang JQ, Zhang XZ, Zhang XM (1992) Observation on the effect of Fujian's *Phyllanthus amarus* in treatment of HBV infection. Chin J Integr Tradit West Med Liver Dis 2:10
220. Cao WZ, Liu JQ, Cao DY, Su F, Xu SG (1998) Clinical study on anti-HBV activity of Phyllanthus herb from Anhui, China. China J Chin Mater Med 23:180
221. Wang M, Cheng H, Li Y, Meng L, Zhao G, Mai K (1995) Herbs of the genus Phyllanthus in the treatment of chronic hepatitis B: observations with three preparations from different geographic sites. J Lab Clin Med 126:350
222. Su X, Chen H, Wang L, Jiang C, Liu J, Zhao M, Ma X, Zhao Y, Han D (1984) The protective effects of *Glycyrrhiza glabra* against Hepatitis. J Tradit Chin Med 4:127
223. Iino S, Tango T, Matsushima T, Toda G, Miyake K, Hino K, Kumada H, Yasuda K, Kuroki T, Hirayama C, Suzuki H (2001) Amino acid substitutions in S region of hepatitis B virus in the sera from patients with acute hepatitis. Hepatol Res 19:31
224. Doshi JC, Vaidya AB, Antarkar DS, Deolalikar R, Antani DH (1994) A two-stage clinical trial of *Phyllanthus amarus* on hepatitis B carriers: failure to eradicate the surface antigen. Indian J Gastroenterol 13:7
225. Tusenius KJ, Spoek JM, Kramers CW (2001) Iscador Qu for chronic hepatitis C: an exploratory study. Complement Ther Med 9:12
226. van Rossum TGJ, Vulto AG, Hop WCJ, Brouwer JT, Niesters HGM, Schalm SW (1999) Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double blind randomized placebo-controlled phase I/II trial. J Gastroenterol Hepatol 14:1093
227. Tsubota A, Kumada H, Arase Y, Chayama K, Saitoh S, Ikeda K, Kobayashi M, Suzuki Y, Murashima N (2002) Combined ursodeoxycholic acid and glycyrrhizin therapy forchronic hepatitis infection: a randomized control trial in 170 patients. Eur J Gastroenterol Hepatol 11:1077
228. van Rossum TGJ, Vulto AG, Hop WCJ, Schalm SW (2001) Glycyrrhizin-induced reduction of ALT in European patients with chronic hepatitis. Am J Gastroenterol 96:2432

229. Mehrotra R, Rawat S, Kulshreshtha DK (1990) In vitro studies on the effect of certain natural products against hepatitis B virus. Indian J Med Res 92:133
230. Stauder G, Kabil S (1997) Oral enzyme therapy in hepatitis C patients. J Immunother 13:153
231. Suzuki H, Ohta Y, Tekino T, Fujisawa K, Hirayama C (1983) Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis-double blind trial. Asian Med J 26:423
232. Miyake K, Tango T, Ota Y, Mitamura K, Yoshiba M, Kako M, Hayashi S, Ikeda Y, Hayashida N, Iwabuchi S, Sato Y, Tomi T, Funaki N, Hashimoto N, Umeda T, Miyazaki J, Tanaka K, Endo Y, Suzuki H (2002) Efficacy of Stronger Neo-Minophagen C compared between two doses administered three times a week on patients with chronic viral hepatitis. J Gastroenterol Hepatol 17:1198
233. Vailaii A, Arista I, Sozze E (1993) Randomized open study of the dose-effect relationship of a short course of IdB1016 in patients with viral or alcoholic hepatitis. Fitoterapia 64:219
234. Narendranathan M, Remla A, Mini PC, Satheesh P (1999) A trial of *Phyllanthus amarus* in acute viral hepatitis. Trop Gastroenterol 20:164
235. Parés A, Planas R, Torres M, Caballería J, Viver JM, Acero D, Panés J, Rigau J, Santos J, Rodés J (1998) Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial. J Hepatol 28:731
236. Ferenci P, Dragosics B, Dittrich H, Frank H, Benda L, Lochs H, Meryn S, Base W, Schneider B (1989) Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. J Hepatol 9:105
237. Thom E, Zakay-Rones Z, Wollan T Wadstein J (2002) Randomised study on the efficacy and safety of an oral elderberry extract in the treatment of influenza A and B virus infection. In Proceedings of the 15th international conference on antiviral research, Prague, Czech Republic, March 2002.
238. Zakay-Rones Z, Varsano N, Zlotnik M, Manor O, Regev L, Schlesinger M, Mumcuoglu M (1995) Inhibition of several strains of influenza virus in vitro and reduction of symptoms by an elderberry extract (*Sambucus nigra* L) during an outbreak of influenza B Panama. J Altern Complement Med 1:361
239. Melchoir J, Palm S, Wikman G (1996) Controlled clinical study of standardized *Andrographis paniculata* extract (Kan-Jang tablet) in common cold a pilot trial. Phytomedicine 3:315
240. Caceres DD, Hancke JL, Burgos RA, Sandberg F, Wikman GK (1999) Use of visual analogue scale measurements (VAS) to asses the effectiveness of standardised *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomized double blind placebo study. Phytomedicine 6:217
241. Hancke J, Burgos R, Caceres D, Wikman G (1995) A double-blind study with a new monodrug Kan Jang: decrease of symptoms and improvement in the recovery from common colds. Phytother Res 9:559
242. Josling P (2001) Preventing the common cold with a garlic supplement: a double-blind placebo-controlled survey. Adv Ther 18:189
243. Phillpotts RJ, Wallace J, Tyrrell DA, Freestone DS, Shepherd WM (1983) Failure of oral 4′, 6-dichloroflavan to protect against rhinovirus infection in man. Arch Virol 75:115
244. Tanamly MD, Tadros F, Labeeb S (2004) Randomised double-blinded trial evaluating silymarin for chronic hepatitis C in an Egyptian village: study description and 12-month results. Dig Liver Dis 36:752
245. Gordon A, Hobbs DA, Bowden DS (2006) Effects of *Silybum marianum* on serum hepatitis C virus RNA alanine aminotransferase levels and well-being in patients with chronic hepatitis C. J Gastroenterol Hepatol 21:275
246. Melhem A, Stern M, Shibolet O, Israeli E, Ackerman Z, Pappo O, Hemed N, Rowe M, Ohana H, Zabrecky G, Cohen R, Ilan Y (2005) Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial. J Clin Gastroenterol 39:737
247. Strickland GT, Tanamly MD, Tadros F (2005) Two-year results of a randomised double-blinded trial evaluating silymarin for chronic hepatitis C. Dig Liver Dis 37:542
248. El-Zayadi AR, Attia M, Badran HM (2005) Non-interferon-based therapy: an option for amelioration of necro-inflammation in hepatitis C patients who cannot afford interferon therapy. Liver Int 25:746
249. Koytchev R, Alken RG, Dundarov S (1999) Balm mint extract (Lo-701) for topical treatment of recurring herpes labialis. Phytomedicine 6:225

250. Wobling RH, Leonhardt K (1994) Local therapy of herpes simplex with dried extract from *Melissa officinalis*. Phytomedicine 1:25
251. Syed TA, Cheema KM, Ashfaq AS, Holt AH Jr (1996) *Aloe vera* extracts 0.5% in a hydrophilic cream versus *Aloe vera* gel for the management of genital herpes in males. A placebo-controlled, double-blind, comparative study. J Eur Acad Dermatol Venereol 7:294
252. Syed TA, Afzal M, Ashfaq AS, Holt AH, Ali AS, Ahmad SH (1997) Management of genital herpes in men with 0.5% *Aloe vera* extract in a hydrophilic cream: a placebo-controlled double-blind study. J Dermatol Treat 8:99
253. Jayavasu C, Balachandra K, Sangkitporn S, Maharungraungrat A, Thavatsupa P, Bunjob M, Chavalittumrong P, Dechatiwongse N, Ayudhaya T, Sittisomwong N (1992) Clinical trial in the treatment of genital herpes patients. Commun Dis J 18:152
254. Carson CF, Ashton L, Dry L, Smith DW, Riley TV (2001) *Melaleuca alternifolia* (tea tree) oil gel (6%) for the treatment of recurrent herpes labialis. J Antimicrob Chemother 48:450
255. Saller R, Buechi S, Meyrat R, Schmidhauser C (2001) Combined herbal preparation for topical treatment of Herpes labialis. Forsch Komplementarmed Klass Naturheilkd 8:373
256. Sangkitporn S, Chaiwat S, Balachandra K, Na-Ayudhaya TD, Bunjob M, Jayavasu C (1995) Treatment of herpes zoster with *Clinacanthus nutans* (bi phaya yaw) extract. J Med Assoc Thai 178:624
257. Charuwichitratana S, Wongrattanapasson N, Timpatanapong P, Bunjob M (1996) Herpes zoster: treatment with *Clinacanthus nutans* cream. Int J Dermatol 35:665
258. Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M, Wells K, Wenner CA, Standish LJ (2000) A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytother Res 14:333
259. Durant J, Chantre G, Gonzalez G, Vandermander J, Halfon P, Rousse B, Guedon D, Rahelinirina V, Chamaret S, Montagnier L, Dellamonica P (1998) Efficacy and safety of *Buxus sempervirens* L. Preparation (SPV-30) in HIV infected asymptomatic patients randomized, double-blind, placebo-controlled trial. Phytomedicine 5:1
260. Gotoh Y, Tada K, Yamada K, Minamitani M, Negishi M, Fujimaki M, Ikematsu S, Hada M, Mori K, Ito M, Shigeta S, Nakashima H, Yamamoto N, Shiokawa Y (1987) Administration of glycyrrhizin to patients with human immunodeficiency virus infection. Igaku no Ayumi 140:619
261. Gaspar-Marques C, Simões MF, Valdeira ML, Rodríguez B (2008) Terpenoids and phenolics from *Plectranthus strigosus*, bioactivity screening. Nat Prod Res 22(2):167–177

25 Cocoa and the Immune System and Proliferative Disorders

Francisco J. Pérez-Cano, Àngels Franch, Cristina Castellote, and Margarida Castell

Key Points

- Cocoa is an important source of antioxidant flavonoids such as epicatechin, catechin, and procyanidins.
- Cocoa flavonoids affect innate and acquired immunity and proliferative disorders.
- *In vitro* experiments on innate immunity have focused on the secretion of inflammatory mediators, and the results are controversial.
- *In vivo* studies have demonstrated that cocoa flavonoid administration can ameliorate several models of inflammation.
- Intake of a cocoa diet by rats reduces the proportion of Th cells and increases that of B cells.
- These immunoregulatory actions may be beneficial in reducing certain states of autoimmunity and hypersensitivity.
- Many *in vitro* studies and some pre-clinical evidence have shown that cocoa flavonoids exert biological activities related to antitumoral effects.
- Although data suggest some negative associations between a flavonoid-rich diet and cancer, significant relationships are not always found.
- Further preclinical and clinical trials are needed to investigate the mechanisms involved in cocoa actions and to justify cocoa's usage as a therapy for the prevention and treatment of immune-mediated diseases and cancer.

Key Words: Cocoa, flavonoids, inflammation, lymphocytes, proliferation, cancer.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_25

25.1 INTRODUCTION

Cocoa, the basic component of chocolate, is a product obtained by the fermentation, drying, and roasting of the seeds of the *Theobroma cacao* tree. Roasted nibs without shells are milled to obtain cocoa liquor. Cocoa powder is prepared by removing from the cocoa liquor the majority of the lipid fraction (cocoa butter); whereas, chocolate is made of cocoa liquor, cocoa butter, and sugar (1). Cocoa was already known to ancient civilizations such as the Mayans and Aztecs (2, 3). They prepared a beverage called *xocolatl*, which was reserved for members of the highest social orders only. Cocoa was also used to cure fatigue, fever, infections, and heart pain. By the seventeenth and eighteenth centuries, chocolate and cocoa were appreciated throughout Europe and the Americas both for their taste and as foods capable of treating various disorders (4). At present, there is a revival of the health properties of cocoa.

Cocoa is a rich source of fiber, proteins, carbohydrates, and lipids, as well as a source of minerals (potassium, phosphorus, calcium, magnesium, etc.) (1). In addition, cocoa has become the subject of research in the last few years due to its wealth of antioxidant flavonoids. Polyphenols can reach up to 85 mg/g in dried cocoa beans (5) and up to 70 mg/g in cocoa powder (6); however, polyphenol content depends on factors such as geography, climate, and storage (7). Moreover, manufacturing processes influence flavonoid amounts, and, in some conditions, these can be increased up to four times greater than their content in conventional cocoa powder (5).

Cocoa includes many flavonoids that differ chemically in oxidation levels and in the substitution pattern of the C-ring in the benzo-γ-pirone molecule (Fig. 25.1). Cocoa mainly contains flavan-3-ols as (−)-epicatechin and (+)-catechin and procyanidins or proanthocyanidins, which are polymers derived from these flavanols. The procyanidins present in cocoa are unique because they exist as long polymers, constituted by two, three, or up to ten linked units of catechin or epicatechin (8). Procyanidins are the major cocoa flavonoids, with levels ranging from 2.16 to 100 mg/g; however, whereas monomer content ranges from 0.20 to 3.50 mg/g, epicatechin is more abundant than catechin in most cocoa products (5, 9). Flavonols such as quercetin and isoquercitrin, flavones like luteolin, and flavanones such as naringenin are also present in lower quantities (1). Methylxanthines are also present in cocoa and might be responsible for some of the health effects ascribed to cocoa (10).

Cocoa flavonoid monomers are absorbed in the intestine of animals and humans. In rats, catechin and epicatechin seem to be absorbed competitively in the gastrointestinal tract, and the bioavailability of epicatechin is higher than that of catechin (11). Dimer and trimer procyanidins are also absorbed in the small intestine and rapidly detected in plasma (12–14); whereas larger procyanidins are less efficiently absorbed. However, these polymers are metabolized by the colonic microflora into phenolic acids that are absorbed later (7).

Flavonoids have important effects on plants as antioxidants and defenses against infection, among other functions (15); hence, the intake of flavonoid-enriched foods can exert healthy effects on animals and humans. In the last few years, the health effects of cocoa intake have been ascribed to its antioxidant qualities. However, other studies have pointed out the beneficial actions of cocoa on circulation, blood pressure, and platelet activation (4, 16–19). Moreover, cocoa intake has been shown to improve some

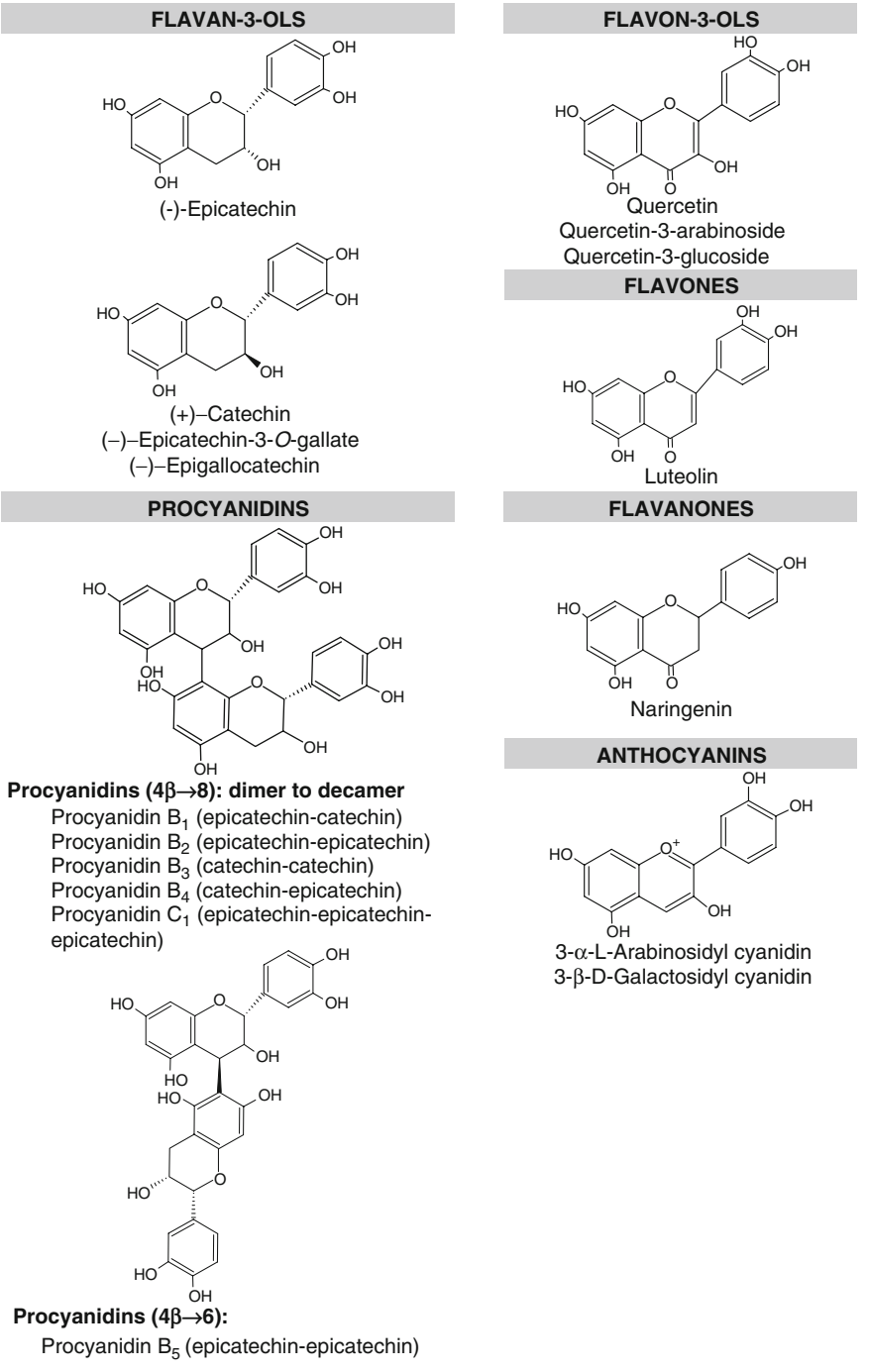


Fig. 25.1. Main flavonoids in cocoa: subclasses and structure (1, 15).

functions in the brain, such as promoting cerebral blood flow and attenuating neuron loss (20). This chapter focuses only on the effects of cocoa on the immune system and on proliferative disorders.

25.2 EFFECTS OF COCOA ON THE IMMUNE SYSTEM

Until now, most of the studies referred to regarding the effects of cocoa on the immune system are *in vitro* experiments, and a great number of these are centered on the actions of single cocoa flavonoids. However, some *in vivo* studies are emerging. Herein, the studies of cocoa's effects on the immune system are compiled and classified under both innate and acquired immunity.

25.2.1 Effects of Cocoa on Innate Immunity and Inflammatory Response

One of the first defenses of our body is the surface epithelia, which provide a physical barrier between the internal milieu and the external world of pathogens (21). If this defense is overcome, macrophages constitute one of the most important cellular types involved in innate immunity. The activation of macrophages involves an oxidant burst causing reactive oxygen species (ROS) and nitric oxide (NO) production and the release of a myriad of inflammatory mediators such chemokines like interleukin (IL)-8, macrophage inflammatory proteins (MIP) 1α and 1β, and cytokines such as tumor necrosis factor (TNF) α, IL-1 and IL-6. Another arm of innate immunity is constituted by certain lymphocytes such as natural killer (NK) cells and T cells with the receptor TCR γδ. NK cells are a subpopulation of lymphocytes that spontaneously recognize and kill cells infected by viruses and tumor cells. And γδ T cells represent a minor subset in most lymphoid compartments, preferentially in those associated with the intestinal epithelium. Due to their cytotoxic characteristics, γδ T cells are more similar to innate immune effectors than to adaptative immune effectors (22).

25.2.1.1 *In Vitro* Effects of Cocoa on Cells Involved in Innate Immunity

Many of the *in vitro* studies of cocoa flavonoids have focused on the secretion of inflammatory mediators by macrophages, and the results are controversial (Table 25.1). It has been reported that the addition of monomers or polymeric fractions of cocoa flavanols to the culture of peripheral blood mononuclear cells (PBMC) activated with lipopolysaccharide (LPS) or phytohemagglutinin (PHA) increases the secretion of TNFα, IL-1β, and IL-6 (23–25). However, an entire cocoa flavonoid-enriched extract and the epicatechin and isoquercitrin monomers decrease TNFα production and the secretion of the monocyte chemoattractant protein (MCP)-1 from LPS-stimulated macrophages (26). Moreover, the addition of a flavonoid-rich cocoa extract decreases the secretion of NO from LPS-stimulated macrophages (26, 27). Other studies show that PBMC treated *in vitro* with cocoa liquor or epicatechin decreases the production of ROS in a dose-dependent manner (28). Moreover, in stimulated macrophages, quercetin and naringenin – minor cocoa flavonoids – are able to reduce NO production (29), and quercetin also decreases the expression of TNFα and IL-1β (30). Similarly, luteolin, another minor cocoa flavonoid, has shown anti-inflammatory properties *in vitro*, decreasing the production of NO and prostaglandin E_2 (PGE_2), as well as the expression of inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), TNFα, and IL-6 from mouse macrophages (31).

Besides the *in vitro* results described above, which are focused on the effects of cocoa flavonoids added to cell culture, similar effects have been reported in the behavior of peritoneal macrophages obtained from rats fed cocoa-enriched diets. This *ex vivo*

Table 25.1
Effects of cocoa flavonoids or cocoa extract on *in vitro* innate immune response

Flavonoids treatment	*Cell target (stimulus)*	*Effect*	*References*
Monomer–dimer procyanidins	Human PBMC (PHA)	~TNFα (protein)	(23)
Tetramer–decamer procyanidins		↑ TNFα (protein)	
Monomer–pentamer procyanidins	Human PBMC (LPS)	↑ TNFα (protein) ↑ IL-1β (protein) ↑ IL-6 (protein)	(24)
Hexamer-decamer procyanidins		↑ TNFα (protein) ↑ IL-1β (protein) ~IL-6 (protein)	
Catechin Epicatechin	Rabbit PBMC (LPS)	↑ TNFα (protein)	(25)
Dimer procyanidins		~TNFα (protein)	
Cocoa extract Epicatechin Isoquercitrin	Rat NR8383 macrophage line (LPS) Murine RAW 264.7 macrophage line (LPS)	↓ TNFα (protein and RNA) ↓ MCP-1 (protein) ↓ IL-1α (RNA) ↓ IL-6 (RNA) ↓ NO	(26)
Cocoa extract	Murine J774.1 macrophage line (LPS + IFNγ)	↓ NO	(27)
Cacao liquor polyphenolic fraction Epicatechin	Human granulocytes and lymphocytes (PMA)	↓ ROS	(28)
Quercetin Naringenin	Murine J774.1 macrophage line (LPS)	↓ NO ↓ iNOS (protein and RNA)	(29)
Quercetin	Bone marrow derived macrophages (LPS)	↓ TNFα (protein) ↓ IL-1β (protein) ↓ iNOS (protein)	(30)
Quercitrin		~TNFα (protein) ~IL-1β (protein) ~iNOS (protein)	
Luteolin	Murine alveolar macrophage MH-S (LPS) Murine RAW 264.7 macrophage line (LPS)	↓ TNFα (protein and RNA) ↓ IL-6 (protein and RNA) ↓ iNOS (protein and RNA) ↓ COX (protein and RNA) ↓ NO ↓ PGE_2	(31)

(continued)

Table 25.1
(continued)

Flavonoids treatment	*Cell target (stimulus)*	*Effect*	*References*
In vivo cocoa administration (p.o.)	Peritoneal macrophages (*ex vivo*)	↓ TNFα (protein) ↓ IL-6 (protein) ↓ NO ↓ ROS	(32, 33)

COX cyclooxygenase; *IL* interleukin; *iNOS* inducible nitric oxide synthase; *LPS* lipopolysaccharide; *MCP* monocyte chemoattractant protein; *NO* nitric oxide; *PBMC* peripheral blood mononuclear cells; *PGE2* prostaglandin E_2; *PHA* phytohemagglutinin; *PMA* phorbol myristate acetate; *p.o.* per os (oral route); *ROS* reactive oxygen species; *TNF* tumor necrosis factor

approach is closer to the physiological effects of cocoa flavonoids since cells receive only the flavonoids absorbed by the intestine. Peritoneal macrophages LPS-stimulated from rats fed cocoa produce lower amounts of TNFα, IL-6, NO, and ROS than those obtained from animals fed standard diets (32, 33).

25.2.1.2 *In Vivo* Effects of Cocoa on Innate Immunity and Inflammatory Response

There are no studies focused on the *in vivo* effects of cocoa on the first barriers of the immune system. However, colonic metabolites of tea polyphenols inhibit the growth of pathogenic bacteria in the intestine (34). These results should promote the research of cocoa flavonoids' influence on microbiota and the intestinal barrier.

Considering the inflammatory response, some studies carried out in animals show the effects of cocoa flavonoid administration in several models of inflammatory response (Table 25.2). It has, in fact, been reported that two subcutaneous (s.c.) injections of catechin and epicatechin produce a significant reduction of carrageenin-induced paw edema in rats (35). Similarly, quercetin inhibits carrageenin-induced paw edema (36) as well as cotton pellet-induced granuloma, adjuvant-carrageenin-induced arthritis (36), and air pouch (37) models in rats. In addition, oral (p.o.) administration of rutin or quercetin (both quercetin glycosides), ameliorates two models of colitis in rats (30, 38). More interestingly, p.o. administration of a cocoa polyphenolic fraction to mice can inhibit ear edema in a dose-dependent manner (39), and, similarly, the p.o. administration of cocoa over a week decreases carrageenin-induced hind-paw inflammation in rats (33, 40).

These results agree with those obtained from other natural products rich in similar flavonoids, such as tea. A green tea extract with a 75% content of polyphenols, some of them also present in cocoa, ameliorates an experimental colitis when administered i.p. to rats (41). In addition, i.p. administration of epigallocatechin-3-gallate ameliorates experimental arthritis in mice and rats (42, 43). In a similar way, oligomeric procyanidins from Jatoba (either p.o. or i.p.) reduce the severity of collagen-induced arthritis in mice (44), and the flavonoids of *Litsea coreana* inhibit adjuvant-induced arthritis in rats (45).

Finally, in terms of innate immune responses, there are very few studies, particularly regarding NK cells and γδ T lymphocytes. The effects of cocoa on NK cell activity have not been studied yet, but there are promising results with flavonoids from other origins. For example, the administration of the hydroxyflavone chrysin increases NK cell activity in a model of abdominal surgery (46). Concerning γδ T lymphocytes, a high cocoa intake

Table 25.2
Effects of cocoa flavonoids, extract or powder on *in vivo* innate immune response

Treatment (doses and route)	*Experimental model*	*Effects*	*References*
Catechin (2 × 200 mg/kg, s.c.) Epicatechin (2 × 100 mg/kg, s.c.)	Paw edema induced by carrageenin in rats	↓ Paw edema	(35)
Quercetin			(36)
(1 × 75 mg/kg, i.p.)	Paw edema induced by carrageenin in mice	↓ Paw edema	
(1 × 0.25 M/kg, i.p.)	Xylene-induced ear edema in mice	~Ear edema	
(6 × 25 mg/kg/day, s.c.)	Cotton pellet granuloma in rats	↓ Granuloma	
(21 × 75 mg/kg, i.p.)	Adjuvant-carrageenan-induced arthritis in rats	↓ Paw edema	
Quercetin (1 × 10–100 mg/kg, local) Isoquercitrin (1 × 10 mM, local) Rutin (1 × 10 mM, local)	Air pouch in rats	↓ Inflammation ↓ Content of PGE_2, TNF-α, RANTES, MIP-2, COX-2	(37)
Quercetin (6 × 10–100 μM/300 μL/day, i.r.) Rutin (6 × 10 mg/kg/day, p.o.)	TNBS-colitis in rats	↓ Inflammation	(38)
Quercetin (10 × 1 mg/kg/day, p.o.) Quercitrin (10 × 1 mg/kg/day, p.o.)	DSS-colitis in rats	↓ Inflammation ↓ Colonic content of TNF-α, IL-1β, iNOS	(30)
Cocoa extract (p.o.) (1 × 4–200 mg, p.o.)	Ear edema in mice	↓ Ear edema	(39)
Cocoa powder (7 × 2.4–4.8 g/kg/day, p.o.)	Paw edema induced by carrageenin in rats	↓ Paw edema	(33)
Cocoa powder (7 × 4.8–9.6 g/kg/day, p.o.)	Paw edema induced by carrageenin in rats	↓ Paw edema	(40)
Cocoa powder (10% food intake)	Healthy young rats	↑ γδ T cell proportion in Peyer's patches and mesenteric lymph nodes	(47)

DSS dextran sulfate sodium; *i.p.* intraperitoneal; *i.r.* intrarectal; *i.v.* intravenous; *p.o.* per os (oral route); *s.c.* subcutaneous; *TNBS* trinitrobenzene sulfonic acid

by young rats increased the proportion of these cells both in Peyer's patches (PP) and in mesenteric lymph nodes (MLN) (47). These findings are also described after the intake of apple polyphenols by healthy mice and rats (48, 49). It is also worth remarking on the role of intestinal γδ T lymphocytes in oral tolerance, mucosal tissue repair, and immunity against viral antigens and tumor cells (50). In murine food-allergy models, apple polyphenols prevented the development of oral sensitization, and this inhibition correlated with a rise in intestinal γδ T-cell populations (48). In addition, polyphenols from green tea are able to increase the expression of CD11b (molecule involved in leukocyte adhesion and migration) on γδ T cells, which likely enhances γδ T-cell migration and function at sites of inflammation (51). These results suggest that diets rich in flavonoids from cocoa or other sources may be capable of increasing γδ T-cell functionality.

All these promising results on inflammation and innate immune response should lead to further investigations for elucidating the mechanism behind cocoa actions and encourage clinical trials. There are two human studies on the relation of cocoa and innate immune responses, both of which have controversial results. For instance, whereas supplementation with cocoa products in one group of healthy humans did not affect inflammation markers (52), regular intake of dark chocolate by a healthy population in southern Italy was inversely related to serum C-reactive protein (CRP) concentration (53).

25.2.2 Effects of Cocoa on Acquired Immune Response

Lymphocytes orchestrate the acquired immune response. The influence of diet on this arm of immunity also has been studied both *in vitro*, on cultured lymphoid cells or PBMC, and *in vivo*, by determining lymphoid tissue composition and function.

25.2.2.1 *In Vitro* Effects of Cocoa on Lymphoid Cells

Some *in vitro* models allow the study of the activation of T or B lymphocytes by determining proliferative responses, the expression of certain surface molecules, or the secretion of cytokines (Table 25.3). Stimulated T lymphocytes proliferate through the enhancement of the IL-2/IL-2 receptor (IL-2R) system (54). In this context, epicatechin, procyanidins, and a cocoa extract are able to reduce lymphocyte proliferation, which is demonstrated to be related to a decrease of IL-2 secretion and/or IL-2R surface expression (28, 55–57). Moreover, quercetin also diminishes the surface expression of IL-2R (58) and inhibits lymphocyte activation and proliferation without inducing apoptosis (59). Overall, the *in vitro* effects of cocoa flavonoids on lymphocyte proliferation may comprise controlling T-cell stimulation by means of downregulating IL-2 production.

Activated Th lymphocytes can result in Th1 or Th2 subsets, effector cells with different functions and cytokine secretion profiles (60). The Th1 subset mainly secretes interferon γ (IFNγ) and IL-2, responsible for the promotion of both T-cell-mediated immunity and phagocytosis, thus enhancing actions against intracellular pathogens. The Th2 subset is characterized by the production of IL-4 and IL-5, which are powerful activators of IgE and IgA production and eosinophil recruitment, which then motivate antibody-mediated responses against parasites (60). It has been reported that quercetin (58) and a cocoa extract (61) decrease the *in vitro* production of IFNγ from stimulated lymphocytes, thus downregulating Th1 responses. On the contrary, the cocoa extract and, in a stronger way, epicatechin increase IL-4 secretion from stimulated lymphocytes (56). However, the effect of

cocoa flavonoids on the secretion of Th2-related cytokines seems to be related to the degree of procyanidin polymerization, because long-chain procyanidins reduce the levels of both IL-4 and IL-5 (57, 62). In addition, certain procyanidins can induce the secretion of IL-10 from stimulated human, peripheral blood Th lymphocytes (24). IL-10, a cytokine also related to the Th2-subset, has anti-inflammatory properties inhibiting macrophage activation and exerting regulatory functions (63). Taking together all these results, it can be postulated that, *in vitro*, cocoa flavonoids favor Th2 responses (IL-4, IL-10) but decrease

Table 25.3
Effects of cocoa flavonoids or cocoa extract on *in vitro* acquired immune response

Flavonoids treatment	*Cell target (stimulus)*	*Effect*	*References*
Cacao liquor polyphenolic fraction	Human PBMC (PHA, PWM)	↓ Lymphocyte proliferation ↓ IL-2 (protein and RNA) ↓ Serum IgG	(28)
Pentamer, hexamer, heptamer procyanidins	Human PBMC (PHA)	↓ IL-2 (protein and RNA)	(55)
Cocoa extract epicatechin	Murine EL4.BU.OU6 lymphoid line (PMA)	↓ IL-2R ↓ IL-2 (protein) ↑ IL-4 (protein)	(56)
Monomer-tetramer procyanidins	Human PBMC (PHA)	~IL-2 (RNA) ~IL-4 (protein and RNA)	(57)
Pentamer-heptamer procyanidins hexamer-octamer procyanidins		↓ IL-2 (RNA) ↓ IL-4 (protein and RNA)	
Quercetin	Human PBMC (SEB + anti-CD28)	↓ Lymphocyte proliferation	(59)
Quercetin	Isolated Th cells from mice (anti-CD3 + anti-CD28)	↓ IL-2R ↓ IL-2 (protein and RNA) ↓ IFN-γ (protein and RNA)	(58)
Rutin		~IL-4 (protein and RNA) IL-2: ~protein; ↑ RNAγ IFN-γ: ~protein; ↑ RNA ↑ IL-4 (RNA)	
Cocoa extract	Human PBMC (PHA)	↓ IFN-γ (protein)	(61)
Monomer-hexamer and octamer-decamer procyanidins	Human PBMC (PHA)	~IL-5 (protein)	(62)
Heptamer procyanidins		↓ IL-5 (protein)	
Procyanidins	Human PBMC (LPS)	↑ IL-10 (protein)	(24)

IL interleukin; *IFN* interferon; *LPS* lipopolysaccharide; *PBMC* peripheral blood mononuclear cells; *PHA* phytohemagglutinin; *PMA* phorbol myristate acetate; *PWM* pokeweed mitogen; *SEB* staphylococcal enterotoxin B

the proliferation and Th1 responses (IL-2, IFNγ). The mechanism by which cocoa exerts these opposite effects on Th1/Th2 cytokines remains to be established, but it is likely that a reciprocal inhibition between Th1/Th2 cytokines may be involved. In addition, cocoa flavonoids modulate other regulatory cytokines, such as transforming growth factor β (TGFβ). In this sense, cocoa procyanidins enhance the secretion of TGFβ from non-stimulated immune cells but reduce the release of this cytokine from stimulated cells (64).

In spite of these results obtained *in vitro*, it must be noted that, due to metabolic factors, the cocoa compounds that reach immune organs *in vivo* are not identical to those used under culture conditions. Moreover, their actions on the lymphoid cells are submitted to numerous interactions and regulatory conditions present in the immune system. Therefore, it is necessary to perform *in vivo* approaches.

25.2.2.2 *In Vivo* Effects of Cocoa on Acquired Immunity

Although multiple studies refer to the effects of cocoa on immune cells, few go beyond the *in vivo* cocoa influence, considering lymphoid organs and immune cell functionality (Table 25.4).

In the thymus, where T-lymphocyte maturation occurs, changes in lymphocyte composition induced by a cocoa-enriched diet have been described (65). Long-term cocoa intake promotes the progress of immature thymocytes (CD4 + C8+ or CD4-CD8-cells with TCRαβ^{low} expression) toward more mature T-cell stages (specifically CD4 + CD8-cells with TCRαβ^{high}) (65). Moreover, the cocoa diet increases the proportion of CD4-CD8-cells in the thymus (65). Since this double-negative subset has multi-lineage potential (as B, T, myeloid, NK and dendritic cells) (66), cocoa intake may promote their differentiation.

The influence of cocoa intake on T cells is not only constrained to the thymus, but it also affects other lymphoid organs. For instance, in the spleen, a continuous intake of a high cocoa diet by young rats increases the proportion of B cells while reducing that of Th cells (32). These changes, however, do not positively affect the secretion of antibodies since, in these rats, the blood concentration of IgG, IgM, and IgA decreases (32). Moreover, the cocoa diet can exert a direct effect on the composition of gut-associated lymphoid tissue (GALT), which includes organized lymphoid structures such as PP and MLN (67). In both GALT compartments, the continuous intake of a rich cocoa diet produces changes in lymphocyte composition in young rats (47). Cocoa intake reduces the proportion of TCRαβ + T cells (mainly Th subset) by increasing the B-cell percentage in both PP and MLN (47), similar to what occurs in the spleen (32). Likewise, the concentration of IgA and IgM secreted to the intestinal lumen decreases in rats (47). Therefore, the high and continuous intake of a cocoa diet globally produces a relative reduction of Th cells in secondary lymphoid tissues as well as decreasing antibody synthesis, which may be a consequence of the attenuation of T-helper functions. It remains to be clarified which mechanisms are involved, but a possible explanation may be the influence of cocoa on lymphocyte proliferation. As reported above, *in vitro* studies show an inhibitory effect of cocoa on Th activation (28, 56). However, the study of proliferative response and IL-2 secretion by the spleen and MLN from rats fed high doses of cocoa does not reveal any reduction. Nevertheless, and interestingly, there is a decrease of IL-4 secretion in these tissues (32, 47), suggesting that the intake of high doses of cocoa by young rats can down-regulate antibody synthesis and Th2 responses. These results agree with those obtained after administering quercetin in a murine model of asthma: this treatment reduces the increased levels of IL-4 and IL-5 and produces a

significant inhibition of all asthmatic reactions (68). Similarly, the administration of quercitrin and a flavonoid extract of *Kalanchoe pinnata* protects mice from anaphylactic shock and death and reduces the production of specific IgE antibodies, eosinophilia, and the synthesis of IL-5 and IL-10 (69).

Table 25.4
Effects of cocoa flavonoids, extract, or powder on *in vivo* acquired immune response

Treatment (doses and route)	*Experimental model*	*Effects*	*References*
Cocoa powder (10% food intake)	Thymus of healthy young rats	↓ Proportion of CD4 + CD8+ TCRαβ^{low} cells ↓ Proportion of CD4-CD8-TCRα–β^{low} cells ↑ Proportion of CD4 + CD8-TCRαβ^{high} cells ↑ Proportion of CD4-CD8-TCRαβ-cells	(65)
Cocoa powder (10% food intake)	Spleen of healthy young rats	↑ Proportion of B cells ↓ Proportion of Th cells ↓ Serum IgG, IgM and IgA ~Spleen ability to secrete Ig ↑ Proliferative response ~IL-2 secretion ~IFNγ secretion ↓ IL-4 secretion	(32)
Cocoa powder (10% food intake)	GALT (PP and MLN) of healthy young rats	↑ B cell proportion ↓ TCRαβ (Th) cell proportion ↓ Gut IgAs and IgMs ~Proliferative response ↑ IL-2 secretion ~IFNγ secretion ↓ IL-4 secretion ~IL-10 secretion	(47)
Quercetin (3 × 8–16 mg/kg/day, i.p.)	Asthma induced in mice	↓ Asthmatic reaction ~IFNγ secretion ↓ IL-4 secretion ↓ IL-5 secretion	(68)
Cocoa powder (4–10% food intake)	OVA-immunized rats	↓ Serum anti-OVA IgM, IgG1, IgG2a, IgG2c ↑ Serum anti-OVA IgG2b ↓ Anti-OVA IgG-secreting cells ~OVA-induced proliferative response ↑ IFNγ secretion ↓ IL-4 secretion	(70)

GALT gut-associated lymphoid tissue; *IFN* interferon; *i.p.* intraperitoneal; *MLN* mesenteric lymph nodes; *OVA* ovalbumin; *p.o. PP* Peyer's patches; *TCR* T cell receptor

Recently, researchers have investigated the specific immune response developed in adult rats fed two long-lasting cocoa-diets (70). Serum concentrations of IgM, IgG1, IgG2a, IgG2b, and IgG2c antibodies specific to ovalbumin (OVA) have been determined after 4 weeks of immunization. It is noteworthy that IgG isotypes can be associated with Th1 or Th2 immune responses. In rats, IgG1 and IgG2a are associated with the Th2 response, while IgG2b depends on the Th1 response (71–74). Cocoa diets decrease the serum anti-OVA IgM, IgG1, IgG2a, and IgG2c antibodies, but interestingly, anti-OVA IgG2b does not decrease but rather increases with a cocoa diet (70). Moreover, the number of cells that secrete anti-OVA IgG antibodies diminishes in lymph nodes, while the secretion of IL-4 and IFNγ decreases and increases, respectively, although the proliferative response does not change (70). Consequently, a cocoa diet seems to downregulate the Th2 antibody response, whereas it can maintain Th1 antibody production. These results agree with those obtained in mice fed the isoflavone genistein (75) or apple polyphenols (48) or treated with flavonoid glycosides of *Sedum sarmentosum* (76). Similarly, a diet including two flavones (apigenin and chrysin) causes a decrease in the serum IgE (77). Taking together all these results, it can be concluded that dietary flavonoids can attenuate the synthesis of Th2-related antibodies. The mechanism responsible for this action remains to be established. Some studies concerning the effect of cocoa diets on cytokine expression indicate that this diet produces a certain imbalance in the Th1/Th2 ratio in favor of Th1 (32, 70). These results are similar to those obtained with other flavonoids such as apigenin, chrysin, centaurein, and centaureidin (77, 78). This imbalance would involve, among other effects, a decrease in the number of antibody-secreting cells, as it has been found in rats after ingesting a long-term cocoa diet (70). On the other hand, apigenin suppresses the synthesis of pathogenic autoantibodies in a mouse model of lupus, which is attributed to an inhibition of autoreactive Th1, Th17, and B cells (79). However, the administration of some flavonoids in a mouse model of disseminated candidiasis promotes Th1 responses (IFNγ and IL-2) and thus protects the mice against infection (80). All these results may suggest that cocoa-enriched diets followed over a long period of time can produce an attenuating effect on the Th2 response.

25.3 ANTIPROLIFERATIVE EFFECTS OF COCOA

To date, many studies performed *in vitro* or in experimental models have shown that flavonoids exert a wide range of biological activities related to antitumoral effects. These activities include the inhibition of several kinases and transcription factors, among other effects. Therefore, flavonoids seem to exert antiproliferative actions, apoptosis induction, and angiogenesis inhibition. However, few reports have analyzed the antiproliferative effects induced by whole cocoa powder. Some flavonoids present in cocoa as monomers or procyanidins have been studied *in vitro* and are likely to be effective as anticancer agents (81–84). However, a lack of literature occurs regarding animal research, cocoa administration, and cancer incidence. There are only some approaches associating flavonoid intake (including cocoa as a source) and the incidence of different types of cancer (85). Finally, it is summarized that the main preventive and therapeutic effects of cocoa and its major flavonoids on various pathways and molecular targets as well as evidence in animals and humans allow us to consider cocoa as an antiproliferative agent (Fig. 25.2).

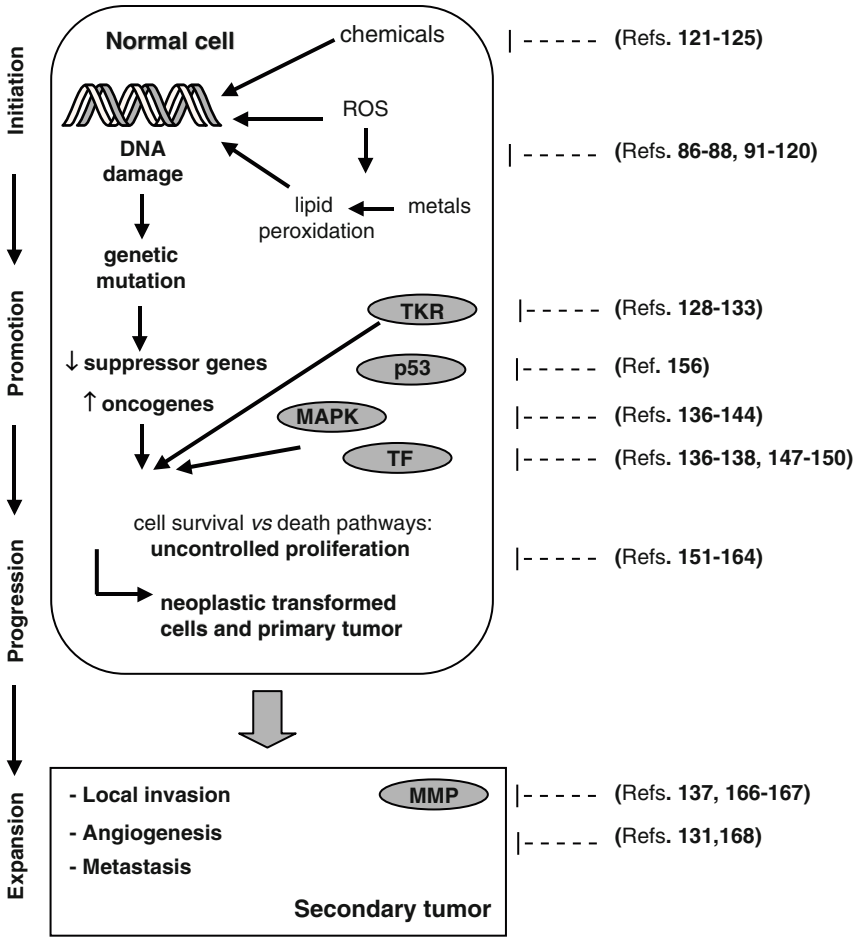


Fig. 25.2. Simplified diagram of the events during carcinogenesis and dissemination processes in which cocoa or its flavonoids have shown modulatory effects. First action takes place in the induction step, controlling DNA mutation. Later, promotion and progression are counteracted by cocoa action on some oncogenes and suppressor genes. Finally, the formation of secondary tumors may be prevented by controlling the expansion of the primary tumor formed in the neoplastic transformation. *MAPK* mitogen activated protein kinases; *MMP* matrix metalloproteinases; *ROS* reactive oxygen species; *TF* transcription factors; *TKR* tyrosine kinase receptors.

25.3.1 Antioxidant, Free-Radical Scavenging and Metal Ion Chelating Activities of Cocoa

Cancer includes a large number of diseases with distinct etiological factors that act as genotoxic agents. The oxidation of DNA by oxidant chemicals or ROS plays a role in mutagenesis and carcinogenesis. In contrast, the antioxidant effects of cocoa flavonoids delineate their putative beneficial action in controlling cell damage and tumor progression.

The cocoa procyanidins, epicatechin, and catechin, have an important antioxidant power (86–88). It has been estimated that a serving of dark chocolate or a cup of cocoa provides a high antioxidant activity greater than that of jasmine, black tea, or blueberries

(6, 9, 89, 90). The antioxidant activity of cocoa could be explained partially by a direct neutralizing action on free radicals. Epicatechin, catechin, and procyanidins scavenge oxidant radicals (39, 91–94), while cocoa flavonoids chelate metals and inhibit enzymes involved in ROS production (39, 95). Cocoa flavonoids also enhance other antioxidant compounds. Thus, epicatechin regenerates α-tocopherol from its corresponding radical (96), and quercetin increases glutathione concentration and the gene expression of superoxide dismutase (SOD) (97). The antioxidant effect of cocoa flavonoids has been determined *in vitro* in an oxidant-induced erythrocyte haemolysis model (98, 99) and in a lymphocyte cell line (100). Epicatechin also reduces the oxidative stress in HepG2 cells (101, 102), and procyanidins protect intestinal Caco-2 cells from integrity loss induced by oxidative stress (103). The cocoa polyphenolic extract inhibits ROS formation and xanthine oxidase activity in stimulated myelocytic leukemia HL-60 cells (39) besides enhancing the activity of antioxidant enzymes in human HepG2 cells treated with tert-butylhydroperoxide (104). Recently, it has been shown that cocoa procyanidins decrease ROS production in rat pheochromocytoma cells (105), and cocoa extract and epicatechin reduce ROS production in a neuronal cell line exposed to oxidative stress (106).

Knowledge of the *in vivo* antioxidant effects of cocoa is rather limited. Some experimental studies have evidenced that cocoa intake increases total antioxidant capacity and decreases lipid oxidation products in plasma or serum (12, 107–109). Studies in rats have shown that a cocoa-enriched diet increases the antioxidant capacity of lymphoid tissues, affecting the thymus more than the spleen (65). Moreover, cocoa intake in rats promotes antioxidant defense by boosting catalase and SOD activities in the thymus (65).

Cocoa intake also improves the *in vivo* antioxidant defense under stress situations. A long-term cocoa-fiber diet reduces lipid peroxidation in the serum and liver of hypercholesterolemic rats (110). Moreover, cocoa intake reduces plasma 8-isoprostane in obese diabetic rats (111), and the intake of cocoa polyphenols increases the resistance of LDL to oxidation in rabbits (112). Although less is known about these effects in humans, similar results regarding LDL oxidation have been found (113–115), and in one study, a two-week consumption of chocolate with a high flavanol content counteracted oxidative stress in soccer players (116).

Some *in vivo* approaches have associated the antistress oxidative action of cocoa with cancer modulation. Thus, the daily administration of 400 mg/kg of cocoa to rats reduces liver injury, hepatic necrosis, steatosis, and inflammation induced by oxidative stress (117). More recently, the intake of a cocoa-rich diet has shown a partial attenuation of liver injury induced with *N*-nitrosodiethylamine in rats by the induction of antioxidant defenses and the modulation of signals related to cell death (118). In addition, the protective effect of cocoa *in vivo* also has been described in rodent models of colorectal and lung cancer (119, 120).

25.3.2 Effects of Cocoa on Metabolizing Enzymes

The body has a complex and extensive system of metabolizing enzymes in charge of converting lipophilic compounds – which may be xenobiotics – to more hydrophilic compounds that are more easily excreted. The objective of metabolism is the deactivation of these chemicals, and therefore detoxification; but this action sometimes can result in products that are more active.

It has been posited that flavonoids may affect phase I and II of drug-metabolizing enzymes. Flavonoids are able to produce both the inhibition of phase I enzymes and the activity increase of phase II enzymes. Thus, flavonoids can, respectively, decrease the metabolic activation and increase detoxification of pro-carcinogens in both animals and humans (121, 122).

In this regard, the antimutagenic properties of cocoa-liquor polyphenols have been described. Studies performed by Yamagishi and coworkers have shown that the p.o. administration of cocoa powder or cocoa liquor diminishes the mutagenic action of heterocyclic amines in rats (123, 124). They also described the positive effect of cocoa procyanidins on mammary and pancreatic tumorigenesis in female Sprague–Dawley rats (125) and on lung carcinogenesis in male F344 rats (126).

25.3.3 Inhibition of Growth-Factor Activities by Cocoa

The overexpression and mutation of some tyrosine kinase receptors (TKRs) can induce key signals for tumor growth and metastasis, such as cellular survival, proliferation, and migration. Some of these TKRs are specific for growth factors like epidermal growth factor (EGF), insulin growth factor (IGF), platelet-derived growth factor (PDGF), vascular-endothelial growth factor (VEGF), fibroblast growth factor (FGF), and c-Kit or c-Met. Research has shown that flavonoids are able to inhibit TKRs by acting as a mimetic in their ATP-binding sites and therefore conferring antiproliferative effects (127).

Among the flavonoids present in cocoa with TKR signaling inhibition ability, the minor compounds quercetin and luteolin are the most studied. Quercetin inhibits the EGF-dependent growth in breast cancer (MCF-7), hepatocarcinoma (HepG2), epidermoid carcinoma (A431), and colon tumor (WiDr) cell lines by decreasing EGF-receptor phosphorilation (128, 129). Quercetin also blocks the EGF-stimulated growth and migration activities of pancreatic carcinoma cells (MiaPaCA-2), leading to cell apoptosis (129–131). This flavonoid also reduces the growth of a mouse fibroblastic cell line (NIH3T3) that expresses surface receptors of PDGF and FGF (128). Similarly, luteolin suppresses the growth of an epidermoid carcinoma (A431) cell line, probably by inhibiting the EGF-receptor signaling pathway (129, 130).

On the other hand, although epicatechin has not shown any inhibitory effect on some TKR pathways, epicatechin-3-O-gallate partially reduces the phosphorilation of some intracellular kinases on a breast cancer cell line (MCF-104) (132). In addition, in endothelial cells (HAEC), cocoa procyanidins decrease the expression of a human EGF-receptor, the erythroblastic leukemia viral oncogene homolog 2 (ErbB2), which is a TKR that usually mutates in patients with breast and ovarian cancer (133).

25.3.4 Modulation of MAPK Pathways by Cocoa

The family of mitogen-activated protein kinases (MAPK) is involved in conserved signal transduction pathways activated by extracellular stimuli. MAPK activation leads to cell proliferation and differentiation by controlling the activities of downstream transcription factors (134). Modulation of their pathways by cocoa flavonoids has been reported (135).

The extract of cocoa, procyanidin and procyanidin B2 bind and inhibit the activity of the mitogen-activated kinase–kinase (MEK) – 1 in a mouse epidermal cell line and in

vascular smooth-muscle cells (136, 137). MEK-1 phosphorylates the extracellular signal-regulated protein-kinase (ERK), which promotes cell survival through antiapoptotic signaling pathways (138). After activation, ERK levels decline; therefore, there is an enhancement of cell apoptosis and death (139). Some studies have reported variability in the effects of cocoa on ERK, but overall, either cocoa intake or the *in vitro* addition of cocoa procyanidin B2 modulates ERK activity (138–141). On the other hand, a cocoa polyphenolic extract on Caco-2 cells reduces the expression of MAPK-kinase-1 (MAPKK1), kinase that also phosphorilates ERK (142). In addition, both the flavanols and procyanidins added separately to human dermal microvascular endothelial cells have shown an inhibitory activity on MAPKK1 gene expression (133).

Otherwise, c-jun N-amino terminal kinase (JNK) and p38MAPK, members of the MAPK superfamily, are involved in differentiation, inflammatory responses, and cell death (138). These kinases are down-modulated both *in vitro* and *in vivo* by cocoa extract and by pure fractions but not by epicatechin alone (101, 105, 106, 138). Finally, the pathway of protein kinase b (AKT) regulates the activity of numerous molecular targets and is activated in various types of cancer (143, 144). Epicatechin and cocoa polyphenolic extract activate this survival pathway on HepG2 cell lines (139, 141), but a cocoa diet seemed to prevent this activation in an experimental rat model of hepatotoxic injury (118).

25.3.5 Influence of Cocoa on Transcription Factors

Nuclear factor-κB (NF-κB) is a transcription factor (TF) involved in inflammation, cell proliferation, and oncogenic processes (145). Activator protein-1 (AP-1) is a heterodimeric protein complex involved as a TF in apoptosis and cancer development, among other biological processes (146). Both TFs are modulated by MAPK pathways, and as some cocoa flavonoids modulate these pathways, they can also affect the down-stream consequences of these pathways. Nevertheless, flavonoids could directly modulate these TFs (147).

In vitro, epicatechin, catechin, and quercetin, as well as the extract of cocoa procyanidin and B-type dimeric procyanidins, down-modulate NF-κB and AP-1 in different cancer cell lines (136, 138, 148–150). The effect on NF-κB seems to be mediated at early stages by decreasing the activation of IκB-kinase (IKK), consequently diminishing the concentration of phosphorilated IkBα (150). Moreover, in the late pathway of NF-κB activation, flavonoids may inhibit NF-κB binding to its DNA consensus sequence (147, 148). On the other hand, regarding AP-1 activation, only little effect is produced by catechin, epicatechin, or B1 and B2 procyanidins in a human cell line of colon carcinoma (HT-29) (146).

The expression of other TFs, such as the signal transducer and activator of transcription (STAT) 1, is downregulated by cocoa and epicatechin in Caco-2 cells as shown by cDNA arrays (142). However, procyanidin B2 fails to inhibit the STAT3 transcription factor, which also is altered in some cancers (148).

25.3.6 Antiproliferative Activity of Cocoa

Cocoa flavonoids have also demonstrated antiproliferative activity in several tumor cell lines, probably due to the influence on some of the above-described molecular targets.

One study shows that catechin regulates the cell growth of human colorectal cancer cells (HCT-116) through a novel mechanism of post-transcriptional suppression (151). However, other studies are controversial and show that epicatechin and catechin appear to be ineffective in controlling the proliferation of other cancer cell lines, such as mouse myeloma cells (Sp2/O-Ag14). This lack of effect may be due to the low hydrophobicity of these flavonoids and therefore their minor membrane effects (152). For that reason, some chemical modifications of flavonoid monomers have been synthesized to better achieve antiproliferative effects. Thus, a synthetic epicatechin derivative has high activity as an irreversible inhibitor of the dihydrofolate reductase (153). Moreover, some procyanidin derivatives of epicatechin and catechin show a high inhibition of proliferation and therefore high cytotoxicity on gastrointestinal-tract and prostate cell lines (154).

Focusing on cocoa, both the powder itself and a procyanidin-enriched extract have shown *in vitro* antiproliferative effects on a human cell line of colon cancer (Caco-2) by causing non-apoptotic cell death, affecting cell cycle progression, and inhibiting polyamine biosynthesis (155). Pentameric procyanidins isolated from *Theobroma cacao* selectively inhibit the proliferation of several human cell lines of breast cancer (156). In addition, the hexameric procyanidin fraction isolated from cocoa has shown a protective effect by reducing induced cytotoxicity after cell-membrane interaction (103).

Considering the association between inflammatory and carcinogenic processes, improper upregulation in the expression of COX-2 increases cell proliferation and angiogenesis, which implies its involvement in the progression of cancer (157). Cocoa procyanidins inhibit the expression of COX-2 in a dose-dependent manner that may be due to an upstream action in the MAPK pathway and/or to NF-κB suppression (39, 136, 139). However, the inhibitory activity on COX-2 has also been attributed to other phytochemicals present in cocoa, such as caffedymine and its analogs, in addition to the flavonoids (158).

25.3.7 Apoptosis Regulation by Cocoa

A broad array of pro-apoptotic and antiapoptotic effector molecules controls the equilibrium between cell death and the renewal of damaged or abnormal cells. Flavonoids have been shown to control apoptosis in both *in vitro* and *in vivo* studies (105, 159).

Some flavonoid monomers can up- and down-regulate the expression of p53 and Bcl-2, respectively, promoting *in vitro* cancer cell apoptosis (160). In addition, epicatechin has an arresting effect on the cell cycle of esophageal adenocarcinoma cells (161). Interestingly, the induction of apoptosis in cancer cell lines is more pronounced with oligomer/polymer than with monomer flavonoids (162, 163). In this sense, procyanidins inhibit the growth of several breast-cancer cell lines by inducing the arrest of their cell cycle in the G0-G1 phase (163). Procyanidins also induce apoptosis and necrosis on a human hormone-resistant prostate-cancer cell line (PC-13) through a mitochondrion-dependent mechanism (164). Regarding the effect of cocoa on apoptosis, cocoa pentameric procyanidins induce the dephosphorilation of p53 and then control the proliferation of human breast cancer cells (156).

Considering antiapoptotic proteins, the cocoa procyanidin B2 inhibits the gene expression of Bcl-xL, Bcl-2, XIAP, and cFLIP in different Hodgkin's lymphoma cell lines (148). In one rat pheocromocytoma PC12 cell model, not only procyanidin B2 but

also the cocoa procyanidin fraction downregulated Bcl-xL and Bcl-2 (105). To date, Bcl-2 has been shown to induce mitochondrial dysfunction by releasing cytochrome c from the mitochondria, which leads to the activation of the caspase pathways. A decrease in caspase-3 activity has been shown to occur in cocoa added both *in vitro* (105) and in animals fed cocoa in a carcinogenic rat model (118).

25.3.8 Role of Cocoa on Transformation, Migration, and Angiogenesis

Cell neoplastic transformation, migration, and invasion are considered major events occurring during carcinogenic processes. Some of these steps are modulated by cocoa and its flavonoids. In this sense, the cocoa procyanidin fraction and procyanidin B2 inhibit the *in vitro* neoplastic transformation of JB6P + cells, which involves the inhibition of MEK/ERK signal-transduction pathways (136). On the other hand, MMPs are able to degrade the extracellular matrix proteins, allowing cancer progression and metastatic spread. The activity of these enzymes seems to be modulated by the MAPK and NF-κB pathways (159, 165). As cocoa modulates these upstream factors, it can also influence MMP and hence the invasive activity of some cancers. Thus, cocoa procyanidins inhibit the activity of MMP-2 (137), whereas quercetin has similar effects on MMP-2 and MMP-9 (166, 167). Furthermore, oligomer procyanidins selectively inhibit chemotaxis, the G0-G1 phase transition, and the MMP activity of the human astrocytoma cell line U-87, reducing tumor progression, cell invasion, and metastasis (168). Finally, the cocoa flavonoid luteolin blocks the invasive and migration activities of pancreatic carcinoma cells (MiaPaCA-2) by inhibiting the EGF-receptor tyrosin activity (131).

Uncontrolled angiogenesis is a key process contributing to tumor growth involving endothelial cell proliferation (with the participation of some cell cycle regulators such as the MAPK pathways). Although very few studies on cocoa are available, purified flavanol and procyanidin fractions from cocoa have been shown to inhibit endothelial signaling and growth through the downregulation of ErbB2 (133).

25.3.9 Human Evidence Associating Cocoa Intake and Cancer

Although the *in vitro* and experimental preclinical studies above demonstrated a protective effect of cocoa on proliferative disorders, at present there are limited studies evaluating the relationship between human cancer development and cocoa intake. Nevertheless, some observational studies have evaluated the association between cancer incidence and diets (169–172). These studies consider flavonoid consumption as flavones, flavonols, flavan-3-ols, procyanidins, flavanones, and isoflavones by using the dietary history method or the food frequency questionnaire and its association to lung (173–175), colorectal (176, 177), or breast (178) cancers. Although these data suggest some association between a flavonoid-rich diet and protection from cancer, significant relationships have not always been found. In a cancer-prevention study cohort of 24,111 healthy male smokers, only stratified analysis could link the intake of these compounds with the reduction of pancreatic cancer risk (179). Another interesting report is a large case-control study that demonstrated the differential effectiveness of flavonoid intake on gastric cancer development between females and males: an inverse association was found between plasma flavonoids and disease in women, but no such association was described for men (180).

A recent case-control study involving 1,456 cases per group (85) evaluated the major dietary flavonoids quercetin, catechin, epicatechin, naringenin, and hesperitin, and their relationships to colorectal cancer. Except for hesperitin, the other four flavonoids are present in cocoa products. This study examined the consumption of chocolate as a source of flavonoids, ascribing 6% of epicatechin intake coming from cocoa. The main finding of this study was that patients with colorectal cancer consumed lower amounts of flavonols and procyanidins, particularly quercetin, catechin, and epicatechin than matched control individuals, in a dose-dependent relationship (85). On the other hand, the cocoa-protective effect has been described in some other cohort and case-control studies as showing an inverse relationship between quercetin, catechin, and epicatechin intake and lung cancer (170, 175, 181).

Finally, this chapter cannot end without talking about the Kuna Indians of Panama. The population residing in the indigenous area of the San Blas islands has the highest flavonoid intake, compared to any other community. Their flavonoid source is a flavanol-rich cocoa beverage, which contributes more than 900 mg flavonoids/day to their diet (182). The frequency of cancer death within the Kuna population living in San Blas is significantly lower than that of people living in mainland Panama, which can be the result of cocoa consumption, although other possible explanations may exist (182).

25.4 CONCLUSIONS AND PERSPECTIVES

Cocoa is a food with a high nutritional value and with bioactive compounds that have demonstrated to be effective on some physiological and pathophysiological processes. In this sense, cocoa and its flavonoids modulate innate and acquired immune responses. *In vivo* results suggest that cocoa can produce an attenuating effect on both inflammation and acquired Th2 responses. These immunoregulatory actions may be beneficial in reducing certain states of autoimmunity and hypersensitivity. In addition, accumulated evidence suggests the efficacy of cocoa as a potential antiproliferative food. Cocoa flavonoids act at different molecular and cellular levels, but the precise underlying mechanism and the specific active components remain unknown. Further preclinical studies and clinical trials are needed to investigate the mechanism involved in cocoa actions and to justify their usage as adjuvant or combination therapy for the prevention and treatment of immune-mediated diseases and in cancer development and metastasis.

Acknowledgments The present study was supported by the *Ministerio de Ciencia y Tecnología* (AGL2005-02823 and AGL2008-02790) and the *Generalitat de Catalunya* (SGR2009118).

REFERENCES

1. Borchers AT, Keen CL, Hannum SM, Gershwin ME (2000) Cocoa and chocolate: composition, bioavailability and health implications. J Med Food 3:77–105
2. Hurst WJ, Tarka SM Jr, Powis TG, Valdez F Jr, Hester TR (2002) Cacao usage by the earliest Maya civilization. Nature 418:289–290
3. Pucciarelli DL, Grivetti LE (2008) The medicinal use of chocolate in Early North America. Mol Nutr Food Res 52:1215–1227
4. Keen CL (2001) Chocolate: food as medicine/medicine as food. J Am Coll Nutr 20:436S–439S

5. Tomas-Barberan FA, Cienfuegos-Jovellanos E, Marín A, Muguerza B, Gil-Izquierdo A, Cerda B, Zafrilla P, Morillas J, Mulero J, Ibarra A, Pasamar MA, Ramón D, Espín JC (2007) A new process to develop a cocoa powder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. J Agric Food Chem 55:3926–3935
6. Vinson JA, Proch J, Bose P, Muchler S, Taffera P, Shuta D, Samman N, Agbor GA (2006) Chocolate is a powerful ex vivo and in vivo antioxidant, an antiatherosclerotic agent in an animal model, and a significant contributor to antioxidants in the European and American Diets. J Agric Food Chem 54:8071–8076
7. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747
8. Natsume M, Osakabe N, Yamagishi M, Takizawa T, Nakamura T, Miyatake H, Hatano T, Yoshida T (2000) Analyses of polyphenols in cacao liquor, cocoa, and chocolate by normal-phase and reversed-phase HPLC. Biosci Biotechnol Biochem 64:2581–2587
9. Gu L, House SE, Wu X, Ou B, Prior RL (2006) Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. J Agric Food Chem 54:4057–4061
10. McShea A, Ramiro-Puig E, Munro SB, Casadesus G, Castell M, Smith MA (2008) Clinical benefit and preservation of flavonols in dark chocolate manufacturing. Nutr Rev 66:630–641
11. Baba S, Osakabe N, Natsume M, Muto Y, Takizawa T, Terao J (2001) In vivo comparison of the bioavailability of (+)-catechin, (−)-epicatechin and their mixture in orally administered rats. J Nutr 131:2885–2891
12. Baba S, Osakabe N, Natsume M, Yasuda A, Takizawa T, Nakamura T, Terao J (2000) Cocoa powder enhances the level of antioxidative activity in rat plasma. Br J Nutr 84:673–680
13. Deprez S, Brezillon C, Rabot S, Philippe C, Mila I, Lapierre C, Scalbert A (2000) Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. J Nutr 130:2733–2738
14. Holt RR, Lazarus SA, Sullards MC, Zhu QY, Schramm DD, Hammerstone JF, Fraga CG, Schmitz HH, Keen CL (2002) Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. Am J Clin Nutr 76:798–804
15. Middleton E, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751
16. Cooper KA, Donovan JL, Waterhouse AI, Williamson G (2008) Cocoa and health: a decade of research. Br J Nutr 99:1–11
17. Buijsse B, Feskens EJM, Kok FJ, Kromhout D (2006) Cocoa intake, blood pressure, and cardiovascular mortality: The Zutphen Elderly Study. Arch Int Med 166:411–417
18. Corti R, Flammaer AJ, Hollenberg NK, Lüscher TF (2009) Cocoa and cardiovascular health. Circulation 119:1433–1441
19. Lippi G, Franchini M, Montagnana M, Favaloro EJ, Guidi GC, Targher G (2009) Dark chocolate: consumption for pleasure or therapy? J Thromb Thrombolysis 28:482–488
20. Ramiro-Puig E, Castell M, McShea A, Perry G, Smith MA, Casadesús G (2009) Food antioxidants and Alzheimer's disease. In: Packer L, Sies H, Eggersdorfer M, Cadenas E (eds) Micronutrients and brain health. CRC Press, Boca Raton, FL, USA
21. Murphy KM, Travers P, Walport M (2007) Janeway's immunobiology, vol 7. Garland Science, England
22. Meresse B, Cerf-Bensussan N (2009) Innate T cell responses in human gut. Sem Immunol 21:121–129
23. Mao TK, van de Water J, Keen CL, Schmitz HH, Gershwin ME (2002) Modulation of TNF-alpha secretion in peripheral blood mononuclear cells by cocoa flavanols and procyanidins. Clin Dev Immunol 9:135–141
24. Kenny TP, Keen CL, Schmitz HH, Gershwin ME (2007) Immune effects of cocoa procyanidin oligomers on peripheral blood mononuclear cells. Exp Biol Med (Maywood) 232:293–300
25. Wisman KN, Perkins AA, Jeffers MD, Hagerman AE (2008) Accurate assessment of the bioactivities of redox-active polyphenolics in cell culture. J Agric Food Chem 56:7831–7837
26. Ramiro E, Franch A, Castellote C, Pérez-Cano F, Permanyer J, Izquierdo-Pulido M, Castell M (2005) Flavonoids from *Theobroma cacao* down-regulate inflammatory mediators. J Agric Food Chem 53:8506–8511
27. Ono K, Takahashi T, Kamei M, Mato T, Hashizume S, Kamiya S, Tsutsumi H (2003) Effects of an aqueous extract of cocoa on nitric oxide production of macrophages activated by lipopolysaccharide and interferon-gamma. Nutrition 19:681–685

28. Sanbongi C, Suzuki N, Sakane T (1997) Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans in vitro. Cell Immunol 177:129–136
29. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E (2007) Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm 2007:45673–45683
30. Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Gálvez J, Zarzuelo A (2005) In vivo quercitrin antiinflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. Eur J Immunol 35:584–592
31. Chen CY, Peng WH, Tsai KD, Hsu SL (2007) Luteolin suppresses inflammation-associated gene expression by blocking NF-κB and AP-1 activation pathway in mouse alveolar macrophages. Life Sci 81:1602–1614
32. Ramiro-Puig E, Pérez-Cano FJ, Ramírez-Santana C, Castellote C, Izquierdo-Pulido M, Permanyer J, Franch A, Castell M (2007) Spleen lymphocyte function modulated by a cocoa-enriched diet. Clin Exp Immunol 149:535–542
33. Castell M, Franch A, Ramos-Romero S, Ramiro-Puig E, Pérez-Cano FJ, Castellote C (2009) Effect of a diet rich in cocoa flavonoids on experimental acute inflammation. In: Flavonoids: biosynthesis, biological effects and dietary sources, Nova Publishers, Huntington, NY, USA, pp. 213–229
34. Lee HC, Jenner AM, Low CS, Lee YK (2006) Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Res Microbiol 157:876–884
35. Matsuoka Y, Hasegawa H, Okuda S, Muraki T, Uruno T, Kubota K (1995) Ameliorative effects of tea catechins on active oxygen-related nerve cell injuries. J Pharmacol Exp Ther 274:602–608
36. Rotelli AE, Guardia T, Juárez AO, de la Rocha NE, Pelzer LE (2003) Comparative study of flavonoids in experimental models of inflammation. Pharmacol Res 48:601–606
37. Morikawa K, Nonaka M, Narahara M, Torii I, Kawaguchi K, Yoshikawa T, Kumazawa Y, Morikawa S (2003) Inhibitory effect of quercetin on carrageenan-induced inflammation in rats. Life Sci 74:709–721
38. Kim H, Kong H, Choi B, Yang Y, Kim Y, Lim MJ, Neckers L, Jung Y (2005) Metabolic and pharmacological properties of rutin, a dietary quercetin glycoside, for treatment of inflammatory bowel disease. Pharm Res 22:1499–1509
39. Lee KW, Kundu JK, Kim SO, Chun KS, Lee HJ, Surh YJ (2006) Cocoa polyphenols inhibit phorbol ester-induced superoxide anion formation in cultured HL-60 cells and expression of cyclooxygenase-2 and activation of NF-κB and MAPKs in mouse skin in vivo. J Nutr 136:1150–1155
40. Ramos-Romero S, Ramiro-Puig E, Pérez-Cano FJ, Castellote C, Franch A, Castell M (2008) Anti-inflammatory effects of cocoa in rat carrageenin-induced paw oedema. Proc Nutr Soc 67:65
41. Mazzon E, Muià C, Paola RD, Genovese T, Menegazzi M, De Sarro A, Suzuki H, Cuzzocrea S (2005) Green tea polyphenol extract attenuates colon injury induced by experimental colitis. Free Radic Res 39:1017–1025
42. Morinobu A, Biao W, Tanaka S, Horiuchi M, Jun L, Tsuji G, Sakai Y, Kurosaka M, Kumagai S (2008) (−)-Epigallocatechin-3-gallate suppresses osteoclast differentiation and ameliorates experimental arthritis in mice. Arthritis Rheum 58:2012–2018
43. Lin SK, Chang HH, Chen YJ, Wang CC, Galson DL, Hong CY, Kok SH (2008) Epigallocatechin-3-gallate diminishes CCL2 expression in human osteoblastic cells via up-regulation of phosphatidylinositol 3-kinase/Akt/Raf-1 interaction: a potential therapeutic benefit for arthritis. Arthritis Rheum 58:3145–3156
44. Miyake M, Ide K, Sasaki K, Matsukura Y, Shijima K, Fujiwara D (2008) Oral administration of highly oligomeric procyanidins of Jatoba reduces the severity of collagen-induced arthritis. Biosci Biotechnol Biochem 72:1781–1788
45. Wang TY, Li J, Ge JF, Li CY, Jin Y, Lü XW, Cheng WM, Tang JH (2008) Preliminary study of total flavonoids from *Litsea coreana* Levl., on experimental adjuvant-induced arthritis in rats. Am J Chin Med 36:899–912
46. Beaumont DM, Mark TM Jr, Hills R, Dixon P, Veit B, Garrett N (2008) The effects of chrysin, a *Passiflora incarnata* extract, on natural killer cell activity in male Sprague-Dawley rats undergoing abdominal surgery. AANA J 76:113–117

47. Ramiro-Puig E, Pérez-Cano FJ, Ramos-Romero S, Pérez-Berezo T, Castellote C, Permanyer J, Franch A, Izquierdo-Pulido M, Castell M (2008) Intestinal immune system of young rats influenced by cocoa-enriched diet. J Nutr Biochem 19:555–565
48. Akiyama H, Sato Y, Watanabe T, Nagaoka MH, Yoshioka Y, Shoji T, Kanda T, Yamada K, Totsuka M, Teshima R, Sawada J, Goda Y, Maitani T (2005) Dietary unripe apple polyphenol inhibits the development of food allergies in murine models. FEBS Lett 579:4485–4491
49. Holderness J, Hedges JF, Daughenbaugh K, Kimmel E, Graff J, Freedman B, Jutila MA (2008) Response of γδ T cells to plant-derived tannins. Crit Rev Immunol 28:377–402
50. Boismenu R (2000) Function of intestinal γδ T cells. Immunol Res 21:123–127
51. Graff JC, Jutila MA (2007) Differential regulation of CD11b on γδ T cells and monocytes in response to unripe apple polyphenols. J Leukoc Biol 82:603–607
52. Mathur S, Devaraj S, Grundy SM, Jialal I (2002) Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. J Nutr 132:3663–3667
53. di Giuseppe R, di Castelnuovo A, Centritto F, Zito F, de Curtis A, Costanzo S, Vohnout B, Sieri S, Krogh V, Donati MB, de Gaetano G, Iacoviello L (2008) Regular consumption of dark chocolate is associated with low serum concentrations of C-reactive protein in a healthy Italian population. J Nutr 138:1939–1945
54. Stoeck M, Lees R, Szamel M, Pantaleo G, MacDonald HR (1989) Comparison of phorbol-12-myristate-13-acetate and dioctanoyl-sn-glycerol in the activation of EL4/6.1 thymoma cells. J Cell Physiol 138:541–547
55. Mao TK, Powell JJ, Van de Water J, Keen CL, Schmitz HH, Greshwin ME (1999) The influence of cocoa procyanidins on the transcrition of interleukin 2 in peripheral blood mononuclear cells. Int J Immunother 15:23–29
56. Ramiro E, Franch A, Castellote C, Andrés-Lacueva C, Izquierdo-Pulido M, Castell M (2005) Effect of *Theobroma cacao* flavonoids on immune activation of a lymphoid cell line. Br J Nutr 93:859–866
57. Mao T, Van de Water J, Keen CL, Schmitz HH, Gershwin ME (2000) Cocoa procyanidins and human cytokine transcription and secretion. J Nutr 130:2093S–2099S
58. Yu ES, Min HJ, An SY, Won HY, Hong JH, Hwang ES (2008) Regulatory mechanisms of IL-2 and IFNγ suppression by quercetin in T helper cell. Biochem Pharmacol 76:70–78
59. Lugli E, Ferraresi R, Roat E, Troiano L, Pinti M, Nasi M, Nemes E, Berteoncelli L, Gibellini L, Salomoni P, Cooper EL, Cossarizza A (2009) Quercetin inhibits lymphocyte activation and proliferation without inducing apoptosis in peripheral mononuclear cells. Leuk Res 33:140–150
60. Constant SL, Bottomly K (1997) Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. Annu Rev Immunol 15:297–322
61. Jenny M, Saner E, Klein A, Ledochowski M, Schennach H, Ueberall F, Fuchs D (2009) Cacao extracts suppress tryptophan degradation of mitogen-stimulated peripheral blood mononuclear cells. J Ethnopharmacol 122:261–267
62. Mao TK, Van de Water J, Keen CL, Schmitz HH, Gershwin ME (2002) Effect of cocoa flavanols and their related oligomers on the secretion of interleukin-5 in peripheral blood mononuclear cells. J Med Food 5:17–22
63. Couper KN, Blount DG, Riley EM (2008) IL-10: the master regulator of immunity to infection. J Immunol 180:5771–5777
64. Mao TK, Van de Water J, Keen CL, Schmitz HH, Gershwin ME (2003) Cocoa flavonols and procyanidins promote transforming growth factor-β_1 homeostasis in peripheral blood mononuclear cells. Exp Biol Med 228:93–99
65. Ramiro-Puig E, Urpí-Sardà M, Pérez-Cano FJ, Franch A, Castellote C, Andrés-Lacueva C, Izquierdo-Pulido M, Castell M (2007) Cocoa-enriched diet enhances antioxidant enzyme activity and modulates lymphocyte composition in thymus from young rats. J Agric Food Chem 55:6431–6438
66. Bhandoola A, Sambandam A (2006) From stem cell to T cell: one route or many? Nat Rev Immunol 6:117–126
67. Mowat AM (2003) Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol 3:331–341
68. Park HJ, Lee CM, Jung ID, Lee JS, Jeong YI, Chang JH, Chun SH, Kim MJ, Choi IW, Ahn SC, Shin YK, Yeom SR, Park YM (2009) Quercetin regulates Th1/Th2 balance in a murine model of asthma. Int Immunopharmacol 9:261–267

69. Cruz EA, Da-Silva SAG, Muzitano MF, Silva PMR, Costa SS, Rossi-Bergmann B (2008) Immunomodulatory pretreatment with *Kalanchoe pinnata* extract and its quercitrin flavonoid effectively protects mice against fatal anaphylactic shock. Int Immunopharmacol 8:1616–1621
70. Pérez-Berezo T, Ramiro-Puig E, Pérez-Cano FJ, Castellote C, Permanyer J, Franch A, Castell M (2009) Influence of a cocoa-enriched diet on specific immune response in ovalbumin-sensitized rats. Mol Nutr Food Res 53:389–397
71. Graser BJ (1995) Downregulation of intragraft IFN-gamma expression correlates with increased IgG1 alloantibody response following intrathymic immunomodulation of sensitized rat recipients. Transplantation 60:1516–1524
72. Gracie JA, Bradley JA (1996) Interleukin-12 induces interferon-gamma dependent switching of IgG alloantibody subclass. Eur J Immunol 26:1217–1221
73. Saoudi A, Bernard I, Hoedemaekers A, Cautain B, Martínez K, Druet P, De Baets M, Guéry JC (1999) Experimental autoimmune myasthenia gravis may occur in the context of a polarized Th1- or Th2-type immune response in rats. J Immunol 162:7189–7197
74. Bridle BW, Wilkie BN, Jevnikar AM, Mallard BA (2007) Deviation of xenogeneic immune response and bystander suppression in rats fed porcine blood mononuclear cells. Transpl Immunol 17:262–270
75. Kogiso M, Sakai T, Mitsuya K, Komatsu T, Yamamoto S (2006) Genistein suppresses antigen-specific immune responses through competition with 17beta-estradiol for estrogen receptors in ovalbumin-immunized BALB/c mice. Nutrition 22:802–809
76. Qin F, Sun HX (2008) Immunosuppressive activity of the ethanol extract of *Sedum sarmentosum* and its fractions on specific antibody and cellular responses to ovalbumin in mice. Chem Biodivers 5:2699–2709
77. Yano S, Umeda D, Yamashita T, Ninomiya Y, Sumida M, Fujimura Y, Yamada K, Tachibana H (2007) Dietary flavones suppresses IgE and Th2 cytokines in OVA-immunized BALB/c mice. Eur J Nutr 46:257–263
78. Chang SL, Chiang YM, Chang CLT, Yeh HH, Shyur LF, Kuo YH, Wu TK, Yang WC (2007) Flavonoids, centaurein and centaureidin, from *Bidens pilosa*, stimulate IFN-γ expression. J Ethnopharmacol 112:232–236
79. Kang HK, Ecklund D, Liu M, Datta SK (2009) Apigenin, a non-mutagenic dietary flavonoid, suppresses lupus by inhibiting autoantigen presentation for expansion of autoreactive Th1 and Th17 cells. Arthritis Res Ther 11(2):R59. doi:10.1186/ar2682
80. Lee JY, Lee JH, Park JH, Kim SY, Choi JY, Lee SH, Kim YS, Kang SS, Jang EC, Han Y (2009) Liquiritigenin, a licorice flavonoid, helps mice resist disseminated candidiasis due to *Candida albicans* by Th1 immune response, whereas liquiritin, its glycoside form, does not. Int Immunopharmacol 9:632–638
81. Faria A, Calhau C, de Freitas V, Mateus N (2006) Procyanidins as antioxidants and tumor cell growth modulators. J Agric Food Chem 54:2392–2397
82. Zhao J, Wang J, Chen Y, Agarwal R (1999) Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3′-gallate as the most effective antioxidant constituent. Carcinogenesis 20:1737–1745
83. Shoji T, Masumoto S, Moriichi N, Kobori M, Kanda T, Shinmoto H, Tsushida T (2005) Procyanidin trimers to pentamers fractionated from apple inhibit melanogenesis in B16 mouse melanoma cells. J Agric Food Chem 53:6105–6111
84. Eng ET, Ye J, Williams D, Phung S, Moore RE, Young MK, Gruntmanis U, Braunstein G, Chen S (2003) Suppression of estrogen biosynthesis by procyanidin dimers in red wine and grape seeds. Cancer Res 63:8516–8522
85. Theodoratou E, Kyle J, Cetnarskyj R, Farrington SM, Tenesa A, Barnetson R, Porteous M, Dunlop M, Campbell H (2007) Dietary flavonoids and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 16:684–693
86. Miller KB, Stuart DA, Smith NL, Lee CY, McHale NL, Flanagan JA, Ou B, Hurst WJ (2006) Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. J Agric Food Chem 54:4062–4068

87. Jonfia-Essien WA, West G, Alderson PG, Tucker G (2008) Phenolic content and antioxidant capacity of hybrid variety cocoa beans. Food Chem 108:1155–1159
88. Belščak A, Komes D, Horžić D, Ganić KK, Karlović D (2009) Comparative study of commercially available cocoa products in terms of their bioactive composition. Food Res Int 42:707–716
89. Kwon DY, Choi KH, Kim SJ, Choi DW, Kim YS, Kim YC (2009) Comparison of peroxyl radical scavenging capacity of commonly consumed beverages. Arch Pharm Res 32:283–287
90. Lee KW, Kim YJ, Lee HJ, Lee CY (2003) Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem 51:7292–7295
91. Hatano T, Miyatake H, Natsume M, Osakabe N, Takizawa T, Ito H, Yoshida T (2002) Proanthocyanidin glycosides and related polyphenols from cacao liquor and their antioxidant effects. Phytochemistry 59:749–758
92. Counet C, Collin S (2003) Effect of the number of flavanol units on the antioxidant activity of procyanidin fractions isolated from chocolate. J Agric Food Chem 51:6816–6822
93. Yilmaz Y, Toledo RT (2004) Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. J Agric Food Chem 52:255–260
94. Pollard SE, Kuhnle GG, Vauzour D, Vafeiadou K, Tzounis X, Whiteman M, Rice-Evans C, Spencer JP (2006) The reaction of flavonoid metabolites with peroxynitrite. Biochem Biophys Res Commun 350:960–968
95. Morel I, Lescoat G, Cogrel P, Sergent O, Pasdeloup N, Brissot P, Cillard P, Cillard J (1993) Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem Pharmacol 45:13–19
96. Pazos M, Andersen ML, Medina I, Skibsted LH (2007) Efficiency of natural phenolic compounds regenerating alpha-tocopherol from alpha-tocopheroxyl radical. J Agric Food Chem 55:3661–3666
97. Alía M, Mateos R, Ramos S, Lecumberri E, Bravo L, Goya L (2006) Influence of quercetin and rutin on growth and antioxidant defense system of a human hepatoma cell line (HepG2). Eur J Nutr 45:19–28
98. Zhu QY, Holt RR, Lazarus SA, Orozco TJ, Keen CL (2002) Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. Exp Biol Med 227:321–329
99. Zhu QY, Schramm DD, Gross HB, Holt RR, Kim SH, Yamaguchi T, Kwik-Uribe CL, Keen CL (2005) Influence of cocoa flavonols and procyanidins on free radical induced human erythrocyte hemolysis. Clin Dev Immunol 12:27–34
100. Verstraeten SV, Mackenzie GG, Oteiza PI, Fraga CG (2008) (−)-Epicatechin and related procyanidins modulate intracellular calcium and prevent oxidation in Jurkat T cells. Free Radic Res 42:864–872
101. Granado-Serrano AB, Martín M, Goya L, Bravo L, Ramos S (2009) Time-course regulation of survival pathways by epicatechin on HepG2 cells. J Nutr Biochem 20:115–124
102. Martín MA, Ramos S, Mateos R, Izquierdo-Pulido M, Bravo L, Goya L (2009) Protection of human HepG2 cells against an oxidative stress by the flavonoid epicatechin. Phytotherapy Res 24:503–509
103. Erlejman AG, Fraga CG, Oteiza PI (2006) Procyanidins protect Caco-2 cells from bile acid- and oxidant-induced damage. Free Radic Biol Med 41:1247–1256
104. Martín MA, Ramos S, Mateos R, Granado-Serrano AB, Izquierdo-Pulido M, Bravo L, Goya L (2008) Protection of human HepG2 cells against oxidative stress by cocoa phenolic extract. J Agric Food Chem 56:7765–7772
105. Cho ES, Jang YJ, Kang NJ, Hwang MK, Kim YT, Lee KW, Lee HJ (2009) Cocoa procyanidins attenuate 4-hydroxynonenal-induced apoptosis of PC12 cells by directly inhibiting mitogen-activated protein kinase kinase 4 activity. Free Radic Biol Med 46:1319–1327
106. Ramiro-Puig E, Casadesús G, Lee HG, Zhu X, McShea A, Perry G, Pérez-Cano FJ, Smith MA, Castell M (2009) Neuroprotective effect of cocoa flavonoids on in vitro oxidative stress. Eur J Nutr 48:54–61
107. Wang JF, Schramm DD, Holt RR, Ensunsa JL, Fraga CG, Schmitz HH, Keen CL (2000) A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. J Nutr 130:2115S–2119S
108. Orozco TJ, Wang JF, Keen CL (2003) Chronic consumption of a flavanol- and procyanindin-rich diet is associated with reduced levels of 8-hydroxy-2′-deoxyguanosine in rat testes. J Nutr Biochem 14:104–110

109. Lecumberri E, Mateos R, Ramos S, Alía M, Rúperez P, Goya L, Izquierdo-Pulido M, Bravo L (2006) Characterization of cocoa fiber and its effect on the antioxidant capacity of serum in rats. Nutr Hosp 21:622–628
110. Lecumberri E, Goya L, Mateos R, Alía M, Ramos S, Izquierdo-Pulido M, Bravo L (2007) A diet rich in dietary fiber from cocoa improves lipid profile and reduces malondialdehyde in hypercholesterolemic rats. Nutrition 23:332–341
111. Jalil AM, Ismail A, Pei CP, Hamid M, Kamaruddin SH (2008) Effect of cocoa extract on glucometabolism, oxidative stress, and antioxidant enzymes in obese-diabetic rats. J Agric Food Chem 56:7877–7884
112. Kurosawa T, Itoh F, Nozaki A, Nakano Y, Katsuda S, Osakabe N, Tsubone H, Kondo K, Itakura H (2005) Suppressive effects of cacao liquor polyphenols (CLP) on LDL oxidation and the development of atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic rabbits. Atherosclerosis 179:237–246
113. Kondo K, Hirano R, Matsumoto A, Igarashi O, Itakura H (1996) Inhibition of LDL oxidation by cocoa. Lancet 348:1514
114. Osakabe N, Baba S, Yasuda A, Iwamoto T, Kamiyama M, Takizawa T, Itakura H, Kondo K (2001) Daily cocoa intake reduces the susceptibility of low-density lipoprotein to oxidation as demonstrated in healthy human volunteers. Free Radic Res 34:93–99
115. Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, Fukuda K, Muto Y, Kondo K (2007) Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. Am J Clin Nutr 85:709–717
116. Fraga CG, Actis-Goretta L, Ottaviani JI, Carrasquedo F, Lotito SB, Lazarus S, Schmitz HH, Keen CL (2005) Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. Clin Dev Immunol 12:11–17
117. McKim SE, Konno A, Gäbele E, Uesugi T, Froh M, Sies H, Thurman RG, Arteel GE (2002) Cocoa extract protects against early alcohol-induced liver injury in the rat. Arch Biochem Biophys 406:40–46
118. Granado-Serrano AB, Martín MA, Bravo L, Goya L, Ramos S (2009) A diet rich in cocoa attenuates *N*-nitrosodiethylamine-induced liver injury in rats. Food Chem Toxicol 47:2499–2506
119. Weyant MJ, Carothers AM, Dannenberg AJ, Bertagnolli MM (2001) (+)-Catechin inhibits intestinal tumor formation and suppresses focal adhesion kinase activation in the min/+ mouse. Cancer Res 61:118–125
120. Gu Q, Hu C, Chen Q, Xia Y, Feng J, Yang H (2009) Development of a rat model by 3, 4-benzopyrene intra-pulmonary injection and evaluation of the effect of green tea drinking on p53 and bcl-2 expression in lung carcinoma. Cancer Detect Prev 32:444–451
121. Hoensch HP, Kirch W (2005) Potential role of flavonoids in the prevention of intestinal neoplasia: a review of their mode of action and their clinical perspectives. Int J Gastrointest Cancer 35:187–195
122. Luceri C, Caderni G, Sanna A, Dolara P (2002) Red wine and black tea polyphenols modulate the expression of cycloxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced F344 rat colon tumors. J Nutr 132:1376–1379
123. Yamagishi M, Natsume M, Nagaki A, Adachi T, Osakabe N, Takizawa T, Kumon H, Osawa T (2000) Antimutagenic activity of cacao: inhibitory effect of cacao liquor polyphenols on the mutagenic action of heterocyclic amines. J Agric Food Chem 48:5074–5078
124. Yamagishi M, Osakabe N, Natsume M, Adachi T, Takizawa T, Kumon H, Osawa T (2001) Anticlastogenic activity of cacao: inhibitory effect of cacao liquor polyphenols against mitomycin C-induced DNA damage. Food Chem Toxicol 39:1279–1283
125. Yamagishi M, Natsume M, Osakabe N, Nakamura H, Furukawa F, Imazawa T, Nishikawa A, Hirose M (2002) Effects of cacao liquor proanthocyanidins on PhIP-induced mutagenesis in vitro, and in vivo mammary and pancreatic tumorigenesis in female Sprague-Dawley rats. Cancer Lett 185:123–130
126. Yamagishi M, Natsume M, Osakabe N, Okazaki K, Furukawa F, Imazawa T, Nishikawa A, Hirose M (2003) Chemoprevention of lung carcinogenesis by cacao liquor proanthocyanidins in a male rat multi-organ carcinogenesis model. Cancer Lett 191:49–57
127. Teillet F, Boumendje A, Boutonnat J, Ronot X (2008) Flavonoids as RTK inhibitors and potential anticancer agents. Inc Med Res Rev 28:715–745

128. Stevens MF, McCall CJ, Lelieveld P, Alexander P, Richter A, Davies DE (1994) Structural studies on bioactive compounds: synthesis of polyhydroxylated 2-phenylbenzothiazoles and a comparison of their cytotoxicities and pharmacological properties with genistein and quercetin. J Med Chem 37:1689–1695
129. Huang YT, Hwang JJ, Lee PP, Ke FC, Huang JH, Huang CJ, Kandaswami C, Middleton E, Lee MT (1999) Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. Br J Pharmacol 128:999–1010
130. Lee LT, Huang YT, Hwang JJ, Lee AY, Ke FC, Huang CJ, Kandaswami C, Lee PP, Lee MT (2004) Transinactivation of the epidermal growth factor receptor tyrosine kinase and focal adhesion kinase phosphorylation by dietary flavonoids: effect on invasive potential of human carcinoma cells. Biochem Pharmacol 67:2103–2114
131. Lee LT, Huang YT, Hwang JJ, Lee PP, Ke FC, Nair MP, Kanadaswam C, Lee MT (2002) Blockade of the epidermal growth factor receptor tyrosine kinase activity by quercetin and luteolin leads to growth inhibition and apoptosis of pancreatic tumor cells. Anticancer Res 22:1615–1627
132. Bigelow RL, Cardelli JA (2006) The green tea catechins, (−)-epigallocatechin-3-gallate (EGCG) and (−)-epicatechin-3-gallate (ECG), inhibit HGF/Met signaling in immortalized and tumorigenic breast epithelial cells. Oncogene 25:1922–1930
133. Kenny TP, Keen CL, Jones P, Kung H, Schmitz HH, Gershwin ME (2004) Pentameric procyanidins isolated from *Theobroma cacao* seeds selectively downregulate ErbB2 in human aortic endothelial cells. Exp Biol Med 229:255–263
134. Kamata H, Hirata H (1999) Redox regulation of cellular signalling. Cell Signal 11:1–14
135. Briviba K, Pan L, Rechkemmer G (2002) Red wine polyphenols inhibit the growth of colon carcinoma cells and modulate the activation pattern of mitogen-activated protein kinases. J Nutr 132:2814–2818
136. Nam JK, Ki WL, Dong EL, Rogozin EA, Bode AM, Hyong JL, Dong Z (2008) Cocoa procyanidins suppress transformation by inhibiting mitogen-activated protein kinase kinase. J Biol Chem 283:20664–20673
137. Lee KW, Kang NJ, Oak MH, Hwang MK, Kim JH, Schini-Kerth VB, Lee HJ (2008) Cocoa procyanidins inhibit expression and activation of MMP-2 in vascular smooth muscle cells by direct inhibition of MEK and MT1-MMP activities. Cardiovasc Res 79:34–41
138. Zhang WY, Liu HQ, Xie KQ, Yin LL, Li Y, Kwik-Uribe CL, Zhu XZ (2006) Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] suppresses the expression of cyclooxygenase-2 in endotoxin-treated monocytic cells. Biochem Biophys Res Commun 345:508–515
139. Martín MA, Serrano AB, Ramos S, Izquierdo-Pulido M, Bravo L, Goya L (2010) Cocoa flavonoids up-regulate antioxidant enzyme activity via the ERK1/2 pathway to protect against oxidative stress-induced apoptosis in HepG2 cells. J Nutr Biochem 21:196–205
140. Ramos S, Moulay L, Granado-Serrano AB, Vilanova O, Muguerza B, Goya L, Bravo L (2008) Hypolipidemic effect in cholesterol-fed rats of a soluble fiber-rich product obtained from cocoa husks. J Agric Food Chem 56:6985–6993
141. Granado-Serrano AB, Martín MA, Izquierdo-Pulido M, Goya L, Bravo L, Ramos S (2007) Molecular mechanisms of (−)-epicatechin and chlorogenic acid on the regulation of the apoptotic and survival/proliferation pathways in a human hepatoma cell line. J Agric Food Chem 55:2020–2027
142. Noé V, Peñuelas S, Lamuela-Raventós RM, Permanyer J, Ciudad CJ, Izquierdo-Pulido M (2004) Epicatechin and a cocoa polyphenolic extract modulate gene expression in human Caco-2 cells. J Nutr 134:2509–2516
143. Clarke RB (2003) p27KIP1 phosphorylation by PKB/Akt leads to poor breast cancer prognosis. Breast Cancer Res 5:162–163
144. Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, Franklin RA, McCubrey JA (2003) Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. Leukemia 17:590–603
145. Sun B, Karin M (2008) NF-kB signaling, liver disease and hepatoprotective agents. Oncogene 27:6228–6244
146. Jeong WS, Kim IW, Hu R, Kong AN (2004) Modulation of AP-1 by natural chemopreventive compounds in human colon HT-29 cancer cell line. Pharm Res 21:649–660

147. Mackenzie GG, Carrasquedo F, Delfino JM, Keen CL, Fraga CG, Oteiza PI (2004) Epicatechin, catechin, and dimeric procyanidins inhibit PMA-induced NF-kappaB activation at multiple steps in Jurkat T cells. FASEB J 18:167–169
148. Mackenzie GG, Adamo AM, Decker NP, Oteiza PI (2008) Dimeric procyanidin B2 inhibits constitutively active NF-kB in Hodgkin's lymphoma cells independently of the presence of IkB mutations. Biochem Pharmacol 75:1461–1471
149. Paur I, Austenaa LM, Blomhoff R (2008) Extracts of dietary plants are efficient modulators of nuclear factor kappa B. Food Chem Toxicol 46:1288–1297
150. García-Mediavilla V, Crespo I, Collado PS, Esteller A, Sánchez-Campos S, Tuñón MJ, González-Gallego J (2007) The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. Eur J Pharmacol 557:221–229
151. Sukhthankar M, Yamaguchi K, Lee SH, McEntee MF, Eling TE, Hara Y, Baek SJ (2008) A green tea component suppresses posttranslational expression of basic fibroblast growth factor in colorectal cancer. Gastroenterology 134:1972–1980
152. Tsuchiya H, Tanaka T, Nagayama M (2008) Antiproliferative effects associated with membrane lipid interactions with green tea catechins. J Health Sci 54:576–580
153. Sánchez-del-Campo L, Tárraga A, Montenegro MF, Cabezas-Herrera J, Rodríguez-López JN (2009) Melanoma activation of 3-o-(3, 4, 5-trimethoxybenzoyl)-(−)-epicatechin to a potent irreversible inhibitor of dihydrofolate reductase. Mol Pharm 6:883–894
154. Actis-Goretta L, Romanczyk LJ, Rodriguez CA, Kwik-Uribe C, Keen CL (2008) Cytotoxic effects of digalloyl dimer procyanidins in human cancer cell lines. J Nutr Biochem 19:797–808
155. Carnesecchi S, Schneider Y, Lazarus SA, Coehlo D, Gossé F, Raul F (2002) Flavanols and procyanidins of cocoa and chocolate inhibit growth and polyamine biosynthesis of human colonic cancer cells. Cancer Lett 175:147–155
156. Ramljak D, Romanczyk LJ, Metheny-Barlow LJ, Thompson N, Knezevic V, Galperin M, Ramesh A, Dickson RB (2005) Pentameric procyanidin from *Theobroma cacao* selectively inhibits growth of human breast cancer cells. Mol Cancer Ther 4:537–546
157. Peng G, Dixon DA, Muga SJ, Smith TJ, Wargovich MJ (2006) Green tea polyphenol (−)-epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. Mol Carcinog 45:309–319
158. Park JB (2007) Caffedymine from cocoa has COX inhibitory activity suppressing the expression of a platelet activation marker, P-selectin. J Agric Food Chem 55:2171–2175
159. Nandakumar V, Singh T, Katiyar SK (2008) Multi-targeted prevention and therapy of cancer by proanthocyanidins. Cancer Lett 269:378–387
160. Ganguly C, Saha P, Panda CK, Das S (2005) Inhibition of growth, induction of apoptosis and alteration of gene expression by tea polyphenols in the highly metastatic human lung cancer cell line NCI-H460. Asian Pac J Cancer Prev 6:326–331
161. Pierini R, Kroon PA, Guyot S, Johnson IT, Belshaw NJ (2008) The procyanidin-mediated induction of apoptosis and cell-cycle arrest in esophageal adenocarcinoma cells is not dependent on p21Cip1/WAF1 R. Cancer Lett 270:234–241
162. Pierini R, Kroon PA, Guyot S, Ivory K, Johnson IT, Belshaw NJ (2008) Procyanidin effects on oesophageal adenocarcinoma cells strongly depend on flavan-3-ol degree of polymerization. Mol Nutr Food Res 52:1399–1407
163. Kozikowski AP, Tückmantel W, Böttcher G, Romanczyk LJ (2003) Studies in polyphenol chemistry and bioactivity. 4. Synthesis of trimeric, tetrameric, pentameric, and higher oligomeric epicatechin-derived procyanidins having all-4β, 8-interflavan connectivity and their inhibition of cancer cell growth through cell cycle arrest. J Org Chem 68:1641–1658
164. Shang XJ, Yao G, Ge JP, Sun Y, Teng WH, Huang YF (2009) Procyanidin induces apoptosis and necrosis of prostate cancer cell line PC-3 in a mitochondrion-dependent manner. J Androl 30:122–126
165. Mojzis J, Varinska L, Mojzisova G, Kostova I, Mirossay L (2008) Antiangiogenic effects of flavonoids and chalcones. Pharmacol Res 57:259–265
166. Guo Y, Wang S, Hoot DR, Clinton SK (2007) Suppression of VEGF-mediated autocrine and paracrine interactions between prostate cancer cells and vascular endothelial cells by soy isoflavones. J Nutr Biochem 18:408–417

167. Tan WF, Lin LP, Li MH, Zhang YX, Tong YG, Xiao D, Ding J (2003) Quercetin, a dietary-derived flavonoid, possesses antiangiogenic potential. Eur J Pharmacol 459:255–262
168. Zhang FJ, Yang JY, Mou YH, Sun BS, Ping YF, Wang JM, Bian XW, Wu CF (2009) Inhibition of U-87 human glioblastoma cell proliferation and formyl peptide receptor function by oligomer procyanidins (F2) isolated from grape seeds. Chem Biol Interact 179:419–429
169. Arts IC, Hollman PC (2005) Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 81:317S–325S
170. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T, Aromaa A (2002) Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 76:560–568
171. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P (2001) Flavonol and flavone intake and the risk of cancer in male smokers (Finland). Cancer Causes Control 12:789–796
172. Arts IC, Jacobs DR, Gross M, Harnack LJ, Folsom AR (2002) Dietary catechins and cancer incidence among postmenopausal women: the Iowa Women's Health Study (United States). Cancer Causes Control 13:373–382
173. Garcia-Closas R, Agudo A, Gonzalez CA, Riboli E (1998) Intake of specific carotenoids and flavonoids and the risk of lung cancer in women in Barcelona, Spain. Nutr Cancer 32:154–158
174. Stefani ED, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Carzoglio JC, Ronco A, Olivera L (1999) Dietary antioxidants and lung cancer risk: a case control study in Uruguay. Nutr Cancer 34:100–110
175. Le ML, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN (2000) Intake of flavonoids and lung cancer. J Natl Cancer Inst 92:154–160
176. Rossi M, Negri E, Talamini R, Bosetti C, Parpinel M, Gnagnarella P, Franceschi S, Dal Maso L, Montella M, Giacosa A, La Vecchia C (2006) Flavonoids and colorectal cancer in Italy. Cancer Epidemiol Biomarkers Prev 15:1555–1558
177. Lin J, Zhang SM, Wu K, Willett WC, Fuchs CS, Giovannucci E (2006) Flavonoid intake and colorectal cancer risk in men and women. Am J Epidemiol 164:644–651
178. Peterson J, Lagiou P, Samoli E, Lagiou A, Katsouyanni K, La Vecchia C, Dwyer J, Trichopoulos D (2003) Flavonoid intake and breast cancer risk: a case-control study in Greece. Br J Cancer 89:1255–1259
179. Bobe G, Weinstein SJ, Albanes D, Hirvonen T, Ashby J, Taylor PR, Virtamo J, Stolzenberg-Solomon RZ (2008) Flavonoid intake and risk of pancreatic cancer in male smokers (Finland). Cancer Epidemiol Biomarkers Prev 17:553–562
180. Sasazuki S, Inoue M, Miura T, Iwasaki M, Tsugane S (2008) Plasma tea polyphenols and gastric cancer risk: a case-control study nested in a large population-based prospective study in Japan. Cancer Epidemiol Biomarkers Prev 17:343–351
181. Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao JT, Cai L, Cozen W, Mack TM, Lu QY, Zhang ZF (2008) Dietary flavonoid intake and lung cancer – a population-based case-control study. Cancer 112:2241–2248
182. Bayard V, Chamorro F, Motta J, Hollenberg NK (2007) Does flavanol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, stroke, diabetes mellitus, and cancer in Panama. Int J Med Sci 4:53–58

26 Maturation and Activation of Dendritic Cells by Botanicals Used in Traditional Chinese Medicine: Role in Immune Enhancement

Xin Chen

Key Points

- The therapeutic effects of traditional Chinese medicines (TCM) are usually attributed to its up- or downregulation of immune responses.
- Dendritic cells (DCs) as professional antigen-presenting cells (APCs) play a central role in the initiation and regulation of immune responses.
- A number of TCM herbal medicines or their components have *in vitro* and *in vivo* activity in promoting major functions of DCs.
- The promotion of DC function may underlie the efficacy of TCM in the treatment of immunosuppressive diseases, such as cancer and viral infection.
- These findings also provide the basis for the development of novel therapeutic agents from traditional medicinal botanicals.

Key Words: Immunology, dendritic cells, traditional chinese medicine, polysaccharide, host resistance.

26.1 INTRODUCTION

Traditional Chinese medicine (TCM) has been used for a long time in China and other Asian nations for the treatment of a wide spectrum of diseases and disorders. The clinical effects of TCM are usually attributed to its capacity in upregulating or down-regulating immune responses (1, 2) with support from recent laboratory studies. For example, our group found that Ying Zhi Huang, a multiple component TCM product with anti-inflammatory efficacy, potently inhibited the activation of T lymphocytes (3). Furthermore, we identified another two immunosuppressive TCM products with the

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_26

capacity to inhibit chemokine-induced leukocyte chemotaxis (4). This study led us to identify a number of chemical compounds from TCM with inhibitory activity on chemokines/chemokine receptors interactions (5–10).

More recently, studies aimed at understanding the action of TCM on dendritic cells (DCs) have been gaining increasing attention. Accumulating data indicate that some immunosuppressive TCM have the capacity to suppress the development and function of DCs, while other TCM that enhance host resistance to infection and tumor are able to promote the activation of DCs. The inhibitory effect of TCM on maturation and biological function of DCs, including trafficking (11), has been previously reviewed by us (12). We will therefore focus our discussion in this review to studies to botanicals or their components in TCM with the capacity to promote the maturation and antigen-presentation function of DCs.

DCs are a heterogeneous population of bone-marrow-derived myelocytic leukocytes that specialize in the uptake, processing, transport, and presentation of antigens to effector cells participating in both innate and adaptive immune systems. By constantly sampling the environmental antigens, immature DCs distributed in the peripheral tissues act as sentinels. After encounter with microbial products or damaged tissue, DCs are induced to mature and as a result upregulate their expression of CCR7 which enable them to migrate away from inflammatory sites to draining LNs. The microbial products are processed by DC and then presented on the surface of matured DC as antigenic peptides by major histocompatibility complex (MHC) molecules. In addition, the surface expression of co-stimulatory molecules is upregulated during the course of maturation, which enables DCs to become effective activators of effector lymphocytes. DCs thus initiate immunity by activating effector T and B cells to mount adaptive immune responses and by the concomitant production of cytokines which activate cells of innate immune system such as natural killer (NK) cells and macrophages (13–15). The stimulation of maturation or promotion of biological function of DCs by pharmacological agents has the potential to boost immune responses against cancer and pathogens as well as to enhance the efficacy of vaccines. It is therefore not surprising that TCM with activity to stimulate maturation and activation of DCs are mainly "Qi-enhancing" herbs with the capacity to strengthen host resistance and many of which are historically used for the treatment of cancer, as reviewed below.

26.1.1 Amomi Semen (Sha Ren)

Fuhui and colleagues screened 99 Chinese herbal medicines on the activation of DCs which led to the discovery that an extract of *amomi semen* (*amomum* seed, known in China as Sha Ren) had both *in vitro* and *in vivo* activity of activating DCs. Initially, the effects of ethanol extracts of 99 herbal medicines on MHC II expression by XS106, a murine epidermal-derived Langerhans cell line, was examined. *Amomi semen*, *Polyporus* (*Polyporus sclerotium*, Zhu Ling in Chinese), and *plantaginis semen* (*Plantago* seed, Che Qian Zi in Chinese) were found to upregulate the expression of MHC II on XS106 cells. The effects of these extracts on the phenotype (MHC class II, CD80, and CD86) and IL-12p70 production by mouse bone marrow-derived DCs (BMDCs) were examined. *Amomi semen* extract markedly induced the phenotypic maturation and production of IL-12p70 by BMDCs, which was comparable to the effect of LPS stimulation. The

immunological adjuvant effect of *amomi semen* extract was studied in the E.G7-OVA (ovalbumin) tumor model. The treatment of mice with a subcutaneous injection of BMDCs pre-incubated with *amomi semen* extract and pulsed with OVA peptide significantly inhibited the growth of tumor cells and prolonged survival compared to controls. Furthermore, therapeutic effects were also observed on established tumors. The inhibition rates for both the prophylactic and therapeutic protocols were comparable to those of lipopolysaccharides. These results indicate that *amomi semen* extract potently activates DCs and they become potentially therapeutically useful (16). The effect of extract of *amomi semen* on the activation of DCs was not based on its contamination with LPS, since the effect could not be abrogated by the treatment of polymyxin B (16). Identification of active component(s) contained in ethanol extract of *amomi semen* responsible for the activation of DCs should be addressed in a future study.

26.1.2 Astragalus Membranaceus (Huang Qi)

The root of *Astragalus membranaceus* (known in China as Huang Qi) is used in TCM to enhance host resistance and for cancer therapy. Its effects were attributed to boosting anti-tumor immunity (for review, see ref. (17)). Dong and colleagues reported that *Astragulus* Injection (AGI), an injectable preparation comprising the extract of Huang Qi, enhanced the DC-based vaccine-mediated inhibition of metastasis in a mouse tumor model. In their study, myeloid DCs from C57BL/6 mice were pre-sensitized by Mut1 (a MHC class I-restricted polypeptide tumor antigen expressed by Lewis lung cancer). These DCs were then used to treat mice with metastatic lung cancer in combination with AGI or IL-2. After being treated with tumor antigen polypeptide sensitized DCs plus AGI or IL-2, the size of lung cancer modules decreased, the proportion of subsets $CD4^+$ and $CD8^+$ T cells in mouse spleen increased, and the IL-2/IL-4 ratio in serum also increased significantly. During the observation period, the growth rate of tumor in mice treated with DCs combined with either IL-2 or AGI, was lower than that in mice treated with DCs alone (18).

The pharmacological activity of *Astragulus* is likely due to polysaccharide fractions (19, 20). Shao and colleagues reported that treatment with *Astragulus* polysaccharide (ASP, 10–250 μg/ml) upregulated the surface expression of CD11c and MHC II and increased the production of IL-12, while decreasing the phagocytic capacity of murine BMDC (21). Polysaccharides from *astragalus* also are reported to bind directly to TLR4 on macrophages (22). Thus, activation of DC by this polysaccharide may also be mediated by TLR4.

26.2 BU ZHONG YI QI TANG (OR HOCHU-EKKI-TO)

Bu Zhong Yi Qi Tang (BZYQT), also known as Hochu-ekki-to (HOT) in Japan, is a TCM herbal preparation extracted from ten herbal plants by boiling them in water. BZYQT is one of the most popular traditional herbal remedy used in Southeast Asian nations and is prescribed mainly for chronic fatigue, immune deficiency, and malnourished patients (23). BZYQT has various biological effects, including enhancing immune response. Preliminary clinical data suggest that BZYQT may be beneficial for tumor patients, presumably by boosting anti-tumor immunity (24). BZYQT was reported to

promote antitumor immune responses in mice by inducing tumor-specific type 1 cytokine production (25, 26) and to stimulate GM-CSF and TNF production by peripheral blood mononuclear cells (PBMC) from both healthy subjects and hepatocellular carcinoma patients (27). This TCM was also reported to increase IL-18-induced ICAM-1 and B7.2/CD86 expression as well as TNF and IFNγ production by human PBMC (28).

Nabeshima and colleagues examined the effect of this herbal remedy on the maturation of DC. In their study, immature human monocyte-derived DCs (MoDCs) were stimulated with BZYQT, TNF, or LPS (BZYQT-DC, TNF-DC, and LPS-DC, respectively) for 2 days. Flow cytometric analysis showed that BZYQT dose-dependently stimulated DC to express the surface maturation markers CD80, CD83, and CD86 and was comparable to the effects of TNF and LPS. Similar to LPS-DC, BZYQT-DC reduced their albumin uptake capacity and exhibited potent allogeneic stimulatory activity. However, IL-12 p70 production by BZYQT-DC and TNF-DC was lower than that by LPS-DC (23). Thus BZYQT has the capacity to stimulate the maturation of DC and therefore may be beneficial for the establishment of anti-tumor immunity.

26.2.1 Ganoderma Lucidum (Ling Zhi or Reishi)

Ganoderma lucidum (GL), known in China as Ling Zhi and Japan as Reishi, is a medicinal mushroom used in Asia as a tonic in promoting longevity and health. GL has been reported to boost immune responses and consequently has anti-tumor and antiviral effects (29). Various components isolated from GL, such as polysaccharides, protein (LZ-8), and triterpene were found to promote the maturation of DCs.

26.2.1.1 Polysaccharides

The polysaccharides present in GL have been extensively studied by the immunologists, and its capacities to activate antigen-presenting cells (APCs), mononuclear phagocytes as well as T and B lymphocytes have been documented (for review, see ref. (30)).

Lin and colleagues reported that the development of MoDC with polysaccharide from GL (PS-G) enhanced the surface expression of CD80, CD86, CD83, CD40, CD54, HLA-DR and the production of IL-12p70, p40, and IL-10. PS-G treatment inhibited endocytosis by DC and increased the capacity of DC to activate T cells. Antibody against TLR4 inhibited the PS-G-induced activation of DC, suggesting a vital role of TLR4 in the effect of PS-G. Furthermore, PS-G treatment resulted in the activation of IκB kinase, NF-κB, and the increase of MAP kinase phosphorylation. The inhibition of NF-κB by helenalin and p38 MAPK by SB98059 prevented PS-G-induced activation of DC (31). Thus, PS-G is able to activate DC by a NF-κB and MAPK pathway. The evidence that toll-like receptors are utilized by PS-G is supported by studies showing that TLR4 was used by PS-G to activate macrophages (32–34), and TLR4/TLR2 was used by PS-G to activate B cells (34, 35). Furthermore, these effects of PS-G were reportedly not mediated by any contaminating LPS (32). The same group further examined the effects of PS-G on human MoDC with microarray analysis. In comparing mean signal values between PS-G-treated DCs with untreated DCs, 3,477 (17%) probe sets were upregulated, and 4,418 (19%) probe sets were downregulated by PS-G treatment. These results demonstrate that genes associated with phagocytosis (CD36, CD206, and CD209) are decreased and genes associated with proinflammatory chemokines (CCL20,

CCL5, and CCL19), cytokines (IL-27, IL-23A, IL-12), and co-stimulatory molecules (CD40, CD54, CD80, and CD86) were increased. To confirm the microarray data, they further investigated the effect of PS-G on antigen-specific antibody and cytokine production. Immunization with ovalbumin (OVA)/PS-G showed that the anti-OVA IgG2a levels were increased compared with OVA alone in BALB/c mice (36). These data demonstrate that PS-G could effectively promote the activation and maturation of immature DCs which have the capacity to preferentially stimulate Th1 responses.

Cao and colleagues examined the effect of GL-P on the generation of tumor-specific cytotoxic T lymphocytes (CTL) by DCs. Mouse BMDCs were pulsed with P815 tumor cell lysate in the presence of GL-PS (0.8–12.8 μg/ml). P815 specific CTL were induced by splenic lymphocytes stimulated with mature DC, as shown by the increased activity of Lactate dehydrogenase (LDH) and enhanced production of IFNγ and Granzyme B (37).

Chan and colleagues compared the effects of GL mycelium extract (GL-M) and spore extracts (GL-S) on human PBMC and MoDC. They found that GL-M (10 μg/ml, 72 h) induced ~fourfold increase in the proliferation of PBMCs as compared with an untreated control. In contrast, GL-S suppressed the proliferation of PBMCs and induced cellular apoptosis. Both extracts stimulated Th1 and Th2 cytokine mRNA expression, but GL-M was a relatively more potent Th1 stimulator. Unlike GL-S, GL-M enhanced the maturation of DCs including upregulation of CD40, CD80, and CD86, and reduction in endocytosis of fluorescein isothiocyanate-dextran. However, GL-M-treated DCs only modestly enhanced lymphocyte proliferation in allogeneic mixed lymphocyte culture with a low degree of enhancement in the effector function of T cells (38). This group further compared the effects of different sources of polysaccharides. They found that pure GL mycelium polysaccharides (GL-P) markedly enhanced the proliferation of PBMCs and induced the phenotypic and functional maturation of MoDC with the production of high levels of IL-12 and IL-10. In contrast, GL spore and barley polysaccharides-derived pure glucan did not show stimulatory activity on DC (39). Thus, polysaccharides from GL mycelium have a unique stimulatory effect on DC maturation.

26.2.1.2 Zhi-8

LZ-8 is a protein derived from GL with immunomodulatory capacities (40, 41). Lin and colleagues examined the effects of rLZ-8 derived from *yeast* on human monocyte-derived DCs. The treatment of DC with rLZ-8 (5–50 μg/ml, 24–48 h) enhanced cell-surface expression of CD80, CD86, CD83, and HLA-DR, as well as the production of IL-12 p40, IL-10, and IL-23, and was associated with the suppression of endocytosis. The effect of rLZ-8 was not due to contamination by LPS, since proteinase K abrogated the effect of rLZ-8 on DCs. In addition, the treatment of DCs with rLZ-8 increased allolymphocyte stimulatory capacity. Naïve T cells stimulated with rLZ-8-treated DCs produced higher levels of IFNγ and IL-10. Neutralization with antibodies against TLR4 inhibited the rLZ-8-induced production of IL-12 p40 and IL-10 in DCs. Furthermore, rLZ-8 can stimulate TLR4 or TLR4/MD2-transfected HEK293 cells to produce IL-8, suggesting that TLR4 is used by LZ-8 to stimulate DCs. rLZ-8 was able to augment IKK, NF-κB activity, and also IκBα and MAPK phosphorylation. Further, the inhibition of NF-κB by helenalin prevented the effects of rLZ-8 to various degrees. To confirm the *in vitro* data, the effect of rLZ-8 on antigen-specific antibody and cytokine production in BALB/c mice was investigated. Immunization with OVA/rLZ-8 resulted

in markedly higher levels of anti-OVA IgG2a, IFNγ, and IL-2, as compared with OVA alone (42). Thus, in addition to polysaccharides, GL-derived proteins such as LZ-8 also promote the activation and maturation of immature DCs.

In addition, Wang and colleagues reported that ganoderma triterpene (40–200 μg/ml) stimulated the proliferation of DC derived from mouse spleens. Furthermore, ganoderma triterpene enhanced the GM-CSF/IL-4-mediated development of DCs (43) and thus may promote the differentiation of DCs.

26.2.2 Panax Ginseng (Ren Shen)

Ginseng (in China known as Ren Shen) is one of the most widely used herbal medicines in TCM and other traditional medicines and reportedly has a wide range of therapeutic and pharmacological activities, including the capacity to boost immune responses (17, 44). The metabolites of saponin and polysaccharides present in *ginseng* were shown to have the capacity to activate DCs.

26.2.2.1 Ginsenoside

Ginsenosides is the major pharmacologically active ingredient of *ginseng* and is responsible for most of the activities of *ginseng* (44). Orally administered ginsenosides are promptly metabolized in the digestive tract and absorbed into circulation (45). The inhibition of lung metastasis by ginsenosides is actually mediated by the intestinal bacterial metabolite M1 derived from protopanaxadiol saponins of *ginseng* (45). Takei and colleagues investigated the effects of M1 and M4, the end products of metabolized steroidal *ginseng* saponins in digestive tracts, on the maturation of DCs *in vitro*. Human monocytes were cultured with GM-CSF and IL-4 for 6 days, followed by another 2 days in the presence of M1, M4, or TNF as a maturation stimulus. Stimulation with 1–20 μM of M1 or M4 increased the expression levels of HLA-DR, CD1a, CD80, CD83, and CD86, accompanied with decreased endocytotic activity. DCs primed with M1 and M4 produced high levels of IL-12 and IL-6 upon CD40-L stimulation. M1- and M4-treated DCs acquired the expression of CCR7 and migrated in response to MIP-3β. M4-matured DCs also displayed enhanced T cell stimulatory capacity in an MLR (mixed lymphocyte reaction), as measured by T cell proliferation. Furthermore, M1- and M4-mediated maturation of DCs endows them with the capacity to polarize $CD4^+CD45RA^+$ naive T cells toward Th1 cells, as shown by the production of high levels of IFNγ and low levels of IL-4, IL-5, and IL-10. M4-treated DCs had the capacity to increase the cytotoxicity of CD8 cells (46). The effect of M1 and M4 on the maturation of DCs was reportedly not based on LPS contamination (46). These results suggest that the effect of metabolites of ginsenosides on the maturation of DCs and promotion of Th1 responses may contribute to their anticancer activity (45).

26.2.2.2 Polysaccharides

Polysaccharides isolated from *Panax ginseng* have been shown to activate immune cells such as macrophages, B cells, and T cells (47), and promote the differentiation of anti-tumor Th1 responses (48, 49). Kim and colleagues found that the *in vitro* treatment of *ginseng* polysaccharides (0.1–10 μg/ml) increased the viability of mouse bone marrow cells following gamma radiation. Furthermore, *ginseng* polysaccharides also enhanced the production of IL-12, expression of MHC II, co-stimulatory molecule

(CD86) and allostimulatory activity. *In vivo* administration of *ginseng* polysaccharides (100 mg/kg) to mice markedly increased the number of bone marrow cells in gamma-irradiated mice. These bone marrow cells from *ginseng* polysaccharide-treated mice included DC progenitors, since DCs could develop *in vitro* culture following irradiation (50). Presumably, the crude extract of *ginseng* comprises both ginsenosides and polysaccharides, and these two components may have additive or even a synergistic effect in the activation of DCs.

26.2.3 Lycium Barbarum (Guo Ji Zi)

The fruit of *Lycium Barbarum* L. (*L. Barbarum*, also known as wolfberry, and Guo Ji Zi in China), is a commonly used Chinese herbal medicine as well as tonic food dietary supplement and is used in the treatment of diseases such as insomnia, liver dysfunction, diabetes, visual degeneration, and male infertility (44, 51). The polysaccharide–protein complex (LBP) is a major active component of *L. barbarum* (52, 53). Laboratory studies revealed a wide spectrum of pharmacological activities of LBP. For example, it has been reported that LBP has antioxidative activity (42), antagonizes glutamate excitotoxicity in rat cortical neurons (54), attenuates radio- and chemotherapy-induced myelosuppression (51), induces human hepatoma apoptosis, and inhibits hepatoma proliferation (55). Nevertheless, the *in vitro* and *in vivo* stimulatory activity on immune competent cells appears to be the most profound biological activity of LBP (52, 56, 57). It has been shown that BPL affects the activation of different lineage of immune cells, including lymphocytes (58), DCs (44) and macrophages (59). Duan and colleagues found four polysaccharides derived from LBP-enhanced splenocyte proliferation induced by Con A (concanavalin A). Polysaccharides containing an alpha-(1→4)-d-polygalacturonans backbone showed greater immunostimulatory activity (60).

Chen and colleagues separated LBP into five homogeneous fractions, designated LBPF1, LBPF2, LBPF3, LBPF4, and LBPF5 and found that LBP, LBPF4, and LBPF5 markedly stimulated the proliferation of mouse splenic $CD3^+$ T cells, but not $CD19^+$ B cells in concentration ranges of 1–300 μg/ml ($P < 0.05$–0.01), thus excluding the effect of potentially contaminating LPS, which is a potent stimulant of B cell proliferation (58). In addition, LBP (50 mg/kg, i.p.) markedly upregulated the expressions of CD40, CD80, CD86, and MHC class II molecules on peritoneal macrophages (59). The same group also found that all five fractions of LBP (LBPF1–5) and LBP itself were able to stimulate the phenotypic and functional maturation of DCs in a dose-dependent manner (1–1,000 μg/ml). BMDC treated with LBP and its fractions *in vitro* resulted in the upregulation of surface expression of MHC II and co-stimulatory molecules (CD40, CD80 and CD86), in association with an increase in allo-stimulatory activity and decreased endocytotic capacity. LBP treatment markedly stimulated the production of IL-12 (p40 and p70) and upregulated IL-12p40 mRNA on BMDCs. DCs matured by LBP *in vitro* acquired the capacity to enhance Th1 and Th2 responses *in vitro* as well as *in vivo* after transfer to mice. Importantly, the administration of LBP *in vivo* (s.c., i.p., or p.o.) also markedly increased the expression of MHC II and co-stimulatory molecules on splenic DCs and primed Th1 responses in Balb/c mice. The extremely low levels of endotoxin (<1.0 EU/ml) found in LBP and its fractions mitigate against a possible role of contaminating LPS in the maturation of DCs (44).

The anticancer activity of LBP is associated with its stimulatory activity on immune cells. Gan and colleagues reported that that LBP3p, the third fraction of LBP, at an optimal dosage of 10 mg/kg (p.o., 10 day once daily) inhibited the growth of sarcoma S180 solid tumor by 43.05%, which was comparable to the 47.77% inhibition exerted by cyclophasphamide (20 mg/kg, p.o., 10 day once daily) (61). Moreover, S180 tumor inoculation inhibited phagocytosis by macrophages, Ab production, lymphocyte proliferation, CTL activity as well as IL-2 expression, which could be restored by the administration of LBP3p (61). This group also reported that LBP3p at 5–40 μg/ml markedly stimulated the production of IL-2 and TNF and gene expression by *in vitro* cultured human PBMC (up to 14-fold) (62). He and colleagues examined the effect of LBP on tumor-infiltrating lymphocytes and DCs in the mouse H22 hepatoma model. Tumor-bearing mice given low dose (0.625 g/kg) or high dose (1.25 g/kg) of LBP orally for 2 weeks showed 23.79% and 41.12% of tumor growth inhibition. LBP treatment also resulted in an increase of tumor-infiltrating CD4 and CD8 cells ($P < 0.05$). The administration of LBP also increased the number of tumor-infiltrating DCs and their expression of CD80 (63). Presumably, the activation of CD4 and CD8 T cells was triggered by LBP-activated DCs.

Zhu and colleagues examined the direct effect of LBP on the maturation of *in vitro* cultured mouse BMDCs. $CD11c^+$ cells were MACS purified from GM-CFS/IL-4-treated BMDC and treated with LBP (100 μg/ml) for 2 days. LBP treatment increased the expression of MHC II, CD11c and the production of IL-12p40. The functional maturation of DCs was also promoted by LBP-treatment, since LBP-treated DCs exhibited a reduced endocytic capacity and increased proliferative effect on naive allogeneic T cells (64). Thus, LBP promotes the generation of functionally active, mature DC. Since tumor-infiltrating DCs usually have a reduced expression of co-stimulatory molecules and cytokine production (65), and a reduced number of the tumor-infiltrating DCs are associated with poor prognosis (66), the stimulatory effect of LBP on DCs presumably has beneficial adjuvant anti-tumor effects.

26.2.4 Plantago Asiatica L. (Che Qian Zi)

Plantago asiatica L. leaves have been used as a wound-healing remedy and for the treatment of diseases involved in the infection of skin, respiratory tract, digestive tract, reproduction in TCM, and other traditional medical systems (67). The seeds of *Plantago asiatica* L. (Che Qian Zi in Chinese) have antipyretic, diuretic (68), and expectorant (67) activity. Huang and colleagues examined the effect of an ethanol and aqueous extract of the seeds of *Plantago asiatica* L. (ES-PL) on the maturation of mouse BMDCs. The results showed that at concentration ranges of 10–200 μg/ml, ES-PL dose-dependently upregulated the expression of MHC II and co-stimulatory molecules (CD80 and CD86) on DCs. The functional maturation of DCs treated with ES-PL was confirmed by decreased mannose receptor-mediated endocytosis and increased antigen-presenting stimulation of allogeneically naïve or syngeneically T lymphocytes. CCR7 mRNA expression by DCs treated with ES-PL was also enhanced. The ES-PL was pretreated with polymyxin B to exclude the potential contamination of LPS (69). The same groups further examined the phenylethanoid glycosides and polysaccharides isolated from the seeds of *Plantago asiatica L* which contained only trace levels of LPS

(≤ 0.0625 EU/mg). Compared with untreated cells, dendritic cells treated with acteoside, isoacteoside, or polysaccharides (50 μg/ml, each, 48 h) expressed a higher level of MHC class II molecule and the costimulatory molecule CD86 (B7-2). Furthermore, DCs activated by these compounds exhibited a decreased capacity to endocytose and increased ability to stimulate the activation of naïve T cells. These results showed that acteoside, isoacteoside, and polysaccharides from the seed of *Plantago asiatica* L. are responsible for the ability of crude extract to mature dendritic cells (70). The result of these studies are consistent with results from another group obtained by testing ethanol extract from seeds of *Plantago asiatica* L (16).

26.3 NON-TCM HERBAL MEDICINE

Some herbal medicine derived from medical traditions other than TCM were also reported to activate DCs. For example, *Echinacea purpurea*, a naïve North American herbal medicine which is popularly used as a food supplement in the US for enhancing immune response (for review, see ref. (17)). Wang and colleagues found that plant extracts from root [R] and stem plus leaf [S + L] tissues of *Echinacea purpurea* exhibited opposing (enhancing vs. inhibitory) effects on the expression of the CD83 marker of human DCs. DNA microarray analysis revealed that [S + L]-treated DCs exhibited decreased mRNA expression of specific chemokines (e.g., CCL3 and CCL8) and their receptors (e.g., CCR1 and CCR9). Other chemokines and regulatory molecules (e.g., CCL4 and CCL2) involved in the c-Jun pathway were upregulated in [R]-treated DCs (71). These results suggest that *Echinacea purpurea* extracts can promote DC differentiation and expression of specific immune-related genes. Thus, besides TCM, herbal medicines derived from other traditional medical systems also have the capacity to affect the biological function of DCs.

26.4 CONCLUSIONS AND PERSPECTIVES

Accumulating evidence presented in this review indicate that numerous TCM and their components have the capacity to stimulate the maturation and function of antigen-presenting DCs, as summarized in the Table 26.1. These *in vitro* and laboratory animal studies reveal that TCM has the capacity to rapidly boost immune responses by activating DCs, suggesting the therapeutic potential of Chinese herbal remedies in immunosuppressive diseases such as cancer and viral infection. These findings also provide the basis for the discovery of novel therapeutic agents from botanicals used in TCM.

Polysaccharides from various traditional medicinal herbs have been shown to boost immune responses both *in vitro* as well as *in vivo* (10, 19, 21, 72–78) in animal models, including the activation of DCs as discussed in this review. Polysaccharides and polysaccharide–protein complexes isolated from mushrooms, fungi, yeasts, algae, lichens, and plants, have been shown to have immune stimulatory and anti-tumor effects (79). These naturally occurring polysaccharides consist of a class of macromolecules that can profoundly affect the immune system and therefore have the potential to act as immunostimulators (80). It is well known that beta-glucans is responsible for the immune effects of many herbal-derived polysaccharides complexes. For example, LBP is a beta-glucan in chemical nature (44). Beta-glucans are ubiquitously found in both

Table 26.1
Effects of botanicals used in traditional Chinese medicine on the activation of dendritic cells

TCM herb	*Compound*	*LPS level or treatment*	*Action on DC*	*Putative receptor*
Amomi semen (Sha Ren)	Ethanol extract (16)	Polymyxin B	1. Increase MHC II expression on a murine epidermal-derived Langerhans cell line (XS106) 2. Upregulate expression of MHC II and co-stimulatory molecules on BMDC 3. Stimulate production of IL-12 4. Enhance efficacy of DC-based tumor vaccine and inhibit tumor growth	
Astragalus membranaceus (Huang Qi)	*Astragulus* injection (18)		Enhanced DC-based vaccine-mediated inhibition of metastasis in a mouse tumor model	
	Polysaccharides (21)		1. Upregulate MHC and co-stimulatory molecules 2. Stimulate production of cytokines and chemokine	
Bu Zhong Yi Qi Tang (Hochu-ekki-to) (23)			1. Induces expression of co-stimulatory molecules 2. Enhances allogeneic stimulatory activity 3. Reduces the capacity of endocytosis 4. Induce modest production of IL-12	

Ganoderma lucidum (Ling Zhi or Reishi)	Mycelium extract (GL-M) (38)	0.00003% of 1 pg/ml (10 μg/ml of GL-M)	1. Stimulate proliferation of PBMCs and production of Th1 cytokine 2. Upregulation of CD40, CD80, and CD86 3. Decrease endocytosis 4. Modestly enhances lymphocyte stimulatory capacity	
	Polysaccharides	<1 ng/25 μg; polymyxin B (32) <1 pg/ml (39)	1. Upregulates MHC and co-stimulatory molecules (31, 36, 39) 2. Stimulates production of cytokines and chemokines (31, 36, 39) 3. Decreases endocytosis (31, 36, 39) 4. Stimulates proliferation of DCs (43) 5. Promotes Th1 responses *in vivo* (36) 6. Activates NF-κB and MAPK signaling pathway (31)	TLR4 (31–35)
	LZ-8 (85)	<1.0 EU/μg	1. Upregulate MHC and co-stimulatory molecules 2. Stimulate production of cytokines 3. Enhance lymphocyte stimulatory capacity 4. Increase IKK, NF-κB activity, and IκBα and MAPK phosphorylation 5. *In vivo* administration stimulate production of Ag-specific Ab and cytokines (IL-2, IFNγ)	TLR4
	Triterpene (43)		Stimulate proliferation and differentiation of DCs	

(continued)

Table 26.1
(continued)

TCM herb	*Compound*	*LPS level or treatment*	*Action on DC*	*Putative receptor*
Panax ginseng (Ren Shen)	Saponin metabolites (M1, M4) (46)	<1.0 EU/ml	1. Upregulate MHC and co-stimulatory molecules 2. Promote CD40L-mediated production of cytokines 3. Increase expression of CCR7 and migration to MIP-3β 4. Enhance lymphocyte stimulatory capacity 5. Polarize naïve CD4 T cells towards Th1 6. Increase cytotoxicity of CD8 cells	
	Polysaccharides (50)		1. Upregulate MHC and co-stimulatory molecules 2. Stimulate production of IL-12 3. *In vivo* administration increase the number of DC progenitors in bone marrow	
Lycium barbarum (Guo Ji Zhi)	Polysaccharide–protein complex (LBP)		1. Upregulate MHC II and co-stimulatory molecules (44, 63) 2. Stimulate production of IL-12 (44) 3. Enhance lymphocyte stimulatory capacity (44) 4. Decrease endocytosis (44) 5. Have *in vivo* activity in the maturation of DCs and prime Th1 responses (44) 6. Increase DC infiltration in tumor (63)	

Plantago asiatica L. (Che Qian Zi)	Ethanol extract (16)	Polymyxin B	1. Increase MHC II expression on a murine epidermal-derived Langerhans cell line (XS106) 2. Upregulate expression of MHC II and co-stimulatory molecules on BMDC	
	Ethanol and aqueous extract (69)	Polymyxin B	1. Upregulate expression of MHC II and co-stimulatory molecules 2. Decrease endocytosis 3. Increase allostimulation 4. Upregulate expression of CCR7 mRNA	
	Phenylethanoid glycosides and polysaccharides (70)	≤0.0625 EU/mg	1. Upregulate expression of MHC II and co-stimulatory molecules 2. Decrease endocytosis 3. Increase allostimulation	
Polyporus sclerotium (Zhu Ling)	Ethanol extract (16)	Polymyxin B	1. Increase MHC II expression on a murine epidermal-derived Langerhans cell line (XS106) 2. Upregulate expression of MHC II and co-stimulatory molecules on BMDC	

bacterial or fungal cell walls and have been implicated in the initiation of antimicrobial immune response (81, 82). This compound acts on several immune receptors including Dectin-1, complement receptor (CR3) and TLR-2/6 and activates a group of immune cells including macrophages, neutrophils, monocytes, natural killer cells, and dendritic cells (83). Therefore, future studies should examine whether these immune receptors used by beta-glucan to activate DCs are also used by TCM-derived polysaccharides. If that is the case, the advantage of using TCM-derived beta-glucan/polysaccharides as stimulators of DCs should be compared with similar compounds from other sources. Furthermore, whether DCs are the primary target of these herbal polysaccharides should be more carefully studied, since beta-glucan receptors are widely distributed on other immune competent cells. In addition, more mechanistic studies will be needed to elucidate the molecular basis of DC-activating action of TCM.

DCs represent a heterogeneous population of antigen-presenting cells, and species differences exist, therefore it is crucial to characterize the action of TCM on a defined subset of human DC. Studies aimed at documenting the stimulatory action of TCM and their components on the activation of DCs need to be carefully controlled to rule out the potential contamination of LPS in the tested samples, especially for those in which the TLR4 pathway is proposed to be involved. Furthermore, the relevant physiological or pharmacological concentrations of TCM should be used in *in vitro* studies and appropriate *in vivo* modeling system should be developed to verify the *in vivo* relevance of the observations. In addition to initiating the immune response, mature DCs also have the capacity to expand $CD4^{+}FoxP3^{+}$ regulatory T cells (Tregs) (84). Thus, whether TCM-activated DCs stimulate the expansion of Tregs and as a result induce immune tolerance should be addressed carefully in future studies.

Acknowledgments The author thanks Dr. Joost J Oppenheim for his critical review of the manuscript. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN26120080001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the U.S. Government. This Research was supported [in part] by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

REFERENCES

1. Borchers AT, Hackman RM, Keen CL, Stern JS, Gershwin ME (1997) Complementary medicine: a review of immunomodulatory effects of Chinese herbal medicines. Am J Clin Nutr 66(6):1303–1312
2. Borchers AT, Sakai S, Henderson GL et al (2000) Shosaiko-to and other Kampo (Japanese herbal) medicines: a review of their immunomodulatory activities. J Ethnopharmacol 73(1–2):1–13
3. Chen X, Krakauer T, Oppenheim JJ, Howard OM (2004) Yin zi huang, an injectable multicomponent chinese herbal medicine, is a potent inhibitor of T-cell activation. J Altern Complement Med 10(3):519–526
4. Chen X, Howard OM, Yang X, Wang L, Oppenheim JJ, Krakauer T (2002) Effects of Shuanghuanglian and Qingkailing, two multi-components of traditional Chinese medicinal preparations, on human leukocyte function. Life Sci 70(24):2897–2913
5. Chen X, Beutler JA, McCloud TG et al (2003) Tannic acid is an inhibitor of CXCL12 (SDF-1alpha)/CXCR4 with antiangiogenic activity. Clin Cancer Res 9(8):3115–3123

6. Chen X, Oppenheim J, Howard OM (2001) Shikonin, a component of antiinflammatory Chinese herbal medicine, selectively blocks chemokine binding to CC chemokine receptor-1. Int Immunopharmacol 1(2):229–236
7. Chen X, Oppenheim JJ, Howard OM (2004) Chemokines and chemokine receptors as novel therapeutic targets in rheumatoid arthritis (RA): inhibitory effects of traditional Chinese medicinal components. Cell Mol Immunol 1(5):336–342
8. Chen X, Yang D, Shen W et al (2000) Characterization of chenodeoxycholic acid as an endogenous antagonist of the G-coupled formyl peptide receptors. Inflamm Res 49(12):744–755
9. Chen X, Yang L, Oppenheim JJ, Howard MZ (2002) Cellular pharmacology studies of shikonin derivatives. Phytother Res 16(3):199–209
10. Chen X, Yang L, Zhang N et al (2003) Shikonin, a component of chinese herbal medicine, inhibits chemokine receptor function and suppresses human immunodeficiency virus type 1. Antimicrob Agents Chemother 47(9):2810–2816
11. Chen X, Murakami T, Oppenheim JJ, Howard OM (2005) Triptolide, a constituent of immunosuppressive Chinese herbal medicine, is a potent suppressor of dendritic-cell maturation and trafficking. Blood 106(7):2409–2416
12. Chen X, Yang L, Howard OM, Oppenheim JJ (2006) Dendritic cells as a pharmacological target of traditional Chinese medicine. Cell Mol Immunol 3(6):401–410
13. Steinman RM (1991) The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 9:271–296
14. Banchereau J, Briere F, Caux C et al (2000) Immunobiology of dendritic cells. Annu Rev Immunol 18:767–811
15. Hart DN (1997) Dendritic cells: unique leukocyte populations which control the primary immune response. Blood 90(9):3245–3287
16. Fukui H, Mitsui S, Harima N et al (2007) Novel functions of herbal medicines in dendritic cells: role of Amomi Semen in tumor immunity. Microbiol Immunol 51(11):1121–1133
17. Block KI, Mead MN (2003) Immune system effects of echinacea, ginseng, and astragalus: a review. Integr Cancer Ther 2(3):247–267
18. Dong JC, Dong XH (2005) Comparative study on effect of Astragulus injection and interleukin-2 in enhancing anti-tumor metastasis action of dendrite cells. Zhongguo Zhong Xi Yi Jie He Za Zhi 25(3):236–239
19. Shimizu N, Tomoda M, Kanari M, Gonda R (1991) An acidic polysaccharide having activity on the reticuloendothelial system from the root of Astragalus mongholicus. Chem Pharm Bull (Tokyo) 39(11):2969–2972
20. Bedir E, Pugh N, Calis I, Pasco DS, Khan IA (2000) Immunostimulatory effects of cycloartane-type triterpene glycosides from astragalus species. Biol Pharm Bull 23(7):834–837
21. Shao P, Zhao LH, Zhi C, Pan JP (2006) Regulation on maturation and function of dendritic cells by Astragalus mongholicus polysaccharides. Int Immunopharmacol 6(7):1161–1166
22. Shao BM, Xu W, Dai H, Tu P, Li Z, Gao XM (2004) A study on the immune receptors for polysaccharides from the roots of *Astragalus membranaceus*, a Chinese medicinal herb. Biochem Biophys Res Commun 320(4):1103–1111
23. Nabeshima S, Murata M, Hamada M, Chong Y, Yamaji K, Hayashi J (2004) Maturation of monocyte-derived dendritic cells by Hochu-ekki-to, a traditional Japanese herbal medicine. Int Immunopharmacol 4(1):37–45
24. Satoh H, Ishikawa H, Ohtsuka M, Sekizawa K (2002) Japanese herbal medicine in patients with advanced lung cancer: prolongation of survival. J Altern Complement Med 8(2):107–108
25. Harada M, Seta K, Ito O et al (1995) Concomitant immunity against tumor development is enhanced by the oral administration of a kampo medicine, Hochu-ekki-to (TJ-41: Bu-Zhong-Yi-Qi-Tang). Immunopharmacol Immunotoxicol 17(4):687–703
26. Utsuyama M, Seidlar H, Kitagawa M, Hirokawa K (2001) Immunological restoration and anti-tumor effect by Japanese herbal medicine in aged mice. Mech Ageing Dev 122(3):341–352
27. Kao ST, Yang SL, Hsieh CC, Yang MD, Wang TF, Lin JG (2000) Immunomodulation of Bu-Zhong-Yi-Qi-Tang on in vitro granulocyte colony-stimulating-factor and tumor necrosis factor-alpha production by peripheral blood mononuclear cells. Immunopharmacol Immunotoxicol 22(4):711–720

28. Tamura R, Takahashi HK, Xue D et al (2004) Enhanced effects of combined bu-zhong-yi-qi-tang (TJ-41) and interleukin-18 on the production of tumour necrosis factor-alpha and interferon-gamma in human peripheral blood mononuclear cells. J Int Med Res 32(1):25–32
29. Miyazaki T, Nishijima M (1981) Studies on fungal polysaccharides. XXVII. Structural examination of a water-soluble, antitumor polysaccharide of *Ganoderma lucidum*. Chem Pharm Bull (Tokyo) 29(12):3611–3616
30. Lin ZB (2005) Cellular and molecular mechanisms of immuno-modulation by *Ganoderma lucidum*. J Pharmacol Sci 99(2):144–153
31. Lin YL, Liang YC, Lee SS, Chiang BL (2005) Polysaccharide purified from *Ganoderma lucidum* induced activation and maturation of human monocyte-derived dendritic cells by the NF-kappaB and p38 mitogen-activated protein kinase pathways. J Leukoc Biol 78(2):533–543
32. Hsu HY, Hua KF, Lin CC, Lin CH, Hsu J, Wong CH (2004) Extract of Reishi polysaccharides induces cytokine expression via TLR4-modulated protein kinase signaling pathways. J Immunol 173(10):5989–5999
33. Chen HS, Tsai YF, Lin S et al (2004) Studies on the immuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. Bioorg Med Chem 12(21):5595–5601
34. Shao BM, Dai H, Xu W, Lin ZB, Gao XM (2004) Immune receptors for polysaccharides from *Ganoderma lucidum*. Biochem Biophys Res Commun 323(1):133–141
35. Lin KI, Kao YY, Kuo HK et al (2006) Reishi polysaccharides induce immunoglobulin production through the TLR4/TLR2-mediated induction of transcription factor Blimp-1. J Biol Chem 281(34):24111–24123
36. Lin YL, Lee SS, Hou SM, Chiang BL (2006) Polysaccharide purified from *Ganoderma lucidum* induces gene expression changes in human dendritic cells and promotes T helper 1 immune response in BALB/c mice. Mol Pharmacol 70(2):637–644
37. Cao LZ, Lin ZB (2003) Regulatory effect of *Ganoderma lucidum* polysaccharides on cytotoxic T-lymphocytes induced by dendritic cells in vitro. Acta Pharmacol Sin 24(4):321–326
38. Chan WK, Lam DT, Law HK et al (2005) *Ganoderma lucidum* mycelium and spore extracts as natural adjuvants for immunotherapy. J Altern Complement Med 11(6):1047–1057
39. Chan WK, Law HK, Lin ZB, Lau YL, Chan GC (2007) Response of human dendritic cells to different immunomodulatory polysaccharides derived from mushroom and barley. Int Immunol 19(7):891–899
40. Kino K, Sone T, Watanabe J et al (1991) Immunomodulator, LZ-8, prevents antibody production in mice. Int J Immunopharmacol 13(8):1109–1115
41. Haak-Frendscho M, Kino K, Sone T, Jardieu P (1993) Ling Zhi-8: a novel T cell mitogen induces cytokine production and upregulation of ICAM-1 expression. Cell Immunol 150(1):101–113
42. Lin CL, Wang CC, Chang SC, Inbaraj BS, Chen BH (2009) Antioxidative activity of polysaccharide fractions isolated from *Lycium barbarum* Linnaeus. Int J Biol Macromol 45(2):146–151
43. Wang B, Hu Y, Li J (2005) Effect of Ganoderma triterpene on proliferation of dendritic cells from mouse spleen. Zhong Yao Cai 28(7):577–579
44. Chen Z, Lu J, Srinivasan N, Tan BK, Chan SH (2009) Polysaccharide-protein complex from *Lycium barbarum* L. is a novel stimulus of dendritic cell immunogenicity. J Immunol 182(6):3503–3509
45. Wakabayashi C, Hasegawa H, Murata J, Saiki I (1997) In vivo antimetastatic action of ginseng protopanaxadiol saponins is based on their intestinal bacterial metabolites after oral administration. Oncol Res 9(8):411–417
46. Takei M, Tachikawa E, Hasegawa H, Lee JJ (2004) Dendritic cells maturation promoted by M1 and M4, end products of steroidal ginseng saponins metabolized in digestive tracts, drive a potent Th1 polarization. Biochem Pharmacol 68(3):441–452
47. Lee YS, Chung IS, Lee IR, Kim KH, Hong WS, Yun YS (1997) Activation of multiple effector pathways of immune system by the antineoplastic immunostimulator acidic polysaccharide ginsan isolated from *Panax ginseng*. Anticancer Res 17(1A):323–331
48. Kim KH, Lee YS, Jung IS et al (1998) Acidic polysaccharide from *Panax ginseng*, ginsan, induces Th1 cell and macrophage cytokines and generates LAK cells in synergy with rIL-2. Planta Med 64(2):110–115
49. Han SK, Song JY, Yun YS, Yi SY (2005) Ginsan improved Th1 immune response inhibited by gamma radiation. Arch Pharm Res 28(3):343–350

50. Kim HJ, Kim MH, Byon YY, Park JW, Jee Y, Joo HG (2007) Radioprotective effects of an acidic polysaccharide of *Panax ginseng* on bone marrow cells. J Vet Sci 8(1):39–44
51. Luo Q, Li Z, Huang X, Yan J, Zhang S, Cai YZ (2006) *Lycium barbarum* polysaccharides: Protective effects against heat-induced damage of rat testes and H2O2-induced DNA damage in mouse testicular cells and beneficial effect on sexual behavior and reproductive function of hemicastrated rats. Life Sci 79(7):613–621
52. Huang L, Lin Y, Tian G, Ji G (1998) Isolation, purification and physico-chemical properties of immunoactive constituents from the fruit of *Lycium barbarum* L. Yao Xue Xue Bao 33(7):512–516
53. Peng X, Tian G (2001) Structural characterization of the glycan part of glycoconjugate LbGp2 from *Lycium barbarum* L. Carbohydr Res 331(1):95–99
54. Ho YS, Yu MS, Yik SY, So KF, Yuen WH, Chang RC (2009) Polysaccharides from wolfberry antagonizes glutamate excitotoxicity in rat cortical neurons. Cell Mol Neurobiol 29(8):1233–1244
55. Zhang M, Chen H, Huang J, Li Z, Zhu C, Zhang S (2005) Effect of *Lycium barbarum* polysaccharide on human hepatoma QGY7703 cells: inhibition of proliferation and induction of apoptosis. Life Sci 76(18):2115–2124
56. Luo Q, Yan J, Zhang S (1999) Effects of pure and crude *Lycium barbarum* polysaccharides on immunopharmacology. Zhong Yao Cai 22(5):246–249
57. Xu Y, He L, Xu L, Liu Y (2000) Advances in immunopharmacological study of *Lycium barbarum* L. Zhong Yao Cai 23(5):295–298
58. Chen Z, Kwong Huat Tan B, Chan SH (2008) Activation of T lymphocytes by polysaccharide-protein complex from *Lycium barbarum* L. Int Immunopharmacol 8(12):1663–1671
59. Chen Z, Soo MY, Srinivasan N, Tan BK, Chan SH (2009) Activation of macrophages by polysaccharide-protein complex from *Lycium barbarum* L. Phytother Res 23(8):1116–1122
60. Duan CL, Qiao SY, Wang NL, Zhao YM, Qi CH, Yao XS (2001) Studies on the active polysaccharides from *Lycium barbarum* L. Yao Xue Xue Bao 36(3):196–199
61. Gan L, Hua Zhang S, Liang Yang X, Bi Xu H (2004) Immunomodulation and antitumor activity by a polysaccharide-protein complex from *Lycium barbarum*. Int Immunopharmacol 4(4):563–569
62. Gan L, Zhang SH, Liu Q, Xu HB (2003) A polysaccharide-protein complex from *Lycium barbarum* upregulates cytokine expression in human peripheral blood mononuclear cells. Eur J Pharmacol 471(3):217–222
63. He YL, Ying Y, Xu YL, Su JF, Luo H, Wang HF (2005) Effects of *Lycium barbarum* polysaccharide on tumor microenvironment T-lymphocyte subsets and dendritic cells in H22-bearing mice. Zhong Xi Yi Jie He Xue Bao 3(5):374–377
64. Zhu J, Zhao LH, Zhao XP, Chen Z (2007) *Lycium barbarum* polysaccharides regulate phenotypic and functional maturation of murine dendritic cells. Cell Biol Int 31(6):615–619
65. Gabrilovich DI, Corak J, Ciernik IF, Kavanaugh D, Carbone DP (1997) Decreased antigen presentation by dendritic cells in patients with breast cancer. Clin Cancer Res 3(3):483–490
66. Tsuge T, Yamakawa M, Tsukamoto M (2000) Infiltrating dendritic/Langerhans cells in primary breast cancer. Breast Cancer Res Treat 59(2):141–152
67. Samuelsen AB (2000) The traditional uses, chemical constituents and biological activities of Plantago major L. A review. J Ethnopharmacol 71(1–2):1–21
68. Doan DD, Nguyen NH, Doan HK et al (1992) Studies on the individual and combined diuretic effects of four Vietnamese traditional herbal remedies (Zea mays, Imperata cylindrica, Plantago major and Orthosiphon stamineus). J Ethnopharmacol 36(3):225–231
69. Huang DF, Xie MY, Yin JY et al (2009) Immunomodulatory activity of the seeds of *Plantago asiatica* L. J Ethnopharmacol 124(3):493–498
70. Huang DF, Tang YF, Nie SP, Wan Y, Xie MY, Xie XM (2009) Effect of phenylethanoid glycosides and polysaccharides from the seed of *Plantago asiatica* L. on the maturation of murine bone marrow-derived dendritic cells. Eur J Pharmacol 620(1-3):105–111
71. Wang CY, Chiao MT, Yen PJ et al (2006) Modulatory effects of *Echinacea purpurea* extracts on human dendritic cells: A cell- and gene-based study. Genomics 88(6):801–808
72. Han SB, Kim YH, Lee CW et al (1998) Characteristic immunostimulation by angelan isolated from Angelica gigas Nakai. Immunopharmacology 40(1):39–48

73. Sonoda Y, Kasahara T, Mukaida N, Shimizu N, Tomoda M, Takeda T (1998) Stimulation of interleukin-8 production by acidic polysaccharides from the root of *Panax ginseng*. Immunopharmacology 38(3):287–294
74. Liu M, Li J, Kong F, Lin J, Gao Y (1998) Induction of immunomodulating cytokines by a new polysaccharide-peptide complex from culture mycelia of Lentinus edodes. Immunopharmacology 40(3):187–198
75. Gao Y, Zhou S, Jiang W, Huang M, Dai X (2003) Effects of ganopoly (a *Ganoderma lucidum* polysaccharide extract) on the immune functions in advanced-stage cancer patients. Immunol Invest 32(3):201–215
76. Yoon YD, Han SB, Kang JS et al (2003) Toll-like receptor 4-dependent activation of macrophages by polysaccharide isolated from the radix of Platycodon grandiflorum. Int Immunopharmacol 3(13–14):1873–1882
77. Ando I, Tsukumo Y, Wakabayashi T et al (2002) Safflower polysaccharides activate the transcription factor NF-kappa B via Toll-like receptor 4 and induce cytokine production by macrophages. Int Immunopharmacol 2(8):1155–1162
78. Cui R, He J, Wang B et al (2003) Suppressive effect of *Astragalus membranaceus* Bunge on chemical hepatocarcinogenesis in rats. Cancer Chemother Pharmacol 51(1):75–80
79. Ooi VE, Liu F (2000) Immunomodulation and anticancer activity of polysaccharide-protein complexes. Curr Med Chem 7(7):715–729
80. Tzianabos AO (2000) Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clin Microbiol Rev 13(4):523–533
81. Kataoka K, Muta T, Yamazaki S, Takeshige K (2002) Activation of macrophages by linear (1right-arrow3)-beta-D-glucans. Impliations for the recognition of fungi by innate immunity. J Biol Chem 277(39):36825–36831
82. Brown GD, Gordon S (2003) Fungal beta-glucans and mammalian immunity. Immunity 19(3):311–315
83. Chan GC, Chan WK, Sze DM (2009) The effects of beta-glucan on human immune and cancer cells. J Hematol Oncol 2:25
84. Yamazaki S, Iyoda T, Tarbell K et al (2003) Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J Exp Med 198(2):235–247
85. Lin YL, Liang YC, Tseng YS et al (2009) An immunomodulatory protein, Ling Zhi-8, induced activation and maturation of human monocyte-derived dendritic cells by the NF-κB and MAPK pathways. J Leukoc Biol 86(4):877–889

27 Microalgae and Immune Potential

Rathinam Raja and
Shanmugam Hemaiswarya

Key Points

- Immunopotentiating activities have been found in whole cells such as bacteria, mushrooms, algae, lichens, and higher plants.
- Microalgae with its long history of food use, easy cultivation, and high nutritional content make it a valuable source for immunomodulating studies.
- One of the main components that possesses the immunomodulating activity is the polysaccharides of the cell.
- The basic mechanism of the immunostimulatory activity is through the stimulation of the macrophages and modulation of the complement system.

Key Words: Microalgae, immunomodulators, *spirulina,* immunoglobulin, allergy, metabolic diseases.

27.1 INTRODUCTION

One of the most promising recent alternatives to classical antibiotic treatment is the use of immunomodulators for enhancing host defense response (1). Several types of immunomodulators have been identified including mammalian proteins such as interferon gamma (IFN-γ) (2), granulocyte colony-stimulating factor (3), and granulocyte macrophage colony-stimulating factor (GM-CSF) (4), as well as substances isolated and purified from microorganisms (1). Immunopotentiating activities have also been found in whole cells like bacteria, mushrooms, algae, lichens, and higher plants. Microalgae with its added advantages such as a long history of its food use, easy cultivation, and high nutritional content make it a valuable source for immunomodulating studies. Scientists are increasingly turning their attention to algae as ingredient factories, particularly the nutritional components. The omega-3 fatty acid DHA extracted from marine algae is already in market, while several companies are offering the carotenoid and astaxanthin (AX) from other algal sources. *Spirulina*, *Chlorella*, and *Aphanizomenon*

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_27

flos-aquae provide cellular protection with exceptional amounts of β-carotene (provitamin A) and chlorophyll, whereas *Dunaliella* is the highest known natural source of β-carotene (5–7). Some microalgae have favorable nutritional profiles for cancer and immune therapies. *Chlorella*, the alga to emphasize stimulates immunity in the treatment of all degenerative diseases by means of *Chlorella* Growth Factor (CGF).

27.2 *SPIRULINA*

The blue green alga (Cyanobacterium), *Spirulina* (*Arthrospira*) *platensis* Geitler (Fig. 27.1a, b) has a soft cell wall made up of complex sugars and protein which is easily digestible. It has 62% amino acid which is a richest natural source of pseudo-vitamin B_{12} with (Vitamins B_1 and B_2) and a whole spectrum of carotenoids and xanthophyll phytopigments (8). Hence, a considerable attention has been paid for the cultivation of *Spirulina*. Current world production of *Spirulina* for human consumption is more than 1,000 metric tons annually (9). The USA leads the world for *Spirulina* products in the form of pills and spray-dried powder followed by China, India, Israel, Japan, Mexico, Taiwan, and Thailand in the healthy food market (10). Phycocyanin (PC) extracted from *Spirulina*, which is commercially known as lima blue, is used as a blue colorant for food and cosmetics. The phycobiliproteins (phycoerythrins and phycocyanin) extracted from *Spirulina* is used as fluorescence tag in connection with the detection of particular biological molecules viz., a fluorescent color marker coupled to antibiotics.

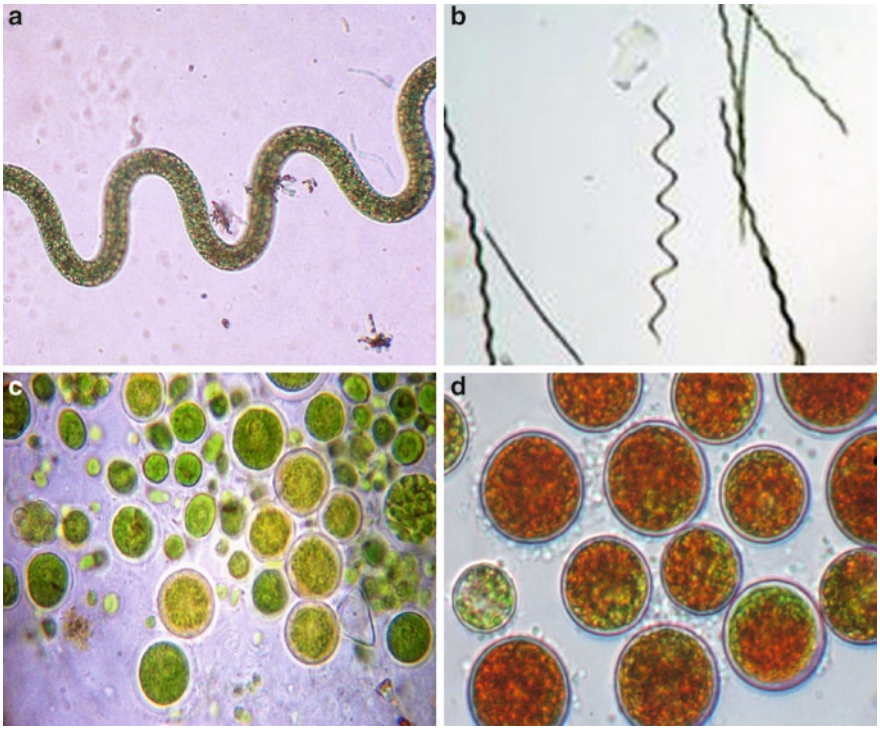


Fig. 27.1. (a–d) Morphological structures of some microalgae. (a) *Spirulina* sp. ×100. (b) *Spirulina* sp. ×45. (c) *Chlorella* sp. ×100. (d) *Haematococcus* sp. ×100.

Spirulina inhibits viral replication and strengthens cellular and humoral immune system causing regression and inhibition of cancers (11). Several reports revealed that the extract unique to *Spirulina* named as *Calcium-Spirulan* (a polymerized sugar molecule) inhibits replication of HIV-1, herpes simplex, human cytomegalovirus, influenza A virus, mumps virus, and measles virus under *in vitro* conditions and are very safe to human cells (12). Hamsters treated with this water soluble extract had better recovery rates when infected with lethal herpes virus. A recent *in vitro* study showed *in vitro* that an aqueous extract of *S. platensis* inhibited HIV-1 replication in human T-cells, peripheral blood mononuclear cells, and Langerhan cells (11). The advantage of using algal products with proven antiviral properties in fighting certain viruses is that they can immunomodulate even when an infection is developed. Studies on mice, hamsters, chickens, turkeys, cats, and fish reveal that *Spirulina* consistently improves their immune system functions. The rich brilliant blue polypeptide, Phycocyanin from *Spirulina,* stimulates hematopoiesis and emulates the effect of hormone erythropoetin (EPO). Phycocyanin also regulates the production of white blood cells, even when bone marrow stem cells are damaged. Although early interest in commercial production of *Spirulina* was focused mainly on its nutrient content, recent attention has been given to its therapeutic properties such as antioxidant effects, immunomodulation, anticancer potency, antiviral, and cholesterol regulatory properties.

27.3 IMMUNOMODULATION OF MACROPHAGES BY *SPIRULINA*

Monocytes and macrophages are phagocytes, acting in both nonspecific defense (and innate immunity) or help to initiate specific defense mechanisms (or adaptive immunity) in vertebrate animals. Their role is to phagocytose (engulf and then digest) cellular debris and pathogens either as stationary or as mobile cells, and to stimulate lymphocytes and other immune cells to respond to the pathogen. In response to antigen, macrophages secrete mediators such as nitric oxide and cytokines. Cytokines play an important role in the inflammatory cascade and include proinflammatory cytokines such as TNF, IL-1, IL-8, and anti-inflammatory cytokines like IL-10 (13). The proinflammatory cytokines are responsible for initiating an effect against exogenous pathogens and anti-inflammatory cytokines are crucial for downregulating the incremented inflammatory process and maintaining homeostasis for the correct functioning of vital organs.

Mice fed with microalgal diet showed an increase in the number of splenic antibody producing cells in response to sheep red blood cells (SRBC). *Spirulina* enhanced the macrophage functions and IL-1 production, but there was no change in the IgG-antibody production (9). IL-1 is a proinflammatory cytokine that has numerous biological effects, including activation of many inflammatory processes, induction of expression of acute-phase proteins, an important function in neuroimmune response, and direct effects on the brain itself (14). Similarly, in chickens fed with the *Spirulina* diet had macrophages which exhibited enhanced phagocytic activity and increased nitric oxide synthase activity (13). The enhanced macrophage phagocytic function has also been shown in cats (15), dogs (16) as well as in humans (17, 18) fed with *Spirulina platensis* extract. The immunomodulatory action has been suggested by some researchers to be mediated through the innate immune system. All the above studies used *Spirulina* as powder or hot water extract and the active components considered were phycocyanin and water

Table 27.1
Microalgal polysaccharides with immunomodulating activity

Microalgae	*Polysaccharides*	*Effect*	*References*
Aphanizomenon flos-aquae	Immunon	Increase NF-κB activation and IL-1β and TNF-α signaling	(19, 22)
Chlorella pyrenoidosa	Immurella		(22, 23)
Spirulina platensis	Immulina		(22)
Chlorella stigmatophora	Hydrosoluble extracts	Anti-inflammatory, analgesic and free radical scavenging activities	(24)
Phaeodactylum tricornutum			

soluble polysaccharides. These components have been known to cause immunomodulation via increased proliferation of erythrocytes, granulocyte-monocyte, and fibroblast lineage cells derived from bone marrow cells of mice (18).

A high molecular weight polysaccharide fraction (Immulina) from *Spirulina* was a potent activator of NF-κB and induced both IL-1β and TNF-α mRNAs in THP-1 human monocytes (19). Immulina dose-dependently increased the expression of chemokines, namely, interleukin (IL)-8, MCP-1, MIP-1α, MIP-1β, IL-10 as well as the expression of TNF-α, IL-1β, and COX-2. Thymidine uptake experiments verified that Immulina did not affect the viability and growth rate of THP-1 cells. The activity *in vitro* was more potent than the *in vivo* effects after oral administration (20). NF-κB activation by Immulina is suppressed by antibodies to CD14 and TLR2 but not by antibodies to TLR4. Similarly, NF-κB directed luciferase expression was enhanced by Immulina treatment when cells were cotransfected with vectors expressing proteins supporting TLR2- (CD14 and TLR2) but not TLR4-(CD14, TLR4, and MD-2) dependent activation (21). In addition to Immulina, there are several other microalgae which produce polysaccharides with immunomodulating activities especially on the macrophages (Table 27.1).

27.4 *SPIRULINA* AND CELL MEDIATED IMMUNITY

In mice, *S. fusiformis* (400 or 800 mg/kg body wt.) administration significantly inhibited the humoral immune response, cell-mediated immune response delayed-type hypersensitivity reaction (DTH) and TNF-alpha in a dose dependent manner (Fig. 27.2). *In vitro*, *S. fusiformis* (50 or 100 μg/mL) decreased the mitogen (phytohemagglutinin) induced T-lymphocyte proliferation in a concentration dependent manner when compared with control cells. These observations clearly suggest that *S. fusiformis* has a remarkable immunosuppressive effect which provides a scientific validation for the popular use of this drug (25).

The number of hemagglutinating antibodies in serum was reduced after *S. fusiformis* (400 or 800 mg/kg body wt.) dose-related treatment. During the cell-mediated immune response, the sensitized T-lymphocytes, on being challenged with the antigen, secrete a number of lymphokines. These lymphokines attract scavenger cells to the site of the reaction, where they are then immobilized to promote defensive (inflammatory) reaction (26). Therefore, the inhibition of inflammation observed in our present study indicates that there might be an inhibition of release of lymphokines on *S. fusiformis*

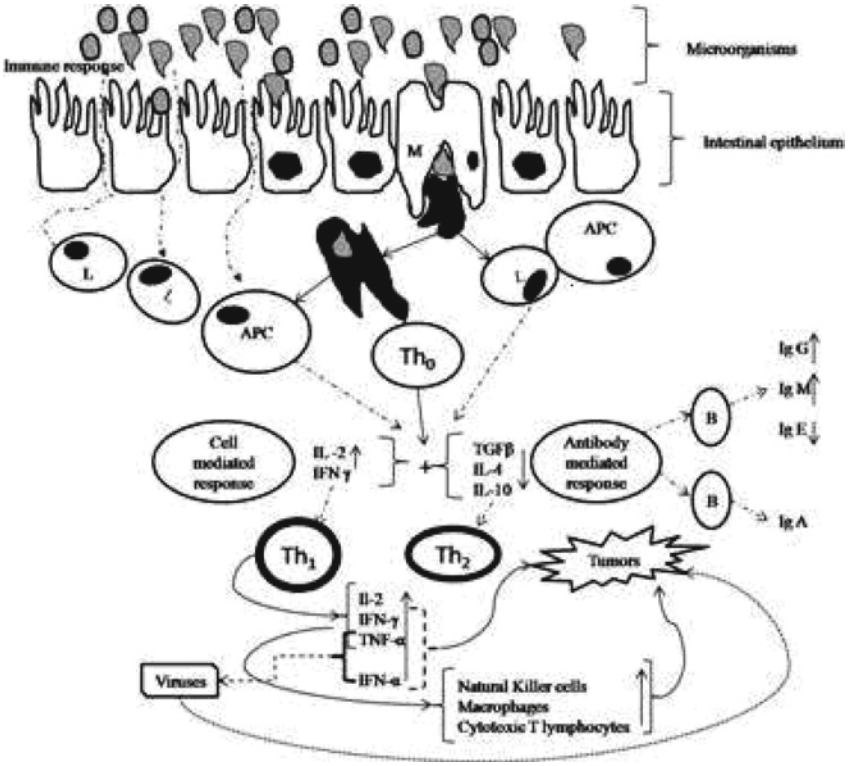


Fig 27.2. Hypothesized mechanism of immunomodulation.

administration. A moderate level of inflammatory mediators is essential for host survival from infection, whereas overproduction has deleterious effects. As a result, synthesis of inflammatory mediators must be tightly governed. The cytokine TNF-α induces the production of proinflammatory cytokines, active oxygen species, nitric oxide and matrix metalloproteinases, thereby implicating TNF-α as a therapeutic target for treating the pain, swelling, and progressive joint destruction caused by rheumatoid arthritis (27). In the present study, the abundance of TNF-α in arthritic mice joints provides evidence of its involvement in the disease pathology, which is supported by studies demonstrating that neutralization of TNF-α leads to decreased production of other inflammatory cytokines (28). The *S. fusiformis* significantly decreased the production of TNF-α in adjuvant-induced arthritic mice in dose dependent manner.

IFN-γ is a macrophage activating cytokine that promotes Th1 biased responses associated with cell mediated immunity (29). The Th1/Th2 balance is critical in determining whether an immune response is to be dominated by macrophage activation or by antibody production. An increased spleen cell production of IFN-γ in mice fed with *Spirulina* extract, suggests a shift toward Th1 type cell mediated immunity. It was also indicated a Th1 bias (increased production of IFN-γ) in mice fed with when *Spirulina* (30) or when added to cultures of human peripheral blood mononuclear cells (increased production of IFN-γ). In addition, consumption of a hot water extract of *Spirulina* for two months by human volunteers resulted in greatly enhanced production of IFN-γ by NK cells in response to IL-12 and IL-18 (17).

27.5 ENHANCED IMMUNOGLOBULIN PRODUCTION BY *SPIRULINA*

Spirulina is known to immunomodulate by activating the IgA secretion. IgA secreted at mucosal surfaces function to protect against various viral and bacterial pathogens by its unique nature of agglutination of microorganisms, neutralization of bacterial enzymes, toxins and inhibition of antigens (31). Several investigators have observed an increased mucosal IgA response by long-term treatment with *Spirulina* or its components or after antigen stimulation. Mice that consumed a chemically defined chow mixed with an extract containing Immulina exhibited changes in several immune parameters. The *ex vivo* production of IgA and IL-6 from Peyer's patch cells was enhanced two fold and (IFN-γ) production from spleen cells was increased four fold in immulina treated mice. Oral consumption of this polysaccharide can enhance components within both the mucosal and systemic immune systems (21). Ingestion of *S. platensis* by men for more than a year enhanced salivary S-IgA levels while treatment for less than a year had no effect. Mice that ingested this extract exhibited enhanced spleen cell IFN-γ production *ex vivo*. Interestingly, the enhanced production of IFN-γ by spleen cells followed a time course similar to that exhibited for production of IL-6 and IgA by Peyer's patch cells. The similar time course may indicate that a common cell type is initially targeted by the extract and is mediating both the effects locally on Peyer's patches as well as systemically on spleen cells. The influence of *Spirulina* on IgA levels in human saliva and demonstrated that it enhances IgA production, indicating an important role of microalga in mucosal immunity (32).

27.6 *SPIRULINA* IN ALLERGY AND RHINITIS

It has been well documented that *Spirulina* exhibits anti-inflammatory properties by inhibiting the release of histamine from mast cells (33, 34). In a recent randomized double blind placebo-controlled trial (35), individuals with allergic rhinitis was fed daily, either with placebo or *Spirulina* for 3 months. Peripheral blood mononuclear cells were isolated before and after the *Spirulina* feeding and levels of cytokines [IL-4, IFN- and IL-2] which are important in regulating immunoglobulin (Ig)E-mediated allergy were measured. The study showed that high dose of *Spirulina* significantly reduced IL-4 levels by 32%, demonstrating the protective effects of this microalgae toward allergic rhinitis.

A Japanese team identified the molecular mechanism of the human immune capacity of *Spirulina* by analyzing blood cells of volunteers with pre- and post-oral administration of hot water extract of *Spirulina platensis*. IFN-γ production and Natural Killer (NK) cell damage were increased after administration of the microalgal extracts to male volunteers (17). In a recent double blind, placebo controlled study from Turkey evaluating the effectiveness and tolerability of *Spirulina* for treating patients with allergic rhinitis, *Spirulina* consumption significantly improved the symptoms and physical findings compared with placebo, including nasal discharge, sneezing, nasal congestion, and itching (36). It is well understood that deficiency of nutrients is responsible for changes in immunity which manifests as changes in production of T-cells, secretory IgA antibody response, cytokines, and NK-cell activity. The above

studies suggest that *Spirulina* may modulate the immune system by its role in covering nutritional deficiencies.

27.7 PHYCOCYNANIN FROM *SPIRULINA*

Phycocyanin (PC), a water soluble protein pigment, is one of the major constitutes of *Spirulina platensis*. It is in association with the outer face of the photosynthetic II light harvesting apparatus. Some characteristics of PC make it well suitable for fluorescence analyses in flow cytometry, histochemistry, immunoassay, and detection of reactive oxygen species (37). PC can be used as natural dyes and its medicinal and pharmaceutical properties especially antioxidant (38), antitumor, and immunity boosting effects are the focus of research (39) for a long time. Recent studies suggested that PC inhibited cancer cell growth via the induction of apoptosis (40). It has been known already that *S. platensis* is a good candidate for selenium (Se) enrichment and also serves as a promising source of dietary Se supplementation (41, 42). However, very limited information on the antiproliferative activity of selenium containing phycocyanin (Se-PC) and the underlying mechanism is available. An influence of selenium enriched phycocyanin on anaphylactic reaction severity and circulating antibody response against model allergen – hen's egg white ovalbumin was studied in rats. Rats receiving Se-PC demonstrated significantly increased specific IgG response (43). Phycocyanin can promote the expression of CD59 protein, reduce the reproduction of Hela cells. With an ascendance of phycocyanin concentration, the expression quantities of CD59 protein and apoptosis inducing Fas protein increased and the multiplication activity of Hela cells declined (44).

Human CD59 is a plasma membrane anchored glycoprotein containing a 4 kDa N-linked carbohydrate chain that functions as an inhibitor of the CD5b-9 membrane attack complex (MAC) of human complement (45, 46). Thereby, it restricts the cytolytic activity of the CD5b-9 complex to protect human blood and vascular cells from autologous complement attack (47, 48). CD59 plays a pivotal role in regulating immunity and suppressing the hyperacute rejection (HAR) in xenograft transplantation (49). It was reported that CD59 has close relationship with the occurrence of hurt, inflammation, and tumor (50, 51). PC has an antitumor activity, and probably it acts as a kind of mitosis depressor able to combine with the receptor of mitosis depressor on the surface of tumor cells. Through the linkage of receptor and the activation of cell death promoting protein kinases by complicated pathway, the transcription and expression of CD59 gene was promoted. Meanwhile excessively expressed CD59 protein can induce Fas protein expression on the surface of Hela cells and then combine with Fas antigen which can activate death domain and activate the conduction of proapoptosis signal in tumor cells. In the end, the proliferation of tumor cells is held back and cells go to die. The studies provide us a new idea about the molecular mechanism of the antitumor activity of PC (44). In another study, after two weeks the white blood cells (lymphocyte activity) of a PC group were higher than the control group and higher than or equal to a normal group without cancer. This suggests that PC raises lymphocyte activity (21). These results imply that phycocyanin has anticancer activity and also strengthens the body's resistance through increasing general immunity. Experts deem that taking a small dosage of PC daily can prevent generation of malignant cancer or can inhibit its recurrence (29).

27.8 *CHLORELLA*

One of the most powerful algae against cancer is *Chlorella* (Fig. 27.1c) – an unicellular, green algae containing the highest chlorophyll level per ounce of any plant, as well as protein (nearly 58%), carbohydrates, all of the vitamins B, C, and E, amino acids, enzymes, and rare trace minerals (9). In a study, lab mice were supplemented with *Chlorella* for ten days, and injected the mice with three types of cancer. According to Moss, over 70% of the *Chlorella* strengthened mice did not develop cancer while all of the untreated mice died within twenty days. Research regarding *Chlorella*'s immune boosting effect is not limited to animal studies. According to another study, 15 glioblastoma patients were treated with powdered and liquid *Chlorella* along with standard chemotherapy or radiation therapy. Although glioblastoma patients normally display a 2 year survival rate of 10%, the 15 *Chlorella* treated patients exhibited a survival rate of 40%. This is only one of the many successful studies linking *Chlorella* to strengthened immune response thus making *Chlorella* a necessary component of effective and well-rounded cancer treatment. It acts as both a powerful nutrient and a detoxifying food.

An experimental study was designed to assess the effects of oral administration of *Chlorella* protein hydrolysate (Cv-PH) on the recovery of both innate and specific immune responses of undernourished mice (52). The facts in support of the hypothesis are as follows: the treatment of starved mice with Cv-PH provided benefits in terms of (a) hemopoiesis (recovery of bone marrow cellularity and the lymphocyte pool), (b) macrophage activation and phagocytosing capacity, and (c) stimulation of both humoral and cell immune functions such as antibody response and the reconstitution of delayed-type hypersensitivity response. Different immune cell populations might be induced after activation in the gut-associated lymphoid tissue. *Chlorella* and its hydrophilic extracts have been shown to possess many physiological functions, including immune system improvement, hypoglycemic effects, lowering hyperlipidemic state in high fat fed animals, etc. However, lipophilic extract of *Chlorella* (LEC) is less appreciated in terms of its physiological actions. In the concentration ranges that were devoid of cytotoxicty, LEC produced a dose dependent (between 0.25 and 0.0315 mg/mL) inhibition on LPS-induced nitric oxide production. The study shows LEC effectively block LPS-induced nitric oxide production, is through blockage of expression of iNOS mRNA.

Chlorella powder was tested in 118 *in vitro* enzyme assay systems. The powder showed potent inhibitions of peptidase cathepsin S, thromboxane A (2) synthase, and cyclooxygenase-2 in a dose concentration manner. Other activities observed were inhibitions of tumor necrosis factor alpha converting enzyme, protein tyrosine phosphatase (SHP-2), calpain, protein kinases, and protein tyrosine phosphatases. *Chlorella* powder had no significant effect on cyclooxygenase-1 (53). These actions to inhibit cyclooxygenase-2 and thromboxane synthase could contribute to the purported anti-inflammatory and antithrombotic effects of *Chlorella*.

27.9 *HAEMATOCOCCUS*

The green alga, *Haematococcus pluvialis* (Fig. 27.1d) accumulates high amounts of AX under adverse environmental conditions, and it is the world's richest source of astaxanthin (54). AX plays an important role in protecting the alga against UV

light damage and photo-oxidation of the polyunsaturated AX. It exhibits strong free radical scavenging activity, protects against lipid peroxidation and oxidative damage of LDL-cholesterol, cell membranes, cells, and tissues. Several studies have clearly shown the effectiveness of astaxanthin as a cancer preventive agent in rats and mice. The effect of AX on colon cancer in male rats is well executed (55). A recent study with rats indicated that astaxanthin is effective at ameliorating retinal injury, and it is effective in protecting photoreceptors from degeneration. AX was also found to easily cross the blood–brain barrier (unlike β-carotene) and did not form any crystals in the eye (56).

Immune response cells are particularly sensitive to oxidative stress and membrane damage by free radicals because they rely heavily on cell to cell communications via cell membrane receptors. Furthermore, the phagocytic action of some of these cells releases free radicals that can rapidly damage these cells if they are not neutralized by antioxidants (57). AX significantly influences immune function in several *in vitro* and *in vivo* assays using animal models. Other evidences also pointout to the immunomodulating activity of astaxanthin on the proliferation and functions of murine immuno competent cells (58). Finally, studies on human blood cells *in vitro* have demonstrated enhancement by AX of immunoglobulin production in response to T-dependent stimuli. AX increases the production of T-helper cell antibody and increases the number of antibody secretory cells from primed spleen cells (59). The above study discovered the effect of AX in the production of immunoglobulins *in vitro* by human blood cells and found that it increases the production of IgA, IgG, and IgM in response to T-dependent stimuli (60). Other studies performed *in vivo* using mice have shown the immunomodulating action of AX and other carotenoids for humoral responses to T-dependent antigens and suggested that the supplementation with carotenoids may be useful to restore immune responses (61). In agreement with the above results, various foods and drinks with added AX have been prepared to increase the immune response mediated by T-lymphocytes and NK cells to alleviate or prevent the decrease of immunological functions caused by stress (62). Due to its immunomodulating action, AX has also been utilized as a medication for the treatment of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and Crohn's disease (63).

Malnutrition induced by dietary restriction produces a series of metabolic changes that lead to depression of immunocompetence and several studies have assessed the effects of nutritional support on immunity (64). Algal protein hydrolysates possess various biological activities and have been administered to patients with different protein metabolic diseases (65), but there are no reports on its immunomodulating properties. Immunomodulation by using natural products can provide an alternative to conventional therapy for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of impaired immune response or when a selective immunosuppression is desired in situations like autoimmune disorders. Though enhancement of immune function has been claimed by various natural products, very few have been subjected to randomized clinical trails. Recently, novel vaccine delivery system has been developed using single-celled alga, *Chlamydomonas reinhardtii*. Microalgae in particular, *Chlamydomonas* have many features that are desirable for vaccine delivery systems, including its ease for genetic manipulation,

inexpensive to produce and nontoxic. The first antigen to be tested was the p57 antigen, the causing agent of bacterial kidney disease. It is caused by *Renibacterium salmoninarum* and infects all wild and farmed salmonids. The vaccine was well expressed in the algae and effects in the immune functions were noted in the immunized animals such as fish and rabbits. Issues that remain to be addressed include expression of the antigen, posttranslational modifications, antigen immunogenicity, and effective means of production.

REFERENCES

1. Tzianabos AO (2000) Polysaccharide immunomodulators as therapeutic agents: structural aspects and biological function. Clin Microbiol Rev 13:523–533
2. Murray HW (1996) Current and future clinical applications of interferon-gamma in host antimicrobial defense. Intensive Care Med 22:S456–S461
3. Nemunaitis J (1997) A comparative review of colony-stimulating factors. Drugs 54:709–729
4. Hamilton JA, Anderson GP (2004) GM-CSF biology. Growth Factors 22:225–231
5. Raja R (2003) Studies on *Dunaliella salina* (Dunal) Teod. with special reference to its anticancer properties. Ph.D. Thesis, University of Madras, Chennai, India
6. Raja R, Hemaiswarya S, Balasubramanyam D, Rengasamy R (2007) Protective effect of *Dunaliella salina* (Volvocales, Chlorophyta) against experimentally induced fibrosarcoma on wistar rats. Microbiol Res 162:177–184
7. Raja R, Hemaiswarya S, Rengasamy R (2007) Exploitation of *Dunaliella* for β-carotene production. Appl Microbiol Biotechnol 74:517–523
8. Richmond A (1988) *Spirulina*. In: Borowitzka A, Borowitzka L (eds) Microalgal biotechnology. Cambridge University Press, Cambridge, pp 83–121
9. Raja R, Hemaiswarya S, Ashok Kumar N, Sridhar S, Rengasamy R (2008) A perspective on the biotechnological potential of microalgae. Crit Rev Microbiol 34:77–88
10. Spolaore P, Joannis-Cassen C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96
11. Ayehunie S, Belay A, Hu Y, Baba T, Ruprecht R (1996) Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*), 7th IAAA Conference, Knysna, South Africa, 17 April
12. Hayashi T, Hayashi K (1996) Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J Nat Prod 59:83–87
13. Al-Batshan HA, Al-Mufarrej SI, Al-Homaidan AA, Qureshi MA (2001) Enhancement of chicken macrophage phagocytic function and nitrite production by dietary *Spirulina platensis*. Immunopharmacol Immunotoxicol 23:281–289
14. Mosmann TR, Coffman RL (1989) Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145–173
15. Qureshi MA, Ali RA (1996) *Spirulina platensis* exposure enhances macrophage phagocytic function in cats. Immunopharmacol Immunotoxicol 18:457–463
16. Zhang HQ, Lin AP, Sun Y, Deng YM (2001) Chemo and radio protective effects of polysaccharide of *Spirulina platensis* on hemopoietic system of mice and dogs. Acta Pharmacol Sin 22:1121–1124
17. Hirahashi T, Matsumoto M, Hazeki K, Saeki Y, Ui M, Seya T (2002) Activation of the human innate immune system by *Spirulina*: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. Int Immunopharmacol 2:423–434
18. Hayashi O, Ishii K, Kawamura C, YenHei S, YeBao N et al (2004) Enhancement of mucosal immune functions by dietary *Spirulina platensis* in human and animals. Nutr Sci 7:31–34
19. Pugh N, Pasco DS (2001) Characterization of human monocyte activation by a water soluble preparation of *Aphanizomenon flosaquae*. Phytomedicine 8:445–453

20. Grzanna R, Polotsky A, Phan PV, Pugh N, Pasco D, Frondoza CG (2006) Immolina, a high-molecular-weight polysaccharide fraction of *Spirulina*, enhances chemokine expression in human monocytic THP-1 cells. J Altern Complement Med 12:429–435
21. Balachandran P, Nirmal D, Pugh Guoyi M, David S (2006) Toll-like receptor 2-dependent activation of monocytes by *Spirulina* polysaccharide and its immune enhancing action in mice. Int Immunopharmacol 6:1808–1814
22. Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS (2001) Isolation of three new polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. Planta Med 67:737–742
23. Suárez ER, Syvitski R, Kralovec JA, Noseda MD, Barrow CJ, Ewart HS, Lumsden MD, Grindley TB (2006) Immunostimulatory polysaccharides from *Chlorella pyrenoidosa*. A new galactofuranan. Measurement of molecular weight and molecular weight dispersion by DOSY NMR. Biomacromolecules 7:2368–2376
24. Guzmán S, Gato A, Lamela M, Freire-Garabel J, Calleja M (2001) Antiinflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phytother Res 17:665–670
25. Rasool M, Sabina EV (2009) Appraisal of immunomodulatory potential of *Spirulina fusiformis*: an *in vivo* and *in vitro* study. J Nat Med 63:169–175
26. Mediratta PK, Sharma KK, Surender S (2001) Evaluation of immunomodulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action. J Ethnopharmacol 80:15–20
27. Beutler B (1995) TNF, immunity and inflammatory disease lesions of the past decade. J Investig Med 43:227–235
28. Haworth C, Brennan FM, Chantry D, Turner M, Maini RN, Feldman M (1991) Expression of granulocyte-macrophage colony-stimulating factor in rheumatoid arthritis: regulation by tumor necrosis factor-alpha. Eur J Immunol 21:2575–2579
29. Boehm U, Klamp T, Groot M, Howard JC (1997) Cellular responses to interferon-gamma. Ann Rev Immunol 15:749–795
30. Mao TK, Gershwin ME, De Water V (2000) Effect of *Spirulina* on the secretion of cytokines from peripheral blood mononuclear cells. J Med Food 3:135–140
31. Kilian M, Russell MW (1994) Function of mucosal immunoglobulins. In: Ogra PL, Mestecky J, Lamm ME, Strober W, McGhee JR, Bienenstock J (eds) Handbook of mucosal immunology. Academic, New York, pp 127–137
32. Ishii K, Katoch T, Okuwaki Y, Hayashi O (1999) Influence of dietary *Spirulina platensis* on IgA level in human saliva. J Kagawa Nutr Univ 30:27–33
33. Yang HN, Lee EH, Kim HM (1997) *Spirulina platensis* inhibits anaphylactic reaction. Life Sci 61:1237–1244
34. Kim HM, Lee EH, Cho HH, Moon YH (1998) Inhibitory effect of mast cell-mediated immediate-type allergic reactions in rats by *Spirulina*. Biochem Pharmacol 55:1071–1076
35. Pao TK, Van de Water J, Gershwin ME (2005) Effects of a *Spirulina*-based dietary supplement on Cytokine production from allergic rhinitis patients. J Med Food 8:27–30
36. Cingi C, Conk-Dalay M, Cakli H, Bal C (2008) The effects of *Spirulina* on allergic rhinitis. Eur Arch Otorhinolaryngol 265:1219–1223
37. Glazer AN (1994) Phycobiliprotein – a family of valuable, widely used fluorophores. J Appl Phycol 6:105–112
38. Romay C, Gonzalez R (2000) Phycocyanin is an antioxidant protector of human erythrocytes against by peroxyl radicals. J Pharm Pharmacol 52:367–368
39. Li B, Zhang XC, Gao MH, Yu H (2004) Study on the anti-tumor immune activity of phycocyanin and polysaccharides from *Spirulina platensis*. J Ocean Univ China 34:396–402
40. Reddy MC, Subhashini J, Mahipal SV, Bhat VB, Srinivas Reddy P, Kiranmai G, Madyastha KM, Reddanna P (2003) C-Phycocyanin, a selective cyclooxygenase-2 inhibitor, induces apoptosis in lipopolysaccharide-stimulated RAW 264.7 macrophages. Biochem Biophys Res Commun 304:385–392
41. Chen T, Zheng W, Yang F, Bai Y, Wong YS (2006) Mixotrophic culture of high selenium-enriched *Spirulina platensis* on acetate and the enhanced production of photosynthetic pigments. Enzyme Microb Technol 39:103–107

42. Chen T, Zheng W, Wong YS, Yan F, Bai Y (2006) Accumulation of selenium in mixotrophic culture of *Spirulina platensis* on glucose. Bioresour Technol 97:2260–2265
43. Egorova EA, Gmoshinskiĭ IV, Zorin SN, Mazo VK (2006) Studies of immunomodulation caused by selenium-enriched phycocyanin. Vopr Pitan 75:19–21
44. Li B, Zhang X, Gao Chu X et al (2005) Effects of CD59 on antitumoral activities of phycocyanin from *Spirulina platensis*. Biomed Pharmacother 59:551–560
45. Ninomiya H, Stewart BH, Rollins SA, Zhao J, Bothwell AL, Slims PJ (1992) Contribution of the N-linked carbohydrate of erythrocyte antigen CD59 to its complement-inhibitory activity. J Biol Chem 267:8404–8410
46. Davies A, Lachmann PJ (1993) Membrane defence against complement lysis: the structure and biological properties of CD59. Immunol Res 12:258–275
47. Rollins SA, Sims PJ (1990) Complement-inhibitor activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. J Immunol 144:3478–3483
48. Meri S, Morgan BP, Davies A, Daniels RH, Olavesen MG, Waldmann H et al (1990) Human protectin (CD59), an 18,000–20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. Immunology 71:1–9
49. Menret S, Plat M, Blancho G, Martinat-Botte F, Bernard P, Karam G et al (2004) Characterization of human CD55 and CD59 transgenetic pigs and kidney xenotransplantation in the pig-to-baboo combination. Transplantation 77:1468–1471
50. McGeer PL, Walker DG, Akiyama H, Kawamata T, Guan AL, Parker CJ et al (1991) Detection of the membrane inhibitor of reactive lysis (CD59) in diseased neurons of Alzheimer brain. Brain Res 544:315–319
51. Uesu T, Mizuno M, Inoue H, Tomoda J, Tsuji T (1995) Enhanced expression of decay accelerating factor and CD59/homologous restriction factor 20 on the colonic epithelium of ulcerative colitis. Lab Invest 72:587–591
52. Morris HJ, Carrillo O, Almarales A et al (2007) Immunostimulant activity of an enzymatic protein hydrolysate from green microalga *Chlorella vulgaris* on undernourished mice. Enzyme Microb Technol 40:456–460
53. Cheng FC, Feng JJ, Chen KH, Imanishi H, Fujishima M, Takekoshi H, Naoki Y, Shimoda M (2008) *Chlorella* powder inhibits the activities of peptidase cathepsin S, PLA2, cyclooxygenase-2, thromboxane synthase, tyrosine phosphatases, tumor necrosis factor-alpha converting enzyme, calpain and kinases. Int J Food Sci Nutr 23:1–10
54. Lorenz RT, Cysewski GR (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol 18:160–167
55. Tanaka T, Kawamori T, Ohnishi M, Makita H, Mori H, Satoh K, Hara A (1995) Suppression of azoxymethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during post initiation phase. Carcinogenesis 16:2957–12963
56. Tso MO, Lam TT (1996) Method of retarding and ameliorating central nervous system and eye damage, U.S. Patent #5527533. Board of trustees of the University of Illinois, United States of America
57. Hughes DA (1999) Effects of dietary antioxidants on the immune function of middle-aged adults. Proc Nutr Soc 58:79–84
58. Okai Y, Higashi-Okai K (1996) Possible immunomodulating activities of carotenoids in *in vitro* cell culture experiments. Int J Immunopharmacol 18:753–758
59. Jyonouchi H, Sun S, Mizokami M, Gross MD (1996) Effects of various carotenoids on cloned, effector-stage T-helper cell activity. Nutr Cancer 26:313–324
60. Jyonouchi H, Sun S, Tomita Y, Gross MD (1995) Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and suboptimal doses of antigen. J Nutr 124:2483–2492
61. Jyonouchi H, Zhang L, Gross M, Tomita Y (1994) Immunomodulating actions of carotenoids: enhancement of *in vivo* and *in vitro* antibody production to T-dependent antigens. Nutr Cancer 21:47–58
62. Asami S, Zhi-bo Y, Yamashita E, Otoze H (2001) Anti-stress composition. Patent US6265450
63. Lignell A, Bottiger P (2001) Use of xanthophylls, astaxanthin e.g. for treatment of autoimmune diseases, chronic viral and intracellular bacterial infections. Patent WO01/24787 A1

64. Chandra RK (1997) Nutrition and the immune system: an introduction. Am J Clin Nutr 66:4605–4635
65. Tchorbanov B, Bozhkova M (1988) Enzymatic hydrolysis of cell proteins in green algae *Chlorella* and *Scenedesmus* after extraction with organic solvents. Enzyme Microb Technol 10:233–238

28 Mushrooms: Immunomodulating Activity and Role in Health Promotion

Ken-ichiro Minato

Key Points

- *Lentinula edodes*, *Grifola frondosa*, *Pleurotus cornucopiae*, *Pholiota nameko*, and many other kinds of edible mushrooms have shown their potent and unique immunomodulating activities.
- It has mainly been recognized that their activities result from the action of their polysaccharides or polysaccharide–protein complexes.
- The polysaccharides appear to stimulate the human immune response and to be effective in preventing immunodeficiency diseases via activation of cytokine networks.
- In this chapter, these properties of the immunomodulating polysaccharides from *L. edodes*, *G. frondosa*, *P. nameko*, *P. cornucopiae*, and others are described.

Key Words: Biological response modifiers (BRM), *grifola rondosa,* immunomodulators, *lentinula edodes, pleurotus cornucopiae, pholiota nameko*, polysaccharides.

28.1 INTRODUCTION

Mushrooms have recently become attractive as functional foods, and their extracts are widely sold as nutritional supplements and touted as beneficial for health. Approximately 15,000 species of mushrooms have been identified all over the world, and some 650 species have been reported to be shown as medicinal materials. Many medicinal mushrooms have become attractive as source materials for antitumor agents, immunomodulators, antibiotics, and antihypertensive drugs, to mention some of their uses. The market value of the mushrooms provided as source of dietary supplement products is approximately $5–6 billion per year worldwide (1). There is an increasing medicinal and pharmaceutical interest in many species mushrooms.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_28

Most of the medicinal mushrooms have been utilized as traditional oriental therapeutic agents in China, Japan, Korea, and other Asian countries. Recently, many scientific and medicinal studies were increasingly performed in the United States as well as Asian countries. It has been described that some of the isolated and identified substances of higher Basidiomycetes mushrooms origin expressed promising antitumor, immunomodulating, antihypercholesteroletic, antiviral, antibacterial, and antiparasitic effects (2). Edible mushrooms, including *Lentinus*, *Flammulina*, *Grifola*, *Pleurotus*, and *Agaricus*, have traditionally shown that they possessed potent immunomodulating activities. Thus, there are many species of mushrooms that have been found to markedly inhibit the growth of different kinds of tumors by modulating the immune system in the host. There have been reports of antitumor activity of some edible mushrooms such as *Lentinula edodes*, *Flammulina velutipes*, *Pholiota nameko*, *Agaricus bisporus*, *Hypsizigus marmoreus*, *Tricholma giganteum*, and *Tricholoma lobayense*. Antitumor activities of *Ganoderma lucidum*, *Grifola frondosa*, *Albatrellus confluens*, *Cryptoporus volvatus*, and *Stellera chamaejasme*, B cell activation of *Phellinus linteus*, and mitogenic activity of *Boletus satanas* have been also reported. These mushrooms have been expected to become source materials for the development of drugs.

There is considerable research and information on assessing the biological activities of the components of medicinal mushrooms. The gross compositions of mushrooms are protein, fat, carbohydrate, fiber, and ash, except for water which occupy 90% in the fruiting body. Polysaccharides such as β-glucan are well known for their pharmacological values as antitumor agents or immunomodulators. In addition, they are a good source of rare minerals, amino acids, and trace elements such as copper, zinc, iron, etc. Moreover, a variety of mushrooms possess lots of secondary metabolites. These substances are known to be derived from many intermediates in primary metabolism. These are amino acid-derived pathways, the shikimic acid pathway for the biosynthesis of aromatic compounds, the acetate malonate pathway from acetyl coenzyme A, and the mevalonic acid pathway from acetyl coenzyme A for the synthesis of sterols. It has been reported that these compounds possessed unique and potent bioactivities such as the modulating activity for NF-κB activation, inhibitory activity for protein kinases, alkylating effect on proteins and DNA, inhibitory activity for the proliferation of cancer cells via modulating check points in cell cycle, and inhibitory activity for oxidative stress, and so on (3).

As mentioned above, many compounds from the mushrooms demonstrate their potent and unique properties as biological response modifiers (BRM). They are known to enhance or suppress immune response through a lot of factors, which are a dose, a way of administrating them, the mechanism of immunomodulating action, and their active sites. It especially was polysaccharides, such as β-glucans, and polysaccharide–peptide complex that were isolated from medicinal and edible mushrooms as potent antitumor agents or immunomodulators in animals. Imunomodulating polysaccharides isolated from more than 30 species have shown potent antitumor action in animals. It is considered to augment or complement the desired immune system to maintain the host's health condition. Many scientific reports have shown the function of the polysaccharides which could be efficient in the possible treatment of immune diseases, including allergic asthma, food allergy, atopic dermatitis, inflammation, autoimmune joint inflammation, atherosclerosis, hyperglycemia, thrombosis, human immunodeficiency virus infection,

listeriosis, tuberculosis, and septic shock (4). Moreover, it has been studied that isolated polysaccharides from the mushrooms stimulated or suppressed specific components of the immune system.

Cytokines are recognized as important components secreted from immunocomplement cells. They play a pivotal role in the regulation of immune responses via cytokine networks and signaling pathways. Also, they stimulate inflammatory responses by inducing the proliferation and activation of lymphocyte and monocyte. $CD4^+$ T cells and macrophages have been considered pivotal cytokines in immune responses. When they are stimulated with immunomodulators such as the polysaccharides, interleukins (ILs), interferons (IFNs) and tumor necrosis factor (TNF) are secreted from them and support $CD4^+$ Th cell differentiation toward Th1 and Th2 cells. The direction of this differentiation depends on the kind of cytokines and signal transduction through their receptor. The immune response via $CD4^+$ Th cells depends on regulating the balance of the production of antigen-specific Th1 and Th2 cells. The polysaccharides as immunomodulators appear to stimulate the human immune response and to be effective in preventing it from cancer and immunodeficiency diseases via the activation of cytokine networks and signaling pathways. In this chapter, the properties of the immunomodulating polysaccharides from edible mushrooms, which were *L. edodes*, *G. frondosa*, *P. nameko*, *Pleurotus cornucopiae*, and others were described.

28.2 ANTITUMOR AND IMMUNOMODULATING POLYSACCHARIDES FROM THE MUSHROOMS

At first, immunomodulatery strategies of the mushrooms were to augment or suppress properties of the host immune response in prevention from cancers of the stomach, esophagus, lungs, etc. Many investigators have then reported that the antitumor substances were isolated and identified from some kinds of mushrooms, and an antitumor activity of mushrooms appeared to be attributable to the polysaccharides and polysaccharide-protein complexes contained in themselves. Ikekawa et al. (5) reported that the hot water extracts from some kinds of mushrooms showed significant antitumor activities against implanted tumor of Sarcoma 180 through host-mediated mechanism. It has been well known that the antitumor polysaccharides in these mushrooms enhance the immunomodulating effects in the host. Hence, these polysaccharides have been recognized as BRM. It has been well known that lentinan, which was β-1, 6-blanched β-1, 3-glucan, from *L. edodes* possessed a strong antitumor effect via its immunopotentiating activity (6). Grifolan has been isolated and purified from *G. frondosa* mycelium (7). And, the antitumor β-glucan (designated as D-fraction) was extracted from *G. frondosa* fruiting body by Nanba et al. (8). These glucans *in vivo* enhanced immunoreactivity, inducing the activation of macrophages, cytotoxic T cells, and NK cells. In addition, schizophyllan from *Schizophyllum commune*, and PSK from *Trametes versicolor* were approved as pharmaceutical agents in Japan. *T. versicolor* also contained bioactive polysaccharide-peptide, PSP. It has also been reported that antitumor polysaccharides such as SSG from *Sclerotinia sclerotiorum* and many kinds of bioactive polysaccharides and polysaccharide–protein complexes from *Agaricus blazei*, *F. velutipes*, *Auricularia auricular-judae*, *G. lucidum*, *Amanita muscaria*, *Polyporous confluens*, *T. giganteum*, *Ganoderma tsugae*, *Pleurotus sajor-caju*, *C. volvatus*, and *Sarcodn aspratus* were isolated and purified.

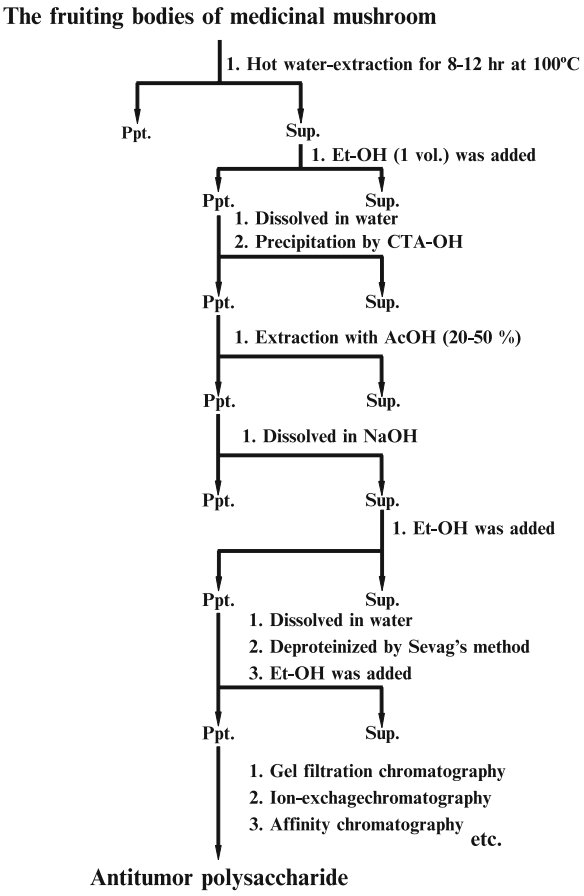


Fig. 28.1. Extraction of an antitumor polysaccharide from the mushroom fruiting body by traditional method.

On the other hand, it is considered that searching for new antitumor and other medicinal substances from the mushrooms and studying their several medicinal values have become a matter of great significance. Many reports showed that most of the antitumor polysaccharides played many important roles in strengthening the immune system for host defense. Recently, it has been reported that these polysaccharides stimulated immunocomplement cells such as T lymphocytes, natural killer cell, and macrophage and could induce the productions of some kinds of cytokines from these cells (9–11). Cytokines, which are bioactive peptides such as ILs, TNF and IFNs etc., are secreted from stimulated lymphocytes and macrophages, and organize and regulate cytokine networks to maintain the immune system. It has been considered that the immune responses depending on $CD4^+$ helper T (Th) cells are controlled via maintaining the balance of Th1 and Th2 cells (12). Th1 cells produce IFN-γ and TNF-α, whereas Th2 cells produce IL-4, IL-5, and IL-13. In addition, a type of macrophages producing IL-1, IL-12, IL-23, TNF-α, reactive oxygen species (ROS), and nitrogen reactive species (NOS) support Th1 response. In contrast, other types of macrophages secreting IL-10 contribute to Th2 response. Liu et al. (9) and Jin et al. (13) showed that polysaccharides

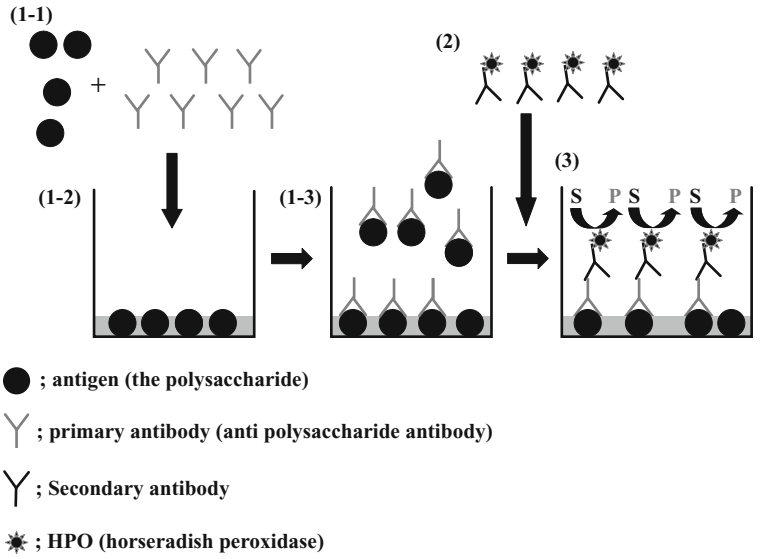


Fig. 28.2. ELISA inhibition assay for the determination of an immunomodulating polysaccharide. (1) Primary anti-polysaccharide antibody incubation. (1-1) Incubate the sample in diluted primary antibody for 30 min at 4°C. (1-2) Incubate the antigen (the polysaccharide) coating well with the mixture of sample and the primary antibody for 1 h at 25°C. (1-3) Rinse the well to remove the conjugations of the sample and the antibody. (2) Secondary antibody incubation. Incubate the well in the diluted HRP labeled secondary antibody for 1.5 h at 25°C. (3) Detection. After coloration with H_2O_2-*O*-phenilenediamine for 10 min at 25°C, absorbance at 492 nm is measured.

from *L. edodes* induced the production of some kinds of cytokines, including TNF-α, IL-1β, IL-10, IL-12, and IFN-γ, from stimulated human peripheral blood mononuclear cells (PBMC), peritoneal exudates cells, and splenocytes. *G. frondosa* also contained some β-glucan preparations that led macrophages, T lymphocyte, and NK cell, or spleen and lymph node to produce IL-1, IL-6, IL-12, IL-18, IFN-γ, and TNF-α. These agents increased production of IL-1, IL-2, IL-6, IL-8, IFN-γ, and TNF-α from stimulated PBMC and human monocytes (3).

Although the analytical methods of polysaccharides have improved considerably, it is still difficult to purify and quantify the polysaccharides as summarized in Fig. 28.1. It is very tedious to obtain the polysaccharides reproducibly, and difficult to determine them exactly. This seems a reason why no paper had reported how the contents of antitumor polysaccharides changed in the mushrooms during growth and storage of the fruiting bodies. To detect the exact and simple analysis for the antitumor polysaccharides in mushrooms, we have prepared a few antipolysaccharide antibodies, and then applied the enzyme linked immunosorbent assay (ELISA) to determine the polysaccharides in the mushrooms using these antibodies (Fig. 28.2), (14, 15).

28.3 LENTINAN FROM *L. EDODES*

Chihara et al. (6) isolated and purified lentinan from the hot water extract of *L. edodes* called as "Shiitake" in Japan. Lentinan has two branches for every five d-glucopyranosyl residues at *O*-6. The average molecular weight of lentinan was deduced as

400,000. The antitumor mechanism of this glucan has been thought to be by activation of immunoresponses in the host. Lentinan had a strong antitumor effect; it markedly inhibited the growth of Sarcoma 180 implanted subcutaneously in mice, inducing an almost complete regression of tumors at doses of 1 $mg \cdot kg^{-1}$ without toxicity. It has also been reported that lentinan showed prominent antitumor activity in murine allogeneic and syngeneic host. It is widely accepted that the activated immunocompetent cells, such as macrophages, cytotoxic T cells, and natural killer (NK) cells, in host usually play important roles in tumor immunity. These reports suggest that the lentinan acts as an immunomodulator to develop tumor immunity against allogeneic and some syngeneic tumors. In addition, Nanba et al. (16) reported that the extract containing lentinan from *L. edodes* fruiting bodies showed antitumor effects on allogeneic and syngeneic tumors after oral administration. It is thought that lentinan augments the immune response through modulation of the function of phagocytes such as macrophages. It has been also reported that lentinan possesses an immunomodulating effect which is seen in the activation of a variety of macrophage functions, for example, TNF-α, IL-1, NO and superoxide anion production, phagocytosis, and cytotoxicity.

We detected a change in lentinan from *L. edodes* fruiting body during growth (17). The fruiting bodies were harvested at early formation period of fruiting body (Stage I; the cap closed, 4 days after the fruiting body started to form), at middle stage of the formation period (Stage II; the cap opened moderately, 6 days after), and at last stage of the formation period (Stage III; the cap opened completely, 7 days after). It was recognized that the fruiting body at Stage II was suitable as a commercial product. During this period, the weight of the fruiting body increased from 9 to 18 g during growth, and the content of lentinan increased from 1.4 to 1.5 $mg \cdot g^{-1}$ fw assessed by ELISA using the anti-lentinan antibodies. Thereafter, lentinan content decreased to 1.1 $mg \cdot g^{-1}$ fw at Stage III. Moreover, we investigated their immunomodulating activities in TNF-α and NO production from murine peritoneal macrophages. The effect of *L. edodes* increased from approximately 500 $pg \cdot ml^{-1}$, which was the activity of the mushroom at Stage I, to 700 $pg \cdot ml^{-1}$ (at Stage II) as TNF-α productions, and from 4.3 μM (at Stage I) to 7.1 μM (at Stage II) as NO productions. However, the activity, which the mushroom at Stage III showed, decreased to 500 $pg \cdot ml^{-1}$ as TNF-α production and to 4.8 μM as NO production. These results suggested that the contents of antitumor polysaccharides, which were synthesized in mushrooms, change through the growth periods; thereby, a suitable period in which to harvest the mushroom with strong antitumor activity was found. And, the change reflected a change in the potency of an immunomodulating activity of *L. edodes*. Therefore, it is necessary to determine an amount of an antitumor polysaccharide in a medicinal mushroom and harvest it at the period when the mushroom is effectively available as functional food and a source of nutritional supplement.

28.4 ANTITUMOR POLYSACCHARIDES FROM *G. FRONDOSA*

G. frondosa, called as "Maitake", is also a very popular edible mushroom in Japan. Antitumor glucan fractions, which were designated grifolan and D-fraction, were obtained from *G. frondosa* liquid-cultured mycelium (7) and fruiting bodies (8), respectively. Grifolan consisted of a β-1, 3-polyglucose backbone with a branch for every three D-glucopyranosyl residues at *O*-6. D-fraction was mainly composed of a backbone

of β-1, 6-linked glucose residues with side chains of β-1, 3-glucose residues. There have been reports that they showed enhanced immunomodulating activities, including the activation of macrophages, cytotoxic T cells and NK cells. It had been demonstrated that grifolan enhanced to produce a pleiotropic cytokine, IL-6. It should activate the host defense system by IL-6 exhibiting broad immunomodulating activities, involving the induction of IL-2 production from T cells and the induction of acute phase proteins in hepatocytes. In addition, the polysaccharide augmented productions of IL-1 and TNF-α, proinflammatory cytokines from stimulated murine macrophages (RAW264.7). On the other hand, it had been shown that the glucan from the fruiting body suppressed tumor growth by significantly increasing the production of TNF-α and IFN-γ from spleen cells or NK cells, and IL-12 from macrophages (18). We investigated a change in the amount and activity of immunomodulating glucan from the fruiting body during growth as well as lentinan in *L. edodes* (17). *G. frondosa* fruiting bodies were harvested at four different stages during growth from Stage I to IV. The cap of the mushroom at Stage I (5 days after the fruiting body started to form) and II (10 days after) remained closed, and then they opened moderately at Stage III (15 days after) and completely at Stage IV (18 days after). The weight of *G. frondosa* fruiting bodies also increased from approximately 135 g to 200 g during the growth of the mushroom. It was recognized that the fruiting body at Stage III was suitable as a commercial product. The amount of the glucan in the fruiting body increased from 1.7 $mg \cdot g^{-1}$ fw to 2.7 $mg \cdot g^{-1}$ fw through the period from Stage I to III. However, its content decreased rapidly to 1.3 $mg \cdot g^{-1}$ fw at the growth stage IV. These results suggested that the contents of antitumor polysaccharides in mushrooms changed during the growth of the fruiting bodies. Moreover, the changes in the effect of *G. frondosa* fruiting bodies during growth in TNF-α and NO productions from murine macrophages were investigated. The amount of TNF-α which was released from macrophages stimulated with the extract from the fruiting body at growth stage I was approximately 300 $pg \cdot ml^{-1}$. And, at growth stage III, its effect on TNF-α production significantly increased to 500 $pg \cdot ml^{-1}$. Thereafter, the amount of TNF-α was decreased to 260 $pg \cdot ml^{-1}$at growth stage IV. The amount of NO produced from the macrophages was also examined. Its amount increased from 5.2 to 7.1 μM in the extracts from *G. frondosa* during growth from I to III. Thereafter, the amount of NO from macrophages stimulated with the extract at stage IV of the mushroom decreased to 3.7 μM. The results in this study suggested that the content of an antitumor polysaccharide in a mushroom changed during the growth period, and this change would affect an immunomodulating activity of a medicinal mushroom.

28.5 IMMUNOMODULATING ACTIONS OF *P. CORNUCOPIAE* VAR. *CITRINOPILEATUS,* OYSTER MUSHROOMS, AND *P. NAMEKO*

The basidiomycete fungi *P. cornucopiae* var. *citrinopileatus* called 'Tamogitake' and *P. nameko* c 'nameko' are also delicious and popular edible mushrooms in Japan. We suggest that these mushrooms can provide source materials for the development of a novel immunomodulator. The basidiomycete fungi *P. cornucopiae* is also called "oyster mushroom" and is considered very delicious. The production and consumption of this edible mushroom have rapidly increased every year. Therefore, it is believed that this

mushroom was effectively utilized. Recently, it has been reported that this mushroom possesses various pharmacological functions. It was reported that D-mannitol and a hot water extract from this mushroom showed significant antihypertensive effects. However, little has been reported regarding the immunomodulating effects of a polysaccharide from the fruiting body of *P. cornucopiae* var. *citrinopileatus*. To investigate the immunomodulating action of the mushroom, we chromatographically isolated an immunomodulating polysaccharide fraction possessing a molecular mass of 448 kDa from a hot water soluble extract of this fruiting body, and measured the cytokine production from macrophage cell lines, RAW264 (mouse) and U937, THP-1 (human), that were treated with the fraction. It showed high activities of productions of tumor necrosis factor (TNF)-α, IP-10, nitric oxide (NO) from the treated macrophages. However, Th2-cytokine, such as IL-10, could not be produced from them (19, 20). In addition, the immunomodulating activity of the polysaccharide was enhanced by a guanylic acid (5′-GMP-Na), and suppressed by vitamin D_2 (unpublished). This result suggested that the immunomodulating activity of a functional polysaccharide would be changed by interactive action with other food factors.

P. nameko also possess potent immunomodulating activities as a result of cytokine production from murine human macrophages (21). This edible mushroom had shown significant strong inhibition action against the Sarcoma 180 ascites tumor growth (5). To determine an immunomodulating activity of the *P. nameko* fruiting body, the pattern of cytokine production and the subset analysis of splenic lymphocytes in BALB/c mice administered the freeze dried powder of the mushroom for 2–6 weeks. The powder was found to enhance the immunomodulating effects on the production of TNF-α from peritoneal exudates macrophages. Moreover, the *P. nameko* fruiting body significantly increased $CD3^+CD4^+$ T cells subset in spleen from the mice. And, it was demonstrated to enhance secreting of a Th1 type cytokine IFN-γ, and to attenuate production of a Th2 type cytokine IL-4 from splenic lymphocytes (unpublished).

These results suggested that the two mushrooms *P. cornucopiae* and *P. nameko* activated immunocompetent cells such as macrophages and lymphocytes, and could provide source materials for the development of a Th1-polarizing immunomodulator (Fig. 28.3). Thus, these mushrooms also could be used as effective materials of functional foods and immmunomodulators.

28.6 OTHERS

Many edible mushrooms have already become attractive as health-beneficent foods and as source materials for immunomodulators, antitumor agents, antibiotics, and antihypertensive. Therefore, medical and pharmaceutical interest in the mushrooms has strongly increased worldwide. In 1969, Ikekawa et al. (5) reported on the antitumor activities of aqueous extracts of edible mushrooms, including *L. edodes, F. velutipes*, *P. ostreatus, P. spodoleucus, P. nameko, T. matsutake,* and *A. auricula-judae*. Recently, immunomodulators from more than 30 species of mushrooms have been isolated and their pharmaceutical activities were shown.

The glucan and the glucan–protein complex from *A. blazei* Murill possessed remarkable antitumor activity by enhancing T cell activities, and immunomodulating activities that promoted the secretion of IL-8, TNF-α and nitric oxide from macrophages and the

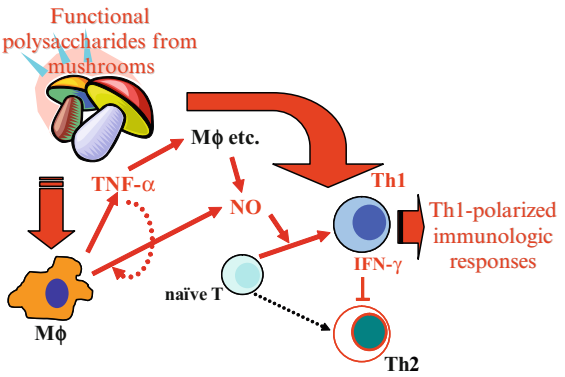


Fig. 28.3. The hypothesis for immunomodulating action of a functional polysaccharide from edible mushrooms. The mushrooms can provide source materials for the development of a Th1-polarizing immunomodulator.

synthesis of proinflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α in human monocytes (22). *Tricholoma* species also showed a strong antitumor activity via stimulation for immunocomplement cells. They have the ability to enhance phagocytic function of peritoneal exudate cells, and mitogenic activity of T cells in tumor-bearing mice. *A. bisporus* is a major mushroom cultivated globally. In the Western countries, the production of this mushroom accounts for 80–98% of total mushroom production. The white button and the crimini stimulated macrophages to produce TNF-α. *A. bisporus* fruiting body possessed antitumor activity through enhancing cytotoxic activity of T cells (23). Moreover, the other mushrooms involving *S. aspratus* (24) and *Isaria japonica* (25) showed potent immunomodulating actions and antitumor activities.

We believe that the success of these studies could lead to an increase in utilization of these mushrooms as a common food, a nutritional or functional food, and a source material for drugs or immunomodulators.

28.7 CONCLUSIONS AND PERSPECTIVES

In oriental traditional medicine, it has been believed that medicine and food have the same origin, which was called "Ishoku-Dougen" in Japanese. Many edible mushrooms have already become attractive as health-beneficent foods, and as source materials for immunomodulators, antitumor agents, antibiotics, and antihypertensive, etc. Therefore, the medical and pharmaceutical interest in the mushrooms has become increasingly strong worldwide. Historically, the polysaccharides or glucan had been investigated in their immunomodulating actions and roles in the immune system. And, most data showed that the polysaccharides from the mushrooms can provide source materials for the development of Th1-polarizing immunomodulators, which thereby might be expected to prevent the disease caused by an increase in Th2 responses, such as allergy (Fig. 28.3). We have already reported that extracts from some kinds of edible mushrooms such as *L. edodes*, *G. frondosa*, *S. aspratus*, *P. cornucopiae*, *P. nameko*, and *A. blazei* showed significant immunomodulating actions. However, recently, a variety of small molecule compounds such as epoxy compounds, flavonoids, and terpene compounds, have shown

properties to inhibit and stimulate molecular targets in the immune system. Further, we must clarify their role in the immune system as well as the active site of the polysaccharide. Many kinds of mushrooms are expected to become useful materials of functional food or immunomodulators. It is necessary that the researchers must elucidate what is an active site of a functional compound, and how it plays a pivotal role in the immune system. The success of our work would help us lead a healthier life.

REFERENCES

1. Wasser SP, Nevo E, Sokolov D, Reshetnikov S, Timor-Tismenetsky M (2000) Dietary supplements from medicinal mushrooms: diversity of types and variety of regulations. Int J Med Mushrooms 2:1–19
2. Wasser SP, Weis AL (1999) Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. Int J Med Mushrooms 1:31–62
3. Zaidman BZ, Yassin M, Mahajna J, Wasser SP (2005) Medicinal mushroom modulators of molecular targets as cancer therapeutics. Appl Microbiol Biotechnol 67:453–468
4. Lull C, Wichers HJ, Savelkoul HFJ (2005) Antiinflammatory and immunomodulating properties of fungal metabolites. Mediat Inflamm 2:63–80
5. Ikekawa T, Uehara N, Maeda Y, Nakanishi M, Fukuoka F (1969) Antitumor activity of aqueous extracts of edible mushrooms. Cancer Res 29:734–735
6. Chihara G, Maeda Y, Hamuro J, Sasaki T, Fukuoka F (1969) Inhibition of mouse Sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) sing. Nature 222:687–688
7. Ohno N, Iino K, Takeyama T, Suzuki I, Sato K, Oikawa S, Miyazaki T, Yadomae T (1985) Structural characterization and antitumor activity of the extracts from matted mycelium of cultured *Grifola frondosa*. Chem Pharm Bull 33:3395–3401
8. Nanba H, Hamaguchi A, Kuroda H (1987) The chemical structure of an antitumor polysaccharide in fruit bodies of *Grifola frondosa* (Maitake). Chem Pharm Bull 35:1162–1168
9. Liu F, Ooi VEC, Fung MC (1999) Analysis of immunomodulating cytokine mRNAs in the mouse induced by mushroom polysaccharides. Life Sci 64:1005–1011
10. Okazaki M, Adachi Y, Ohno N, Yadomae T (1995) Structure-activity relationship of (1 → 3)-β-d-glucans in the induction of cytokine production from macrophages, in vitro. Biol Pharm Bull 18:1320–1327
11. Sakurai T, Ohno N, Yadomae T (1994) Changes in immune mediators in mouse lung produced by administration of soluble (1 → 3)-β-d-glucan. Biol Pharm Bull 17:617–622
12. Mosmann TR, Coffman RL (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145–173
13. Jin M, Jung HJ, Choi JJ, Jeon H, Oh JH, Kim B, Shin SS, Lee JK, Yoon K, Kim S (2003) Activation of selective transcription factors and cytokines by water-soluble extract from *Lentinus lepideus*. Exp Bio Med 228:749–758
14. Mizuno M, Minato K, Tsuchida H (1996) Preparation and specificity of antibodies to an anti-tumor β-glucan, lentinan. Biochem Mol Biol Int 39:679–685
15. Mizuno M, Yamakawa A, Minato K, Kawakami S, Tatsuoka S, Tsuchida H (1999) Preparation of antibody against antitumor β-glucan *in Grifola frondosa* and its application. Food Sci Technol Res 5:398–401
16. Nanba H, Kuroda H (1987) Antitumor mechanisms of orally administered shiitake fruit bodies. Chem Pharm Bull 35:2459–2464
17. Minato K, Mizuno M, Kawakami S, Tatsuoka S, Denpo Y, Tokimoto K, Tsuchida H (2001) Changes in immunomodulating activities and content of antitumor polysaccharides during the growth of two medicinal mushrooms, *Lentinus edodes* (Berk.) Sing. and *Grifola frondosa* (Dicks.: Fr) S. F. Gray. Int J Med Mushrooms 3:1–7
18. Kodama N, Komuta K, Sakai N, Nanba H (2002) Effects of D-fraction, a polysaccharide from *Grifola frondosa* on tumor growth involve activation of NK cells. Biol Pharm Bull 25:1647–1650

19. Minato K, Abe C (2008) An immunomodulating action of a polysaccharide fraction from an edible mushroom, *Pleurotus cornucopiae* var. *citrinopileatus*. J Clin Biochem Nutr 43:359–362
20. Minato K (2008) A polysaccharide fraction from an edible mushroom, *Pleurotus cornucopiae* var. *citrinopileatus* stimulates an immunomodulating action of murine macrophage, RAW264, on TNF-α and NO productions in vitro. Int J Med Mushrooms 10:235–244
21. Minato K, Kasahara S (2006) Immunomodulating action of edible mushrooms, *Pleurotus cornucopiae* var. *citrinopileatus* and *Pholiota nameko*. 8th International Mycological Congress, Medimond S. r. l., Italy, pp 219–224
22. Sorimachi K, Akimoto K, Ikehara Y, Inafuku K, Okubo A, Yamazaki S (2001) Secretion of TNF-α, IL-8 and nitric oxide by macrophages activated with *Agaricus blazei* Murill fractions in vitro. Cell Struct Funct 26:103–108
23. Nanba H, Kuroda H (1988) Potentiation of host-mediated antitumor activity by orally administered mushroom (*Agaricus bispora*) fruit bodies. Chem Pharm Bull 36:1437–1444
24. Mizuno M, Shiomi Y, Minato K, Kawakami S, Ashida H, Tsuchida H (2000) Fucogalactan isolated from *Sarcodon aspratus* elicites release of tumor necrosis factor-α and nitric oxide from murine macrophages. Immunopharmacology 46:113–121
25. Kawakami S, Minato K, Imamura T, Aizono Y, Mizuno M (2003) Stimulation of TNF-α and NO production from murine macrophage by water-soluble polysaccharides from *Isaria japonica*. In: Shahidi F, Ho CT, Watanabe S, Osawa T (eds) Food factors in health promotion and disease prevention: ACS Symposium Series 851. American Chemical Society, Washington, DC, pp 152–162

29 Immunological Functions of Polysaccharides from Soy Sauce

Makio Kobayashi

Key Points

- Soy sauce is a traditional fermented seasoning of Japan, and now available throughout the world. Ever since the biological functions of the ingredients in soy sauce have been elucidated, soy sauce is considered to be not only a traditional seasoning but also a functional food for human health.

Key Words: Allergy, antiallergic activity, clinical study, immunomodulation, intestinal immune system, *shoyu* polysaccharides (SPS), soy sauce.

29.1 INTRODUCTION

Soy sauce, traditionally used in Japan and several oriental countries, is a liquid seasoning currently used in cooking worldwide (1). The daily consumption of soy sauce in Japan is estimated at about 30 ml per person, according to data from the Japan Soy Sauce Brewers Association, 1988. Studies suggest that soy sauce contains certain bioactive components in addition to taste and aroma compounds, and has various biological functions including anticarcinogenic (2, 3), antimicrobial (4), antioxidative (5–7), and antiplatelet (8) activities and the inhibition of an angiotensin I-converting enzyme (9). Therefore, soy sauce is considered to be not only a traditional seasoning but also a functional food. Further study is needed to elucidate the biological functions of soy sauce ingredients. In this chapter, new immunological functions of soy sauce are described with respect to allergies, such as antiallergic and immunomodulating activities of polysaccharides from soy sauce.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_29

29.2 BREWING OF JAPANESE SOY SAUCE

Shoyu is the Japanese name for soy sauce. Many varieties of *shoyu* are produced in Japan and other oriental countries. Their characteristics depend on the various types and different ratios of raw materials used, the types of microorganisms employed, and the conditions of fermentation. In Japan, naturally brewed *shoyu* is produced as shown in Fig. 29.1 (1, 10–12). Moistened soybeans are cooked under both high pressure and temperature, while the wheat is roasted and crushed. These two materials are mixed with a small amount of seed mold, *Aspergillus oryzae* or *A. sojae*. This mixture is placed on a large porous plate through which temperature- and moisture-controlled air is passed to provide the appropriate conditions for mold growth and enzyme production. The mold-cultured material is termed *koji*, and the *koji* is mixed with high NaCl in water to make a mash called *moromi*. The *moromi*, containing about 15% (w/v) NaCl, is stored in large tanks for several months at room or elevated temperature. The *moromi* is fermented with lactobacilli and yeasts and then well aged. The aged *moromi* is pressed, and the liquid part (raw soy sauce) is pasteurized to obtain the final product, soy sauce (*shoyu*).

29.3 POLYSACCHARIDES FROM SOY SAUCE

In soy sauce, the proteins of the raw materials are completely degraded into peptides and amino acids by microbial proteolytic enzymes after fermentation (13), and so no allergens are present (13–15). In contrast, polysaccharides originating from the cell wall of soybeans are resistant to enzymatic hydrolysis, and are present even after fermentation (16, 17). In 1972, Kikuchi and Yokotsuka (16) purified polysaccharides from soy sauce and investigated their properties in detail. The cell wall polysaccharides of soybeans, one of the main raw materials of soy sauce, contain a large amount of galacturonic acid and are only slightly hydrolyzed by mold enzymes during the *koji* and *moromi* stages (16, 17). These polysaccharides make up approximately 1% (w/v) of the soy sauce. Although it has been more than 30 years since polysaccharides such as pectic substances were first reported to be present in soy sauce (16, 17), their biological activities have still not been investigated. Therefore, here we examine the biological functions, including the antiallergic and immunomodulating activities of *Shoyu* polysaccharides (SPS) obtained from either the dialysate or the ethanol precipitate of raw soy sauce.

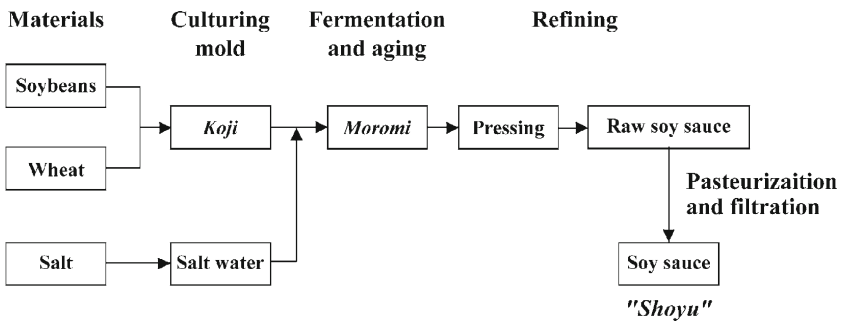


Fig. 29.1. Brewing process of soy sauce from raw materials to final product *shoyu*.

29.4 ANTIALLERGIC ACTIVITY OF SPS

Allergic responses can be divided into four general categories, based on the mechanism of immunological involvement (18–20). A type I allergy, which causes various symptoms of asthma, rhinitis, atopic dermatitis, and anaphylactic shock, is the most common (18–20). The pathological mechanism of a type I allergy has been explained as the degranulation of mast cells and the release of chemical mediators such as histamine, leukotrienes, and prostaglandins from these cells (18–20). Mast cell degranulation occurs in response to immunological stimuli in which the antigen-immunoglobulin E (IgE) antibody reaction predominates on the cell membrane (18–20).

Recently, it has been reported that many substances derived from natural plants and foods showed antiallergic activities *in vitro* and *in vivo*. Therefore, as a first screening *in vitro*, we examined the inhibitory effects of SPS on hyaluronidase, which are usually measured to evaluate the antiallergic activity (21–27). Hyaluronidase, a mucopolysaccharide-splitting enzyme, has been implicated in allergic reactions, the migration of cancer cells, inflammation, and an increase in the permeability of the vascular system (21–27). Kakegawa et al. (28–30) have reported a good correlation between the inhibitory effect on hyaluronidase and the release of histamine from mast cells in response to acidic antiallergy agents. In previous studies (21–27), extracts of vegetables, fruits and herbs were evaluated for inhibitory effects, and several antiallergic compounds were identified. Notably, in 1992, Sawabe et al. (22) showed that pectic substances such as those in apple and citrus inhibit both activated hyaluronidase and the release of histamine from mast cells. This was the first report on the antiallergic activity of polysaccharides. In 1997, it was also reported that alginic acids from brown algae have inhibitory effects (24). In 2004, we demonstrated that SPS from raw soy sauce have the same potent inhibitory effect on the activation of hyaluronidase as the antiallergic agent disodium cromoglycate (31). In contrast to the above pectic substances (22), interestingly, polysaccharides prepared from heat-treated materials of soy sauce such as cooked soybeans and roasted wheat, have no inhibitory effects (31). Therefore, antiallergic SPS originate from partially degraded polysaccharides of the materials in the *koji* stage, and are stable even during the *moromi* stage. Soy sauce contains about 1% (w/v) SPS, the novel antiallergic substances similar to disodium cromoglycate (31).

Furthermore, we examined the antiallergic activities of SPS by assaying the release of histamine from rat basophilic leukemia (RBL-2H3) cells, which had been induced by the antigen. RBL-2H3 cells release histamine when exposed to various stimuli *in vitro* (32, 33), and have the same function as mast cells and basophils, which are involved in allergic type I reactions (22–25, 32, 34–39). SPS that inhibit hyaluronidase also significantly inhibit the release of histamine from RBL-2H3 cells (31). The inhibitory effect of SPS on the release is concentration-dependent, and was estimated to be about one-tenth that of ketotifen, an antiallergic drug (38). However, the cytotoxicity of SPS is at least tenfold lower than that of ketotifen (31). Therefore, SPS from soy sauce would be safe and is expected to act as an antiallergic seasoning for foods.

Finally, we evaluated the antiallergic effect of orally administered SPS by inducing a passive cutaneous anaphylaxis (PCA) reaction in the ears of mice, as an animal model for a type I allergy. Although many antiallergic substances inhibit hyaluronidase and the release of histamine from basophils or mast cells *in vitro* (22–25), there were no studies

on the effects of these substances on the PCA reaction *in vivo*. SPS had a significant suppressive effect on the PCA reaction when 12 mg/kg body weight per day was administered orally for 3 days (31). The effective concentration of SPS is lower than that of antiallergic substances described previously (40–43). For example, the minimum effective oral doses for suppressing the PCA reaction were reported as follows: 100 mg/kg of persimmon leaf extract (40), 125 mg/kg of *Perilla frutescens* extract (41, 42), and 100 mg/kg of buckwheat grain extract (43). In contrast, 12 mg/kg of SPS per day corresponded to only about 1 ml/kg of raw soy sauce per day (31).

29.5 IMMUNOMODULATING ACTIVITIES OF SPS

Recently, it has been reported that immunoregulatory effects are related to the incidence or prevention of infectious diseases and allergies. In addition, it has been shown that certain foods have a host defense function related to the immune system (44–47). In particular, several polysaccharides, such as dietary fibers (48), β-glucan (49, 50) and marine algae (51, 52), have been shown to have immunomodulatory activities.

Immunological functions are generally divided into two systems, natural and adaptive. In natural immunity, macrophages play diverse roles in the host defense mechanism against invasive insults. Their functions include the presentation of antigens to T and B cells, destruction of intracellular microbial pathogens, and generation and secretion of monokines (20). It is accepted that $CD4^+$ T cell clones can be classified into two distinct populations, type 1 helper T cells (Th1) and type 2 helper T cells (Th2), based on the patterns of cytokine production (18, 19). Th1 cells are mainly responsible for cell-mediated immunity, while Th2 cells are responsible for humoral immunity. Each subset produces cytokines for self-amplification and cross-regulation. Interferon (IFN)-γ, produced by Th1 cells, has two functions. First, it activates macrophages, enhancing their ability to both phagocytize and destroy microbes. Second, it stimulates the production of immunoglobulin G (IgG) antibodies. Th2 cells produce interleukin (IL)-4 and IL-5. IL-4 is the major inducer of IgE production in B cells. Studies have pointed out the importance of the balance of Th1/Th2 cells in disorders such as infectious and allergic diseases (18).

In order to clarify the immunomodulatory effects of SPS in systemic immunity, we investigated the effect of SPS on the activation of macrophages as well as the regulation of the balance of Th1/Th2 cell responses *in vitro* and *in vivo* (53). SPS from soy sauce enhanced glucose consumption in peritoneal macrophages treated with glycogen *in vitro*, and oral administration of the SPS to mice increased the capacity of peritoneal macrophages to consume glucose *in vivo* (53). These results suggest that SPS activate peritoneal macrophages. It is accepted that glucose consumption increases as a result of the activation of macrophages at the priming stage (54). Since glucose is used to produce ATP and NADPH for the expression of active oxygen species and phagocytosis (49, 55), it is likely that SPS enhance the pentose phosphate pathway in peritoneal macrophages. Belardelli (56) reported that all cytokines produced by Th1 cells have some positive role in the activation of macrophages, whereas cytokines produced by Th2 cells such as IL-4 exhibit suppressive effects on macrophage functions. Thus, the balance of Th1 and Th2 cell responses is cross-linked with macrophage function and SPS might directly enhance macrophages and/or cause a shift to predominantly Th1 cell responses in mice.

Furthermore, it was reported that the Th1/Th2 cell response was shifted to a predominantly Th2 cell response in allergic diseases, and the production of IgE by B cells was thus increased in these diseases (57). Lim et al. (48) reported that pectin could alleviate type I allergic reactions by enhancing lymphocyte function. Shida et al. (58) and Fujiwara et al. (59) showed that certain lactic acid bacteria inhibited the rise in serum IgE levels by improving the Th1/Th2 balance. Recently, Matsushita et al. (53) demonstrated that SPS from soy sauce significantly suppressed the production of IL-4 and enhanced that of IFN-γ, and thus in mice treated with SPS, there was a shift toward predominantly Th1 cell responses. Therefore, SPS may play a role in the suppression of IgE production by B cells through adaptation of the balance of Th1/Th2. In conclusion, SPS exerted part of their antiallergic effect through the regulation of immune function, and effectively enhanced both macrophage and lymphocyte function.

29.6 STIMULATORY EFFECT OF SPS ON INTESTINAL IMMUNE SYSTEM

The mucosal immune system in the gut has attracted much attention, and elucidation of the system is underway. It has become clear that the mucosal immune system in the gut responds differently from the systemic immune system. The intestinal tract is one of those internal organs that are always exposed to various kinds of pathogens and antigens, including disease-causing germs, viruses, and allergens. Orally administered antigens interact with the gut-associated lymphoid tissue (GALT), a well-developed immune network that is involved not only in the protection of the host from pathogens but also in preventing unusual reactions to ingested proteins. This immune response is mainly a humoral immune response mediated by immunoglobulin A (IgA)-producing cells and results in the secretion of IgA which constitutes almost 80% of all the antibodies produced in mucosal-associated tissue (60, 61). Mucosal IgA prevents the adhesion of bacteria or viruses, and reduces the absorption of food antigens in the intestine (62–65). Antigen uptake occurs through a specialized system represented by the M cells overlying Peyer's patch (PP) (66). PP is the major site in the intestine for inducing an active immune response between macrophages and dendritic cells and T and B cells.

Yasui et al. (67) reported that certain lactic acid bacteria, which generate large quantities of IgA, could prevent infections of the rotavirus. Recently, Matsushita et al. (68) demonstrated that SPS from soy sauce enhanced IgA production in PP cells *in vitro* and *in vivo*. In fact, compared to that of the control group of mice, the IgA concentration in the intestine of the mice that were fed SPS was significantly higher. These results suggest that SPS enhance IgA production through the stimulation of the intestinal immune system and that SPS could be expected to prevent allergic reactions by interfering with the absorption of allergens and microbial infections. As mentioned above, SPS exhibit strong antiallergic activities, and this effect was exerted through the Th1/Th2 balance. Together with previous findings, these results confirm that SPS effectively enhance not only the systemic immune system but also the mucosal one and provide new insight into the physiological role of SPS.

Furthermore, Matsushita et al. (68) evaluated the absorption of SPS to epithelial cells using a Caco-2 cell model. It has been reported that Caco-2 cells undergo spontaneous differentiation in culture and exhibit the characteristics of mature enterocytes by

forming polarized monolayers with a brush border and functionally competent tight junctions (69). Caco-2 cells can serve as a screen since compounds with high permeability in this model are typically well absorbed *in vivo* (70). Using this model, it was shown that SPS might be transported across cell monolayers because the intestinal absorption of uronic acid occurred and digested SPS were distributed within the body.

29.7 CLINICAL STUDIES ON SPS

Allergic rhinitis is a common allergic disease in industrialized nations, characterized by sneezing, watery rhinorrhea, itching in the nose, eyes, and palate, and nasal congestion (71). In many instances, patients also experience headaches and fatigue, with a significant effect on their quality of life (QOL). Recently, the incidence of both perennial allergic rhinitis (PAR) (72–77) and seasonal allergic rhinitis (SAR) (78–83) has been increasing worldwide, leading to a marked increase in direct and indirect treatment costs. Antihistamines reduce allergic symptoms but can cause nervousness, irritability, dizziness, and sleepiness. So, many allergic patients desperately desire the development of safe dietary components not having side effects.

Despite steady advances in conventional therapies for allergic symptoms, natural products such as phytochemicals and herbal extracts have been widely used by consumers as alternatives to prescription drugs without definitive clinical evidence. However, few placebo-controlled clinical trials have examined the efficacy and safety of these natural products. In randomized, double-blind, placebo-controlled clinical studies of PAR using an 11-herb mixture (Biminne) (74), and SAR using grapeseed extract (80), *Perilla frutescens* extract (82), and a Chinese herbal medicine (containing 18 herbs) (81), it was demonstrated that Biminne, the *P. frutescens* extract, and the Chinese herbal medicine are significantly effective, but grapeseed extract is not effective. Therefore, we determined whether oral supplementation of SPS is an effective intervention for patients with PAR (84) and SAR (85). Recently, it was reported that polysaccharides derived from black currant improved the symptoms of Japanese cedar pollinosis in a randomized double-blind, placebo-controlled study (83).

29.8 IMPROVEMENT OF ALLERGIC SYMPTOMS OF PATIENTS WITH PAR

PAR causes major discomfort in affected patients, seriously degrades work and learning performances as well as quality of sleep, and has perturbing physical, psychological, and social consequences (72–77). Therefore, effective management should be directed not only toward rapid and adequate relief of the clinical symptoms but also toward a sustained improvement in the patients' daily living. QOL is a concept comprising physical and psychological characteristics that can be used to assess problems in the social aspect of a person's lifestyle. Generic questionnaires make it possible to measure these factors.

In a 4-week randomized, double-blind, placebo-controlled parallel group study (84), patients with PAR were treated with 600 mg of SPS ($n = 11$) or a placebo ($n = 10$) each day. After 4 weeks of treatment with SPS, symptom scores for runny nose, sore throat, and itchy eyes significantly decreased from the baseline within the group ($p < 0.05$), but no changes in these scores were observed after 4 weeks of treatment in the placebo group

(84). The total symptom score, calculated from the sum of individual scores, showed a significant difference between the two groups after 4 weeks of treatment ($p < 0.05$).

29.9 IMPROVEMENT OF ALLERGIC SYMPTOMS OF PATIENTS WITH SAR

SAR also causes major discomfort in affected patients, for example, in Japan, those with an allergy to Japanese cedar (*Cryptomeria japonica*) pollen in spring (78–83). SAR may occur at any age and affects between 10% to 30% of children (78). SAR is episodic and can cause fatigue, absenteeism, and irritability, adversely affecting the child's adjustment and achievement in school (78). Furthermore, children with SAR may subsequently develop PAR (78).

In an 8-week randomized, double-blind, placebo-controlled parallel group study (85), patients with SAR due to Japanese cedar pollen were treated with 600 mg of SPS ($n = 25$) or a placebo ($n = 26$) each day. After 4 weeks of treatment with SPS, scores of symptoms such as sneezing, nasal stuffiness, and hindrance in daily life were significantly different ($p < 0.05$) from those in the placebo-treated groups (85). The total symptom score, calculated from the sum of individual scores, showed a significant difference ($p < 0.05$) between the two groups after 4–8 weeks.

In two double-blind placebo-controlled clinical studies (84, 85), the patients with PAR received SPS after past sensitization with allergens (84), whereas the patients with SAR due to Japanese cedar pollen received it before sensitization with the allergens (85). It was demonstrated that SPS significantly improved the QOL of patients with PAR and SAR, making it clear that the oral supplementation of SPS is effective for both the therapeutic and preventive treatment of allergies. However, SPS did not affect levels of histamine, specific IgE, and Th1/Th2 in serum, which is not consistent with the findings of an animal study (31). The discrepancy might be explained in part by differences in species, allergen, or period of SPS administration. Furthermore, routine blood tests, including complete blood cell counts, hepatic and renal function tests, and concentrations of proteins and lipids, showed the safety of SPS at a daily dose of 600 mg for 8 weeks (85). The SPS supplementation (600 mg of SPS per day) corresponded to 60 ml of soy sauce per day. This alternative treatment with SPS originating from Japanese soy sauce might reduce therapy costs for allergic diseases.

29.10 CONCLUSIONS AND PRESPECTIVES

In the brewing of soy sauce, proteins of the raw materials are completely degraded into peptides and amino acids by microbial proteolytic enzymes after fermentation, and no allergens remain. In contrast, degraded polysaccharides from the cell wall of soybeans, termed SPS, are resistant to enzymatic hydrolysis, and are found in soy sauce even after fermentation. We have demonstrated that SPS have potent antiallergic activities *in vitro* and *in vivo*, and the oral supplementation of SPS was an effective intervention for patients with allergic rhinitis in two double-blind placebo-controlled clinical studies. Furthermore, we have found that SPS have a regulatory effect on both the balance of Th1/Th2 cell responses and the intestinal immune system. From these results, the immunological functions of SPS are summarized in Fig. 29.2. Recently, we

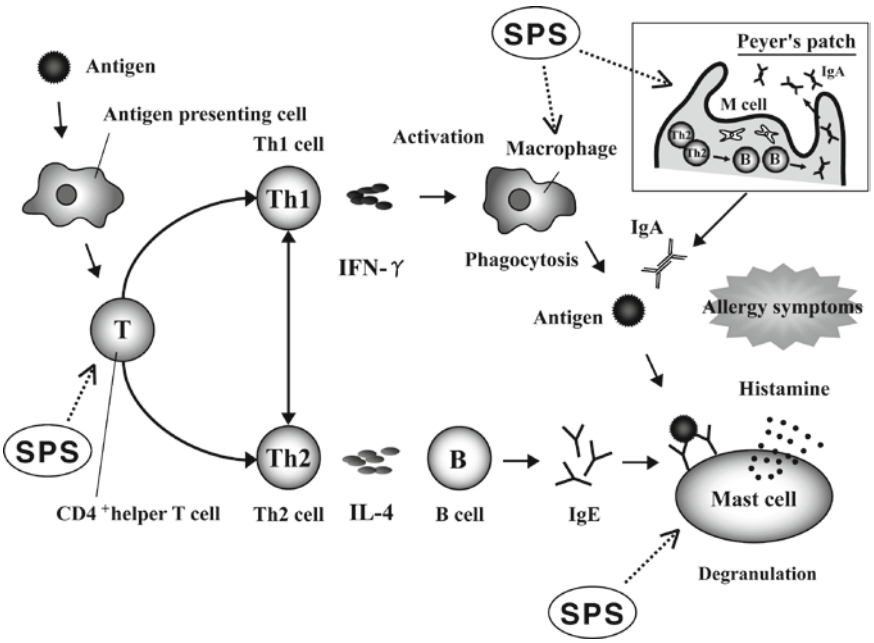


Fig. 29.2. Mechanism of antiallergic and immunomodulating effects of SPS.

demonstrated that SPS enhanced the absorption of iron and reduced elevated levels of triacylglycerol in animal and human studies (86, 87). Therefore, soy sauce is a potentially promising functional food.

REFERENCES

1. Yokotsuka T (1986) Soy sauce biochemistry. Adv Food Res 30:195–329
2. Benjamin H, Storkson J, Nagahara A, Pariza MW (1991) Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by dietary soy sauce. Cancer Res 51:2940–2942
3. Kataoka S, Liu W, Albright K, Storkson J, Pariza M (1997) Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia and reduction of H_2O_2 concentration in human polymorphonuclear leucocytes by flavour components of Japanese-style fermented soy sauce. Food Chem Toxicol 35:449–457
4. Masuda S, Hara-Kudo Y, Kumagai S (1998) Reduction of *Escherichia coli* O157:H7 populations in soy sauce, a fermented seasoning. J Food Prot 61:657–661
5. Long LH, Kwee DC, Halliwell B (2000) The antioxidant activities of seasonings used in Asian cooking. Powerful antioxidant activity of dark soy sauce revealed using the ABTS assay. Free Radic Res 32:181–186
6. Harada K, Ando M, Kitao S, Sakamoto Y, Kobayashi M, Tamura Y (2002) Measurement of antioxidative capacity of fish sauce using chemiluminescence method. Fish Sci 68(Suppl 2):1437–1440
7. Ando M, Harada K, Kitao S, Kobayashi M, Tamura Y (2003) Relationship between peroxyl radical scavenging capability measured by the chemiluminescence method and an aminocarbonyl reaction product in soy sauce. Int J Mol Med 12:923–928
8. Tsuchiya H, Sato M, Watanabe I (1999) Antiplatelet activity of soy sauce as functional seasoning.J Agric Food Chem 47:4167–4174
9. Kinoshita E, Yamakoshi J, Kikuchi M (1993) Purification and identification of an angiotensin I-converting enzyme inhibitor from soy sauce. Biosci Biotechnol Biochem 57:1107–1110

10. Kobayashi M, Hayashi S (1998) Modeling combined effects of temperature and pH on the growth of *Zygosaccharomyces rouxii* in soy sauce mash. J Ferment Bioeng 85:638–641
11. Kobayashi M, Hayashi S (1998) Supplementation of NaCl to starter culture of the soy yeast *Zygosaccharomyces rouxii*. J Ferment Bioeng 85:642–644
12. Nagai H, Kobayashi M, Tsuji Y, Nakashimada Y, Kakizono T, Nishio N (2002) Biological and chemical treatment of the solid waste from the process of soy sauce manufacture. Water Sci Technol 45:335–338
13. Kobayashi M, Hashimoto Y, Taniuchi S, Tanabe S (2004) Degradation of wheat allergen in Japanese soy sauce. Int J Mol Med 13:821–827
14. Tsuji H, Okada N, Yamanishi R, Bando N, Kimoto M, Ogawa T (1995) Measurement of Gly m Bd 30 K, a major soybean allergen, in soybean products by a sandwich enzyme-linked immunosorbent assay. Biosci Biotechnol Biochem 59:150–151
15. Ogawa T, Samoto M, Takahashi K (2000) Soybean allergens and hypoallergenic soybean products. J Nutr Sci Vitaminol 46:271–279
16. Kikuchi T, Yokotsuka T (1972) Studies on the polysaccharides from soy sauce. Part I. Purification and properties of two acidic polysaccharides. Agric Biol Chem 36:544–550
17. Kikuchi T, Sugimoto H (1976) Detailed structure of an acidic polysaccharide in soy sauce, confirmed by use of two kinds of purified pectinases. Agric Biol Chem 40:87–92
18. Metcalfe DD, Baram D, Mekori YA (1997) Mast cells. Physiol Rev 77:1033–1079
19. Lorentz A, Schwengberg S, Sellge G, Manns MP, Bischoff SC (2000) Human intestinal mast cells are capable of producing different cytokine profiles: role of IgE receptor cross-linking and IL-4. J Immunol 164:43–48
20. Borish L (2003) Allergic rhinitis: systemic inflammation and implications for management. J Allergy Clin Immunol 112:1021–1031
21. Maeda Y, Yamamoto M, Masui T, Sugiyama K, Yokota M, Okada N, Sugiyama K, Katayama H, Nakagomi K (1991) Hyaluronidase inhibitor in the fruit of *Citrus reticulata* Blanco. Eisei Kagaku 37:205–210
22. Sawabe Y, Nakagomi K, Iwagami S, Suzuki S, Nakazawa H (1992) Inhibitory effects of pectic substances on activated hyaluronidase and histamine release from mast cells. Biochim Biophys Acta 1137:274–278
23. Kakegawa H, Matsumoto H, Satoh T (1992) Inhibitory effects of some natural products on the activation of hyaluronidase and their anti-allergic actions. Chem Pharm Bull 40:1439–1442
24. Asada M, Sugie M, Inoue M, Nakagomi K, Hongo S, Murata K, Irie S, Takeuchi T, Tomizuka N, Oka S (1997) Inhibitory effect of alginic acids on hyaluronidase and on histamine release from mast cells. Biosci Biotech Biochem 61:1030–1032
25. Ito H, Miyazaki T, Ono M, Sakurai H (1998) Antiallergic activities of rabdosiin and its related compounds: chemical and biochemical evaluations. Bioorg Med Chem 6:1051–1056
26. Ippoushi K, Yamaguchi Y, Itou H, Azuma K, Higashio H (2000) Evaluation of inhibitory effects of vegetables and herbs on hyaluronidase and identification of rosmarinic acid as a hyaluronidase inhibitor in lemon balm (*Melissa officinalis* L.). Food Sci Technol Res 6:74–77
27. Fujitani N, Sakaki S, Yamaguchi Y, Takenaka H (2001) Inhibitory effects of microalgae on the activation of hyaluronidase. J Appl Phycol 13:489–492
28. Kakegawa H, Mitsuo N, Matsumoto H, Satoh T, Akagi M, Tasaka K (1983) Hyaluronidase-inhibitory and anti-allergic activities of the photo-irradiated products of tranilast. Chem Pharm Bull 33:3738–3744
29. Kakegawa H, Momoi Y, Tada K, Mitsuo N, Matsumoto H, Taira Z, Endo K, Satoh T, Terada H (1984) Studies on methodology of finding the anti-allergic agent with the guidance of anti-hyaluronidase activity. J Pharm Dyn 7:s-96
30. Kakegawa H, Matsumoto H, Satoh T (1985) Activation of hyaluronidase by metallic salts and compound 48/80, and inhibitory effect of anti-allergic agents on Hyaluronidase. Chem Pharm Bull 33:642–646
31. Kobayashi M, Matsushita H, Yoshida K, Tsukiyama R, Sugimura T, Yamamoto K (2004) *In vitro* and *in vivo* anti-allergic activity of soy sauce. Int J Mol Med 14:879–884
32. Teshima R, Ikebuchi H, Sekita S, Natori S, Terao T (1985) Effects of various cytochalasins on the IgE-mediated serotonin release from rat basophilic leukemia cells. Int Archs Allergy Appl Immunol 78:237–242

33. Teshima R, Suzuki K, Ikebuchi H, Terao T (1986) Possible involvement of phosphorylation of a 36,000-dalton protein of rat basophilic leukemia (RBL-2H3) cell membranes in serotonin release. Mol Immunol 23:279–284
34. Joly F, Bessou G, Benveniste J, Ninio E (1987) Ketotifen inhibits paf-acether biosynthesis and β-hexosaminidase release in mouse mast cells stimulated with antigen. Eur J Pharmacol 144:133–139
35. Tanaka Y, Takagaki Y, Nishimune T (1991) Effects of metal elements on β-hexosaminidase release from rat basophilic leukemia cells (RBL-2H3). Chem Pharm Bull 39:2072–2076
36. Kataoka M, Takagaki Y (1992) Screening of the crude drugs having the antiallergic effect using rat basophilic leukemia cells (RBL-2H3). Shoyakugaku Zasshi 46:25–29 (in Japanese)
37. Hoffmann A, Vieths S, Haustein D (1997) Biologic allergen assay for *in vivo* test allergens with an *in vitro* model of the murine type I reaction. J Allergy Clin Immunol 99:227–232
38. Kanda T, Akiyama H, Yanagida A, Tanabe M, Goda Y, Toyoda M, Teshima R, Saito Y (1998) Inhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells. Biosci Biotechnol Biochem 62:1284–1289
39. Hoffmann A, Jamin A, Foetisch K, May S, Aulepp H, Haustein D, Vieths S (1999) Determination of the allergenic activity of birch pollen and apple prick test solutions by measurement of β-hexosaminidase release from RBL-2H3 cells. Comparison with classical methods in allergen standardization. Allergy 54:446–454
40. Kotani M, Matsumoto M, Fujita A, Higa S, Wang W, Suemura M, Kishimoto T, Tanaka T (2000) Persimmon leaf extract and astragalin inhibit development of dermatitis and IgE elevation in NC/Nga mice. J Allergy Clin Immunol 106:159–166
41. Makino T, Furuta Y, Fujii H, Nakagawa T, Wakushima H, Saito K, Kano Y (2001) Effect of oral treatment of *Perilla frutescens* and its constituents on type-I allergy in mice. Biol Pharm Bull 24:1206–1209
42. Makino T, Furuta Y, Wakushima H, Fujii H, Saito K, Kano Y (2003) Anti-allergic effect of *Perilla frutescens* and its active constituents. Phytother Res 17:240–243
43. Kim CD, Lee W-K, No K-O, Park S-K, Lee M-H, Lim SR, Roh S-S (2003) Anti-allergic action of buckwheat (*Fagopyrum esculentum* Moench) grain extract. Int Immunopharmacol 3:129–136
44. Tanaka T, Sugiura H, Inaba R, Nishikawa A, Murakami A, Koshimizu K, Ohigashi H (1999) Immunomodulatory action of citrus auraptene on macrophage functions and cytokine production of lymphocytes in female BALB/c mice. Carcinogenesis 20:1471–1476
45. Oka H, Emori Y, Kobayashi N, Hayashi Y, Nomoto K (2001) Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. Int Immunopharmacol 1:521–532
46. Hosono A, Otani H, Yasui H, Watanuki M (2002) Impact of fermented milk on human health: cholesterol-lowering and immunomodulatory properties of fermented milk. Animal Sci J 73:241–256
47. Ike K, Uchida Y, Nakamura T, Imai S (2005) Induction of interferon-gamma (IFN-γ) and T helper 1 (Th1) immune response by bitter gourd extract. J Vet Med Sci 67:521–524
48. Lim BO, Yamada K, Nonaka M, Kuramoto Y, Hung P, Sugano M (1997) Dietary fibers modulate indices of intestinal immune function in rats. J Nutr 127:663–667
49. Kiho T, Matsushita M, Usui S, Ukai S (1998) Biological activities of (1-3)-β-D-glucans with reducing glucose side chains. Biosci Biotechnol Biochem 62:570–572
50. Kawaguchi M, Shiomi R, Tabata K, Kosuge Y, Mizumachi K, Kurisaki J (2004) Enhancement of Th1 immune response in mice by the ingestion of Suehirotake (*Schizophyllum commune* Fries) mycelium. Nippon Shokuhin Kagaku Kogaku Kaishi 51:102–105 (in Japanese)
51. Yoshizawa Y, Enomoto A, Todoh H, Ametani A, Kaminogawa S (1993) Activation of murine macrophages by polysaccharide fractions from marine algae (*Porphyra yezoensis*). Biosci Biotechnol Biochem 57:1862–1866
52. Yoshizawa Y, Ametani A, Tsunehiro J, Nomura K, Itoh M, Fukui F, Kaminogawa S (1995) Macrophage stimulation activity of the polysaccharide fraction from a marine alga (*Porphyra yezoensis*): Structure-function relationships and improved solubility. Biosci Biotechnol Biochem 59:1933–1937
53. Matsushita H, Kobayashi M, Tsukiyama R, Yamamoto K (2006) *In vitro* and *in vivo* immunomodulating activities of *Shoyu* polysaccharides from soy sauce. Int J Mol Med 17:905–909
54. Ryan JL, Glode LM, Rosenstreich DL (1979) Lack of responsiveness of C3H/HeJ macrophages to lipopolysaccharide: the cellular basis of LPS-stimulated metabolism. J Immunol 122:932–935

55. Sugiura H, Sugiura H, Ueya E, Ueya S, Mirbod SM (2000) Enhanced macrophage functions and cytokine production of lymphocytes after ingestion of bon narine in female BALB/c mice. Life Sci 68:505–515
56. Belardelli F (1995) Role of interferons and other cytokines in the regulation of the immune response. Acta Pathol Microbiol Immunol Scand 103:161–179
57. Abbas AK, Murphy KM, Sher A (1996) Functional diversity of helper T lymphocytes. Nature 383:787–793
58. Shida K, Makino K, Morishita A, Takamizawa K, Hachimura S, Ametani A, Sato T, Kumagai Y, Habu S, Kaminogawa S (1998) *Lactobacillus casei* inhibits antigen-induced IgE secretion through regulation of cytokine production in murine splenocyte cultures. Int Arch Allergy Immunol 115:278–287
59. Fujiwara D, Inoue S, Wakabayashi H, Fujii T (2004) The antiallergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. Int Arch Allergy Immunol 135:205–215
60. McGhee JR, Mestecky J, Dertzbaugh MT, Eldridge JH, Hirasawa M, Kiyono H (1992) The mucosal immune system: from fundamental concepts to vaccine development. Vaccine 10:75–88
61. Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM (2000) A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 288:2222–2226
62. Andre C, Lambert R, Bazin H, Heremans JF (1974) Interference of oral immunization with the intestinal absorption of heterologous albumin. Eur J Immunol 4:701–704
63. Stokes CR, Soothill JF, Turner MW (1975) Immune exclusion is a function of IgA. Nature 255:745–746
64. Silbart LK, Keren DF (1989) Reduction of intestinal carcinogen absorption by carcinogen-specific secretory immunity. Science 243:1462–1464
65. Lamm ME (1997) Interaction of antigens and antibodies at mucosal surfaces. Annu Rev Microbiol 51:311–340
66. Kraehenbuhl JP, Neutra MR (2000) Epithelial M cells: differentiation and function. Annu Rev Cell Dev Biol 16:301–332
67. Yasui H, Kiyoshima J, Ushijima H (1995) Passive protection against rotavirus-induced diarrhea of mouse pups born to and nursed by dams fed *Bifidobacterium brebe* YIT4064. J Infect Dis 172:403–409
68. Matsushita H, Kobayashi M, Tsukiyama R, Fujimoto M, Suzuki M, Tsuji K, Yamamoto K (2008) Stimulatory effect of *Shoyu* polysaccharides from soy sauce on the intestinal immune system. Int J Mol Med 22:243–247
69. Hidalgo IJ, Raub TJ, Borchardt RT (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 96:736–749
70. Balimane PV, Chong S, Morrison RA (2000) Current methodologies used for evaluation of intestinal permeability and absorption. J Pharmacol Toxicol Methods 44:301–312
71. Thomet OAR, Schapowal A, Heinisch IVWM, Wiesmann UN, Simon H-U (2002) Anti-inflammatory activity of an extract of *Petasites hybridus* in allergic rhinitis. Int Immunopharmacol 2:997–1006
72. Burtin B, Duchateau J, Pignat JC, Donnelly F, Bousquet J (2000) Further improvement of quality of life by cetirizine in perennial allergic rhinitis as a function of treatment duration. J Invest Allergol Clin Immunol 10:66–70
73. Gerth Van Wijk R, Terreehorst IT, Mulder PGH, Garrelds IM, Blom HM, Popering S (2000) Intranasal capsaicin is lacking therapeutic effect in perennial allergic rhinitis to house dust mite. A placebo-controlled study. Clin Exp Allergy 30:1792–1798
74. Hu G, Walls RS, Bass D, Bullock R, Grayson D, Jones M, Gebski V (2002) The Chinese herbal formulation Biminne in management of perennial allergic rhinitis: a randomized, double-blind, placebo-controlled, 12-week clinical trial. Ann Allergy Asthma Immunol 88:478–487
75. Chervinsky P, Casale T, Townley R, Tripathy I, Hedgecock S, Fowler-Taylor A, Shen H, Fox H (2003) Omalizumab, an anti-IgE antibody, in the treatment of adults and adolescents with perennial allergic rhinitis. Ann Allergy Asthma Immunol 91:160–167
76. Potter PC, Van Niekerk CH, Schoeman HS (2003) Effects of triamcinolone on quality of life in patients with persistent allergic rhinitis. Ann Allergy Asthma Immunol 91:368–374

77. Ciprandi G, Tosca MA, Cirillo I, Vizzaccaro A (2003) The effect of budesonide on the cytokine pattern in patients with perennial allergic rhinitis. Ann Allergy Asthma Immunol 91:467–471
78. Pearlman DS, Lumry WR, Winder JA, Noonan MJ (1997) Once-daily cetirizine effective in the treatment of seasonal allergic rhinitis in children aged 6 to 11 years: a randomized, double-blind, placebo-controlled study. Clin Pediatr 36:209–215
79. Hordijk GJ, Antvelink JB, Luwema RA (1998) Sublingual immunotherapy with a standardized grass pollen extract: a double-blind placebo-controlled study. Allergol Immunopathol 26:234–240
80. Bernstein DI, Bernstein CK, Deng C, Murphy KJ, Bernstein IL, Bernstein JA, Shukla R (2002) Evaluation of the clinical efficacy and safety of grapeseed extract in the treatment of fall seasonal allergic rhinitis: a pilot study. Ann Allergy Asthma Immunol 88:272–278
81. Xue CCL, Thien FCK, Zhang JJS, Da Costa C, Li CG (2003) Treatment for seasonal allergic rhinitis by Chinese herbal medicine: a randomized placebo controlled trial. Altern Ther Health Med 9:80–87
82. Takano H, Osakabe N, Sanbongi C, Yanagisawa R, Inoue K, Yasuda A, Natsume M, Baba S, Ichiishi E, Yoshikawa T (2004) Extract of *Perilla frutescens* enriched for rosmarinic acid, a polyphenolic phytochemical, inhibits seasonal allergic rhinoconjunctivitis in humans. Exp Biol Med 229:247–254
83. Dejima K, Ohshima A, Yanai T, Yamamoto R, Takata R, Yoshikawa T (2007) Effects of polysaccharide derived from black currant on relieving clinical symptoms of Japanese cedar pollinosis: a randomized double-blind, placebo-controlled trial. Biosci Biotechnol Biochem 71:3019–3025
84. Kobayashi M, Matsushita H, Shioya I, Nagai M, Tsukiyama R, Saito M, Sugita T, Sugimura T, Yamamoto K (2004) Quality of life improvement with soy sauce ingredients, *Shoyu* polysaccharides, in perennial allergic rhinitis: a double-blind placebo-controlled clinical study. Int J Mol Med 14:885–889
85. Kobayashi M, Matsushita H, Tsukiyama R, Saito M, Sugita T (2005) *Shoyu* polysaccharides from soy sauce improve quality of life for patients with seasonal allergic rhinitis: a double-blind placebo-controlled clinical study. Int J Mol Med 15:463–467
86. Kobayashi M, Nagatani Y, Magishi N, Tokuriki N, Nakata Y, Tsukiyama R, Imai H, Suzuki M, Saito M, Tsuji K (2006) Promotive effect of *Shoyu* polysaccharides from soy sauce on iron absorption in animals and humans. Int J Mol Med 18:1159–1163
87. Kobayashi M, Magishi N, Matsushita H, Hashimoto T, Fujimoto M, Suzuki M, Tsuji K, Saito M, Inoue E, Yoshikawa Y, Matsuura T (2008) Hypolipidemic effect of *Shoyu* polysaccharides from soy sauce in animals and humans. Int J Mol Med 22:565–570

30 Cinnamon and Immune Actions: Potential Role in Tristetraprolin-Mediated Inflammatory Diseases

Heping Cao

Key Points

- Inflammatory diseases place a heavy burden on the American health care system.
- Tristetraprolin, a zinc-dependent mRNA binding protein decreases the stability of mRNAS coding for some proinflammatory cytokines.
- Tristetraprolin-deficient mice develop a profound inflammatory syndrome.
- Tristetraprolin is a potential cancer therapy due to its control of vascular endothelial growth factor mRNA stability.
- Cinnamon extract stimulates the expression of antiinflammatory tristetraprolin.
- Bioactive compound(s) in cinnamon extract define its molecular mechanisms.
- Cinnamon is potentially important in tristetraprolin-mediated inflammatory diseases.

Key Words: Cancer, cinnamon, immunity, inflammation, insulin, macrophage, obesity, tristetraprolin.

30.1 INTRODUCTION

Inflammation and associated diseases have placed a heavy burden on the American health care system. Drug treatment for reducing inflammation and related diseases has not been satisfactory. Complementary and alternative approaches need to be evaluated. Bioactive plant extracts have historically been used as alternative medicines for the prevention, alleviation, and cure of various diseases. The mechanisms of how bioactive plant extracts work are poorly understood due in part to the lack of knowledge in the structures of bioactive components in most of the extracts.

Anti-inflammatory activities are proposed to play an important role in the mediation of various health conditions by these alternative therapies; however, the anti-inflammatory mechanisms are not completely understood. Recent results indicate

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_30

that tristetraprolin (TTP[1]) is an anti-inflammatory protein that binds to the unstable elements of mRNAs coding for inflammation-related factors such as tumor necrosis factor-alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), cyclooxygenase-2 (COX2), and some interleukins (ILs) mRNAs, and decreases their stability. The mRNA binding and destabilizing activities of TTP are zinc-dependent and regulated by phosphorylation. TTP-deficient mice develop a severe inflammatory syndrome with arthritis, autoimmunity, cachexia, dermatitis, and myeloid hyperplasia. The expression of TTP is reduced in fats of obese people with the metabolic syndrome and the brains of suicide victims. TTP is also proposed as a molecular target for cancer therapy due to its control in vascular endothelial growth factor (VEGF) mRNA stability.

Cinnamon extract (CE), like insulin, stimulates TTP gene expression in mouse adipocytes. Unlike insulin, CE also stimulates TTP expression in mouse macrophages. Given the importance of TTP in biology and diseases, and the benefits of cinnamon, it is important to identify the bioactive compound(s) in this botanical extract. This chapter reviews the biological, medical, and nutritional significance of TTP, and the potentials of cinnamon in TTP-mediated inflammatory diseases.

30.2 TRISTETRAPROLIN IS AN ANTI-INFLAMMATORY PROTEIN

TTP, or zinc-finger protein 36 (ZFP36), is an anti-inflammatory protein. TTP deficiency in knockout mice causes a complex inflammatory syndrome with arthritis, autoimmunity, cachexia, dermatitis, and myeloid hyperplasia (1, 2). This is largely due to excessive production of TNF-α and GM-CSF from the corresponding mRNAs that are normally degraded after binding TTP, but stabilized and persistent in cells from TTP knockout mice (3, 4). Transforming growth factor-beta (TGF-β) is a pleiotropic cytokine that plays a critical role in modulating immune response and inflammation. TTP expression is upregulated by TGF-β, suggesting a potential role of TTP in mediating the immune suppressive action of TGF-β *in vivo* (5).

30.3 TRISTETRAPROLIN IS A ZINC-DEPENDENT mRNA BINDING AND DESTABILIZING PROTEIN

TTP is a zinc-binding protein with two zinc-finger binding motifs (6, 7). The biochemical function of TTP is binding to AU-rich elements (AREs) with high binding specificity for class II AREs within the 3′-untranslated regions of mRNA molecules (3, 4, 8–15). The binding of TTP to AREs results in the subsequent removal of the poly(A) tails and degradation of the RNA bodies (3, 13). Most of the TTP-targeted mRNAs identified so far code for proinflammatory factors such as TNF-α (3, 10, 11, 13), GM-CSF (4), COX2 (16, 17), IL2 (18), IL3 (14), IL8 (19), IL10 (20), cytokine signaling 3 (21), E47 (22), and plasminogen activator inhibitor type 2 (23).

The mRNA binding activity of TTP is dependent on zinc. The removal of zinc from binding reaction mixtures destroys TTP's ability to bind to TNF-α mRNA ARE (10, 11). TTP mRNA and protein are detected in a number of tissues including

spleen, thymus, lymph node, lung, liver, and intestine (24, 25). The expression levels of TTP mRNA and protein in mammalian cells are increased in response to several kinds of stimuli, including insulin and other growth factors, and in response to stimulators of innate immunity, such as the bacterial endotoxin lipopolysaccharide (LPS) (24, 25).

30.4 TTP IS A LOW-ABUNDANCE, INDUCIBLE, CYTOSOLIC, AND HYPER-PHOSPHORYLATED PROTEIN

TTP is a hyper-phosphorylated protein in extraordinarily low abundance and is inducible, stable, and cytosolic (10–12, 24, 26). We have produced high-titer antibodies and used them successfully in characterizing the patterns of TTP expression in mouse tissues and cells. TTP is a very low-abundance protein in normal mouse tissues (24); however, it is induced several hundredfold by LPS in mouse RAW264.7 cells (Fig. 30.1) and in the rat spleen (24).

TTP is phosphorylated extensively *in vivo* (11, 24, 26) and is a substrate for multiple protein kinases (10, 11, 27). We have identified multiple phosphorylation sites in mammalian TTP by mass spectrometry and site-directed mutagenesis; some of which are predicted by motif scanning to be phosphorylated by several protein kinases (7, 26, 28).

30.5 TRISTETRAPROLIN IS ASSOCIATED WITH CANCER

TTP is widely present in human cancer cell lines (11, 29). TTP binds to the 3′-untranslated region of COX2 mRNA in human colorectal adenocarcinoma cell lines (16). Recent studies provide evidence that VEGF, an angiogenic cytokine, is a target of TTP (19, 30). These studies suggest that TTP is a new target for antiangiogenic therapies. VEGF has been proposed as the most important regulator of physiological and pathological angiogenesis (31). VEGF mRNA level is regulated by hypoxia, growth factors and hormones through both transcriptional and posttranscriptional mechanisms. VEGF mRNA has a short half-life and its abundance is regulated by stabilizing proteins HuR (32) and hRNP-L (33) and destabilizing proteins such as TTP and its family members (34), which bind to the 3′-untranslated region of VEGF mRNA. TTP binds to VEGF mRNA 3′-untranslated region and plays a key role by inducing VEGF mRNA degradation (30). TTP decreases RasVal12-dependent VEGF expression and the development of vascularized tumors in nude mice (30). TTP is ubiquitously expressed in the tissues and cell lines of primary malignant glioma (a highly aggressive tumor of the central nervous system) (19). Conditional over expression of TTP as a transgene in malignant glioma cells leads to RNA destabilization of IL8 and VEGF and down-regulation of IL8 and VEGF protein production. *In vivo* RNA binding indicates a shift of mRNA toward ectopic TTP and away from endogenous HuR. The biochemical phenotype is associated with a decrease in cell proliferation, loss of cell viability, and apoptosis (19). Taking together, these results suggest that TTP represents a novel antiangiogenic and antitumor agent acting through its destabilizing activity on VEGF mRNA.

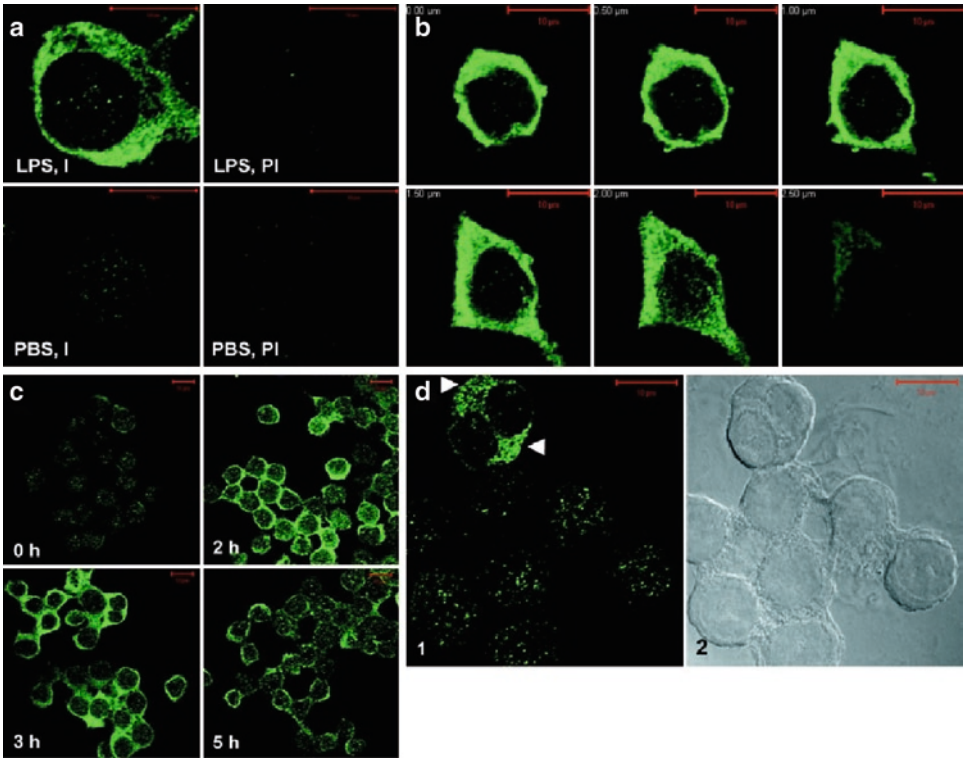


Fig. 30.1. TTP is a extraordinarily low-abundance protein that is inducible and cytosolic in mammalian cells. (a) Induction of TTP in RAW macrophage cells. Cells were treated with LPS (0.1 µg/ml) or PBS for 3 h, then fixed and stained with either the anti-MBP-mTTP serum (I) or preimmune serum (PI). (b) Cytosolic localization of TTP in RAW cells. Cells were stimulated with LPS (0.1 µg/ml) for 2 h and stained with anti-MBP-mTTP serum. Serial optical sections of the same cell were collected at 0.5 µm intervals. (c) Time-course of TTP induction in RAW cells. Cells were stimulated with LPS (0.1 µg/ml) for 0, 2, 3, and 5 h as indicated and stained with anti-MBP-mTTP serum. (d) TTP immunostaining during cell division in RAW cells. Unstimulated cells were stained with the anti-MBP-mTTP serum. Immunoreactive TTP was visible in the cytoplasm of the dividing cell indicated by the *arrowheads* in (d1), whereas no cytoplasmic staining was visible in the other cells in the field. A light microscopic image of the same field of cells is also shown (d2).

30.6 TRISTETRAPROLIN EXPRESSION IS REDUCED IN FATS OF OBESE PEOPLE WITH THE METABOLIC SYNDROME

Several lines of evidence suggest that TTP is involved in obesity. First, TTP mRNA levels are four to fivefold lower in visceral fat of obese people with the metabolic syndrome compared to those without the metabolic syndrome (35). Second, TTP gene is located in regions of linkage for the metabolic syndrome (35). Third, TTP mRNA levels in visceral adipose tissue in women are negatively correlated with fasting insulin levels, the insulin resistance index, and 2-h postglucose insulinemia, and positively correlated with adiponectinemia, suggesting that TTP gene expression in omental adipose tissue may contribute to partial protection against the development of insulin resistance and diabetes (36). Finally, TTP is abundantly expressed and increased by insulin in mouse

3T3 fibroblasts over expressing normal human insulin receptors (25) and 3T3-L1 adipocytes (37, 38), and induced by fetal bovine serum and differentiation mixtures during the differentiation of preadipocytes (39). These studies suggest that TTP may be an important factor for the physiological control of obesity-associated metabolic disorders.

30.7 TRISTETRAPROLIN EXPRESSION IS REDUCED IN BRAINS OF SUICIDE VICTIMS

TTP is potentially involved in normal brain activity because TTP gene expression is reduced in the brains of suicide victims. The cause of suicidal behavior is multifactorial, involving multiple genes, environmental factors, and gene-environment interactions. Thalmeier et al. studied the transcriptomic expression profile of postmortem brain tissue of suicide victims to identify new candidate genes and biological patterns for suicidal behavior (40). The authors analyzed the expression of over 23,000 mRNAs in postmortem orbitofrontal cortex tissue derived from 11 suicide victims and 10 nonpsychiatric controls. 124 transcripts are significantly changed with 59 underexpressed and 65 overexpressed in the suicide group (40). Both microarray and quantitative RT-PCR assays show that TTP gene expression is reduced by approximately twofold in postmortem orbitofrontal cortex of violent suicide victims (40). These results suggest that reduced TTP expression is involved in the pathophysiology of suicide.

30.8 TRISTETRAPROLIN IS ASSOCIATED WITH BLOOD PRESSURE

Microarray and function studies show that immediate-early response gene 3 mRNA turnover is decreased in cells derived from TTP-knockout mice (41). Since immediate-early response 3 protein is related to the regulation of blood pressure, TTP is therefore potentially involved in this important process.

30.9 TRISTETRAPROLIN EXPRESSION IS INCREASED BY MICRONUTRIENTS

The micronutrient, zinc (Zn), is the first metal shown to regulate TTP gene expression in mammalian cells. TTP gene expression is markedly increased by 50–100 μM $ZnSO_4$ in intact TK-L cells within 1 h, reached peak levels in 4 h, and remained constant to 12 h (42). Similar Zn effects are reported in Swiss 3T3 fibroblasts, primary mouse embryonic fibroblasts, H4 cells, normal human skin fibroblasts, and 1321-N1 human astrocytoma cells (42). TTP gene expression is also increased in liver, lung, kidney, and brain of mice 3 h after injection of $ZnSO_4$ (50 mg/kg) but not in mice after the consumption of 50 mM $ZnSO_4$ in water for 14 days (42). TTP gene expression is also increased by 50–100 μM $CdCl_2$ and $AgNO_3$ in TK-L1 cells (42).

Microarray and quantitative PCR technologies were used to investigate Zn responsiveness of known genes that influence Zn homeostasis and to identify genes that may relate to phenotypic outcomes of altered dietary Zn intake (43). Human monocytic/macrophage THP-1 cells were either acutely Zn depleted, using a cell-permeable Zn-specific chelator or were supplemented with Zn to alter intracellular Zn concentrations. Microarrays composed of approximately 22,000 elements were used to identify

those genes responsive to either Zn depletion, Zn supplementation, or both conditions. Approximately 5% or 1,045 genes are Zn responsive. Among them, 104 genes respond to Zn linearly in a positive mode (i.e., increased expression as cellular Zn increases). Of the 104 genes in this group, TTP is the most Zn-responsive gene and its expression exhibits a 14-fold reduction in Zn-depleted cells and approximately twofold increase in Zn-supplemented cells (43).

30.10 INSULIN INCREASES TTP AND DECREASES VEGF GENE EXPRESSION IN ADIPOCYTES

Insulin was shown to increase TTP mRNA levels in HIR3.5 preadipocytes (25) and during the differentiation of preadipocytes (39). TTP mRNA is also rapidly induced in adipocytes by 10 and 100 nM insulin treatment, with a 30-min induction resulting in an approximately five- and sevenfold increase over the control, respectively (38). TTP protein is barely detected in untreated cells, but is significantly induced by 10 and 100 nM of insulin treatment for 3 h (38). In contrast, ZFP36L1 (a TTP homologue) protein levels are not significantly affected by insulin treatment using ZFP36L1 antibodies produced with the method similar to that for TTP antibodies (24, 38, 44).

To test the functional consequences of elevated TTP protein levels in mouse adipocytes, RT-PCR was used to screen the expression of 42 other genes in insulin-treated adipocytes (38). VEGFA mRNA levels are decreased approximately 30–50% by 10 and 100 nM insulin treatments for 30–120 min (38), and that VEGFB mRNA levels are also significantly decreased by the same treatments (38). VEGF mRNAs code for VEGF, a proangiogenic factor important for the development of obesity (45, 46) and cancers (47), whose stabilities are known to be destabilized by TTP family proteins in cancer cells (19, 30, 34).

30.11 CINNAMON EXTRACT, LIKE INSULIN, INCREASES TTP AND DECREASES VEGF GENE EXPRESSION IN ADIPOCYTES

Cinnamon and other spices including cloves, turmeric, and bay leaves have insulin-like activity *in vitro* (48), and are proposed to be effective in the treatment of diabetes (49). However, not all studies have reported positive effects of cinnamon in patients with diabetes (50, 51). This discrepancy may be due to the selection of patients, level of glucose control, oral hypoglycemic agents, and/or diet or type of cinnamon used.

We prepared a water-soluble CE and HPLC-purified cinnamon polyphenols (CP) from CE (52). The structure of a trimer is shown in Fig. 30.2a. These polyphenols have been shown to be absorbed (53) and to increase the activity and sensitivity of insulin (37, 52, 54, 55). Microscopic observation indicated that approximately 80–90% of the differentiated mouse 3T3-L1 fibroblasts cells accumulated lipid drops (an indication of differentiation from preadipocytes to adipocytes) (Fig. 30.2b). The cells were serum-starved for 3–4 h before treatments.

TTP mRNA levels in 10 and 100 μg/ml CE-treated adipocytes are up to two- and sixfold, respectively, those of the controls (37). TTP protein is barely detected in untreated cells but is significantly induced by 10 and 100 μg/ml of CE in 3T3-L1 adipocytes after 3 h treatment (37). The purified CP at 10 and 100 μg/ml also increases the

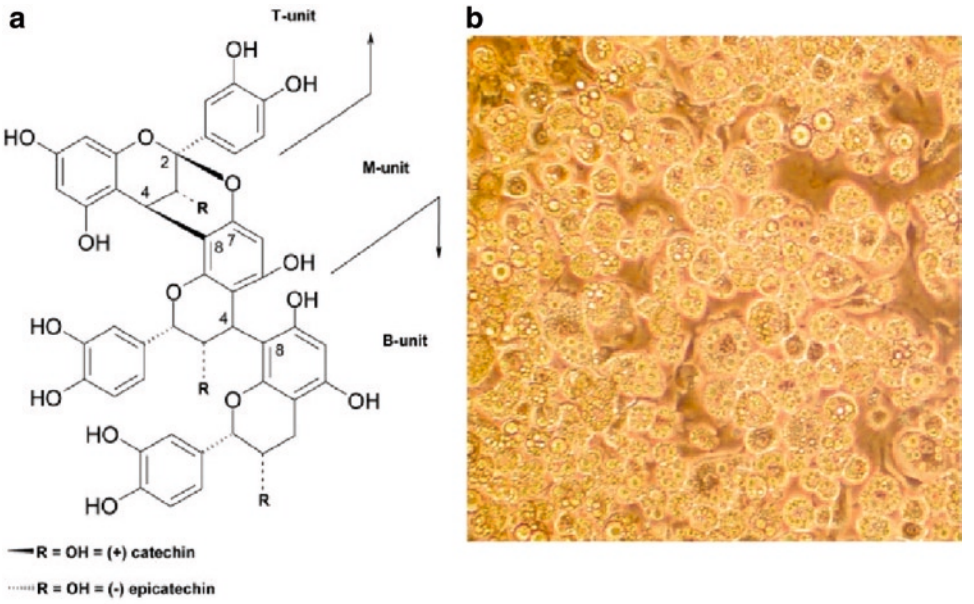


Fig. 30.2. Cinnamon polyphenol and differentiated adipocytes. (a) The structure of a CP purified from CE. The CP structure was determined as a doubly linked procyanidin type-A polymer by nuclear magnetic resonance, mass spectroscopy, and infrared spectroscopy as described (52). (b) Differentiated mouse 3T3-L1 adipocytes (37).

amount of TTP in the adipocytes after 3 h treatment. Higher concentrations of CE and CP treatments result in more TTP in the adipocytes (37).

30.12 CINNAMON EXTRACT, UNLIKE INSULIN, INCREASES TTP GENE EXPRESSION IN MACROPHAGES

Mouse RAW264.7 macrophages are widely used as a cell model for inflammation research. TTP gene expression has been investigated extensively using this cell line (24, 56). TTP protein is rapidly induced by LPS and accumulated in the cytosol of these cells (24, 57). We utilized this model to evaluate the effect of CE on TTP gene expression in RAW macrophages (58). CE rapidly increases TTP mRNA levels in mouse RAW cells. TTP mRNA levels in cells treated with 100 μg/ml CE for 30–240 min are 150–200% of those in the corresponding controls (Fig. 30.3a). Insulin does not exhibit any significant effect on TTP mRNA levels in RAW cells (Fig. 30.3a). LPS possesses a much more potent effect on TTP gene expression in RAW cells. TTP mRNA levels in cells treated with 0.1 μg/ml LPS for 30–240 min are 9–39-fold of the controls, respectively (Fig. 30.3b).

TTP protein is increased in cells treated with 100 μg/ml CE for 90–180 min (Fig. 30.3c, lanes 6–8). LPS increases TTP protein levels in RAW cells much earlier and with a greater magnitude than CE induction (Fig. 30.3c, lanes 9–14). However, TTP protein levels are below detection in cells treated with insulin for the same length of time (58).

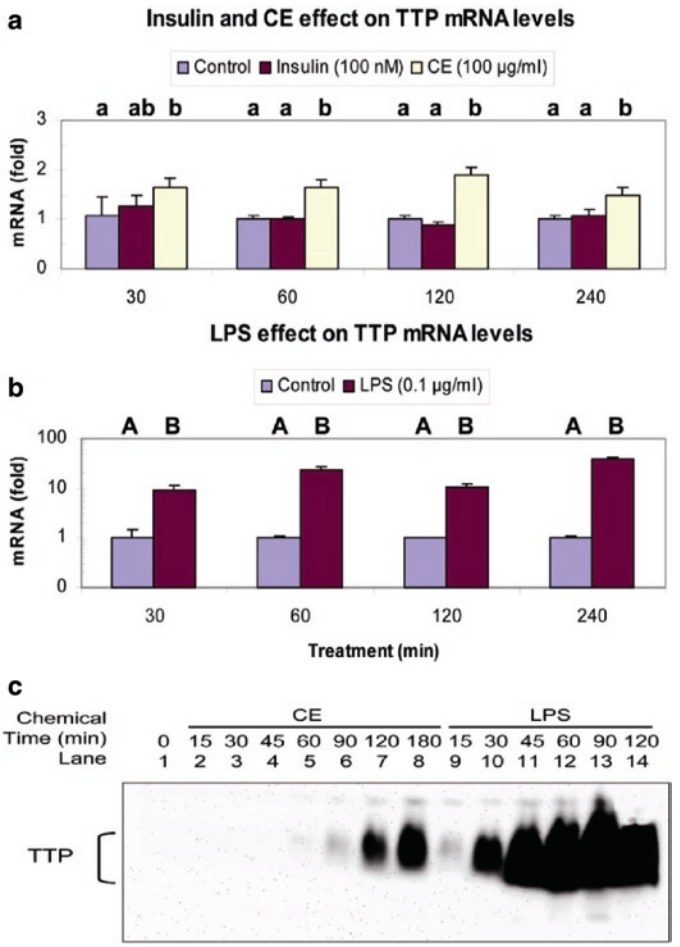


Fig. 30.3. CE and LPS, but not insulin, increased TTP mRNA and protein levels in RAW macrophages. (a) DMSO control versus CE and insulin treatments on TTP mRNA levels. (b) DMSO control versus LPS treatments on mRNA levels. Total RNAs were isolated from RAW cells treated with DMSO (0.1%), insulin (100 nM), CE (100 µg/ml), and LPS (0.1 µg/ml) for 30–240 min. Values with different *lower* and *upper case letters* displayed above the columns of the figure are significantly different at $p < 0.05$ or $p < 0.01$, respectively. (c) DMSO control versus CE and LPS treatments on TTP protein levels. Proteins were separated by 10% SDS-PAGE. TTP was detected by immunoblotting with anti-MBP-TTP serum (24). *Lane 1*, DMSO control; *lanes 2–8*, CE treatment; *lanes 9–14*, LPS treatment (100 µg of protein/lane) (58).

Further analyses showed that: (1) CE induces TTP gene expression more rapidly than those of proinflammatory cytokine mRNAs encoding TNFα, COX2, and IL6 in mouse macrophages; (2) the net increases of TTP mRNA levels are larger than those of proinflammatory cytokines; (3) CE increases more GLUT1 gene expression than LPS; and (4) CE effects on the expression pattern of these genes are different from those of LPS in RAW macrophages during the initial treatment (58). These results indicate that CE is capable of affecting inflammatory responses by regulating anti- and proinflammatory as well as the major GLUT gene expression in macrophages.

30.13 CONCLUSIONS AND PERSPECTIVES

Diet and lifestyle play major roles in disease prevention. The consumption of a nutritious diet is important for maintaining long-term health and decreasing the risk of chronic diseases. Research is urgently needed to determine dietary means, including consumption of bioactive food components that may alleviate or prevent diseases; however, there is a lack of sound evidence at the molecular level to support this practice.

Cinnamon extract (CE) exhibits insulin-like activity in cells, animals, and people with type 2 diabetes (49, 54, 55, 59–62). This is supported by several lines of evidence including (1) CE increases glucose metabolism in a fat cell assay (52); (2) CE increases insulin receptor β auto-phosphorylation and decreases tyrosine phosphatase activity *in vitro* (54); (3) CE increases glucose uptake and glycogen biosynthesis, activates glycogen synthase, and inhibits glycogen synthase kinase-3β (55); (4) CE potentiates *in vivo* insulin-regulated glucose utilization in rats fed a high-fructose diet (60); (5) CE decreases serum glucose levels and increases insulin in rats (62) and decreases blood pressure (59); (6) cinnamon powder decreases the levels of glucose, triglycerides, and LDL cholesterol in people with type 2 diabetes (49, 63); and (7) CE, like insulin (38), increases TTP

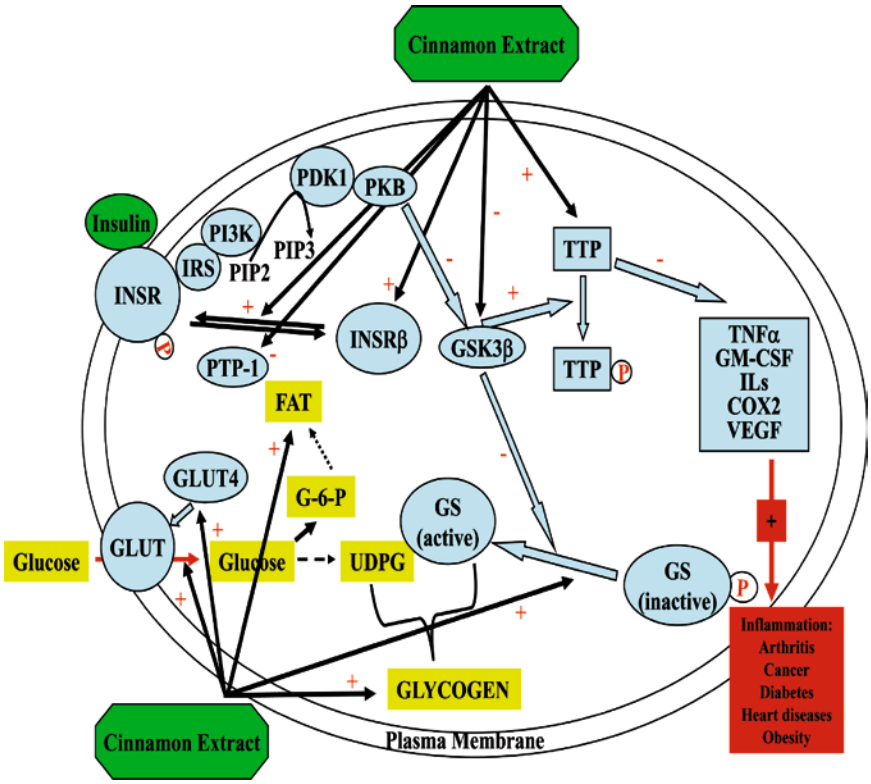


Fig. 30.4. A model that links cinnamon extract, insulin, TTP, and cytokines to inflammation and associated diseases. Like insulin, CE increases TTP gene expression in mouse adipocytes. However, unlike insulin, CE also increases TTP gene expression in mouse macrophages. The detailed evidence is described in the text ("+" represents positive effect and "–" represents negative effect). The model is modified from Cao et al. (37, 64, 65).

expression in mouse adipocytes (37, 38). However, unlike insulin, CE also increases TTP expression in mouse macrophages (58). Based on these results, we have proposed a model to link cinnamon polyphenols, insulin, TTP, and cytokines to inflammatory diseases and inflammation in a wide variety of related diseases (Fig. 30.4). More detailed studies are required to fully understand the health benefits of CE.

REFERENCES

1. Phillips K, Kedersha N, Shen L, Blackshear PJ, Anderson P (2004) Arthritis suppressor genes TIA-1 and TTP dampen the expression of tumor necrosis factor alpha, cyclooxygenase 2, and inflammatory arthritis. Proc Natl Acad Sci USA 101:2011–2016
2. Taylor GA, Carballo E, Lee DM et al (1996) A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Immunity 4:445–454
3. Carballo E, Lai WS, Blackshear PJ (1998) Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. Science 281:1001–1005
4. Carballo E, Lai WS, Blackshear PJ (2000) Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood 95:1891–1899
5. Ogawa K, Chen F, Kim YJ, Chen Y (2003) Transcriptional regulation of tristetraprolin by transforming growth factor-beta in human T cells. J Biol Chem 278:30373–30381
6. Blackshear PJ (2002) Tristetraprolin and other CCCH tandem zinc-finger proteins in the regulation of mRNA turnover. Biochem Soc Trans 30:945–952
7. Cao H, Deterding LJ, Blackshear PJ (2007) Phosphorylation site analysis of the anti-inflammatory and mRNA-destabilizing protein tristetraprolin. Expert Rev Proteomics 4:711–726
8. Bakheet T, Frevel M, Williams BR, Greer W, Khabar KS (2001) ARED: human AU-rich element-containing mRNA database reveals an unexpectedly diverse functional repertoire of encoded proteins. Nucleic Acids Res 29:246–254
9. Blackshear PJ, Lai WS, Kennington EA et al (2003) Characteristics of the interaction of a synthetic human tristetraprolin tandem zinc finger peptide with AU-rich element-containing RNA substrates. J Biol Chem 278:19947–19955
10. Cao H, Dzineku F, Blackshear PJ (2003) Expression and purification of recombinant tristetraprolin that can bind to tumor necrosis factor-alpha mRNA and serve as a substrate for mitogen-activated protein kinases. Arch Biochem Biophys 412:106–120
11. Cao H (2004) Expression, purification, and biochemical characterization of the antiinflammatory tristetraprolin: a zinc-dependent mRNA binding protein affected by posttranslational modifications. Biochemistry 43:13724–13738
12. Carballo E, Cao H, Lai WS, Kennington EA, Campbell D, Blackshear PJ (2001) Decreased sensitivity of tristetraprolin-deficient cells to p38 inhibitors suggests the involvement of tristetraprolin in the p38 signaling pathway. J Biol Chem 276:42580–42587
13. Lai WS, Carballo E, Strum JR, Kennington EA, Phillips RS, Blackshear PJ (1999) Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. Mol Cell Biol 19:4311–4323
14. Lai WS, Blackshear PJ (2001) Interactions of CCCH zinc finger proteins with mRNA: tristetraprolin-mediated AU-rich element-dependent mRNA degradation can occur in the absence of a poly(A) tail. J Biol Chem 276:23144–23154
15. Worthington MT, Pelo JW, Sachedina MA, Applegate JL, Arseneau KO, Pizarro TT (2002) RNA binding properties of the AU-rich element-binding recombinant Nup475/TIS11/tristetraprolin protein. J Biol Chem 277:48558–48564
16. Sawaoka H, Dixon DA, Oates JA, Boutaud O (2003) Tristetraprolin binds to the 3′-untranslated region of cyclooxygenase-2 mRNA. A polyadenylation variant in a cancer cell line lacks the binding site. J Biol Chem 278:13928–13935

17. Sully G, Dean JL, Wait R et al (2004) Structural and functional dissection of a conserved destabilizing element of cyclo-oxygenase-2 mRNA: evidence against the involvement of AUF-1 [AU-rich element/poly(U)-binding/degradation factor-1], AUF-2, tristetraprolin, HuR (Hu antigen R) or FBP1 (far-upstream-sequence-element-binding protein 1). Biochem J 377:629–639
18. Ogilvie RL, Abelson M, Hau HH, Vlasova I, Blackshear PJ, Bohjanen PR (2005) Tristetraprolin down-regulates IL-2 gene expression through AU-rich element-mediated mRNA decay. J Immunol 174:953–961
19. Suswam E, Li Y, Zhang X et al (2008) Tristetraprolin down-regulates interleukin-8 and vascular endothelial growth factor in malignant glioma cells. Cancer Res 68:674–682
20. Stoecklin G, Tenenbaum SA, Mayo T et al (2008) Genome-wide analysis identifies interleukin-10 mRNA as target of tristetraprolin. J Biol Chem 283:11689–11699
21. Ehlting C, Lai WS, Schaper F et al (2007) Regulation of suppressor of cytokine signaling 3 (SOCS3) mRNA stability by TNF-{alpha} involves activation of the MKK6/p38MAPK/MK2 cascade. J Immunol 178:2813–2826
22. Frasca D, Landin AM, Alvarez JP, Blackshear PJ, Riley RL, Blomberg BB (2007) Tristetraprolin, a negative regulator of mRNA stability, is increased in old B cells and is involved in the degradation of e47 mRNA. J Immunol 179:918–927
23. Yu H, Stasinopoulos S, Leedman P, Medcalf RL (2003) Inherent instability of plasminogen activator inhibitor type 2 mRNA is regulated by tristetraprolin. J Biol Chem 278:13912–13918
24. Cao H, Tuttle JS, Blackshear PJ (2004) Immunological characterization of tristetraprolin as a low abundance, inducible, stable cytosolic protein. J Biol Chem 279:21489–21499
25. Lai WS, Stumpo DJ, Blackshear PJ (1990) Rapid insulin-stimulated accumulation of an mRNA encoding a proline-rich protein. J Biol Chem 265:16556–16563
26. Cao H, Deterding LJ, Venable JD et al (2006) Identification of the anti-inflammatory protein tristetraprolin as a hyperphosphorylated protein by mass spectrometry and site-directed mutagenesis. Biochem J 394:285–297
27. Cao H, Lin R (2008) Phosphorylation of recombinant tristetraprolin in vitro. Protein J 27:163–169
28. Chrestensen CA, Schroeder MJ, Shabanowitz J et al (2004) MAPKAP kinase 2 phosphorylates tristetraprolin on in vivo sites including Ser178, a site required for 14-3-3 binding. J Biol Chem 279:10176–10184
29. Carrick DM, Blackshear PJ (2007) Comparative expression of tristetraprolin (TTP) family member transcripts in normal human tissues and cancer cell lines. Arch Biochem Biophys 462:278–285
30. Essafi-Benkhadir K, Onesto C, Stebe E, Moroni C, Pages G (2007) Tristetraprolin inhibits Ras-dependent tumor vascularization by inducing VEGF mRNA degradation. Mol Biol Cell 18:4648–4658
31. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9:669–676
32. Goldberg-Cohen I, Furneauxb H, Levy AP (2002) A 40-bp RNA element that mediates stabilization of vascular endothelial growth factor mRNA by HuR. J Biol Chem 277:13635–13640
33. Shih SC, Claffey KP (1999) Regulation of human vascular endothelial growth factor mRNA stability in hypoxia by heterogeneous nuclear ribonucleoprotein L. J Biol Chem 274:1359–1365
34. Ciais D, Cherradi N, Bailly S et al (2004) Destabilization of vascular endothelial growth factor mRNA by the zinc-finger protein TIS11b. Oncogene 23:8673–8680
35. Bouchard L, Tchernof A, Deshaies Y et al (2007) ZFP36: a promising candidate gene for obesity-related metabolic complications identified by converging genomics. Obes Surg 17:372–382
36. Bouchard L, Vohl MC, Deshaies Y, Rheaume C, Daris M, Tchernof A (2007) Visceral adipose tissue zinc finger protein 36 mRNA levels are correlated with insulin, insulin resistance index, and adiponectinemia in women. Eur J Endocrinol 157:451–457
37. Cao H, Polansky MM, Anderson RA (2007) Cinnamon extract and polyphenols affect the expression of tristetraprolin, insulin receptor, and glucose transporter 4 in mouse 3T3-L1 adipocytes. Arch Biochem Biophys 459:214–222
38. Cao H, Urban JF Jr, Anderson RA (2008) Insulin increases tristetraprolin and decreases VEGF gene expression in mouse 3T3-L1 adipocytes. Obesity (Silver Spring) 16:1208–1218
39. Lin NY, Lin CT, Chen YL, Chang CJ (2007) Regulation of tristetraprolin during differentiation of 3T3-L1 preadipocytes. FEBS J 274:867–878

40. Thalmeier A, Dickmann M, Giegling I et al (2008) Gene expression profiling of post-mortem orbitofrontal cortex in violent suicide victims. Int J Neuropsychopharmacol 11:217–228
41. Lai WS, Parker JS, Grissom SF, Stumpo DJ, Blackshear PJ (2006) Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts. Mol Cell Biol 26:9196–9208
42. Taylor GA, Blackshear PJ (1995) Zinc inhibits turnover of labile mRNAs in intact cells. J Cell Physiol 162:378–387
43. Cousins RJ, Blanchard RK, Popp MP et al (2003) A global view of the selectivity of zinc deprivation and excess on genes expressed in human THP-1 mononuclear cells. Proc Natl Acad Sci USA 100:6952–6957
44. Cao H, Lin R, Ghosh S, Anderson RA, Urban JF Jr (2008) Production and characterization of ZFP36L1 antiserum against recombinant protein from *Escherichia coli*. Biotechnol Prog 24:326–333
45. Nishimura S, Manabe I, Nagasaki M et al (2007) Adipogenesis in obesity requires close interplay between differentiating adipocytes, stromal cells and blood vessels. Diabetes 56:1517–1526
46. Tilg H, Moschen AR (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6:772–783
47. Ferrara N, Kerbel RS (2005) Angiogenesis as a therapeutic target. Nature 438:967–974
48. Broadhurst CL, Polansky MM, Anderson RA (2000) Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. J Agric Food Chem 48:849–852
49. Khan A, Safdar M, Ali Khan MM, Khattak KN, Anderson RA (2003) Cinnamon improves glucose and lipids of people with type 2 diabetes. Diab Care 26:3215–3218
50. Vanschoonbeek K, Thomassen BJ, Senden JM, Wodzig WK, van Loon LJ (2006) Cinnamon supplementation does not improve glycemic control in postmenopausal type 2 diabetes patients. J Nutr 136:977–980
51. Baker WL, Gutierrez-Williams G, White CM, Kluger J, Coleman CI (2008) Effect of cinnamon on glucose control and lipid parameters. Diab Care 31:41–43
52. Anderson RA, Broadhurst CL, Polansky MM et al (2004) Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem 52:65–70
53. Shoji T, Masumoto S, Moriichi N et al (2006) Apple procyanidin oligomers absorption in rats after oral administration: analysis of procyanidins in plasma using the porter method and high-performance liquid chromatography/tandem mass spectrometry. J Agric Food Chem 54:884–892
54. Imparl-Radosevich J, Deas S, Polansky MM et al (1998) Regulation of PTP-1 and insulin receptor kinase by fractions from cinnamon: implications for cinnamon regulation of insulin signalling. Horm Res 50:177–182
55. Jarvill-Taylor KJ, Anderson RA, Graves DJ (2001) A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. J Am Coll Nutr 20:327–336
56. Zhu W, Brauchle MA, Di Padova F et al (2001) Gene suppression by tristetraprolin and release by the p38 pathway. Am J Physiol Lung Cell Mol Physiol 281:499–508
57. Deleault KM, Skinner SJ, Brooks SA (2007) Tristetraprolin regulates TNF TNF-alpha mRNA stability via a proteasome dependent mechanism involving the combined action of the ERK and p38 pathways. Mol Immunol 56:2160–2169
58. Cao H, Urban JF Jr, Anderson RA (2008) Cinnamon polyphenol extract affects immune responses by regulating anti- and proinflammatory and glucose transporter gene expression in mouse macrophages. J Nutr 138:833–840
59. Preuss HG, Echard B, Polansky MM, Anderson R (2006) Whole cinnamon and aqueous extracts ameliorate sucrose-induced blood pressure elevations in spontaneously hypertensive rats. J Am Coll Nutr 25:144–150
60. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y (2003) Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. Diabetes Res Clin Pract 62:139–148
61. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y (2004) Cinnamon extract prevents the insulin resistance induced by a high-fructose diet. Horm Metab Res 36:119–125
62. Verspohl EJ, Bauer K, Neddermann E (2005) Antidiabetic effect of *Cinnamomum cassia* and *Cinnamomum zeylanicum* in vivo and in vitro. Phytother Res 19:203–206

63. Hlebowicz J, Darwiche G, Bjorgell O, Almer LO (2007) Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in healthy subjects. Am J Clin Nutr 85:1552–1556
64. Cao H, Urban JF Jr, Anderson RA (2007) Prevention of obesity by plant polyphenols via tristetraprolin-mediated cytokine mRNA instability. Obesity 15[9], A90
65. Cao H, Hininger-Favier I, Kelly MA et al (2007) Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet. J Agric Food Chem 55:6372–6378

31 Immunotoxicology and Foods

Rodney R. Dietert and Janice M. Dietert

Key Points

- Foods play important roles in determining both the risk of immunotoxicity and the potential for adverse health outcomes linked to immunotoxicity-based immune dysfunction.
- Foods (1) can be a source of immunotoxicity-inducing contaminants and endogenous chemicals, (2) can protect against some immunotoxicants, and (3) can correct or repair some forms of immune damage. In this chapter, we discuss each of the aspects that connect foods with immunotoxicology.

Key Words: Foods, immune dysfunction, developmental immunotoxicity, inflammaging, disease matrix, immunomodulation, contaminants, nutraceuticals.

31.1 INTRODUCTION

Immunotoxicology can be defined as immune system effects that result from exposure to chemicals and drugs, biological materials, physical factors (such as ultraviolet and ionizing radiation), medical devices, and additional physiological factors, collectively referred to as agents (1). It is important to recognize that immunotoxicity and immunosuppression are not synonymous. Immunosuppression is only one of many different adverse outcomes that can result from immunotoxicity. In fact, any condition in the environment that leads to significant deviation from a normal and balanced immune response capability is considered to be immunotoxicity.

Immunotoxicity is just as likely to appear as inappropriately enhanced immune responses as it is to appear as suppressed immune responses. It is the enhanced responses that are associated with many of the diseases of growing concern today (e.g., allergies, autoimmune and inflammatory diseases). In fact, most of the best characterized immunotoxicants (e.g., dioxin, lead, mercury, tobacco smoke) do not produce a single type of adverse outcome. Instead, they produce immunosuppression for some types of responses, immune enhancement for other responses, and misregulated inflammation

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_31

during microbial or tumor challenge. The latter often produces collateral damage and disease affecting the respiratory, neurological, cardiovascular, gastrointestinal, and hepatic systems.

The variety of immune dysfunction produced by environmental insults increases the risk of a wide variety of diseases affecting virtually every tissue and organ of the body. These diseases include not only those most obviously connected to the immune system (allergy, autoimmunity, leukemia, repeated infections) but also those more recently identified as connected to immune insult and dysfunction (inflammatory bowel disease, lung cancer, Parkinson's disease) and chronic inflammation (atherosclerosis, myalgic encephalomyelitis). In fact, many of the diseases that were originally labeled under a "systems" medical heading (neurological, cardiovascular, endocrine, reproductive) are increasingly recognized as having immune dysfunction as part of their basis (2).

Recently, the emphasis on immunotoxicity and health risks has shifted to a focus on early life environmental factors that influence later-life health risk (3) and even longevity (4, 5). The epigenetic control of inflammation appears to be a significant piece in determining healthy aging (6). Therefore, it is not surprising that many adult and geriatric diseases have their basis in early-life immune insult and dysfunction (7–9).

Life-long patterns of diseases have been identified that are connected to early life immune dysfunction (10). Many entry diseases such as atopic dermatitis, asthma, Kawasaki disease, type 1 diabetes, recurrent infections, pediatric celiac, inflammatory bowel disease, and multiple sclerosis arise during childhood or in the young adult. But despite the seriousness of these initial immune-based diseases and conditions, they are often merely the first in a progressive sequence of lifelong health challenges (10). Environmental risk factors that promote the underlying early-life immune dysfunction can serve both as causes and triggers of these immune-based diseases (11).

The reason that foods are such an important consideration for immunotoxicity and disease is that among the spectrum of environmental risk factors for the immune system, foods represent one of the most readily controllable sources of immunomodulatory factors. While foods can impact immune status at all life stages, attention to foods early in life can reduce the risk of pediatric immune dysfunction and immune-based diseases (12–14). Such management interventions are likely to pay health dividends in the adult (7, 15).

31.2 HOW FOODS AFFECT IMMUNOTOXICITY AND IMMUNE-MEDIATED DAMAGE

Foods, or more accurately the integral chemical constituents and contaminants of foods, can affect immunotoxicity in at least four different ways. First, food-associated chemicals can be immunotoxic with the capacity to damage the developing or fully-matured immune system. Examples of food-based exposure to immunotoxicants in the human population include exposure to aflatoxin (16), alcohol (17), methyl mercury (18) and PCBs (19).

Other food components have the potential to counteract the effects of immunotoxicants. This can happen in at least three different ways: (1) Some foods have factors that protect the immune system against specific immunotoxic insult. These foods contain compounds that are able to prevent or minimize exposure of the target immune system to orally-ingested immunotoxicants. Food constituents either prevent the toxicant from reaching the immune cells or tissues or prohibit critical molecular interactions between

the toxicant and the immune cells avoiding immune damage. Chemicals in food such as zinc, glutamine, polyphenols, and flavonoids like quercetin not only aid immunity but also provide a "barrier effect" for epithelial cells in which cells are more protected against some toxicants (20). (2) Various nutraceuticals and functional foods contain chemicals that can reverse some of the immune dysfunction that results from environmental insult (21, 22). The chemicals act through their specific immunomodulatory and anti-inflammatory activities enabling these foods and food supplements to provide direct repair of the immune system long after the initial toxic insult has occurred. (3) Finally, chemicals in foods may affect the adverse health outcomes of immunotoxicity by repairing tissues damaged by the dysfunctional immune system. For example, take a scenario where immunotoxicity produced misregulated inflamamation and this led to hepatic glutathione depletion as the front line problem for liver damage. Specific foods may be able to restore glutathione levels to normal levels, even if they do not affect the immune dysfunction. These foods may minimize the health risk of immunotoxicity even if they did not act directly on the immune system. Figure 31.1 illustrates two examples of the induction of immunotoxicity by food contaminants (2,3,7,8, tetrachlorodibenzo-*p*-dioxin and the heavy metal, lead). Examples are also shown of suggested inhibition of the pathways leading to immunotoxicity or potential correction of the adverse effects of immunotoxicity by chemicals in foods.

31.3 HOW FOODS AFFECT PATTERNS OF IMMUNE-DYSFUNCTION-LINKED DISEASE

Immune dysfunction resulting from exposure to immunotoxicants including those found in foods can elevate the risk of infectious disease, cancer, autoimmunity, allergic disease, and inflammatory conditions. By themselves, these are serious conditions and several are life threatening. Others severely impact family economics and individual quality of life. In fact, immune dysfunction-based diseases affect more than a quarter of all children in developed countries (10). But it is important to recognize that the underlying immune dysfunction can contribute to extensive disease comorbidity. Often a primary immune-based disease is only the tip of the iceberg when it comes to the lifetime of environmentally induced health risks. The original immune insult and/or ramifications of the primary disease can lead to secondary diseases and conditions that may not be as obviously linked to a common immune dysfunction (10).

A matrix or pattern of immune-based diseases includes later-life diseases that commonly arise after a pediatric immune-based disease has been diagnosed. For example, many diseases associated with misregulated inflammation and aberrant proinflammatory cytokine production lead to a higher risk of clinical depression (23, 24). Additionally, reduced sensory function (hearing loss and loss of taste) is often a later outcome of certain autoimmune and inflammatory conditions (25, 26). Tissue-focused immune problems that feature chronic inflammation (such as in asthma and inflammatory bowel disease) can contribute to an elevated risk of later-life cancer in the affected tissue (27, 28). In fact, the entire issue of misregulated inflammation, later life "inflammaging" and adult inflammatory-based disease is probably most easily addressed prior to disease onset before immune imprinting (7) has been established (e.g., during early childhood).

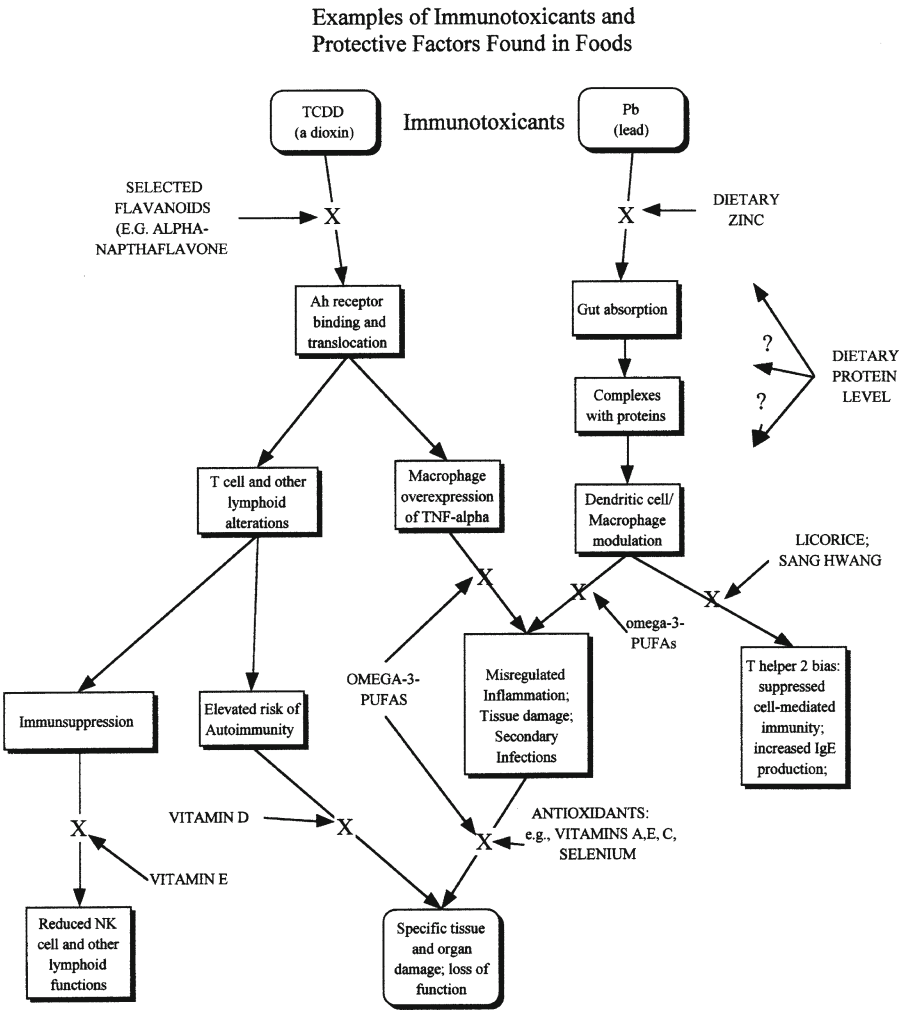


Fig. 31.1. The diagram depicts the range of interactions between foods and immunotoxicity. Chemicals in foods can both cause and protect against immunotoxicity. Two examples of immunotoxic contaminants of foods [2,3,7,8,tetrachlorodibenzo-*p*-dioxin (TCDD) and the heavy metal Pb (lead)] are illustrated at the top of the flow chart. Inappropriate exposure to these toxicants via food (as well as other routes) can result in immune dysfunction and/or tissue damage. However, other chemicals found in foods including nutrients, nutraceuticals, and components of functional foods have been reported to inhibit one or more steps in the pathways leading to immunotoxicity and tissue damage. Some examples of these are illustrated. The "X" symbol is used to indicate probable points of inhibition along pathways of immunotoxicity.

This is an area where foods are likely to play a critical role. Obviously, prevention of the underlying immune dysfunction through healthier management of the baby's environment is the best strategy whenever possible. But once an immune-based disease such as childhood asthma or type 1 diabetes is diagnosed, foods should be used intelligently as part of the strategy to minimize the risk of an allergy or autoimmune web of multiple diseases. Often, the initial pediatric diseases can be successfully managed. But effective use of foods for (1) correcting the underlying immune dysfunction and/or (2) preventing

the secondary immune-linked diseases within a web are likely to be the determining factors of adult longevity and quality of life.

Using dietary components to optimize immune function is discussed throughout the chapters of this book. The take-home message from this current chapter is that even single toxicant exposures, particularly if they are in early life, are capable of promoting a lifetime of immune-based diseases. Foods provide an excellent opportunity to break an immune dysfunction cycle and to minimize the risk of the diseases that may emerge as the individual ages.

31.4 AGE-RELATED AND GENDER-SPECIFIC CONSIDERATIONS

Among the important considerations of foods and risk of immunotoxicity are the factors of age and sex. For virtually all comparisons to date, including those that are food-born, the developing immune system is more sensitive to immunotoxicity than that of the adult (3, 29, 30). This is not surprising given that the developing immune system is particularly susceptible to environmentally-determined imprinting, and once a dysfunctional immune response pattern is established, it can persist well into adulthood (7).

Foods play an important role in determining the childhood immune status (31, 32). This extends both to the specific nature of the diet (composition and contaminants) (19, 33) as well as to undernutrition (34). Interrelated to the issue of foods is the development of the gastrointestinal tract, the gut-associated lymphoid tissue (GALT), and the natural microflora. In fact, gut microbial development and maintenance appears to play an important factor in immune function versus dysfunction (35, 36) linked to the risk of pediatric disease (37). For this reason, functional foods containing prebiotics, probiotics, or their combination (synbiotics) may provide a useful aid for the protection of the immature immune system against environmental insult (38).

The increased sensitivity of the young is based, at least in part, on critical windows of vulnerability linked to unique prenatal and neonatal developmental processes (1, 30, 39, 40). This translates into significant health risks over a lifetime (15, 41). The increased age-related sensitivity of the young is demonstrated by the fact that lower exposure levels are required to produce adverse outcomes, immunotoxicity persists following early life exposure, a broader spectrum of adverse effects is seen and the immature immune system is more likely to be imprinted for later-life environmental-immune problems (7, 29, 30, 42).

Most immunotoxicants are capable of exerting effects in both males and females. However, the actual health risk that is posed by exposure to an immunotoxicant can vary widely between sexes. Both dose sensitivity and the nature of the adverse outcome have been reported to differ between males and females. For example, the reports of immunotoxicants for the developing immune system that produce differential effects based on sex include such categories as: heavy metals, drugs, phytoestrogens, alcohol, polychlorinated compounds, pesticides, and herbicides (reviewed in 43). While some of these effects may be related to the endocrine-disrupting capacity of some immunotoxicants, it is not clear that this is the basis for all sex-related differences among immunotoxicants. The implications of these differences are significant for health risk. For example, on the basis of comparisons in animal models, a problematic exposure to a food-associated immunotoxicant in early life (e.g., genistein, alcohol) may present very different immune-based health risks for male versus female offspring (43–45).

31.5 EXPOSURE TO IMMUNOTOXICANTS VIA FOODS

31.5.1 Alcohol

Among the well-defined immunotoxicants associated with foods is alcohol. As with all toxicants, the dose is critical in determining the risk of adverse immune effects. The effects of alcohol on the immune system are numerous, and the range of adverse outcomes depends on exposure level, duration, age, and gender. Among the targets of alcohol exposure in the fetus as well as in the adult is mucosal immunity of the airways that is compromised and unable to provide needed antioxidant defenses (46, 47). Excessive alcohol use increases the risk of serious airway infections such as tuberculosis (48). In the same vein, alcohol ingestion can impair intestinal mucosal immune defenses by reducing bacterial defense and inhibiting repair processes (49). Other evidence also suggests that immune-related healing and repair is suppressed by alcohol (50).

The impact of alcohol on the liver is well known. Liver damage produced by alcohol appears, at least in part, to be immune mediated. Alcohol-induced hyperactive Kupffer cells contribute to chronic inflammation and hepatic necrosis (51). In fact, some investigators have proposed that immune-mediated mechanisms account for the high occurrence of fibrosis, cirrhosis, and cancer across many different forms of liver disease (52, 53).

In animal models, fetal exposure to alcohol has been reported to impair innate immune function and to increase the neonate's susceptibility to various infections (17, 54). In humans, alcohol-induced immune dysfunction includes a decrease in Th1/Th2 ratios and an increase in susceptibility to certain infections (55). This is probably based in part on its effects on dendritic cells as well as its capacity to disrupt cytokine networks during host challenge (56). Alcohol intoxication has been reported to impair the maturation of dendritic cells (57) and the capacity of these cells to promote T cell responses during antigen presentation (58). At least one mechanistic observation is that alcohol acts on macrophages via impact on heat shock proteins to cause the dysregulation of TNF-alpha production (59).

31.5.2 Pesticides, Insecticides and Herbicides

Chemicals related to food production that may enter the food chain as contaminants can include pesticides, insecticides, herbicides, fungicides, rodenticides, and antimicrobial compounds. These usually contaminate foods during production and residues can be passed along to the consumer. Investigations suggest that several of these chemicals affect the immune system. Among the herbicides that are of primary interest for potential immunotoxicity are atrazine and 3,4-dichloropropionanalide (propanil).

The developing immune system appears to be particularly sensitive to atrazine exposure. Mice that were exposed in utero were found to have altered acquired immune responses. The changes were highly gender-dependent in terms of both the direction and spectrum of the immune alterations (60, 61). Propanil has the capacity to suppress innate immunity (via natural killer cells and macrophages) and to alter the activity of cells involved in acquired immunity (T and B lymphocytes). The disruption of cell signaling (e.g., Nuclear Factor-kappa B, calcium flux) appears to be one possible mechanism for immune disruption as was recently reviewed by Salazar et al. (62). Human evidence also suggests that propanil has the potential to disrupt lymphoid responses via interference with cell signaling (63).

Over time, there has been a gradual shift away from the more persistent organochlorine pesticides to organophosphates. Some evidence suggests that certain organophosphate pesticides deserve more attention as to their potential to be immunotoxicants. For example, Navarro et al. (64) reported that the neonatal exposure of rats to the organophosphate chlorpyrifos produced pronounced T cell immunotoxicity that appeared after the animals aged to adulthood. Other investigators also reported the suppression of T cell responses in rats following chlopryrifos exposure (65). Malathione has also been reported to produce immunotoxicity in a variety of animal models (66).

31.5.2.1 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are among the significant concerns of food-born pollutants. These are persistent environmental pollutants, and they can reside in the environment as well as in body fat for a lengthy period of time. Much of the food-borne exposure to PCBs comes through the bioconcentration of PCBs in the food chain particularly in marine animals. Fish can accumulate PCB well in excess of the concentration in the water in which they live. Larger fatty-type species such as salmon and tuna can be a reservoir for both PCBs and mercury.

PCBs are known as important immunotoxicants capable of altering both host defenses to infectious challenge and immune responses to vaccinations. Heilmann et al. (19) reported that Faroe Island children consuming a largely marine-based diet had childhood vaccination responses that were inversely correlated with their exposure to PCBs. In fact, the immunotoxicity was so extensive that 28% of the children had such poor responses to vaccination that they were unprotected. The best predictor of the vaccine response in seven-year-old children was the PCB concentration of cord blood at the time of their birth. This suggests that prenatal development was the most sensitive critical window for PCB-induced immunotoxicity.

In similar studies on an Inuit population of children in northern Canada, investigators found that elevated PCB levels were associated with higher rates of acute otitis media and respiratory infections. A study among Dutch school children also found higher rates of recurrent middle ear infections among children with elevated levels of PCBs (67). Additional studies in Sweden found that prenatal exposure to PCBs increased the risk of childhood infections (68). In a study of older women, PCB body burden was associated with an increased risk of rheumatoid arthritis (69). The results pertaining to childhood and adult immune function are not surprising in light of a report that prenatal PCB levels can impact thymus size at birth (70).

31.5.2.2 Perfluorooctanoic Acid (PFOA)

Perfluorooctanoic acid (PFOA), also known as C8, is one of the more recent food contaminants to cause immunotoxic and other health concerns. PFOA is one of the chemicals used in certain nonstick cooking wares as a bonding agent. Such kitchen ware is often a prominent tool used in low-fat cooking. Ironically, exposure to PFOA has the potential to counteract some of the health benefits of pursuing low-fat cooking. PFOA is detectable in the bloodstream of most Americans including newborns. The chance of the release of PFOA from cookware seems to be increased under prolonged high-heat conditions. Both inhalation exposure from cooking fumes and PFOA residues on cooked food are potential routes of exposure.

Evidence suggests that PFOA is immunotoxic at sufficient doses. At high doses in mice, it produces thymic and splenic atrophy (71). A lower range of exposures to PFOA causes the suppression of T-dependent antibody responses in mice (72). The scope and range of PFOA effects on the immune system were covered in a recent review by DeWitt et al. (73).

31.5.3 Heavy Metals

Heavy metals represent one of the categories of food contaminants with significant immunotoxic activity. Among the most prominent contaminants of immunotoxic concern are arsenic, cadmium, lead, and mercury. These metals can contaminate not only foods but also drinking water. They each produce a wide array of adverse outcomes for the exposed immune system and share or overlap in the spectrum of changes they can induce. As detailed in the following, exposure to these metals produces skewed immune response capabilities that concomitantly increase susceptibility to certain infections while increasing the risk of allergic and autoimmune diseases.

31.5.3.1 Arsenic

Arsenic exposure via foods is likely to come from seafood, particularly that found in brackish water. Arsenic interferes with signal transduction in lymphocytes resulting in apoptosis (74–77). It also targets macrophages and interferes with their differentiation, maturation, and functional responses (78, 79). In particular, arsenic has been proposed as a significant immunotoxic risk for children (41, 74).

31.5.3.2 Mercury

Mercury intake from foods occurs largely from fish with additional contributions from fruits and vegetables. It is among the most significant food-associated toxicants for the immune system. The developing immune system is particularly vulnerable to mercury-induced immunotoxicity and special care is needed to avoid exposure.

Mercury is thought to produce an elevated risk of autoimmunity (80–82). A couple of possible mechanisms that have been suggested for the link of mercury to autoimmunity is its capacity to inhibit the death-induced signaling complex associated with CD95-mediated T cell apoptosis (83, 84) and the recently reported interference of mercury with T cell receptor signaling (85).

An additional effect of mercury exposure is similar to that of lead. Depending upon the form of mercury (e.g., inorganic vs. organic), it has the capacity to skew immune responses and cytokine production toward Th2 and away from Th1. This has been reported both for humans (86) as well as in rodents (87).

31.5.3.3 Cadmium

Cadmium contamination is most likely to be found in vegetables and cereals. The metal is a potent immunotoxicant causing damage to the thymus and thymocytes. At least part of the process of cadmium-induced immunotoxicity appears to involve excessive production of oxidative radicals, which produces thymic damage, the induction of lymphoid apoptosis (88), and macrophage cell death (89). Th1 cells appear to be more sensitive than Th2 cells to the toxic effects of cadmium. There is a shift in cytokine production ratios that biases immune responses toward Th2 (90). Cadmium-induced

skewing toward Th2 responses has been reported by other researchers (91) and is similar to the adverse Th biasing effects seen with lead and mercury.

31.5.3.4 Lead

Lead appears to be most often encountered in foods via wine, game, fish, and meat. Among the most sensitive targets for lead are macrophages, dendritic cells, and T lymphocytes (30). Among the hallmarks of lead-induced immunotoxicity is a bias in immune responses that effectively decreases Th1-driven cell-mediated immune responses (92, 93) and increases the likelihood of Th2-dependent allergic reactions (94, 95). This skewing of immune responses has been observed in numerous species of animal models as well as among wildlife species (96).

In recent studies on mice, lead was found to produce the Th functional shift by affecting dendritic cell maturation, skewing antigen presentation by these cells toward Th2-driven responses and away from Th1 responses (97). This is consistent with the observation in animals (92, 95) and humans (98–101) that lead exposure is associated with increased IgE production driven at least in part by increased production of IL-4 and reduced production of interferon-gamma.

Additionally, the usage of the T cell receptor appears to be disrupted following exposure to lead and this along with other observations of autoantibody production suggests that lead exposure is likely to increase the risk of autoimmune reactions (94, 96, 102). Macrophage function is impaired by lead in such a way as to decrease the overall antimicrobial activity of the phagocytes, increase susceptibility to certain bacteria infections (e.g., *Listeria*), and cause the overproduction of proinflammatory cytokines such as TNF-alpha and IL-6 (reviewed in 97).

31.5.3.5 Multiple Metals

Of particular importance for food-related immunotoxicity is the observation that multi-metal exposure can pose an even greater risk of immunotoxicity than exposure to single metals. Bishayi and Sengupta (103) showed that combined arsenic and lead exposure of mice resulted in a synergy of immunotoxicity potential with combined metal exposure producing a more serious suppression of macrophage function, including decreased resistance to bacteria, than was observed with exposure to individual metals.

31.5.4 Contaminants of Foods from Plastic Products

Several components of plastics used in food wrapping, storage, and preparation have the capacity to leech into food. This can increase during storing or heating foods in plastic containers. While a single exposure may be modest, chronic exposure to chemicals found in plastics, many of which are known to be endocrine disruptors (e.g., estrogenic, antiandrogenic, thyroid interfering chemicals), can present an immunological risk. Among the chemicals of concern are bisphenol A (BpA) and the phthalates.

31.5.4.1 BpA

BpA is a component of plastic products used in food storage and preparation. As a result, exposures can occur that are both significant and in the range of health concerns (104). BpA has been reported to reduce the production of Th1-related cytokines in rats (105) and mice (106), to reduce regulatory T cells (Tregs) in mice (107), and to selectively

elevate production of Th2 cytokines in mice (107). Additional investigation also reported a decline in Tregs following transmaternal exposure to BpA that was associated with a loss of oral tolerance to dietary proteins (108). However, other laboratories reported elevated Th1 responses in mice following prenatal exposure to BpA (109). It seems likely that developmental timing, exposure dose, and gender all play a significant role in determining the precise nature of the immune insult that results from BpA exposure.

31.5.4.2 Phtalates

Phthalates are an integral component of many plastic products designed to provide flexibility. Like BpA, phthalates can leech out of the products into food resulting in exposure to children and adults. Phthalates have a controversial role relative to immunotoxicity and immune modulation. Some studies have suggested they can act as adjuvants increasing the levels of certain immune responses (110).

But even that effect may be dependent upon the route and levels of exposure. For example, a recent study found that in individuals allergic to dust mites, inhaled phthalates did not aggravate nasal responses (111). Likewise, the topical application of phthalates to mice had no effect on induced allergic responses to proteins (112). In a comparative study, Ohnishi et al. (113) showed that certain endocrine disruptors including BpA can disrupt toll-like receptor 4 (TLR4) signaling in macrophages. A limited number of phthalates also produced this effect but most of those examined did not. In a study designed to examine the effects of phthalates on Th2 cytokines and IgE production in mice, Butala et al. (114) reported that phthalate exposure produced no effects. Similarly, Piepenbrink et al. (115) showed that while prenatal exposure of female rats to di-(2-ethylhexyl) phthalate produced alterations in developmental and reproductive parameters, there was little evidence of persistent immunotoxicity in the adult offspring.

Thus far the evidence suggests that BpA is likely to represent the more serious immunotoxic concern compared with phthalates. However, the latter can exert effects on other physiological systems that suggest the need for caution relative to exposure and health risks (116).

31.5.4.3 Polycyclic Aromatic Hydrocarbons

Polyxcyclic aromatic hydrocarbons (PAHs) are the products of incomplete combustion. They include established immunotoxicants such as benzo(a)pyrene (BP), 3-methycholanthrene (3MC) and dimethylbenzanthracene (DMBA). PAHs are found in combustion-related air pollution including cigarette smoke. They can occur in food either via absorption from pollution in the air or through high-heat cooking such as grilling. PAHs are more often found in grilled and smoked meats as opposed to vegetables. They are also produced in the vapor from high-heated cooking oils.

PAHs appear to produce dysregulation of immune function that can vary as to the type of effects depending upon the timing (e.g., early life vs. adult) and level of exposure. Exposure in humans has been reported to suppress T cell responses and at some doses to elevate the numbers of natural killer cell activity (117). In studies on human monocytes and macrophages, BP was found to inhibit macrophage maturation (118).

Animal studies confirmed that PAHs are highly immunotoxic and are capable of suppressing both antibody production and cell-mediated host defenses (119). They disrupt cell signaling in lymphocytes (120) and impair thymocyte differentiation (121). In particular, PAH exposure can impair: (1) the presentation of antigens by profession

antigen-presenting cells during immune responses and (2) the responsiveness of primed lymphocytes to control antigen presentation signals during the course of host-pathogen defenses-acquired immune responses (122). These combined results suggest that PAHs are capable of causing immune dysfunction affecting several aspects of acquired and innate immunity and can impact host resistance to disease.

31.5.5 Mycotoxins

Mold-derived toxins form an important group of food contaminants that are potent immunotoxicants. Immunotoxic metabolites of fungi can contaminate raw food crops and make their way into processed foods as well. They have been recognized to cause health problems for more than 100 years. However, detailed studies of their immunotoxicity are much more recent.

Among the groups of mycotoxins that are important contaminants of food are the aflatoxins (in nuts and cereals), the fusmonisins (corn and cereals), ochratoxin (beans and cereals), patulin (fruit), and the trichothecenes (corn and cereals) (123). Of these, the trichothecenes are metabolites of fungi such as *Fusariaum* and *Cephalosporium*. These go by other names such as T-2 toxin and vomitoxin. Most of these occur via fungal growth on cereals and grain during wet, damp storage. These toxins target not only the immune system (124) but also the neurological and gastrointestinal systems.

31.5.5.1 Trichothecenes

The effects of trichothecenes are highly dose dependent. They can dramatically affect macrophages both by stimulating inflammation and skewing immune response capabilities at low-to-moderate dose ranges (125, 126). Of importance is the finding that mycotoxins can elevate the inflammatory response to endotoxin (bacterial lipopolysaccaride, LPS). This is significant because many chronic diseases feature the misregulation of inflammation, which is a feature of diseases of aging and a component of higher risk of autoimmunity. Therefore, exposure to these fungal metabolites appears capable of contributing to problematic immune dysfunction. The trichothecene, deoxynivalenol, has been reported to alter dendritic cell function and may be one route through which immunosuppression is induced (127).

At higher doses they can cause leukocyte cell death through apoptosis.

31.5.5.2 Alflatoxin B1

Additional studies suggest that another storage toxin from fungi, alflatoxin B1 (produced by certain species of *Aspergillus*) can significantly impair cell-mediated immune responses, increase proinflammatory cytokine production, and decrease vaccine responses in animals (128, 129).

31.5.5.3 Ochratoxin

Ochratoxin causes nephrotoxicity in addition to immunotoxicity with early life exposure being of particular concern (130). However, the immune system may not be the most sensitive target at least in adults (131). Ochratoxin can be passed to the infant through human milk (132). Similar to the effects of other mycotoxins, exposure to ochratoxin has been reported to increase inflammatory responses (133), decrease certain acquired immune responses (134), and increase the susceptibility to secondary bacterial infections (135).

31.5.5.4 Patulin

Patulin is a mycotoxin that is a metabolite produced by certain species of *Penicillium* and *Aspergillus* growing on fruit. It is most likely to be encountered in foods through the consumption of apple juice or products with apple juice ingredients from mold-damaged fruit. However, because the mold-damage is not always visible, complete elimination of patulin-laded fruit from the food chain can be challenging.

31.6 FOODS AND THE REDUCTION OF IMMUNOTOXICITY-RELATED PROBLEMS

Other chapters in this book discuss in considerable detail the wide variety of dietary factors that affect the immune system. Major categories include: fatty acids, vitamins and minerals, amino acids (ratios and absolute levels), flavanoids, and various nutraceuticals. In many cases, the effects of these dietary components are considered therapeutically without the necessity of an overt connection to the underlying cause of immune dysfunction. This section describes the potential benefits of applying specific components of foods as part of the solution to both immune dysfunction and resulting tissue damage linked to immunotoxicity.

There are several ways in which foods can play a critical role in reducing the adverse impact of exposure to immunotoxicants and thereby reducing the risk of immune-related disease. Components of food can (1) reduce exposure of cells and tissues to insult by scavenging or blocking the availability of toxicants for the immune system, (2) interfere with normal metabolic pathways used to produce indirect-acting toxicants and/or the toxic metabolites of parent compounds, (3) alter the chemical environment tied to an adverse outcome thereby reducing the impact of the immunotoxic change and (4) help to shift the dysfunctional immune system (moving it toward a more well-balanced host defense system) or repair the resulting tissue damage.

31.6.1 Preventing Immunotoxicity Versus Correcting Adverse Outcomes

Obviously, preventing environmentally-induced immune insult and the resulting immune dysfunction is preferable whenever possible. This provides a frontline opportunity to minimize health risks. But in many cases, this is not an option. Instead, protection against the adverse effects of environmentally-induced immune dysfunction (e.g., causing overproduction of inflammatory mediators) and/or correction of immune imbalances themselves (e.g., correcting T helper imbalances) are the more common intervention points involving foods and food-related natural products. Promising food-related sources that have the potential to correct common adverse outcomes of developmental immunotoxicity (DIT) were recently discussed (22). Natural products showing the greatest promise for reversing DIT outcomes included extracts of *Astragalus membranaceus*, *Dioscorea alata*, *Echinacea purpurea*, *Ganoderma lucidum*, *Grifola frondosa*, *Lentinus edodes*, *Nigella sativa*, *Panax ginseng*, *Phellinus linteus*, *Sophora flavescens* Ait., and *Trigonella foenum graecum L.*

31.6.2 Inhibition of Immunotoxicity and/or Immune-Inflicted Damage

Food sources can contain both direct inhibitors of immunotoxicants and inhibitors of the immune cell-produced toxins. Among the important categories of food chemicals

are free radical scavengers and metabolic inhibitors. Such protective activity has been associated with: vitamin E (136, 137), selenium (138), vitamin C (139), green tea extracts (140), grape seed proanthocyanidins (141), polyphenols (142), triterpenoids (143), Tunisian radish extract (144), chicory leaf extract (145), whole blueberry powder (146), and melatonin (147). Other components of food can help to replenish or spare the critical biochemical targets of immunotoxicity. Among these are curcumin and N-acetylcysteine (148). The former has been used to reduce the susceptibility of children to recurrent viral infections (149).

Additionally, food components can act indirectly by either providing or reducing the availability of substrates involved in producing adverse outcomes of immunotoxicity. For example, diets or supplements that affect arginine availability, use, and metabolism can be expected to influence the nitric oxide production pathway particularly among some genotypes of individuals (150, 151). Other diets high in saturated fat establish a permissible environment for macrophages to promote tissue inflammation (particularly following various pollutant exposures), metabolic disorders, and inflammatory-based diseases (152–154).

In contrast, lower fat diets and/or those rich in *n*-3 fatty acids that ensure a better balance of *n*-3 versus *n*-6 fatty acids can reduce the substrate availability for promoting inflammation (155) and can help to protect against the misregulated inflammation so often found with chemical- or drug-induced immunotoxicity (156). Dietary fat intake can be a major determinant in risk of adverse outcomes like atherosclerosis (157). With increasing concern about misregulated inflammation, one of the food components that appears to inhibit oxidative damage and intestinal inflammation is l-carnitine (158). l-carnitine appears to help intervene in the inappropriate inflammation associated with metabolic syndrome (159). It is particularly promising in reducing the age-related decline in acquired immune function that is associated with chronic inflammation (160).

Other components of foods also play important roles. Dietary protein levels, particularly in early life, can affect the impact of immunotoxicity under some circumstances (161, 162). In Chen et al. (162), the maternal dietary protein level was shown to affect the extent of lead-induced immunotoxicity in the offspring with low but adequate protein intake resulting in partial protection. Dietary protein–carbohydrate balance is also important (163). These investigators found that the ratio of dietary protein–carbohydrate could alter the immune system potential of rats. The effects (immune potentiation vs. immune suppression including age-related decline) were different for young versus old rats depending upon not only the age of dietary exposure, but also the duration rats consumed a given diet. Of additional note is the fact that specific diet-immune interactions can vary significantly with age and gender. A protective diet against environmentally-mediated immune insult at one age and in one gender may not have the same effect across all ages and genders (163).

31.7 CONCLUSIONS AND PERSPECTIVES

Foods play an important role in the status of the immune system and overall health. The impact of foods on the immune system extends to every manifestation of immunotoxicity (adverse immunological outcomes). Immunotoxicity can be induced by chemicals that are endogenous components of foodstuffs as well as by chemical and microbiological contaminants of foods. Exposure level can often determine a beneficial

from a harmful immunological outcome. In the endogenous chemical category are amino acids, fatty acids, carbohydrates, micronutrients, and vitamins as well as other chemicals such as flavanoids and naturally-occurring toxins in foods (e.g., alkaloids). Immunotoxic food contaminants can occur at virtually any place in the food production process between the farm and the table. Oral exposure to toxicants represents a significant immunotoxic concern, and its assessment requires a focus not only on systemic immunity but also on the more specialized gut-associated lymphoid tissue (GALT). Other foods have chemicals that are (1) protective against immunotoxicants, (2) have the potential to correct, at least in part, the immune dysfunction induced by immunotoxicants, or (3) restore tissue function commonly damaged by the dysfunctional immune system. These food-related factors are discussed in detail in other chapters.

The key to a practical understanding of foods and the risk of immunotoxicity begins first and foremost with the recognition that age- and gender-based groups can differ widely in their vulnerability to food-associated immunotoxicants. As a result, the risk of adverse outcomes and even the range of immunological changes may be highly group-specific. For this reason, the immunological risk of exposure of the fetus to given levels of marine-based mercury, PCBs, or PFOAs can be dramatically different from that of an adult. Therefore, the immunotoxicity of foods must be approached with a clear understanding of both the immunological hazards that exist and the populations most at risk for food-associated immune dysfunction.

Finally, because food choices often exist and foods can have immunotoxicity-inducing and/or immune-protecting capacities, they offer one of the best opportunities for immunological-based health risk reduction and effective immune management over a lifetime. In some cases, the use of alternative foodstuffs or food sources by the most at-risk populations not only enable individuals to passively avoid immunotoxicants, but it can also help to actively protect them against certain types of damage to the immune system (e.g., protection against oxidative damage). For this reason, an integrated goal of foods and the immune system should be toxicant avoidance, protection against toxicant damage, and the restoration of effective immune balance.

REFERENCES

1. Luster MI, Dietert RR, Germolec DR, Luebke RW, Makris SL (2009) Developmental immunotoxicology. In: Sonawane B, Brown R (eds) Encyclopedia of Environmental Health. Elsevier, Oxford, UK
2. Suttles J, Stout RD (2009) Macrophage CD40 signaling: a pivotal regulator of disease protection and pathogenesis. Semin Immunol 21:257–264
3. Dietert RR (2009) Developmental immunotoxicology: focus on health risks. Chem Res Toxicol 22:17–23
4. Lowell WE, Davis GE Jr (2008) The light of life: evidence that the sun modulates human lifespan. Med Hypotheses 70:501–507
5. Vallejo AN, Michel JJ, Bale LK, Lemster BH, Borghesi L, Conover CA (2009) Resistance to age-dependent thymic atrophy in long-lived mice that are deficient in pregnancy-associated plasma protein A. Proc Natl Acad Sci USA 106:11252–11257
6. Balistreri CR, Colonna-Romano G, Lio D, Candore G, Caruso C (2009) TLR4 polymorphisms and ageing: implications for the pathophysiology of age-related diseases. J Clin Immunol 29:406–415
7. Bellinger DL, Lubahn C, Lorton D (2008) Maternal and early life stress effects on immune function: relevance to immunotoxicology. J Immunotoxicol 5:419–444

8. Palinski W, Yamashita T, Freigang S, Napoli C (2007) Developmental programming: maternal hypercholesterolemia and immunity influence susceptibility to atherosclerosis. Nutr Rev 65:S182–S187
9. Rooney AA, Yang Y, Makris SL (2008) Recent progress and diverse effects in developmental immunotoxicology: overview of a symposium at the 46th Annual SOT Meeting, Charlotte, NC. J Immunotoxicol 5:395–400
10. Dietert RR, Zelikoff JF (2009) Pediatric immune dysfunction and health risks following early life immune insult. Curr Pediatr Rev 5:36–51
11. Dietert RR (2009) Distinguishing environmental causes of immune dysfunction from pediatric triggers of disease. The Open Pediatr Med J 3:38–44
12. Björkstén B (2008) Environmental influences on the development of the immune system: consequences for disease outcome. Nestle Nutr Workshop Ser Pediatr Program 61:243–254
13. Enke U, Seyfarth L, Schleussner E, Markert UR (2008) Impact of PUFA on early immune and fetal development. Br J Nutr 100:1158–1168
14. Nesterenko TH, Aly H (2009) Fetal and neonatal programming: evidence and clinical implications. Am J Perinatol 26:191–198
15. Dietert RR, Piepenbrink MS (2008) The managed immune system: protecting the womb to delay the tomb. Hum Exp Toxicol 27:129–134
16. Jiang Y, Jolly PE, Preko P, Wang JS, Ellis WO, Phillips TD, Williams JH (2008) Aflatoxin-related immune dysfunction in health and in human immunodeficiency virus disease. Clin Dev Immunol 2008:790309
17. McGill J, Meyerholz DK, Edsen-Moore M, Young B, Coleman RA, Schlueter AJ, Waldschmidt TJ, Cook RT, Legge KL (2009) Fetal exposure to ethanol has long-term effects on the severity of influenza virus infections. J Immunol 182:7803–7808
18. Bilrha H, Roy R, Moreau B, Belles-Isles M, Dewailly E, Ayotte P (2003) In vitro activation of cord blood mononuclear cells and cytokine production in a remote coastal population exposed to organochlorines and methyl mercury. Environ Health Perspect 111:1952–1957
19. Heilmann C, Grandjean P, Weihe P, Nielsen F, Budtz-Jørgensen E (2006) Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med 3:e311
20. Amasheh M, Andres S, Amasheh S, Fromm M, Schulzke JD (2009) Barrier effects of nutritional factors. Ann N Y Acad Sci 1165:267–273
21. Shepherd DM (2007) Immunomodulation by nutraceuticals and functional foods. In: Luebke R, House R, Kimber I (eds) Immunotoxicology and Immunophamacology, 3rd edn. CRC Press, Inc, Boca Raton, FL, pp 185–206
22. Dietert RR, Dietert JM (2007) Early-life immune insult and developmental immunotoxicity (DIT)-associated diseases: potential of herbal- and fungal-derived medicinals. Curr Med Chem 14:1075–1085
23. Gold SM, Irwin MR (2009) Depression and immunity: inflammation and depressive symptoms in multiple sclerosis. Immunol Allergy Clin North Am 29:309–320
24. Dhabhar FS, Burke HM, Epel ES, Mellon SH, Rosser R, Reus VI, Wolkowitz OM (2009) Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression. J Psychiatr Res 43:962–969
25. Yehudai D, Shoenfeld Y, Toubi E (2006) The autoimmune characteristics of progressive or sudden sensorineural hearing loss. Autoimmunity 39:153–158
26. Wang H, Zhou M, Brand J, Huang L (2007) Inflammation activates the interferon signaling pathways in taste bud cells. J Neurosci 27:10703–10713
27. Sherman PW, Holland E, Sherman JS (2008) Allergies: their role in cancer prevention. Q Rev Biol 83:339–362
28. Lin OS (2009) Acquired risk factors for colorectal cancer. Methods Mol Biol 472:361–372
29. Luebke RW, Chen DH, Dietert R, Yang Y, King M, Luster MI, Immunotoxicology Workgroup (2006) The comparative immunotoxicity of five selected compounds following developmental or adult exposure. J Toxicol Environ Health B Crit Rev 9:1–26
30. Dietert RR, Piepenbrink MS (2006) Perinatal immunotoxicity: why adult exposure assessment fails to predict risk. Environ Health Perspect 114:477–483
31. Lucas RM, Ponsonby AL, Pasco JA, Morley R (2008) Future health implications of prenatal and early-life vitamin D status. Nutr Rev 66:710–720

32. Hanson LA, Zaman S, Werner B, Håversen L, Motas C, Moisei M, Mattsby-Baltzer I, Lange S, Banasaz M, Midtvedt T, Norin E, Silfverdal SA (2008) Growth and nutrition: the first six months. Nestle Nutr Workshop Ser Pediatr Program 61:123–134
33. Prescott SL, Dunstan JA (2007) Prenatal fatty acid status and immune development: the pathways and the evidence. Lipids 42:801–810
34. Desai M, Gayle DA, Casillas E, Boles J, Ross MG (2009) Early undernutrition attenuates the inflammatory response in adult rat offspring. J Matern Fetal Neonatal Med 1:1–5
35. Isolauri E, Kalliomäki M, Laitinen K, Salminen S (2008) Modulation of the maturing gut barrier and microbiota: a novel target in allergic disease. Curr Pharm Des 14:1368–1375
36. Conroy ME, Shi HN, Walker WA (2009) The long-term health effects of neonatal microbial flora. Curr Opin Allergy Clin Immunol 9:197–201
37. Björkstén B (2004) Effects of intestinal microflora and the environment on the development of asthma and allergy. Springer Semin Immunopathol 25:257–270
38. Saulnier DM, Kolida S, Gibson GR (2009) Microbiology of the human intestinal tract and approaches for its dietary modulation. Curr Pharm Des 15:1403–1414
39. Dietert RR, Etzel RA, Chen D, Halonen M, Holladay SD, Jarabek AM, Landreth K, Peden DB, Pinkerton K, Smialowicz RJ, Zoetis T (2000) Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. Environ Health Perspect 108:483–490
40. Dietert RR, Dietert JM (2008) Potential for early-life immune insult including developmental immunotoxicity in autism and autism spectrum disorders: focus on critical windows of immune vulnerability. J Toxicol Environ Health B Crit Rev 11:660–680
41. Selgrade MK (2007) Immunotoxicity: the risk is real. Toxicol Sci 100:328–332
42. Dietert RR (2008) Developmental immunotoxicology (DIT): windows of vulnerability, immune dysfunction and safety assessment. J Immunotoxicol 5:401–412
43. Guo TL, White KL Jr, Brown RD, Delclos KB, Newbold RR, Weis C, Germolec DR, McCay JA (2002) Genistein modulates splenic natural killer cell activity, antibody-forming cell response, and phenotypic marker expression in F(0) and F(1) generations of Sprague-Dawley rats. Toxicol Appl Pharmacol 181:219–227
44. Guo TL, Germolec DR, Musgrove DL, Delclos KB, Newbold RR, Weis C, White KL Jr (2005) Myelotoxicity in genistein-, nonylphenol-, methoxychlor-, vinclozolin- or ethinyl estradiol-exposed F1 generations of Sprague-Dawley rats following developmental and adult exposures. Toxicology 211:207–219
45. Weinberg J, Jerrells TR (1991) Suppression of immune responsiveness: sex differences in prenatal ethanol effects. Alcohol Clin Exp Res 15:525–531
46. Boé DM, Vandivier RW, Burnham EL, Moss M (2009) Alcohol abuse and pulmonary disease.J Leukoc Biol 86:1097–1104
47. Sozo F, O'Day L, Maritz G, Kenna K, Stacy V, Brew N, Walker D, Bocking A, Brien J, Harding R (2009) Repeated ethanol exposure during late gestation alters the maturation and innate immune status of the ovine fetal lung. Am J Physiol Lung Cell Mol Physiol 296:L510–L518
48. Lönnroth K, Williams BG, Stadlin S, Jaramillo E, Dye C (2008) Alcohol use as a risk factor for tuberculosis – a systematic review. BMC Public Health 8:289
49. Choudhry MA, Chaudry IH (2008) Alcohol, burn injury, and the intestine. J Emerg Trauma Shock 1:81–87
50. Hapa O, Cakici H, Gideroğlu K, Ozturan K, Kükner A, Buğlayici G (2009) The effect of ethanol intake on tendon healing: a histological and biomechanical study in a rat model. Arch Orthop Trauma Surg 129:1721–1726
51. Nath B, Szabo G (2009) Alcohol-induced modulation of signaling pathways in liver parenchymal and nonparenchymal cells: implications for immunity. Semin Liver Dis 29:166–177
52. Cubero FJ, Urtasun R, Nieto N (2009) Alcohol and liver fibrosis. Semin Liver Dis 29:211–221
53. Corazza N, Badmann A, Lauer C (2009) Immune cell-mediated liver injury. Semin Immunopathol 31:267–277
54. Gauthier TW, Young PA, Gabelaia L, Tang SM, Ping XD, Harris FL, Brown LA (2009) In utero ethanol exposure impairs defenses against experimental group B streptococcus in the term Guinea pig lung. Alcohol Clin Exp Res 33:300–306

55. Lau A, von Dossow V, Sander M, MacGuill M, Lanzke N, Spies C (2009) Alcohol use disorder and perioperative immune dysfunction. Anesth Analg 108:916–920
56. Watson RR, Borgs P, Witte M, McCuskey RS, Lantz C, Johnson MI, Mufti SI, Earnest DL (1994) Alcohol, immunomodulation, and disease. Alcohol Alcohol 29:131–139
57. Buttari B, Profumo E, Mancinelli R, Cesta Incani U, Tosti ME, Attilia ML, Ceccanti M, Riganò R (2008) Chronic and acute alcohol exposure prevents monocyte-derived dendritic cells from differentiating and maturing. Int J Immunopathol Pharmacol 21:929–939
58. Siggins RW, Bagby GJ, Molina P, Dufour J, Nelson S, Zhang P (2009) Alcohol exposure impairs myeloid dendritic cell function in rhesus macaques. Alcohol Clin Exp Res 33:1524–1531
59. Mandrekar P, Catalano D, Jeliazkova V, Kodys K (2008) Alcohol exposure regulates heat shock transcription factor binding and heat shock proteins 70 and 90 in monocytes and macrophages: implication for TNF-alpha regulation. J Leukoc Biol 84:1335–1345
60. Rowe AM, Brundage KM, Barnett JB (2008) Developmental immunotoxicity of atrazine in rodents. Basic Clin Pharmacol Toxicol 102:139–145
61. Rooney AA, Matulka RA, Luebke RW (2003) Developmental atrazine exposure suppresses immune function in male, but not female Sprague–Dawley rats. Toxicol Sci 76:366–375
62. Salazar KD, Ustyugova IV, Brundage KM, Barnett JB, Schafer R (2008) A review of the immunotoxicity of the pesticide 3, 4-dichloropropionanalide. J Toxicol Environ Health B Crit Rev 11:630–645
63. Corsini E, Codecà I, Mangiaratti S, Birindelli S, Minoia C, Turci R, Viviani B, Facchi A, Vitelli N, Lucchi L, Galli CL, Marinovich M, Colosio C (2007) Immunomodulatory effects of the herbicide propanil on cytokine production in humans: in vivo and in vitro exposure. Toxicol Appl Pharmacol 222:202–210
64. Navarro HA, Basta PV, Seidler FJ, Slotkin TA (2001) Neonatal chlorpyrifos administration elicits deficits in immune function in adulthood: a neural effect. Brain Res Dev Brain Res 130:249–252
65. Blakley BR, Yole MJ, Brousseau P, Boermans H, Fournier M (1999) Effect of chlorpyrifos on immune function in rats. Vet Hum Toxicol 41:140–144
66. Banerjee BD, Pasha ST, Hussain QZ, Koner BC, Ray A (1998) A comparative evaluation of immunotoxicity of malathion after subchronic exposure in experimental animals. Indian J Exp Biol 36:273–282
67. Weisglas-Kuperus N, Vreugdenhil HJ, Mulder PG (2004) Immunological effects of environmental exposure to polychlorinated biphenyls and dioxins in Dutch school children. Toxicol Lett 149:281–285
68. Glynn A, Thuvander A, Aune M, Johannisson A, Darnerud PO, Ronquist G, Cnattingius S (2008) Immune cell counts and risks of respiratory infections among infants exposed pre- and postnatally to organochlorine compounds: a prospective study. Environ Health 7:62
69. Lee DH, Steffes M, Jacobs DR (2007) Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. Environ Health Perspect 115:883–888
70. Park HY, Hertz-Picciotto I, Petrik J, Palkovicova L, Kocan A, Trnovec T (2008) Prenatal PCB exposure and thymus size at birth in neonates in Eastern Slovakia. Environ Health Perspect 116:104–109
71. Qazi MR, Xia Z, Bogdanska J, Chang SC, Ehresman DJ, Butenhoff JL, Nelson BD, Depierre JW, Abedi-Valugerdi M (2009) The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high-dose phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARalpha). Toxicology 260:68–76
72. DeWitt JC, Copeland CB, Luebke RW (2009) Suppression of humoral immunity by perfluorooctanoic acid is independent of elevated serum corticosterone concentration in mice. Toxicol Sci 109:106–112
73. DeWitt JC, Shnyra A, Badr MZ, Loveless SE, Hoban D, Frame SR, Cunard R, Anderson SE, Meade BJ, Peden-Adams MM, Luebke RW, Luster MI (2009) Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. Crit Rev Toxicol 39:76–94
74. Soto-Peña GA, Vega L (2008) Arsenic interferes with the signaling transduction pathway of T cell receptor activation by increasing basal and induced phosphorylation of Lck and Fyn in spleen cells. Toxicol Appl Pharmacol 230:216–226

75. Bustamante J, Dock L, Vahter M, Fowler B, Orrenius S (1997) The semiconductor elements arsenic and indium induce apoptosis in rat thymocytes. Toxicology 118:129–136
76. Muscarella DE, Bloom SE (2002) Differential activation of the c-Jun N-terminal kinase pathway in arsenite-induced apoptosis and sensitization of chemically resistant compared to susceptible B-lymphoma cell lines. Toxicol Sci 68:82–92
77. Cheng HY, Li P, David M, Smithgall TE, Feng L, Lieberman MW (2004) Arsenic inhibition of the JAK-STAT pathway. Oncogene 23:3603–3612
78. Lemarie A, Morzadec C, Bourdonnay E, Fardel O, Vernhet L (2006) Human macrophages constitute targets for immunotoxic inorganic arsenic. J Immunol 177:3019–3027
79. Arkusz J, Stańczyk M, Lewińska D, Stepnik M (2005) Modulation of murine peritoneal macrophage function by chronic exposure to arsenate in drinking water. Immunopharmacol Immunotoxicol 27:315–330
80. Silbergeld EK, Silva IA, Nyland JF (2005) Mercury and autoimmunity: implications for occupational and environmental health. Toxicol Appl Pharmacol 207:282–292
81. Pilones K, Lai ZW, Gavalchin J (2007) Prenatal HgCl(2) exposure alters fetal cell phenotypes. J Immunotoxicol 4:295–301
82. Abedi-Valugerdi M (2009) Mercury and silver induce B cell activation and anti-nucleolar autoantibody production in outbred mouse stocks: are environmental factors more important than the susceptibility genes in connection with autoimmunity. Clin Exp Immunol 155:117–124
83. McCabe MJ Jr, Whitekus MJ, Hyun J, Eckles KG, McCollum G, Rosenspire AJ (2003) Inorganic mercury attenuates CD95-mediated apoptosis by interfering with formation of the death inducing signaling complex. Toxicol Appl Pharmacol 190:146–156
84. Laiosa MD, Eckles KG, Langdon M, Rosenspire AJ, McCabe MJ Jr (2007) Exposure to inorganic mercury in vivo attenuates extrinsic apoptotic signaling in *Staphylococcal aureus* enterotoxin B stimulated T-cells. Toxicol Appl Pharmacol 225:238–250
85. Ziemba SE, Menard SL, McCabe MJ Jr, Rosenspire AJ (2009) T-cell receptor signaling is mediated by transient Lck activity, which is inhibited by inorganic mercury. FASEB J 23:1663–1671
86. de Vos G, Abotaga S, Liao Z, Jerschow E, Rosenstreich D (2007) Selective effect of mercury on Th2-type cytokine production in humans. Immunopharmacol Immunotoxicol 29:537–548
87. Gorrie MJ, Qasim FJ, Whittle CJ, Gillespie KM, Szeto CC, Nicoletti F, Bolton EM, Bradley JA, Mathieson PW (2000) Exogenous type-1 cytokines modulate mercury-induced hyper-IgE in the rat. Clin Exp Immunol 121:17–22
88. Pathak N, Khandelwal S (2007) Role of oxidative stress and apoptosis in cadmium induced thymic atrophy and splenomegaly in mice. Toxicol Lett 169:95–108
89. Kim J, Kim SH, Johnson VJ, Sharma RP (2005) Extracellular signal-regulated kinase-signaling-dependent G2/M arrest and cell death in murine macrophages by cadmium. Environ Toxicol Chem 24:3069–3077
90. Pathak N, dn Khandelwal S (2008) Impact of cadmium in T lymphocyte subsets and cytokine expression: differential regulation by oxidative stress and apoptosis. Biometals 21:179–187
91. Carey JB, Allshire A, van Pelt FN (2006) Immune modulation by cadmium and lead in the acute reporter antigen-popliteal lymph node assay. Toxicol Sci 91:113–122
92. Miller TE, Golemboski KA, Ha RS, Bunn T, Sanders FS, Dietert RR (1998) Developmental exposure to lead causes persistent immunotoxicity in Fischer 344 rats. Toxicol Sci 42:129–135
93. McCabe MJ Jr, Singh KP, Reiners JJ Jr (1999) Lead intoxication impairs the generation of a delayed type hypersensitivity response. Toxicology 139:255–264
94. Heo Y, Lee WT, Lawrence DA (1997) In vivo the environmental pollutants lead and mercury induce oligoclonal T cell responses skewed toward type-2 reactivities. Cell Immunol 179:185–195
95. Snyder JE, Filipov NM, Parsons PJ, Lawrence DA (2000) The efficiency of maternal transfer of lead and its influence on plasma IgE and splenic cellularity of mice. Toxicol Sci 57:87–94
96. Dietert RR, McCabe MJ Jr (2007) Lead immunotoxicity. In: Luebke R, House R, Kimber I (eds) Immunotoxicology and immunopharmacology, 3rd edn. CRC Press, Inc, Boca Raton, FL, pp 207–224
97. Gao D, Mondal TK, Lawrence DA (2007) Lead effects on development and function of bone marrow-derived dendritic cells promote Th2 immune responses. Toxicol Appl Pharmacol 222:69–79
98. Lutz PM, Wilson TJ, Ireland J, Jones AL, Gorman JS, Gale NL, Johnson JC, Hewett JE (1999) Elevated immunoglobulin E (IgE) levels in children with exposure to environmental lead. Toxicology 134:63–78

99. Karmaus W, Brooks KR, Nebe T, Witten J, Obi-Osius N, Kruse H (2005) Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study. Environ Health 4:5
100. Sun L, Hu J, Zhao Z, Li L, Cheng H (2003) Influence of exposure to environmental lead on serum immunoglobulin in preschool children. Environ Res 92:124–128
101. Heo Y, Lee BK, Ahn KD, Lawrence DA (2004) Serum IgE elevation correlates with blood lead levels in battery manufacturing workers. Hum Exp Toxicol 23:209–213
102. Hudson CA, Cao L, Kasten-Jolly J, Kirkwood JN, Lawrence DA (2003) Susceptibility of lupus-prone NZM mouse strains to lead exacerbation of systemic lupus erythematosus symptoms. J Toxicol Environ Health A 66:895–918
103. Bishayi B, Sengupta M (2006) Synergism in immunotoxicological effects due to repeated combined administration of arsenic and lead in mice. Int Immunopharmacol 6:454–464
104. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007) Human exposure to bisphenol A (BPA). Reprod Toxicol 24:139–177
105. Miao S, Gao Z, Kou Z, Xu G, Su C, Liu N (2008) Influence of bisphenol a on developing rat estrogen receptors and some cytokines in rats: a two-generational study. J Toxicol Environ Health A 71:1000–1008
106. Sawai C, Anderson K, Walser-Kuntz D (2003) Effect of bisphenol A on murine immune function: modulation of interferon-gamma, IgG2a, and disease symptoms in NZB X NZW F1 mice. Environ Health Perspect 111:1883–1887
107. Yan H, Takamoto M, Sugane K (2008) Exposure to Bisphenol A prenatally or in adulthood promotes T(H)2 cytokine production associated with reduction of CD4CD25 regulatory T cells. Environ Health Perspect 116:514–519
108. Ohshima Y, Yamada A, Tokuriki S, Yasutomi M, Omata N, Mayumi M (2007) Transmaternal exposure to bisphenol a modulates the development of oral tolerance. Pediatr Res 62:60–64
109. Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y (2004) Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. Immunology 112:489–495
110. Larsen ST, Nielsen GD (2008) Structure-activity relationship of immunostimulatory effects of phthalates. BMC Immunol 9:61
111. Deutschle T, Reiter R, Butte W, Heinzow B, Keck T, Riechelmann H (2008) A controlled challenge study on di(2-ethylhexyl) phthalate (DEHP) in house dust and the immune response in human nasal mucosa of allergic subjects. Environ Health Perspect 116:1487–1493
112. Dearman RJ, Beresford L, Bailey L, Caddick HT, Betts CJ, Kimber I (2008) Di-(2-ethylhexyl) phthalate is without adjuvant effect in mice on ovalbumin. Toxicology 244:231–241
113. Ohnishi T, Yoshida T, Igarashi A, Muroi M, Tanamoto K (2008) Effects of possible endocrine disruptors on MyD88-independent TLR4 signaling. FEMS Immunol Med Microbiol 52:293–295
114. Butala JH, David RM, Gans G, McKee RH, Guo TL, Peachee VL, White KL Jr (2004) Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F1 mice. Toxicology 201:77–85
115. Piepenbrink MS, Hussain I, Marsh JA, Dietert RR (2005) Developmental immunotoxicology of Di-(2-Ethylhexyl)phthalate (DEHP): age-based assessment in the female rat. J Immunotoxicol 2:21–31
116. Mahood IK, Scott HM, Brown R, Hallmark N, Walker M, Sharpe RM (2007) In utero exposure to di(n-butyl) phthalate and testicular dysgenesis: comparison of fetal and adult end points and their dose sensitivity. Environ Health Perspect 115:55–61
117. Karakaya A, Ates I, Yucesoy B (2004) Effects of occupational polycyclic aromatic hydrocarbon exposure on T-lymphocyte functions and natural killer cell activity in asphalt and coke oven workers. Hum Exp Toxicol 23:317–322
118. van Grevenynghe J, Rion S, Le Ferrec E, Le Vee M, Amiot L, Fauchet R, Fardel O (2003) Polycyclic aromatic hydrocarbons inhibit differentiation of human monocytes into macrophages. J Immunol 170:2374–2381
119. Dean JH, Ward EC, Murray MJ, Lauer LD, House RV (1985) Mechanisms of dimethylbenzanthracene-induced immunotoxicity. Clin Physiol Biochem 3:98–110
120. Davila DR, Davis DP, Campbell K, Cambier JC, Zigmond LA, Burchiel SW (1995) Role of alterations in Ca(2+)-associated signaling pathways in the immunotoxicity of polycyclic aromatic hydrocarbons. J Toxicol Environ Health 45:101–126
121. Holladay SD, Smith BJ (1995) Alterations in murine fetal thymus and liver hematopoietic cell populations following developmental exposure to 7, 12-dimethylbenz[a]anthracene. Environ Res 68:106–113

122. Johnson BE, Bell RG, Dietert RR (1990) 3-Methylcholanthrene-induced immunosuppression in mice to *Trichinella spiralis* antigens. Immunopharmacol Immunotoxicol 12:237–256
123. Richard JL (2007) Some major mycotoxins and their mycotoxicoses – an overview. Int J Food Microbiol 119:3–10
124. Pestka JJ (2007) Modulation of inflammatory gene expression by trichothecene mycotoxins. In: Luebke R, House R, Kimber I (eds) Immunotoxicology and immunophamracology, 3rd edn. CRC Press, Inc, Boca Raton, FL, pp 291–306
125. Li M, Harkema JR, Islam Z, Cuff CF, Pestka JJ (2006) T-2 toxin impairs murine immune response to respiratory reovirus and exacerbates viral bronchiolitis. Toxicol Appl Pharmacol 217:76–85
126. Kankkunen P, Rintahaka J, Aalto A, Leino M, Majuri ML, Alenius H, Wolff H, Matikainen S (2009) Trichothecene mycotoxins activate inflammatory response in human macrophages. J Immunol 182:6418–6425
127. Bimczok D, Döll S, Rau H, Goyarts T, Wundrack N, Naumann M, Dänicke S, Rothkötter HJ (2007) The Fusarium toxin deoxynivalenol disrupts phenotype and function of monocyte-derived dendritic cells in vivo and in vitro. Immunobiology 212:655–666
128. Dietert RR, Qureshi MA, Nanna UC, Bloom SE (1985) Embryonic exposure to aflatoxin B_1: mutagenicity and influence on development and immunity. Environ Mutagen 7:715–725
129. Meissonnier GM, Pinton P, Laffitte J, Cossalter AM, Gong YY, Wild CP, Bertin G, Galtier P, Oswald IP (2008) Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicol Appl Pharmacol 231:142–149
130. Walker R, Larsen JC (2005) Ochratoxin A: previous risk assessments and issues arising. Food Addit Contam 22:6–9
131. Dortant PM, Peters-Volleberg GW, Van Loveren H, Marquardt RR, Speijers GJ (2001) Age-related differences in the toxicity of ochratoxin A in female rats. Food Chem Toxicol 39:55–65
132. Dostal A, Jakusova L, Cajdova J, Hudeckova H (2008) Results of the first studies of occurrence of ochratoxin A in human milk in Slovakia. Bratisl Lek Listy 109:276–278
133. Ferrante MC, Raso GM, Bilancione M, Esposito E, Iacono A, Meli R (2008) Differential modification of inflammatory enzymes in J774A.1 macrophages by ochratoxin A alone or in combination with lipopolysaccharide. Toxicol Lett 181:40–46
134. Al-Anati L, Petzinger E (2006) Immunotoxic activity of ochratoxin A. J Vet Pharmacol Ther 29:79–90
135. Stoev SD, Goundasheva D, Mirtcheva T, Mantle PG (2000) Susceptibility to secondary bacterial infections in growing pigs as an early response in ochratoxicosis. Exp Toxicol Pathol 52:287–296
136. Fernandez-Cabezudo MJ, Hasan MY, Mustafa N, El-Sharkawy RT, Fahim MA, Al-Ramadi BK (2003) Alpha tocopherol protects against immunosuppressive and immunotoxic effects of lead. Free Radic Res 37:437–445
137. Wang XH, Zhou XQ, Xu JP, Wang Y, Lu J (2009) The effects of vitamin E on NK cell activity and lymphocyte proliferation in treated mice by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin. Immunopharmacol Immunotoxicol 4:1–7
138. Sodhi S, Sharma A, Brar RS (2006) A protective effect of vitamin E and selenium in ameliorating the immunotoxicity of malathion in chicks. Vet Res Commun 30:935–942
139. Koner BC, Banerjee BD, Ray A (1998) Organochlorine pesticide-induced oxidative stress and immune suppression in rats. Indian J Exp Biol 36:395–398
140. Emara AM, El-Bahrawy H (2008) Green tea attenuates benzene-induced oxidative stress in pump workers. J Immunotoxicol 5:69–80
141. Bagchi D, Bagchi M, Stohs S, Ray SD, Sen CK, Preuss HG (2002) Cellular protection with proanthocyanidins derived from grape seeds. Ann N Y Acad Sci 957:260–270
142. López D, Pavelkova M, Gallova L, Simonetti P, Gardana C, Lojek A, Loaiza R, Mitjavila MT (2007) Dealcoholized red and white wines decrease oxidative stress associated with inflammation in rats. Br J Nutr 98:611–619
143. Haridas V, Hanausek M, Nishimura G, Soehnge H, Gaikwad A, Narog M, Spears E, Zoltaszek R, Walaszek Z, Gutterman JU (2004) Triterpenoid electrophiles (avicins) activate the innate stress response by redox regulation of a gene battery. J Clin Invest 113:65–73

144. Ben Salah-Abbès J, Abbès S, Houas Z, Abdel-Wahhab MA, Oueslati R (2008) Zearalenone induces immunotoxicity in mice: possible protective effects of radish extract (*Raphanus sativus*). J Pharm Pharmacol 60:761–770
145. Kim JH, Mun YJ, Woo WH, Jeon KS, An NH, Park JS (2002) Effects of the ethanol extract of *Cichorium intybus* on the immunotoxicity by ethanol in mice. Int Immunopharmacol 2:733–744
146. DeFuria J, Bennett G, Strissel KJ, Perfield JW II, Milbury PE, Greenberg AS, Obin MS (2009) Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. J Nutr 139:1510–1516
147. Kim YO, Ahn YK, Kim JH (2000) Influence of melatonin on immunotoxicity of cadmium. Int J Immunopharmacol 22:275–284
148. Ahmed T, Tripathi AK, Suke SG, Kumar V, Ahmed RS, Das S, Banerjee BD (2009) Role of HSP-27 and reduced glutathione in modulating malathion-induced apoptosis of human peripheral blood mononuclear cells: Ameliorating effect of N-acetylcysteine and curcumin. Toxicol In Vitro 23:1319–1325
149. Zuccotti GV, Trabattoni D, Morelli M, Borgonovo S, Schneider L, Clerici M (2009) Immune modulation by lactoferrin and curcumin in children with recurrent respiratory infections. J Biol Regul Homeost Agents 23:119–124
150. Böger RH (2007) The pharmacodynamics of l-arginine. J Nutr 137:1650S–1655S
151. Zeng X, Wang F, Fan X, Yang W, Zhou B, Li P, Yin Y, Wu G, Wang J (2008) Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. J Nutr 138:1421–1425
152. De Taeye BM, Novitskaya T, McGuinness OP, Gleaves L, Medda M, Covington JW, Vaughan DE (2007) Macrophage TNF-alpha contributes to insulin resistance and hepatic steatosis in diet-induced obesity. Am J Physiol Endocrinol Metab 293:E713–E725
153. Subramanian V, Ferrante AW Jr (2009) Obesity, inflammation, and macrophages. Nestle Nutr Workshop Ser Pediatr Program 63:151–159
154. La Merrill M, Baston DS, Denison MS, Birnbaum LS, Pomp D, Threadgill DW (2009) Mouse breast cancer model-dependent changes in metabolic syndrome-associated phenotypes caused by maternal dioxin exposure and dietary fat. Am J Physiol Endocrinol Metab 296:E203–E210
155. Mena MP, Sacanella E, Vazquez-Agell M, Morales M, Fitó M, Escoda R, Serrano-Martínez M, Salas-Salvadó J, Benages N, Casas R, Lamuela-Raventós RM, Masanes F, Ros E, Estruch R (2009) Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. Am J Clin Nutr 89:248–256
156. Calderón-Garcidueñas L, Macías-Parra M, Hoffmann HJ, Valencia-Salazar G, Henríquez-Roldán C, Osnaya N, Monte OC, Barragán-Mejía G, Villarreal-Calderon R, Romero L, Granada-Macías M, Torres-Jardón R, Medina-Cortina H, Maronpot RR (2009) Immunotoxicity and environment: immunodysregulation and systemic inflammation in children. Toxicol Pathol 37:161–169
157. He K, Liu K, Daviglus ML, Jenny NS, Mayer-Davis E, Jiang R, Steffen L, Siscovick D, Tsai M, Herrington D (2009) Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). Am J Cardiol 103:1238–1243
158. Fortin G, Yurchenko K, Collette C, Rubio M, Villani AC, Bitton A, Sarfati M, Franchimont D (2009) l-carnitine, a diet component and organic cation transporter OCTN ligand, displays immunosuppressive properties and abrogates intestinal inflammation. Clin Exp Immunol 156:161–171
159. Cave MC, Hurt RT, Frazier TH, Matheson PJ, Garrison RN, McClain CJ, McClave SA (2008) Obesity, inflammation, and the potential application of pharmaconutrition. Nutr Clin Pract 23:16–34
160. Thangasamy T, Subathra M, Sittadjody S, Jeyakumar P, Joyee AG, Mendoza E, Chinnakkanu P (2008) Role of l-carnitine in the modulation of immune response in aged rats. Clin Chim Acta 389:19–24
161. Handy RD, Abd-El Samei HA, Bayomy MF, Mahran AM, Abdeen AM, El-Elaimy EA (2002) Chronic diazinon exposure: pathologies of spleen, thymus, blood cells, and lymph nodes are modulated by dietary protein or lipid in the mouse. Toxicology 172:13–34
162. Chen S, Golemboski K, Piepenbrink M, Dietert R (2004) Developmental immunotoxicity of lead in the rat: influence of maternal diet. J Toxicol Environ Health A 67:495–511
163. Pal S, Poddar MK (2008) Dietary protein-carbohydrate ratio: exogenous modulator of immune response with age. Immunobiology 213:557–566

Section F
Prebiotics and Probiotics

32 Probiotics and Inflammatory Immune Responses

Corinne Grangette

Key Points

- Probiotic microorganisms can provide a certain number of health benefits.
- Their capacity to modulate the immune system and the different mechanisms of action involved will be discussed in the present review.

Key Words: Probiotics, lactic acid bacteria, microbiota, immune-modulation, inflammatory bowel diseases, allergy, dendritic cells.

32.1 INTRODUCTION

Almost immediately after birth, the intestinal tract progresses from sterility to extremely dense microbial colonization, ending up with a microbial ecosystem in an adult that comprises ten times more bacteria than human cells (1, 2). The human colonic ecosystem alone is constituted of more than 1,500 bacterial species, belonging to a limited number of broad taxonomic divisions, the collective genome of which is estimated to contain 100 times more genes than the human genome (3). In the intestine, this microbiota is in permanent contact and reciprocal interaction with the host cells and with nutrients, composing an extremely complex and highly regulated ecosystem whose contribution to gastrointestinal health and disease is now well recognized. This microbiota is considered to be essential in priming the immune system during ontogeny by the development and maturation of both mucosal and systemic immune systems. As such, this complex ecosystem plays an important role in normal gut functioning and in the maintenance of intestinal homeostasis (4, 5). This bacteria–host mutualism serves numerous important functions for the host, including the maintenance of a physical barrier against colonization or invasion by pathogens, the facilitation of digestion and assimilation of nutrients, and an early warning system, providing immunological surveillance signals at the gut mucosa-lumen interface.

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_32

The emergence of a number of immunological disorders such as allergic diseases, inflammatory bowel diseases, type 1 diabetes and multiple sclerosis has been attributed to a modern lifestyle, resulting in certain dietary, hygiene, and medical habits. A number of possible hypotheses have been formulated to explain these observations. The hygiene hypothesis indicates that the lack of challenges for the immune system of the newborn will result in an immune system that can show certain deficiencies at later age. More recently, some of these disorders have been linked to a possible dysbalance in the development of the regulatory immune system. Regulatory pathways are mainly triggered by harmless microorganisms and the lack of exposure to these "old friends" might explain the increasing numbers of immunoregulatory disorders observed today (6). Notably, defects in immunoregulatory processes such as the development of tolerance against the endogenous commensal microflora, have been shown to be associated with the pathogenesis of inflammatory bowel disease (IBD) (7).

A critical feature of the mucosal immune system is the ability to discriminate between harmful pathogens and harmless members of the commensal flora. This is achieved in part by an evolutionary-conserved family of cell surface and cytosolic receptors of the innate immunity, referred to as pattern recognition receptors (PRRs). These molecules, such as toll-like receptors (TLRs), are able to recognize conserved microbial components (microbe-associated molecular pattern, MAMP's) (8). It was recently suggested that the disruption of the mucosal barrier can lead to the exposure of a multitude of these MAMPs that could interact with TLRs-expressing immune cells, consequently leading to potent inflammatory responses (9). Paradoxically, although both commensal and harmful bacteria express these MAMP's and are able to trigger PRRs, commensal bacteria do not induce inflammatory responses. In order to explain this apparent contradiction, it has been suggested that whereas pathogenic bacteria can pass through the epithelial barrier and activate the TLR-dependent inflammatory cascade (notably by inducing NFκB translocation), commensals would be sequestrated at the epithelial cell surface (10, 11). Moreover, it has been shown that the recognition of commensal bacteria by TLRs at the host cell surface plays a crucial role in maintaining intestinal homeostasis (12). Indeed, some commensal bacteria are able to dampen intestinal inflammation by inhibiting the NFκB signaling pathway, either by inhibiting ubiquitination and degradation of IκB, thus blocking the transactivation of NFκB-mediated genes (13), or by promoting the nuclear export of NFκB subunit relA through a PPAR-γ-dependent pathway (14).

Recent findings reported a notable influence of the microbiota composition on the incidence of emerging intestinal as well as nonintestinal pathologies, and microbial dysbiosis has clearly been implicated in IBD (15) and obesity (16). With increasing evidence supporting the crucial role of the microbiota in maintaining health, strategies to manipulate intestinal bacteria using probiotics are actively investigated. Indeed, substituting antibiotics or immunosuppressive drugs (which provide considerable side effects) with probiotics can be an attractive alternative or addition to these traditional therapies. The concept is not recent at all and was already introduced by Elie Metchnikoff in 1907. In his book "the prolongation of life" (17), he proposed that the long and healthy life of Bulgarian peasants was due to their consumption of fermented milk products and suggested that the ingestion of certain microbes, such as the fermenting

Bacillus (*Lactobacillus*) could have beneficial effects on human health. Nearly a century later, these beneficial microorganisms were termed probiotics (18). The current definition, drafted in 2001 by an expert panel commissioned by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO), identifies probiotics as "Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Most of the available probiotics are represented by lactobacilli or bifidobacteria, but certain strains of *Escherichia coli* (*E. coli* Nissle 1917) or *Enterococcus* (i.e. *Enterococcus faecium* SF68) have also been selected for their probiotic properties, as well as some yeasts (i.e. *Saccharomyces boulardii*). Indeed, the regular intake of probiotic bacteria has been shown to maintain the gut immune homeostasis by altering microbial balance or by interacting with the gut immune system, explaining their potential effect in gastro-intestinal diseases. Probiotics have proven benefits in the treatment or prevention of lactose intolerance, certain types of diarrhea either induced by antibiotherapy, bacterial or viral infections (19), inflammatory bowel diseases (20), some cancers (21), food allergy, and atopic eczema in children (22). Although there is now considerable body of information concerning the clinical efficiency of probiotics, their mechanisms of action remain unclear. Their beneficial effects can be exerted through different means, such as the remodeling of microbial communities (production of antimicrobial metabolites, competitive exclusion of enteric pathogens), promotion of intestinal barrier function, neutralization of procarcinogenic enzymatic activities, and metabolic activities (Fig. 32.1). Their capacity to modulate the local and systemic immune responses is regarded as one of the most obvious beneficial properties (Fig. 32.2).

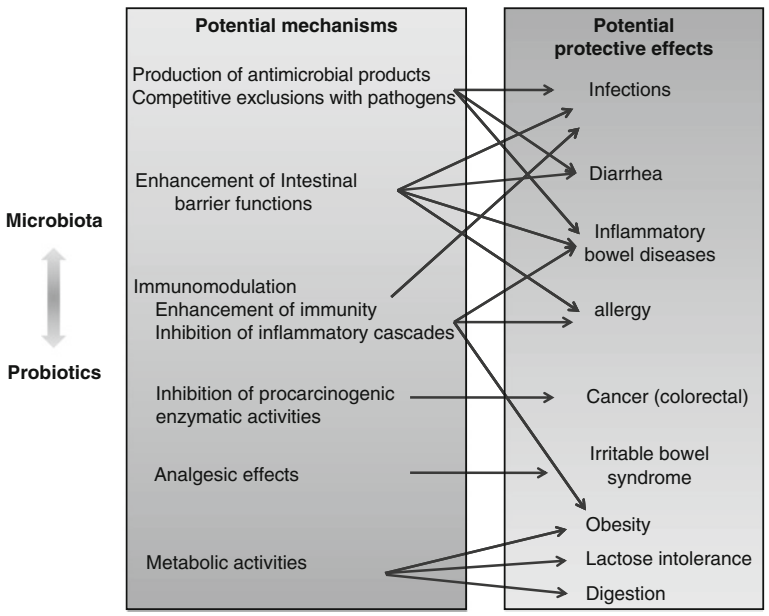


Fig. 32.1. Potential mechanisms of action of probiotics and beneficial impact on various diseases.

32.2 STIMULATION OF THE IMMUNE SYSTEM BY PROBIOTICS

There is now a body of evidence that probiotics can exert immunostimulatory properties leading to better immune protection in the different mucosal immune compartments. First, they can enhance gut immune function by different mechanisms, including the production of low pH, organic acids, carbon dioxide, hydrogen peroxide, bacteriocins, the depletion of nutrients; and competition for available living space. The mucosal lymphoid tissue of the gastrointestinal tract (GALT) plays an important role as a first line of defense against ingested pathogens. By interacting with the mucosal epithelial cells lining the gut, as well as with the lymphoid cells residing in the vicinity, probiotics are able to modulate the local and systemic immune responses (Fig. 32.2). Such microorganisms

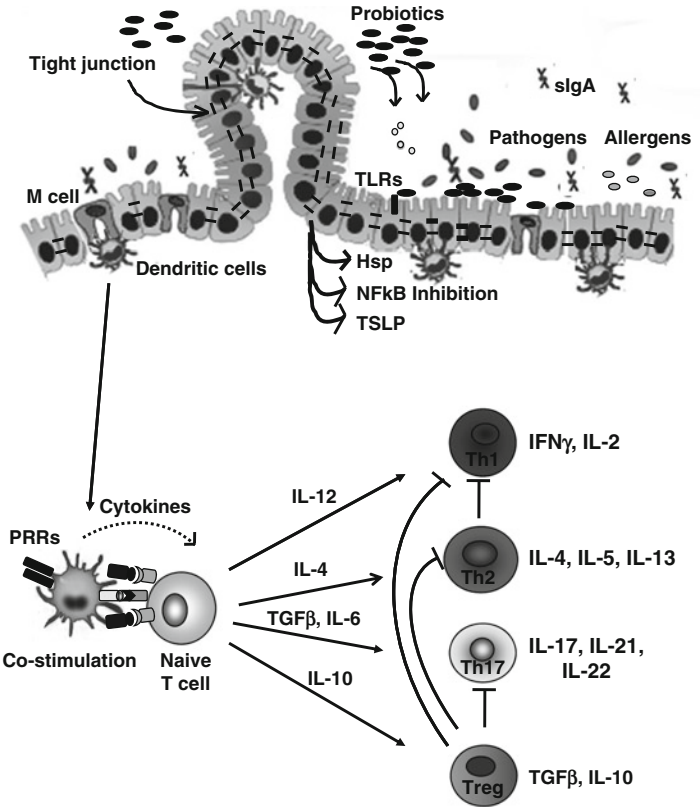


Fig. 32.2. Differential mechanisms and immune cells targeted by probiotics during their cross-talk with the gut mucosal immune system. Probiotic microorganisms can first limit pathogen infection and microbial overgrowth by 1) microbial exclusion, 2) through the release of anti-microbial factors and 3) the induction of secretory IgA. They also exert their beneficial effect by the reinforcement of the epithelial barrier, notably through the induction of cytoprotective factors (i.e. Hsps) and the activation of tight junction expression. By the release of cytokines and chemokines by epithelial cells, the sampling of bacteria or bacterial components by M cells or directly by dendritic cells, innate immune responses are initiated that allow, according to the anti-inflammatory/ pro-inflammatory environment, differential activation of DCs, leading to induction of either Th1, Th2 or Treg immune responses. Probiotics can also dampen intestinal inflammation by inhibiting the NFkB signaling pathways, notably through the interaction with PRRs (i.e. TLRs).

can indeed stimulate the cells of the innate immunity. As described above, intestinal epithelial cells (IEC), which make up the actual barrier that separates the host from the gut luminal environment, express different receptors of the innate immunity (PRRs). Most of these molecules are in part transcriptionally regulated by the transcription factor nuclear factor NFκB leading to the production of a wide range of inflammatory and chemoattractant cytokines. Some probiotic bacteria seem to be able to transiently trigger innate signal transduction and proinflammatory gene expression in the intestinal epithelium, such as IL-6 (23), allowing the recruitment and activation of different cells of the innate immunity such as macrophages, dendritic (DC) and Natural Killer (NK) cells. These effects are strain-specific and it has been largely shown that lactobacilli can differently modulate macrophages and DCs. Certain strains are able to induce proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-12, tumor necrosis factor alpha (TNF-α), and gamma interferon (IFN-γ) (24, 25). Such cytokines (IFN-γ and IL-12) potently augment the functions of macrophages and NK cells, which may be a possible mechanism of their anti-infectious and antitumor activities (26, 27). As IL-12 plays a critical role in inducing a Th1-dominant immune response and in enhancing cellular immunity, this could also explain the immune-stimulatory (28) and antiallergic (29) potential of certain strains. A number of studies have addressed the effects of supplementation with various probiotic strains in healthy volunteers. Increases in NK activity and macrophage phagocytic activity have been observed after supplementation with *Lactobacillus casei* Shirota (30), *L. casei* DN114001 (31), *L. rhamnosus* HN001 (32) or *Bifidobacterium lactis* HN019 (33) or with different strain mixtures. Several human studies have also confirmed the capacity of probiotics to increase resistance to infection through the augmentation of phagocytic capability of peripheral blood (34). This is associated with the increased production of proinflammatory (IL-12, IL-6, TNF-α and IFN-γ) and anti-inflammatory (IL-10) mediators.

Dendritic cells (DCs) are antigen-presenting cells that play a central role in orchestrating immune responses to self and foreign antigens. It has been largely reported that lactobacilli can differentially modulate DC maturation (35, 36). While some strains are able to induce regulatory DCs (37), others seem to be able to activate DCs that allow the polarization of $CD4^+$ and $CD8^+$ T cells toward T helper 1 and Tc1 (cytotoxic T cells) pathways (38). Considering the pivotal role of DCs in priming adaptive immunity, these results could explain the adjuvant effects of certain probiotics. The best-defined effector component of the mucosal adaptive immune system is secretory immunoglobulin A (sIgA). sIgA is the main immunoglobulin of the humoral immune response, which together with the innate mucosal defenses provides protection against microbial antigens at the intestinal mucosal surface. Some probiotic bacteria influence immunoglobulin production, notably by increasing the numbers of IgA- and IgM-producing cells in the intestinal mucosa with enhanced secretion of sIgA (39). In clinical human studies, the enhancement of specific anti-IgA titers to *Salmonella typhimurium* was observed in subjects fed with fermented milk containing *L. acidophilus* (40). Very interesting results have been obtained in infants suffering of rotavirus-associated diarrhea. Oral administration of *L. rhamnosus* GG (LGG) has been shown to stimulate rotavirus-specific IgA antibody responses (41). The supplementation of infant formula with *B. bifidum* and *Streptococcus thermophilus* was also able to reduce the incidence of acute diarrhea and rotavirus shedding in infants admitted to hospital (42). The use of probiotic strains as a

mucosal adjuvant to enhance ongoing immune responses or vaccine has also been reported. It has, for example been shown that the oral administration of LGG improved immunogenicity of an oral rotavirus vaccine (43). In mice, the adjuvant effect of *L. casei* in a model of gluten sensitivity was recently also shown, suggesting the potential role of certain strains as vaccine adjuvant, mainly by promoting T cell responses toward a Th1 phenotype (44).

32.3 PROBIOTICS AND REGULATION OF THE ALLERGIC IMMUNE RESPONSE

A considerable portion of the Western population suffers from allergy, and the incidence is still rising. As stated above, the lack of challenge for the immune system as a result of our hygienic lifestyle has been suggested as one of the possible causes. Indeed, at birth, the immune system of an infant is not fully developed and tends to be directed toward "pro-allergic" T helper type 2 (Th2)-biased immune responses, characterized by a capacity to produce Th2 cytokines, such as IL-4, IL-5, and IL-13. That leads to the stimulated production of IgE by B cells and thus the increase of the risk for allergic reactions through activation of mast cells. Dysbiosis has been observed in allergic children showing an aberrant microbiota even before the onset of allergy; with higher levels of clostridia and lower levels of bifidobacteria (45). The concept of using probiotics as a possible means for antiallergic therapy has emerged from there. It has then been suggested that probiotics may provide the safe microbial stimulation needed for the development of the immune system in infants. A limited number of strains have been tested for their efficacy in the treatment and prevention of allergy in infants. Such clinical trials have shown that the standard treatment of infants with atopic eczema, an extensively hydrolyzed infant formula, can be significantly improved through the addition of LGG or *B. lactis* Bb-12. The administration of LGG prenatally to 159 pregnant women for 2 weeks and postnatally to babies for 6 months halved subsequent occurrence of eczema in 2-year-old at-risk infants (22), and this effect extended beyond infancy (46). In murine models of allergy, some probiotic strains have been also shown to prevent allergic disease by promoting oral tolerance induction (47). The precise mechanisms behind these beneficial effects are not entirely known. Several mechanisms have been evoked, such as the improvement of the intestinal barrier function, modulation of the intestinal microbiota, or enzymatic degradation of dietary antigens by enzymes from probiotics. The modulation of the immune system may be the main mechanism by which probiotics could regulate the Th2 dysbalance observed in allergy. Evidence is accumulating that probiotic microorganisms preferentially elicit two substantial T cell lineages, able to counterbalance the proallergic T helper 2-directed immune response: the T helper 1 cells and the T regulatory suppress-1 cells. Some lactic acid bacteria (LAB) have been shown to display stimulatory properties on the innate immune system via cells such as macrophages and dendritic cells, inducing the production of IL-12. This, in turn, allows the stimulation of IFNγ production to promote the Th1 phenotype (see Fig. 32.2). We have demonstrated *in vitro* that certain LAB can highly reduce the capacity of allergen-specific human T cells to produce Th2 cytokines (IL-4, IL-5), but, in contrast largely favor the production of IFN-γ (48). This possible antiallergic effect seems to be dependent on antigen-presenting cells (APC) and requires IL-12 (49). This effect was also

confirmed *in vivo* in two murine models of allergy to birch pollen and to the major house dust mite allergens of *Dermatophagoides pteronyssinus* (Der p 1), respectively (29, 50). Notably, the coapplication of *L. plantarum* and the allergen induced a T-helper type 1 (Th1)-biased allergen-specific IgG response, reduced specific IgE response, and favored the production of INF-γ upon allergen restimulation. We also demonstrated that such selected lactobacilli can modulate DC from allergic patients to instruct autologous naïve CD4+ T cells to produce more interferon-γ (51).

Probiotics may also decrease allergic diseases by promoting immunosuppressive cytokines (TGF-β, IL-10) (52). This could be achieved by the induction of regulatory DCs that could promote regulatory cytokine production and the induction of regulatory T cells (Tregs) (see Fig. 32.2). This counter-regulatory hypothesis could be emphasized by the negative correlation observed between Th2-associated parasitic helminth infections and the prevalence of allergy. Indeed, it was shown in a murine model of allergy, that helminth infection elicits a regulatory T cell population able to downregulate allergen-induced lung pathology *in vivo* (53). By their capacity to enhance sIgA production, probiotics could also contribute to allergen exclusion and thereby reduce the exposure of the immune system to dietary antigens. We must remember that not all probiotics have the same immunological properties, and we have yet to define and select the best strains to exploit with optimal efficacy of the immunomodulatory functions of these micro organisms.

32.4 PROBIOTICS AND INFLAMMATORY BOWEL DISEASES

It is now well accepted that homeostasis versus chronic intestinal inflammation is determined by the presence or absence of appropriate control mechanisms that could be linked to a balance between protective (“good”) and aggressive (“bad”) luminal bacteria. In genetically susceptible individuals (54), an inappropriate mucosal immune response against the intestinal flora appears to be the principal mechanism leading to the pathogenesis of inflammatory bowel disease (IBD), mainly characterized by ulcerative colitis (UC), Crohn’s disease (CD), and pouchitis. The evidence for the validity of this precept is due to the fact that all of the many experimental murine models of mucosal inflammation are dependent on the presence of a normal microflora, that is, inflammation does not occur if the mice are reared and maintained in a germfree environment. The requirement for the presence of a normal flora is also supported in IBD patients, by the fact that the symptoms are sometimes ameliorated by the administration of antibiotics or by the diversion of the enteric stream away from the area of inflammation.

Metagenomic analysis indicates that the microflora of IBD patients is unstable and presents a reduced complexity of the bacterial phylum Firmicutes. While 43 distinct ribotypes of Firmicutes were identified in healthy microbiota, only 13 ribotypes were detected in CD patients, indicating a serious degree of microbial dysbiosis 15. In addition, a reduced number of lactobacilli and bifidobacteria was observed in the mucosa of IBD patients (55). Other studies reported that *Faecalibacterium prausnitzii*, one of the major bacterium of the *Clostridium leptum* group was particularly depleted in IBD patients’ ileocolonic mucosa-associated microbiota (56, 57). The anti-inflammatory capacity of this bacterium was then demonstrated in murine experimental models (58).

While most therapeutic strategies target the suppression or modulation of the inflammation, novel complementary therapeutic tools are needed in order to overcome problems linked to secondary side effects, as well as commonly observed nonresponsiveness of patients, leading to frequent relapses. The proposal of probiotics as such an alternative approach has recently emerged (59). Consequently, a number of clinical intervention studies have been carried out to address the beneficial potential of probiotics in patients suffering from pouchitis, Crohn disease, or ulcerative colitis. Most of these studies are summarized in Table 32.1, showing conflicting results. The greatest efficacy is observed for the prevention of pouchitis relapse and a potential effectiveness in UC. Evidence for the use of probiotics in CD is less persuasive, with only a limited number of studies reporting positive outcomes. Clearly larger trials using standardized protocols and better selected strains are required to confirm their possible role as therapeutic tools.

In contrast to the human situation, a large number of experimental animal models of IBD have reported protective effects of selected microorganisms. These models use IL-10 or IL-2 deficient mice, or are based on the transfer of $CD4^+$ CD45 RB^{hi} or $CD4^+$ $CD62L^+$ T cells into severe combined immunodeficiency (SCID) mice. Chemically-induced models of colitis have also been extensively used. An intrarectal administration of trinitrobenzene-sulfonic acid (TNBS) or the administration of dextran sodium sulfate (DSS) in drinking water is the most frequently used example. In all of these models a large number of probiotic strains have been tested for the prevention of colitis, often showing a significant capacity to reduce inflammation. In IL-10 knockout (KO) mice, different strains of *L. salivarius* (60, 61), *L. plantarum* (62), *L. reuteri* (63), *B. infantis* (60) were shown to dampen inflammation. The protective effect of the VSL#3 cocktail was also demonstrated in different models such as the DSS model and in $IL10^{-/-}$ KO mice, while its protective effect in the TNBS-induced colitis model was controversial (64, 65). Although there is now a considerable body of information concerning the efficiency of probiotics, their mechanisms of action remain largely unresolved. Probiotics may impair the "pro-inflammatory" activity of other luminal bacteria via the secretion of antimicrobial products, competitive exclusion, or by the induction of defensins. It has also been shown well that certain probiotics are able to enhance epithelial barrier function (66, 67). Notably, the probiotic *E. coli* Nissle 1917 was shown to counteract the disruptive effect of enteropathopathic *E. coli* (EPEC) on epithelial cell monolayer *in vitro*, by altering protein kinase C signaling (PKCζ) and causing the redistribution of the zonula occludens-2 (ZO-2), one of the main members of the tight junction protein complexes (68). Commensal bacteria can actively modulate signaling in mammalian IECs. While pathogenic bacteria induce TLR- and NLR-mediated NF-κB activation, *in vitro* studies have demonstrated that commensal bacteria such as *Lactobacillus* spp. can actively inhibit this pathway. It was notably shown that VSL#3 produces soluble factors able to Inhibit NF–κB and to induce cytoprotective heat shock proteins in colonic epithelial cells through proteasome inhibition (69) (see Fig. 32.2). Similarly, soluble factors released by *Bifidobacterium breve* C50 alleviated the secretion by epithelial cells of the TNF-α-induced CXCL8 chemokine, by decreasing the early phosphorylation steps of both the AP-1 and NF-κB signaling cascades (70). In the same way, *in vitro* coculture of inflamed ileal explants from CD patients with viable selective strains(*L. casei* DN114001) significantly reduced the release of TNF-α by the inflamed mucosa and the expression of TNF-α protein by intraepithelial lymphocytes (IEL).

Table 32.1
Summary of the main human clinical trials of probiotics in inflammatory bowel diseases

	Disease/activity	*n*	*Duration*	*Probiotic used*	*Type of study*	*Result*	*References*
Ulcerative colitis	Active/inactive	19	nd	*L. plantarum*		6/9 with active	Niedzielin (104)
	Active	116	12 month	*E. coli* Nissle	RCT, vs. mesalazine	Similar remission and relapse	Rembacken (105)
	Inactive	108	12 weeks	*E. coli* Nissle	vs. mesalazine	Similar relapse	Kruis (106)
	Inactive	20	12 month	VSL3	OL	75% in remission	Venturi (107)
	Inactive	327	12 month	*E. coli* Nissle	RCT, vs. mesalazine	Similar relapse rate 55%	Kruis (108)
	Inactive	21	12 month	Yakult femented Milk	RCT, vs. placebo	73% remission vs. 10%	Ishikawa (109)
	Active	25	1 month	*Saccharomyces boulardii*	Open-label	63% remission	Guslandi (109)
	Inactive	187	12 month	LGG	RCT ± mesalazine	No difference	Zocco (110)
Pouchitis	Chronic relapsing	40	9 month	VSL3	RCT, vs. placebo	85% remission vs. 0%	Gionchetti (111)
	Acute active	20	3 month	LGG	RCT, vs. placebo	0%	Kuisma (112)
	Acute active	10	1 month	*L. acidphilus*, *B. lactis*	Open-label	50% endoscopic improvement	Laake (113)
	Chronic relapsing	36	12 month	VSL3	RCT, vs. placebo	85% remission vs. 6%	Mimura (114)

(continued)

Table 32.1 (continued)

	Disease/activity	*n*	*Duration*	*Probiotic used*	*Type of study*	*Result*	*References*
Crohn's disease	Active	28	12 month	*E. coli* Nissle + prednisone	RCT, vs. placebo	70% vs. 30% (NS)	Malchow (115)
	Active	4	6 month	LGG	OL	73% lower	Gupta (116)
	Maintenance of remission	40	12 month	VSL3 + rifaximin	RCT, vs. mesalamine	80% vs. 60%	Campieri (117)
	Active	25	3 month	*L. salivarius*	OL	76% avoid other treatment	Mccarthy (118)
	Maintenance of remission	37	12 month	LGG	RCT, vs. mesalamine	No difference	Prantera (119)
	Active	11	6 month	LGG	RCT, vs. placebo	No difference	Schultz (120)
	Maintenance of remission	75	24 month	LGG	RCT, vs. pacebo	31% vs. 17%	Bousvaros (121)
	Maintenance of remission	98	6 month	*L. johnsonii*	RCT, vs. placebo	No difference	Marteau (122)
	Maintenance of remission	30	24 month	Symbiotic 2000	RCT, vs. placebo	No difference	Chermersh (123)
	Active	10	13 month	Symbiotic Psyllium + Bifidobact + Lactobacillus	RCT	Remission improvement of CDAI	Fujimori (124)

OL open label, *RCT* randomized control trial, *LGG L. rhamnosus* GG

In addition, coculture with this *L. casei* reduced the number of T cells displaying the α chain of IL-2R (CD25), an activation marker highly expressed by lamina propria lymphocytes (LPL) in Crohn's disease, while it increased the percentage of lymphocytes undergoing apoptosis, which may contribute to its ability to diminish the number of activated T cells in the lamina propria (71). Conversely, Rakoff-Nahoum et al. (12), reported that activation of TLRs by commensal bacteria is critical for protection against gut injury, notably through the induction of factors involved in tissue repair, such as IL-6, KC-1, and heat-shock proteins. Moreover, lactobacilli and the VSL#3 bacterial mixture strengthen intestinal barrier functions through the upregulation of β-defensins (i.e. hBD-2) via induction of proinflammatory pathways, including NF-κB and AP-1 (72). It was also recently shown that NF-κB signaling in the gut epithelium is a critical regulator of epithelial integrity and intestinal immune homeostasis, since the ablation of NEMO (subunits essential for NF-κB activation) spontaneously caused severe chronic intestinal inflammation in mice (73). All these results seem to indicate that commensal bacteria could maintain intestinal homeostasis by differential control of such signaling pathways following the signals involved. IECs are much more than a simple physical barrier to the external environment since they are in close contact with the intestinal immune system. The differential capacity of commensal/probiotic bacteria to interact with IECs will clearly influence the secretion of cytokines (Fig. 32.2). The follicle-associated epithelium that overlies Peyer's patches contains specialized M cells (microfold) which facilitate luminal sampling. Specialized dendritic cells (DCs) located in the lamina propria could also express tight junction proteins and extend their dendrites between IECs to directly sample bacteria (Fig. 32.2). The maturation state of DCs (i.e., the level of costimulatory molecules expression) within the gastrointestinal tract is currently regarded as a crucial factor governing the type and direction of a mucosal immunological response (Fig. 32.2). It has been shown recently that IECs can condition DCs through the release of thymic stromal lyphopoietin (TSLP), resulting in the induction of "non-inflammatory DCs," which could be crucial for the maintenance of gut homeostasis. Defects in this mechanism may allow exacerbated Th1 or Th17 inflammatory responses, resulting in IBD. Several reports have shown that nonpathogenic commensal bacteria, including probiotics, have the ability to elicit a characteristic cytokine response in *in vitro* coculture models of leucocyte-sensitized epithelial cells and can deliver a distinct signal to underlying immunocompetent cells. Notably, monocytes acquire immunoregulatory functions in the presence of probiotic-activated IEC (74, 75). Probiotics, in particular lactobacilli, seem to exert different effects on DCs. Substantial differences were found among strains in the capacity to induce IL-12 and TNF-α production in the DC. Interestingly some strains (i.e. *L. reuteri*) were able to inhibit the DC activation induced by others (i.e. *L. casei*), suggesting that the composition of the gut microflora, including ingested probiotics could influence the differential T effector/T regulator-driving capacities of the gut DCs (35). Indeed, some *Lactobacillus* species were able to activate DCs to induce a strong T cell immune response, indicating their potential as efficient immune stimulators (38), while others, such as VSL#3, were more potent inducers of IL-10 on DCs and as such able to inhibit the generation of Th1 cells (36). We indeed showed that protection by lactic acid bacteria against symptoms of IBD, induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) in mice, was strain-specific and correlated to their *in vitro* immuno-modulatory properties (76). Interestingly, we

observed that the strains that exhibited anti-inflammatory potentials led to the generation of partially matured regulatory DCs, able to rescue mice from colitis after adoptive transfer (37). The preventive effect of these probiotic-pulsed DCs required the induction of $CD4^+$ $CD25^+$ regulatory cells and seemed to involve the immunosuppressive pathway involving the indolamine 2, 3 dioxgenase (IDO) enzyme. Two other studies reported also that probiotics can improve murine colitis by inducing TGF-β-bearing regulatory T cells and can induce *in vivo* peripheral T cell hyporesponsiveness, suggesting a modulation through DC function (77, 78). It was also shown that lactobacilli, in a strain-specific manner, were able to prime DCs to promote the development of Treg cells through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) interaction (79). All these findings suggested that specific interaction between DCs and T cells in the gut are necessary for probiotic induction of regulatory cells. The precise mechanisms remain, however, unclear. Moreover, as the immunomodulatory/immunoregulatory capacities of probiotics are strain-specific, it is now very important to unravel in detail the molecular and cellular interaction of probiotics with the hosts' mucosal immune system in order to define more solid criteria for their selection as effective and safe immune intervention tools. The precise identification of active components involved in their beneficial effects is also needed. As previously described, some reports have suggested that probiotics could exert anti-inflammatory effects through the release of soluble factors (69, 70). Some authors have suggested that the protective effects of probiotics are mediated by their own DNA via TLR9 signaling. However, this effect seemed not to be restricted to probiotic DNA since the administration of other microbial DNA (i.e., from a non-probiotic *E. coli* strain) also ameliorated experimental colitis (64). By using cell wall mutants of lactobacilli, we could show some years ago that the composition of the cell wall can greatly influence the protective effect of the bacteria in a TNBS-induced colitis model 80. We recently showed that the protective effect of probiotic-conditioned DCs was TLR2- and NOD2-dependent, confirming the potential role of peptidoglycan (PGN) (37). Indeed, we could demonstrate that the oral or systemic administration of PGN purified from a protective strain is able to rescue mice from colitis in a NOD2-dependent way (Macho-Fernandez, submitted). Watanabe et al. 81 have recently shown that commercial/synthetic muramyl dipeptide (MDP), the common and minimal key motif of PGN detected by NOD2, is able to protect mice from colitis in a NOD2-dependent manner. They previously showed that the MDP activation of NOD2 is able to negatively regulate TLR2 responses, heightening the Th1 responses (82). They also reported that NOD2 transgenic mice are resistant to the development of experimental colitis (83). These findings could indeed support the hypothesis suggesting that NOD2 signaling has downregulatory activity and that mutations in NOD2 contribute to IBD by leading to excessive TLR2 cytokine responses. Since the mutations in the *Card15* gene (encoding for NOD2) have been involved in patients suffering from CD (54), we can speculate that the anti-inflammatory potential of LAB could be mediated through PGN and NOD2 signaling. Interestingly, the immune-modulation capacities of a certain number of lactobacilli were found to be largely modified after treating the bacteria with *N*-acetyl-muramidase (M1 enzyme). Interestingly, the sensibility of *Lactobacillus* strains to this treatment was negatively correlated with their *in vitro* IL-12 inducing capacity in murine splenocytes (84). Therefore, we hypothesize that all modification of the cell wall structure that can affect the PGN turnover, and the release of biological active muropep-

tides could have a strong impact on the immune-modulation capacities of the bacteria and could help to explain the strain-specificity generally observed. Such results could also explain the low efficacy of probiotics in CD patients. We could expect that the selection of designer probiotics able to bypass the loss of NOD2 function in such patients could allow best efficacy of probiotics in CD.

It is becoming evident that a better knowledge about the probiotic mechanisms of action is necessary to select strains with an optimal efficacy improving the potential of probiotics in the treatment of IBD.

32.5 PROBIOTICS AND OBESITY

The intestinal microbiota is a complex symbiotic ecosystem which has the capacity to digest luminal components and to synthesize useful host nutrients, while stimulating immune defense mechanisms. Therefore, an equilibrium between microbial nutrient utilization and host nutrient production should be achieved (85). Given the worldwide epidemic in obesity, there is a growing interest in the interaction of the microbiota with the host in obese state. Colonization of germ-free mice with an "obese" microbiota resulted in a significant greater increase in total body fat than colonization with a "lean" microbiota. These findings suggested that the microbiota of obese individuals may be more efficient at extracting nutritional value from a given diet than the microbiota of lean individuals and that this trait is transmissible by the microbiota (16, 86). Moreover, dysbiosis has been reported both in human obese patients and in leptin-deficient obese (*ob*/*ob*) mice, showing a significant decrease of the main Bacteroidetes phylum (16, 87). Additionally, when obese patients lost weight over a one-year period, the proportion of Firmicutes became similar to that of lean individuals. Such modification of microbiota composition could therefore affect microbial fermentation of dietary polysaccharides that will influence the intestinal absorption of monosaccharides and short-chain fatty acids and consequently their conversion to more complex lipids in the liver and the deposit of lipids in adipocytes.

Regarding obesity, only few studies have addressed the potential effects of probiotics in the management of this disease. It has been shown that supernatants from lactobacilli-treated adipocytes decreased the inflammatory-type response of lymphocytes, in correlation with a reduction of leptin production (88). Also, a selected strain of *Lactobacillus rhamnosus*, able to produce conjugated linoleic acid, has been reported to protect mice from diet-induced obesity (89). Interestingly, the administration of prebiotics dietary fiber (oligofructose [OFS]), known to modulate the gut microbiota typified by increased bifidobacteria, was shown to improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia (90). Since obesity is presently viewed as an inflammatory disease, affecting both innate and acquired immune systems (91, 92), we could speculate that probiotics with potential anti-inflammatory properties could counteract the development of complications associated with this pathology. Human microbiome projects are in progress all around the word and could allow moving forward to the understanding of the complexity of the interaction of dietary factors, microbiota and their impact on metabolic diseases. It is now apparent that a multidisciplinary approach is required and it would be useful to line out specific strategies for the modification of the gut microbiota in order to impact on the occurrence of such diseases.

32.6 CONCLUSIONS AND PERSPECTIVES

It is clear that the activities of probiotics are multifaceted and could either stimulate the immune system or regulate imbalanced immune disorders. Of the various mechanisms that might be involved, the one that has devoted the most attention is the impact on immune regulation. This happens through specific interaction with dendritic cells and the induction of regulatory pathways. Promising results have been obtained in the treatment of certain diseases, in particular, allergy and certain types of IBD. However, their effectiveness is still quite variable and seems to be largely strain-specific. It has now become extremely urgent to unravel the possible mechanism of actions in more detail and to identify active components involved. The results will help to define the best criteria for selecting the most suitable probiotic strains, applicable in a variety of therapeutic or prophylactic approaches.

A better understanding of how diet influences each individual's genetic potential, its overall performance and its susceptibility to disease, will greatly prepare the field for future applications. However, in parallel, a general legislation is required, regulating the use of both dietary supplements and functional foods in a medical and nutritional context. In the field of nutritional science, current challenges include fundamental knowledge and concrete regulations, allowing to provide efficient and safe strains to consumers (93). The rapidly evolving research field of probiotics is closely matched by an increased familiarity of probiotics to the general public. Functional food legislations are in evolution worldwide and should largely be considering two different applications of probiotics: their use in foods or their use in medical applications. The FAO/WHO Working Group for the Evaluation of Probiotics in Food (94, 95) specifies that probiotics must be alive when administered, must have documented health benefits in the target host (96–99), must be taxonomically defined at the genus, species, and strain level, and must be safe for its intended use. For foods, the United States specifies two types of claims, both requiring substantiation: the "disease/substance relationship" claim and the "disease/risk reduction" claim, described in detail in the "US Nutrition Labeling and Education Act" of 1990 [codified into regulations on January 6, (100)]. In addition, claims which relate the food to the normal structure or functioning of the human body are also allowed.

In contrast, in Europe, the "Council Directive 2000/13/EC on labeling, presentation and advertising of foodstuffs" (101) prohibits to link foods to any property related to the prevention, the treatment or the curing of a human disease. However, the regulation "EC 1924/2006 on Nutrition and Health Claims made on Food" (102) specifies that health claims could describe or refer (1) to the effect of a nutrient or other substance on bodily functions, including psychological and behavioral functions, as well as slimming and weight control, and (2) to the reduction of disease risk and to children's development and health. In Europe, the International Life Sciences Institute (ILSI, http://europe.ilsi.org/passclaim) also defined some general principles and requirements. In addition, the PASSCLAIM document delivers criteria to assess the scientific support for claims on foods (103).

In Japan, probiotics can be submitted under the Foods for Specified Health Uses (FOSHU). When approved, the producer is allowed to put a special label on the package, proving that the health claim made was approved by the ministry of health, based on a scientific dossier.

For nonfood use, the FAO/WHO definition was sufficiently broad to also deal with a range of probiotic applications which go beyond what is expected from a food or a dietary supplement: a probiotic microorganism(s) may be used orally in (1) drug applications (often referred to as "live biotherapeutics"), (2) microbial feed (animal use), (3) genetically modified organisms, and (4) live vaccines. Requirements for efficacy and safety evaluation are different for these different applications. For example, a probiotic used as a drug must not only fulfill the general FAO conditions stipulated above, but also be in agreement with the existing national regulations and guidelines on good clinical practices.

REFERENCES

1. Stark PL, Lee A (1982) The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. J Med Microbiol 15(2):189–203
2. Palmer C, Bik EM, Digiulio DB, Relman DA, Brown PO (2007) Development of the human infant intestinal microbiota. PLoS Biol 5(7):e177
3. Xu J, Gordon JI (2003) Inaugural article: honor thy symbionts. Proc Natl Acad Sci USA 100(18): 10452–10459
4. Hooper LV (2004) Bacterial contributions to mammalian gut development. Trends Microbiol 12(3):129–134
5. Kelly D, Conway S (2005) Bacterial modulation of mucosal innate immunity. Mol Immunol 42(8):895–901
6. Guarner F, Bourdet-Sicard R, Brandtzaeg P et al (2006) Mechanisms of disease: the hygiene hypothesis revisited. Nat Clin Pract Gastroenterol Hepatol 3(5):275–284
7. Duchmann R, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Buschenfelde KH (1995) Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). Clin Exp Immunol 102(3):448–455
8. Akira S, Hemmi H (2003) Recognition of pathogen-associated molecular patterns by TLR family. Immunol Lett 85(2):85–95
9. Strober W, Fuss IJ, Blumberg RS (2002) The immunology of mucosal models of inflammation. Annu Rev Immunol 20:495–549
10. Gewirtz AT, Navas TA, Lyons S, Godowski PJ, Madara JL (2001) Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol 167(4):1882–1885
11. Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303(5664):1662–1665
12. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118(2):229–241
13. Neish AS, Gewirtz AT, Zeng H et al (2000) Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science 289(5484):1560–1563
14. Kelly D, Campbell JI, King TP et al (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 5(1):104–112
15. Manichanh C, Rigottier-Gois L, Bonnaud E et al (2006) Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 55(2):205–211
16. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444(7122):1027–1031
17. Metschnikoff E (1908) The prolongation of life, optimistic studies. London
18. Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66(5):365–378
19. Arvola T, Laiho K, Torkkeli S et al (1999) Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics 104(5):e64

20. Gionchetti P, Rizzello F, Venturi A et al (2000) Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 119(2):305–309
21. Rafter J (2003) Probiotics and colon cancer. Best Pract Res Clin Gastroenterol 17(5):849–859
22. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E (2001) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 357(9262):1076–1079
23. Hoffmann M, Rath E, Holzlwimmer G et al (2008) *Lactobacillus reuteri* 100-23 transiently activates intestinal epithelial cells of mice that have a complex microbiota during early stages of colonization. J Nutr 138(9):1684–1691
24. Hessle C, Hanson LA, Wold AE (1999) Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. Clin Exp Immunol 116(2):276–282
25. Miettinen M, Matikainen S, Vuopio-Varkila J et al (1998) Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. Infect Immun 66(12):6058–6062
26. Perdigon G, Fuller R, Raya R (2001) Lactic acid bacteria and their effect on the immune system. Curr Issues Intest Microbiol 2(1):27–42
27. Takagi A, Matsuzaki T, Sato M, Nomoto K, Morotomi M, Yokokura T (2001) Enhancement of natural killer cytotoxicity delayed murine carcinogenesis by a probiotic microorganism. Carcinogenesis 22(4):599–605
28. Shida K, Kiyoshima-Shibata J, Nagaoka M, Watanabe K, Nanno M (2006) Induction of interleukin-12 by *Lactobacillus* strains having a rigid cell wall resistant to intracellular digestion. J Dairy Sci 89(9):3306–3317
29. Hisbergues M, Magi M, Rigaux P et al (2007) In vivo and in vitro immunomodulation of Der p 1 allergen-specific response by *Lactobacillus plantarum* bacteria. Clin Exp Allergy 37(9):1286–1295
30. Nagao F, Nakayama M, Muto T, Okumura K (2000) Effects of a fermented milk drink containing *Lactobacillus casei* strain Shirota on the immune system in healthy human subjects. Biosci Biotechnol Biochem 64(12):2706–2708
31. Parra MD, Martínez de Morentin BE, Cobo JM, Mateos A, Martinez JA (2004) Daily ingestion of fermented milk containing *Lactobacillus casei* DN114001 improves innate-defense capacity in healthy middle-aged people. J Physiol Biochem 60(2):85–91
32. Sheih YH, Chiang BL, Wang LH, Liao CK, Gill HS (2001) Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium *Lactobacillus rhamnosus* HN001. J Am Coll Nutr 20(2 Suppl):149–156
33. Gill HS, Rutherfurd KJ (2001) Immune enhancement conferred by oral delivery of *Lactobacillus rhamnosus* HN001 in different milk-based substrates. J Dairy Res 68(4):611–616
34. Cebra JJ (1999) Influences of microbiota on intestinal immune system development. Am J Clin Nutr 69(5):1046S–1051S
35. Christensen HR, Frokiaer H, Pestka JJ (2002) Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J Immunol 168(1):171–178
36. Hart AL, Lammers K, Brigidi P et al (2004) Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut 53(11):1602–1609
37. Foligne B, Zoumpopoulou G, Dewulf J et al (2007) A key role of dendritic cells in probiotic functionality. PLoS One 2(3):e313
38. Mohamadzadeh M, Olson S, Kalina WV et al (2005) Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. Proc Natl Acad Sci USA 102(8):2880–2885
39. Perdigon G, Alvarez S, Rachid M, Aguero G, Gobbato N (1995) Immune system stimulation by probiotics. J Dairy Sci 78(7):1597–1606
40. Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM (1994) Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol 10(1):55–63
41. Kaila M, Isolauri E, Saxelin M, Arvilommi H, Vesikari T (1995) Viable versus inactivated lactobacillus strain GG in acute rotavirus diarrhoea. Arch Dis Child 72(1):51–53
42. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH (1994) Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344(8929):1046–1049

43. Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T (1995) Improved immunogenicity of oral D × RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. Vaccine 13(3):310–312
44. D'Arienzo R, Maurano F, Luongo D et al (2008) Adjuvant effect of *Lactobacillus casei* in a mouse model of gluten sensitivity. Immunol Lett 119(1–2):78–83
45. Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E (2001) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J Allergy Clin Immunol 107(1):129–134
46. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E (2003) Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet 361(9372):1869–1871
47. Prioult G, Fliss I, Pecquet S (2003) Effect of probiotic bacteria on induction and maintenance of oral tolerance to beta-lactoglobulin in gnotobiotic mice. Clin Diagn Lab Immunol 10(5):787–792
48. Pochard P, Gosset P, Grangette C et al (2002) Lactic acid bacteria inhibit TH2 cytokine production by mononuclear cells from allergic patients. J Allergy Clin Immunol 110(4):617–623
49. Pochard P, Hammad H, Ratajczak C et al (2005) Direct regulatory immune activity of lactic acid bacteria on Der p 1-pulsed dendritic cells from allergic patients. J Allergy Clin Immunol 116(1):198–204
50. Repa A, Grangette C, Daniel C et al (2003) Mucosal co-application of lactic acid bacteria and allergen induces counter-regulatory immune responses in a murine model of birch pollen allergy. Vaccine 22(1):87–95
51. Ratajczak C, Duez C, Grangette C, Pochard P, Tonnel AB, Pestel J (2007) Impact of lactic acid bacteria on dendritic cells from allergic patients in an experimental model of intestinal epithelium. J Biomed Biotechnol 2007(1):71921
52. Pessi T, Sutas Y, Hurme M, Isolauri E (2000) Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. Clin Exp Allergy 30(12):1804–1808
53. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM (2005) Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med 202(9):1199–1212
54. Hugot JP, Chamaillard M, Zouali H et al (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411(6837):599–603
55. Favier C, Neut C, Mizon C, Cortot A, Colombel JF, Mizon J (1997) Fecal beta-d-galactosidase production and bifidobacteria are decreased in Crohn's disease. Dig Dis Sci 42(4):817–822
56. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 104(34):13780–13785
57. Martinez-Medina M, Aldeguer X, Gonzalez-Huix F, Acero D, Garcia-Gil LJ (2006) Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. Inflamm Bowel Dis 12(12):1136–1145
58. Sokol H, Pigneur B, Watterlot L et al (2008) *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA 105(43):16731–16736
59. Sartor RB (2004) Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology 126(6):1620–1633
60. McCarthy J, O'Mahony L, O'Callaghan L et al (2003) Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut 52(7):975–980
61. Sheil B, McCarthy J, O'Mahony L et al (2004) Is the mucosal route of administration essential for probiotic function? Subcutaneous administration is associated with attenuation of murine colitis and arthritis. Gut 53(5):694–700
62. Schultz M, Veltkamp C, Dieleman LA et al (2002) *Lactobacillus plantarum* 299 V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. Inflamm Bowel Dis 8(2):71–80
63. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN (1999) *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology 116(5):1107–1114
64. Rachmilewitz D, Katakura K, Karmeli F et al (2004) Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology 126(2):520–528
65. Shibolet O, Karmeli F, Eliakim R et al (2002) Variable response to probiotics in two models of experimental colitis in rats. Inflamm Bowel Dis 8(6):399–406

66. Madsen K, Cornish A, Soper P et al (2001) Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121(3):580–591
67. Southcott E, Tooley KL, Howarth GS, Davidson GP, Butler RN (2008) Yoghurts containing probiotics reduce disruption of the small intestinal barrier in methotrexate-treated rats. Dig Dis Sci 53(7): 1837–1841
68. Zyrek AA, Cichon C, Helms S, Enders C, Sonnenborn U, Schmidt MA (2007) Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. Cell Microbiol 9(3):804–816
69. Petrof EO, Kojima K, Ropeleski MJ et al (2004) Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology 127(5):1474–1487
70. Heuvelin E, Lebreton C, Grangette C, Pot B, Cerf-Bensussan N, Heyman M (2009) Mechanisms involved in alleviation of intestinal inflammation by *Bifidobacterium breve* soluble factors. PLoS One 4(4):e5184
71. Carol M, Borruel N, Antolin M et al (2006) Modulation of apoptosis in intestinal lymphocytes by a probiotic bacteria in Crohn's disease. J Leukoc Biol 79(5):917–922
72. Schlee M, Harder J, Koten B, Stange EF, Wehkamp J, Fellermann K (2008) Probiotic lactobacilli and VSL#3 induce enterocyte beta-defensin 2. Clin Exp Immunol 151(3):528–535
73. Nenci A, Becker C, Wullaert A et al (2007) Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature 446(7135):557–561
74. Haller D, Serrant P, Peruisseau G et al (2002) IL-10 producing CD14low monocytes inhibit lymphocyte-dependent activation of intestinal epithelial cells by commensal bacteria. Microbiol Immunol 46(3):195–205
75. Parlesak A, Haller D, Brinz S, Baeuerlein A, Bode C (2004) Modulation of cytokine release by differentiated CACO-2 cells in a compartmentalized coculture model with mononuclear leucocytes and nonpathogenic bacteria. Scand J Immunol 60(5):477–485
76. Foligne B, Nutten S, Grangette C et al (2007) Correlation between in vitro and in vivo immunomodulatory properties of lactic acid bacteria. World J Gastroenterol 13(2):236–243
77. Braat H, van den Brande J, van Tol E, Hommes D, Peppelenbosch M, van Deventer S (2004) *Lactobacillus rhamnosus* induces peripheral hyporesponsiveness in stimulated CD4+ T cells via modulation of dendritic cell function. Am J Clin Nutr 80(6):1618–1625
78. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M (2005) Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-beta-bearing regulatory cells. J Immunol 174(6):3237–3246
79. Smits HH, Engering A, van der Kleij D et al (2005) Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 115(6):1260–1267
80. Grangette C, Nutten S, Palumbo E et al (2005) Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. Proc Natl Acad Sci USA 102(29):10321–10326
81. Watanabe T, Asano N, Murray PJ et al (2008) Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. J Clin Invest 118(2):545–559
82. Watanabe T, Kitani A, Murray PJ, Strober W (2004) NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 5(8):800–808
83. Yang Z, Fuss IJ, Watanabe T et al (2007) NOD2 transgenic mice exhibit enhanced MDP-mediated down-regulation of TLR2 responses and resistance to colitis induction. Gastroenterology 133(5):1510–1521
84. Sashihara T, Sueki N, Ikegami S (2006) An analysis of the effectiveness of heat-killed lactic acid bacteria in alleviating allergic diseases. J Dairy Sci 89(8):2846–2855
85. Hooper LV, Xu J, Falk PG, Midtvedt T, Gordon JI (1999) A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc Natl Acad Sci USA 96(17):9833–9838
86. Backhed F, Ding H, Wang T et al (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 101(44):15718–15723
87. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444(7122):1022–1023

88. Bleau C, Lamontagne L, Savard R (2005) New *Lactobacillus acidophilus* isolates reduce the release of leptin by murine adipocytes leading to lower interferon-gamma production. Clin Exp Immunol 140(3):427–435
89. Lee HY, Park JH, Seok SH et al (2006) Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. Biochim Biophys Acta 1761(7):736–744
90. Cani PD, Neyrinck AM, Fava F et al (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 50(11):2374–2383
91. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95(5):2409–2415
92. Rajala MW, Scherer PE (2003) Minireview: the adipocyte – at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144(9):3765–3773
93. Gorbach SL (2002) Probiotics in the third millennium. Dig Liver Dis 34(Suppl 2):S2–S7
94. Food and Agriculture Organization of the United Nations (FAO) (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf
95. Food and Agriculture Organization of the United Nations (FAO) (2002) Guidelines for the evaluation of probiotics in food. ftp://ftp.fao.org/es/esn/food/wgreport2.pdf
96. International Life sciences Institute Europe (ILSI) (2004) Process for the Assessment of Scientific Support for Claims on Food (PASSCLAIM). http://europe.ilsi.org/publications/ecprojects/passclaim-phase2.htm
97. U.S. Food and Drug Administration (FDA) (2007) Evidence based review system for the scientific evaluation of health claims. http://www.cfsan.fda.gov/~dms/hclmgui6.html
98. U.S. Food and Drug Administration (FDA) (2004) Substantiation for dietary supplement claims made under Section 403(r) (6) of the Federal Food, Drug, and Cosmetic Ac. http://www.cfsan.fda.gov/~dms/dsclmgui.html
99. Evidence for Health Claims on Food: How Much is Enough? Canadian Nutrition Congress meeting, Winnipeg, CANADA. 2007. http://jn.nutrition.org/cgi/content/full/138/6SI/1189S
100. Guide to Nutrition Labeling and Educational Act (NLEA) Requirements http://www.fda.gov/ora/inspect_ref/igs/nleatxt.html#EXEMPTIONS%20AND%20SPECIAL
101. Council Directive 2000/13/EC on labeling, presentation and advertising of foodstuffs http://www.fsai.ie/legislation/food/eu_docs/Labelling/General%20Labelling%20Provisions%20for%20Foodstuffs/Dir%202000.13%20EC.pdf
102. Regulation (EC) 1924/2006 on Nutrition and Health Claims made on Food http://www.fsai.ie/legislation/food/eu_docs/Labelling/Cor_Reg1924_2006.pdf
103. Aggett PJ, Antoine JM, Asp NG et al (2005) PASSCLAIM: consensus on criteria. Eur J Nutr 44(Suppl 1):i5–i30
104. Niedzielin K, Kordecki H, Birkenfeld B (2001) A controlled, double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299 V in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol 13(10):1143–1147
105. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT (1999) Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet 354(9179):635–639
106. Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M (1997) Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 11(5):853–858
107. Venturi A, Gionchetti P, Rizzello F et al (1999) Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther 13(8):1103–1108
108. Kruis W, Fric P, Stolte M (2001) Maintenance of remission in ulcerative colitis is equally effective with *Escherichia coli* Nissle 1917 and with standard mesalamine. Gastroenterology 120 (Suppl 1):A127

109. Ishikawa H, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T (2003) Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. J Am Coll Nutr 22(1):56–63
110. Zocco MA, dal Verme LZ, Cremonini F et al (2006) Efficacy of *Lactobacillus* GG in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 23(11):1567–1574
111. Gionchetti P, Amadini C, Rizzello F, Venturi A, Poggioli G, Campieri M (2003) Probiotics for the treatment of postoperative complications following intestinal surgery. Best Pract Res Clin Gastroenterol 17(5):821–831
112. Kuisma J, Mentula S, Jarvinen H, Kahri A, Saxelin M, Farkkila M (2003) Effect of *Lactobacillus rhamnosus* GG on ileal pouch inflammation and microbial flora. Aliment Pharmacol Ther 17(4):509–515
113. Laake KO, Line PD, Aabakken L et al (2003) Assessment of mucosal inflammation and circulation in response to probiotics in patients operated with ileal pouch anal anastomosis for ulcerative colitis. Scand J Gastroenterol 38(4):409–414
114. Mimura T, Rizzello F, Helwig U et al (2004) Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut 53(1):108–114
115. Malchow HA (1997) Crohn's disease and *Escherichia coli*. A new approach in therapy to maintain remission of colonic Crohn's disease? J Clin Gastroenterol 25(4):653–658
116. Gupta P, Andrew H, Kirschner BS, Guandalini S (2000) Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. J Pediatr Gastroenterol Nutr 31(4):453–457
117. Campieri M, Rizzello F, Venturi A (2000) Combination of antibiotic and probiotic treatment is efficacious in prophylaxix of post-operative recurrence of Crohn's disease: a randomized controlled study vs mezalamine. Gastroenterology 118:A781
118. McCarthy J, OMahony L, Dunne C (2001) An open trial of a novel probiotic as an alternative to steroids in mild/moderately active Crohn's disease. Gut 49(Suppl 3):2447
119. Prantera C, Scribano ML (2002) Probiotics and Crohn's disease. Dig Liver Dis 34(Suppl 2):S66–S67
120. Schultz M, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, Rath HC (2004) *Lactobacillus* GG in inducing and maintaining remission of Crohn's disease. BMC Gastroenterol 4:5
121. Bousvaros A, Guandalini S, Baldassano RN et al (2005) A randomized, double-blind trial of *Lactobacillus* GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. Inflamm Bowel Dis 11(9):833–839
122. Marteau P, Lemann M, Seksik P et al (2006) Ineffectiveness of *Lactobacillus johnsonii* LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo conrolled GETAID trial. Gut 55(6):842–847
123. Chermesh I, Tamir A, Reshef R et al (2007) Failure of Synbiotic 2000 to prevent postoperative recurrence of Crohn's disease. Dig Dis Sci 52(2):385–389
124. Fujimori S, Tatsuguchi A, Gudis K et al (2007) High dose probiotic and prebiotic cotherapy for remission induction of active Crohn's disease. J Gastroenterol Hepatol 22(8):1199–1204

33 Prebiotics in Immuno-Modulation for Treatment of Acute Pancreatitis

Refaat A. Hegazi

Key Points

- Intestinal mucosal immune system plays a significant role in the pathogenesis of severe acute pancreatitis and its associated complications.
- Prebiotics restore intestinal bacteria flora in patients with severe acute pancreatitis. Moreover, they exert beneficial anti-infective and metabolic effects. Earlier studies show that prebiotics improve clinical outcome in patients with severe acute pancreatitis.
- More research is warranted to study the effects of different prebiotics and optimal doses of prebiotics on the clinical outcome in patients with acute pancreatitis.

Key Words: Acute pancreatitis, prebiotics, enteral nutrition, nutritional immunomodulation.

33.1 INTRODUCTION

Within the last decade, the concept of modulating the intestinal mucosal immune system by altering the intestinal bacterial flora with probiotics and prebiotics has grown significantly. Moreover, scientific interest in the potential therapeutic use of probiotics and prebiotics in the management of different gastrointestinal diseases, including acute pancreatitis, has equally expanded. Probiotics are broadly defined as live microorganisms which when administered in adequate amounts confer a health benefit to the host. Consistently, recent literature fosters the hypothesis that probiotics confer a wide range of immune- and disease-modulating effects that make these microorganisms a favorable therapeutic target of a variety of diseases, both in animal models and in humans. Prebiotics are indigestible food products that promote the growth and/or the functional metabolic activities of beneficial intestinal bacteria. In order to meet the definition of prebiotics, a substance should have three properties. It should resist degradation by

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_33

gastric acid as well as enzymatic degradation by pancreatic and intestinal enzymes. Lastly, it is selectively fermented by intestinal beneficial bacteria (e.g., lactobacilli and bifidobacteria), thus promoting the proliferation and/or activity of these bacterial strains. Examples of prebiotics are fibers (e.g., oat, gum arabic) inulin-like prebiotics (e.g., inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS)), polydextrose, and polyols like lactulose and lactitol. Examples of food sources rich in prebiotics are whole grain, honey, banana, garlic, onions, leeks, and artichokes. The use of prebiotics to modulate the intestinal flora and the gut immune system in patients with acute pancreatitis is a novel area of investigation that warrants further studies. In this chapter, we will review the pathogenesis of the disease and the potential applications of using prebiotics in patients with acute pancreatitis.

33.2 PATHOGENESIS AND DIAGNOSIS OF ACUTE PANCREATITIS

Acute pancreatitis continues to be an increasingly prevalent disease. It contributes to 200,000 hospital admissions each year. Approximately 70–80% of cases are mild to moderate while the disease is severe and associated with life-threatening complications in 20–30% of cases.

Clinically, patients with acute pancreatitis present with acute postprandial abdominal pain usually in the epigastric area or left upper quadrant that radiates to the back. The exact onset of the pain is often missed by the patient. Physical signs are few; upper abdominal tenderness may be palpated. In some patients, when peri-pancreatic exudates from pancreatic necrosis leak along the falciform ligament and into the retroperitoneum, skin discoloration in the periumbilical region is known as Cullen's sign. In addition to the abdominal pain, elevation of serum levels of the pancreatic enzymes, amylase and lipase, remains to be an important diagnostic test of acute pancreatitis. Serum lipase levels are especially important in the follow up of the course of the disease.

Major causes are gall stones and alcohol, attributing to approximately 70% of the cases of acute pancreatitis. Other causes include hypertriglyceridemia, ERCP, hypercalcemia, and drugs. In most of cases, the etiology of the disease can be identified by a comprehensive clinical history (e.g., alcohol abuse), physical examination, laboratory measurements (e.g., hypertriglyceridemia), and radiological methods (e.g., gall stones). However, a clear etiology cannot be identified in many cases.

Pathologically, acute pancreatitis is inflammation of the pancreatic acinar cells. The exact pathogenic mechanism of acute pancreatitis remains to be determined. However, it is related to the unregulated activation of the pancreatic enzyme trypsin within the pancreatic acinar cells. Normally, trypsin activation is regulated by several protective mechanisms. These include the synthesis of the enzyme in its inactive enzyme trypsinogen form, enzyme compartmentalization, synthesis of trypsin inhibitors such as the serine peptidase inhibitor, Kazal type 1 (SPINK1) protein, and low levels of intracellular ionized calcium ion concentrations. Failure of these regulatory mechanisms (e.g., SPINK1 genetic mutations, hypercalcemia) leads to the uncontrolled activation of trypsin which can result in auto-digestion of the pancreatic gland tissue, triggering the initial local pancreatic inflammatory hit. This local inflammation is mediated by the release of several proinflammatory mediators and enzymes which perpetuates the inflammatory cycle. For instance, there is an associated stimulated secretion of

the proinflammatory cytokines, tumor necrosis factor (TNF), interlekin-1 (IL-1) and interleukin-6 (IL-6) as well as the chemokine interleukin-8 by local inflammatory neutrophils, macrophages, and lymphocytes. Concomitantly, the activation of other tissue injury mediators such as the enzymes elastase and phospholipase A2, the complement and kinin pathways also takes place. This proinflammatory response is associated with increased secretion of the anti-inflammatory cytokine interleukin-10 in an attempt to limit the excess inflammatory response. Failure of regulating these anti-inflammatory and cellular cascade results in possibly an exaggerated inflammatory response that characterizes acute pancreatitis. This initial cellular injury is characterized by local peri-pancreatic inflammatory cellular and fluid accumulation.

In severe acute pancreatitis, systemic inflammatory and cellular changes occur in other body parts leading to distant organ dysfunction (severe inflammatory response syndrome, SIRS). The pathogenesis of severe acute pancreatitis-associated SIRS is thought to be a result of a "two hit" disease. The first hit is driven by the pancreatic acinar cellular reaction to an initial insult (e.g., gall stones, alcohol). The result of the first hit is a leak of the highly proteolytic pancreatic enzymes resulting in local inflammatory reaction, with the recruitment of inflammatory cells to the pancreatic injury site. A more exacerbated inflammatory response could result in pancreatic acinar cellular necrosis, the formation of pseudocysts and abscess formation. Consequently, the "second hit" is mainly induced by distant organ inflammatory reaction attributed to the leak of inflammatory molecules to distant organs via the systemic circulation. In a severe form of the disease, these systemic inflammatory changes could be life threatening as a result of multiorgan failure (MOF), especially the lungs, kidneys, and hematopeotic system. Studies have shown that reversal of MOF eliminates the mortality risk associated with severe acute pancreatitis.

33.3 ROLE OF THE GUT IN ACUTE PANCREATITIS

The intestinal tract plays a significant role in the pathogenesis of acute pancreatitis and its associated complications. Importantly, the septic complications of severe acute pancreatitis are thought to be related to the translocation of microbes to the pancreas from other organs (bacterial translocation), primarily the intestinal tract.

Experimental studies and aspirate culture studies of peri-pancreatic fluid collections suggest that the gut is the major site of bacterial translocation to the pancreatic site causing peri-pancreatic infections and infected bacterial necrosis. In critically ill patients with SIRS (either due to severe acute pancreatitis, burns or trauma), the gut is considered the "undrained abscess" responsible for multiorgan failure (1).

Studies have highlighted the importance of the "intestinal barrier" mediating bacterial translocation. Intestinal epithelial cells do not merely function as a physical barrier against invading pathogenic bacteria but also exert powerful immunomodulatory effects on the gut-associated lymphoid tissue. The intestinal epithelial cells are coated by a "glycocalyx" layer consisting of a mix of glycoproteins and mucin that serves as the first check-point against pathogen invasion and penetration. The intestinal epithelial cell membrane provides an extra layer of epithelial resistance against bacterial penetrance. The joining intestinal epithelial cells "tight junction" also plays an essential role in maintaining the integrity of the "intestinal barrier".

In patients with acute pancreatitis, three interrelated pathological changes of the intestinal tract that have been described: intestinal mucosal barrier dysfunction, lack of homeostatic effects of enteral nutrients, and changes in the composition of intestinal luminal bacteria.

33.3.1 Intestinal Mucosal Barrier Changes

Studies of experimental models of severe acute pancreatitis have consistently showed a significant intestinal mucosal dysfunction that correlates with the onset and progression of the disease. For instance, Rahman et al. (2), showed decreased intestinal blood flow in animal models of acute pancreatitis. This intestinal hypoperfusion could be attributed to several factors like increased plasma viscosity and significantly slower blood sedimentation as a result of vascular changes concomitant with acute pancreatitis. Moreover, Wang et al. (3) showed increased apoptosis of intestinal cells in severe acute pancreatitis. Consistent with these experimental studies, changes of intestinal permeability "leaky gut" have been also described in patients with acute pancreatitis, especially the severe form of the disease. For instance, in an important study by Liu et al. (4), intestinal permeability, as measured by the differential lactulose–mannitol absorption was significantly impaired in patients with severe acute pancreatitis. Interestingly, the severity of intestinal dys-permeability was positively associated with the severity of the disease. Ammori et al. (5) have also shown that intestinal permeability changes in patients with severe acute pancreatitis correlate with endotoxemia in these patients.

33.3.2 Role of Enteral Nutrients in Patients with Acute Pancreatitis

Compared to parenteral, enteral nutrition is associated with a lower rate of infectious complications, shorter hospital stay, and less mortality rates in patients with severe acute pancreatitis. The exact mechanism of these protective effects of enteral nutrition remains to be elucidated. Interestingly, mechanistic studies of markers of systemic inflammatory response failed to show differences between the two nutritional support methods. For instance, Powell and colleagues (6) studied the serum levels of the inflammatory marker C-reactive protein (CRP) and cytokines (IL-6 and TNF) of two groups of patients with severe acute pancreatitis either enterally or parenterally fed. No significant differences were detected between the two groups. These results suggest that the immunomodulatory effects of enteral nutrition may have been mediated by its effects on the intestinal mucosal functions. Due to difficult sampling, the intestinal mucosa in critically ill patients with acute pancreatitis, the exact effects of enteral nutrition on intestinal mucosal functions, and structure in acute pancreatitis is difficult to discern in human subjects. However, compelling evidence suggests that enteral nutrients possess trophic effects on the enterocytes, stabilizing the structure and/or function of the intestinal barrier and maintaining the functional integrity of both intestinal and systemic immune compartments which ultimately decreases the risk of bacterial translocation. As patients with acute pancreatitis experience food-induced abdominal pain and food-stimulated pancreatic secretion, they decrease their food intake, before they even seek medical care. Therefore, it may be hypothesized that the intestinal functional and structural changes associated with acute pancreatitis could be attributed to the lack of trophic

effects of nutrients on enterocytes. In the next section, we will highlight the molecular and immunological effects that dietary factors exert on the intestinal mucosal immune system and how these effects mediate the trophic effects of enteral nutrients.

Nutrients exert homeostatic effects on both the intestinal immune system by regulating the expression of intestinal cytoprotective and inflammatory molecules. For instance, dietary glutamine has been shown to induce the expression of hemoxygenase-1 enzyme, a major cytoprotective molecule against intestinal injury and inflammation (7) by the duodenal enterocytes. In addition to its homeostatic effects on the intestinal epithelial cells, diet exerts immunomodulatory effects on the GALT. For instance, Ma and colleagues reported that mice fed a high-fat diet were more susceptible to chemically induced colitis. These mice also had higher numbers of non-CD1d-restricted natural killer T cells in colonic intraepithelial lymphocytes (IELs) compared with mice fed a normal diet. This subset of intestinal T-cells attained a very active phenotype expressing the proinflammatory T-helper-1 (Th1) cytokines, TNF-α and interferon-γ, having been up-regulated by a high-fat diet. Interestingly, mice fed the high-fat diet also had decreased levels of colonic regulatory T-cells (8). Consistently, dietary fat induces toll-like receptor-4 (TLR-4)-mediated signaling both in macrophages and adipocytes (9). TLR-4 receptors are transmembrane pattern recognition receptors that are also activated by the bacterial product lipopolysaccharide. Collectively, these studies advance our understanding that IELs are a subset of intestinal mucosal cells that are very responsive to dietary antigens.

Interestingly, a network of inflammatory cytokines allows an immunologic dialogue between dietary nutrients, IELs and another important subset of intestinal mucosal cells, the intestinal epithelial cells (IECs). IECs are potent immune cells that modulate the immune system by secreting cytokines and chemokines (e.g., IL-6, IL-8 and IL-15). The function of these cytokines is mainly to recruit immune cells from the systemic circulation to the site of tissue injury. In addition, IECs secrete another important pluripotent growth factor for the survival of T-cells, interleukin-7 (IL-7). For instance, IL-7 stimulates the expression of Glut-1 transporter, necessary for glucose utilization by the T-cells. Interestingly, IL-7 administration enhances both intestinal epithelial and peripheral lymphocyte numbers and function.

Interestingly, new evidence suggests that IEC-derived IL-7 and IELs play a major role in intestinal mucosal adaptation to nutritional changes. For instance, a recent animal study showed that mice receiving total parenteral nutrition (TPN) but no enteral feeding showed significantly inhibited IL-7 secretion by the IEC compared with mice fed regular chow and intravenous solution (10). The study also showed that this inhibited IL-7 secretion by IECs in the TPN group was associated with significant changes in the IEL phenotype (decreased IEL proliferation and number). Moreover, animal studies show that TPN-administered mice have significantly decreased IEL-derived secretion of the anti-inflammatory cytokine IL-10 and significantly increased secretion of the proinflammatory cytokine IFN-γ. These immunologic changes were associated with declined epithelial barrier function.

Collectively, these findings implicate the important role of dietary factors in activating and maintaining the functional integrity of intestinal inflammatory cells.

In addition to these significant effects of dietary factors on the intestinal immune system, diet is also a major homeostatic factor of systemic immune responses. For

instance, glutamine and glucose constitute the main nutrient sources for peripheral lymphocytes. In patients with acute pancreatitis, the lack of these homeostatic effects of nutrients could add to the immuno-compromised state and susceptibility for infectious complications. Consistently, recent studies have shown that patients with severe acute pancreatitis exhibit a characteristic depletion of subsets of T- and B-lymphocytes early in the course of the disease, which could be explained by the lack of trophic effects of nutrients on lymphocytes.

Another important dietary factor that plays a major role in modulating the immunologic effects of GALT is the undigested carbohydrate that stimulates the growth of beneficial intestinal bacteria or the prebiotics. Studies have shown that prebiotics activate immunologically active cells leukocytes in the GALT. Prebiotics also enhance the secretion of antimicrobial molecules, bacteriocins, and IgA. They also acidify the intestinal luminal contents via increased production of short chain fatty acids. Importantly, one of the short chain fatty acids (butyrate) has been shown to exert anti-inflammatory effects in models of intestinal inflammation.

33.3.3 Role of Intestinal Bacterial Flora in Patients with Acute Pancreatitis

The intestinal tract is bathed with intestinal bacteria with the total number of intestinal luminal bacteria averaging ten times more than the mammalian cells. Immunologically, intestinal intraluminal bacteria are essential for maintaining the function of the intestinal epithelial cells. For instance, luminal bacteria are essential for the optimal expression of intestinal cellular cytoprotective enzymes. Rakoff-Nahoum et al. (11) have shown that the intestinal expression of the cytoprotective protein heat shock protein-70 (HSP-70) is less in mice treated with an oral broad spectrum antibiotic as compared to its expression in the intestine of the control mice. This study highlighted the importance of intestinal bacteria as a crucial mechanism of maintaining the integrity of the intestinal mucosa and modulating the extent of intestinal mucosal inflammation. In agreement, animal models of inflammatory bowel disease do not develop colitis when bred in germ-free conditions. These experimental studies had human implications. For instance, modulating the intestinal luminal bacteria with probiotics have been shown to improve the intestinal permeability and induce the expression of tight junction proteins. Interestingly, recent *in vitro* studies suggest that a degree of selectivity of the intestinal immunomodulatory effects of probiotics exists and that these effects are strain- or even species-specific. For instance, the binding of intestinal epithelial cells to the pathogenic bacteria, salmonella, was competitively inhibited by some probiotics strains, not others. Consistently, probiotics have been used effectively in the management of some intestinal diseases like pouchitis, antibiotics-associated diarrhea and irritable bowel syndrome.

Recent studies have affirmed that characteristic changes exist in the composition of intestinal bacteria of patients with severe acute pancreatitis. These include depletion of the beneficial intestinal bacteria such as lactobacillus. These changes correlate with the onset of pancreatitis (12, 13). In addition to the depletion of the beneficial intestinal luminal bacteria, an overgrowth of colonic pathogens occurs in patients with severe acute pancreatitis. Collectively, these results implicate the significant role of intestinal luminal bacteria in the pathogenesis of the acute pancreatitis.

33.4 MODULATING THE LUMINAL INTESTINAL BACTERIA IN ACUTE PANCREATITIS

Given the aforementioned imbalance of intestinal bacteria flora characteristic of patients with acute pancreatitis, the use of probiotics, prebiotics, and synbiotics (a combination of probiotics and prebiotics) to modulate the intestinal luminal bacteria had been investigated as a therapeutic intervention especially in patients with severe acute pancreatitis, with mixed results (Table 33.1).

Probably, the first prospective clinical study to investigate the effect of prebiotics, not supplemented with probiotics in patients with acute pancreatitis was by Karakan et al. (14). The authors randomized 30 patients with severe acute pancreatitis into two groups. The two groups received 2,000 kcal per day of isocaloric and isonitrogenous jejunal feeding (35% lipids and 20% proteins) supplemented with peripheral parenteral nutrition solution containing 120 g/L of glucose and 50 g/L aminoacid and 20% lipid solution. One group received Multifibers-supplemented tube feeding formula including 0.7 g/100 ml of soluble fibers and 0.8 g/100 ml insoluble fibers, a total of 24 g of fiber per day and compared to the other group (15 patients in each group). Patients in the two groups were similar in age, sex, BMI, the interval between the onset of symptoms and admission and the severity of disease as measured by APACHE II score and Balthazar CT scores. The study showed that the duration of hospital stay was shorter in the prebiotics group as compared to the control group (10 ± 4 vs. 15 ± 6, respectively). Overall complications rate was less in the prebiotics group than in the control group (46.6 vs. 60%, $p < 0.05$). Importantly, markers of systemic inflammatory disease and disease severity (CRP and APACHE II and CT scores) were normalized earlier in the prebiotics group than in the control group. In conclusion, the supplementation of jejunal feeding with the high dose of prebiotics mix (insoluble and soluble fibers) improved clinical outcome and dampened systemic inflammatory disease in patients with severe acute pancreatitis. Unfortunately, this study did not investigate the effects of prebiotics supplementation on the composition of the intestinal bacterial flora. It would be interesting to correlate clinical findings with the expansion of a specific strain or species of intestinal bacteria, which could be enhanced by this prebiotics mix.

The potential benefit of probiotics in patients with severe acute pancreatitis has also been investigated. Recently, in a large multicenter double blind placebo-control clinical trial, the effectiveness of probiotics in patients with predicted severe acute pancreatitis was tested. Besselink and collegues (15) recruited 298 patients with predicted severe acute pancreatitis. Patients were randomized into two groups: one received a multispecies probiotic mix and the other group received placebo. However, both groups received fiber-supplemented (49% prebiotic fiber inulin and oligosaccharides) enteral feeding formula-fed jejunally via a nasojejunal tube. The probiotic mix was a combination of six lactobacillus and bifidobacterium species. These probiotics were selected on the basis of their capacity to inhibit the growth of the pathogens most often cultured from infected necrotizing pancreatitis *in vitro*. The authors stated that probiotics species reported to have been associated with an infectious complication irrespective of the disease were excluded. The authors used a daily dose of 10^{10} bacteria per patient. Patients were assigned to receive the probiotics or placebo at the first occasion after randomization but no later than 72 h after the onset of symptoms of pancreatitis. The primary study end-

Table 33.1
Summary of studies using prebiotics- or synbiotics-supplemented enteral feeding in patients with acute pancreatitis

Study	*Study patients*	*Prebiotics/probiotics*	*Control*	*Results*
Karakan (13)	30 patients with severe acute pancreatitis	Jejunal formula supplemented with both soluble and insoluble fibers	Nonsupplemented jejunal formula[a]	Shorter ICU stay & less inflammatory markers in the prebiotics group
Bessilink (14)	296 patients with predicted severe acute pancreatitis	Multifibers/six strains of probiotics supplemented jejunal feeding	Multifibers supplemented jejunal feeding	Increased mortality in the synbiotics group
Olah (19)	62 patients with severe acute pancreatitis	Four plant fibers/four *Lactobacillus* genera	Four plants fibers	Less incidence of SIRS, MOF and complications in the synbiotics group
Olah (17)	45 patients with acute pancreatitis	Oat fiber/*Lactobacillus plantarum*	Oat fiber	Less infected pancreatic necrosis and abscesses in the synbiotics group

[a]The only study that used prebiotics-supplemented enteral feeding formula

points were the composite of infectious complications (including infected pancreatic necrosis, bacteremia, pneumonia, urosepsis or infected ascites) during admission and 90 days follow-up. The study showed that there was no difference in the rate of infectious complications between the probiotics group as compared to placebo (30 vs. 28%, respectively, $p > 0.05$). However, patients in the group of this selection of probiotics experienced more mortality and bowel ischemia than placebo group (mortality of 16 vs. 6% and bowel ischemia of 6 vs. 0%, respectively). The study concluded that prophylaxis with this specific mix of probiotics should not be administered in patients with predicted severe acute pancreatitis. The aforementioned study suffered few methodological concerns which were extensively discussed by other investigators (16). To summarize, questions were raised regarding the use of this specific mix of probiotics and the lack of clinical studies demonstrating safety of the mix as well as lack of studies on doses of this mix. Given the lack of difference in the rate of infectious complications between the two groups and the fact that gut ischemia was more common in the probiotics group, some investigators raised the concern that the observed increased mortality was indeed a reflection of poor randomization. A closer look at the study design reveals another important observation; both the study and control groups had used fiber-supplemented tube-feeding formula. Accordingly, it could be hypothesized that the addition of prebiotics to the tube feeding could have an effect on the composition of the patients' intrinsic intestinal bacterial flora which may have confounded the clinical effects of the tested probiotics. One of the questions that arise is whether the bifidogenic effects of prebiotics added to the probiotics increased the total intestinal bacterial count in the experimental group, creating a situation of "iatrogenic bacterial overgrowth". Similarly, it could be further questioned whether the clinical outcomes of the aforementioned study was biased by a more favorable one in the prebiotics-supplemented enteral feeding group. In agreement with this postulation, the aforementioned studies showing the favorable effects of prebiotics supplementation of enteral feeding on clinical outcome in patients with severe acute pancreatitis as compared to nonsupplemented enteral feeding formula foster our observation. Collectively, with the lack of fecal microbiological analysis in the aforementioned studies testing the role of combining probiotics and prebiotics in patients with severe acute pancreatitis, it is difficult to relate observed clinical effects to the administered probiotics or to the patients' own intrinsic bacteria being stimulated by the used prebiotics. For such studies, we suggest the use of three study groups, a nonsupplemented enteral feeding group, an only prebiotics group and a symbiotic group.

Based on the previous study, prebiotics would have the potential advantage of promoting patients' own commensal beneficial intestinal bacteria without undesired risks of introducing exogenous bacterial species.

Earlier studies have shown that the use of synbiotics reduces bacterial translocation and inhibit the extent of cellular injury in different models of stress (17). For these reasons, the effectiveness of synbiotics in the management of patients with acute pancreatitis has been investigated. For instance, Olah and collegues randomized 45 patients with acute pancreatitis into two groups (18–20). Both groups received jejunal feeding of isocaloric feeding formulas supplemented with 10 g of oat fiber. The treatment group (22 patients) received live lactobacillus plantarum 299, 10^9 organisms during the first 7 days and the control group (23 patients) received a similar dose of heat-inactivated lactobacillus plantarum 299. Severe acute pancreatitis was diagnosed in 15 patients in the control

group and in 17 patients in the synbiotics group. The outcome variables studied were rates of pancreatic necrosis and infection (diagnosed by culture and gram staining of samples obtained by CT or ultrasonographically guided fine-needle aspiration or through direct samples from the pancreatic tissue if laparotomy was required), organ failure, septic complications requiring surgical procedures, length of hospital stay, and death. Of all the outcome measures, the rate of pancreatic infection was significantly lower in the synbiotics group than the control group (30 vs. 5%, $p < 0.05$). Moreover, patients requiring surgical interventions were fewer in the synbiotics group as compared to the control group (22 vs. 5%, $p < 0.05$). The same group recently studied the effectiveness of another synbiotics (a mix of four different lactobacillus genera and four different plant fibers)-supplemented jejunal feeding in patients with severe acute pancreatitis. The study included 62 patients and feeding was initiated within 24 h after admission. Consistently, the study showed that septic complications related to pancreatitis (infected pancreatic necrosis or abscesses) were significantly lower in the synbiotics group.

33.5 CLINICAL EFFECTS OF PREBIOTICS IN PATIENTS WITH ACUTE PANCREATITIS

Interestingly, beyond their inherent advantage of restoring intestinal luminal bacteria prebiotics exert metabolic effects that could add to their clinical benefits in patients with acute pancreatitis.

33.5.1 Metabolic Effects

Patients with acute pancreatitis in need of enteral feeding experience a variety of other concomitant ailments that may associate or predispose to increased morbidity and/or mortality. Besides their ability to promote bacterial proliferation and activity, prebiotics possess distinct metabolic and endocrinal effects. In the next paragraph, we will discuss the clinical effects of prebiotics that could affect the course of the disease added to their inherent effects of stimulating the proliferation of intestinal bacterial flora and possibly inhibiting bacterial translocation.

For instance, inulin-like prebiotics possess low calorie content and get degraded distally in the intestinal tract by colonic bacteria making them a favorable source of carbohydrate for critically ill patients with stress-induced hyperglycemia, patients with insulin resistance, and in diabetic patients as well.

Similarly, studies have shown that prebiotics exert antihypertriglyceridemic effect. These interesting findings were described initially in animal studies when rats were fed significant amounts of FOS (50–200 g/kg of rat chow) supplemented in their diet (21). The study showed that FOS significantly lowers fasting triglyceride concentration. The same group of investigators later suggested that glucagon-like peptide1 (GLP-1) may mediate the lipid-lowering properties of FOS in rats (22). A recent meta-analysis by Brighenti pooling the human studies of inulin-like prebiotics, especially inulin and FOS, on serum triglycerides, the intake of inulin-like prebiotics was associated with significant decreases in serum triglycerides by 0.17 mmol/L (23).

Prebiotics also stimulate the absorption of electrolytes especially magnesium and calcium making them favorable in patients with acute pancreatitis who are at risk of

nutritional depletion as part of the disease process. However, conflicting results of the effects of different types and doses of prebiotics on mineral absorption make it difficult to give specific recommendations until more research is conducted.

33.5.2 Anti-infective Effects

Critically ill patients with acute pancreatitis, especially the severe form of the disease are prone to increased risks of superimposed infection. For instance, Bessilink and colleagues recently showed in their study of 173 patients with acute pancreatitis that infections occur early in the course of disease and that 80% of patients who died of the disease had infections. They also reported that in patients with parenchymal pancreatic necrosis, bacteremia was associated with increased risk of infected necrosis. The authors concluded that infections have a significant impact on mortality and that prophylactic measures should focus on early intervention (24).

Studies have shown that prebiotics promote the proliferation and/or the metabolic activities of beneficial intestinal bacteria which may competitively inhibit the binding of pathogenic bacteria to the intestinal epithelial cells. For instance, et al studied the effects of dietary supplementation of FOS on the intestinal bacterial composition. They have shown that FOS promotes the growth of bifidobacteria. In addition to the inulin-like prebiotics some fibers like oat and gum arabic exert the same prebiotic effect. Consistently, both *in vitro* and *in viv*o studies show that prebiotics could be used effectively in the management of intestinal infections. For instance, FOS has been shown to induce colonization resistance to *C. difficile*. In addition, in a large well-designed randomized clinical trial, Lewis and colleagues randomized 142 patients with *C. difficile* after initial treatment with antibiotics into two groups: one group received FOS (12 g/day) and was compared to a placebo group receiving sucrose. Both groups were followed for a period of 30 days. The study showed a significant reduction in the relapse rate of diarrhea in the prebiotics group as compared to placebo (8.3 vs. 34.3%, $p < 0.001$, respectively). Importantly, stool culture showed increase in fecal bifidobacteria from baseline in the prebiotics group (25). It could be hypothesized that the favorable effects of the prebiotic fibers on the intestinal function and their ability to reduce intestinal infections could partly explain their favorable clinical outcome in patients with acute pancreatitis, especially in the severe form of the disease. In agreement, a recent study in 342 infants compared the effects of prebiotics (galacto- and fructo-oligosaccharides) supplemented formula on the incidence of intestinal and respiratory tract infections as compared to nonsupplemented formula. The study showed that the incidence of gastroenteritis and the percentage of children with multiple antibiotic courses/year was lower in children receiving prebiotics as compared to the control group (26).

33.6 SIDE EFFECTS OF PREBIOTICS IN PATIENTS WITH SEVERE ACUTE PANCREATITIS

Generally, reports of harmful effects of inulin like fructans and soluble fiber prebiotics are scarce. However, case reports related the use of insoluble fiber-supplemented tube feedings to the increased risk of intestinal ischemia. There is no controlled randomized study to substantiate such findings. The use of a blend of soluble and insoluble fibers may

mimic the normal dietary fiber intake. Interestingly, the aforementioned study by Karakan and colleagues is probably the first controlled study to use of a blend of soluble and insoluble fibers in critically ill patients with severe acute pancreatitis (14). In contrast, the study showed improved clinical outcomes in the group receiving the fiber-supplemented jejunal feeding. Moreover, fiber-supplemented feeding associated with reduced mortality was reported in this. No incidents of intestinal ischemia were reported.

A clinically relevant issue is whether the use of fiber-supplemented tube feeding formulas may potentially increase the osmolality of the feeding formula which may be associated with any unwanted gastrointestinal symptoms of intolerance like flatulence, bloating, and possibly diarrhea in patients with acute pancreatitis. Consistently, an earlier study by Sobotka et al. (27) investigated the gastrointestinal effects of inulin-supplemented formula on 9 patients with gastrointestinal diseases (3 inflammatory bowel disease, 2 chronic pancreatitis, and 1 duodeno-pancreatectomy). Patients received jejunal feeding formula for 1 week and the then inulin was dissolved in water and added to the formula for another week. The study showed that inulin supplementation of jejunal feeding increased flatulence and increased stool frequency and consistency, though not statistically different before the use of inulin. This increased intolerance associated with the supplementation of inulin in large doses (30–37.5 g/day/patient) dissolved in water and added to the feeding formula may have been explained by substantial increases in formula osmolality and potential formula contamination. Importantly, this study suggests that the gastrointestinal effects of prebiotics may be dose-dependent. Consistently, in the early mentioned study by Lewis et al. (25) demonstrating beneficial effects of FOS on *C. difficile* relapse, the authors reported in a pilot work of their own that when patients received higher doses of FOS, they experienced bloating and discomfort. Therefore, the appropriate dose of and the differences between prebiotics strains should be thoroughly considered when comparing the gastrointestinal effects of different prebiotics. Consistently, a meta-analysis of the clinical and physiological effects of fiber-containing enteral feeding formulas indicate that supplementing enteral formulas with mixtures of fiber may mimic the dietary recommendations and be more favorable than with single fibers (28).

33.7 DISTINCT EFFECTS OF DIFFERENT PREBIOTICS?

Akin to probiotics, the clinical effects of prebiotics may differ based on the disease setting and on the probiotics species used in the study. For instance, while the use of the probiotics strain *Saccharomyces bolardii* was consistently shown to prevent recurrent *C. difficile* colitis, the effectiveness of another probiotics strain, *Lactobacillus* GG, is inconsistent. Whether there are differences in clinical effects of different types of prebiotics used in the nutritional management of patients with acute pancreatitis is still to be elucidated. Before we address the question whether all prebiotics exert the same immunomodulatory effects in patients with severe acute pancreatitis, let us review the available data on the prebiotics types used to supplement enteral feeding formulas. The main sources of prebiotics supplemented to the tube-feeding formulas belong to either fibers (like oat, gum Arabic), a group of prebiotics called inulin-like prebiotics or commonly a mix of more than one type. The inulin-like prebiotics includes a group of oligo-saccharides that vary depending on the degree of polymerization (PD). For instance, FOS has a shorter PD than inulin with the assumption that the shorter the PD the more chances these will be fer-

mented proximally in the colon without causing gastrointestinal intolerance. The scientific evidence of this assumption is still needed. Although studies have compared the bifidogenic effects of different prebiotics, comparative studies of clinical effects are still missing. For instance, earlier work by Gibson and Wang (29) showed that FOS possesses a significant bifidogenic effects as compared to sucrose and inulin. However, *in vivo* studies supplementing human subjects with either fiber did not reveal any significant differences, although a trend of higher bifidogenic effect of FOS as compared to inulin. Importantly, the bifidogenic effect of different prebiotics in human subjects is certainly confounded by the composition of the individual's own intestinal bacterial flora. Interestingly, current evidence suggests that the host original intestinal bacterial composition content may play a critical role determining the bacterial enhancing effects of prebiotics. However, whether these differences in physical characteristics translate into distinct clinical effects especially in patients with acute pancreatitis remain to be investigated.

33.8 CONCLUSIONS AND PERSPECTIVES

In conclusion, prebiotics restore the balance of the beneficial and pathogenic intestinal bacteria. They also restore the homeostatic functions of the gut-associated lymphoid tissue. These effects of prebiotics make them a very exciting nutritional intervention in patients with acute pancreatitis. Future areas of investigation are the selectivity and optimum doses of different prebiotics.

REFERENCES

1. Marshall JC, Charbonney E, Gonzalez PD (2008) The immune system in critical illness. Clin Chest Med 29(4):605–616
2. Rahman SH, Ammori BJ, Holmfield J, Larvin M, McMahon MJ (2003) Intestinal hypoperfusion contributes to gut barrier failure in severe acute pancreatitis. J Gastrointest Surg 7(1):26–35
3. Wang GJ, Gao CF, Wei D, Wang C, Ding SQ (2009) Acute pancreatitis: etiology and common pathogenesis. World J Gastroenterol 15(12):1427–1430
4. Liu H, Li W, Wang X, Li J, Yu W (2008) Early gut mucosal dysfunction in patients with acute pancreatitis. Pancreas 36(2):192–196
5. Ammori BJ, Leeder PC, King RFGJ et al (1999) Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J Gastrointest Surg 3:252–262
6. Powell JJ, Murchison JT, Fearon KC, Ross JA (2000) Siriwardena AK.Randomized controlled trial of the effect of early enteral nutrition on markers of the inflammatory response in predicted severe acute pancreatitis. Br J Surg 87(10):1375–1381
7. Hegazi RA, Rao KN, Mayle A, Sepulveda AR, Otterbein LE, Plevy SE (2005) Carbon monoxide ameliorates chronic murine colitis through a heme oxygenase 1-dependent pathway. J Exp Med 202(12):1703–1713
8. Ma X, Torbenson M, Hamad AR, Soloski MJ, Li Z (2008) High-fat diet modulates non-CD1d-restricted natural killer T cells and regulatory T cells in mouse colon and exacerbates experimental colitis. Clin Exp Immunol 151(1):130–138
9. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116(11):3015–3025
10. Yang H, Sun X, Haxhija EQ (2007) Teitelbaum DH.Intestinal epithelial cell-derived interleukin-7: A mechanism for the alteration of intraepithelial lymphocytes in a mouse model of total parenteral nutrition. Am J Physiol Gastrointest Liver Physiol 292(1):G84–G91

11. Rakoff-Nahoum S, Hao L, Medzhitov R (2006) Role of toll-like receptors in spontaneous commensal-dependent colitis. Immunity 25(2):319–329
12. Andersson R, Wang XD (1999) Gut barrier dysfunction in experimental acute pancreatitis. Ann Acad Med Singapore 28(1):141–146
13. Leveau P, Wang X, Soltesz V, Ihse I, Andersson R (1996) Alterations in intestinal motility and microflora in experimental acute pancreatitis. Int J Pancreatol 20(2):119–125
14. Karakan T, Ergun M, Dogan I, Cindoruk M, Unal S (2007) Comparison of early enteral nutrition in severe acute pancreatitis with prebiotic fiber supplementation versus standard enteral solution: a prospective randomized double-blind study. World J Gastroenterol 13(19):2733–2737
15. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Nieuwenhuijs VB, Bollen TL, van Ramshorst B, Witteman BJ, Rosman C, Ploeg RJ, Brink MA, Schaapherder AF, Dejong CH, Wahab PJ, van Laarhoven CJ, van der Harst E, van Eijck CH, Cuesta MA, Akkermans LM (2008) Gooszen HG; Dutch Acute Pancreatitis Study Group. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 371(9613):651–659
16. Reid G, Gibson G, Sanders ME, Guarner F, Versalovic J, International Scientific Association for Probiotics and Prebiotics (2008) Probiotic prophylaxis in predicted severe acute pancreatitis. Lancet 372(9633):112–113
17. Bengmark S (2001) Nutritional modulation of acute- and "chronic"-phase responses. Nutrition 17(6):489–495
18. Oláh A, Belágyi T, Issekutz A, Gamal ME, Bengmark S (2002) Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. Br J Surg 89(9):1103–1107
19. Oláh A, Belágyi T, Issekutz A, Olgyai G (2005) Combination of early nasojejunal feeding with modern synbiotic therapy in the treatment of severe acute pancreatitis (prospective, randomized, double-blind study). Magy Seb 58(3):173–178
20. Oláh A, Belágyi T, Pótó L, Romics L Jr, Bengmark S (2007) Synbiotic control of inflammation and infection in severe acute pancreatitis: a prospective, randomized, double blind study. Hepatogastroenterology 54(74):590–594
21. Roberfroid M (1993) Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. Crit Rev Food Sci Nutr 33(2):103–148
22. Kok NN, Morgan LM, Williams CM, Roberfroid MB, Thissen JP, Delzenne NM (1998) Insulin, glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide and insulin-like growth factor I as putative mediators of the hypolipidemic effect of oligofructose in rats. J Nutr 128(7):1099–1103
23. Brighenti F (2007) Dietary fructans and serum triacylglycerols: a meta-analysis of randomized controlled trials. J Nutr 137(11 Suppl):2552S–2556S
24. Besselink MG, van Santvoort HC, Boermeester MA, Nieuwenhuijs VB, van Goor H, Dejong CH, Schaapherder AF, Gooszen HG, Dutch Acute Pancreatitis Study Group (2009) Timing and impact of infections in acute pancreatitis. Br J Surg 96(3):267–273
25. Lewis S, Burmeister S, Brazier J (2005) Effect of the prebiotic oligofructose on relapse of Clostridium difficile-associated diarrhea: a randomized, controlled study. Clin Gastroenterol Hepatol 3(5):442–448
26. Bruzzese E, Volpicelli M, Squeglia V, Bruzzese D, Salvini F, Bisceglia M, Lionetti P, Cinquetti M, Iacono G, Amarri S, Guarino A (2009) A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. Clin Nutr 28(2):156–161
27. Sobotka L, Brátova M, Slemrová M, Manák J, Vizd'a J, Zadák Z (1997) Inulin as the soluble fiber in liquid enteral nutrition. Nutrition 13(1):21–25
28. Elia M, Engfer MB, Green CJ, Silk DB (2008) Systematic review and meta-analysis: the clinical and physiological effects of fibre-containing enteral formulae. Aliment Pharmacol Ther 27(2):120–145
29. Gibson GR, Wang X (1994) Enrichment of bifidobacteria from human gut contents by oligofructose using continuous culture. FEMS Microbiol Lett 118(1–2):121–127

34 Probiotics and Immunomodulation

O.N. Donkor, T. Vasiljevic, and H.S. Gill

Key Points

- The human gastrointestinal tract harbors a vast ensemble of microbes representing over 500 different species.
- In a healthy state, a fine balance exists between the populations of health-promoting and potentially harmful bacteria.
- Gut microflora plays an important role in the development, maturation and functioning of the immune system.
- Disruptions in the intestinal homeostasis result in increased susceptibility to infectious diseases, cancers and immunoinflammatory disorders.
- Many factors such as enteric infections, intake of antibiotics and stress could disturb the intestinal microbial homeostasis.
- Intake of probiotic organisms have been shown to restore/optimize gut microbial balance and promote health and well-being.
- Lactobacilli and bifidobacteria are commonly used as probiotics.
- Specific strains of probiotics are able to modulate the functioning of the immune system in health and disease. Probiotics enhance immune function in healthy individuals, but down-regulate dysfunctional immune responses in subjects suffering from immunoinflammatory disorders such as allergies and inflammatory bowel diseases.
- Probiotic-induced immune stimulation is associated with increased protection against intestinal and extra-intestinal infections and cancers, and improved efficacy of vaccines.
- The mechanisms by which probiotics mediate their disparate immunological effects in health and disease are not fully understood.

Key Words: Probiotics, immune enhancement, immunoregulation, gastrointestinal tract, intestinal flora, *lactobacillus, bifidobacterium.*

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_34

34.1 INTRODUCTION

Colonization of the mammalian gastrointestinal tract (GIT) begins immediately after birth, where the establishment and maintenance of the microbial community is influenced by the method of birth, health status, the organism's age, diet and the environment (1, 2). Bacterial populations in the infant colon progressively change from primary colonizing facultative anaerobes, such as *Escherichia ecoli* and enterococci, to communities that contain increasingly diverse assemblages of strict anaerobes, until by about 2 years of age when they begin to resemble adult-like microbiotas (3). Some organisms are able to persist in the gut and become dominant populations, while in contrast other species are transient and may only be detectable for a few days (4). In order for the intestine to function optimally, however, the "balance" of the bacterial flora must be maintained, and this appears to be increasingly difficult as lifestyles have changed. An increase in stress and modern day living, which makes a consequential demand on the immune system, can disrupt homeostasis in the gut. Similarly, the direct effects of a change in dietary patterns and eating habits can affect overall gut functionality. Another contributory factor includes the consumption of pharmaceutical compounds, in particular antibiotics, which by design destroy bacteria, and therefore can have a harmful effect on the balance of the gut microbiota. All of these combine to shift the balance of the gut microflora away from potentially beneficial or health-promoting bacteria such as the lactobacilli and bifidobacteria, towards an increase in potentially harmful or pathogenic micro-organisms, like the clostridia, sulfate-reducers and proteolytic *Bacteroides* species. The predominance of the latter may pre-dispose towards a number of clinical disorders, including bowel cancer and inflammatory bowel diseases such as ulcerative colitis, while making the host more susceptible to infections by transient enteropathogens such as *Salmonella*, *Campylobacter*, certain species of *E. coli* and *Listeria*. It is of considerable benefit to the host, therefore, to maintain a good microbial community structure, through increased levels of bacteria such as lactobacilli and bifidobacteria, preferably at the expense of more harmful organisms. This gives justification to the use of probiotics as a way forward for the prophylactic treatment of enteropathogenic infections, as well as contributing towards protection against various intestinal diseases and disorders – especially those mediated by individual pathogens. What remains to be established is the extent to which probiotic organisms can be beneficial, to determine how any benefits may be manifested and to recognize any limitations (5–7).

Probiotics are defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (8). A wide variety of species and genera could be considered potential probiotics (9). Examples of commercially important probiotic strains include *Lactobacillus* (Lb) *rhamnosus* GG, *Lb. casei* Shirota, *Lb. johnsonii* La1 and *B. lactis* Bb12. While many health benefits of probiotics have been reported (10, 11), only a few including modulation of the immune system have been well documented (12).

The GIT comprises a highly dynamic environment, where nutritional, medicinal, hormonal, and endogenously synthesized molecules are utilized and metabolized by both the host and microbiota alike. However, evidence indicates that one should not consider gut microbiota as simply autonomous residents of the host GIT, but rather important contributors in defining the gut ecosystem (13). Previous research has implicated nonpathogenic bacteria as factors regulating mucosal inflammation (14, 15) and host defense (1). Enteric bacteria have also been demonstrated to supply energy

substrates to epithelial cells, such as short-chain fatty acids produced through bacterial fermentation (14). Indeed, an essential partnership has evolved between gastrointestinal epithelial cells, immune cells, and resident bacteria for which the normal function and activity of each is dependent on the other two components (2). However, it is poorly understood to what extent these microbial species can influence the development and functions of the host GIT. Although the focus here will be on commensal microbiota, that is, those microbial species present in the healthy gut, emerging evidence suggests that many microbial species (pathogenic, symbiotic, and commensal) rely on and/or exploit this partnership to survive. With over 400 different species thought to inhabit the adult gut, of which only a small percentage have been successfully cultured *in vitro*, it is clear that the complete understanding for the presence and function of all bacterial species is far from ascertained (10, 16, 17).

In addition to the differences in intestinal morphology and immune functions, the presence/absence of microbiota modulates water absorption, luminal pH, the concentration of deconjugated bile acids and the oxidation–reduction potentials of the gut (18). Furthermore, significant evidence indicates that the gut microbiota plays an important role in the metabolism and bioavailability of nutritional compounds, such as flavonoids (19), phytoestrogens (20, 21) and carbohydrates (22).

As hundreds of different microbial species may be present at any given time in the GIT, it has yet to be described how this complex bacterial community interacts with the host to modulate function along the length of the GIT. Certain differentially regulated genes contribute directly to significantly modified biological functions displaying regional specificity; however, the most significant biological processes altered in the host GIT by the presence of microbiota are immune response and water transport. Furthermore, immune response and water transport are consistently modulated in all GIT regions examined, reinforcing the concept that the normal healthy gut is, in part, dependent on the molecular relationship between the host and commensal microflora (23).

34.2 THE IMMUNE SYSTEM

In humans, the immune system is partially open to the external influences and its role becomes evident when it is defective. Thus, inherited and acquired immunodeficiency states are characterized by increased susceptibility to infections, sometimes caused by commensal organisms not normally considered to be pathogenic. The immune system can be divided into two general classes: innate (natural or non-specific) and adaptive (acquired or specific) immunity, which work in concert. The adaptive immune system develops late in the phylogeny, and most species survive without it. However, this is not true for mammals, which have an extremely sophisticated adaptive immune system of both systemic and mucosal (local) type. There appears to be great redundancy of mechanisms in both systems providing robustness to ensure that essential defense functions are preserved (24).

34.3 INNATE IMMUNE SYSTEM

An infectious agent entering the body is immediately recognized and counteracted by the innate immune system, which comprises surface barriers, soluble factors, specialized phagocytes and dendritic cells (DCs). DCs along with macrophages and

monocytes provide an interface between the innate and adaptive immune systems as they act as professional "antigen-presenting cells" (APCs). This "bridging" role is crucial in initiating the adaptive immune response, as T cells do not respond to free-antigen but only to antigen that is presented by APCs. Together, these functions constitute a primary layer of natural defense against invading microorganisms, with the common goal of restricting their entry into the body by providing a physical or structural hindrance and clearance mechanisms such as epithelial linings of skin and mucosae, mucus, ciliary function and peristalsis. Chemical factors such as the pH of body fluids, numerous antimicrobial peptides and proteins as well as phagocytic cells, for example, neutrophils, eosinophils, monocytes/macrophages and DCs constitute further innate protective mechanisms. Challenges to the innate system often lead to the activation of the adaptive immune system, which aids substantial recovery from infection (24).

34.3.1 Activation of the Innate System

Germline encodes for the recognition molecules involved in the innate immunity. This system is very similar among healthy individuals with no apparent memory effect; therefore, re-exposure to the same antigen will normally elicit more or less the same type of response. These receptors perceive particular molecular structures essential for microbial survival and ubiquitous in many types of bacteria, including endotoxin or lipopolysaccharide, teichoic acids and unmethylated cytosine and guanine separated by a phosphate (CpG) motifs of DNA (25). Although such structures are generally called pathogen-associated molecular patterns (PAMPs), they also occur in commensal bacteria (26). However, the intestinal microflora may induce quiet different molecular programming of the innate immune system, which likely explains why the indigenous microbiota is normally tolerated by the host (27). The cellular receptors of the innate immune system that recognize PAMPs as "danger signals" are called pattern recognition receptors (PRRs), many of them belonging to the so-called Toll-like receptors (TLRs). They are expressed mainly by macrophages and DCs, but also by a variety of other cell types such as B- and epithelial cells (26). The engagement of PRRs causes cellular activation. In the case of professional APCs, which present the antigen to B and T cells, this leads to maturation accompanied by the production of cytokines and up-regulation or down-regulation of cell-surface molecules according to strictly defined kinetics (28). Such signaling molecules will critically influence further induction of both innate and adaptive immunity with regard to effector potency, particularly the polarization of T-cell responses in terms of cytokines. Engagement of other types of receptors on phagocytic cells such as Ig Fc and complement receptors triggers phagocytosis and the elimination of invading microorganisms (29). Although pathogens have evolved mechanisms to evade the innate immunity (e.g. bacterial capsules), they usually cannot persist within the body when an adaptive immune response reinforces the innate immunity by providing specific antibodies directed against the invading pathogens or their toxins. Thus, the innate and adaptive immune systems are not independent; the innate immunity influences the character of the adaptive response, and the effector arm of the adaptive response support several innate defense mechanisms (24).

34.4 ADAPTIVE IMMUNE SYSTEM

The adaptive system, also termed specific immunity, is acquired through interactions with the environment. The innate and adaptive systems are highly integrated and interdependent (30) with the adaptive system subject to induction, anticipation (immune memory) and clonal expansion. Responses of the adaptive immune system are central to the body's ability to eliminate bacterial, viral and parasitic infections; therefore, understanding these responses is the key to understanding the mechanisms of allergy, autoimmunity, vaccination, carcinogenicity and organ graft rejection or acceptance. Humans as mammals have developed an extremely sophisticated adaptive immune system of both systemic and mucosal (local) type (31).

The cells involved in adaptive responses are thymus (T)-derived cytotoxic T lymphocytes (CTL) and helper T (Th) lymphocytes, bone (B) marrow-derived lymphocytes and assorted accessory cells (DCs, macrophages and stromal cells). Lymphocytes (along with several of their accessory cells) circulate to and through the spleen, lymph nodes, specialized lymphoid tissue in the gut (Peyer's patches and appendix), bronchi and oropharynx (adenoids and tonsils). These "secondary" sites serve as clonal generating stations for T and B lymphocytes, once the appropriate antigen (from bacterial, viral or parasitic sources) has been encountered. Thus, an army of identical and antigen-specific lymphocytes is uniquely directed at the offensive antigen, ensuring its elimination in normal animals, including humans. Cytotoxic T lymphocytes destroy virus-infected cells by making direct contact with the latter in order to induce cytolysis. Th lymphocytes can be classified as Th1, Th2 or T regulatory (Treg)/Th3 cells, based upon their cytokine profiles. Treg cells produce IL-10 and TGF-β and are able to down-regulate skewed Th1/Th2 immune response. Although there are three subsets of Th lymphocytes, our focus will be on Th1 and Th2. Th1 cells as opposed to CTL, "activate" the appropriate accessory cell, and it is the latter that then delivers the cytolytic blow. Th1 cells are the target of the AIDS virus, and when the vital Th1 cells are crippled throughout the body (advanced AIDS) opportunist infections ultimately kill the infected host (human or animal). Th2 lymphocytes, on the other hand, activate B lymphocytes whose function is to produce an appropriate antibody against the offensive antigen, leading to its destruction and elimination (30).

34.4.1 Adaptive Immune Responses

The purpose of adaptive immunity is primarily to combat infections by preventing colonization of pathogens and keep them out of the body (immune exclusion), and to seek out specifically and destroy invading microorganisms (immune elimination). In addition, specific immune responses are, through regulatory mechanisms, involved in the avoidance of overreaction (hypersensitivity or allergy) against harmless antigens as well as discrimination between components of "self" and "non-self". Autoimmunity occurs when the latter control mechanism breaks down (32). It follows from the previous section that an adaptive immune response includes every aspect of cellular activation, differentiation and all other biological mechanisms induced when specific immunity is elicited. Both the primary and secondary stimulation depends on professional APCs, which express major histocompatibility complex (MHC) class II determinants as genetically determined restriction elements for $CD4^+$ Th cells (29). In this

manner, the T cell receptors specifically recognize short immunogenic peptide sequences of the antigen that each is presented in the polymorphic grove of an MHC molecule. The ability of the adaptive immune system to distinguish self from non-self likewise depends largely on the structure of the MHC molecules, which are slightly different in each individual except for homozygous (identical) twins. The immune response may also involve polymorphic MHC class I molecules and $CD8^+$ T cells with cytotoxic and/or suppressive potential (29). All of these cell categories are present in secondary lymphoid organs and at immunological effector sites where the primed immune cells extravasate by means of "homing molecules", which differ markedly between the systemic and the mucosal immune system. A long-lasting secondary immune response gives rise to abundant differentiation of effector cells and release of biologically active substances, aiming at the neutralization and elimination of antigens through a variety of targeted strategies. Such immunological effector mechanisms, and the non-specific biological amplification often triggered by them via hyperactivation of innate immunity, are collectively referred to as immune reactions (24). Adaptive immunity is thus based on specific immune responses but expressed by an array of cellular and humoral immune reactions. The effector cells of the B-cell system are the terminally differentiated Ig (immunoglobulin)-producing plasma cells. These immunocytes constitute the basis for so-called humoral immunity, which is mediated by circulating antibodies comprising five Ig classes (IgG, IgA, IgM, IgD and IgE). The antigen-specific receptor on the surface of the B lymphocyte is a membrane-bound form of Ig produced by the same cell (29, 32). The engagement of surface Ig by corresponding antigen will, in co-operation with "help" provided by cognate Th cells, initiate B-cell differentiation and clonal expansion. The resulting effector B cells can then transform into plasma cells that secrete large amounts of antibody with the same specificity as that of the antigen receptor expressed by the progenitor B lymphocyte. Whereas IgM (primary response) and IgG (secondary response) dominate systemic humoral immunity, IgA is normally the dominating antibody class of mucosal immunity (24).

When adaptive immunity is mainly mediated by activated effector T cells and macrophages, the reaction is referred to as cell-mediated or delayed-type hypersensitivity (DTH). Whether humoral or cell-mediated immunity will dominate, depends largely on the cytokine profile of the activated Th cells. Cytokines are polypeptide messenger substances that stimulate cellular growth, differentiation and functional development via specific receptors on the producer cell itself (autocrine function) or on immediately adjacent cells (paracrine function). Cytokines derived from leukocytes are traditionally designated by the prefix interleukin (IL). Notably, however, cytokine action is not confined to the immune system; such peptides may also influence the central nervous system and the neuroendocrine system (24, 33).

34.4.2 $CD4^+$ T Cell Differentiation

As previously stated, Th cells are mainly found in four distinct cell types: Th1, Th2 or Treg/Th3. Treg cells produce IL-10 and TGF-β and are able to down-regulate skewed Th1/Th2 immune response. However, the recent discovery of Th17 cells confirmed earlier evidence that helper T cells may adopt phenotypes other than Th1 and Th2 (34). The Th17 cells are characterized by the production of IL-17 that drives rapid neutrophil

recruitment in response to bacterial and fungal infections (35, 36) and is thought to play a role in various autoimmune disorders (37). The Th17 phenotype does not only provide convincing evidence for the existence of more than two helper T phenotypes but also links the fairly separate fields of Th1/Th2 and Treg immunology to each other. As a result, the Th1/Th2 paradigm is currently being extended into a framework that includes at least Th1, Th2, Th17 and Treg cells (38), which functionally correspond to promoting the cellular, humoral, rapid antibacterial/antifungal and anti-inflammatory response, respectively. A balance between Th1/Th2 is pivotal for immunological homeostasis, and the polarization of immune responses towards Th1 or Th2 underlies the development of various immunoinflammatory disorders. For example, allergies are driven by the over-activation of Th2 immune responses and inflammatory bowel disease (IBD) and autoimmune disorders such as Type 1 diabetes are predominantly driven by Th1 type immune responses. Evidence from recent studies suggests that defective Treg cell activity may be the central cause for the concurrent rise in Th1 and Th2-mediated diseases observed over the recent decades; patients with Type 1 diabetes, multiple sclerosis and with a predisposition to allergy development are known to exhibit deficient Treg cell function (39). Th1 cells produce pro-inflammatory cytokines such as IFN-γ, TNF-α and IL-2, while Th2 cells produce the cytokines IL-4, IL-5, IL-6 and IL-13. The cytokines produced by Th1 cells stimulate phagocytosis and the destruction of microbial pathogens while Th2 cytokines such as IL-4 generally stimulate the production of antibodies directed towards large extracellular parasites. IL-5 stimulates eosinophil responses, also part of the immune response towards large extracellular parasites.

On the negative side, Th1 pathway seems to be involved in organ-specific autoimmune diseases such as arthritis and multiple sclerosis when it is overreactive, while the overexpressed Th2 pathway has been indicated as an underlying allergy. The differentiation of naive T cell into either Th1 or Th2 cells is generally dependent on environmental conditions (DCs, cytokines in the milieu, nature and dose of antigen, etc.). The differentiation proceeds within a few days of direct contact of naive T cells with APCs. The process by which commitment develops is called polarization. The naive T cells may pass through a transient state (Th_o) on their way to becoming activated cells (Fig. 34.1). The polarization of the Th cells could be indicative of a more profound polarization of the immune system as a whole. In fact, there is good evidence that this polarization already begins with those cells having the primary contact with antigens, including DCs, macrophages and other APCs. Immunological memory involves the fast recall of cytokine expression by Th lymphocytes. Two distinct profiles of cytokine expression, Th1 and Th2, can be induced by antigen and polarizing signals during the activation of naive Th cells and can subsequently be reexpressed on stimulation by the antigen alone. It now seems that Th1 differentiation is reliant on IFN-γ and IL-12 whereas Th2 development relies on IL-4. The presence of IL-12 induces the signal transducers and activators of transcription-1 (STAT) dependent signaling cascade that up-regulates the expression of the T bet transcription factor, a master regulator that coordinates expression towards Th1 differentiation. T-bet induces IFN-γ and IL-12R β chain expression, which enables STAT-4-mediated signaling and a further increase in IFN-γ production. T-bet also prevents differentiation towards Th2 by suppressing the expression of the factors required for the Th2 subset differentiation process. The transcription factor GATA-3 induces Th2 development. GATA-3 is activated by the Th2-polarizing stimulus, IL-4, IL-5 and IL-13

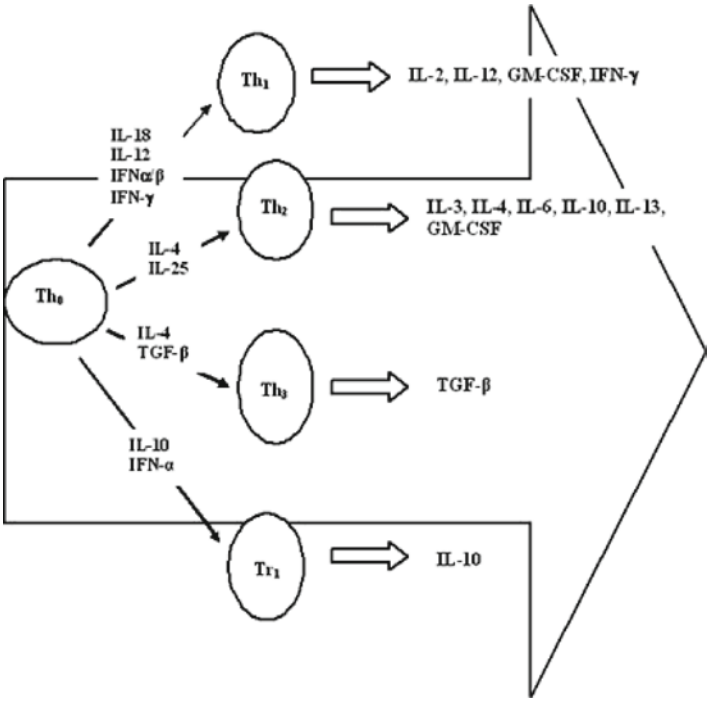


Fig. 34.1. T-cell sub-populations and the cytokines required for their differentiation (adapted from Meyers (33)).

and has recently been observed to autoactivate its transcription (40, 41). GATA-3 also suppresses the critical elements for the Th1 differentiation process (e.g. STAT-4 and IL-12R). Factors involved in the Th1 response negatively impact the Th2 response and vice-versa. This regulatory loop allows full differentiation towards one subset or the other once the decisional process has been initiated.

To summarize, the IL-12 and IFN-γ cytokines and the STAT-1, STAT-4 and T-bet transcription factors are associated with a Th1 response, whereas the IL-4, IL-5 and IL-13 and the STAT-6 and GATA-3 transcription factors are linked to a Th2 response (42). Other cytokines have been associated with Th1 type responses (e.g. IL-2, lymphotoxin) or Th2 type responses (e.g. IL-6, IL-9, IL-10), but their production does not necessarily characterize the Th1 or Th2 type response (43).

34.5 THE MUCOSAL IMMUNE SYSTEM

Mucosal immunity is the first line of protection that reduces the need for systemic immunity, which is principally proinflammatory. The mucosal immune system consists of two non-inflammatory layers of defense namely immune exclusion, performed by secretory antibodies to inhibit surface colonization of microorganisms and dampen the penetration of potentially dangerous soluble substances, and immunosuppressive mechanisms to avoid local and peripheral hypersensitivity to antigens that are normally innocuous (44). The latter mechanism is referred to as “oral tolerance” when induced

via the gut and is likely a reason for persistent food protein allergy to be relatively rare (24). This oral tolerance is provided through the suppression of Th1 cells by IL-4, IL-10 and TGF-β when exposed to low concentrations of antigens. High doses cause clonal allergy; T cells are in a state of cellular unresponsiveness which makes them incapable of secreting IL-2 or proliferating (45). A similar down regulatory tone of the immune system normally develops against antigenic components of the commensal microbial flora (46).

Although the immune response of the intestinal mucosa exhibits several common features with the immune responses produced by other organs, it is characterized by certain distinctive properties. The immune properties of the digestive mucosa are provided by the gut-associated lymphoid tissue (GALT). The GALT is composed of both organised (e.g., Peyer's patches) and diffusely distributed lymphoid cells. Additional protection against pathogens is provided by the mucus layer covering the epithelium (33, 44).

The intestinal immune system must sensor all antigens in order to properly assess which ones require an immune response. Intestinal antigens are acquired through different mechanisms. First, enterocytes transport antigens from the intestinal lumen to the lamina propria. Enterocytes can even act as APCs, since these cells express MHC class II molecules as well. Antigen sampling also occurs in Peyer's patches, where a specialized epithelium, known as the follicular-associated epithelium (FAE), covers one or many lymphoid follicles composed of B and T cells, DCs and macrophages. The FAE is made up of enterocytes and M cells. Differing from enterocytes by the absence of a brush border and by the absence of mucus production, M cells have the important task of transporting antigens across the epithelium to the lymphoid follicle, a structure commonly referred to as the dome epithelium (33, 44). An enormous variety of pathogens take advantage of the properties of M cells to penetrate the intestinal mucosa and invade the host. Finally, DCs, using their dendrites, also act as guard cells in the intestinal lumen without disturbing the integrity of their tight surface junctions. The intestinal epithelium is also characterized by the presence of intraepithelial lymphocytes (IEL) located between the enterocytes. Most IELs have a $CD8^+$ T cell phenotype. Within the lymphocyte subsets that populate the intestinal mucosa are found $CD4^+$ T helper cells (Th1 and Th2), cytotoxic $CD8^+$ T, and a wide variety of regulatory T cells such as Th3, T_R1 and thymic-derived $CD4^+CD25^+$ (33).

With the help of the $CD4^+$ T cells and cytokines (IL-10 and TGF-β) present in the gut, B cells differentiate into dimeric IgA isotype antibody-secreting plasma cells. The dimeric IgAs are then secreted in the intestinal lumen by the enterocytes, where they aid in binding antigens and neutralizing viruses, bacteria and toxins, thereby contributing to the host protection against infectious agents and toxic substances (47–50). The intestinal immune system is the subject of complex regulation processes allowing the elimination of pathogenic micro-organisms, while maintaining a tolerance towards food antigens and endogenous flora. In chronic intestinal inflammatory diseases such as Crohn's disease (48, 51) and hemorrhagic rectocolitis, a deregulation of the intestinal immune system would lead to an appropriate response against one or more endoluminal antigens. The mucosal immune system normally maintains itself in a state that favors tolerance and IgA production, showing a slight deviation towards the Th2 response over Th1 response. However, this deviation is not absolute, since some chronic inflammatory

bowel diseases such as ulcerative colitis are somewhat Th2-driven, whereas others like Crohn's disease show a predominantly Th1-mediated cytokine profile (52, 53). Thus, the cytokine profile plays an important role in the maintenance of intestinal immune homeostasis.

34.5.1 Antibody-Mediated Mucosal Defense

The intestinal mucosa contains about 80% of the body's activated B cells, which are terminally differentiated to Ig-producing blasts and plasma cells. Most of these immunocytes produce dimeric IgA, which along with pentameric IgM, can be actively transported through secretory epithelia by the polymeric Ig receptor (pIgR), also known as membrane secretory component. The binding site for this receptor depends on a small peptide called "joining" (J) chain incorporated selectively into dimeric IgA and pentameric IgM (54). Immune exclusion is then mediated by the generated secretory IgA (SIgA) and secretory IgM (SIgM) antibodies in cooperation with innate non-specific defense mechanisms. In addition, some serum-derived or locally produced IgG antibodies may be transferred passively to the gut lumen. Importantly however, because IgG is complement-activating, its contribution to surface defense is potentially proinflammatory, which could jeopardize the epithelial barrier function (24).

34.6 PROBIOTICS AND THE IMMUNE SYSTEM

34.6.1 Probiotic-Intestinal Epithelium Interaction

Human intestine is colonized by a great number of microorganisms living in the intestinal area and supporting a variety of physiological functions. The intestinal microbial colonization has a sequential pattern. It starts at birth and continues during the subsequent phases of life to form an individual intestinal microbiota. This process facilitates the formation of a physical and immunological barrier between the environment and the gastro-intestinal tract. The composition of the commensal colon microbiota is likely influenced by a combination of food practices and other factors like the geographical localization, various levels of hygiene or various climates. The host-microbe interaction is of primary importance during the neonatal period. The establishment of a normal microbiota provides the most substantial antigenic challenge to the immune system, thus helping the GALT maturation. At birth, the intestinal immune system is inexistent and will develop at the same time as the intestinal microbiota. It becomes more and more complex as food practices change. Approximately at 2 years of age, the intestinal microbiota appears comparable in composition to that of an adult and the intestinal immune system is considered mature. The intestinal microbiota contributes to the anti-inflammatory character of the intestinal immune system. Several immunoregulatory mechanisms, including regulatory cells, cytokines, apoptosis among others, participate in the control of immune responses by preventing the pathological processes associated with excessive reactivity. Many inflammatory diseases are due to disregulation of these mechanisms. An interesting premise for probiotic physiological action is their capacity to modulate the immune system. Consequently, many studies have focused on the effects of probiotics on the diverse aspects of the immune response.

Following the consumption of probiotic products, the interaction of these bacteria with intestinal enterocytes initiates a host response, since intestinal cells produce various immunomodulatory molecules when stimulated by bacteria.

The interaction between probiotic strains and enterocytes is important for the controlled production of cytokines and chemokines secreted by epithelial cells (Table 34.1). Some probiotic organisms can modulate *in vitro* expression of pro- and anti-inflammatory cytokines in a strain-dependent manner. For instance, *Lb. sakei* induced the expression of IL-1β, IL-8 and TNF-α, whereas *Lb. johnsonii* stimulated the production of TGF-β in Caco-2 cells. This apparently required some form of communication between the epithelial cells and the underlying leucocytes (55). The interaction of probiotics and M cells located in Peyer's patches has been established, and considerable attention has been placed on the importance of these cells in the transport of antigens across the intestinal epithelium (56, 57). A study using fluorescent-tagged lactobacilli in mice showed that, 10 min after oral feeding of probiotics, fluorescence was detected in immune cells in Peyer's patches and the lamina propria in the small intestine as well as

Table 34.1
Probiotic-induced cytokine production: *in vitro* effects

Cell culture *In vitro studies*	*Bacteria*	*Cytokine*	*Reference*
Peripheral blood mononuclear cells (PBMCs)	*B. longum* BB536	IFN-γ, IL-12	Odamaki et al. (137)
PBMCs Monocytes	*Lb. plantarum*	IL-10, IL-12, TNF-α, IFN-γ	Grangette et al. (138)
Human monocytes or PBMCs	*B. adolescentis*, *Cl. perfringens*, *Corynebacterium minutissimum* *Lb. plantarum*, *St. aureus*, *St. mitis*	IL-12, IFN-γ, and TNF-α	Hessle et al. (139)
PBMC	*Lb. casei* and *B. breve*	IL-1, IL-6 and IL-10	Lammers et al. (140)
Macrophage derived cell line	*Lb. paracasei strains*, *B. longum*	INF-γ, and IL-6	Ivec et al. (141)
Human myeloid DCs	*Lb. gasseri*, *Lb. johnsonii*, *Lb. reuteri*	IL-12 and IL-18, IFN-γ, Tc1	Mohamadzadeh et al. (102)
Monocyte/macrophage	*Escherichia coli Nissle 1917*; *E. coli K12* *Lb. casei strain Shirota*	IL-10, IL-12 and TNF-α	Cross et al. (142)
ATCC (T84 and HT-29) cells	*E. coli Nissle* *Lactobacillus* *Bifidobacterium* *Streptococcus*	IL-8	Otte and Podolsky (60)
Monocyte-derived DCs	*Lb. reuteri*, *Lb. casei*	Treg cells IL-10	Smits et al. (97)

in immune cells in the crypt and lymph nodules in the colon (57). In addition, fluorescence associated with the presence of *Lb. casei* persevered in Peyer's patches for about 2–3 days (58). These findings demonstrate that probiotics, or at least their bacterial products, can access the intestinal mucosal immune system, persist for a certain amount of time and initiate a specific immune response.

34.6.2 Probiotic-Induced Cytokine Production by Enterocytes

Probiotics can stimulate cytokine expression in enterocytes in a strain-dependent manner. For example, the probiotic *E. coli* Nissle 1917 induced the production of IL-8 in HT-29 cells whereas the lactobacilli and bifidobacteria contained in the probiotic VSL#3 did not (59, 60). *Lb. plantarum* 299v also increased IL-8 mRNA levels in HT-29 epithelial cells previously stimulated by TNF-α. This IL-8 production required the presence of live bacteria, and was not observed when the adhesion between *Lb. plantarum* 299v and HT-29 cells was inhibited (61). This study provided no evidence to further elaborate on this observation. However, IL-8 mRNA clearly increased in the presence of TNF-α, while the secretion of IL-8 in the supernatant actually decreased. Nevertheless, the importance of this landmark study is in suggesting that probiotic adhesion to intestinal cells is an important mechanistic component in their action. The quality and dose of probiotic preparations may also impact the IL-8 production by enterocytes. When incubated with higher doses of *Lb. rhamnosus* GG, Caco-2 cells produced less IL-8 induced by TNF-α stimulation (62). IL-8 appears to be a major cytokine produced by enterocytes following an encounter with a probiotic organism. The IL-8 cytokine primarily functions as a neutrophil chemoattractant. Probiotic strains, however, differ in their capacity to augment IL-8 expression and some strains appear to rather decrease epithelial cell production of IL-8. For instance, *Lb. reuteri* exerted an anti-inflammatory effect on T84 and HT-29 cell lines by diminishing their IL-8 production. *Lb. reuteri* also induced the production of nerve growth factor (NGF), an anti-inflammatory molecule (63). Since all these findings were based on the use of cell lines as experimental models, they did not necessarily represent the actual *in vivo* situation. Aside from IL-8, enterocytes can excrete other cytokines such as IL-6 in the presence of probiotic organisms, as demonstrated with more physiological models. By studying rats whose intestinal tract were colonized by only one organism, *B. lactis* Bb12, it was shown that intestinal epithelial cells could produce IL-6 up to 5 days following bacterial colonization (64). After co-incubation of primary intestinal cells with probiotics, it was found that *Lb. casei* CRL 431 and *Lb. helveticus* R389 also increased IL-6 secretion (65). *Lb. casei* subs. *casei*, *Lb. paracasei* and *Lb. acidophilus* probiotics induced the production of IL-15, a multifunctional cytokine, in Caco-2 cells as well (66). Finally, the analysis of mRNA microarrays revealed that *Lb. casei* DN-114 01 reduced the expression of chemokines that attract macrophages (CXCL1 and CXCL2) and DCs (CCL20) in Caco-2 cells exposed to *Shigella flexneri* (67). It was also found that some lactobacilli strains modulated the IFN-γ induced expression of HLA-DR, CD45 and ICAM-1 (intercellular adhesion molecule-1) on HT-29 cells (68). Lastly, it was shown that *Lb. rhamnosus* GG inhibited cytokine-induced apoptosis (TNF-α, IL-1α or IFN-γ) of intestinal epithelial cells by activating the antiapoptotic molecule AKT/protein kinase B and by inhibiting the pro-apoptotic p38/mitogen-activated protein kinase signaling pathway (69).

Altogether, these studies suggest that the interaction of probiotic bacteria with the intestinal epithelium is a key determinant for cytokine production by enterocytes and probably the initiating event in the immune system.

34.7 PROBIOTICS AND INNATE IMMUNITY

34.7.1 Phagocytic Cell Function

Many probiotic strains can influence innate defense mechanisms such as phagocytosis (Table 34.3). For example, *Lb. acidophilus* and *Lb. casei* induced a systemic immunostimulation by increasing the phagocytosis capacity of murine peritoneal macrophages (70, 71). In addition, *Lb. acidophilus* La1 increased the phagocytosis capacity of leucocytes isolated from the blood of humans fed probiotics, which was consistent with the adhesion potential of this bacterium (72–74). Moreover, bacteria like *B. lactis* Bb12, with slightly lower adhesion potential, also substantially increased phagocytosis (73). Additionally, *B. lactis* HN019 considerably augmented the phagocytosis of peripheral blood mononuclear cells (PBMC) (75–77). Similarly, a milk product containing *Lb. rhamnosus* GG significantly up-regulated the expression of important phagocytosis receptors like CR1, CR3, FcγRIII and FCαR in the neutrophils of healthy individuals (78). It is noteworthy that patients suffering from milk-hypersensitivity also showed an increased expression of these receptors and that the expression of these molecules was reduced when they consumed milk containing *Lb. rhamnosus* GG. As suggested *Lb. rhamnosus* GG stimulated immune response in the phagocytes of healthy individuals and inhibited phagocytosis in allergic individuals (78). *Lb. johnsonii* La1 was shown to increase the respiratory burst of phagocytes isolated from human blood following probiotic consumption (74). Moreover, the *ex vivo* phagocytic activity of mononuclear and polymorphonuclear phagocytes was enhanced following the consumption of *Lb. rhamnosus* HN001 and *B. lactis* HN109 (77, 79).

34.7.2 Natural Killer Cell Activity

Probiotic organisms also regulate the activity of natural killer (NK) cells (Table 34.3). *Lb. rhamnosus* HN001 and *B. lactis* HN109, for example, significantly increased the cytotoxic potential of NK cells, which subsequently decreased after the cessation of probiotic consumption, although remained above baseline (76, 79). The use of the probiotic *Lb. casei* subsp. *casei* in combination with dextran, a prebiotic, also enhanced the cytotoxic efficiency of NK cell (66). *Lb. casei* Shirota administration was also able to enhance NK cell activity. This activity was correlated to IL-12 production, another cytokine implicated in NK cells activity (80). These studies suggest that probiotics may play a major role in boosting the immunosurveillance of NK cells, which may aid in the prevention of malignant tumors. Furthermore, the positive effects of probiotics on phagocytosis and NK cell function appear to be greater in immune-deficient elderly persons (76, 77). Thus, the consumption of probiotics may favor innate immune defenses in aging individuals. Finally, the oral intake of *Lb. fermentum* CECT5716 was able to potentate the immunologic response of an anti-influenza vaccine by increasing the proportion of NK cells (81).

34.8 PROBIOTICS AND ADAPTIVE IMMUNITY

34.8.1 Responses to Vaccines

Many probiotic strains are apparently able to stimulate the production of antigen-specific IgA antibodies well, which helps maintain intestinal humoral immunity (Table 34.3). For example, subjects who consumed fermented milk containing *B. bifidum* and *Lb. acidophilus* La1 following vaccination against *Salmonella typhi* Ty21 showed a significant increase in IgA serum concentration (82). In addition, children 2–5 year old who received *Lb. rhamnosus* GG concomitantly with a rotavirus vaccination showed an increased number of IgA secreting cells (83). Moreover, *Lb. rhamnosus* GG dramatically increased IgA seroconversion during the remission phase in children suffering from acute rotavirus-induced diarrhea (84, 85). One such study also reported that only live probiotics induced the IgA specific response, which helped in preventing reinfection (84). Bifidobacteria can also promote IgA production, since children who consumed a preparation containing *B. lactis* Bb12 for a few months after receiving their polio vaccine showed an increase in the total amount of IgA in the feces and, more particularly, antipoliovirus IgA (86). Some of these studies have emphasized the fact that total serum IgA level was enhanced following the oral consumption of probiotics. However, there is a basic difference between the IgAs found in the serum and that present in the intestine, as the latter have a dimeric or polymeric form and contain a secretory component required for export. Thus, measuring serum IgA may not reflect actual digestive tract conditions. Accordingly, Park et al. (87) studied IgA production by intestinal mucosal lymphoid cells in mice. They showed that *B. bifidum* significantly induced IgA production in Peyer's patches and mesenteric lymph nodes, with optimal secretion obtained with probiotics encapsulated in alginate microparticles. Surprisingly, rather than inducing a specific humoral immune response, *B. bifidum* apparently had a more systemic immune effect (87). Another study demonstrated that a peptide fraction derived from *Lb. helveticus*-fermented milk contributed to induce local mucosal and systemic IgA immune responses in mice that were infected with *E. coli* O157:H7. Results indicated that the metabolites produced by probiotics might influence host immunity, and would therefore be highly appropriate for use in a food matrix (88). Moreover, it appears that the influence of probiotics on humoral immunity may be partially determined by the colonizing properties of the probiotic organisms. Indeed, although both *Lb. johnsonii* and *Lb. paracasei* displayed similar adhesion properties to Caco-2 cells, *Lb. johnsonii* was a better colonizer in the intestines of gnotobiotic mice, and a more efficient inducer of intestinal IgA production than *Lb. paracasei* (89). A recent study reported on a possible interaction between probiotics and breastfeeding on the number of Ig-secreting cells, suggesting that probiotics during breastfeeding may positively influence gut immunity (90). Similarly, 28 critically ill patients admitted to the intensive care unit received live probiotics (VSL#3) for a week in a double-blind, randomized controlled trial (91). Patients had a significantly larger increase in systemic IgA and IgG concentrations than in the patients who received placebo or sonicates, which indicated a greater enhancement in immune activity.

34.8.2 Dendritic Cell Function and Regulatory T Cell (Treg) Responses

APCs, and more particularly DCs, are key players in both, attaining the Th1/Th2 balance and the development of tolerance. There are several types of DCs with the

ability to orient the immune response according to activation environment, specific DC subset, or their activation kinetics (92). Given the importance of DCs in the orchestration of the immune response, it has been hypothesized that probiotic organisms modulate the immune response by influencing DC maturation. DCs may thus instruct naive $CD4^+$ T cells to differentiate into Th1, Th2 or even Th3 (Fig. 34.1). Using DCs derived from human monocytes, Braat et al. (93) showed that DCs, allowed to mature in the presence of *Lb. rhamnosus*, reduced both the proliferation of T cells (naive and memory) and the secretion of IL-2, IL-4 and IL-10 upon anti-CD3/anti-CD28 stimulation. In addition, using T cells isolated from healthy and Crohn's patients, it was found that the oral consumption of *Lb. rhamnosus* induced the same unresponsive state in $CD4^+$ Th1 and Th2 cells *in vivo* (93). Another study examined bone marrow-derived murine DCs exposed to different irradiated lactobacilli (*Lb. reuteri*, *Lb. plantarum* Lb20, *Lb. casei* subsp. *alactus*, *Lb. plantarum* 299v and *Lb. johnsonii* La1). All the strains were able to induce the maturation of DCs. *Lb. casei* subsp. *alactus* was characterized as an inducer of pro-inflammatory cytokines (IL-12, IL-6, TNF-α) in DCs, whereas *Lb. reuteri* appeared to be a poor stimulator of IL-12. Surprisingly, *Lb. reuteri* inhibited the production of IL-12, IL-6 and TNF-α and the expression of B7.2 (CD86) in DCs induced by *Lb. casei* subsp. *alactus*, while maintaining steady DC production of IL-10 (94). This study emphasized that the differentiation process for DC of the gut could be modulated according to the composition of gut microflora, including ingested probiotics, alone or in combination. Further study in mice demonstrated that the probiotic preparation VSL#3 increased the expression of B7.1 (CD80), B7.2 (CD86), CD40 and MHC class II molecules. In addition, when the DCs were incubated in the presence of probiotics, they were unable to induce T cell proliferation. A substantial increase in IL-10 levels was, however, observed in the supernatant when DCs were incubated with the probiotics for 3 days (95). These results demonstrated that probiotics possessed the ability to modulate DC surface phenotype and cytokine release by blood DCs.

The regulation of DC cytokines by probiotics may contribute to the benefit of these molecules in treatment of intestinal diseases. However, the DCs isolated from the blood likely differed from those derived from bone marrow and, consequently, those present in the intestine. Hart et al. (96) compared changes in the expression of DC differentiation markers and cytokine production upon incubation with VSL#3 in human DCs obtained from blood or intestinal tissue following biopsy. VSL#3 diminished pro-inflammatory effects of LPS by decreasing LPS (lipopolysaccharide)-induced production of IL-12 while maintaining IL-10 production. VSL#3 was also a potent inducer of IL-10 by DCs from blood and intestinal tissue, and inhibited the generation of Th1 cells. Of all the probiotics in the VSL#3 preparation, bifidobacteria were the most potent IL-10 inducers. They were also more effective in decreasing surface expression of B7.1 (CD80) in DCs, and inhibiting T cell production of IFN-γ as well (96). Some probiotics influenced monocyte-derived DCs to drive the development of Treg cells. These Treg cells produced increased levels of IL-10. Thus, when human monocyte-derived DCs were incubated in the presence of *Lb. reuteri* and *Lb. casei*, they induced T cell differentiation into regulatory T cells that produced large amounts of IL-10. *Lb. plantarum*, on the other hand, was incapable of inducing the regulatory T cell differentiation. It appears that the ability of these probiotics to induce regulatory T cells by the DCs may be due to their ability to bind to the lectin DC (DC specific intercellular adhesion molecule 3-grabbing nonintegrin; DC-SIGN) (97). In addition, in an animal model of inflammatory bowel disease caused by Th1 cells, the

probiotic VSL#3, when administered to mice for 3 weeks, reduced colitis severity. This beneficial effect was associated with the production of IL-10 and, in particular, the generation of greater numbers of Treg cells expressing TGF-β at the surface of the cell membrane of the lamina propria. It is significant that the transfer of mononucleated lamina propria cells from mice treated with VSL#3 to naive mice impeded colitis development, and that this effect depended on regulatory $CD4^+$ cells, since the depletion of regulatory $CD4^+$ T cells impeded the very protecting effect generated by the transfer (98). Finally, it was shown that the probiotic *Lb. paracasei* NCC2461 induced the development of a $CD4^+$ T cell subset characterized by a low proliferation potential but a marked ability to secrete IL-10 and TGF-β. This subset is very similar to a population of regulatory cells that participate in the oral tolerance process required to maintain gastro-intestinal stability (99). *Lb. paracasei* NCC2461 also participated in the β-lactoglobulin (β-LG) oral tolerance process in mice, attributable to the hydrolysis of β-LG into peptides, which stimulated production of IL-10 (100, 101). Thus, metabolites generated by the breakdown of food by probiotic organisms may have immunomodulatory effects. These studies in general indicated that many probiotic organisms act as anti-inflammatory agents by influencing DCs to induce a non-response state, more particularly by encouraging the development of T cells with immunoregulatory properties (Table 34.1). Meanwhile, another study suggested that some lactobacilli strains promote DCs to regulate T cell responses towards Th1 pathway by stimulating the secretion of high levels of IL-12 and IL-18, but not IL-10 (102). Almost all strains belonging to the *Lb. casei* group were able to induce a high level of IL-12 via macrophages stimulation.

34.9 PROBIOTICS AND IMMUNOREGULATION

Most of the knowledge on how probiotics affect the immune system comes from profile analyses of cytokines produced by a wide variety of immune cells in response to the consumption of probiotic organisms (Tables 34.1 and 34.2). It has been proposed by numerous studies that probiotics exert an immunomodulatory effect by influencing the cytokine production of the various effector cells in the intestine and especially enterocytes. However, the effects appear to be strain-dependent. Moreover, depending on the strain, either pro-inflammatory or anti-inflammatory effects on the immune system have been observed. The interaction between probiotic strains and enterocytes is important for the controlled production of cytokines and chemokines secreted by epithelial cells. In view of the importance of the Th1/Th2 paradigm to our understanding of the immune response, it has been suggested that consumption of probiotic products could produce an immunomodulatory effect by disrupting the T $CD4^+$ and helper cell differentiation process by skewing the Th1/Th2 balance (103).

The balance between Th1 and Th2 cytokine production may direct the immune response and its outcome. A true balance between Th1 and Th2 profiles can be difficult to maintain, as Th1 and Th2 cells inhibit each other. For example, IL-4 promotes Th2 cell expansion and limits the proliferation of Th1 cells as opposed to IFN-γ, which enhances the growth of Th1 cells but decreases Th2-cell development (33, 104). In fact, the cytokine microenvironment clearly represents a potent determinant of Th1/Th2 polarization, with IL-4 and IL-12 as the initiating key factors derived principally from the innate immune responses during the T-cell priming. Activated macrophages and DCs are the main source

Table 34.2
Some recent studies on immunomodulatory effects of probiotics: animal models

Strain	*In vivo animal model*	*Response*	*Reference*
Lb. paracasei ssp. *paracasei* F19, *Lb. acidophilus* NCFB 1748	Germ free and normal microflora mice	Upregulation of *Mmp7* – gene implicated in regulation of intestinal mucosal defense	Nerstedt et al. (143)
Lb. pentosus S-PT84	BALB/c mice	Skewed Th1/Th2 balance towards Th1 and lowered serum IgE levels, suppressed IL-4 production, upregulated IL-10 production	Nonaka et al. (144)
Lb. gasseri NC1500 and NC1501	IL-10-deficient mouse model	Significant anti-inflammatory activity that reduces the severity of colitis	Carroll et al. (145)
Lb. plantarum	Intact and immunocompromized BALB/c mice	Attenuation of suppressive effect of cyclophosphamide on the LPS-driven lymphoproliferation	Bujalance et al. (146)
Lb. casei CRL 431	Malnourished Swiss albino mice	Restoration of cytokine profile, upregulation of IL-10, recovery of the defense mechanisms against pneumococci	Aguero et al. (147)
Lb. casei Shirota, *B. breve* Yakult	Germ-free BALB/c mice	Strains differentially affected epithelial gene expression in the small intestine and colon	Shima et al. (148)
Lb. johnsonii La1	Aged 57BL6/n mice	Enhanced intestinal IgA production and aided in recovering nutritional status and systemic immune responses	Kaburagi et al. (149)
Lb. paracasei NCC2461	Aged male C57BL/6J mice	Th1 cell-dependent immune responses (IgG2a levels and delayed type hypersensitivity response) increased significantly	Vidal et al. (150)
Enterococcus faecalis FK-23	Weaning BALB/c mice	Decreased ratio of serum total IgE to IgG2a levels – may improve the intestinal ecosystem disturbed by antibiotic use	Shimada et al. (151)
E. coli Nissle 1917	Young pigs	Only minor effects on the distribution of mucosal immune cells	Duncker et al. (152)
Lb. acidophilus LAFTI L10 and *Lb. paracasei* LAFTI L26		Enhanced Interleukin 10 and interferon γ production from splenocytes	Paturi et al. (153)

of IL-12, in contrast to an early burst of IL-4 which may come from NKT cells, mast cells, basophiles or already matured Th2 cells (105, 106). Altogether, exogenous stimuli such as pathogen-derived products, the maturational stage of APCs and cytokines will influence Th1/Th2 differentiation on a background of genetic factors. In addition, there is an impact from complex interactions between antigen dose, T-cell receptor (TCR) engagement and MHC antigen affinities. Influential antigenic properties include the nature of the antigen, with bacteria and viruses promoting Th1-cell differentiation and helminths, the Th2 subset. Th2 differentiation also appears to be promoted by small soluble proteins characteristic of allergens (24, 33). However, other immune cells, such as regulatory T cells, can also intervene to block either Th1 or Th2 activity or in some cases even both. Several subsets of T cells with immunoregulatory properties with distinct phenotypes and distinct mechanisms of action have been identified. These include the antigen-induced Type 1 Tr cells which secrete high levels of IL-10 and low-to-moderate levels of transforming growth factor (TGF-β) (107) and Type 3 T cells (Th3), which primarily secrete TGF-β, as well as the naturally-occurring, thymic-derived $CD4^+$ $CD25^+$ T cells, which inhibit immune response through cell–cell contact (106, 108).

34.9.1 Alleviating Th1/Th2 Imbalance: Downregulation of Th2

The lactobacilli, such as *Lb. plantarum*, *Lb. lactis*, *Lb. casei* and *Lb. rhamnosus* GG all appear to inhibit Th2 response in allergic patients. These bacteria significantly reduce IL-4 and IL-5 production by peripheral blood mononuclear cells (PBMC) when human cells are preincubated with lactobacilli prior to stimulation with specific allergens. This mechanism requires the presence of monocytes and depends on Th1 cytokines (IL-12 and IFN-γ) (109). Using a food allergy model to study Th2 response in mice, Shida et al. (110) showed that the peritoneal injection of heat-killed *Lb. casei* Shirota induced an increase in serum IL-12 and a skewing of the cytokine profile from Th2 to Th1 with less IL-4 and IL-5 and more IFN-γ produced. This caused lower secretions of IgE and IgG1 antibodies by splenocytes, thereby preventing the systemic anaphylactic reaction. This phenomenon was specific to *Lb. casei* Shirota, since no effect was found with the *Lb. johnsonii* JCM 2012 injection. A shortcoming of this study was that the authors injected probiotic organisms into the peritoneum, which in no way reflects the real-life setting of the ingested probiotic organisms. In a study using a mouse model of lactoglobulin tolerance, Prioult et al. (100) provided evidence that probiotics modulate the oral tolerance response to the milk protein with also strain-dependent effect. A double-blind clinical study in children who were allergic to cow's milk also showed that the administration of *Lb. rhamnosus* GG for 4 weeks increased IFN-γ production in PBMCs after stimulation with anti-CD3/anti-CD28. At the same time, it suppressed the secretion of IL-4, normally produced in large quantities after stimulation of the $CD4^+$ T cells in allergic children (Table 34.3, (111)). This Th2 down-regulation by *Lb. rhamnosus* GG could be explained by the presence of bacterial proteases, which degrade casein and consequently influence the Th1/Th2 balance. In fact, an earlier study on allergic patients demonstrated that casein degraded by this probiotic organism reduced the production of IL-4 by blood-derived T cells after *in vitro* stimulation with anti-CD3 antibodies (112). These studies suggest that some probiotics function to down-regulate the Th2 subset and skew the immune response towards Th1.

Table 34.3
Examples of immunomodulatory effects of probiotics: human studies

Species	*Strain*	*Effect*	*Reference*
B. lactis	HN019	Increased production of IFN-α and enhanced bactericidal activity	Arunachalam et al. (75)
	HN019	Increased phagocytosis of polymorphonuclear cells and lysis of NK cells	Chiang et al. (154)
	HN019	Increase expression of CD3+, CD25+ and CD56+ cells, increased number of mononuclear and polymorphonuclear cells with phagocytic activity	Gill et al. (77)
	HN019	Increased expression of CD56+ and antitumor activity	Gill et al. (76)
	BB12	Decrease in TGF-β and IL-2	Isolauri et al. (155)
Lb. GG	ATCC 53103	Increased serum TGF-β	Isolauri et al. (155)
	ATCC 53103	Increased serum TGF-β2	Rautava et al. (156)
	ATCC 53103	Secretion of IFN-γ increased	Pohjavuori et al. (111)
	ATCC 53103	Total number of IgG, IgM and IgA secreting cells increased	Rinne et al. (90)
	ATCC 53103	Plasma levels of IL-6 and IL-10 increased	Viljanen et al. (157)
Lb. rhamnosus	HN001	Increased phagocytic activity of mononuclear and polymorphonuclear cells	Gill and Rutherfurd (12)
	HN001	Increased expression of CD56+ and antitumor activity	Gill et al. (76)
Lb. acidophilus	L-92	No effect on ration Th1/Th2	Ishida et al. (158)
Lb. gasseri	TMC0356	Lower concentration of IgE and increased Th1/Th2 ratio	Morita et al. (159)
Mixture of *Lb. acidophilus*, *Lb. delbrueckii*, *S. thermophilus*		Decreased peripherial traffic of CD34+ cells	Mastrandrea (160)
Mixture of LGG, *Lb. rhamnosus* LC705, *B. breve* Bbi99, *P. freudenreichii* ssp. *shermanii* JS		Significant increase in secretion of IL-4	Pohjavuori et al. (111)

(continued)

Table 34.3
(continued)

Species *Strain*	*Effect*	*Reference*
Mixture of *Lb. gasseri* CECT 5714, *Lb. coryniformis* CECT 5711, *S. thermophilus*	Increased concentration of NK cells, monocytes, neutrophiles; increased phagocytic activity of granulocytes and monocytes	Olivares et al. (161)
Probiotic mix – VSL#3	Significant increase in IgG and IgA concentrations	Alberda et al. (91)
Mixture of *Lb. acidophilus* W55, *Lb. casei* W56, *Lb. salivarius* W57, *L. lactis* W58, *B. infantis* W52, *B. lactis* W18, *B. longum* W51	Increased T and B cells proliferation, decreased IgE induction	Flinterman et al. (162)
Mixture of *LGG*, *Lb. rhamnosus* LC705, *B. breve* Bb99; *P. freudenreichii* ssp. *shermanii* JS	Increased levels of plasma C-reactive protein, IL-10, total plasma IgA and IgE	Marschan et al. (163, 164)

Many studies have reported that probiotic strains exert their immunostimulatory effect by stimulating the pro-inflammatory production of cytokines. Probiotics can also increase the production of IFN-α. For example, ingested *Lb. brevis* subsp. *coagulans* (Labre) and *B. lactis* HN019 induced the production of IFN-α by subjects' PBMCs (75, 113). Miettinen et al. (114) also demonstrated that *Lb. rhamnosus* E509, *Lb. rhamnosus* GG and *Lb. bulgaricus* E585 strongly induced productions of IL-1β, IL-6, IL-18 and TNF-α in PBMCs and induced a moderate increase in both RNA and protein production of IL-12 and IL-10. However, other studies suggested that live probiotic bacteria may not be required to influence the immune system (115, 116). Using immunohistochemical analyses of mice intestines, it was shown that orally administered probiotics also influenced the local production of pro-inflammatory cytokines. Thus, the productions of TNF-α, IL-2 and IL-1β were enhanced when *Lb. reuteri* ML1 and *Lb. brevis* ML12 were ingested (117). *Lb. casei*, *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. acidophilus* also induced an increase in producer cells for TNF-α and IFN-γ, whereas only *Lb. acidophilus* increased the number of IL-2 and IL-12 producing cells. Finally, both *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. casei* induced an increase in the number of IL-4 and IL-10 producing cells (118). These two studies presented a strong argument for the existence of a wide variability in the strains that modulate local intestinal cytokine production. Finally, Miettinen et al. (119) tested the immune system stimulation effects of dead versus live lactobacilli, concluding that live bacteria produced more TNF-α, IL-6 and IL-10 in PBMCs than bacteria incubated with glutaraldehyde-fixed cells.

34.9.2 Alleviating Th1/Th2 Imbalance: Down-regulation of Th1

A Th1/Th2 balance is pivotal for immunological homeostasis and skewing towards Th1 due to defective Treg cell activity would, for example, lead to autoimmune disorders such as Type 1 diabetes. As indicated by many *in vitro* and *in vivo* studies, the primary mechanism in this case lies in the induction of regulatory T cells that secrete IL-10 and TGF-β. A study in children with atopic dermatitis found that the consumption of *Lb. rhamnosus* GG increased IL-10 production in the serum (120). IL-10 is also a critical cytokine for the maintenance of tolerance to commensal intestinal bacteria. In its absence, mice developed severe intestinal inflammation (121). The administration of *Lb. reuteri* to IL-10-deficient mice reduced the development of colitis suggesting that probiotics exert an anti-inflammatory action in the intestine (122). Similarly, when IL-10 knockout mice were administered with *Lb. salivarius* and *B. infantis*, the anti-inflammatory effects of these probiotics were thought to be caused by a reduced production of the pro-inflammatory cytokines IL-12, IFN-γ and TNF-α by splenocytes and Peyer's patches cells (123, 124). Interestingly, *Lb. salivarius* 118 also diminished intestinal inflammation in IL-10-deficient mice by down-regulating the production of pro-inflammatory Th1 cytokines, even when bacteria were injected subcutaneously indicating that it may not always be necessary to administer probiotics orally (125).

The role of Treg cells was further supported by a study that showed an increase in Treg numbers following probiotic administration (98). This probiotic administration was associated with an early increase in the production of IL-10 and an increased number of regulatory $CD4^+$ T cells bearing surface TGF-β in the form of latency-associated protein (LAP). The expression of Treg cells was dependent on the IL-10

production since the application of anti-IL-10R mAb blocked their appearance. Furthermore, *Lb. reuteri* and *Lb. paracasei* attenuated intestinal inflammation caused by *Helicobacter hepaticus* in IL-10-deficient mice by reducing the expression of TNF-α and IL-12 in the colon, which did not affect the number of pathogenic *Helicobacter hepaticus* present in their digestive tract (126). Similarly, the consumption of *Lb. rhamnosus* GG relieved symptoms of atopic dermatitis likely due to a reduction in the production of intestinal TNF-α as measured in the feces (127). Mucosa samples isolated from patients with Crohn's disease and incubated with *Lb. casei* DN114001 or *Lb. bulgaricus* LB10 also produced substantially less TNF-α. This phenomenon, however, required the presence of live bacteria (128). Similarly, in another recent study (129), when intestinal mucosal extracts isolated from Crohn's patients were co-cultured with *Lb. casei* DN114001, the production of IL-6 and TNF-α was significantly reduced. Nevertheless, some probiotic strains, such as *Lb. johnsonii*, were unable to decrease TNF-α production by macrophages upon stimulation. This again suggests a strain-dependent effect for probiotics used to control inflammatory reactions (130). In other Th1-mediated autoimmune disease models, it has been shown that probiotics can alleviate inflammatory symptoms. For example, in a murine rheumatoid arthritis model, *Lb. casei* Shirota mitigated arthritis development by reducing the Th1 response (131). Lactobacilli also exhibited an effect in the experimental autoimmune encephalomyelitis (EAE) animal model of multiple sclerosis. However, the effect was strain-dependent. For example, *Lb. reuteri* aggravated EAE, whereas *Lb. casei* and *Lb. murines* were beneficial to inducted mice (132). To summarize, study findings using animal models suggest that the administration of probiotics can affect individuals afflicted with autoimmune disease. However, further studies should be conducted on humans to assess whether the consumption of probiotics might aggravate autoimmune disease. The risk is that a probiotic that steers the immune system towards a Th1 inflammatory response could prove harmful to patients suffering from Th1-mediated autoimmune disease (133).

34.10 DIFFERENTIAL STIMULATION OF TH1 OR TH2 RESPONSE IN HEALTH AND DISEASE

Several studies have shown that specific strains of probiotics are able to selectively stimulate either Th1 or Th2 responses (Table 34.1). It has also been shown that some probiotic strains are able to exert both pro-inflammatory and anti-inflammatory effects depending upon the health status of an individual. For example, supplementation with *Lb. rhamnosus* HN001 has been shown to enhance phagocytic and NK cell cytolytic capacity and IFN-α production in healthy individuals (76, 77) and suppress the development of atopic dermatitis in high-risk children (134) through the induction of immunoregulatory responses (135). Similarly, *Lactobacillus* GG supplementation was reported to increase the expression of phagocytosis receptors (CR1, CR3, FcγRI and FcαR) on neutrophils in healthy subjects but had an opposite effect in milk-hypersensitive subjects (78). Similarly, observations in healthy volunteers and patients with atopic dermatitis have been made by Roessler et al. (136). In summary, many studies have shown that each probiotic appears to influence the immune system in a particular fashion. In other words, immunomodulation properties are bacteria-specific.

34.11 CONCLUSIONS AND PERSPECTIVES

Probiotic consumption is associated with a range of health benefits including the modulation of the immune system. Numerous animal model and human clinical studies have shown that specific strains of probiotics are able to exert immunostimulatory and immunoregulatory effects depending upon the health status of an individual. In a healthy state, probiotic intake has been shown to enhance phagocytic and natural killer cell functions, and augment specific antibody responses to both bacterial and viral vaccines and infections. This enhanced immune responsiveness has been suggested to play a key role in probiotic-mediated protection against infectious diseases (gastrointestinal and respiratory tract infections) and cancers. In a disease state, associated with the perturbation of immunological homeostasis (polarization of immune responses towards Th1 or Th2 phenotype), probiotics have been found to confer health benefits by restoring the Th1versus Th2 balance. For example, specific strains of probiotics have been shown to be effective in reducing the incidence and/or relieve the symptoms of atopic eczema and in maintaining remission in patients with pouchitis or ulcerative colitis. Recent studies, mainly in experimental animals, have also highlighted the potential of using probiotics for the prevention/management of autoimmune disorders such as rheumatoid arthritis, multiple sclerosis and Type 1diabetes mellitus. However, these effects still remain to be validated in properly designed clinical trials. The exact mechanisms by which probiotics mediate their disparate effects are not fully known. Emerging evidence suggests that probiotics may mediate these immunoregulatory effects through the induction of regulatory T cells. It is important to note that the nature of immunomodulatory effects of probiotics are strain-dependent and the viability of organisms is critical for their maximal effect. Furthermore, most of the health benefits associated with probiotics have been demonstrated only for a small number (and in some cases only for a single strain) of probiotic strains.

REFERENCES

1. Gilmore MS, Ferretti JJ (2003) Microbiology. The thin line between gut commensal and pathogen. Science 299:1999–2002
2. McCracken VJ, Lorenz RG (2001) The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. Cell Microbiol 3:1–11
3. Macfarlane S, Macfarlane GT (2004) Bacterial diversity in the human gut. Adv Appl Microbiol 54:261–289
4. Kimura K, McCartney AL, McConnell MA, Tannock GW (1997) Analysis of fecal populations of bifidobacteria and lactobacilli and investigation of the immunological responses of their human hosts to the predominant strains. Appl Environ Microbiol 63:3394–3398
5. Fooks LJ, Gibson GR (2002) Probiotics as modulators of the gut flora. Br J Nutr 88(Suppl 1):S39–S49
6. Satokari RM, Vaughan EE, Akkermans ADL, Saarela M, de Vos WM (2001) Polymerase chain reaction and denaturing gel electrophoresis monitering of fecal *Bifidobacterium* populations in prebiotics and probiotics feeding trial. Syst Appl Microbiol 24:227–231
7. Tannock GW, Munro K, Harmsen HJM, Welling GW, Smart J, Gopal PK (2000) Analysis of the fecal microflora of human subjects consuming a probiotic containing *Lactobacillus rhamnosus* DR20. Appl Environ Microbiol 66:2578–2588
8. FAO/WHO (2002) Guidelines for the evaluation of probiotics in food. Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. London, ON, Canada

9. Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JHJ (1998) Overview of gut flora and probiotics. Int J Food Microbiol 41:85–101
10. Guarner F, Malagelada J-R (2003) Gut flora in health and disease. Lancet 361:512–519
11. Gill HS (2003) Probiotics to enhance anti-infective defenses in the gastrointestinal tract. Best Pract Res Clin Gastroenterol 17:755–773
12. Gill HS, Rutherfurd KJ (2001) Probiotic supplementation to enhance natural immunity in the elderly. Effect of a newly characterized immunostimulatory strain *Lactobacillus rhamnosus* HN001 (DR20™) on leucocytes phagocytosis. Nutr Res 21:183–189
13. Kelly D, Conway S (2001) Genomics at work: the global gene response to enteric bacteria. Gut 49:612–613
14. Fukushima K, Ogawa H, Takahashi K, Naito H, Funayama Y, Kitayama T, Yonezawa H, Sasaki I (2003) Non-pathogenic bacteria modulate colonic epithelial gene expression in germ-free mice. Scand J Gastroenterol 38:626–634
15. Ogawa H, Fukushima K, Sasaki I, Matsuno S (2000) Identification of genes involved in mucosal defense and inflammation associated with normal enteric bacteria. Am J Physiol Gastrointest Liver Physiol 279:G492–G499
16. Hooper LV, Gordon JI (2001) Commensal host-bacterial relationships in the gut. Science 292:1115–1118
17. Vaughan EE, Schut F, Heilig HG, Zoetendal EG, de Vos WM, Akkermans AD (2000) A molecular view of the intestinal ecosystem. Curr Issues Intest Microbiol 1:1–12
18. Wostmann BS (1996) Germfree and gnotobiotic animal models: background and applications. CRC, Boca Raton, FL
19. Spencer JP (2003) Metabolism of tea flavonoids in the gastrointestinal tract. J Nutr 133:3255S–3261S
20. Rowland I, Faughnan M, Hoey L, Wahala K, Williamson G, Cassidy A (2003) Bioavailability of phyto-oestrogens. Br J Nutr 89:S45–S58
21. Turner NJ, Thomson BM, Shaw IC (2003) Bioactive isoflavones in functional foods: the importance of gut microflora on bioavailability. Nutr Rev 61:204–213
22. Blaut M (2002) Relationship of prebiotics and food to intestinal microflora. Eur J Nutr 41:I11–I16
23. Mutch DM, Simmering R, Donnicola D, Fotopoulos G, Holzwarth JA, Williamson G, Irene Corthesy-Theulaz I (2004) Impact of commensal microbiota on murine gastrointestinal tract gene ontologies. Physiol Genomics 19:22–31
24. Cummings JH, Antoine JM, Azpiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC, Gibson GR, Guarner F, Isolauri E, Pannemans D, Shortt C, Tuijtelaars S, Watzl B (2004) PASSCLAIM-gut health and immunity. Eur J Nutr 43(Suppl 2):118–173
25. Beutler B, Rietschel ET (2003) Timeline: innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 3:169–176
26. Medzhitov R (2001) Toll-like receptors and innate immunity. Nat Rev Immunol 1:135–145
27. Nagler-Anderson C (2001) Man in the barrier! Strategic defenses in the intestinal mucosa. Nat Rev Immunol 1:59–67
28. Ricciardi-Castagnoli P, Granucci F (2002) Opinion: interpretation of the complexity of innate immune responses by functional genomics. Nat Rev Immunol 2:881–889
29. Devereux G (2002) The immune system: an overview. In: Calder PC, Field CJ, Gill HS (eds) Nutrition and immune function. CAB International Publishing, Oxon, UK, pp 1–20
30. Hoebe K, Janssen E, Beutler B (2004) The interface between innate and adaptive immunity. Nat Immunol 5:971–974
31. Haddad PS, Azar GA, Groom S, Boivin M (2005) Natural health products, modulation of immune function and prevention of chronic diseases. Adv Access Publ 2:513–520
32. Roitt I, Brostoff J, Male D (1998) Immunology, 5th edn. Mosby, London, UK, p. 423
33. Meyers RA (2007) Immunology from cell biology to disease. Wiley-VCH Verlag GmbH & Co, Weinheim, Germany
34. Kelso A, Troutt AB, Maraskovsky E, Gough NM, Morris L, Pech MH, Thomson JA (1991) Heterogeneity in lymphokine profiles of CD4+ and CD8+ T cells and clones activated in vivo and in vitro. Immunol Rev 123:85–114

35. Ley K, Smith E, Stark MA (2006) IL-17A-producing neutrophil-regulatory Tn lymphocytes. Immunol Res 34:229–242
36. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM (2006) Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 24:677–688
37. Bettelli E, Oukka M, Kuchroo VK (2007) T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol 8:345–350
38. Deenick EK, Tangye SG (2007) Autoimmunity: IL-21: a new player in Th17-cell differentiation. Immunol Cell Biol 85:503–505
39. Guarner F, Bourdet-Sicard R, Brandtzaeg P, Gill HS, McGuirk P, van Eden W, Versalovic J, Weinstock JV, Rook GA (2006) Mechanisms of disease: the hygiene hypothesis revisited. Nat Clin Pract Gastroenterol Hepatol 3:275–284
40. Hofer T, Nathansen H, Lohning M, Radbruch A, Heinrich R (2002) GATA-3 transcriptional imprinting in Th2 lymphocytes: a mathematical model. Proc Natl Acad Sci USA 99:9364–9368
41. van den Ham H-J, de Boer RJ (2008) From the two-dimensional Th1 and Th2 phenotypes to high-dimensional models for gene regulation. Int Immunol 20:1269–1277
42. Murphy KM, Reiner SL (2002) The lineage decisions of helper T cells. Nat Rev Immunol 2:933–944
43. Leonard WJ (2003) Type I cytokines and interferons and their receptors. In: Paul WE (ed) Fundamental immunology. Lippincott Williams & Wilkins, Philadelphia, USA, pp 701–747
44. Macpherson AJ, Harris NL (2004) Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 4:478–485
45. Spiekermann GM, Walker WA (2001) Oral tolerance and its role in clinical disease. J Pediatr Gastroenterol Nutr 32:237–255
46. Helgeland L, Brandtzaeg P (2000) Development and function of intestinal B and T cells. Microbiol Ecol Health Dis 12:110–127
47. Lefrancois L, Puddington L (2006) Intestinal and pulmonary mucosal T cells: local heroes fight to maintain the status quo. Annu Rev Immunol 24:681–704
48. Macdonald TT, Monteleone G (2005) Immunity, inflammation, and allergy in the gut. Science 307:1920–1925
49. Mestecky J, Blumberg RS, Kiyono H, McGhee JR (2003) The mucosal immune system. In: Paul WE (ed) Fundamental immunology. Lippincott Williams & Wilkins, Philadelphia, PA, pp 965–1021
50. Mowat AM (2003) Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol 3:331–341
51. MacDonald TT, Monteleone G, Pender SL (2000) Recent developments in the immunology of inflammatory bowel disease. Scand J Immunol 51:2–9
52. Korzenik JR, Podolsky DK (2006) Evolving knowledge and therapy of inflammatory bowel disease. Nat Rev Drug Discov 5:197–209
53. Strober W, Fuss IJ, Blumberg RS (2002) The immunology of mucosal models of inflammation. Annu Rev Immunol 20:495–549
54. Brandtzaeg P, Farstad IN, Johansen FE, Morton HC, Norderhaug IN, Yamanaka T (1999) The B-cell system of human mocosae and exocrine glands. Immunol Rev 171:45–87
55. Haller D, Bode C, Hammes WP, Pfeifer AM, Schiffrin EJ, Blum S (2000) Nonpathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. Gut 47:79–87
56. Claassen E, Van Winsen R, Posno M, Boersma WJ (1995) New and safe "oral" live vaccines based on *lactobacillus*. Adv Exp Med Biol 371:1553–1558
57. Muscettola M, Massai L, Tanganelli C, Grasso G (1994) Effects of lactobacilli on interferon production in young and aged mice. Ann N Y Acad Sci 717:226–232
58. Galdeano CM, Perdigon G (2004) Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. J Appl Microbiol 97:673–681
59. Lammers KM, Helwig U, Swennen E, Rizzello F, Venturi A, Caramelli E, Kamm MA, Brigidi P, Gionchetti P, Campieri M (2002) Effect of probiotic strains on interleukin 8 production by HT29/19A cells. Am J Gastroenterol 97:1182–1186
60. Otte JM, Podolsky DK (2004) Functional modulation of enterocytes by gram-positive and gramnegative microorganisms. Am J Physiol Gastrointest Liver Physiol 286:613–626

61. McCracken VJ, Chun T, Baldeon ME, Ahrne S, Molin G, Mackie RI, Gaskins HR (2002) TNF alpha sensitizes HT-29 colonic epithelial cells to intestinal lactobacilli. Exp Biol Med 227:665–670
62. Zhang L, Li N, Caicedo R, Neu J (2005) Alive and dead *Lactobacillus rhamnosus* GG decrease tumor necrosis factor-alpha-induced interleukin-8 production in Caco-2 cells. J Nutr 135:1752–1756
63. Ma D, Forsythe P, Bienenstock J (2004) Live *Lactobacillus reuteri* is essential for the inhibitory effect on tumor necrosis factor alpha-induced interleukin-8 expression. Infect Immun 72:5308–5314
64. Ruiz PA, Hoffmann M, Szcesny S, Blaut M, Haller D (2005) Innate mechanisms for *Bifidobacterium lactis* to activate transient pro-inflammatory host responses in intestinal epithelial cells after the colonization of germ-free rats. Immunology 115:441–450
65. Vinderola G, Matar C, Perdigon G (2005) Role of intestinal epithelial cells in immune effects mediated by gram-positive probiotic bacteria: involvement of toll-like receptors. Clin Diagn Lab Immunol 12:1075–1084
66. Ogawa T, Asai Y, Tamai R, Makimura Y, Sakamoto H, Hashikawa S, Yasuda K (2006) Natural killer cell activities of synbiotic *Lactobacillus casei* ssp. *casei* in conjunction with dextran. Clin Exp Immunol 143:103–109
67. Tien MT, Girardin SE, Regnault B, Le Bourhis L, Dillies MA, Coppee JY, Bourdet-Sicard R, Sansonetti PJ, Pedron T (2006) Antiinflammatory effect of *Lactobacillus casei* on Shigella-infected human intestinal epithelial cells. J Immunol 176:1228–1237
68. Delneste Y, Donnet-Hughes A, Schiffrin EJ (1998) Functional foods: mechanisms of action on immunocompetent cells. Nutr Rev 56:S93–S98
69. Yan F, Polk DB (2002) Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J Biol Chem 277:50959–50965
70. Perdigon G, de Macias ME, Alvarez S, Oliver G, de Ruiz Holgado AP (1988) Systemic augmentation of the immune response in mice by feeding fermented milks with *Lactobacillus casei* and *Lactobacillus acidophilus*. Immunology 63:17–23
71. Gill HS, Rutherfurd KJ, Prasad J, Gopal PK (2000) Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019). Br J Nutr 83:167–176
72. Schiffrin EJ (1994) Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J Dairy Sci 78:491–497
73. Schiffrin EJ, Brassart D, Servin AL, Rochat F, Donnet-Hughes A (1997) Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. Am J Clin Nutr 66:515S–520S
74. Donnet-Hughes A, Rochat F, Serrant P, Aeschlimann JM, Schiffrin EJ (1999) Modulation of nonspecific mechanisms of defense by lactic acid bacteria: effective dose. J Dairy Sci 82:863–869
75. Arunachalam K, Gill HS, Chandra RK (2000) Enhancement of natural immune function by dietary consumption of *Bifidobacterium lactis* (HN019). Eur J Clin Nutr 54:263–267
76. Gill HS, Rutherfurd KJ, Cross ML (2001) Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes. J Clin Immunol 21:264–271
77. Gill HS, Rutherfurd KJ, Cross ML, Gopal PK (2001) Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. Am J Clin Nutr 74:833–839
78. Pelto L, Isolauri E, Lilius EM, Nuutila J, Salminen S (1998) Probiotic bacteria down-regulate the milk-induced inflammatory response in milk hypersensitive subjects but have an immunostimulatory effect in healthy subjects. Clin Exp Allergy 28:1474–1479
79. Sheih YH, Chiang BL, Wang LH, Liao CK, Gill HS (2001) Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium *Lactobacillus rhamnosus* HN001. J Am Coll Nutr 20:149–156
80. Takeda K, Suzuki T, Shimada SI, Shida K, Nanno M, Okumura K (2006) Interleukin-12 is involved in the enhancement of human natural killer cell activity by *Lactobacillus casei* Shirota. Clin Exp Immunol 146:109–115
81. Olivares M, Diaz-Ropero MP, Sierra S, Lara-Villoslada F, Fonolla J, Navas M, Rodriguez JM, Xaus J (2007) Oral intake of *Lactobacillus fermentum* CECT5716 enhances the effects of influenza vaccination. Nutrition 23:254–260

82. Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM (1994) Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol 10:55–63
83. Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T (1995) Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. Vaccine 13:310–312
84. Kaila M, Isolauri E, Saxelin M, Arvilommi H, Vesikari T (1995) Viable versus inactivated *Lactobacillus* strain GG in acute rotavirus diarrhoea. Arch Dis Child 72:51–53
85. Majamaa H, Isolauri E, Saxelin M, Vesikari T (1995) Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J Pediatr Gastroenterol Nutr 20:333–338
86. Fukushima Y, Kawata Y, Hara H, Terada A, Mitsuoka T (1998) Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. Int J Food Microbiol 42:39–44
87. Park JH, Um JI, Lee BJ, Goh JS, Park SY, Kim WS, Kim PH (2002) Encapsulated *Bifidobacterium bifidum* potentiates intestinal IgA production. Cell Immunol 219:22–27
88. Leblanc J, Fliss I, Matar C (2004) Induction of a humoral immune response following an *Escherichia coli* O157:H7 infection with an immunomodulatory peptidic fraction derived from *Lactobacillus helveticus*-fermented milk. Clin Diagn Lab Immunol 11:1171–1181
89. Ibnou-Zekri N, Blum S, Schiffrin EJ, von der Weid T (2003) Divergent patterns of colonization and immune response elicited from two intestinal *Lactobacillus* strains that display similar properties in vitro. Infect Immun 71:428–436
90. Rinne M, Kalliomaki M, Arvilommi H, Salminen S, Isolauri E (2005) Effect of probiotics and breastfeeding on the *Bifidobacterium* and *Lactobacillus/Enterococcus* microbiota and humoral immune responses. J Pediatr 147:186–191
91. Alberda C, Gramlich L, Meddings J, Field C, McCargar L, Kutsogiannis D, Fedorak R, Madsen K (2007) Effects of probiotic therapy in critically ill patients: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 85:816–823
92. Moser M (2003) Dendritic cells. In: Paul WE (ed) Fundamental immunology. Lippincott Williams & Wilkins, Philadelphia, PA, pp 455–480
93. Braat H, van den Brande J, van Tol E, Hommes D, Peppelenbosch M, van Deventer S (2004) *Lactobacillus rhamnosus* induces peripheral hyporesponsiveness in stimulated CD4+ T cells via modulation of dendritic cell function. Am J Clin Nutr 80:1618–1625
94. Christensen HR, Frokiaer H, Pestka JJ (2002) Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J Immunol 168:171–178
95. Drakes M, Blanchard T, Czinn S (2004) Bacterial probiotic modulation of dendritic cells. Infect Immun 72:3299–3309
96. Hart AL, Lammers K, Brigidi P, Vitali B, Rizzello F, Gionchetti P, Campieri M, Kamm MA, Knight SC, Stagg AJ (2004) Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut 53:1602–1609
97. Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TMM, Zaat BAJ, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, Kapsenberg ML (2005) Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 115:1260–1267
98. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Monica Boirivant M (2005) Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-β bearing regulatory cells. J Immunol 174(6):3237–3246
99. von der Weid T, Bulliard C, Schiffrin EJ (2001) Induction by a lactic acid bacterium of a population of CD4(+) T cells with low proliferative capacity that produce transforming growth factor beta and interleukin-10. Clin Diagn Lab Immunol 8:695–701
100. Prioult G, Fliss I, Pecquet S (2003) Effect of probiotic bacteria on induction and maintenance of oral tolerance to beta-lactoglobulin in gnotobiotic mice. Clin Diagn Lab Immunol 10:787–792
101. Prioult G, Pecquet S, Fliss I (2004) Stimulation of interleukin-10 production by acidic beta lactoglobulin-derived peptides hydrolyzed with *Lactobacillus paracasei* NCC2461 peptidases. Clin Diagn Lab Immunol 11:266–271

102. Mohamadzadeh M, Olson S, Kalina WV, Ruthel G, Demmin GL, Warfield KL, Bavari S, Klaenhammer TR (2005) Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. Proc Natl Acad Sci USA 102:2880–2885
103. Delcenserie V, Martel D, Lamoureux M, Amiot J, Boutin Y, Roy D (2008) Immunomodulatory effects of probiotics in the intestinal. Curr Issues Mol Biol 10:37–54
104. Van Eden E, van der Zee R, van Kooten P, Berlo SE, Cobelens PM, Kavelaars A, Heijnen CJ, Prakken B, Roord S, Albani S (2002) Balancing the immune system: Th1 and Th2. Ann Rheum Dis 61(Suppl II):25–28
105. Liew FY (2002) TH1 and TH2 cells: a historical perspective. Nat Rev Immunol 2:55–60
106. Kidd P (2003) Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev 8:223–246
107. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG (1997) A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 389:737–742
108. Shevach EM (2000) Suppressor T cells: rebirth, function and homeostasis. Curr Biol 10:572–575
109. Pochard P, Gosset P, Grangette C, Andre C, Tonnel AB, Pestel J, Mercenier A (2002) Lactic acid bacteria inhibit TH2 cytokine production by mononuclear cells from allergic patients. J Allergy Clin Immunol 110:617–623
110. Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T, Habu S, Hachimura S, Kaminogawa S (2002) Lactobacillus casei strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. Clin Exp Allergy 32:563–570
111. Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tiittanen M, Vaarala O, Savilahti E (2004) *Lactobacillus* GG effect in increasing IFN-gamma production in infants with cow's milk allergy. J Allergy Clin Immunol 114:131–136
112. Sutas Y, Hurme M, Isolauri E (1996) Downregulation of anti-CD3 antibody-induced IL-4 production by bovine caseins hydrolysed with *Lactobacillus* GG derived enzymes. Scand J Immunol 43:687–689
113. Kishi A, Uno K, Matsubara Y, Okuda C, Kishida T (1996) Effect of the oral administration of *Lactobacillus brevis* subsp. *coagulans* on interferon-alpha producing capacity in humans. J Am Coll Nutr 15:408–412
114. Miettinen M, Matikainen S, Vuopio-Varkila J, Pirhonen J, Varkila K, Kurimoto M, Julkunen I (1998) Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. Infect Immun 66:6058–6062
115. Pessi T, Sutas Y, Saxelin M, Kallioinen H, Isolauri E (1999) Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. Appl Environ Microbiol 65:4725–4728
116. Amrouche T, Boutin Y, Prioult G, Fliss I (2006) Effects of bifidobacterial cytoplasm, cell wall and exopolysaccharide on mouse lymphocyte proliferation and cytokine production. Int Dairy J 16:70–80
117. Maassen CB, van Holten-Neelen C, Balk F, den Bak-Glashouwer MJ, Leer RJ, Laman JD, Boersma WJ, Claassen E (2000) Strain dependent induction of cytokine profiles in the gut by orally administered *Lactobacillus* strains. Vaccine 18:2613–2623
118. Perdigon G, Maldonado Galdeano C, Valdez JC, Medici M (2002) Interaction of lactic acid bacteria with the gut immune system. Eur J Clin Nutr 56(Suppl 4):S21–S26
119. Miettinen M, Vuopio-Varkila J, Varkila K (1996) Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect Immun 64:5403–5405
120. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765
121. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274
122. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN (1999) *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology 116:1107–1114
123. McCarthy J, O'Mahony L, O'Callaghan L, Sheil B, Vaughan EE, Fitzsimons N, Fitzgibbon J, O'Sullivan GC, Kiely B, Collins JK, Shanahan F (2003) Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut 52:975–980

124. O'Mahony L, Feeney M, O'Halloran S, Murphy L, Kiely B, Fitzgibbon J, Lee G, O'Sullivan G, Shanahan F, Collins JK (2001) Probiotic impact on microbial flora, inflammation and tumor development in IL-10 knockout mice. Aliment Pharmacol Ther 15:1219–1225
125. Sheil B, McCarthy J, O'Mahony L, Bennett MW, Ryan P, Fitzgibbon JJ, Kiely B, Collins JK, Shanahan F (2004) Is the mucosal route of administration essential for probiotic function? Subcutaneous administration is associated with attenuation of murine colitis and arthritis. Gut 53:694–700
126. Pena JA, Rogers AB, Ge Z, Ng V, Li SY, Fox JG, Versalovic J (2005) Probiotic *Lactobacillus* spp. diminish *Helicobacter hepaticus*-induced inflammatory bowel disease in interleukin-10-deficient mice. Infect Immun 73:912–920
127. Majamaa H, Isolauri E (1997) Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 99:179–185
128. Borruel N, Carol M, Casellas F, Antolin M, de Lara F, Espin E, Naval J, Guarner F, Malagelada JR (2002) Increased mucosal tumor necrosis factor alpha production in Crohn's disease can be downregulated ex vivo by probiotic bacteria. Gut 51:659–664
129. Carol M, Borruel N, Antolin M, Llopis M, Casellas F, Guarner F, Malagelada JR (2006) Modulation of apoptosis in intestinal lymphocytes by a probiotic bacteria in Crohn's disease. J Leukoc Biol 79:917–922
130. Pena JA, Li SY, Wilson PH, Thibodeau SA, Szary AJ, Versalovic J (2004) Genotypic and phenotypic studies of murine intestinal lactobacilli: species differences in mice with and without colitis. Appl Environ Microbiol 70:558–568
131. Kato I, Endo-Tanaka K, Yokokura T (1998) Suppressive effects of the oral administration of *Lactobacillus casei* on type II collagen-induced arthritis in DBA/1 mice. Life Sci 63:635–644
132. Maassen CB, van Holten JC, Balk F, Heijne den Bak-Glashouwer MJ, Leer R, Laman JD, Boersma WJ, Claassen E (1998) Orally administered *Lactobacillus* strains differentially affect the direction and efficacy of the immune response. Vet Q 20(Suppl 3):S81–S83
133. Hatakka K, Martio J, Korpela M, Herranen M, Poussa T, Laasanen T, Saxelin M, Vapaatalo H, Moilanen E, Korpela R (2003) Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis pilot study. Scand J Rheumatol 32:211–215
134. Wickens K, Black PN, Stanley TV, Mitchell E, Fitzharris P, Tannock GW, Purdie G, Crane J (2008) A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol 122:788–794
135. Prescott SL, Wickens K, Westcott L, Jung W, Currie H, Black PN, Stanley TV et al (2008) Supplementation with *Lactobacillus rhamnosus* or *Bifidobacterium lactis* probiotics in pregnancy increases cord blood interferon-γ and breast milk transforming growth factor-β and immunoglobin A detection. Clin Exp Allergy 38:1606–1614
136. Roessler A, Friedrich U, Vogelsang H, Bauer A, Kaatz M, Hipler UC, Schmidt I, Jahreis G (2008) The immune system in healthy adults and patients with atopic dermatitis seems to be affected differently by a probiotic intervention. Clin Exp Allergy 38:93–102
137. Odamaki T, Xiao J-Z, Iwabuchi N, Sakamoto M, Takahashi N, Kondo S, Kazuhiro Miyaji K, Iwatsuki K, Togashi H, Enomoto T, Benno Y (2007) Influence of *Bifidobacterium longum* BB536 intake on faecal microbiota in individuals with Japanese cedar pollinosis during the pollen season. J Med Microbiol 56:1301–1308
138. Grangette C, Nutten S, Palumbo E, Morath S, Hermann C, Dewulf J, Pot B, Thomas Hartung T, Hols P, Mercenier A (2005) Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. Proc Natl Acad Sci USA 102:10321–10326
139. Hessle C, Andersson B, Agnes E, World AE (2000) Infect Immun 68:3581–3586
140. Lammers KM, Brigidi P, Vitali B, Gionchetti P, Rizzello F, Caramelli E, Matteuzzi D, Campieri M (2003) Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. FEMS Immunol Med Microbiol 38:165–172
141. Ivec M, Botic T, Koren S, Jakobsen M, Weingartl H, Cencic A (2007) Interactions of macrophages with probiotic bacteria lead to increased antiviral response against vesicular stomatitis virus. Antiviral Res 75:266–274
142. Cross ML, Ganner A, Teilab D, Fray LM (2004) Patterns of cytokine induction by gram-positive and gram-negative probiotic bacteria. FEMS Immunol Med Microbiol 42:173–180

143. Nerstedt A, Nilsson EC, Ohlson K, Håkansson J, Thomas Svensson L, Löwenadler B, Svensson UK, Mahlapuu M (2007) Administration of Lactobacillus evokes coordinated changes in the intestinal expression profile of genes regulating energy homeostasis and immune phenotype in mice. Br J Nutr 97:1117–1127
144. Nonaka Y, Izumo T, Izumi F, Maekawa T, Shibata H, Nakano A, Kishi A, Akatani K, Kiso Y (2008) Antiallergic effects of Lactobacillus pentosus strain S-PT84 mediated by modulation of Th1/Th2 immunobalance and induction of IL-10 production. Int Arch Allergy Immunol 145:249–257
145. Carroll IM, Andrus JM, Bruno-Bárcena JM, Klaenhammer TR, Hassan HM, Threadgill DS (2007) Anti-inflammatory properties of Lactobacillus gasseri expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis. Am J Physiol Gastrointest Liver Physiol 293:G729–G738
146. Bujalance C, Moreno E, Jimenez-Valera M, Ruiz-Bravo A (2007) A probiotic strain of *Lactobacillus plantarum* stimulates lymphocyte responses in immunologically intact and immunocompromized mice. Int J Food Microbiol 113:28–34
147. Aguero G, Villena J, Racedo S, Haro C, Alvarez S (2006) Beneficial immunomodulatory activity of Lactobacillus casei in malnourished mice pneumonia: effect on inflammation and coagulation. Nutrition 22:810–819
148. Shima T, Fukushima K, Setoyama H, Imaoka A, Matsumoto S, Hara T, Suda K, Umesaki Y (2008) Differential effects of two probiotic strains with different bacteriological properties on intestinal gene expression, with special reference to indigenous bacteria. FEMS Immunol Med Microbiol 52:69–77
149. Kaburagi T, Yamano T, Fukushima Y, Yoshino H, Mito N, Sato K (2007) Effect of *Lactobacillus johnsonii* La1 on immune function and serum albumin in aged and malnourished aged mice. Nutrition 23:342–350
150. Vidal K, Benyacoub J, Moser M, Sanchez-Garcia J, Serrant P, Segura-Roggero I, Reuteler G, Blum S (2008) Effect of *Lactobacillus paracasei* NCC2461 on antigen-specific T-cell mediated immune responses in aged mice. Rejuvenation Res 11:957–964
151. Shimada T, Cheng L, Shi HB, Hayashi A, Motonaga C, Tang J, Enomoto K, Enomoto T (2007) Effect of lysed Enterococcus faecalis FK-23 on allergen-induced immune responses and intestinal microflora in antibiotic-treated weaning mice. J Invest Allergy Clin Immunol 17:70–76
152. Duncker SC, Lorentz A, Schroeder B, Breves G, Bischoff SC (2006) Effect of orally administered probiotic E. coli strain Nissle 1917 on intestinal mucosal immune cells of healthy young pigs. Vet Immunol Immunopathol 111:239–250
153. Paturi G, Phillips M, Kailasapathy K (2008) Effect of probiotic strains *Lactobacillus acidophilus* LAFTI L10 and *Lactobacillus paracasei* LAFTI L26 on systemic immune functions and bacterial translocation in mice. J Food Prot 71:796–801
154. Chiang BL, Sheih YH, Wang LH, Gill HS (2000) Enhancement of immunity by *Bifidobacterium lactis*: optimisation and definition of cellular responses. Eur J Clin Nutr 54:849–855
155. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S (2000) Probiotics in the management of atopic eczema. Clin Exp Allergy 30:1604–1610
156. Rautava S, Kalliomäki M, Isolauri E (2002) Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. J Allergy Clin Immunol 109:119–121
157. Viljanen M, Pohjavuori E, Haahtela T, Korpela R, Kuitunen M, Sarnesto A, Vaarala O, Savilahti E (2005) Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema-dermatitis syndrome. J Allergy Clin Immunol 115:1254–1259
158. Ishida Y, Nakamura F, Kanazto H, Sawada D, Yamamoto N, Kagata H et al (2005) Effect of milk fermented with *Lactobacillus acidophilus* strain L-92 on symptoms of Japanese cedar pollen allergy: a randomized placebo-controlled trial. Biosci Biotechnol Biochem 69:1652–1660
159. Morita H, He F, Kawase M, Kubota A, Hiramatsu M, Kurisaki JI, Salminen S (2006) Preliminary human study for possible alteration of serum immunoglobulin E production in perennial allergic rhinitis with fermented milk prepared with *Lactobacillus gasseri* TMC0356. Microbiol Immunol 50:701–706
160. Mastrandrea F (2004) Probiotics reduce the CD34+ hemopoietic precursor cell increased traffic in allergic subjects. Eur Ann Allergy Clin Immunol 36:118–122

161. Olivares M, Díaz-Ropero M, Gómez N, Sierra S, Lara-Villoslada F, Martín R, Rodriguez JM, Xaus J (2006) Dietary deprivation of fermented foods causes a fall in innate immune response. Lactic acid bacteria can counteract the immunological effect of this deprivation. J Dairy Res 73:492–498
162. Flinterman AE, Knol EF, van leperen-van Dijk AG, Timmerman HM, Knulst AC, Bruijnzeel-Koomen CAFM, Pasmans SGMA, van Hoffen E (2007) Probiotics have a different immunomodulatory potential *in vitro* versus *ex vivo* upon oral administration in children with food allergy. Int Arch Allergy Immunol 143:237–244
163. Marschan E, Kuitunen M, Kukkonen K, Poussa T, Sarnesto A, Haahtela T, Korpela R, Savilahti E, Vaarala O (2008) Probiotics in infancy induce protective immune profiles that are characteristic for chronic low-grade inflammation. Clin Exp Allergy 38:611–618
164. Marschan E, Honkanen J, Kukkonen K, Kuitunen M, Savilahti E, Vaarala O (2008) Increased activation of GATA-3, IL-2 and IL-5 of cord blood mononuclear cells in infants with IgE sensitization. Pediatr Allergy Immunol 19:132–139

35 Immunomodulation by Foods and Microbes in Crohn Disease and Ulcerative Colitis

Hitoshi Asakura and Kenji Suzuki

Key Points

- Intestinal mucosa has a single cell layer of epithelial cells that separates the gut lumen harboring the commensal flora and food-born pathogenic antigens from the body.
- Ulcerative colitis and Crohn disease are the chronic nonspecific inflammatory bowel diseases (IBD).
- Recently, the number of patients with CD and UC has been increasing in Asia and the other European countries as well as North America and Western Europe.
- Intestinal environmental factors such as foods and microbes are very important for the pathogenesis of IBD.
- Changes in dietary habits taking high-fat high-sugar Westernized foods and resultant changes in the intestinal microbial community composition may increase incidence in the occurrence of IBD.

Key Words: Crohn disease, elemental diet, environmental factors, westernized foods, IBD, intestinal microbes, metabolic syndrome, parasites, probiotics, ulcerative colitis.

35.1 INTRODUCTION

Intestinal mucosa has a single cell layer of epithelial cells that separates the gut lumen harboring the commensal flora and food-born pathogenic antigens from the body. Normal intestinal mucosa has no hypersensitivity against the commensal flora because of oral tolerance. Gut-associated lymphoid tissue (GALT) protects the intestinal mucosa from the intestinal antigens by producing secretory IgA and Transforming Growth Factor (TGF) β and interleukin (IL)-10 which are immuno-suppressive cytokines. In addition to these cytokines, IL-22, a T helper 17 (Th17) T cell associated

Dietary Components and Immune Function
Edited by: R.R. Watson et al., DOI 10.1007/978-1-60761-061-8_35

cytokine, and one of NK cell subsets may play an important role in the intestinal mucosal protection (1). IL-22 is expressed by Th17 cells, one of NK cell subsets, NKT cells, CD8 cells and γδT cells. However, IL-22 is bifunctional in that it has both proinflammatory and protective effects on tissues depending on the inflammatory conditions. The expression of IL-22 by the colon induces mucus-associated molecules such as MUC1, MUC2, and MUC3, leading to enhanced mucus production due to the restitution of goblet cells and defensin expression in the colonic cells. NK cells develop from hematopoietic stem cells, acquire functional capacities to produce interferon-γ and TNFα, and also perform critical functions in innate and adaptive immune responses. Homeostasis of intestinal NK cells is maintained through signals derived from interaction with the commensal flora. $NKp46^{+}IL22^{+}$ cells express the nuclear hormone receptor retinoic acid receptor-related orphan receptor t(RORγ t) and IL-22. These intestinal $NKp46^{+}IL22^{+}$ cells are generated via a local process that is conditioned by the commensal flora.

Ulcerative colitis (UC) and Crohn disease (CD) are the chronic nonspecific inflammatory bowel diseases (IBD) with unknown etiology. UC was described by Wilks and Moxon in 1859, and CD by Crohn, Ginzburg and Oppenheimer in 1932 with their clinical and pathological analyses, respectively (2). UC is a disease involving mainly the colon and rectum and CD is a disease involving mainly the terminal ileum and/or the colon. Human and murine studies on CD have shown an increased expression of T-helper 1 (Th1) cytokines by intestinal lamina propria lymphocytes characterized by the excessive production of interleukin (IL)-12/IL-23, interferon γ, and tumor necrosis factor (TNF) α. T-helper cells are thought to differentiate into Th1 and Th2, and recently Th17 cells producing IL-17. UC is an atypical Th2 one (3, 4). In these diseases, primary dysregulation of the mucosal immune system leads to excessive immunologic responses to the normal microflora, or changes in the composition of intestinal microflora, and deranged epithelial barrier function may elicit pathologic responses from normal mucosal immune system (5).

Recently, the number of patients with CD and UC has been increasing in Asia and the other European countries as well as North America and Western Europe. These changes in incidence rates may be influenced by various environmental factors, because etiopathogenesis of IBD has been thought to be driven from the mutual reactions among host susceptibility genes (CARD15/NOD2, HLA-class II), environmental factors including intestinal flora and food antigens, and abnormal immune balance (6). There are many environmental factors listed up until now: intestinal microbes and their components, foods, smoking, public hygiene, and so on (7). Geographic and sequential study on the incidence of IBD will give us the clues to clarify its etiopathogenesis.

35.2 INCREASED INCIDENCE OF CD AND UC: WESTERNIZATION OF LIFE STYLE?

UC had not been a common disease before 1945 with an average annual incidence rate of 4 per 100,000 population, but after 1945 it showed a gradual rise in an average annual incidence rate. The incidence of CD and UC in West European countries had started to increase after 1960 and 1950, respectively (7). Ehlin et al.'s study on the

prevalence of gastrointestinal diseases in two British national birth cohorts had shown that the prevalence rates of CD at age 30 years in the 1970 cohort were higher than those in the 1958 cohort, and that those of UC were similar between them (8). In France, the mean annual incidence rate of CD increased from 5.2 per 100,000 inhabitants in 1988-1990 to 6.4 in 1997-1999, but the incidence of UC decreased from 4.2 to 3.5, respectively (9). In a county of USA, the crude rate of UC was 6.3 cases per 100,000 persons a year between 1940 and 1993. However, the adjusted incidence rate of UC increased from 0.6 cases per 100,000 persons a year in 1940–1943 to 8.3 cases in 1984–1993 (10). A peak rate was observed from 1974 to 1983. In other words, the incidence rate of UC seems to have reached a plateau in the Western Europe and North America.

A steep rise in the incidence of CD in Europe began after 1960 with economical development and improved sanitary environment, and a high incidence of CD and a low incidence of UC were reported in the 1980s. However, in Asia, Eastern Europe, and South America, the annual incidence rate of UC and CD had been low before 1990, but it has been steadily increasing in the last 10 years with economical development (11).

In Japan, CD was a rare disease and UC was a not so common until 1975. In Japan, UC and CD patients have been registered as those of the intractable diseases for the national epidemiological survey by the Japanese Department of Ministry of Health, Labour and Welfare since 1973. The data showed that the number of patients with UC and CD has been increasing until now (12). Susceptibility genes and immunological response of Japanese people are thought not to change in the past several decades because of limited immigrants. The number of patients with UC in Japan had started to increase after 1980, followed by CD.

Recent reports from China showed a rising incidence of CD with Westernizing lifestyle. In a district of Korea, an adjusted mean annual incidence rate of UC was 0.63 per 100,000 inhabitants in 1986 and 7.57 in 1997, respectively (13).

35.3 ENVIRONMENTAL FACTORS

The number of patients with CD and UC has been increasing in Western Europe and North America with the Western economic growth after the Second World War and then behind those countries in the other European countries and Far Eastern districts of Asia. Therefore, the environmental factors containing intestinal milieu are suggested to be very important for the pathophysiology of IBD.

Diet, intestinal microbes or their components, appendectomy, breast feeding, public sanitation, early domestic hygiene including running hot water supplies and fixed bath or shower, Helicobacter pylori status, smoking, contraceptives, and Western lifestyle have been proposed as candidates for inducing the abnormal immunological response of the digestive tract (14–17). In terms of the nutritional factors, cola drinks and chocolate consumption were positively associated with the development of CD and UC, while citrus fruit consumption was negatively associated with it (18). The strong evidence for the pathogenesis of UC is that many studies showed an inverse association between previous appendectomy and the development of UC (pooled values of ORs = 0.35, 95% CI, 0.28–0.43) (12). Appendectomy at less than 20 years reduced the occurrence of UC, but not CD (Fig. 35.1). However, there is no relationship

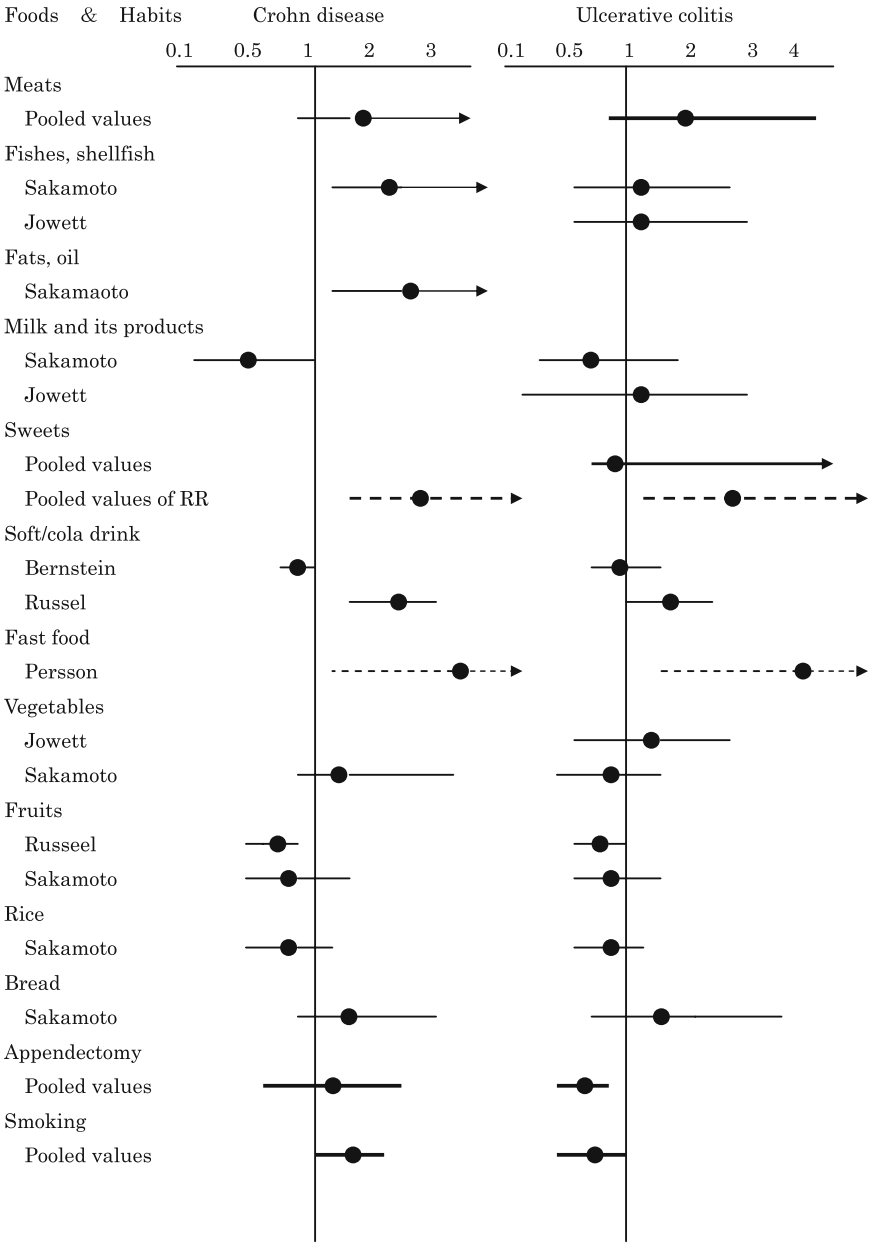


Fig. 35.1. Odds ratio in the relationship between foods and habits and IBD.

between CD and appendectomy (pooled values of ORs = 1.01, 95% CI, 0.39–2.63). The occurrence of UC increased by ceasing smoking (pooled values of ORs = 2.35, 95% CI, 1.69–3.29) and decreased by smoking (pooled values of ORs = 0.34, 95% CI, 0.12–0.93). Moreover, smoking worsened the clinical features of CD (pooled values of ORs = 1.51, 95% CI, 1.2–1.89).

35.3.1 Foods and IBD

The average of daily consumption of dietary animal meats, fish, milk and its products, animal fats, sugar, rice, green vegetables, and fruits taken by Japanese people each year from 1955 to 2006 are shown in Fig. 35.2. Dietary animal meats and fats, and milk products are characteristic of Westernized foods. The daily consumption of dietary animal meats and fats, and milk products has increased since around 1960 and the consumption of rice which is a vegetable protein and starch had rapidly decreased. Short chain fatty acids are the end products of anaerobic bacteria break down of carbohydrates in the colon and they are readily absorbed by intestinal mucosa and trophic to the intestinal mucosa (19). Rice is thought to be a protective food for the colon and rectum.

35.3.1.1 Animal-Derived Foods

Foods significantly associated with the occurrence of CD were meats (Sakamoto; OR = 1.90, 95% CI, 0.95–3.78), fishes/shellfish (Sakamoto; OR = 2.41, 95% CI, 1.18–4.87), fats/oil (Sakamoto; OR = 2.64, 95% CI, 1.29–5.39), sweets/sugar/confectioneries (Persson and Tragnone; pooled values of RR = 2.88, 95% CI, 1.75–4.75, Sakamoto; OR = 2.83, 95% CI, 1.38–5.83), and fast foods (Persson; RR = 3.4, 95% CI, 1.3–9.3). However, there are no significant values in soft/cola drink (Bernstein and Russel; pooled values of OR = 1.23, 95% CI, 0.39–3.92) (20–23).

Foods significantly associated with the occurrence of UC were meats (Jowett and Sakamoto: pooled values of OR = 1.99, 95% CI, 0.84–4.72), sweets/sugar/confectioneries (Jowett and Sakamoto; pooled values of OR = 1.63, 95% CI, 0.55–4.85, Persson and

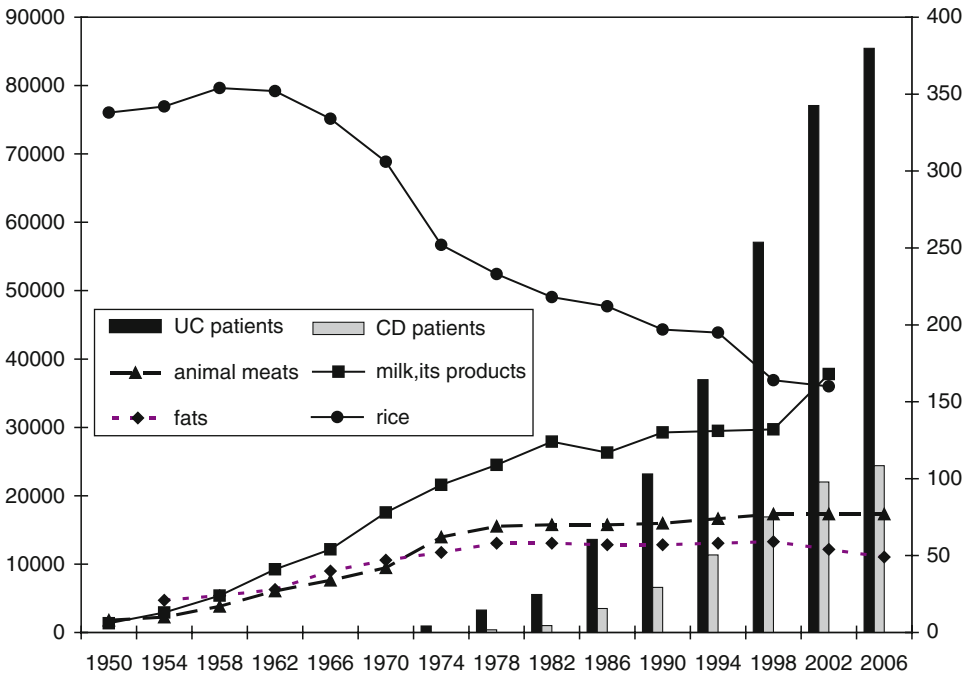


Fig. 35.2. The number of patients with UC and CD (*left Y axis*) and the per day (gm) (*right Y axis*) consumption of Japanese People.

Tragnone; pooled values of RR = 2.52, 95% CI, 1.02–6.23), confectioneries (Persson and Tragnone; pooled values of RR = 2.88, 95% CI, 1.75–4.75, Sakamoto; OR = 2.83, 95% CI, 1.38–5.83), and fast foods (Persson; RR = 3.4, 95% CI, 1.3–9.3). However, there are no significant values in soft/cola drink (Bernstein and Russel; pooled values of OR = 1.23, 95% CI, 0.39–3.92). Persson et al. emphasized that the relative risks of fast foods were 3.4 for CD and 3.9 for UC (23). Jowett et al. reported that dietary factors such as a high meat or alcoholic beverage intake were identified to be associated with an increased likelihood of relapse for UC patients (21). One of the reasons is that hydrogen sulfide which is a bacterially derived cell poison is produced in the large intestine from the dietary animal meats and milk (24, 25).

Fish oils are rich in the long-chain n-3 polyunsaturated fatty acids, eicosapentaenoic and docosahexaenoic acids. Linseed and green plant tissues are rich in the precursor fatty acid, α-linolenic acid. Most of the vegetable oils are rich in the n-6 polyunsaturated fatty acids linoleic acid, the precursor of arachidonic acid. Arachidonic acid-derived eicosanoids (prostaglandin E2) are proinflammatory. Therefore, fish oils are considered anti-inflammatory. Proinflammatory cytokines secretion such as TNF-α, IL-8, and IL-1β by human macrophages was induced by palmitic acid, stearic acid, but not by the shorter chain saturated fatty acids. Linoleic acid upregulated the production of IL-8 by human intestinal smooth muscle cells from patients with CD via arachidonic acid metabolites (26). The ratio of ω3/ω6 is important for the pathogenesis of IBD. A European prospective cohort study showed that the highest quartile of intake of linoleic acid was associated with an increased risk of UC (OR = 2.49) (27).

The number of patients with IBD had increased about 20 years after an initial increase in the daily consumption of dietary animal meats and fats, and milk products and after a decrease in the consumption of rice. However, there are no remarkable changes in the average of daily consumption of green vegetables, bean, and sugar except fruits. The data also suggested the reverse relationship between an increase in the number of IBD patients and a decrease in the consumption of rice as a staple Asian food, implying an increase in the consumption of breads containing yeast organisms. IgG and IgA anti-Saccharomyces cerevisiae (bakers' yeast) were significantly detected in patients with CD (28). These antibodies could be detected in normal healthy control, but their titers were low when compared with those of CD.

35.3.1.2 Elemental Diet and Histidine

It is generally accepted that elemental diet is very effective for the treatment of CD (29). The alteration of bacterial flora, low antigenicity, low fat, and the improvement of nutritional status are postulated as the anti-inflammatory effects of elemental diet. Elemental diet amino acids ameliorated intestinal inflammation of IL-$10^{-/-}$ cell transfer colitis models in a dose-dependent manner. Incubation of intestinal mucosa tissues obtained from CD with elemental diet increased the ratio of interleukin-1 receptor antagonist per interleukin-1 compared with control, but not that from UC (30). In addition, an elemental diet was effective in normalizing an abnormal lactulose breath test in irritable bowel disease subjects, suggesting that elemental diet had an effect in alteration of intestinal bacterial flora (31). However, dietary fat influenced the therapeutic effects of enteral diets in CD. Polymeric enteral diet high in oleate and low in

linoleate lowered the remission rate of CD compared with that high in linoleate and low in oleate.

Recently, several amino acids have been reported to contribute to the modulation of intestinal inflammation in animal colitis models. Dietary glutamine supplement had prophylactic effects on IL-8 and TNFα production in trinitrobenzene sulphonic acid-induced colitis. Dietary glycine prevented chemical-induced experimental colitis in the rats. Histidine-added normal diets but not alanine and glutamine suppressed intestinal inflammation of IL-$10^{-/-}$ cell transfer colitis models in a dose-dependent manner (32). One of the mechanisms to suppress an inflammatory response by histidine will be that dietary histidine showed anti-inflammatory effects on the production of TNFα by macrophages and monocytes. However, histamine and histidine metabolite carnosine hardly suppressed the production of TNFα and IL-6. Histidine has a capacity as a scavenger of the hydroxyl radicals and inhibited the production of IL-8 by intestinal epithelial cell lines treated TNFα or oxidative stress.

When patients with CD will take the conventional diet, they are apt to relapse, suggesting that there may be something in the conventional diet to activate a Th1 immune response. A recent study reported that the elimination of yeasts and milk from diets was beneficial for IBD patients. Cow's milk protein may initiate an immune response in the intestinal mucosa and may be responsible for the activation of cell-mediated immunity after enteric infection or inflammation (33). However, the milk-tolerance theory of UC was denied, but removing foods rich in sulfur amino acids in milk, eggs, and cheese has been proven to be a therapeutic benefit in UC.

35.3.1.3 Metabolic Syndrome

There has been an increasing number of children with metabolic syndrome in the USA and Europe, because they have eaten high calorie foods like sweets containing fat. In addition to it, the number of children suffering from CD has been increasing in the USA. It has been noticed by surgeons that there is a creeping fat containing CD68-positive macrophages around the intestinal mesenterium of CD patients. These adipose tissues produced Peroxisome proliferator activator receptor (PPAR) γ and TNF α, and secreted vascular endothelial growth factor, adiponectin, macrophage colony-stimulating factor, leptin, and migration inhibitory factor (34–36). Unbalance between inflammatory and anti-inflammatory factors produced by adipose tissues may play an important role in the pathogenesis of CD. However, whether or not this phenomenon is a consequence of a kind of abnormal metabolic syndrome induced by Westernized foods is necessary to be clarified.

A high-fat diet increased the proportion of lipopolysacchride (LPS)-expressing bacteria in the gut. Endotoxin/LPS, which are key constituents of gut bacteria play a central role in innate immune-response. Remarkable fatty mice, ob/ob mice showed a reduction in Bacteroidetes and a proportional increase in Firmicutes (primarily Streptococcaceae). The microbiota of these mice promote the absorption of monosaccharides from the gut lumen, resulting in the induction of de novo hepatic lipogenesis. Fasting-induced adipocyte factor (Fiaf), a member of angiopoietin-like family of proteins, is selectively suppressed in the intestinal epithelium of normal mice by conventionalization. The suppression of Fiaf promoted the microbiota-induced deposition of triglycerides in adipocytes (37, 38).

35.3.2 Microbes and IBD

When animals are born, there are no microbes in their intestinal lumen. The intestinal microflora will be established in a week after birth. These intestinal microflora are essential for the development and maturation of the mammalian immune system. When mice are fed under the germ-free conditions, the population of $CD4^+$T cells, IgA-producing B cells, intraepithelial T cells, and dendritic cells in the gut is decreased. Intestinal microflora and lipopolysaccharide contents derived from the diet influenced the development of T cells including regulatory CD4 cells, suggesting that they induced the oral tolerance of the host with production of IL-10 and IFN-γ (39). The human intestinal microflora is estimated to contain 500–2,000 species and their population is estimated to be 10^{13}–10^{15}.

The pathogenesis of UC and CD is not fully understood, but an immunological response to intestinal antigens in those patients is thought to be abnormal when compared with that in control people (5). When mice or rats developing nonspecific enterocolitis similar to UC and CD in the conventional conditions were raised in germ-free conditions, their enterocolitis was not induced or reduced in clinical severity, suggesting that intestinal microbes are very important for the pathogenesis of IBD (39).

35.3.2.1 Specific Pathogens

At first, Mycobacterium sepsis had been thought to be a pathogen of CD, because of the similarities in the diseased sites, granuloma formation, and clinical features between CD and intestinal tuberculosis. Since the pathological features of CD mimics those of intestinal tuberculosis and Johne's disease in cattle, Mycobacterium avium subspecies have been proposed for the pathogens of CD (41–43). There have been available many reports on the detection of serum antibodies against Mycobacterium paratuberculosis with positivity of 40–87%. However, there are reports of conflicting results about the presence of Mycobacterium and Listeria monocytogenes. Combination antibiotic therapy against Mycobacterium was not effective for CD (44).

35.3.2.2 Intestinal Macrophages and Microbes

Bacterial DNA within the granulomas of patients with CD detected by PCR showed a presence of *E. coli* DNA, suggesting nonspecific pathogens for the pathogenesis of CD (45). The molecular characterization of rectal mucosa-associated bacterial flora in IBD showed the reduction in mucosa-associated Bifidobacteria and an increase in *E. coli* and Clostridia.

Intestinal dendritic cells (DCs) extend their transepithelial dendrites into the intestinal lumen and sample intestinal contents for signs of intestinal foreign substances. Macrophages, the major population of tissue-resident mononuclear phagocytes, play a role in bacterial recognition and elimination as well as in the polarization of innate and adaptive immunities. Intestinal macrophages produce several anti-inflammatory cytokines like IL-10 and TGF β and induce the differentiation of $Foxp3^+$ T reg by a mechanism dependent on IL-10 and retinoic acid. Intestinal macrophages also produce the inflammatory cytokines such as TNFα, IFNγ, and IL-6. While a small number of $CD14^+$ cells are present in normal human intestine, these cells are significantly increased in the intestinal mucosa of patients with CD and UC. $CD14^+$ $CD33^+$ cells (CD33 is a marker of intestinal macrophage) produced large amounts of proinflammatory cytokines

such as IL-12/IL-23p40, IL-23, TNFα, and IL-6 in response to commensal bacteria stimuli. These macrophages in CD are derived from the monocytes originated from bone marrow. IFN-γ in the mucosa of CD led to abnormal macrophage differentiation, resulting in hyperproduction of IL-23 (46).

35.3.2.3 Changes in Commensal Floral Clusters

Intestinal microflora can be identified by three methods; culture methods of microflora, quantitative PCR using 16S rRNA gene-targeted group-specific primers, and Terminal Restriction Fragment Length Polymorphism analysis (T-RFLP). The clusters of human fecal microbes profile by T-RFLP method were divided in 7 or 8 clusters using digestion with restriction enzymes of BslI and HhaT and/or MspI, respectively (47, 48). The clusters of fecal microbes in the healthy subjects were included in the clusters I, II, and III when digested with Bs1I and in the cluster I when digested with HhaT. The clusters of the feces of CD patients were included into IV to VII group clusters and those of UC patients were divided into other groups except I. Therefore, the clusters of the fecal microbes in the IBD patients were different from those in healthy subjects. Frank et al.'s study showed that the IBD subset and control subset species of mucosal microbes were composed of distinct microbial communities (49). Andoh et al. reported that the population of Clostridium cluster IV, Clostridium cluster XI, and subcluster XIVa was decreased and Bacteroides significantly increased in CD patients when compared with that of healthy subjects and that Ruminococcus, Eubacterium, Fucobacterium, Gammaproteobacteria, unclassified Bacteroides, and unclassified Lactobacillus were detected in the UC patients, but not in the healthy individuals. However, the specific pathogen was not detected in the intestinal microflora of the IBD patients. On the other hand, Frank et al.'s study showed that UC and CD colonic samples contained fewer sequence type representatives of Bacteroides and the Lachnospiraceae subset of Firmicutes. A reduction of a major member of Firmicutes was associated with a high risk of postoperative recurrence of ileal CD (50). The concentration of propionic and butyric acids in the feces were significantly decreased in patients with IBD (51).

35.3.2.4 Influence of Westernized Foods on Intestinal Microflora

What is the reason for an increase in the number of patients with IBD in the Asian area after that in USA and Western Europe area? Are there any differences in the fecal microflora between Caucasian and Asian people? There is available a report on that the number of Eubacteria, Bifidobacteria, and Veillonellae in the fecal microflora was higher in the Japanese than in the Canadians and that the number of Bacteroide vulgatus, Clostridium coccides, and Bacillus species was lower in the Japanese than in the Canadians (52). The fecal microflora of people living in rural areas (it means eating typical Japanese foods) and of people living in urban areas (it means eating less typical Japanese foods) in Japan was different in the number of Bifidobacteria, which was higher in the rural people (53). A lower number of Clostridium species was observed in people eating brown rice which contains a good amount of food fibers (54). However, there is a report that diets containing high animal fat do not alter the fecal microflora, but influence fecal bile acid excretion (55). 16S ribosomal DNA polymerase chain reaction and temperature gradient gel electrophoresis analyses revealed a profound

modification of the fecal microflora after taking exclusive enteral nutrition in CD patients. These data suggested that some kinds of foods may influence the population of the intestinal microflora.

35.3.2.5 Defensins and toll-like receptors

There have been reported several studies that childhood environmental factors were involved in the development of IBD. Helicobacter pylori seroprevalence was decreased in CD patients but not in UC. Lack of breast feeding was associated with an increased risk of CD and UC. There is a report that there was high prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in CD (45). Caucasian CD patients had mutations in CARD15/NOD2 (nucleotide-binding oligomerization domain 2 56). CARD15/NOD2 function is thought to be an antibacterial factor in human intestinal epithelial cells (57). Single nucleotide polymorphism of NOD2 which activates nuclear factor NF-κ B is one of the candidates for the susceptibility genes of CD, because CARD15/NOD2 is expressed in intestinal epithelial cells and triggers human beta-defensin (HBD)-2 transcription (58, 59). In CD patients with a mutation in the NOD2 gene that is an intracellular peptidoglycan receptor, the ileal Paneth cell defensins, HD-2 and HD-3, which are antimicrobial peptides were diminished.

These immune responses may be induced by defects in the epithelial barrier, an increased intestinal permeability, adherence of bacteria, and a decreased expression of defensins. Intestinal epithelial cells have Toll-like receptors (TLR) to exert direct antibacterial effects via the secretion of antimicrobial peptides and to play an important role in the interrelation between the innate and the adaptive immunity of the intestine.TLR2 is a ligand of muramyl dipeptide (MDP) derived from peptidoglycan (PGN), TLR4, a ligand of LPS, and TLR5, a ligand of flagellin. Bacterial flagellin is a dominant antigen in CD (60). Moreover, there have been many antibodies against intestinal bacterial antigens in the serum of patients with CD. The ligation of TLR5 induces rapid activation of interleukin-1 receptor-associated kinase 4 (IRAK-4) leading to the activation of MAP kinases and IkB kinase, which results in the activation of NF-kB driving the production of proinflammatory genes such as the neutrophil chemoattractant IL-8 (61). NOD2 mutant macrophages were reported to produce larger amounts of IL-12 in response to stimulation with microbial components than wild-type macrophages. Therefore, defects in the innate immune response that are important for immunological protection against intestinal microbes may contribute to the development of CD, especially in the ileal type. However, Japanese and Korean patients with CD had no mutations in CARD15/NOD2 gene. Therefore, there will be many routes between the intestinal microbes and intestinal epithelial cells and lamina propria antigen-presenting cells for the development of CD. Dendritic cells will be activated by TLR signaling, in MyD88-dependent or independent pathway, and then produce interferon γ, TNF-α, IL-6 and IL-12/18. Thus, T cells activated by antigens may undergo distinct developmental pathways, getting properties and effector functions.

35.3.2.6 Probiotics

The alteration of the intestinal microflora by prebiotic or probiotic therapy may induce and maintain remission in colitis mice. VSL#3 probiotic mixture contains 450 billion live lactic acid bacteria that are normally present in the healthy digestive tract.

VSL#3, which contains Lactobacilli species and Bifidobacteria, is good for the intestine and induced remission in patients with active UC (62). In addition, oral administration of Lactobacillus GG induced and maintained remission of some patients with CD. One of the reasons to explain this mechanism is that bacterial flagellin is a dominant antigen in CD. When the intestinal epithelial cells were exposed to flagellin, they produced chemokines that induced the subsequent migration of immature dendritic cells, probably via TLR5.

Ewaschuk et al. listed that *Bacteroides spp, Enterococcus faecalis, Enterobacter cloacae*, intestinal *Helicobacter spp, Fusobacterium spp*, adherent/invasive *E. coli* strains, *Eubacterium*, and *Peptostreptococcus spp*, were selected as the aggressive intestinal microbes and that the beneficial intestinal microbes were *Lactobacillus spp, Bifidobacterium spp, Streptococcus salivarius, Saccharomyces boulardii, Clostridium butyricum, Ruminococci*, and *E. coli* Nissle 1917 (63). The VSL#3 probiotic formula induced mucin gene expression and secretion in colonic epithelial cells (64). This phenomenon may be explained by the fact that soluble proteins produced by probiotic bacteria regulated intestinal epithelial cell survival and growth (65).

Vanderpool proposed mechanisms of probiotic action as follows (66);

1. Probiotics block intestinal pathogenic bacterial effects with competitive exclusion
2. Probiotics produce antibacterial substances such as β-defensins, bacteriocins, heat-shock proteins, and altering luminal pH
3. Probiotics regulate mucosal immune responses via TLR or M cells
4. Probiotics increase anti-inflammatory cytokine production and suppress proinflammatory cytokine production
5. Probiotics enhance epithelial barrier integrity.
6. Probiotics prevent cytokine-induced apoptosis.

Probiotics and prebiotics have been the therapies for ameliorating chronic intestinal inflammation and may be the preventives against IBD for people having disease-susceptibility genes.

There is a difference in incidence of colon cancer between African Americans and Native Africans. The incidence of colorectal cancer is remarkably higher in African Americans than in Native Africans. O'Keefe's study reported that compared with Native Africans, African American consumed more protein and fat, meat, saturated fats, and cholesterol, meaning the Westernized foods (67). Colony counts of 7-alpha dehydroxylating bacteria in African American were higher and those of Lactobacilli were lower. They hypothesized that interactions between the dietary and bacterial environments was important for colorectal cancer risk. Conventional Westernized foods may alter the intestinal microbes, probably resulting in changing intestinal epithelial character.

35.3.3 Parasites: Th2 Response

Another important environmental factor is parasite infection in the digestive tract. A few studies have reported that intestinal parasites inducing a Th2 paradigm had prevented the relapse of CD and UC (68, 69). Lymphocytes from the inflamed intestine of CD produced a Th1 pattern of cytokines. Parasite colonization may provoke a mucosal Th2 response. CD is the most prevalent in highly industrialized countries with temperate

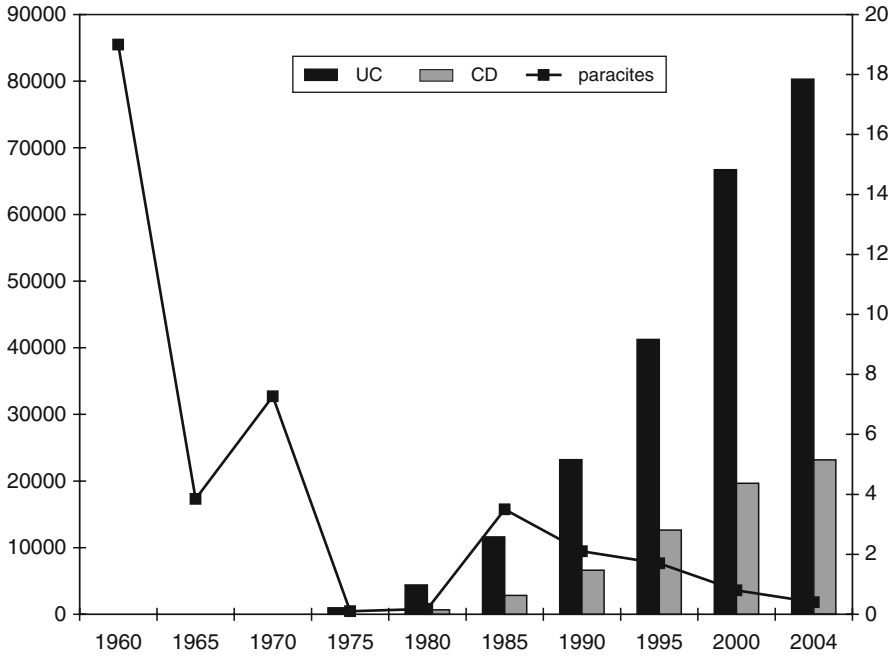


Fig. 35.3. Relationship between detection rate of parasite ova and the number of IBD patients.

climates and it occurs rarely in the tropical third world countries with poor sanitation. The prevalence of helminthes in the United States has been declining for the past 60 years. Since a Th2 immunological response can suppress a Th1 response, Sumers group had thought that parasite infection in the intestinal tract might be effective for the treatment of CD. Their studies showed that administration of Trichuris suis seemed to be safe and effective in the treatment of IBD.

The detection rate of parasite ova consisted of mainly roundworm and pinworm in the stools of the first grade pupils in elementary schools, was obtained from the annual Japanese reports. The relationship between the detection rates of parasite ova in the stools of the first grade pupils in elementary schools and the registered number of patients with CD is shown in Fig. 35.3. After the detection rates of parasite ova were few, the number of patients with IBD had started to increase.

35.4 CONCLUSIONS AND PERSPECTIVES

There is time lag between the increased number of patients and alteration in food consumption. An increase in the number of patients with IBD was observed about 20 years after an increased consumption of animal-derived foods. A high incidence of IBD was observed in two to three decades in CD and in three to fourth decades in UC. It may take about two to three decades to develop the IBD signs and symptoms in humans. Even in the susceptibility gene-bearing animals developing the spontaneous colitis, after birth it took 5–7 weeks in T cell receptor mutant mice, 6–15 weeks in mice with a

disrupted interleukin-2 gene, and 12 weeks in IL-10 knockout mice to develop the spontaneous colitis, (70, 71).

Mizoguchi reported an interesting study that appendectomy at a young age (3–5 week) of T cell receptor-alpha mutant mice suppressed the development of colitis, but appendectomy after that was hard to protect mice from developing colitis (72). This fact may imply that a long-term exposure to intestinal internal and external antigens is needed to develop clinical manifestation of IBD.

This review may support that intestinal environmental factors such as foods and microbes are very important for the pathogenesis of IBD. However, further directly proven study will be needed, because most studies may lack direct evidence linking to the pathogenesis of IBD. The consumption of a high-fat high-sugar Western diet induces obesity and may be associated with the occurrence of CD, resulting in changes of microbial community composition such as reduction in bacteroidetes and a proportional increase in Firmicutes.

In conclusion, changes in dietary habits, taking Westernized foods, and the resultant changes in the intestinal microbial community composition may increase incidence of IBD.

REFERENCES

1. Satoh-Takayama N, Vosshenrich CAJ, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, Mention JJ, Thiam K, Cerf-Bensussan N, Mandelboim O, Ebent G, Santo JP (2008) Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. Immunity 29:958–970
2. Kirsner JB (1985) Inflammatory bowel disease at the university of Chicago – the first 50 years: some personal reflections. Am J Gastroenterol 80:219–227
3. Cobrin GM, Abreu MT (2005) Defects in mucosal immunity leading to Crohn's disease. Immunol Rev 206:277–295
4. Targan SR, Karp LC (2005) Defects in mucosal immunity leading to ulcerative colitis. Immunol Rev 206:296–305
5. Strober W, Fuss J, Mannon P (2007) The fundamental basis of inflammatory bowel disease. J Clin Invest 117:514–521
6. Strober W, Fuss IJ, Blumberg RS (2002) The immunology of mucosal models of inflammation. Annu Rev Immunol 20:495–549
7. Timmer A (2003) Environmental influences on inflammatory bowel disease manifestations. Lessons from epidemiology. Dig Dis 21:91–104
8. Ehlin AGC, Montgomery SM, Ekbom A et al (2003) Prevalence of gastrointestinal diseases in two British national birth cohorts. Gut 52:1117–1121
9. Molinie F, Gower-Rousseau C, Yzet T et al (2004) Opposite evolution in incidence of Crohn's disease and ulcerative colitis in Northern France (1988–1999). Gut 53:843–848
10. Loftus EV, Silverstein MD, Sandborn WJ et al (2000) Ulcerative colitis in Olmsted county, Minnesota, 1940–1993: incidence, prevalence, and survival. Gut 46:336–343
11. Ouyang O, Tandon R, Goh KL et al (2005) The emergence of inflammatory bowel disease in the Asian pacific region. Curr Opin Gastroeneterol 21:408–413
12. Asakura H, Suzuki K, Kitahora T, Morizane T (2008) Is there a link between food and intestinal microbes and the occurrence of Crohn's disease and ulcerative colitis? J Gastroenterol Hepatol 23:1794–1780
13. Yang SK, Hong WS, Min YI et al (2000) Incidence and prevalence of ulcerative colitis in the Songpa-Kangdong district, Seoul, Korea, 1986–1997. J Gastroenterol Hepatol 15:1037–1042

14. Corrao G, Tragnone A, Caprilli R et al (1998) Risk of inflammatory bowel disease attributable to smoking, oral contraception and breast feeding in Italy: a nationwide case-control study. Cooperative investigators of the Italian group for the study of the colon and the rectum (GISC). Int J Epidemiol 27:397–404
15. Duggan AE, Usmani I, Neal KR et al (1998) Appendicectomy, childhood hygiene, Helicobacter pylori status, and risk of inflammatory bowel disease: a case control study. Gut 43:494–498
16. Russel MG, Engels LG, Muris JW et al (1998) Modern life in the epidemiology of inflammatory bowel disease: a case-control study with special emphasis on nutritional factors. Eur J Gastroenterol Hepatol 10:243–249
17. Andersson RE, Olaison G, Tysk C et al (2001) Appendectomy and protection against ulcerative colitis. N Engl J Med 344:808–814
18. Bernstein CN, Rawsthorne P, Cheang M et al (2006) A population-based case control study of potential risk factors for IBD. Am J Gastroenterol 101:993–1002
19. D'Argenio G, Mazzacca G (1999) Short-chain fatty acid in the human colon. Relation to inflammatory bowel diseases and colon cancer. Adv Exp Med Biol 472:149–158
20. Sakamoto N, Kono S, Wakai K et al (2004) Dietary risk factors for inflammatory bowel disease. A multicenter case-control study in Japan. Inflamm Bowel Dis 11:154–163
21. Jowett SL, Seal CJ, Pearce MS et al (2004) Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study. Gut 53:1479–1484
22. Tragnone A, Vaalpiani D, Miglio F et al (1995) Dietary habits as risk factors for inflammatory bowel disease. Eur J Gastroenterol Hepatol 7:47–51
23. Persson PG, Ahlbom A, Hellers G (1992) Diet and inflammatory bowel disease: a case-control study. Epidemiology 3:47–52
24. Roediger WE, Moore J, Babidge W (1997) Colonic sulfide in pathogenesis and treatment of ulcerative colitis. Dig Dis Sci 42:1571–1579
25. Mavee EA, Richardson CJ, Hughes R et al (2000) Contribution of dietary protein to sulfide production in the large intestine: an in vitro and a controlled feeding study in humans. Am J Clin Nutr 72:1488–1494
26. Alzoghaibi MA, Walsh SW, Willey A et al (2003) Linoleic acid induces interleukin-8 production by Crohn's human intestinal smooth muscle cells via arachidonic acid metabolites. Am J Physiol Gastrointest Liver Physiol 286:G528–G537
27. Hart AR (2009) Linoleic acid, a dietary N-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis – a European prospective cohort study. Gut 58:1606–1611
28. Darroch CJ, Barnes RMR, Dawson J (1999) Circulating antibodies to Saccharomyces cerevisiae (baker'/brewers' yeast) in gastrointestinal disease. J Clin Pathol 52:47–53
29. Dray X, Marteau P (2005) The use of enteral nutrition in the management of Crohn's disease in adults. JPEN 29:S166–169
30. Meister D, Bode J, Stand A et al (2002) Anti-inflammatory effects of enteral diet components on Crohn's disease-effected tissues in vitro. Dig Liver Dis 34:430–438
31. Pimentel M, Constantino T, Kong Y et al (2004) A 14-day elemental diet is highly effective in normalizing the lactulose breath test. Dig Dis Sci 49:73–77
32. Andou A, Hisamatsu T, Okamoto S et al (2009) Dietary Histidine ameliorates murine colitis by inhibition of proinflammatory cytokine production from macrophages. Gastroenterology 136:564–574
33. Chung HL, Lee JJ, Kim SG (2003) Cow's milk protein induced changes in the expression of HLA-DR antigens on colonic epithelial cells. Ann Allergy Asthma Immunol 90:348–350
34. Desreumaux P, Ernast O, Geboes K et al (1999) Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. Gastroenterology 117:73–81
35. Schaffler A, Furst A, Buchler C et al (2006) Vascular endothelial growth factor secretion from mesenteric adipose tissue and from creeping fat in Crohn's disease. J Gastroenterol Hepatol 21:1419–1423
36. Paul G, Schaffler A, Neumeier M et al (2006) Profiling adipocytokine secretion from creeping fat in Crohn's disease. Inflamm Bowel Dis 12:471–477
37. Backhed F, Ding H, Wang T et al (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 101:15718–15723

38. Ley RE, Backhed F, Turnbaugh P et al (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 102:11070–11075
39. Hrncir T, Stepankova R, Kozakova H, Hudcovic T, Tlaskalova-Hogenova H (2008) Gut microbiota and lipopolysaccharide content of the diet influence development of regulatory T cells: studies in germ-free mice. BMC Immunol 9:65
40. Schultz M, Tonkonogy SL, Sellon RK, Veltkamp C, Godfrey VL, Kwon J, Grenther WB, Balish E, Horak I, Sartor RB (1999) IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. Am J Physiol Gastrointest Liver Physiol 276:G1461–G1472
41. Naser SA, Ghobrial G, Romero C, Valentine JF (2004) Culture of Mycobacterium avium subspecies paratuberculosis from the blood of patients with Crohn's disease. Lancet 364:1039–1044
42. Autschbach F, Eisold S, Hinz U et al (2005) High prevalence of Mycobacterium avium subspecies paratuberculosis IS900 DNA in gut tissues from individuals with Crohn's disease. Gut 54:944–949
43. Feller M, Huwiler K, Stephan R et al (2007) Mycobacterium avium subspecies paratuberculosis and Crohn's disease: a systematic review and meta-analysis. Lancet Infect Dis 7:607–613
44. Selby W, Pavli P, Crotty B et al (2007) Two-year combination antibiotic therapy with clarithromycin, rifabutin, and ciofazimine for Crohn's disease. Gastroenterology 132:2313–2319
45. Darfeuille-Michaud A, Boudeau J, Bulois P et al (2004) High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. Gastroenterology 127:412–421
46. Kamada N, Hisamatsu T, Okamoto S, Sato T, Matsuoka K, Arai K, Nakai T, Hasegawa A, Inoue N, Watanabe N, Akagawa KS, Hibi T (2005) Abnormally differentiated subsets of intestinal macrophage play a key role in the Th1-dominant chronic colitis through excess production of IL-12 and IL-23 in response to bacteria. J Immunol 175:6900–6908
47. Andoh A, Tsujikawa T, Sasaki M, Mitsuyama K, Suzuki Y, Matsui T, Matsumoto T, Benno Y, Fujiyama Y (2008) Faecal microbiota profile of Crohn's disease determined by terminal restriction fragment length polymorphism analysis. Aliment Pharmacol Ther 29:75–82
48. Andoh A, Sakata S, Koizumi Y, Mitusyama K, Fujiyama Y, Benno Y (2007) Terminal restriction fragment length polumorpfism analysis of the diversity of fecal microbiota in patients with ulcerative colitis. Inflamm Bowel Dis 13:955–962
49. Frank DN, St Amand AL, Feldman RA, Bodeker EC, Harpaz N, Pace NR (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. PNAS 104:13780–13785
50. Sokol H, Pigneur B, Watterlot L et al (2008) Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA 105:6731–16736
51. Takaishi H, Matsuki T, Nakazawa A et al (2008) Imbalance in intestinal microflora constitution could be involved in the pathogenesis of inflammatory bowel disease. Int J Med Microbiol 298:463–472
52. Benno Y, Suzuki K, Suzuki K, Narisawa K, Bruce WR, Mitsuoka T (1986) Comparison of the fecal microflora in rural Japanese and urban Canadians. Microbiol Immunol 30:521–532
53. Benno Y, Endo K, Mizutani T, Namba Y, Komori T, Mitsuoka T (1989) Comparison of fecal microflora of elderly persons in rural and urban areas of Japan. Appl Environ Microbiol 55:1100–1105
54. Benno Y, Endo K, Miyoshi H, Okuda T, Koishi H, Mitsuoka T (1989) Effect of rice fiber on human fecal microflora. Microbiol Immunol 33:435–440
55. Cummings JH, Wiggins HS, Jenkins DJ et al (1978) Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid, and fat excretion. J Clin Invest 61:953–963
56. Ogura Y, Bonen DK, Inohara N et al (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411:603–606
57. Hisamatsu T, Suzuki M, Reinecker HC et al (2003) CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 124:993–1000
58. Wehkamp J, Fellermann K, Stange EF (2005) Human defensins in Crohn's disease. Chem Immunol Allergy 86:41–54
59. Voss E, Wehkamp J, Wehkamp K et al (2006) NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2. J Biol Chem 281:2005–2011
60. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM (2004) Bacterial flagellin is a dominant antigen in Crohn disease. J Clin Invest 113:1296–1306

61. Vijay-Kumar M, Aitken JD, Gewirtz AT (2008) Toll like receptor-5: protecting the gut from enteric microbes. Semin Immunopathol 30:11–21
62. Bibiloni R, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB (2005) VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol 100:1539–1546
63. Ewaschuk JB, Dieleman LA (2006) Probiotics and prebiotics in chronic inflammatory bowel diseases. World J Gastroenterol 12:5941–5950
64. Caballero-Franco C, Keller K, DE Simone C et al (2007) The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol 292:G315–G322
65. Yan F, Cao H, Cover TL et al (2007) Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology 132:62–75
66. Vanderpool C, Yan F, Polk DB (2008) Mechanisms of probiotic action: implication for therapeutic applications in inflammatory bowel diseases. Inflamm Bowel Dis 14:1585–1596
67. O'Keefe SJD, Chung D, Mahmoud N et al (2007) Why do African Americans get more colon cancer than Native Africans? J Nutr 137:175S–182S
68. Sumers RW, Elliott DE, Qadir K et al (2003) Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am J Gastroenterol 98:2034–2040
69. Sumers RW, Elliott DE, Urban JF et al (2005) Trichuris suis therapy in Crohn's disease. Gut 54:87–90
70. Mombaerts P, Mizoguchi E, Grusby MJ et al (1993) Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell 75:274–282
71. Sadlack B, Merz H, Schorle H et al (1993) Ulcerative colitis-like disease in mice with disrupted interleukin-2 gene. Cell 75:253–261
72. Mizoguchi A, Mizoguchi E, Chiba C et al (1996) Role of appendix in the development of inflammatory bowel disease in TCR-alpha mutant mice. J Exp Med 184:707–715

About the Editors

Ronald R. Watson, Ph.D., attended the University of Idaho but graduated from Brigham Young University in Provo, Utah, with a degree in chemistry in 1966. He earned his Ph.D. in biochemistry from Michigan State University in 1971. His postdoctoral schooling in nutrition and microbiology was completed at the Harvard School of Public Health, where he gained 2 years of postdoctoral research experience in immunology and nutrition.

From 1973 to 1974 Dr. Watson was assistant professor of immunology and performed research at the University of Mississippi Medical Center in Jackson. He was assistant professor of microbiology and immunology at the Indiana University Medical School from 1974 to 1978 and associate professor at Purdue University in the Department of Food and Nutrition from 1978 to 1982. In 1982 Dr. Watson joined the faculty at the University of Arizona Health Sciences Center in the Department of Family and Community Medicine of the School of Medicine. He is currently professor of health promotion sciences in the Mel and Enid Zuckerman Arizona College of Public Health.

Dr. Watson is a member of several national and international nutrition, immunology, cancer, and alcoholism research societies. He is presently funded by the National Heart Blood and Lung Institute to study heart disease. In addition he has an NIH grant from NCCAM to study dietary supplements in modulating immune function and thus heart structure and function. For 30 years he was funded by Wallace Research Foundation to study dietary supplements in health promotion. Dr. Watson has edited more than 35 books on nutrition and other 53 scientific books. He has published more than 500 research and review articles.

Dr. Sherma Zibadi received her M.D. degree from the Mashad University of Medical School in 2001. Dr. Zibadi has recently completed her doctorate degree from the Department of Nutritional Sciences at the University of Arizona. Her main scholarly interest and experience involve the study of heart failure and its major risk factors such as hypertension, obesity and metabolic syndrome, finding ways to prevent the undesirable cardiac alternations and extent the healthy heart lifespan. Her current research focus lies in developing animal models of various forms of heart failure to study the potential mediators of cardiac remodeling process, which helps to identify new targets for treatment of heart failure. Dr. Zibadi's research also extends into alternative medicine, exploring the preventive and therapeutic effects of natural dietary supplements on heart failure and its major risk factors in both basic animal and clinical studies, translating lab research finding into clinical practice. Dr. Zibadi is the author of several full-length publications in prestigious journals and books, and manuscripts submitted for publication or in preparation dealing with the underlying mechanisms of heart failure and complementary medicine as novel therapeutics against heart disease.

Professor Victor R. Preedy is currently Professor of Nutritional Biochemistry in the Department of Nutrition and Dietetics, King's College London and Professor of Clinical Biochemistry in the Department of Clinical Biochemistry, King's College London. He is also Director of the Genomics Centre, Kings College London. Professor Preedy graduated in 1974 with a Degree in Biology and Physiology with Pharmacology. He gained his Ph.D. in 1981 in the field of Nutrition and Metabolism, specializing in protein turnover. In 1992 he received his Membership of the Royal College of Pathologists, based on his published works and in 1993 he gained a D.Sc. degree for his outstanding contribution to protein metabolism. At the time, he was one of the university's youngest recipients of this distinguished award. Professor Preedy was elected as a Fellow to the Royal College of Pathologists in 2000. Since then he has been elected as a Fellow to the Royal Society for the Promotion of Health (2004) and The Royal Institute of Public Health (2004). In 2009 he was elected as a Fellow of the Royal Society for Public Health. Professor Preedy has written or edited over 550 articles, which includes over 160 peer-reviewed manuscripts based on original research and 85 reviews and 30 books. He has a wide interested in health related matters, particularly nutrition and diet.

About the Series Editor

Dr. Adrianne Bendich is Director of Medical Affairs at GlaxoSmithKline (GSK) Consumer Healthcare where she is responsible for leading the innovation and medical programs in support of many well-known brands including TUMS and Os-Cal. Dr. Bendich had primary responsibility for GSK's support for the Women's Health Initiative (WHI) intervention study. Prior to joining GSK, Dr. Bendich was at Roche Vitamins Inc. and was involved with the groundbreaking clinical studies showing that folic acid-containing multivitamins significantly reduced major classes of birth defects. Dr. Bendich has co-authored over 100 major clinical research studies in the area of preventive nutrition. Dr Bendich is recognized as a leading authority on antioxidants, nutrition and immunity and pregnancy outcomes, vitamin safety and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich is the editor of ten books including "*Preventive Nutrition: The Comprehensive Guide For Health Professionals*, Fourth Edition" co-edited with Dr. Richard Deckelbaum, and is Series Editor of "*Nutrition and Health*" for Springer/Humana Press which contains 38 published volumes including Vitamin D, Second Edition edited by Dr. Michael Holick; "Iron Deficiency and Overload" edited by Dr. Shlomo Yehuda and Dr. David Mostofsky; "Nutrition Guide for Physicians" edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple and Dr. Mary Struble; "Nutrition and Metabolism" edited by Dr. Christos Mantzoros; "Fluid and Electrolytes in Pediatrics" edited by Leonard Feld and Dr. Frederick Kaskel; "Handbook of Drug-Nutrient Interactions" edited by Dr. Joseph Boullata and Dr. Vincent Armenti; "*Probiotics in Pediatric Medicine*" edited by Dr. Sonia Michail and Dr. Philip Sherman; " *Handbook of Nutrition and Pregnancy*" edited by Dr. Carol Lammi-Keefe, Dr. Sarah Couch and Dr. Elliot Philipson; "*Nutrition and Rheumatic Disease*" edited by Dr. Laura Coleman; "*Nutrition and Kidney Disease*" edited by Dr. Laura Byham-Grey, Dr. Jerrilynn Burrowes and Dr. Glenn Chertow; "*Nutrition and Health in Developing Countries*" edited by Dr. Richard Semba and Dr. Martin Bloem; "*Calcium in Human Health*" edited by Dr. Robert Heaney and Dr. Connie Weaver and "*Nutrition and Bone Health*" edited by Dr. Michael Holick and Dr. Bess Dawson-Hughes.

Dr. Bendich served as Associate Editor for "*Nutrition*" the International Journal; served on the Editorial Board of the *Journal of Women's Health and Gender-based*

Medicine, and was a member of the Board of Directors of the American College of Nutrition.

Dr. Bendich was the recipient of the Roche Research Award, is a Tribute to Women and Industry Awardee and was a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences, 2000–2001. In 2008, Dr. Bendich was given the Council for Responsible Nutrition (CRN) Apple Award in recognition of her many contributions to the scientific understanding of dietary supplements. Dr. Bendich holds academic appointments as Adjunct Professor in the Department of Preventive Medicine and Community Health at UMDNJ and has an adjunct appointment at the Institute of Nutrition, Columbia University P&S, and is an Adjunct Research Professor, Rutgers University, Newark Campus. She is listed in Who's Who in American Women.

Index